Effects of field-applied fungicides, grain moisture, and time on deoxynivalenol during postharvest storage of winter wheat grain

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Abstract: Fusarium head blight, caused mainly by Fusarium graminearum Schwabe, results in major losses in wheat. In two separate field experiments, spikes of winter wheat cultivars ‘Overland’ (moderately resistant) and ‘Overley’ (susceptible) were sprayed at anthesis with the triazole fungicide Prosaro (prothioconazole + tebuconazole) or the strobilurin fungicide Headline (pyraclostrobin) or not sprayed. Following harvest, deoxynivalenol (DON) concentrations were monitored during 120 d of grain storage at 10 °C, 40% relative humidity, and 10%, 16%, or 20% grain moisture. In ‘Overland’, DON decreased significantly at P = 0.05 from an average of 3.6 to 3.0 μg g⁻¹ in the check and decreased from 2.7 to 2.2 μg g⁻¹ in the Prosaro treatment. DON did not significantly decrease (4.4–4.1 μg g⁻¹) in the Headline treatment. DON concentrations did not differ between 16% (3.1 μg g⁻¹) and 20% (3.0 μg g⁻¹) grain moisture. In ‘Overley’, DON increased significantly from 3.1 to 3.6 μg g⁻¹ in the check and from 2.9 to 3.5 μg g⁻¹ in the Headline treatment, but remained the same at 2.2 μg g⁻¹ in the Prosaro treatment. DON concentrations were not different between 16% (3.2 μg g⁻¹) and 20% (3.1 μg g⁻¹) grain moisture but were significantly lower (2.7 μg g⁻¹) at 10% grain moisture. These results indicate that the effects of fungicides applied at anthesis in the field can impact DON concentrations through grain storage. Triazoles are recommended over strobilurins to achieve this extended postharvest protection from DON, and grain moisture during storage should be below the maximum safe level of 13.5% at 10 °C.

Key words: Fusarium head blight, mycotoxin, triazole, strobilurin, grain moisture.

Résumé : La fusariose de l’épe, principalement causée par Fusarium graminearum Schwabe, entraîne d’importantes pertes de blé. Dans le cadre de deux expériences au champ, les auteurs ont pulvérisé ou pas le fongicide Prosaro, à base de triazole (prothioconazole + tebuconazole), ou le fongicide Headline, à base de strobilurine (pyraclostro- bone), à l’anéthese, sur les épis des cultivars de blé d’hiver ‘Overland’ (modérément résistant) et ‘Overley’ (sensible). Après la moisson, ils ont mesuré la concentration de désoxynivalénol (DON) pendant 120 j d’entreposage à 10 °C, 40 % d’humidité relative et une teneur en eau de 10 %, 16 % ou 20 % dans le grain. Chez ‘Overland’, la concentration de DON a significativement diminué (P = 0.05) d’en moyenne 3,6 à 3,0 μg g⁻¹ pour le traitement témoin et de 2,7 à 2,2 μg g⁻¹ le grain traité au Prosaro, mais elle n’a pas diminué significativement (de 4,4 à 4,1 μg g⁻¹) pour celui traité avec Headline. La concentration de DON était similaire, que le grain renferme 16 % (3,1 μg g⁻¹) ou 20 % (3,0 μg g⁻¹) d’eau. Chez ‘Overley’, la concentration de DON a augmenté de façon significative, soit de 3,1 à 3,6 μg g⁻¹ pour le...
traitement témoins et de 2,9 à 3,5 μg g⁻¹ pour l’application de Headline, mais est demeurée la même à 2,2 μg g⁻¹ pour l’application de Prosaro. La concentration de DON était identique, que le grain renferme 16 % (3,2 μg g⁻¹) ou 20 % d’eau (3,1 μg g⁻¹), mais elle était significativement plus faible (2,7 μg g⁻¹) quand il n’en contenait que 10 %. Ces résultats indiquent que l’application d’un fongicide à l’anthesis, au champ, peut avoir influer sur la concentration de DON dans le grain durant l’entreposage. Pour bénéficier cette protection prolongée contre le DON, on préconise l’usage de triazoles plutôt que de strobilurines. En outre, la teneur en eau du grain pendant l’entreposage devrait être inférieure à la teneur maximale jugée sécuritaire (13,5 % à 10 °C). [Traduit par la Rédaction]

Mots-clés : fusariose de l’épi, mycotoxine, triazole, strobilurine, teneur en eau du grain.

