Phytopathologia Mediterranea (2012) 51, 1, 236−246

Research Papers

Effect of prothioconazole-based fungicides on *Fusarium* head blight, grain yield and deoxynivalenol accumulation in wheat under field conditions

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Summary. The effect of triazole-based treatments on *Fusarium* head blight (FHB), grain yields and the accumulation of deoxynivalenol (DON) in harvested wheat kernels was evaluated by means of twenty multi-site field experiments performed during five consecutive growing seasons (from 2004–2005 to 2008–2009) in Italy. Fungicide treatments were carried out on different cultivars of common wheat (cv. Serio, Blasco, Genio and Savio) and durum wheat (cv. Orobel, Saragolla, San Carlo, Levante, Duilio, Karur and Derrik) after artificial inoculation with a mixture of toxigenic *Fusarium graminearum* and *F. culmorum* strains. The application of fungicides containing prothioconazole (Proline® or Prosaro®) at the beginning of anthesis (BBCH 61) resulted in a consistent reduction of FHB disease severity (by between 39 and 93%) and DON levels in wheat kernels (by between 40 and 91%) and increased wheat yields (from 0.4 to 5.6 t ha^{-1}, average 2.2 t ha^{-1}), as compared to the untreated/inoculated control. Fungicides containing tebuconazole (Folicur® SE) and cyproconazole plus prochloraz (Tiptor® Xcell) showed a reduced effectiveness compared with prothioconazole-based treatments. All fungicide treatments were more effective in reducing DON and increasing grain yields of common wheat than durum wheat. Results showed that the application of fungicides containing prothioconazole at the beginning of anthesis provided a strong reduction of FHB disease, allowing both an increase in grain yields and a considerable reduction of DON content in wheat kernels.

Key words: cereals, *Fusarium graminearum, Fusarium culmorum,* fungicides, mycotoxins.

Introduction

Fusarium head blight (FHB) of wheat is a worldwide disease caused mainly by a complex of species belonging to the genus *Fusarium* and by *Microdochium nivale* that, under favourable environmental conditions, can colonize plants during the production cycle and cause serious damage in terms of yields and quality of harvested grains (Parry *et al.*, 1995).

Deoxynivalenol (DON), a trichothecene mycotoxin mainly produced by *Fusarium culmorum* and *F. graminearum*, has been shown to be the most common mycotoxin associated with FHB - infected grains; it causes haematic and anorexic syndromes and neurotoxic and immunotoxic effects in mammals (Visconti, 2001). In order to protect human health from exposure to *Fusarium* toxins, the European Commission (EC) has fixed maximum admissible levels for DON (and other mycotoxins) in cereals, including wheat, and derived products intended for human consumption (European Commission, 2006a, 2007). In addition the EC has set guidance values for *Fusari-*

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ISSN (print): 0031-9465
ISSN (online): 1593-2095

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um mycotoxins in products intended for animal feed, fixing the guidance value of DON at 8 mg kg^{-1} in cereals and cereal products with the exception of maize by-products (European Commission, 2006b).

Several studies have shown that a reduction in FHB severity leads to decreased mycotoxin content in grains both naturally or artificially infected. Strategies for FHB control include the use of good agronomic practices (such as crop rotation, tillage, nitrogen fertilization and seed treatment), resistant varieties and treatments with effective fungicides. Generally, a single strategy fails during epidemic conditions (Edwards, 2004; Beyer *et al.*, 2006; Yuen and Schoneweis, 2007). Applications of fungicides containing triazoles (such as tebuconazole, one of the most widely tested products for FHB and DON control, or prothioconazole, a recently registered broad spectrum fungicide belonging to the new chemical class of triazolinthiones) during anthesis, have been shown to be particularly effective in reducing FHB incidence, disease severity and DON accumulation both in glasshouse and in field trials (Mesterhazy, 2003; Haidukowski *et al.*, 2005; Paul *et al.*, 2007; Muellenborn *et al.*, 2008; Paul *et al.*, 2008). The efficacy of triazole-based fungicides for FHB and DON control in wheat has been sumarized in a recent review work that analyzed over 100 uniform fungicide studies carried out in the USA in the field across 11 years and 14 States. Prothioconazole, metconazole and prothioconazole plus tebuconazole showed a superior efficacy compared to tebuconazole alone (Paul *et al.*, 2008). Nevertheless, in some individual studies, the observed difference in efficacy was not significant, suggesting that specific study factors (different application rates, timing and populations, differences in cultivars susceptibility to FHB and DON accumulation, differences in aggressiveness of fungal populations) or environmental factors (differences in weather conditions) were probably influencing the performances of these fungicides (Paul *et al.*, 2008).

In recent years, severe FHB epidemics have occurred in Italy, particularly in the Northern and Central regions. In addition, in several monitoring programmes carried out in Italy, DON was frequently found in wheat kernels, sometimes at high levels (Pascale *et al.*, 2002; Aureli *et al.*, 2009; Plizzari *et al.*, 2009; Visconti and Pascale, 2010). The purpose of this study was therefore to evaluate the effectiveness of prothioconazole-based fungicides in reducing FHB and DON accumulation in wheat grown in Italy, inoculated under field conditions with a mixture of *Fusarium graminearum* and *F. culmorum* and to determine if the effectiveness of treatment with these fungicides was influenced by the wheat species (durum and common wheat).

