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Effect of fungicides on *Fusarium* head blight and deoxynivalenol content in durum wheat grain

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Abstract

In 1998–99 and 1999–2000 six trials were conducted to evaluate the effect of fungicides on *Fusarium* head blight in the field, on infected kernels and deoxynivalenol (DON) concentration in grain. A single application of prochloraz, tebuconazole, epoxiconazole or bromuconazole, applied to durum wheat varieties at the manufacturer's recommended dose at the beginning of anthesis stage, provided good control of the disease when infective pressure in the field was low to medium, and when the main pathogens were *F. graminearum* and *F. culmorum*. Kresoxim-methyl showed a low efficacy at controlling the disease. Tebuconazole, prochloraz and bromuconazole were effective at controlling *F. graminearum* and *F. culmorum*, while kresoxim-methyl was not effective in reducing *Fusarium* infected kernels. DON concentration in grain of cultivars inoculated with *F. graminearum* and *F. culmorum* was high, averaging 4.2 mg kg^{-1} (untreated control). Tebuconazole, prochloraz and bromuconazole reduced DON concentration by 43%, while epoxiconazole was ineffective. DON concentration in kernels of naturally infected cultivars was 1.95 mg kg^{-1}, a concentration which exceeds the 1 mg kg^{-1} maximum level of contamination allowed in the United States. Furthermore prochloraz, bromuconazole and tebuconazole applications, in the naturally inoculated trials, reduced DON concentration from 73% to 96%, while epoxiconazole showed the lowest effectiveness. Moreover, a positive linear correlation between *Fusarium* infected grains and the DON concentration was observed.

Introduction

Over the last ten years, in many wheat-growing areas of Central and Northern Italy, there has been an increase in severity of *Fusarium* head blight (FHB), also known as scab or ear blight (Pancaldi et al., 1997; Balmas et al., 2000). This is one of the most severe wheat diseases occurring worldwide causing reduced yields, discoloured and shrivelled kernels.

Fusarium graminearum (Schwabe) Group 2 *sensu* Francis and Burgess, *F. culmorum* (Smith) Sacc., *F. avenaceum* (Corda ex Fr.) Sacc., *F. poae* (Peck) Wollenw. and *Microdochium nivale* (Fr.) Samuels & Hallet var. *majus* and var. *nivale* are the major fungal pathogens responsible for head infections in Italy (Casulli et al., 1995; Pancaldi et al., 1997), in the UK (Parry et al., 1995; Turner and Jennings, 1997), in Northern Europe (Elen et al., 1997; Obst et al., 1997; Perkowski et al., 1997; Caron, 2000), in midwestern states of USA and in Canada (Bai and Shaner, 1994; Gilbert et al., 1994; McMullen et al., 1997). Several *Fusarium* species are toxigenic. One of the most common and harmful is deoxynivalenol (DON or vomitoxin), belonging to the B group of trichothecenes. Toxin generation may occur in the field but continues, in favourable conditions during storage. DON is produced mainly by *F. graminearum* and *F. culmorum* (Miller et al., 1991; D'Mello et al., 1997; Bottalico, 1998). This mycotoxin is responsible for haemorrhagic and anorexic syndromes in livestock. Pigs fed with grain contaminated by DON display a considerable loss of body weight, resulting in severe economic problems for farmers (Snijders and Perkowski, 1990; Mirocha et al., 1995; Rotter and Prelusky, 1996).

From a recent survey conducted on wheat from different Italian regions, DON was in concentrations higher than 1 mg kg^{-1}, the maximum level allowed in the United States and CIS (former USSR) for finished products for human consumption. Guidelines have also been set for Austria (0.5 mg kg^{-1}) and Canada (2 mg kg^{-1}) (McMullen et al., 1997; Lops et al., 1998; Pascale et al., 2000).