Introduction

Fusarium head blight (FHB) is a devastating disease of wheat and other small grain cereals that results in major economic losses worldwide (McMullen et al. 2012). It is caused mainly by Fusarium graminearum Schwabe, but other species of Fusarium are known to be causal agents (Parry et al. 1995). Symptoms manifest as premature whitening or bleaching of one or more spikelets on the spike, which results in partial or entire bleaching of the spike (Dill-Macky 2010). Bleached spikelets are infertile or contain shriveled kernels that appear chalky white or pink, referred to as Fusarium-damaged kernels (FDK), scabby kernels, or “tombstones” (McMullen et al. 1997). In addition to poor grain quality, infection by this pathogen also results in contamination of grain with the mycotoxin deoxynivalenol (DON) which is harmful to humans and animals (McMullen et al. 2012). In humans, ingestion of DON-contaminated grain results in food poisoning symptoms including diarrhea, nausea, vomiting, abdominal pain, headache, and dizziness (Desjardins 2006). In animals, symptoms include vomiting, feed refusal, weakness, and emaciation (Pestka 2007).

Due to the low advisory level (1 μg g⁻¹) for DON in finished wheat products intended for human consumption (FDA 2010), it is important to ensure that the mycotoxin does not increase in grain during storage, especially if the crop was affected by FHB during the growing season. An increase of only a fraction of a μg g⁻¹ can lead to a DON concentration that exceeds the advisory level. In addition, it has been shown that DON can increase dramatically during the malting of wheat. Jin et al. (2018) found that when 20 hard red spring wheat samples were malted 2 mo following harvest, DON increased an average of 460% with a range from 113% to 1820%. In 12 of these samples that had original DON concentrations near or below the advisory level of 1 μg g⁻¹, DON increased to unacceptable levels (1.27–6.06 μg g⁻¹) after malting. Therefore, information on the concentration of DON in stored grain and how it changes over time is invaluable in making informed decisions on whether the grain can be used to make products for human consumption or as animal feed, which has higher advisory levels of up to 10 μg g⁻¹ (FDA 2010).

Management strategies and tactics for FHB include cultural practices such as crop rotation and tillage to reduce residue-borne inoculum, use of genetic resistance, and fungicide application timed at anthesis (Parry et al. 1995; Wegulo et al. 2015). Grain quality losses and mycotoxin concentration in grain can be reduced during harvest. FDK can be blown away by adjusting the combine’s fan speed and shutter opening (Salgado et al. 2011, 2014). To further reduce DON contamination in grain, postharvest practices including the use of sieves and specific gravity tables can remove the lighter FDK (Dexter and Nowicki 2003).

Even after pre-storage measures to remove FDK, grain is likely to contain F. graminearum-infected kernels and DON at the time it is stored. The maximum safe moisture level for storage of wheat grain at 10 °C is 13.5% (Maier et al. 2017). Suboptimal storage conditions including higher than ideal moisture and temperature can lead to growth of F. graminearum and an increase of DON during storage (Birzele et al. 2000). High grain moisture content, measured as water activity (a_w), is favorable to fungal growth and mycotoxin formation in grain during storage (Magan et al. 2003, 2014). Water activity is the ratio of the partial pressure of water vapor in the grain to the saturation vapor pressure of pure water under the same environment. It is numerically equivalent to equilibrium relative humidity (RH, expressed as a decimal) and is the major environmental factor, along with other factors including temperature, that influences stability or spoilage of stored food or grain (Pitt and Hocking 2009). Control of a_w is critical to reducing the growth of fungi and their metabolic activities during food or grain storage (Schwabe and Kramer 1995; Comerio et al. 1999). The optimal conditions for in vitro growth of F. graminearum are 25 °C and a_w = 0.98 (Brennan et al. 2003; Hope et al. 2005).