Materials and methods

Fungal inoculum

Fusarium graminearum (ITEM 126) and *F. culmorum* (ITEM 6273) used to inoculate wheat were obtained from the culture collection of the CNR Institute of Sciences of Food Production, Bari, Italy (http://www.ispa.cnr.it/Collection). Both strains were isolated from wheat in Northern Italy and were able to produce DON *in vitro* when grown on autoclaved wheat. Conidia were obtained by growing the fungal strains in shaking cultures in 1 L Erlenmeyer flasks containing 400 mL of V8 juice (Campbell Grocery Products LTD, King's Lynn, Norfolk, UK) liquid medium (V8 juice 200 mL + $CaCO_3$ 3 g brought to 1 L with distilled water) previously sterilised by autoclaving for 15 min at 121°C. After 7 days of incubation in the dark at 25°C and 150 rpm, the flask contents were filtered through two layers of cheesecloth to obtain a conidial suspension. The concentration of the inoculum was measured with a Thoma camera (HBG Henneberg-Sander GmbH, Lutzellinden, Germany) at the light-microscope.

Fungicides

The following commercially available products were used in the field experiments: Proline® (prothioconazole 250 g L^{-1}, application rate 200 g a.i. ha^{-1}), Prosaro® (prothioconazole 125 g L^{-1} + tebuconazole 125 g L^{-1}, application rate 125 + 125 g a.i. ha^{-1}) and Folicur® SE (tebuconazole 43.1 g L^{-1}, application rate 215 g a.i. ha^{-1}) manufactured by Bayer CropScience (Milan, Italy), Tiptor® S (cyproconazole 48 g L^{-1} + prochloraz 360 g L^{-1}, application rate 55 + 425 g a.i. ha^{-1}) or Tiptor® Xcell (cyproconazole 22 g L^{-1} + prochloraz 170 g L^{-1}, application rate 55 + 425 g a.i. ha^{-1}) manufactured by Syngenta Crop Protection (Milan, Italy).

Field trials

Separate field trials were carried out in Northern Italy (Pavia, Mantova, Bologna and Ravenna provinces) and in Central Italy (Perugia province) during five consecutive growing seasons (from 2004–2005 to 2008–2009) using common wheat (*Triticum aestivum* L.) cultivars Serio, Blasco, Genio and Savio (9 uniform fungicide studies) and durum wheat (*Triticum durum* Desf.) cultivars Orobel, Saragolla, San Carlo, Levante, Duilio, Karur and Derrik (11 uniform fungicide studies) (Table 1). Cultivar selection was based on their high or moderate susceptibility to Fusarium head blight and their widespread cultivation in the region where the experiments were performed. All plots were cultivated according to normal agronomic practices which were standardised across sites.

The experimental design was a randomised block with 4 replicate plots for each trial (four fungicide treatments and one inoculated/untreated control). Each plot had a surface area of 12–18 m^2 (1.2 × 10 m or 2 × 9 m or 2.5 × 7 m). Plots were artificially inoculated by spraying on each plot 450–700 mL of a suspension containing a mixture of conidia of *F. culmorum* and *F. graminearum* (about 1.0×10^5 conidia per mL) with an Echo motorized pump (Model SHR 4100, KIORITZ Corporation, Tokyo, Japan) or by hand pump. Delivery pressure at the nozzle (Teejet 8002 VS, TeeJet-LH Agro South Europe, Oilvet Orleans, France) was 2.5 atm, and the distance of the nozzle from the ears was 4–5 cm in order to avoid conidial dispersion. Fungicide treatments were performed within 24–48 hours before inoculation by using the same Echo pump with a delivery pressure at the nozzle (Teejet 8003 VS, TeeJet-LH Agro South Europe, Oilvet Orleans, France) of 3 atm and a delivery rate of 500 L ha^{-1}. Both inoculation and treatments were performed in evening hours with a relative humidity above 75% and in the absence of wind. A single fungicide treatment was applied at the beginning of anthesis (BBCH 61). In total, prothioconazole and prothioconazole plus tebuconazole were tested in 20 studies, tebuconazole in 11 studies and cyproconazole plus prochloraz in 17 studies.

The effectiveness of fungicides in disease control, their influence on grain yield and DON content in kernels were determined by comparison with artificially inoculated untreated plots.

Visual disease assessment

Visual disease assessment and in-field activity of the fungicides were evaluated at late milk stage (BBCH 77) by examining 100 heads collected at random from each plot. The incidence of FHB (percentage of diseased heads) and severity (percentage of infected area of the head) were calculated as a mean value of the experimental plots (n = 4).

Table 1. Cultivars, locations and years of experimentation.

