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Disease control/Moyens de lutte

Evaluation of different fungicides for control of fusarium head blight in wheat inoculated with 3ADON and 15ADON chemotypes of *Fusarium graminearum* in Canada

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Abstract: Fusarium head blight (FHB) continues to threaten the economic sustainability of many small grain producers in Manitoba by causing losses in grain yield and quality. In this 2-year study, four fungicides were tested on two spring wheat cultivars with different levels of resistance to FHB inoculated with 3ADON and 15ADON chemotypes of *Fusarium graminearum*. All fungicides (prothioconazole, tebuconazole, metconazole and prothioconazole+ tebuconazole) reduced FHB index, per cent *Fusarium*-damaged kernels (% FDK) and deoxynivalenol (DON) levels and increased yield compared with the non-sprayed control in the moderately resistant cultivar 'Glenn'. In the highly susceptible cultivar 'Roblin', all fungicides reduced FHB index and increased yield compared with the non-sprayed control; however, inconsistent results were observed for FDK and DON. Differences were observed between the two chemotypes for all variables in both years except for FDK and yield in 2010. This study confirmed that host resistance plays an important role in host–pathogen–fungicide interaction. Therefore, the combined effect of growing moderately resistant cultivars with fungicide application can reduce damage caused by FHB even under high FHB severity.

Keywords: chemotypes, disease control, fungicides, fusarium head blight, wheat

Résumé: La fusariose de l'épi (FE) continue de menacer la viabilité économique de plusieurs petits producteurs de grains manitobains en occasionnant des pertes de rendement et de qualité. Au cours de cette étude de deux ans, quatre agents fongicides ont été testés sur deux cultivars de blé de printemps affichant différents degrés de résistance à la FE, inoculés avec les chimiotypes 3ADON et 15ADON de *Fusarium graminearum*. Tous les agents fongicides (prothioconazole, tébuconazole, metconazole et prothioconazole + tébuconazole) ont réduit l'indice de la FE, le pourcentage *Fusarium*-grains endommagés (% FGE) ainsi que les niveaux de déoxynivalénol (DON). Ils ont également accru le rendement par rapport aux témoins non aspergés. Toutefois, des résultats contradictoires ont été observés quant au FGE et au DON. Des différences ont été observées entre les deux chimiotypes relativement à toutes les variables durant les deux années, sauf pour le FGE et le rendement en 2010. Cette étude a confirmé que la résistance de l'hôte joue un rôle important dans les interactions hôte-agent pathogène-agent fongicide. En conséquence, la culture de cultivars moyennement résistants, combinée à l'application d'agents fongicides, peut réduire les dommages causés par la FE, et ce, même pour des degrés élevés de gravité de la maladie.

Mots clés: agents fongicides, blé, brûlure de l'épi, chimiotype, lutte contre les maladies

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Introduction

Fusarium head blight (FHB) or scab is one of the major fungal diseases of cereals worldwide. This disease can cause severe damage to many economically important crops such as wheat, barley, corn and oats. In North America, *Fusarium graminearum* Schwabe (teleomorph: *Gibberella zeae* (Schwein.) Petch) is considered to be the major causative agent of FHB although other *Fusarium* species are also implicated (McMullen *et al*., 1997; Gilbert & Tekauz, 2000). Since 1980, FHB has caused major yield and quality losses in the Prairies and in eastern Canada (Gilbert *et al.*, 2001). One of the major concerns of FHB is the contamination of grain with the mycotoxin deoxynivalenol (DON) which is produced by the pathogen. DON poses a health hazard in food and feed, causing neurotoxic and immunotoxic effects in mammals (D'Mello *et al*., 1999; Desjardins, 2006). Currently, wheat cultivars that are highly resistant to FHB and DON accumulation are not available. Therefore, integrated FHB management strategies are considered to be the most effective approach to control the disease (Bai & Shaner, 2004). Genetic resistance, cultural practices including crop rotation, tillage, seed treatment, fungicide application and biological control agents are recommended (Parry *et al*., 1995; Edwards *et al*., 2001; Mesterházy, 2003; Gilbert & Tekauz, 2011).

