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Changes of species spectrum associated with Fusarium head blight caused by fungicides

Zmeny druhového spektra fuzariózy klasov spôsobené fungicídmi

Kamil HUDEC[1]*, Jana KIČINOVÁ[2] and Milan MIHÓK[3]

[1]Slovak Agricultural University, Department of Plant Protection, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic, *correspondence: kamil.hudec@uniag.sk

[2]Slovak Agricultural University, Department of Plant Protection, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic

[3]Slovak Agricultural University, Department of Plant Protection, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic

Abstract

Fusarium head blight (FHB) is a dangerous disease of cereals in case of yield losses and mycotoxin production. The aim of this work was to estimate the influence of used fungicides on species spectrum associated with Fusarium head blight (FHB). The study was realised in Slovakia on winter wheat by natural infection of FHB. The ears were sprayed with fungicides at beginning of flowering. After development of disease symptoms, fungicide efficacy was estimated, and *Fusarium* species spectrum were isolated and determined in laboratory. The fungicide efficacy against FHB achieved 60.2 – 88.3%. The highest effectivity was estimated by using of prothioconazole + tebuconazole and prothioconazole + fluoxastrobin, followed by dimoxystrobin + cyproconazole, metconazole, propiconazole + cyproconazole, and prochloraz + tebuconazole. *F. graminearum* was the most frequently isolated from the ears, followed by *M. nivale*, *F. avenaceum*, *F. culmorum*, and *F. poae*. Using of prothioconazole + tebuconazole, metconazole, prothioconazole + fluoxastrobin, and tebuconazole significantly decreased incidence of *F. graminearum* on infected ears. The incidence of *M. nivale* on ears was significantly increased by using of all fungicides in comparison with untreated control. The results suggest that the ear spraying by triazole fungicides could enhance the incidence of less susceptible *Fusarium* species in ears, especially *M. nivale*. The variability in fungicide efficacy of fungicides against FHB in agricultural praxis colud be affected besides multi-pathogen origin of FHB just by selective effect of fungicides on different *Fusarium* species.

Keywords: fungicides, Fusarium head blight, species spectrum, wheat

Abstrakt

Fuzarióza klasu je nebezpečná choroba spôsobujúca straty na úrode obilnín a kontamináciu zrna mykotoxínmi. Práca bola zameraná na hodnotenie vplyvu fungicídov na druhové spektrum fuzárií podieľajúcich sa na prirodzenej infekcii klasov pšenice. V práci bolo hodnotených šesť fungicídov, ktoré boli v poraste pšenice aplikované na začiatku kvitnutia klasov. Po rozvinutí infekcie boli vyhodnotené symptómy fuzariózy klasov, fungicídna účinnosť testovaných prípravkov a vyizolované spektrum fuzárií. Účinnosť testovaných fungicídov proti fuzarióze klasu sa pohybovala v rozpätí 60,2 – 88,3%. Najúčinnejšie boli fungicídy na báze prothioconazole + tebuconazole spolu s prothioconazole + fluoxastrobin, nasledované fungicídmi dimoxystrobin + cyproconazole, metconazole, propiconazole + cyproconazole, prochloraz + tebuconazole. *F. graminearum* bolo najfrekventovanejším pôvodcom fuzariózy klasu v obidvoch rokoch. Ostatné druhy boli podľa klesajúcej frekvencie výskytu zoradené nasledovne: *M. nivale*, *F. avenaceum*, *F. culmorum*, *F. poae*. Aplikácia fungicídov prothioconazole + tebuconazole, metconazole, prothioconazole + fluoxastrobin a tebuconazole preukazne eliminovala z ošetrených a napadnutých klasov výskyt *F. graminearum*. Výskyt *M. nivale* v napadnutých klasoch v porovnaní s neošetrenou kontrolou preukazne zvýšilo použitie všetkých testovaných fungicídov. Výsledky poukazujú na to, že aplikácia triazolových fungicídov môže v napadnutých klasoch pšenice zvyšovať výskyt tých druhov z rodu *Fusarium* resp. *Microdochium*, ktoré sú na použité fungicídy menej citlivé, najmä *M. nivale*. Vzhľadom na široké druhové spektrum pôvodcov fuzariózy klasu sa ukazuje, že rozdielna citlivosť jednotlivých druhov na fungicídy a multipatogénne pozadie choroby sú významné faktory, ovplyvňujúce variabilnú účinnosť fungicídov proti tejto chorobe v poľných podmienkach.