Cultivar	Common wheat	
	Location	Growing season
Serio	Pavia	2004–2005
	Bologna	2004–2005
	Ravenna	2006–2007
	Ravenna	2007–2008
	Ravenna	2008–2009
Genio	Perugia	2006–2007
	Perugia	2007–2008
Blasco	Bologna	2005–2006
Savio	Perugia	2007–2008

Cultivar	Durum wheat	
	Location	Growing season
Saragolla	Bologna	2005–2006
	Bologna	2006–2007
San Carlo	Bologna	2006–2007
	Bologna	2007–2008
Levante	Ravenna	2006–2007
	Ravenna	2007–2008
Duilio	Perugia	2006–2007
	Perugia	2007–2008
Orobel	Bologna	2005–2006
Karur	Mantova	2008–2009
Derrik	Rovigo	2008–2009

Disease severity was assessed by using a scale similar to that of Parry *et al.* (1984) with 8 evaluation classes (0, 2, 5, 10, 25, 50, 75 and 90% area infected) and applying the following formula: ∑(number of heads per evaluation class × evaluation class)/total number of scored heads.

Grain yields

At maturity (BBCH 89), ears were mechanically harvested using a small plot combine harvester (Hege mod. 125B, Maschinenbau, Germany) and the grain yields (t ha^{-1}) were determined at about 13% kernel relative humidity (Infratec^{TM} grain analyzer 1241, Foss, Italy). Subsequently, a homogeneous 1 kg of kernels was taken from each plot for DON analysis.

DON analysis

Deoxynivalenol (DON) concentrations were determined according to the method reported by Haidukowski *et al.* (2005), based on immunoaffinity column clean-up of extracts and toxin determination by HPLC/UV. Appropriate dilutions of sample extracts before loading on immunoaffinity columns were necessary for samples contaminated with DON at levels higher than 2.0 mg kg^{-1} to avoid saturation of the DON-antibody binding sites. The detection limit of the method was 0.05 mg kg^{-1} (signal to noise ratio of 3:1).

Statistical analysis

Data were processed by the Kruskal-Wallis non-parametric test due to failure of Levene's test for evaluating variance homogeneity. Mann-Whitney test was used for pairwise comparison for each variable; control of Type I error across tests was performed by using the Bonferroni approach. Statistical analyses were performed separately for common and durum wheat field trials. All data were processed by the PASW® Statistic 18 software (formerly SPSS).

Results

Mean values of FHB incidence and severity, yields and DON content in inoculated control and fungicide treated plots of common and durum wheat are summarized in Table 2. Figures 1 and 2 show box plots of distribution of FHB incidence and severity and grain yields for inoculated and untreated wheat control (CONTROL) and for plots treated with prothioconazole (PROT), prothioconazole + tebuconazole (PROT+TEBU), tebuconazole (TEBU) and cyproconazole + prochloraz (CYPRO+PROC). Figure 3 shows the effect of fungicide treatments on deoxynivalenol (DON) content in harvested grains (percentage of DON reduction), as compared to the inoculated and untreated control.

Visual assessment of FHB

Common wheat

Fusarium head blight incidence and disease severity in inoculated and untreated control plots ranged from 25.0 to 100% (mean value of 81.2%) and from 29.9 to 90.2% (mean value of 73.7%), respectively. Higher values of disease severity were observed in Ravenna trials (cv. Levante, 2007–2008 and cv. Serio, 2008–2009). The mean disease level was lower in fungicide treated plots. In particular, for prothioconazole treatments, FHB incidence ranged from 3.0 to 68.0% (mean value of 26.0%), and disease severity from 6.9 to 60.3% (mean value of 26.2%). Similar values were observed when a mixture of prothioconazole plus tebuconazole was used (mean values of FHB incidence and severity of 23.8 and 25.6%, respectively). The use of tebuconazole and cyproconazole plus prochloraz reduced both incidence and severity of the disease, although with lesser effects than those obtained with prothioconazole-based fungicides (mean values of 48.2% and 33.8% for tebuconazole, and of 43.5% and 41.2% for cyproconazole plus prochloraz).

Durum wheat

Fusarium head blight incidence and disease severity in inoculated and untreated control plots were variable, ranging from 15.8 to 100% (mean value of 64.9%) and from 5.0 to 90.0% (mean value of 47.4%), respectively. All fungicides treatments reduced both disease incidence and severity. In particular, FHB incidence and disease severity ranged from 2.0 to 38.4%, (mean value of 16.2%) and from 0.5 to 39.2% (mean value of 13.5%), respectively with prothioconazole treatments and from 2.0 to 50.0%, (mean value of 20.3%) and from 1.4 to 40.0% (mean value of 14.9%), respectively with prothioconazole plus tebuconazole treatments. The efficacy of tebuconazole and cyproconazole plus prochloraz treatments against FHB symptoms was less marked than prothi-

Table 2. Mean values and mean rank scores of *Fusarium* head blight incidence (I) and severity (DS), yields and deoxynivalenol (DON) content in inoculated and untreated control plots and in fungicide-treated plots.