In the field, FHB and DON are controlled by applying a fungicide to the wheat spikes at anthesis (Wegulo et al. 2011, 2015). Demethylation inhibitor fungicides, also known as triazoles, slow fungal growth by inhibiting the biosynthesis of sterols which are essential in the maintenance of cell membrane integrity (Hewitt 1998; Chen et al. 2014). Strobilurins are quinone outside inhibitors (Bartlett et al. 2002; Nason et al. 2007; Myung 2015) that interfere with energy production in fungi. In the wheat-FHB pathosystem, triazole fungicides have been shown to be effective in controlling FHB and DON when applied at anthesis (Paul et al. 2008). However, strobilurin fungicides applied at anthesis have been associated with increased DON levels in grain (Ellner 2005; Paul et al. 2018). This increase in DON does not always occur following these treatments (Pirgozliev et al. 2002).
The effects of field-applied fungicides on DON during wheat grain storage have not been investigated in detail. We hypothesized that (a) triazole and strobilurin fungicides applied in the field at anthesis differentially affect DON levels in winter wheat grain, (b) DON production in *F. graminearum*-infected grain continues postharvest if grain moisture is higher than optimal, and (c) the effects of field-applied fungicides on DON persist through storage. We tested these hypotheses by measuring DON during 120 d of postharvest storage in grain from field plots sprayed at anthesis with the triazole fungicide Prosaro (prothioconazole + tebuconazole), the strobilurin fungicide Headline (pyraclostrobin), or not sprayed.

**Materials and Methods**

Grain of hard red winter wheat ‘Overland’ (moderately resistant to FHB, Baenziger et al. 2008) from a 2015 rain-fed field trial and hard red winter wheat ‘Overley’ (susceptible to FHB, Fritz et al. 2004) from a 2016 irrigated field trial were used in postharvest storage experiments. Both field trials were conducted at the Eastern Nebraska Research and Extension Center near Mead, Nebraska, USA (41.228°N, 96.4892°W). Plot size was 1.2 m × 6.1 m (2015) or 1.2 m × 4.6 m (2016). Inoculation was carried out by spreading *F. graminearum*-colonized kernels on the soil surface (67 kernels m⁻²) approximately 4 wk before anthesis followed by spray inoculation at anthesis. The triazole fungicide Prosaro (prothioconazole + tebuconazole, 0.475 L ha⁻¹) and the strobilurin fungicide Headline (pyraclostrobin, 0.658 L ha⁻¹) were applied at label rates during anthesis (mid-spring) to the spikes with a CO₂-powered backpack sprayer equipped with four tee-jet nozzles (Teejet Technologies, Dillsburg, PA, USA) spaced 30.5 cm apart on a boom and set at a pressure of 241 kPa. All plots were spray-inoculated 24 h after fungicide application by spraying a spore suspension of *F. graminearum* (1 × 10⁶ spores mL⁻¹) on the spikes using a hand-pumped backpack sprayer. The spore suspension was prepared from 14- to 21-d-old culture plates of five isolates of *F. graminearum* collected from Nebraska wheat fields. Culture plates were flooded with sterile distilled water (SDW) and spores were dislodged with an L-shaped plastic rod followed by filtration through four layers of cheesecloth and adjustment of spore concentration to 1 × 10⁵ spores mL⁻¹ with the aid of a hemocytometer. Because of the high FHB incidence and severity in 2015 resulting from the inoculation and a severe natural epidemic (Wegulo et al. 2018), FDK levels were very high, resulting in no detectable differences in DON among treatments in a preliminary experiment. Therefore, ‘Overland’ grain from the rain-fed trial was used after cleaning with a modular fractionating aspirator (Carter Day International, Inc., Minneapolis, MN, USA) to remove FDK. In contrast, low levels of FHB developed in 2016, and therefore grain of ‘Overley’ from the irrigated trial was used without removing FDK.

Before initiation of the postharvest grain storage experiments, grain samples were stored in paper bags at room temperature for 264 d (‘Overland’ from the 2015 growing season, experiment 1) or 79 d (‘Overley’ from the 2016 growing season, experiment 2). When the experiments were initiated, moisture content had dropped from 15% (a₃ 0.53) at the time of harvest to 8% (a₃ 0.26) in ‘Overland’ grain, and from 15% to 11% (a₃ 0.40) in ‘Overley’ grain.

At the beginning of storage, the grain was not tempered (10% grain moisture) or was tempered to 16% or 20% grain moisture. To calculate the amount of water needed to hydrate the grain to a desired moisture content (16% and 20%), two hydration curves were generated based on preliminary experiments. For ‘Overland’, the equation used was \( Y = 0.12X + 2.11 \), \( r^2 = 0.70; \) and for ‘Overley’, the equation used was \( Y = 6.47X - 3.85, \) \( r^2 = 0.96; \) where \( X \) is total milliliters of SDW to be added to the grain and \( Y \) is the difference between the initial moisture content of the grain and the desired moisture content.