Kľúčové slová: druhové spektrum, fungicídy, fuzarióza klasu, pšenica

Detailný abstrakt

Fuzarióza klasu je nebezpečná choroba spôsobujúca straty na úrode obilnín a kontamináciu zrna mykotoxínmi. Práca bola zameraná na hodnotenie vplyvu fungicídov na druhové spektrum fuzárií podieľajúcich sa na prirodzenej infekcii klasov pšenice. Poľný pokus bol realizovaný v lokalite Nitra v rokoch 2011-2012 na pšenici ozimnej, odroda Panonia, pestovanej klasickou konvenčnou metódou. V pokuse bolo hodnotených šesť fungicídov (Prosaro 250 EC - prothioconazole + tebuconazole – 0,8 l*ha^{-1}, Fandango 200 EC - fluoxastrobin + prothioconazole – 0,75 l*ha^{-1}, Caramba - metconazole – 1,5 l*ha^{-1}, Swing Top – dimoxystrobin+ cyproconazole – 0,8 l*ha^{-1}, Artea Plus - propiconazole + cyproconazole – 0,5 l*ha^{-1}, Zamir 40 EW - prochloraz + tebuconazole – 1 l*ha^{-1}), ktoré boli v poraste pšenice aplikované na začiatku kvitnutia klasov. Po rozvinutí infekcie boli vyhodnotené symptómy fuzariózy klasov a rozsah napadnutia klasov, prepočítaný na fungicídnu účinnosť testovaných prípravkov. Z klasov boli odobraté vzorky napadnutých pliev so sporuláciou patogéna na neskoršiu laboratórnu analýzu, na základe ktorej bolo vyizolované a stanovené spektrum fuzárií v každom variante.

Účinnosť testovaných fungicídov proti fuzarióze klasu sa pohybovala v rozpätí 60,2 – 88,3%. Najúčinnejšie boli fungicídy na báze prothioconazole + tebuconazole spolu s prothioconazole + fluoxastrobin, nasledované fungicídmi dimoxystrobin + cyproconazole, metconazole, propiconazole + cyproconazole, and prochloraz + tebuconazole. *F. graminearum* bolo najfrekventovanejším pôvodcom fuzariózy klasu v obidvoch rokoch. Ostatné druhy sú podľa klesajúcej frekvencie výskytu zoradené nasledovne: *M. nivale*, *F. avenaceum*, *F. culmorum*, *F. poae*. Aplikácia fungicídov prothioconazole + tebuconazole, metconazole, prothioconazole + fluoxastrobin a tebuconazole preukazne eliminovala z ošetrených a napadnutých klasov výskyt *F. graminearum*. Výskyt *M. nivale* v napadnutých klasoch v porovnaní s neošetrenou kontrolou preukazne zvýšilo použitie všetkých testovaných fungicídov.

Vplyv fungicídov na úrodu bol preukazný iba v roku 2011, kedy bol kvôli vyššiemu úhrnu zrážok oproti roku 2012 zaznamenaný vyšší infekčný tlak listových a klasových chorôb. Všetky fungicídy v roku 2011 preukazne zvýšili úrodu zrna v nasledovnom zostupnom poradí: dimoxystrobin + cyproconazole, prothioconazole + fluoxastrobin, metconazole, propiconazole + cyproconazole, prothioconazole + tebuconazole, prochloraz + tebuconazole. HTZ bolo preukazne zvýšené iba v roku 2011 pri použití fungicídu na báze účinných látok propiconazole + cyproconazole.

Výsledky poukazujú na to, že aplikácia triazolových fungicídov môže v napadnutých klasoch pšenice zvyšovať výskyt tých druhov z rodu *Fusarium* resp. *Microdochium*, ktoré sú na použité fungicídy menej citlivé, najmä *M. nivale*. Vzhľadom na široké druhové spektrum pôvodcov fuzariózy klasu sa ukazuje, že rozdielna citlivosť jednotlivých druhov na fungicídy a multipatogénne pozadie choroby sú významné faktory, spôsobujúce variabilnú účinnosť fungicídov proti tejto chorobe v poľných podmienkach.