Fungicide treatment	Total number of trials	*Fusarium* head blight		Yield (t ha^{-1})	DON (mg kg $^{-1}$)
		I (%)	DS (%)		
Common wheat					
Inoculated and untreated control	9	81.2 (128.9) c^{a}	73.7 (133.8) c	5.44 (29.3) c	13.39 (113.0) c
Prothioconazole	9	26.0 (52.4) a	26.2 (50.1) a	8.27 (104.1) a	2.06 (50.5) a
Prothioconazole + tebuconazole	9	23.8 (48.5) a	25.6 (49.8) a	8.17 (100.7) a	2.29 (53.9) a
Tebuconazole	5	48.2 (88.0) b	33.8 (67.8) ab	7.58 (84.3) ab	5.60 (73.6) ab
Cyproconazole + prochloraz	7	43.5 (79.0) b	41.2 (88.5) b	7.28 (76.3) b	5.92 (83.1) b
Kruskal-Wallis test (total raw data)	-	$P<0.000$ (156)	$P<0.000$ (156)	$P<0.000$ (156)	$P<0.000$ (148)
Durum wheat					
Inoculated and untreated control	11	64.9 (139.6) c*	47.4 (132.0) c	5.00 (70.7) b	13.16 (124.8) c
Prothioconazole	11	16.2 (56.1) a	13.5 (63.6) a	6.78 (116.8) a	4.40 (74.8) a
Prothioconazole + tebuconazole	11	20.3 (65.4) a	14.9 (69.3) a	6.64 (112.1) a	5.26 (79.2) ab
Tebuconazole	6	33.5 (90.2) b	21.1 (83.8) ab	6.07 (98.9) ab	8.06 (93.9) ab
Cyproconazole + prochloraz	10	38.8 (102.3) b	26.3 (99.0) b	5.84 (93.7) b	9.65 (103.2) b
Kruskal-Wallis test (total raw data)	-	$P<0.000$ (180)	$P<0.000$ (180)	$P<0.001$ (196)	$P<0.000$ (189)

[a] Mean rank scores are reported in brackets; values followed by the same letter within the same wheat type (common or durum) are not significantly different at $P=0.05$ according to Mann-Whitney test.

oconazole-based fungicides. Mean values of 33.5% (incidence) and 21.1% (severity) for tebuconazole, and 38.8% (incidence) and 26.3% (severity) for cyproconazole plus prochloraz were observed.

Grain yields

Common wheat

Yield values in inoculated and untreated controls ranged from 3.54 to 7.32 t ha^{-1} (mean value of 5.44 t ha^{-1}). An increase in yield values was observed in all plots when cultivars were treated with fungicides. No significant difference ($P<0.05$) was observed between treatments with prothioconazole and prothioconazole plus tebuconazole (mean values of 8.27 t ha^{-1} and 8.17 t ha^{-1}, respectively). The highest yields were obtained for the cv Serio after prothioconazole treatment (10.10 t ha^{-1} in Ravenna, 2007–2008) and after prothioconazole plus tebuconazole treatment (9.44 t ha^{-1} in Bologna, 2004–2005). The use of tebuconazole and cyproconazole plus prochloraz also showed higher yields with respect to the untreated control. Nevertheless, these values were always inferior to those obtained with the other fungicide treatments (mean values of 7.58 t ha^{-1} and 7.28 t ha^{-1}, respectively).

Durum wheat

Yields in inoculated and untreated controls varied from 1.69 to 7.40 t ha^{-1} (mean value of 5.00 t ha^{-1}). Fungicide treatments increased yield values. In particular, yields obtained after treatments with prothioconazole and prothioconazole plus tebuconazole were always higher (mean values of 6.78 t ha^{-1} and 6.64 t ha^{-1}, respectively) than yields obtained with tebuconazole and cyproconazole plus prochloraz (mean values of 6.07 t ha^{-1} and 5.84 t ha^{-1}, respectively). The highest yields were obtained for the cv Karur after prothioconazole (10.18 t ha^{-1}) and prothioconazole plus tebuconazole (10.03 t ha^{-1}) treatments in Ravenna, 2008–2009.

DON contamination

Common wheat

DON levels in artificially inoculated wheat cultivars (untreated control) ranged from 1.50 mg kg^{-1} (cv. Serio, Pavia, 2004–2005) to 28.55 mg kg^{-1} (cv. Se-