The activity of several fungicides against the pathogens responsible for wheat FHB, tested internationally, gave different results. Some compounds were not very effective in reducing head blight (Milus and Parsons, 1994), whereas some others gave successful control of FHB (Fehrmann and Ahrens, 1984; Mauler-Machnik and Suty, 1997; Matthies and Buchenauer, 2000; Homdork et al., 2000). The use of fungicides in the field may also reduce the presence of DON in kernels. Recently, a relatively new group of fungicides, the strobilurins, have been introduced for use on cereals. These compounds have been shown to be very effective against *M. nivale* and less effective against *F. culmorum* (Bertelsen et al., 1999; Faure and Declercq, 1999). This treatment may also lead to an increase in DON production (Gareis and Ceynowa, 1994; D'Mello et al., 1998; Simpson et al., 2001).

The aim of this research was to evaluate the effect of several fungicides on FHB and DON concentration in durum wheat grains.

Materials and methods

Experimental design

Six trials were carried out over two consecutive years in fields near Bologna (Italy). Susceptible wheat cvs Duilio and Simeto were used in 1998–99, and the cvs Duilio, Simeto, Bracco and Lloyd in 1999–2000. The experimental design was a randomised block with four replications (plots) in both years. Each plot had a surface area of 11.1 m^2 (1.85 × 6 m). In 1998–99, the treatments tested were: tebuconazole at 250 g ha^{-1}; epoxiconazole at 125 g ha^{-1}; kresoxim-methyl at 250 g ha^{-1}; bromuconazole at 250 g ha^{-1} and an untreated control. In 1999–2000, treatments tested were: tebuconazole at 250 g ha^{-1}; epoxiconazole at 125 g ha^{-1}; prochloraz at 585 g ha^{-1}; bromuconazole at 250 g ha^{-1} and an untreated control. These fungicides were tested since in Italy there are few data on their efficacy on FHB in wheat. Furthermore, epoxiconazole and kresoxim-methyl (a strobilurin), are not registered in Italy for wheat. Fungicides were applied at the beginning of anthesis at Zadoks growth stage (GS) 60–61 (Zadoks et al., 1974), using a motorized pump (Solo 422). The delivery pressure at the nozzles (Teejet with inverted cone) was 4 atm and the volume of water delivered was 600 l ha^{-1}. Among the four cultivars tested, Bracco and Lloyd were naturally infected, while Duilio and Simeto were artificially inoculated with a single isolate of *F. graminearum* and *F. culmorum*. The suspension contained conidia of *F. graminearum* (6.5 × 10^5 conidia ml^{-1} in 1998–99 and 4.5 × 10^5 conidia ml^{-1} in 1999–2000) and *F. culmorum* (2.5 × 10^4 conidia ml^{-1} in both years). The difference in conidial concentration was due to *F. culmorum* producing less conidia. Each plot was inoculated with 400 ml of spore suspension, within 24 h following fungicide treatments. The inoculum, sprayed at anthesis under favourable conditions, rapidly colonised the plants.

Head infection determination

Activity of the products in the field was evaluated on 100 heads collected at random from each plot. The percentage of infected heads (incidence) and the infected area of the head (severity) were calculated as a mean for each plot. Severity was assessed using a scale similar to that of Parry et al. (1984), with eight evaluation classes (0%, 2%, 5%, 10%, 25%, 50%, 75% and 90% area infected) and by applying the following formula: number of heads in each class × each evaluation class/total number of heads. Efficacy percentage was determined by applying Abbott's formula: (% infection control − % treated infection/% infection control) × 100.

Percentage of Fusarium *infected kernels*

The trial plots were mechanically harvested and, after threshing, a homogeneous 500 g sample of grain at 13% moisture was taken from each replicate (plot) and bulked to provide a 2 kg sample per treatment. A 50 g sub-sample of grain was randomly selected to determine the weight (g) of 1000 kernels (using an AG 204 Mettler Toledo scale). The incidence of grain infected with *Fusarium* spp., *F. graminearum* and *F. culmorum*, was evaluated on 100 kernels per treatment, from a