Application of fungicides plays an important role in integrated FHB management. Fungicides are applied at anthesis to reduce yield losses and mycotoxin contamination (Mesterházy *et al*., 2003). In general, the DMI (sterol biosynthesis inhibitors) or triazoles (tebuconazole, metconazole and prothioconazole) fungicides are reported to be the most effective chemical compounds against *Fusarium* spp. to reduce FHB incidence and DON accumulation (Edwards *et al*., 2001; Simpson *et al*., 2001; Pirgozliev *et al*., 2002; Mesterházy *et al*., 2003). On the other hand, QoI (Quinone outside inhibitor) class of fungicides which includes the strobilurins can result in increased DON accumulation, even though they partially control FHB incidence (Edwards *et al*., 2001; Simpson *et al*., 2001; Pirgozliev *et al*., 2002; Mesterházy *et al*., 2003; Haidukowski *et al*., 2005). Unfortunately, the overall effect of fungicides in controlling FHB has been limited and results are variable. Most studies reported inadequate or inconsistent control of FHB (Mesterházy *et al*., 2003) rendering fungicides inefficient as a standalone control strategy. Therefore, efficacy of fungicide application depends on factors such as the level of resistance of the cultivar, timing, coverage and rate of application, and aggressiveness of the pathogen populations (Mesterházy *et al*., 2003).

Distribution of chemotype diversity in *F. graminearum* has been studied worldwide. In Canada, 15ADON chemotypes were dominant until 1998, but recently a chemotypic shift from 15ADON to the 3ADON chemotype has been reported (Ward *et al*., 2008). To date, no nivalenol (NIV) chemotypes have been identified in Canada. Recent studies by Guo *et al*. (2008) reported that the 3ADON chemotype is more prevalent in the Red River Valley of Manitoba and it is displacing the 15ADON chemotype, adding a new challenge to current FHB management strategies. This chemotypic shift may have an effect on FHB control using fungicides. The objectives of this study were to: (1) evaluate the efficacy of fungicides against 3ADON and 15ADON chemotypes of *F. graminearum*; and (2) determine correlations between FHB visual disease variables and DON content in infected grain.

Materials and methods

Fungal inoculum

Fusarium graminearum isolates belonging to chemotypes 3ADON (ON-06-39, M8-06-02, M5-06-01 and Q-06-32) and 15ADON (ON-06-05, Q-06-10) were used in the 2009 growing season and DF-Fg-144 (15ADON) was used in the 2010 growing season instead of M8-06-02 (initially M8-06-02 was used as a 15ADON isolate but later, gas chromatography and multiplex PCR confirmed that it produces 3ADON; therefore, it was replaced by a 15ADON isolate in 2010). All the isolates were collected from fusarium-damaged kernels (FDK) of wheat grown by farmers in western and eastern Canada in 2006. A multilocus genotype assay and multiplex PCR assay based on *TRI3* and *TRI12* genes were used to identify each of the isolates as *F. graminearum sensu stricto* and to assess the trichothecene chemotypes of each isolate (O'Donnell *et al*., 2004; Ward *et al*., 2008).

Inoculum preparation

All inocula were produced from single spore cultures of *F. graminearum*. Inoculum was increased by growing the fungal strains in aerated liquid carboxymethylcellulose (CMC) medium. After 7 days of incubation at room temperature (21–24 °C), contents of the flasks were filtered through three layers of cheesecloth to obtain a spore suspension. Conidial concentration was determined using a hemocytometer and adjusted to 5×10^4 conidia mL^{-1}.

Fungicides

Four commercially available fungicides – Proline® (prothioconazole), Prosaro® (prothioconazole +

tebuconazole) and Folicur® (tebuconazole) manufactured by Bayer Crop Science Inc. (Canada), and Caramba® (metconazole) manufactured by BASF Chemical Company (USA), were used in field experiments. Rate of application of prothioconazole was 176.4 mL ha^{-1}, prothioconazole + tebuconazole was 217.12 mL ha^{-1}, tebuconazole was 126.14 mL ha^{-1} and metconazole was 57.80 mL ha^{-1}.

Experimental design

Two spring wheat (*Triticum aestivum* L.) cultivars 'Glenn' (moderately resistant (MR) to FHB) and 'Roblin' (highly susceptible (S) to FHB), were used in these experiments. The experiments were conducted during the 2009 and 2010 growing seasons at the University of Manitoba, Winnipeg. The mean temperatures, relative humidity and precipitation during June, July, August and September are listed in Table 1. A three replicate split-split plot design was used. The main plot effect was four fungicide treatments plus a non-sprayed fungicide control (inoculated with *F. graminearum* but with no fungicide application). Main plots were separated by buffer plots of the wheat cultivar 'Amazon'. Amazon is taller than the two cultivars used in this study. Therefore, it was used to reduce potential fungicide drift among main plots. Sub-plots were the two wheat cultivars and within each wheat cultivar, six isolates of *F. graminearum* and a disease control (sprayed with distilled water) served as the sub-sub plot effect. Plots were 1.5 m wide by 3 m long and seeded at a rate of 1200 seeds per plot. Fungicides were applied at 30% anthesis (Zadoks GS:61) according to the manufacturer's instructions with a CO_2 powered back-pack sprayer. Each fungicide was applied in 200 L ha^{-1} water using a six nozzle boom with 20 cm nozzle spacing. The nozzles were 8002 Teejet flat fan. Two days after fungicide application, at 50% anthesis (Zadoks GS:65), inoculum was applied using a CO_2 powered back-pack sprayer calibrated at 30 psi, at a rate of 1 L of inoculum per plot. The disease control plots were sprayed with distilled water (fungicides applied, no inoculum). A second inoculation of the same plots was performed 3 days after the first inoculation. Plots were mist-irrigated for 12 h at intervals of 5 min h^{-1} using an overhead sprinkler system in the evening of, and the morning after, each inoculation.