Grain was tempered by spreading a 300 g sample of non-sterile grain from each field plot on a plastic tray (50 cm long × 30 cm wide × 3 cm high) and sprinkling evenly with SDW using a heavy-duty manual sprayer (Rubbermaid, Wooster, OH, USA). After tempering, grain samples were separated and homogenized manually and transferred to a hermetically-sealed sterile Microbox micropopagation container (15 cm long × 15 cm wide × 20 cm high; \( \text{SacO}_2 \), Veldeken, Belgium). Microbox containers have a filter in the lid for gas exchange that prevents contamination from external sources (Birzele et al. 2000). Water activity (a₃) was determined using a Pawkit® water activity meter (Decagon Devices, Pullman, WA, USA). Grain moisture content (%) was determined using a grain moisture seed tester (Dickey John Corp., Auburn, IL, USA), model GAC 500-XT. After 14 d of tempering, grain moisture and a₃ were determined, and SDW was added again if the initial tempering was insufficient to adjust to 16% (a₃ 0.60) or 20% (a₃ 0.75) grain moisture. In experiment 2 (‘Overley’ from the 2016 irrigated trial), non-tempered grain at a moisture content of 10% (a₃ 0.40) was added as a third moisture treatment because in experiment 1 there was no significant difference in DON between 16% and 20% grain moisture.

After initial tempering, grain samples in Microbox containers were stored in a seed cooler (Bally Case & Cooler, Inc., Bally, PA, USA) under dark conditions at 10 °C and 40% RH and monitored for changes in moisture content and water activity at monthly intervals. RH and temperature inside the containers were monitored using Watchdog sensors model 1400 (Spectrum Technologies, Thayer Court, IL, USA). Samples were taken at 0, 30, 60, 90, and 120 d after tempering. Each Microbox container
was shaken vigorously to uniformly mix the grain. Then, 20 mL of grain was taken from the container using a centrifuge tube and milled within 2 h of sampling using a cyclone laboratory mill (UDY Corporation, Fort Collins, CO, USA). DON in the milled sample was quantified with a gas chromatograph coupled with a mass selective detector (Agilent 6890/5975, Agilent Technologies, Santa Clara, CA, USA) using published methods (Khatibi et al. 2012, 2014; Berger et al. 2014).

Experimental design and data analysis

In the field, experimental design was a split plot in randomized complete blocks with four replications, with cultivars as the main plots and fungicide treatments as the subplots. In postharvest storage, each experiment was designed as a split-split plot with four replications and was repeated once. The whole plot, subplot, and sub-subplot consisted of field-applied fungicide treatments, grain moisture treatments, and grain storage time, respectively. Data were analyzed with SAS software version 9.4 (SAS Institute Inc., Cary, NC, USA). A combined analysis of the two runs of each experiment was done based on homogeneity of error variances determined from the F-ratio test (Gomez and Gomez 1984). Generalized linear mixed models (PROC GLIMMIX) and the least significant difference test (LSD, \( P = 0.05 \)), were used to demonstrate the effects of \((a)\) fungicide chemical class (Prosaro versus Headline), \((b)\) grain moisture during storage, and \((c)\) grain storage time on DON.

### Results

#### Effects of fungicide treatments and grain storage time on DON

The effects of fungicide and storage time treatments on DON were highly significant in both experiments (Table 1). Two-way or three-way interactions between fungicide, moisture, and time treatments were not significant at \( P = 0.05 \) (Table 1), indicating that differences in DON among fungicide treatments were not significantly affected by moisture levels and vice versa. Therefore, comparison of fungicide treatment means was done by averaging over moisture and storage time treatments, and comparison of moisture treatments was done by averaging over fungicide and storage time treatments during storage.