200 g sub-sample. Grains were cleaned in sterile water, disinfected in a 2% sodium hypochlorite solution for 2 min, rinsed two times in sterile water to eliminate any hypochlorite residues and dried on sterile filter paper, then placed in Petri dishes (5 per dish) containing potato dextrose agar (PDA) (Oxoid) with an addition of $0.5\,g\,l^{-1}$ of PCNB (pentachloronitrobenzene) to slow down the growth of other fungi. Plates were incubated in the dark at 22 °C and after 5 days were placed under near-ultraviolet (NUV) lights (12 h light and 12 h dark) for a further 10 days to favour sporulation. The *Fusarium* species were identified according to Nelson and Burgess (Nelson et al., 1983; Burgess et al., 1988).

Chemical analysis of DON in kernels

DON concentration was determined on three subsamples of 200 g of grain per treatment obtained from the original 2 kg sample. DON was extracted with acetonitrile–water and the mixture was filtered and purified by a column Mycosep 225 Romer. The column eluate was analysed by high-pressure liquid chromatography (HPLC) (Walker and Meier, 1998). In 1998–99 DON concentrations were determined only in tebuconazole treated and the untreated control because of cost restrictions, while in 1999–2000 it was determined in all the treatments.

Statistical analysis

The percentage data, prior to analysis, were arcsine transformed to normalize and homogenize the variance. Data were processed with the variance analysis (ANOVA) according to Fisher's protected least significant difference (PLSD) test at $P = 0.05$ to indicate statistically significant differences between treatments.

Results

Artificially inoculated trials

Head infection determination

In 1998–99, cvs Duilio and Simeto as untreated controls showed an FHB incidence of 84.3% and 87.1%, respectively, and disease severity of 27.9% and 29.6%. Fungicide treatments (tebuconazole, epoxiconazole and bromuconazole) gave a reduction of FHB incidence of over 50%. Severity was reduced with values ranging between 88.2% and 92.8% (Table 1). Kresoxim-methyl proved much less effective in cvs Duilio and Simeto where the incidence of disease was reduced by 10.9% and 14.4%, respectively and severity by 64.9% and 65.2%, respectively. In 1999–2000, the incidence of *Fusarium* infection

Table 1. Effect of fungicides on FHB, infected kernels and DON content in durum wheat, inoculated by spraying a spore suspension of *F. graminearum* (6.5×10^5 conidia ml^{-1}) and *F. culmorum* (2.5×10^4 conidia ml^{-1}). Season 1998–99

Treatment	Infected heads by *Fusarium* spp.				Infected kernels by *Fusarium* spp.		Infected kernels by *F. graminearum* *F. culmorum*		DON ($mg\,kg^{-1}$)
	I (%)[1]	EI (%)[2]	DS (%)[3]	EDS (%)[4]	*I* (%)[1]	EI (%)[2]	*I* (%)[1]	EI (%)[2]	
Cv. Duilio									
Tebuconazole	41.6 c[5]	50.7	3.1 c	88.9	26 b	48	12 c	57	0.38 b
Epoxiconazole	41.2 c	51.1	3.2 c	88.5	34 ab	32	13 bc	54	n.t.[6]
Kresoxim-methyl	75.1 b	10.9	9.8 b	64.9	53 a	0	32 a	0	n.t.
Bromuconazole	38.2 c	54.7	2.0 c	92.8	28 b	44	20 ac	29	n.t.
Untreated control	84.3 a		27.9 a		50 a		28 ab		1.23 a
Cv. Simeto									
Tebuconazole	41.1 c	52.8	2.6 d	91.2	31 b	38	12 b	45	0.30 b
Epoxiconazole	41.8 c	52.0	2.4 d	91.9	33 b	34	10 b	55	n.t
Kresoxim-methyl	74.6 b	14.4	10.3 b	65.2	56 a	0	24 a	0	n.t
Bromuconazole	40.8 c	53.1	3.5 c	88.2	36 b	28	14 ab	36	n.t
Untreated control	87.1 a		29.6 a		50 ab		22 a		0.57 a

[1]Incidence of heads or kernels infected (percentage). [2]Efficacy of fungicides on heads or kernels infected (percentage). [3]Disease severity on area infected (percentage). [4]Efficacy of fungicides on disease severity (percentage). [5]Means followed by the same letter in each column for each cultivar are not significantly different at $P = 0.05$ according to Fisher's PLSD test. [6]Not tested.