Introduction

Fusarium head blight (FHB) is a serious disease causing yield losses and mycotoxin contamination in wheat and other cereals (Beyer et al., 2010). Wheat heads are particularly sensitive to infections by *Fusarium* species at anthesis, when rainfall and moderate temperatures coincide (Klix et al., 2008). During the head and kernel colonization, *Fusarium* species produce toxic secondary metabolites - mycotoxins with negative effects on the other organisms (Bottalico and Perrone, 2002). Deoxynivalenol (DON) is the mycotoxin that was observed at the highest frequency and at the highest concentration in wheat samples (Schollenberger et al., 2002). National and European legislative institutions laid down maximum tolerable levels of selected mycotoxins in wheat and maize products to protect consumers from health risks associated with mycotoxins (European Commission, 2006). Since *Fusarium* damaged kernels are largely responsible for the total DON content of a grain lot, their detection is an issue of food safety (Oerke et al., 2010).

There are several ways to reduce the level of mycotoxins in wheat kernels. Growing wheat cultivars with a low susceptibility towards FHB, using a mouldboard plough, avoiding maize as previous crop (Blandino et al., 2010) and triazole fungicide application at flowering were reported to be effective (Beyer et al., 2006). However, when weather conditions are favourable for infection, mycotoxin accumulation cannot be avoided completely (Yoshida and Nakajima, 2010). Generally, relationships between FHB and DON are characterized by large variability (Kriss et al., 2010), impeding a precise estimation of DON contents from disease severity parameters (Paul et al., 2006).

Fungicides are effective way to control Fusarium head blight, but their efficacy is limited (Ban and Suenaga, 2000). According Milus and Parsons (1994), some active ingredients don't achieve sufficient efficacy in reduction of Fusarium head blight. However, some other fungicides provided high effect against FHB (Homdork et al., 2000). It was found out, that some fungicides have the opposite effect on the production of mycotoxins than on fungal growth. For example, from in vitro test of effect active ingredient tridemorph on T2 toxin prodiction by *Fusarium sporotrichioides*. Results showed, that although higher concentration inhibited mycelial growth, content of T2 toxin increased five times (Pirgozliev et al., 2002).

Based on previous studies (Hudec and Roháčik, 2003) it could be hypothesized that fungicides applied against FHB have influence on associated *Fusarium* species spectrum in ears and kernels. The aim of this study was to estimate the relationship between fungicide efficacy and developed species spectrum on treated ears and kernels of winter wheat.

Materials and methods

Field experiment

The field experiment was conducted in Nitra locality during years 2011-2012. Winter wheat (cv. Panonia, susceptible to FHB infection) was grown under common conventional technology, the size of variants was 20 m^2. The experimental arrangement of variant fields was the Randomised complete blocks design. In particular variants (excepting control variant), six fungicides (Prosaro 250 EC - prothioconazole + tebuconazole – 0.8 l*ha^{-1}, Fandango 200 EC - fluoxastrobin + prothioconazole – 0.75 l*ha^{-1}, Caramba - metconazole – 1.5 l*ha^{-1}, Swing Top – dimoxystrobin + cyproconazole – 0.8 l*ha^{-1}, Artea Plus - propiconazole + cyproconazole – 0.5 l*ha^{-1}, and Zamir 40 EW - prochloraz + tebuconazole – 1 l*ha^{-1}) were applied in both of the years at the beginning of flowering (BBCH 61). The artificial infection was not used. The FHB occurrence was evaluated in the growth stages BBCH 77-87, after symptoms development. The degree of FHB damage as an index of FHB (I_{FHB}) and fungicide efficacy (FE) was expressed according to Haidukowski et al. (2005). The harvested yield from each variant was analysed for moisture, weight, yield (t*ha^{-1}), and weight of thousand seeds. The results obtained were tested by Analysis of variance (ANOVA), Tukey test, P=0.05.

Sampling of heads and identification of *Fusarium* species

From each variant, 4 spikelets from 50 heads infected by FHB were separated in growth stage BBCH 77-87 in the field, during FHB symptoms assessment. Next the spikelets were air dried and stored under laboratory conditions (22 °C) in paper bags for later laboratory analysis.