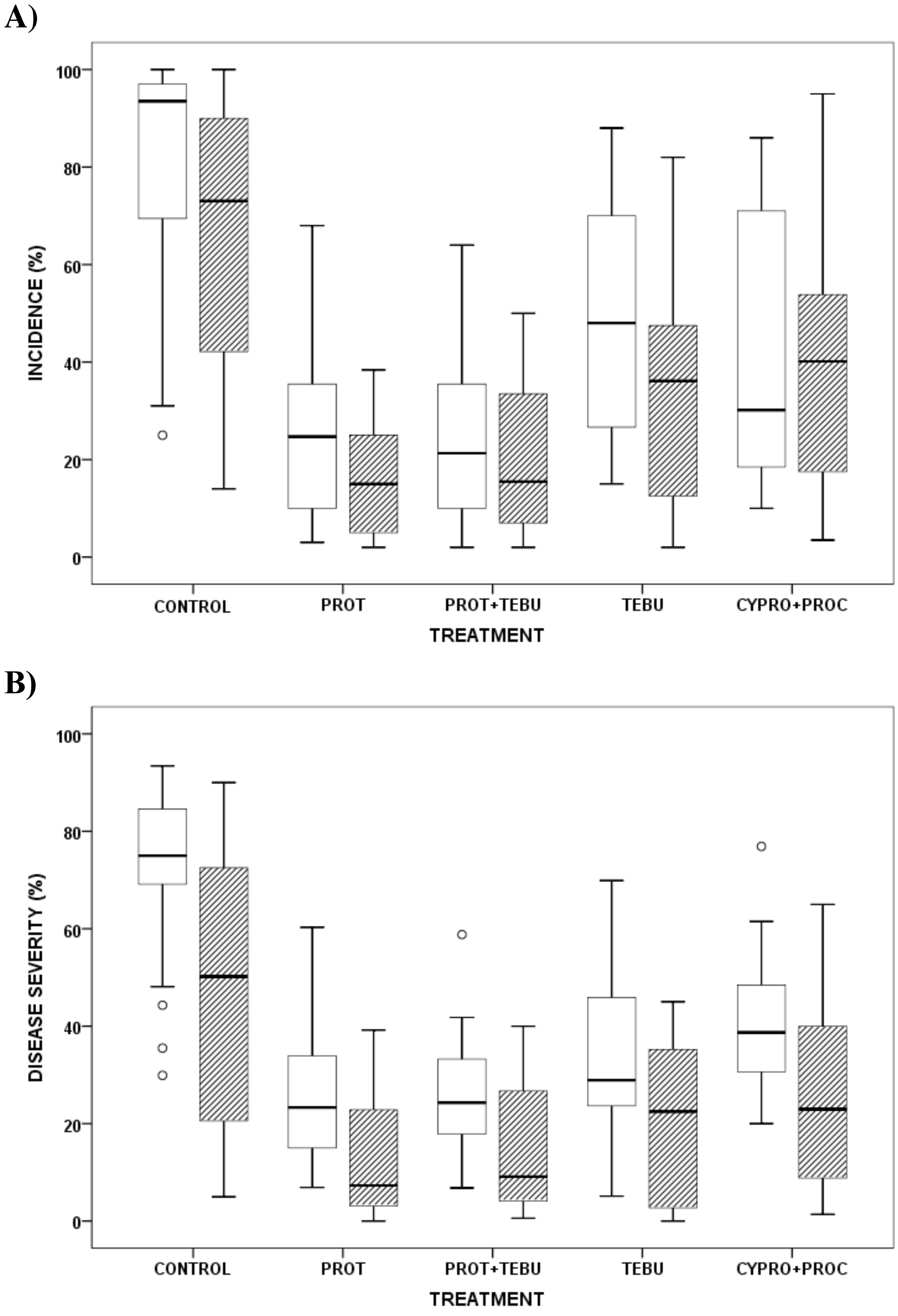

Figure 1. Box plots of data from fungicide-treated plots and untreated inoculated control plots (five-years' experiments) summarizing **A)** distribution of FHB incidence (%) and **B)** FHB severity (%) for common wheat (☐) and durum wheat (▨). Solid lines inside the box represent the median. The length of the box is the interquartile range (IQR) computed from Tukey's hinges representing the 25th and 75th percentile of data. Vertical bars extending beyond the boxes represent the 10th and 90th percentiles. Circles indicate outliers (i.e. values more than 1.5 IQR's but less than 3 IQR's from the end of the box).