| Source of variation | Experiment 1 ('Overland') | Experiment 2 ('Overley') |
|---------------------|---------------------------|--------------------------|
| df                  | \( P > F \)                | df                       | \( P > F \)                |
| Replicated experiments (Runs) | 1 | 0.8294 | 1 | 0.2424 |
| Fungicide treatments (S) | 2 | <0.0001 | 2 | 0.0124 |
| Replications (Reps) | 3 | 0.0035 | 3 | 0.0414 |
| S × Reps | 6 | 0.0011 | 6 | 0.0947 |
| Runs × Reps (S)* | 11 | 0.0049 | 11 | 0.0788 |
| Grain moisture (M) | 1 | 0.8777 | 2 | 0.0168 |
| Linear | — | — | 1 | 0.0001 |
| Quadratic | — | — | 1 | 0.4619 |
| S × M | 2 | 0.0058 | 4 | 0.8778 |
| Reps × M (S)* | 9 | 0.1045 | 18 | 0.1695 |
| Container (Reps × Runs × S × M)* | 12 | 0.0063 | 24 | 0.0044 |
| Storage time (T) | 4 | <0.0001 | 4 | <0.0001 |
| Linear | 1 | 0.1729 | 1 | 0.4623 |
| Quadratic | 1 | <0.0001 | 1 | 0.0779 |
| Cubic | 1 | <0.0001 | 1 | <0.0001 |
| Quartic | 1 | <0.0001 | 1 | 0.0822 |
| S × T | 8 | 0.4360 | 8 | 0.0620 |
| M × T | 4 | 0.2061 | 8 | 0.2542 |
| S × M × T | 8 | 0.1537 | 16 | 0.5218 |
| Reps × T (S)* | 36 | 0.0153 | 36 | 0.2045 |
| Reps × M × T (S)* | 36 | 0.5447 | 72 | 0.4295 |
| Residual | 96 | 143 |
| Total | 243 | 364 |

Note: 'Overland' grain from the 2015 rainfed trial was used in Experiment 1 and P > F 'Overley' grain from the 2016 irrigated trial was used in Experiment 2. Each experiment was repeated once. Treatments were arranged in a split-split plot experimental design with four replications. Nested factors are marked with *. df, degrees of freedom.

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Fig. 1. Deoxynivalenol (DON) concentration in storage averaged over moisture treatments at 0 d after tempering (DAT) compared with 120 DAT in grain of winter wheat ‘Overland’ not treated (check) or treated at anthesis with the strobilurin fungicide Headline or the triazole fungicide Prosaro during the 2015 growing season. Means with the same letter are not significantly different according to the least significant difference test at $P = 0.05$.

Effect of grain moisture treatments on DON

DON concentration did not differ between 16% and 20% grain moisture in ‘Overland’ ($P = 0.878$, Table 1). Similarly, DON concentration was not significantly different between 16% and 20% grain moisture in ‘Overley’. However, in ‘Overley’ in which a 10% moisture treatment was included, a significant effect ($P = 0.0168$, Table 1) was observed, with similar DON concentrations at 16% and 20% grain moisture and a lower concentration at 10% grain moisture (Fig. 3).

Variation in DON over grain storage time

In all treatments (check, Headline, and Prosaro) for ‘Overland’, DON concentration declined during the first 30 d, increased over the next 30 d, then stabilized with slight fluctuations during the last 60 d. During the 120 d of storage, DON significantly decreased over time in the check and Prosaro treatments but not in the Headline treatment (Figs. 1 and 4). In ‘Overley’, DON in the Headline treatment increased over the first 30 d, decreased over the following 60 d, then increased over the last 30 d. In the check, DON concentration increased during the first 60 d, decreased during the following 30 d, and increased during the last 30 d. In the Prosaro treatment, DON concentration increased over the first 30 d, decreased over the following 60 d, and increased over the last 30 d. Over the 120 d of storage, DON significantly increased in the Headline and check treatments but remained the same in the Prosaro treatment (Figs. 2 and 5). The trends in DON concentration over time in moisture treatments averaged over fungicide treatments were similar to those in fungicide treatments averaged over moisture treatments (Figs. 4–6).