Table 1. Mean temperatures, relative humidity and precipitation during June, July, August and September in 2009 and 2010 field seasons at the 'Point' field station, University of Manitoba.

	June		July		August		September	
	2009	2010	2009	2010	2009	2010	2009	2010
Temperature (°C)	16.2	17.0	17.5	20.8	17.7	19.5	17.8	11.9
Relative humidity (%)	62.4	71.6	67.1	66.3	73.9	70.3	70.0	69.9
Precipitation (mm)	61.5	71	114.5	69	56	144	22.5	93

Source: Point Weather Station, Department of Plant Science, University of Manitoba and National Climate Data and Information Archive, Environment Canada.

Disease and yield evaluation

Disease incidence and severity of each plot were rated 22 days after the first inoculation using a FHB disease scale (Stack & McMullen, 1995). Disease incidence was expressed as the percentage of spikes infected in the plot, from 0% (no infection) to 100% (indicating all spikes examined were infected). Disease severity was expressed as the average percentage of each spike which was infected, also on a scale of 0–100%. One hundred spikes from each of five locations (four corners and the middle of the plot) were assessed to measure FHB severity and incidence. FHB index for each plot was calculated using the following formula:

$$\text{FHB index} = (\text{per cent disease incidence} \times \text{per cent disease severity})/100.$$

After maturity, plots were mechanically harvested using a Wintersteiger Elite small plot combine. The wind speed was reduced from normal by 60% to retain as many FDK as possible. This resulted in incomplete threshing and samples were later cleaned up using a belt thresher. Grain yields of each plot were determined (kg ha^{-1}). A 10 g random seed sample was selected from the total seeds harvested from each plot and percentage of FDK was counted as the number of FDKs in the total number of seeds. A FDK was considered to be any seed that was shrivelled, had any mycelial growth or a chalky white or pink discoloration.

DON analysis

Deoxynivalenol analysis for both years was carried out by sampling 10 g of grain randomly from two replicates (replicates 1 and 3, a total of 140 plots). The sample from each plot was ground using a Romer® Mill (Model 2A) and DON was extracted using 50 mL of deionized water, and then quantified using EZ-Quant® Vomitoxin ELISA kit from Diagnostix (www.diagnostix.ca) with a DON quantification limit of 0.5 mg kg^{-1}.

Statistical analysis

Analysis of variance (ANOVA) for FHB index, FDK, yield and DON for each year was performed using

the 'PROC GLM' procedure of the SAS software (SAS version 9.2, SAS Institute Inc., Cary, NC). The model statement used in the analysis was 'variables = fungicide, cultivar, isolate, fungicide*cultivar, fungicide*isolate, cultivar*isolate, fungicide*cultivar*isolate, rep, fungicide*rep, cultivar*rep, fungicide*cultivar*rep'. Replicates and the interaction with replicates were considered random and all the other factors were fixed. The error terms for fungicide, cultivar and fungicide*cultivar, were fungicide*rep, cultivar*rep and fungicide*cultivar*rep, respectively. Other sources of variation were tested against the residuals.

To determine the effect of fungicides on FHB variables, all five fungicide treatments (including the non-sprayed fungicide control) were included in the first analyses. Subsequently, a separate analysis was performed without the non-sprayed fungicide control to identify differences among the four fungicides. The same pattern of analysis was performed for the isolate component. First, an overall analysis was performed for all isolates. Individual analyses were carried out to determine differences among 3ADON isolates and among 15ADON isolates, between 3ADON and 15ADON chemotypes and between the inoculated and disease control samples. FHB index was analysed using disease severity and incidence ratings that were taken at 22 days after the first inoculation. The correlations between the response variables for each year were analysed using the 'PROC CORR' procedure of the SAS (version 9.2) software package considering raw data.

The test of homogeneity of variances indicated that error variances were not homogeneous when data were combined over years. A significant year*treatment interaction was observed; therefore, data for each year were analysed separately (data not shown).