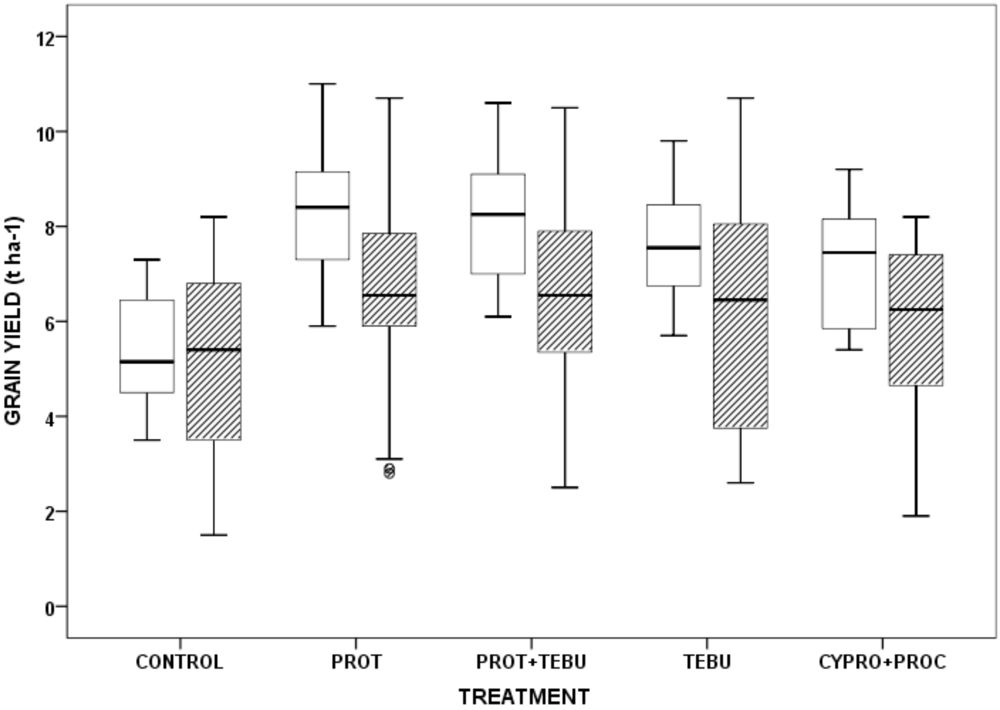

Figure 2. Box plots of data from fungicide-treated plots and untreated inoculated control plots (five-years' experiments) summarizing distribution of grain yields (t ha^{-1}) for common wheat (☐) and durum wheat (▨). Solid lines inside the box represent the median. The length of the box is the interquartile range (IQR) computed from Tukey's hinges representing the 25th and 75th percentile of data. Vertical bars extending beyond the boxes represent the 10th and 90th percentiles. Circles indicate outliers (i.e. values more than 1.5 IQR's but less than 3 IQR's from the end of the box).

rio, Ravenna, 2007–2008). The mean value for the 5-year field experiments was 13.39 mg kg^{-1}. Treatments with fungicides resulted in consistent reductions of DON levels in wheat kernels, by between 34 and 88% (mean value of 4 replicate plots), as compared with levels found in inoculated untreated control samples. Prothioconazole and prothioconazole plus tebuconazole treatments caused the greatest reduction of DON content (by between 68 and 88%) in kernels of the examined cultivars. No significant difference (P<0.05) was observed between the two treatments with overall DON levels, based on means from treated plots, of 2.06 mg kg^{-1} and 2.29 mg kg^{-1}, respectively. Also treatments with tebuconazole and cyproconazole plus prochloraz reduced the DON content (by between 34 and 85%), although they were less effective than prothioconazole-based treatments (DON mean levels of 5.60 mg kg^{-1} and 5.92 mg kg^{-1}, respectively).

Durum wheat

DON levels in artificially inoculated wheat cultivars (untreated control) ranged from 0.78 mg kg^{-1} (cv. Orobel, Bologna, 2005-2006) to 49.86 mg kg^{-1} (cv. Derrik, Rovigo, 2008–2009) with a mean value of 13.16 mg kg^{-1} (5-year experiments). In general, fungicide-treated plots had DON levels lower than inoculated untreated control plots. Prothioconazole led to the greatest reduction of DON levels in wheat kernels, by between 47 and 91% (DON mean level of 4.40 mg kg^{-1}). Similar efficacy was shown by prothioconazole plus tebuconazole treatments (by between 40 and 91%, DON mean level of 5.26 mg kg^{-1}). Also treatments with tebuconazole and cyproconazole plus prochloraz reduced the DON content (by between 10 and 79%), although they were less effective than the prothioconazole-based treatments (DON mean levels of 8.06 mg kg^{-1} and 9.65 mg kg^{-1}, respectively).