The spikelets were surface sterilized with NaOCl (3% for 3 min), rinsed twice with sterile water, dried on filter paper, transferred onto PDA and incubated for 7 days at 21 ± 2 °C, under 12/12 photoperiod to record the percentage of kernels infected by *Fusarium* spp. Fungal cultures were separated and transferred to PDA and SNA to obtain pure cultures for the morphological identification of species according to Nelson et al. (1983). The isolation frequency (IF) was estimated for each variant (Gonzalez et al., 1999), related to year and fungicide treatment. FHB index (IFHB) was calculated by formula = [incidence (%) × severity (%)]/100 (Wegulo et al., 2011), in control variants. The fungicide efficacy (FE) was calculated by classical Abbott formula:

$$FE\ (\%) = \frac{(DEA_C - DEA_T)}{DEA_C} \times 100$$

Where DEA_C = diseased ear area in control variant (%); DEA_T = diseased ear area in treated variant (%) (Gea et al., 2010). FE was calculated for each variant, based on estimation of 50 ears, randomly selected from each field.

Results

The symptoms of FHB (ear bleaching with pink sporulation) appeared two-three weeks after flowering. The FHB damage in control was higher in 2011 (I_{FHB} = 1.76%) than in year 2012 (I_{FHB} = 0.15%). The fungicide efficacy (Table 1) against FHB was the highest by using of prothioconazole + tebuconazole and prothioconazole + fluoxastrobin, followed by dimoxystrobin + cyproconazole, metconazole, propiconazole + cyproconazole, and prochloraz + tebuconazole. The fungicide efficacy against FHB varied between 60.2 – 88.3%.

Mycological analysis of infected ears in untreated control showed, that *Fusarium* species spectrum was very different in both the years. Eight *Fusarium* species were associated with FHB on heads in year 2011 - *F. graminearum*, *M. nivale*, *F. culmorum*, *F. avenaceum*, *F. poae*, *F. sporotrichioides*, *F. semitectum*, and *F. equiseti*. The species spectrum was narrower in year 2012, only five species were isolated: *F. graminearum*, *M. nivale*, *F. avenaceum, F. culmorum. F. poae*
F. graminearum was the most frequent species in both the evaluated years, followed by *M. nivale*, *F. avenaceum*, *F. culmorum*, and *F. poae*.

Table 1. Fungicide efficacy against FHB, winter wheat, cv. Panonia, Nitra, 2011-2012

Tabuľka 1. Účinnosť fungicídov proti fuzarióze klasu, ozimná pšenica, odroda Panonia, Nitra, 2011-2012

Variant Variant	FE – FHB	
	2011	2012
Control Kontrola	-	-
Prothioconazole + tebuconazole	77.3	85.5
Prothioconazole + fluoxastrobin	70.4	88.3
Metconazole	65.1	72.2
Dimoxystrobin + cyproconazole	68.3	75.2
Propiconazole + cyproconazole	62.6	71.7
Prochloraz + tebuconazole	60.2	73.6

FE – FHB – fungicide efficacy (%) against Fusarium head blight on ears.
FE – FHB – fungicídna účinnosť (%) proti fuzarióze klasu.

The changes of species spectrum on ears were recorded in certain variants (Table 2). The other species which are not included into the table 2 were occurred in very low extent. Significant decreasing of occurrence of *F. graminearum* was recorded by 3 fungicides in year 2011 - prothioconazole + tebuconazole, metconazole, and prothioconazole + fluoxastrobin. Occurrence of *F. graminearum* was lower on fungicide treatments in year 2012. All fungicide treatments significantly eliminated *F. graminearum* from infected heads in year 2012. The occurrence of *M. nivale* was very rare in year 2012, but the species was the second most frequent in the year 2011. Occurrence of *M. nivale* in heads was significantly increased by all fungicides. The occurrence of other non-mentioned species was low and the influence of fungicides on their IF was not significant.