Results

Visual assessment of FHB index

Fungicide treatment results varied between the two years of the study; therefore, they were discussed separately. In both years, all fungicide treatments reduced FHB index compared with the non-sprayed fungicide control (Figs 1A, 2A, 3A and 4A). The FHB index across all fungicides and fungal isolates was higher in the S cultivar compared with the MR cultivar (Figs 1A, 2A, 3A and 4A). In both years, fungicides, fungal isolates and cultivars affected FHB index (Table 2). There were differences between 3ADON and 15ADON chemotypes. In the absence of fungicide application, the 3ADON isolates produced higher FHB index on both MR and S cultivars in 2009 and in MR cultivar in 2010 (Figs 1A, 2A and 3A). In 2009, the three-way interaction for FHB index, fungicide by cultivar by isolate, was not significant. The two-way interactions of fungicide by cultivar and cultivar by isolate were significant, but the fungicide by isolate interaction was not (Table 2). The fungicide treatments differed from the non-sprayed fungicide control but no differences were observed among the four fungicide treatments for FHB index. There were no differences either among 3ADON or 15ADON *F. graminearum* isolates but significant differences were found between 3ADON and 15ADON chemotypes and between the inoculated and the disease controls for FHB index (Table 2).

In 2010, unlike the previous year, the three-way interaction was significant. Although the fungicide treatments differed from the non-sprayed fungicide control, no differences were observed among the fungicides (Table 2). As in 2009, differences were observed between 3ADON and 15ADON chemotypes, but unlike 2009, significant differences were also found among 3ADON and 15ADON isolates (Table 2).

FDK percentage

In both 2009 and 2010, fungicides, cultivars and isolates had an effect on FDK (Table 2). In 2009, the four fungicides differed from the non-sprayed fungicide control for FDK, but there were no differences among fungicides. Also, there were differences between the two cultivars and among fungal isolates for FDK (Table 2). Differences were observed between the chemotypes, but no difference was observed among the 3ADON or among the 15ADON isolates (Table 2). In 2010, fungicide, cultivar and isolates all had a significant effect on FDK. All fungicides differed from the non-sprayed fungicide control, but unlike 2009, there were differences among the four fungicides (Table 2). In contrast to 2009, differences were found among the 3ADON and 15ADON isolates but not between the two chemotypes (Table 2). When considering the treatment means for FDK, in the MR cultivar, all fungicide treatments consistently reduced the levels of FDK compared with the non-sprayed fungicide control in both years (Figs 1B, 3B). However, in the S cultivar, no difference was observed between fungicide-treated plots and the non-sprayed fungicide control plots in 2009 (Fig. 2B). In 2010, tebuconazole and metconazole treatments reduced FDK levels in the S cultivar both under 3ADON and 15ADON inoculations (Fig. 4B).

Grain yield

In 2009, differences were not found among the four fungicides for grain yield (Table 2). Cultivars and fungal

