

TIMELY FUNGICIDE APPLICATION: A STRATEGY TO MINIMIZE FUSARIUM HEAD BLIGHT AND ASSOCIATED MYCOTOXIN PRODUCTION IN WINTER WHEAT

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SUMMARY

Re-emergence of *Fusarium* head blight (FHB) on wheat should be taken into account in the global management of cropped fields, especially with respect to fungicide application schemes, due to harmful toxin production. The aim of this study was to assess, in three experimental fields representative of the various topoclimatological zones of Luxembourg, the impact of timing of fungicide spray application on the prevalence and severity of FHB, the concentration of mycotoxins, and *Fusarium* strain pattern in winter wheat. It was found that fungicide treatments and the time of application had a significant impact on the amount of deoxynivalenol (DON) detected ($P=0.027$, ANOVA). In our experimental design, the application of fungicides at 3 different times increased the amount of DON in winter wheat compared to two and single applications. The importance of the timing of fungicide application is discussed in relation to limiting toxin contamination in the field.

Key words: *Fusarium* spp., deoxynivalenol, cereals, chemical treatments

INTRODUCTION

Fungi of the genus *Fusarium* are of great economic significance due to their widespread occurrence and high pathogenicity to all crop species grown throughout the world. *Fusarium* head blight (FHB), mainly caused by *Fusarium avenaceum*, *F. culmorum*, *F. graminearum*, and *F. poae* can be devastating, with an overall decrease in yield reaching 70%. In addition, toxic secondary metabolites produced by *Fusarium* species can be present in contaminated FHB-affected grain (Bullerman and Bianchini, 2007; Dexter *et al.*, 1997; Pirgozliev *et al.*, 2003). The most common among them are trichothecenes, mostly of type B, which include deoxyni-

valenol (DON), nivalenol (NIV) and zearalenone (ZON). The above substances exhibit very strong phytotoxic and zootoxic effects (Gutleb *et al.*, 2002; Rotter *et al.*, 1996; Minervini *et al.*, 2004).

FHB has been increasing in incidence and severity in recent years, due to the implementation of simplified crop rotation (in particular with respect to wheat and maize production), the lack of effective fungicide control for *Fusarium*, thus resulting in the development of resistant strains and the absence of crop varieties resistant to the disease.

Fungicide control of FHB has proved non-constant, and conflicting evidence exists regarding the effect of mycotoxin accumulation in grains contaminated by *Fusarium* spp. (Pirgozliev *et al.*, 2002). Previous studies from Luxembourg showed that type B trichothecene contamination frequently occurs in winter wheat (Giraud *et al.*, 2010) and could be predicted by genetic chemotyping (Pasquali *et al.*, 2010).

The aim of the present study was to assess the impact of the time of fungicide spray application on the concentration of mycotoxins in winter wheat, i.e. in the grains at harvest time. Efficacy of fungicide treatments as measured by FHB prevalence and the occurrence of *Fusarium* mycotoxins were assessed in three experimental fields. In addition, changes in *Fusarium* population composition were investigated by morphological and molecular methods.

MATERIAL AND METHODS

Three fungicide treatments were tested in each of three experimental sites (Burmerange, Reuler and Christnach), representative of the three topoclimatological zones of Luxembourg (south, north and center, respectively).

Treatments were assigned to experimental units, using a randomized complete block split-plot design with four replications for each sub-plot (each site was composed of 16 experimental units). Each sub-plot was 12 m² in size and wheat was harvested in early to mid-July in the southern site (Burmerange) and at the beginning of August in the northern site (Reuler), with a cereal

Table 1. Fungicide treatment and spray application carried out in 2007 in the three experimental sites of Luxembourg. Growing stages were defined according to the BBCH scale (BASF, Bayer, Ciba-Geigy and Hoechst, Landshare *et al.*, 1991).

