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 deoxynivalenol (DON), nivalenol (NIV) and zearalenone (ZON). The above substances exhibit very strong phytotoxic and zootoxic effects (Gutleb et al., 2002; Rotter et al., 1996; M inervini 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-Giegy and Hoechst, Landshare et al., 1991).

<table>
<thead>
<tr>
<th>Exp. code</th>
<th>Stages of fungicide application</th>
<th>Fungicide treatment</th>
<th>Burmerange</th>
<th>Christnach</th>
<th>Reuler</th>
</tr>
</thead>
<tbody>
<tr>
<td>T 0</td>
<td>control</td>
<td>no fungicide application</td>
<td>62</td>
<td>57</td>
<td>65</td>
</tr>
<tr>
<td>T 1</td>
<td>EC 59</td>
<td>1.6 l/ha Input pro set + 1l/ha Bravo</td>
<td>31</td>
<td>17/04/07</td>
<td>30+</td>
</tr>
<tr>
<td>T 2</td>
<td>EC31</td>
<td>0.75l/ha O per team + 1l/ha Bravo</td>
<td>62</td>
<td>23/05/07</td>
<td>65</td>
</tr>
<tr>
<td>T 3</td>
<td>EC37</td>
<td>1.6 l/ha Input pro set + 1l/ha Bravo</td>
<td>37</td>
<td>03/05/07</td>
<td>37</td>
</tr>
</tbody>
</table>

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).

<table>
<thead>
<tr>
<th>Experiment code</th>
<th>Time of spray application</th>
<th>Reuler</th>
<th>Christnach</th>
<th>Burmerange</th>
</tr>
</thead>
<tbody>
<tr>
<td>T 0</td>
<td>Control</td>
<td>10.6±4.4</td>
<td>24.6±21.8</td>
<td>0.9±0.9</td>
</tr>
<tr>
<td>T 1</td>
<td>59</td>
<td>8.2±3.4</td>
<td>16.4±17.3</td>
<td>1.1±0.8</td>
</tr>
<tr>
<td>T 2</td>
<td>31+59</td>
<td>5.9±2.6</td>
<td>9.5±8.3</td>
<td>0.5±0.6</td>
</tr>
<tr>
<td>T 3</td>
<td>31+37+59</td>
<td>7.8±3.6</td>
<td>13.2±11.9</td>
<td>0.7±0.8</td>
</tr>
</tbody>
</table>

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).

<table>
<thead>
<tr>
<th>Experiment code</th>
<th>Time of spray application</th>
<th>Reuler</th>
<th>Christnach</th>
<th>Burmerange</th>
</tr>
</thead>
<tbody>
<tr>
<td>T 0</td>
<td>Control</td>
<td>21.6±10.8</td>
<td>20.4±13.8</td>
<td>31.3±36.4</td>
</tr>
<tr>
<td>T 1</td>
<td>59</td>
<td>21.4±10.1</td>
<td>18.3±11.3</td>
<td>40.3±34.8</td>
</tr>
<tr>
<td>T 2</td>
<td>31+59</td>
<td>18.8±9.0</td>
<td>11.2±8.4</td>
<td>27.1±40.3</td>
</tr>
<tr>
<td>T 3</td>
<td>31+37+59</td>
<td>22.1±10.9</td>
<td>15.4±11.0</td>
<td>28.2±36.4</td>
</tr>
</tbody>
</table>

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 detection.
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

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**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.

<table>
<thead>
<tr>
<th>Experiment code</th>
<th>Location</th>
<th>DON (µg/kg dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Reuler</td>
</tr>
<tr>
<td></td>
<td>Time of spray application</td>
<td>Average</td>
</tr>
<tr>
<td>T 0</td>
<td>Control</td>
<td>261</td>
</tr>
<tr>
<td>T 1</td>
<td>59</td>
<td>136</td>
</tr>
<tr>
<td>T 2</td>
<td>31 + 59</td>
<td>283</td>
</tr>
<tr>
<td>T 3</td>
<td>31+37+59</td>
<td>398</td>
</tr>
</tbody>
</table>

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**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).
(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 trichothecene 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 increased the pathogen’s stress, resulting in a higher toxicogenic response (Reverberi et al., 2010) as recently observed in the laboratory (Audenaert et al., 2010).

Table S1.18. NIV content determined in wheat samples collected from the Christnach site. Data based on means of 6 analyses. Level of quantification or LOQ was 76µg/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.

<table>
<thead>
<tr>
<th>Experiment code</th>
<th>Time of spray application</th>
<th>NIV (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T 0</td>
<td>Control</td>
<td>409</td>
</tr>
<tr>
<td>T 1</td>
<td>59</td>
<td>308</td>
</tr>
<tr>
<td>T 2</td>
<td>31 + 59</td>
<td>279</td>
</tr>
<tr>
<td>T 3</td>
<td>31+37+59</td>
<td>445</td>
</tr>
</tbody>
</table>

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 trichothecene mycotoxins.

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 variable 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.

REFERENCES