Discussion

Previous research has demonstrated higher efficacy of triazole fungicides in controlling FHB and DON in wheat when applied in the field at anthesis compared with strobilurin fungicides, which have been shown in some studies to elevate DON in grain when applied in the field before or during anthesis (Oldenburg et al. 2001; Ellner 2005; Blandino and Reyneri 2009). However, the effects of field-applied triazole and strobilurin fungicides on DON concentrations during storage have not been investigated in detail. This study was designed to fill this knowledge gap. In both experiments at the beginning of grain storage, the higher or similar DON in the field-applied Headline treatment compared with the check and the lower DON in the field-applied Prosaro treatment compared with the Headline and check treatments (Figs. 1 and 2) demonstrated the effectiveness of Prosaro.
in controlling DON and are consistent with previous reports (Oldenburg et al. 2001; Ellner 2005; Blandino and Reyneri 2009). At the end of storage in both experiments, DON was similarly lower in the Prosaro treatment compared with the Headline and check treatments (Figs. 1 and 2). These results indicated that Prosaro was more effective than Headline in controlling DON in the field, and this effectiveness extended through the storage period of 120 d. The effectiveness of the Prosaro treatment is attributed to the fungicide’s efficacy in keeping fungal biomass in grain low compared with the Headline and check treatments.

We observed a significant decrease of 17% in DON in ‘Overland’ over 120 d of grain storage in both the Prosaro and check treatments compared with a non-significant decrease of only 6% in the Headline treatment. In contrast, in ‘Overley’, DON significantly increased by 22% over 120 d of grain storage in the Headline treatment and by 18% in the check but did not increase in the Prosaro treatment. Two non-proven hypotheses have been proposed to explain the elevation of DON in wheat grain following field application of strobilurin fungicides before or during anthesis (Ellner 2005). The first hypothesis is that strobilurin fungicides kill non-pathogenic fungi resident on wheat spikes, thereby reducing competition and supporting the establishment of FHB-causing Fusarium spp. The second hypothesis is that the “greening effect” of strobilurins maintains green leaf area and high moisture content in the kernels, which prolongs the life of wheat spikes and thereby provides a longer period for mycotoxin production.

Fig. 3. Effects of moisture on deoxynivalenol (DON) concentration in storage averaged over fungicide and sampling time treatments in grain of winter wheat cultivars ‘Overland’ and ‘Overley’.

Fig. 4. Deoxynivalenol (DON) concentration in storage over time averaged over moisture treatments in grain of winter wheat ‘Overland’ not treated (check) or treated at anthesis with the strobilurin fungicide Headline or the triazole fungicide Prosaro during the 2015 growing season.

Fig. 5. Deoxynivalenol (DON) concentration in storage over time averaged over moisture treatments in grain of winter wheat ‘Overley’ not treated (check) or treated at anthesis with the strobilurin fungicide Headline or the triazole fungicide Prosaro during the 2016 growing season.
Previous studies in which fungicides were not applied in the field have shown decreases or increases in DON during storage of wheat grain or flour. Variability in the results from these studies were attributed to various reasons. In one experiment, Homdork et al. (2000) found that DON in wheat grain severely infected with Fusarium culmorum (Wm. G. Sm.) Sacc. (>50% infection) remained unchanged during 36 d of storage, whereas DON increased in grain with a 4% or 15% infection level stored under warm and humid conditions (25 °C/73% RH and 25 °C/90% RH). In a second experiment in the same study, DON in wheat grain with a 15% F. culmorum infection level decreased considerably under the same storage conditions and period. The authors acknowledged that they did not have an explanation for the contradictory results from the two experiments, but noted that in the second experiment, DON probably became degraded to other metabolites.

In a study in which DON was measured in flour during 120 d of storage, Kolmanič et al. (2010) found that the level of retention of DON was dependent on the type of packaging material but not on the type of flour or the storage temperature. They found that DON decreased most rapidly when the flour was stored in paper bags that allowed access to the storage atmosphere. In this study, we stored grain used in both experiments in paper bags prior to initiation of storage experiments. During the storage experiments, grain was stored in Microbox containers that allowed gaseous exchange between the containers and the storage atmosphere. Zhang et al. (2016) found that DON in wheat grain significantly decreased whereas DON in wheat flour significantly increased during a 180 d storage period. Both Kolmanič et al. (2010) and Zhang et al. (2016) noted the considerable variability of mycotoxin concentrations in plant material which often makes it difficult to discern treatment effects from the variable effects caused by sampling. Based on their results, they assumed that there are processes that result in slow decomposition of mycotoxins in grain and flour during storage.