Influence of fungicide treatments on yield is shown in Table 3. In year 2011, all fungicides significantly increased the yield, in downward order: dimoxystrobin + cyproconazole, prothioconazole + fluoxastrobin, metconazole, propiconazole + cyproconazole, prothioconazole + tebuconazole, prochloraz + tebuconazole. The weight of thousand seeds (WTS) was significant increased by propiconazole + cyproconazole only. In year 2011, the yield and WTS was not significantly influenced by any fungicide treatment.

Table 2. Influence of fungicide treatments on occurrence of associated *Fusarium* and *Microdochium* species in heads with FHB symptoms, winter wheat, cv. Panonia, Nitra, BBCH 77-87, 2011-2012

Tabuľka 2. Vplyv fungicídov na výskyt druhov z rodu *Fusarium* a *Microdochium* asociovaných s fuzariózou klasu, ozimná pšenica, odroda Panonia, Nitra, BBCH 77-78, 2011-2012

Treatments Ošetrenie	Isolation frequency (%) of *Fusarium (Microdochium)* species Frekvencia izolátov (%) druhov Fuzarium *(Microdochium)*									
	F. graminearum		*M. nivale*		*F. avenaceum*		*F. culmorum*		*F. poae*	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
Control Kontrola	82.6^{a}	91.5^{a}	21.4^{c}	-	2.1^{a}	3.1^{a}	8.6^{c}	2.1^{a}	-	-
Prothioconazole + tebuconazole	22^{c}	11.6^{c}	72.1^{a}	3.2^{a}	-	-	8.2^{c}	-	10.6^{a}	10.1^{a}
Prothioconazole + fluoxastrobin	34.5^{b}	12.1^{c}	65.2^{a}	-	-	-	48.2^{a}	-	-	-
Metconazole	29.2^{c}	-	86.2^{a}	1.9^{a}	3.2^{a}	-	25^{b}	4.2^{a}	-	8.3^{a}
Dimoxystrobin + cyproconazole	75.6^{a}	19.5^{b}	42.1^{b}	-	-	4.6^{a}	8.4^{c}	-	3.2^{a}	-
Propiconazole + cyproconazole	73.3^{a}	11.8^{c}	58.2^{b}	2.1^{a}	4.5^{a}	-	7.5^{c}	-	8.5^{a}	-
Prochloraz + tebuconazole	76.2^{a}	13.3^{c}	79.2^{a}	-	-	-	-	-	-	-

Differences between values signed by the same letter are not significant (Analysis of variance (ANOVA), Tukey test, P=0.05).
Rozdiely medzi hodnotami označenými rovnakým písmom nie sú štatisticky významné (Analýza rozptylu (ANOVA), Tukey test, P=0,05).

Table 3. Influence of fungicide treatment on yield and weight of thousand seeds, winter wheat, cv. Panonia, Nitra, 2011-2012

Tabuľka 3. Vplyv fungicídov na úrodu a hmotnosť tisíc zŕn, ozimná pšenica, odroda Panonia, Nitra, 2011-2012

Treatments Ošetrenie	2011		2012	
	Yield ($t*a^{-1}$)	WTS (g)	Yield ($t*ha^{-1}$)	WTS (g)
Control Kontrola	6.32[b]	35.8[b]	5.19[a]	36.4[a]
Prothioconazole + tebuconazole	8.21[a]	40.9[ab]	6.12[a]	39.1[a]
Prothioconazole + fluoxastrobin	8.62[a]	4.3[ab]	6.21[a]	40.2[a]
Metconazole	8.24[a]	41.1[ab]	6.32[a]	38.9[a]
Dimoxystrobin + cyproconazole	8.91[a]	40.1[ab]	6.36[a]	39[a]
Propiconazole + cyproconazole	8.22[a]	41.3[a]	6.56[a]	40.9[a]
Prochloraz + tebuconazole	8.16[a]	40.2[ab]	6.12[a]	38.7[a]

Differences between values signed by the same letter are not significant (Analysis of variance, Tukey test, P=0.05); WTS – weight of thousand seeds.
Rozdiely medzi hodnotami označenými rovnakým písmom nie sú štatisticky významné (Analýza rozptylu, Tukey test, P = 0,05); HTZ – hmotnosť tisíc zŕn.