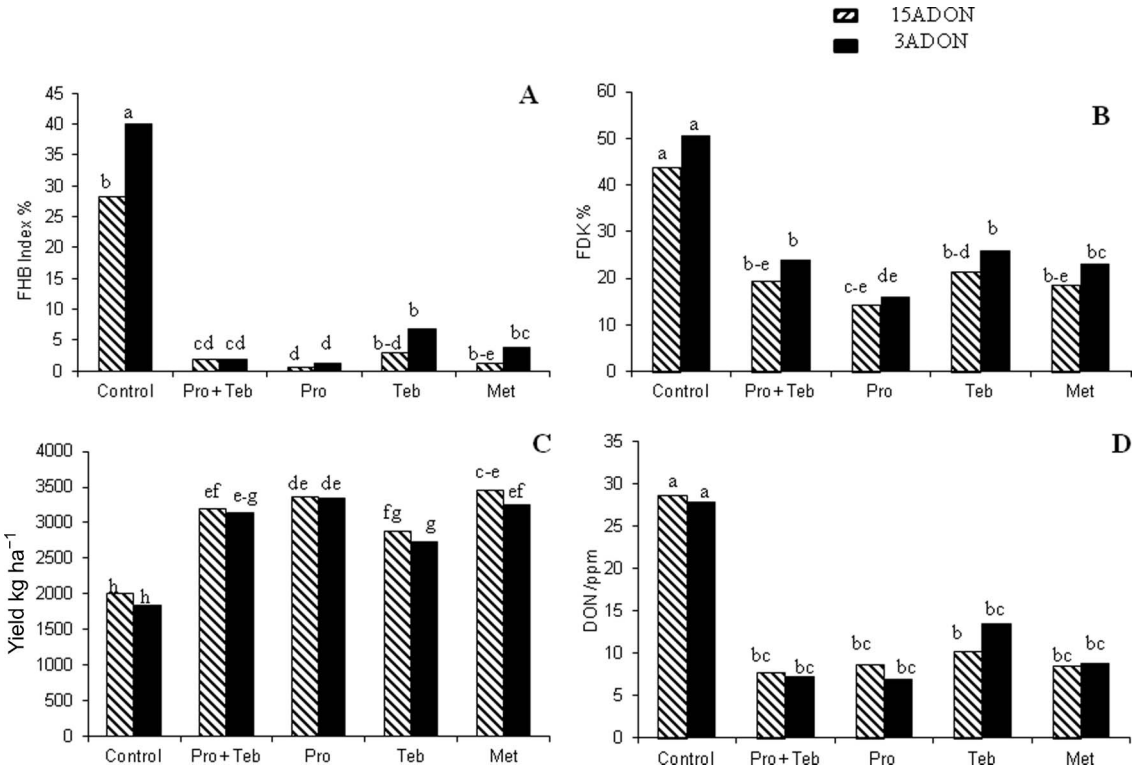

Fig. 1. Fungicide treatment means for (**A**) fusarium head blight index (FHB index), (**B**) percentage of fusarium-damaged kernels (FDK), (**C**) yield and (**D**) concentration of deoxynivalenol (DON) for the 3ADON, 15ADON chemotypes of *Fusarium graminearum* in cultivar 'Glenn' (MR) in 2009. (X-axis represents fungicide treatment means Pro+ Teb = Protioconazole + tebuconazole, Pro = Prothioconazole, Teb = Tebuconazole and Met = Metconazole). Means with the same letter for each fungicide treatment are not significantly different. The 15ADON and 3ADON symbols are common for all figures, box with black stripes represents 15ADON chemotype and filled black box represents 3ADON chemotype.

isolates had a significant effect on yield (Table 2). There were differences between the inoculated treatments and the uninoculated disease control treatments (Table 2). Differences were found between the 15ADON and 3ADON chemotypes and among 3ADON isolates, but no differences were observed among 15ADON isolates (Table 2). In 2010, differences in yield were found among the four fungicides but not between or among 3ADON or 15ADON isolates (Table 2).

DON accumulation in grain

In 2009, differences were not observed in DON levels among fungicide treatments and the non-sprayed fungicide control (Table 2). Also, no differences were observed between cultivars. On the other hand, there were differences in DON content among isolates (Table 2). There were differences among the 3ADON isolates and between the 3ADON and 15ADON fungal isolates, although no differences were found among the 15ADON isolates (Table 2). The overall mean across all treatments for 3ADON was 22.5 ppm and 18.3 ppm for 15ADON chemotypes. In 2010, differences in DON content were found among the four fungicides but there were no significant differences between the two cultivars. There were differences between the 3ADON and 15ADON isolates and among the 3ADON isolates but not among the 15ADON isolates (Table 2). The overall mean across all treatments for 3ADON in 2010 was 8.6 ppm and 5.6 ppm for 15ADON chemotypes. In 2010, prothioconazole+ tebuconazole, prothioconazole and metconazole treatments reduced the DON content in the S cultivar compared with the non-sprayed fungicide control (Fig. 4D).

Correlations among the FHB response variables

In the 2009 field trial, FHB index, yield, FDK and DON content were highly correlated with each other for the two replicates (n = 140). The Pearson correlation coefficients for FHB index-FDK, FHB index-DON and FDK-DON were 0.812, 0.708 and 0.625, respectively. However, in