Exp. code	Stages of fungicide application	Fungicide treatment	Burmerange		Christnach		Reuler	
			Growth stage	Date	Growth stage	Date	Growth stage	Date
T 0	control	no fungicide application						
T 1	EC 59	1.6 l/ha Input pro set + 1l/ha Bravo	62	23/05/07	57	23/05/07	65	05/06/07
T 2	EC31	0.75l/ha Opus team + 1l/ha Bravo	31+	17/04/07	31	17/04/07	30+	18/04/07
	EC59	1.6 l/ha Input pro set + 1 l/ha Bravo	62	23/05/07	57	23/05/07	65	05/06/07
T 3	EC31	0.7l/ha Stereo + 1 l/ha Bravo	31+	17/04/07	31	17/04/07	30+	18/04/07
	EC37	1.6 l/ha Input pro set + 1 l/ha Bravo	37	03/05/2007	37	03/05/07	37	10/05/07
	EC59	0.75l/ha Opus team + 1 l/ha Bravo	62	23/05/2007	57	23/05/07	65	05/06/07

plot combine harvester. Patterns of fungicide treatment were associated with wheat growth stages, and the products used were commercially available (Table 1).

The products Input pro set, Bravo, Opus team, and Stereo contained the active ingredients prothioconazole (250g/l) and spiroxamine (500g/l); chlorothalonil (500g/l); epoxiconazole (84g/l) and fenpropimorphe (250g/l); and cyprodinil (250g/l) and propiconazole (62.5g/l), respectively. Fungicide treatments were not only oriented toward FHB but were aimed also at controlling other leaf and ear diseases.

Prevalence (percentage of infected spikes) and severity (percentage of infected kernels per spike) of the disease, species determination, DON, NIV and ZON quantifications were assessed according to Giraud *et al.* (2010) and Pasquali *et al.* (2010).

Statistical analyses were carried out using SPSS 16.0 (Chicago, USA). Normality of data was tested with Q-Q plots and Kolmogorov-Smirnoff tests, equality of variance by Box-plots and Levene's test. For further analyses, data were transformed by the root mean square operation. Comparison of prevalence and severity between different areas was carried out in a linear mixed model using prevalence, severity, and DON concentration, as dependent variables, and location and field site as independent variables. In a second model, differences between the timing of spray application and *Fusarium* strain composition were assessed for the various locations, with the species *Fusarium* as the dependent vari-

able and treatment and place as the observed variable. Post-hoc tests (Tukey's) were carried out given significant results following the Fisher-F test. *P*-values below 0.05 (2-sided) were considered as significantly different. For correlations between previous crops and FHB, Spearman correlation coefficients were obtained. Unless otherwise stated, all values represent mean±SD (standard deviation).

RESULTS AND DISCUSSION

FHB prevalence was significantly different between locations since, considering all treatments, Christnach (center) showed the highest prevalence, followed by Reuler (north) and Burmerange (south, $P < 0.001$, Fisher-F-Test). (Table 2).

Severity (Table 3) was likewise significantly different between the locations: considering all treatments. Severity was significantly higher in Burmerange (south) compared with both Reuler and Christnach ($P < 0.001$).

Toxin analyses revealed that 100% of the investigated experimental fields were contaminated by DON, with a range of 261-1,588 µg/kg. All three sites were significantly different from one another (Table 4), with Christnach (center) showing the highest contamination, followed by Burmerange (south) and Reuler (north).

Chemical treatments and timing of spray application had a significant impact on the amount of DON detect-

Table 2. Prevalence (% infected wheat spikes) in the 3 experimental sites for 2007. The data are based on the mean of 16 assessments for each condition (4 observations for one replication, 4 replications for one condition).

Experiment code	Time of spray application	Prevalence (mean ± SD)		
		Reuler	Christnach	Burmerange
T 0	Control	10.6±4.4	24.6±21.8	0.9±0.9
T 1	59	8.2±3.4	16.4±17.3	1.1±0.8
T 2	31 + 59	5.9±2.6	9.5±8.3	0.5±0.6
T 3	31+37+59	7.8±3.6	13.2±11.9	0.7±0.8

Table 3. Severity (% infected grains per spike) in the 3 experimental sites for 2007. The data was based on the mean of 16 assessments for each condition (4 observations for one replication, 4 replications for one condition).