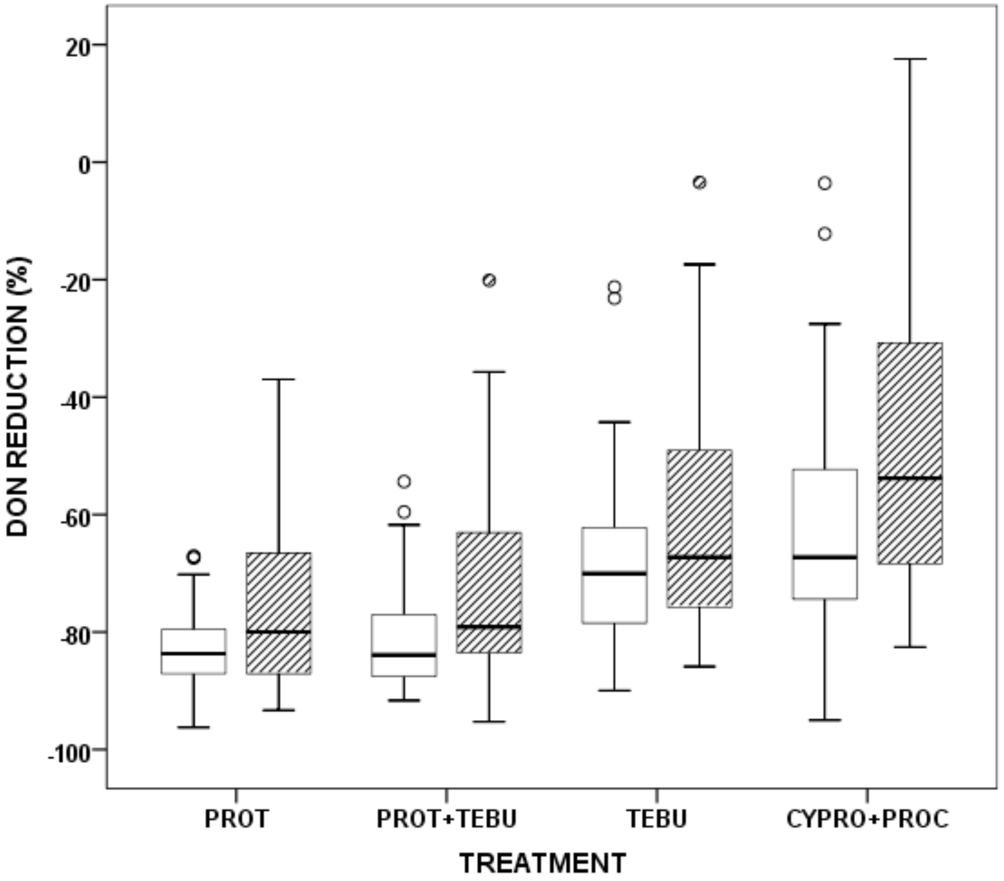

Figure 3. Box plots of data from fungicide-treated plots (five-years' experiments) summarizing the percentage of reduction of DON content of harvested common wheat (☐) and durum wheat (▨), as compared to the inoculated and untreated control (DON contamination range in control samples 1.50–28.55 mg kg^{-1} for common wheat and 0.78–49.86 mg kg^{-1} for durum wheat). Solid lines inside the box represent the median. The length of the box is the interquartile range (IQR) computed from Tukey's hinges representing the 25th and 75th percentile of data. Vertical bars extending beyond the boxes represent the 10th and 90th percentiles. Circles indicate outliers (i.e. values more than 1.5 IQR's but less than 3 IQR's from the end of the box).

Discussion

Recent surveys on the occurrence of DON in wheat collected in Italy indicated a widespread DON contamination in samples grown in Northern and Central Italy with incidence and levels of contamination depending on the growing season. In particular, very high levels of contamination (up to 13.5 mg kg^{-1}) were observed in both durum and common wheat samples collected in 2007–2008 (Aureli *et al.*, 2009; Plizzari *et al.*, 2009; Visconti and Pascale, 2010). In the present study, high levels of DON and FHB incidence and severity were found in wheat samples from all inoculated and untreated plots.

It is well-known that good agricultural practices can reduce the risk of *Fusarium* infection and the consequent accumulation of relevant mycotoxins in harvested kernels (European Commission, 2006c). Nevertheless, when weather conditions are favourable for fungal infection, the use of *Fusarium*-controlling fungicides is necessary to limit FHB and to reduce mycotoxin formation in the field. Over the last few years, proper use of triazole-based fungicides containing tebuconazole, metconazole or prothioconazole at flowering has been shown to be effective in controlling FHB and DON levels in wheat kernels and to increase grain yields (Suty-Heinze and Dutzmann, 2004; Haidukowski *et al.*, 2005; Paul *et al.*, 2007; Muellenborn *et al.*, 2008; Paul *et al.*, 2008; Paul *et al.*, 2010). More recently, Edwards and Godley (2010) showed that prothioconazole application before head emergence, in addition to treatments at flowering, contributed significantly to reduce FHB severity (up to 97%) and DON levels (up to 83%) in harvested grain, as compared to untreated control plots.

The present study evaluated the effect of prothioconazole-based fungicides on Fusarium head blight (FHB) disease, grain yields and DON accumulation in wheat grown in Italy. All fungicide treatments (prothioconazole, prothioconazole plus tebuconazole, tebuconazole and cyproconazole plus prochloraz) decreased incidence and severity of the disease, increasing grain yields as compared with the inoculated and untreated controls, although differences in efficacy of treatments were observed in the different locations and years of experimentation. DON levels were significantly reduced ($P<0.05$) by all fungicide treatments as compared with the inoculated and untreated control (Table 2).

Overall fungicide efficacy on FHB disease, yields and DON content, as compared to the inoculated and untreated control, for common and durum wheat, is shown in Table 3. Prothioconazole-based fungicides were significantly more effective than tebuconazole or cyproconazole plus prochloraz in reducing FHB symptoms and DON levels, with the exception of tebuconazole treatments of durum wheat for which no significant difference was observed in reducing FHB severity. No significant difference was found between treatments with prothioconazole alone or in combination with tebuconazole for both common and durum wheat studies (Table 3). In addition, fungicide treatments increased production for all cultivars, compared with inoculated control plots, mainly due to its capacity to reduce the severity of the disease in most of the cultivars. Increases were more evident when prothioconazole or prothioconazole plus tebuconazole was used (average yield increase of 2.82 and 2.73 t ha^{-1} for common wheat and 1.77 and 1.63 t ha^{-1} for durum wheat, respectively), although no significant difference was observed between treatments with prothioconazole alone or in combination with tebuconazole in comparison with tebuconazole or cyproconazole plus prochloraz (Table 3).