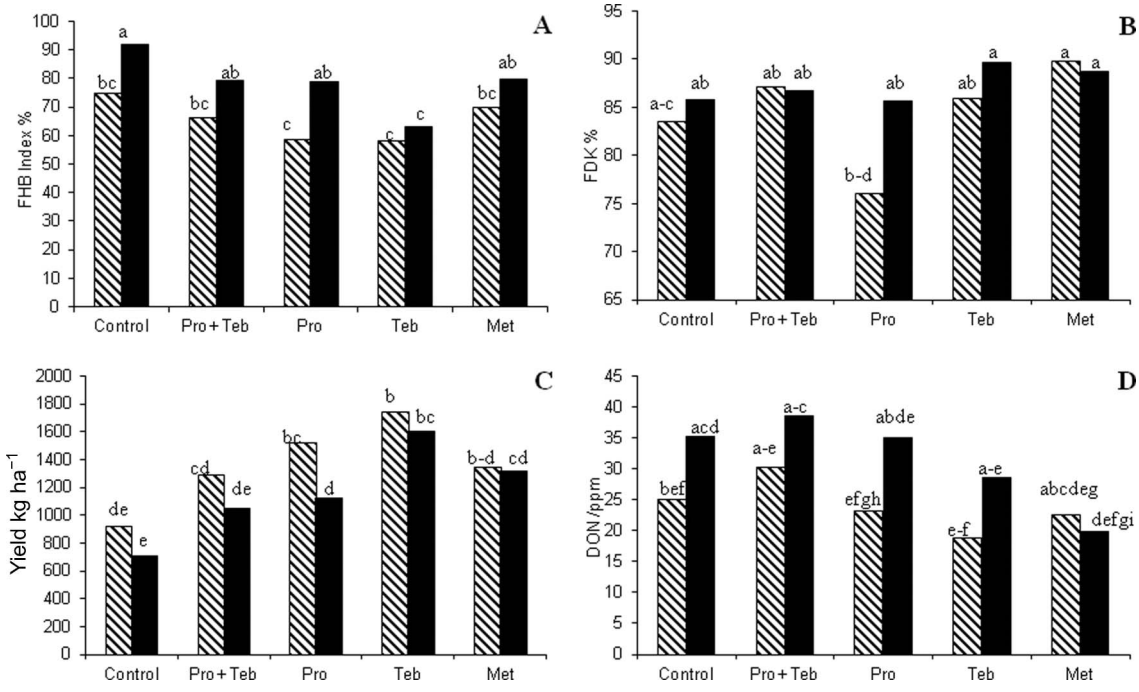

Fig. 2. Fungicide treatment means for (**A**) fusarium head blight index (FHB index), (**B**) percentage of fusarium-damaged kernels (FDK), (**C**) yield and (**D**) concentration of deoxynivalenol (DON) for the 3ADON, 15ADON chemotypes of *Fusarium graminearum* in cultivar 'Roblin' (S) in 2009.

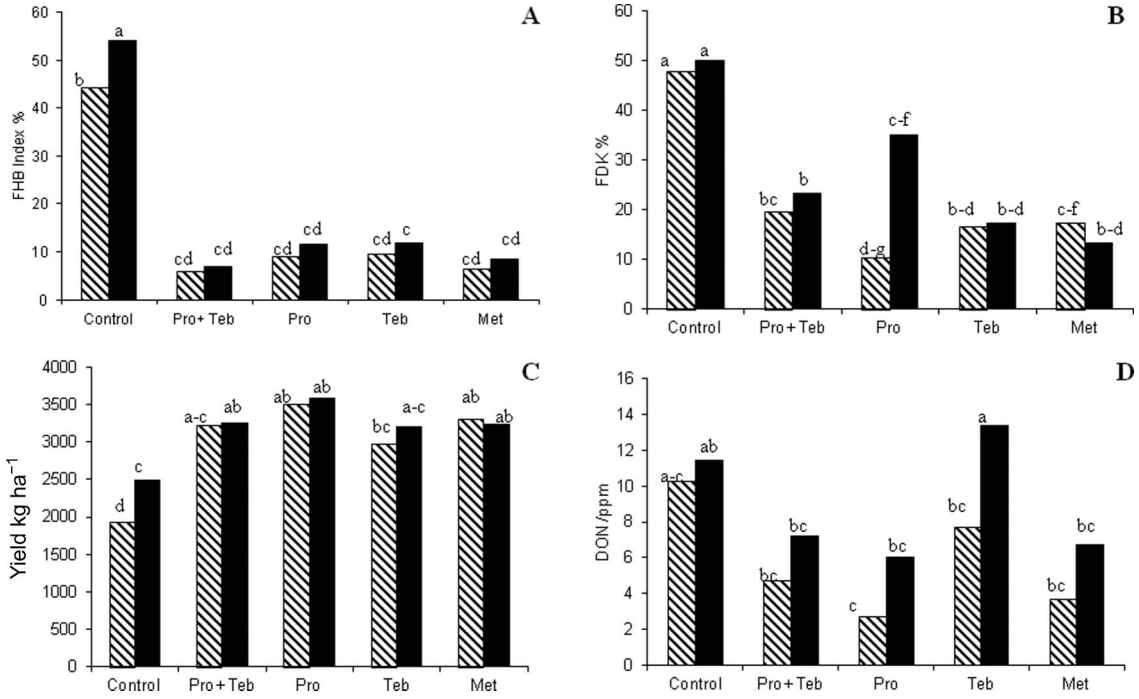

Fig. 3. Fungicide treatment means for (**A**) fusarium head blight index (FHB index), (**B**) percentage of fusarium-damaged kernels (FDK), (**C**) yield and (**D**) concentration of deoxynivalenol (DON) for the 3ADON, 15ADON chemotypes of *Fusarium graminearum* in cultivar 'Glenn' (MR) in 2010.