Experiment code	Time of spray application	Severity (mean ± SD)		
		Reuler	Christnach	Burmerange
T 0	Control	21.6±10.8	20.4±13.8	31.3±36.4
T 1	59	21.4±10.1	18.3±11.3	40.3±34.8
T 2	31 + 59	18.8±9.0	11.2±8.4	27.1±40.3
T 3	31+37+59	22.1±10.9	15.4±11.0	28.2±36.4

Table 4. DON content determined in wheat samples collected from the 3 sites. Data based on means of 3 independent wheat sample analyses. Level of quantification (LOQ) was 76µg/kg.

Experiment code	Location	DON (µg/kg dry weight)					
		Reuler		Christnach		Burmerange	
	Time of spray application	Average	Range	Average	Range	Average	Range
T 0	Control	261	(218-299)	1588	(933-2657)	783	(615-968)
T 1	59	136	(65-265)	808	(425-1134)	614	(479-853)
T 2	31 + 59	283	(92-565)	1465	(818-1919)	693	(493-1074)
T 3	31+37+59	398	(167-871)	934	(432-1244)	857	(571-1253)

ed ($P=0.027$, ANOVA). The single treatment (EC59) showed a trend toward lower DON concentrations compared with the untreated plots (control, $P=0.096$), and differed significantly from the three applications ($P<0.05$). NIV was only detected in one location, Christnach (Table 5). The highest concentration was detected in the plots where three applications were employed. ZON was not detected.

Results of the morphological and molecular identification showed that the most common species isolated from diseased winter wheat spikes collected in the three experimental sites (Fig. 1) were *F. avenaceum* (41.3%) *F. culmorum* (37.9%), *F. graminearum* (16.4%) and *F. poae*

(4.4%). *F. avenaceum* is known for its ability to produce moniliformine while the others species detected are known to be potential trichotecenes producers (e.g. DON and NIV).

The distribution of *Fusarium* species varied strongly from location to location. *F. avenaceum* was the predominant isolated species in Reuler (68.9%) while *F. culmorum* was the strain with the highest incidence in Christnach and in Burmerange (45.4% and 48.3%, respectively). *F. graminearum* was significantly more prevalent (%) in Christnach compared with other locations ($P<0.001$). In terms of treatment, the control resulted in the highest prevalence of *F. graminearum*