Table 4. Average temperature and rainfall in Nitra, 2011-2012

Tabuľka 4. Priemerná teplota a zrážky, Nitra, 2011-2012

Month Mesiac	Average temperature (°C) Priemerná teplota (°C)			Sum of rainfall per month (mm) Úhrn zrážok za mesiac (mm)		
	2011	2012	LTA	2011	2012	LTA
April Apríl	12.6	13.6	10.1	16	13.2	39
May Smieť	15.7	17.2	15	59.3	17.5	61
June Jún	19.5	20.6	17.9	99.6	54.5	66
July Júl	19.6	22.8	19.5	73.9	100.6	51

LTA – long-time average.
LTA – dlhodobý priemer.

Discussion

The natural incidence of FHB was higher in year 2011 than in 2012. The level of FHB in European countries can reach much more than 2% in certain years (Butkute et al., 2008). The species spectrum of the species associated with FHB was broad, whereby *F. graminearum*, *F. culmorum*, *F. avenaceum* a *M. nivale* were the most dominant, which is in agreement with findings of other authors (Blandino et al., 2006; Klix et al., 2008; Müllenborn et al., 2008). A great difference in *M. nivale* occurrence in certain areas is typical for cool and rainy years. It corresponds with results, because the June (time of FHB development) 2011 was more rainy and cool than June 2012 (Table 4). The variable occurrence of *M. nivale* was observed in other studies too and *M. nivale* could be the main pathogen of FHB in some years (Mesterházy et al., 2003).

The FE-FHB of certain fungicides was similar in both years which showed higher efficacy in year 2012. The most effective were fungicides containing prothioconazole and tebuconazole. It is not in agreement with Butkute et al. (2008) who found out good FE by strobilurins. Other authors (Siranidou and Buchenauer, 2001; Cromey et al., 2001) recommended only triazole fungicides against FHB. Otherwise several authors observed suitable FE-FHB of strobilurins (Simpson et al., 2004; Mesterházy et al., 2003), but only in some years and certain conditions, especially by low infection pressure. The seasonal variability in fungicide efficacy could be caused by changing of species spectrum, which caused the FHB symptoms (Blandino et al., 2006). While *F. graminearum* was the most dominant species, *M. nivale* was the second most dominant species in year 2011. It is known, that triazole fungicides have the best efficacy against *Fusarium* spp., while the efficacy against *M. nivale* is lower and vice versa (Mesterházy, 2003). This assumption was confirmed by results of this study, because used fungicides significantly influenced the species spectrum isolated from infected heads in treated variants. The results showed that application of fungicides could increase the occurrence of less susceptible species – in this experiment *M. nivale* namely. Therefore the multi-pathogen origin of FHB could be one of the most important factors causing insufficient or variable FE in agricultural practice.

The influence of fungicide treatment on yield was different in both the years. Particular increasing of yield was not in correspondence with FE-FHB. In the years with low infection pressure of leaf and hed pathogens, the yield used to be increased by strobilurin green effect (Müllenborn et al., 2008).

Conclusions

In wheat crops of Slovakia, the natural incidence of FHB is variable in different years. Achieved level of infection is equivalent to natural infection, which usually oscillate about 2 - 5% of heads. The species spectrum associated with FHB used to be broad, whereby *F. graminearum* is the most dominant species followed by *M. nivale*, *F. avenaceum*, *F. culmorum*, and *F. poae*. A great difference in *M. nivale* occurrence in certain areas is typical for cool and rainy years. The efficacy of tested fungicides against FHB varied between 60.2 – 88.3%. The most effective was prothioconazole + tebuconazole with prothioconazole + fluoxastrobin, followed by dimoxystrobin +

cyproconazole, metconazole, propiconazole + cyproconazole, and prochloraz + tebuconazole. The seasonal variability in fungicide efficacy could be caused by changing of species spectrum, which caused the FHB symptoms. Fungicide treatment by prothioconazole + tebuconazole, metconazole, prothioconazole + fluoxastrobin, tebuconazole significantly eliminated *F. graminearum* from infected heads. Occurrence of *M. nivale* in heads was significantly increased by all fungicides in comparison with untreated control. The results showed that the application of triazole fungicides could increase the occurrence of less susceptible species in ears, especially *M. nivale*. The multi-pathogen origin of FHB and selective efficacy of fungicides could be one of the most important factors causing variable efficacy in agricultural practice.

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