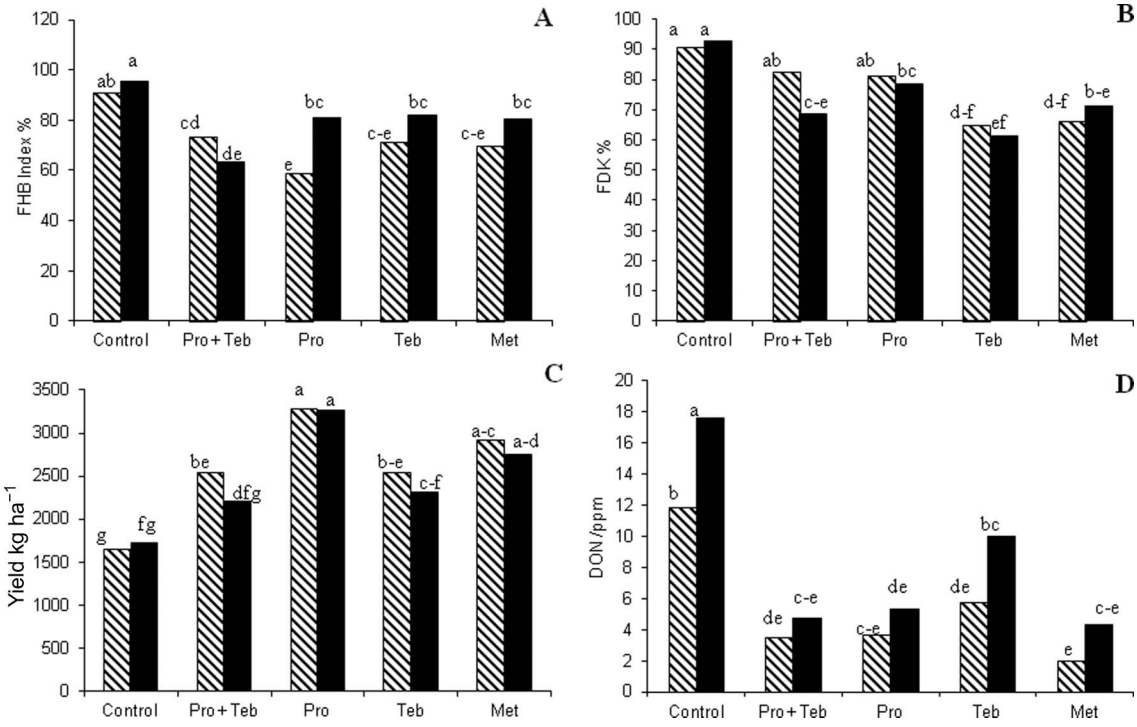

Fig. 4. Fungicide treatment means for (**A**) fusarium head blight index (FHB index), (**B**) percentage of fusarium-damaged kernels (FDK), (**C**) yield and (**D**) concentration of deoxynivalenol (DON) for the 3ADON, 15ADON chemotypes of *Fusarium graminearum* in cultivar 'Roblin' (S) in 2010.

Table 2. Significance of mean square values for fusarium head blight index (FHB index), percentage of fusarium-damaged kernels (FDK), yield and concentration of deoxynivalenol (DON) within each source of variation in 2009 and 2010.

			Significance of mean square values (Type III)							
	df		FHB Index (%)		FDK (%)		Yield (kg ha^{-1})		DON (ppm)	
Source of variance	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010
Overall fungicide	4	4	*	*	*	*	*	*	ns	*
Among fungicides	3	3	ns	ns	ns	*	ns	*	ns	*
Fungicides vs non-sprayed control	1	1	*	*	*	*	*	*	ns	ns
Cultivar	1	1	*	*	*	*	*	*	ns	ns
Fungicide × cultivar	4	4	*	*	*	*	*	ns	*	ns
Isolate	6	6	*	*	*	*	*	*	*	*
Among 3ADON	3	2	ns	*	ns	*	*	ns	*	*
Among 15ADON	1	2	ns	*	ns	*	ns	ns	ns	ns
3ADON vs 15ADON	1	1	*	*	*	ns	*	ns	*	*
Inoculated vs disease control	1	1	*	*	*	*	*	*	*	*
Fungicide × isolate	24	24	ns	*	ns	*	*	ns	ns	ns
Cultivar × isolate	6	6	*	*	ns	*	*	ns	*	ns
Fungicide × cultivar × isolate	24	24	ns	*	ns	*	*	ns	*	ns

Note: *Significant at $P \leq 0.05$; ns = not significant.

2010, weak correlations were observed between the FHB index-DON ($r = 0.367$) and FDK-DON ($r = 0.339$) but the correlation between FHB index-FDK was higher ($r = 0.860$).