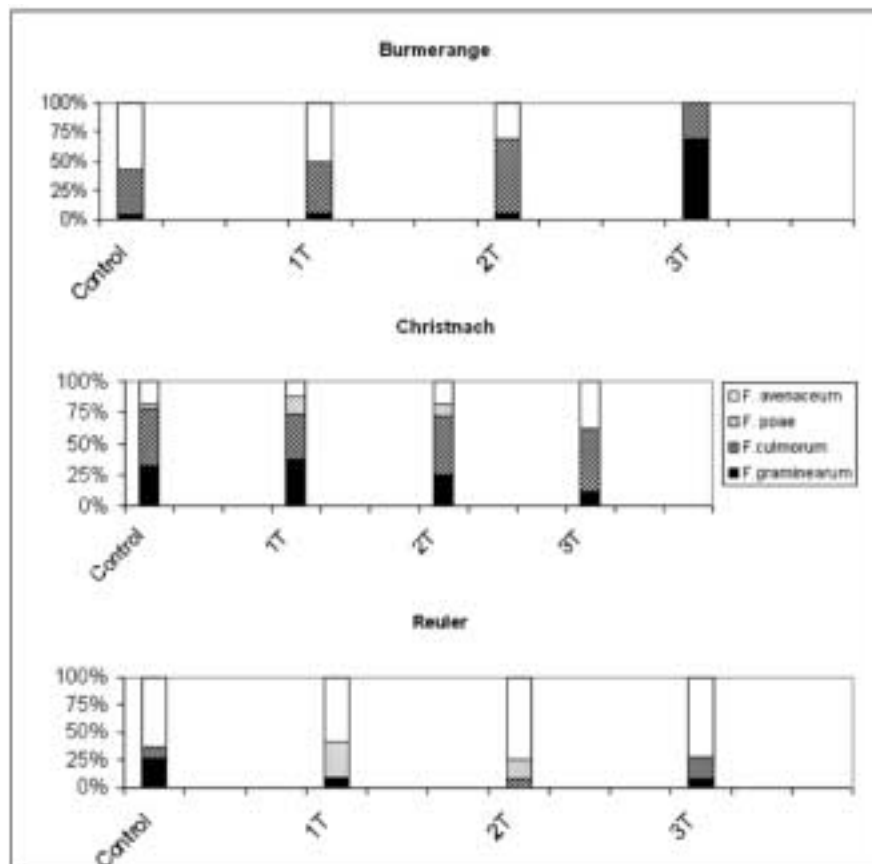
**Fig. 1.** Percentage of species found in each site and parcel according to the 4 treatments (control, 1T for a single treatment at GS 59, 2T for 2 treatments at GS 31 and GS 59 and 3T for 3 treatments at GS 31, GS 37 and GS 59).

Table 5. NIV content determined in wheat samples collected from the Christnach site. Data based on means of 6 analyses. Level of quantification or LOQ was 76 µg/kg (determined for DON and estimated for NIV) in Luxembourg. Even though a trend was found for differences between various treatments effecting NIV ($P=0.061$), this did not reach significance.

Experiment code	Time of spray application	NIV (µg/kg)	
		Average	Range
T 0	Control	409	(237-569)
T 1	59	308	(194-490)
T 2	31 + 59	279	(126-421)
T 3	31+37+59	445	(351-559)

($F<0.001$) compared with all other groups, which differed not significantly. *F. culmorum* was more prevalent in Burmerange, compared to Christnach and Reuler, with all three places being significantly different from one another ($P<0.05$). Treatment 2 resulted in highest prevalence of *F. culmorum*, significantly different from all other treatments, including the control (Fig. 2), with the sequence 2, control, 1, 3. *F. poae* was most prevalent in Christnach followed by Burmerange followed by Reuler, with all three locations being significantly different from one another ($P<0.005$). Treatment 2 was associated with the highest prevalence of this species, followed by treatment 1, control and 3, with only the latter two being non-significantly different from one another, while all others were ($P<0.005$).

Changes in the composition of the *Fusarium* population were observed according to the number of fungicide applications sprayed in the experimental sites. In Burmerange, three applications significantly increased the percentage of *F. graminearum*, while this type of treatment seemed to favor the *F. culmorum* population in Christnach. In these two locations, the three treatments increased the proportion of fungi with the ability to produce trichothecenes. In the north (Reuler), the situation was different for the treatment applied at three stages increased significantly the proportion of isolated *F. avenaceum*, which does not produce trichotecene mycotoxins.

In our experimental design, one surprising result was the negative impact of three applications on the amount of DON in winter wheat. A similar observation has been detected in the case of a NIV contamination although it was not fully significance. A general possible explanation is that the repeated, multiple treatments increase the pathogen's stress, resulting in a higher toxicogenic response (Reverberi *et al.*, 2010) as recently observed in the laboratory (Audenaert *et al.*, 2010).

In conclusion, the results have shown that multiple treatments at several growth stages could result in increased infection by *Fusarium* species, resulting also in increased DON production. However, specific influences due to different region, such as climate, gave vari-

able results with respect to the impact of fungicide application and the effect of *Fusarium* strain population. It is apparent that management strategies based on fungicide application should take into account also the effect that chemical treatments may have on toxin induction by *Fusarium* species.

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