Table 3. Effect of fungicides on *Fusarium* head blight incidence (I) and severity (DS), yields and deoxynivalenol (DON) content in harvested wheat kernels – average values of five-year field trials – as compared to the inoculated and untreated control.

Fungicide treatment	Total number of trials	*Fusarium* head blight reduction		Yield increase (t ha^{-1})	DON reduction (%)
		I (%)	DS (%)		
Common wheat					
Prothioconazole	9	68 (49.0) a[a]	65 (48.9) a	2.82 (67.1) a	81 (40.1) a
Prothioconazole + tebuconazole	9	71 (44.8) a	65 (48.7) a	2.73 (65.4) a	79 (44.1) a
Tebuconazole	5	41 (92.3) b	54 (71.0) b	2.14 (42.3) a	62 (76.0) b
Cyproconazole + prochloraz	7	51 (72.8) b	48 (83.2) b	1.86 (58.6) a	58 (83.4) b
Kruskal-Wallis test (total raw data)	-	$P<0.000$ (120)	$P<0.000$ (120)	$P<0.056$ (120)	$P<0.000$ (114)
Durum wheat					
Prothioconazole	11	72 (50.4) a[*]	72 (52.2) a	1.77 (81.4) a	75 (54.2) a
Prothioconazole + tebuconazole	11	68 (60.2) a	68 (61.6) ab	1.63 (83.0) a	71 (61.0) a
Tebuconazole	6	57 (80.2) b	64 (72.5) b	1.07 (75.6) a	60 (87.1) b
Cyproconazole + prochloraz	10	45 (97.9) c	49 (99.5) c	0.82 (64.5) a	49 (102.4) b
Kruskal-Wallis test (total raw data)	-	$P<0.000$ (140)	$P<0.000$ (140)	$P<0.212$ (152)	$P<0.000$ (146)

[a] Mean rank scores are reported in brackets; values followed by the same letter within the same wheat type (common or durum) are not significantly different at $P=0.05$ according to Mann-Whitney test.

In general, the efficacy of all fungicide treatments to reduce DON levels and to increase grain yields was significantly higher (*P*<0.01) for common wheat (*Triticum aestivum* L.) than durum wheat (*Triticum durum* Desf.), whereas no significant difference was observed in the reduction of disease incidence and severity of the two wheat species. Prothioconazole-based fungicides showed a higher efficacy compared with the other treatments (Table 3). For all treatments, DON reduction percentage was within a narrower range in common wheat studies than in durum wheat studies (Figure 3).

Our finding are in agreement with other investigations carried out on a large number of uniform fungicide studies in the field (multivariate meta-analysis of 11 years of data from 14 U.S. states) proving the effectiveness of some triazole-fungicides, including prothioconazole, for Fusarium head blight and DON control in wheat (Paul *et al.*, 2008). Our data also agree with recent investigations regarding the relationship between FHB disease and yield, showing a negative correlation between these two parameters (Paul *et al.*, 2010).

It has been shown that tebuconazole, the most commonly used fungicide against *Fusarium* disease of wheat, is the most effective against FHB and DON at moderate disease and toxin levels and when applied to moderately resistant cultivars (Mesterhazy *et al.*, 2003; Paul *et al.*, 2007). Despite the forced conditions, i.e. artificial inoculation of highly pathogenic and toxigenic species of *Fusarium*, our findings show that the application of fungicides containing prothioconazole at the beginning of anthesis provides a strong reduction of FHB disease caused by *F. graminearum* and *F. culmorum*, allowing both an increase in grain yield and a considerable reduction of DON content in wheat kernels. The efficacy of treatments was nevertheless less pronounced for durum wheat when visual disease severity was very high. In particular, a lower percentage of reduction of DON (ranging from 40 to 57%) was observed in wheat samples from plots where DON levels in the untreated and inoculated control were higher than 25 mg kg^{-1}, as compared to the other plots containing DON levels in the control samples less than 25 mg kg^{-1} (DON reduction ranging from 70 to 91%).

In conclusion, prothioconazole treatments, alone or in combination with tebuconazole, together with good agricultural management practices, such us tillage, crop rotation and resistant cultivars, are a useful tool for farmers to minimise *Fusarium* infection and to reduce DON levels in harvested grains, guaranteeing at the same time higher yields and grain quality compared to other fungicides treatments. Our results, obtained under field conditions at different years and environmental conditions, provide useful information for wheat protection programs against toxigenic fungi responsible for FHB disease and the consequent DON accumulation in grains, particularly in those years in which environmental conditions could be favorable to cause severe epidemics.

Acknowledgements

The authors would like to thank the Cooperativa Terremerse (Ravenna, Italy), the Consorzio Agrario di Bologna e Modena (Bologna, Italy), the Consorzio Agrario di Ravenna (Ravenna, Italy) and the Section of Agronomy and Ecology of the University of Perugia for their contribution in the field experiments.

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Accepted for publication: November 9, 2011