Discussion

This is the first field study done in Manitoba to compare the efficacy of fungicides in controlling FHB caused by isolates of the 3ADON and 15ADON chemotypes of

F. graminearum. In both years, the environmental conditions were favourable for development of FHB in wheat. Most of the fungicide treatments reduced FHB index, FDK and DON content and increased yield compared with the non-sprayed fungicide controls. These findings are in agreement with other reports, confirming the effectiveness of triazole fungicides in controlling FHB under both greenhouse and field conditions (Homdork *et al*., 2000; Matthies & Buchenauer, 2000; Pirgozliev *et al*., 2002; Menniti *et al*., 2003). However, variability in efficacy of fungicide control also has been reported. Paul *et al*. (2007) reported variable efficacy of tebuconazole on FHB index and DON content of wheat kernels when used on a susceptible cultivar with the fungicide applied at a single growth stage with the same rate of active ingredient per unit area. This study was based on data collected from uniform fungicide trials conducted at multiple locations across US wheat-growing regions.

In our study, the wheat cultivars (MR and S) had a significant effect on all measured variables except DON. All the variables had a significant fungicide–cultivar interaction except for yield and DON in 2010 (Table 2). This implies that the efficacy of fungicides may change based on the level of resistance of the cultivar and that the choice of cultivar plays a key role in the fungicide–cultivar–isolate interaction. Similarly Wegulo *et al*. (2011) reported that fungicide efficacy in reducing FHB and DON was greater in MR cultivars than in S cultivars.

In our study, significant differences were found between the 3ADON and 15ADON chemotypes for FHB index, FDK, yield and DON concentration in 2009 and FHB index and DON in 2010 (Table 2). For some variables, differences were found among the 3ADON isolates or among 15ADON isolates and the fungicide–isolate interactions were not significant for any of the variables, except yield in 2009 and FHB index and FDK in 2010 (Table 2). This could be because 3ADON isolates produce more DON and other toxins in infected kernels than the 15ADON isolates. It has been reported that 3ADON isolates are more aggressive and produce more toxins than 15ADON isolates (Ward *et al*., 2008). Results from our study suggest that the fungicides tested could be used to control FHB caused either by 3ADON or 15ADON chemotypes of *F. graminearum*.

In some fungicide treatments, FDK and DON content increased compared with the non-sprayed fungicide controls. An increase in DON content after application of strobilurin-based fungicides has been noted in other studies (Milus & Parsons, 1994; Matthies & Buchenauer, 2000; Simpson *et al*., 2001; Pirgozliev *et al*., 2002; Menniti *et al*., 2003). To date, there has not been sufficient evidence to explain the role of fungicides in increasing DON in infected grain. One possibility might be that fungicides delayed FHB development in seeds in such a way that more infected kernels were retained during the harvesting process. Heavily infected kernels tend to be shrivelled, lightweight and may be blown out the back of the combine, while heavier less-infected kernels are retained and this may have led to an increase in DON content. In addition, Audenaert *et al*. (2010) reported that hydrogen peroxide induced by sublethal doses of the triazole fungicide prothioconazole can trigger DON biosynthesis. This could be the reason for the elevated levels of DON in some grain samples treated with prothioconazole+ tebuconazole and prothioconazole.

In our study, a strong correlation between FHB visual ratings and DON content was observed in 2009, but in 2010, although significant, the correlations were poor, especially between FHB index-DON and FDK-DON. While several researchers have reported a strong correlation between FHB visual ratings and DON content in infected grain (Homdork *et al*., 2000; Bai *et al*., 2001; Mesterházy *et al*., 2003; Haidukowski *et al*., 2005) others have observed that DON content is either only moderately or not correlated with the FHB visual ratings (Arseniuk *et al*., 1999; Edwards *et al*., 2001; Shaner & Buechley, 2003). The differences in the correlations among the FHB visual disease ratings and DON content between 2009 and 2010 may be due to the variation in weather conditions between the two field seasons. The temperatures in July and August were lower in 2009 than in 2010 (Table 1), which may have affected disease progression. It has been also reported that different environments may have highly significant effects on DON production by individual isolates (Milus & Parsons, 1994). The poor correlations between FHB index-DON and FDK-DON in 2010 could be explained by comparatively high temperatures between flowering and the last rating date in that year, which may have affected the DON synthesis by individual isolates.

The results of this study suggest that fungicides can be used to control FHB efficiently, even under high FHB disease pressure. However, we observed conflicting evidence specifically for the effect of fungicide application on DON accumulation and FDK levels, whereby some fungicides increased DON and FDK compared with the non-sprayed fungicide control. Significant differences were observed between the two *F. graminearum* chemotypes 3ADON and 15ADON for FHB index, FDK, yield and DON. This study confirmed that fungicides were more effective on wheat cultivars that are moderately resistant to FHB than on susceptible cultivars. Therefore, selection of wheat cultivars with the best level of resistance to disease combined with application of fungicides may provide the best protection of the crop against FHB.

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