The lower DON concentration at 10% compared with 16% or 20% grain moisture during storage observed in ‘Overley’ is consistent with results from previous research. Ramirez et al. (2006) showed that the growth rate of and DON production by two isolates of F. graminearum on irradiated wheat grain increased as aw increased. Birzele et al. (2000) found DON concentration in stored wheat grain at 20% moisture to be approximately three times the concentration at 17% moisture by the 5th and 6th week of storage. In contrast, in this study DON concentration at 16% moisture did not differ from that at 20% moisture over a period of 120 d of grain storage. This difference in the results between the two studies is likely due to the temperature at which grain was stored. In the study by Birzele et al. (2000), grain was stored at 20 °C whereas in this study grain was stored at 10 °C. In this study, however, DON concentration at 10% moisture was lower than that at 16% or 20% moisture, which is in agreement with the results of Birzele et al. (2000) that showed higher DON production in Fusarium-contaminated wheat grain stored at higher compared with lower grain moisture. Comerio et al. (1999) and Hope et al. (2005) also showed that more DON was produced by F. graminearum in stored grain at higher than at lower aw values. Because moisture favors germination of F. graminearum spores and growth of the resulting mycelium, fungal growth on or inside the grain at the higher moisture levels (16% and 20% in this study) likely resulted in greater production of DON at these moisture levels compared with the lower moisture level of 10%.

The reasons for the fluctuation in DON concentrations over time (Figs. 4–6) are not known, but may be related to biochemical processes in grain that may be influenced by a range of factors including the environmental conditions (moisture, temperature), the amount of fungal biomass present in the grain, or the level of
expression of trichothecene biosynthesis genes (Kolmanič et al. 2010; Hallen-Adams et al. 2011; Zhang et al. 2016). The fluctuations in DON concentrations over time could also be due to its conversion to other forms such as the acetylated derivative 15-ADON, or its conjugation to masked forms. Conjugation to masked forms is a detoxification process that occurs as a defense reaction of plant tissue to infection by DON-producing fungi (Berthiller et al. 2013). A major detoxification pathway is the conjugation of DON to deoxynivalenol-3-β-D-glucopyranoside (D3G), which has been isolated from DON-contaminated wheat grain (Berthiller et al. 2009). We did not measure masked mycotoxins in this study. However, the results between two replicate experiments for each wheat cultivar were consistent. In addition, data from previous research (Berthiller et al. 2009; Desmarchelier and Seefelder 2011) showed that the relative proportion of D3G to DON was stable at around 20% in 99 wheat and maize samples tested. The consistency between replicate experiments for each cultivar gives us confidence that the differences in DON observed in this study during wheat grain storage were due to the fungicide and moisture treatments we applied. In future similar studies, measurement of masked mycotoxins will help to determine if they influence DON concentration over time during storage of Fusarium-infected wheat grain.

Conclusions

Our research has demonstrated that the reduction in DON observed in the field from applying a triazole fungicide (Prosaro) at anthesis can be extended through the period of grain storage. A strobilurin fungicide (Headline) applied at anthesis in the field was ineffective in reducing DON in grain of the moderately resistant ‘Overland’ stored in the dark at 10 °C for 120 d. In contrast, DON in the grain of the same cultivar treated at anthesis in the field with a triazole fungicide (Prosaro) declined in storage under the same conditions and period. In the susceptible ‘Overley’ grain treated and stored similarly, DON increased during storage in the Headline and check treatments, but in the Prosaro treatment DON was lower at the beginning of storage and did not increase over time.

The decrease in DON in ‘Overley’ grain during storage was likely due to its conjugation to masked forms of the mycotoxin or its conversion to other forms such as the acetylated derivative 15-ADON. In ‘Overley’, the higher DON in the Headline and check treatments compared with the Prosaro treatment at the end of storage indicated that Prosaro was more effective than Headline in controlling DON when applied in the field at anthesis. This effectiveness was extended through the storage period and can be attributed to a smaller F. graminearum biomass in grain from the Prosaro treatment compared with the less effective Headline treatment.

More DON was produced in storage in ‘Overley’ grain tempered to 16% or 20% moisture compared with non-tempered grain at 10% moisture. This was likely due to fungal growth in the grain at the higher moisture levels and indicates the importance of proper drying of grain before storage. Based on these results triazole, but not strobilurin, fungicides are recommended for control of FHB and DON. Grain moisture during storage should be at or below the maximum safe level which is 13.5% at 10 °C.

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