Table 2. Effect of fungicides on FHB, infected kernels, DON content and TGW in durum wheat. Season 1999–2000

Treatment	Infected heads by *Fusarium* spp.				Infected kernels by *Fusarium* spp.		Infected kernels by *F. graminearum* *F. culmorum*		DON (mg kg^{-1})	1000 grain weight (g)
	I (%)[1]	EI (%)[2]	DS (%)[3]	EDS (%)[4]	*I* (%)[1]	EI (%)[2]	*I* (%)[1]	EI (%)[2]		
Cv. Duilio[6]										
Tebuconazole	99.8 a[5]	0.2	24.3 b	56.8	42 a	19.2	22 b	50.0	3.82 bc	41.6 ab
Epoxiconazole	99.5 a	0.5	26.5 b	52.9	44 a	15.4	28 ab	36.4	5.72 ab	46.3 a
Prochloraz	99.5 a	0.5	25.4 b	54.9	30 b	42.3	22 b	50.0	2.98 c	44.0 a
Bromuconazole	99.0 a	1.0	23.9 b	57.5	42 a	19.2	30 ab	31.8	2.47 c	46.4 a
Untreated control	100.0 a		56.3 a		52 a		44 a		7.52 a	35.7 b
Cv. Simeto[6]										
Tebuconazole	85.1 b	14.9	30.7 b	52.7	52 b	27.8	40 b	39.3	5.73 c	47.1 a
Epoxiconazole	86.6 b	13.4	26.2 c	59.6	52 b	27.8	50 ab	24.2	8.96 a	40.5 bc
Prochloraz	86.4 b	13.6	25.9 c	60.1	48 b	33.3	34 b	48.5	6.42 c	40.8 ac
Bromuconazole	87.3 b	12.7	31.9 b	50.8	42 b	41.7	30 b	54.5	6.71 bc	45.1 ab
Untreated control	100.0 a		64.9 a		72 a		66 a		7.65 b	35.0 c
Cv. Bracco[7]										
Tebuconazole	38.4 c[5]	60.2	2.7 b	91.7	18 ab	30.8	0 b	100	0.46 b	51.4 ab
Epoxiconazole	37.4 c	61.2	3.0 b	90.8	30 a	0	0 b	100	0.63 b	54.0 a
Prochloraz	42.0 b	54.4	3.3 b	89.9	8 b	69.2	0 b	100	0.00 c	51.8 ab
Bromuconazole	34.3 d	64.4	3.0 b	90.8	28 a	0	0 b	100	0.00 c	48.8 b
Untreated control	96.4 a		32.7 a		26 a		6 a		1.12 a	48.3 b
Cv. Lloyd[7]										
Tebuconazole	37.3 b	60.4	5.3 b	81.4	42 ab	2.3	6 bc	70	0.34 b	47.4 a
Epoxiconazole	42.7 b	54.7	5.4 b	81.1	50 a	0	10 b	50	0.67 b	47.0 a
Prochloraz	36.3 b	61.5	3.1 b	89.1	16 c	62.8	6 bc	70	0.52 b	42.4 a
Bromuconazole	38.7 b	58.9	3.5 b	87.7	26 bc	39.5	0 c	100	0.20 b	42.6 a
Untreated control	94.2 a		28.5 a		43 ab		20 a		2.75 a	38.9 b

[1]Incidence of heads or kernels infected (percentage). [2]Efficacy of fungicides on heads or kernels infected (percentage). [3]Disease severity on area infected (percentage). [4]Efficacy of fungicides on disease severity (percentage). [5]Means followed by the same letter in each column are not significantly different at $P = 0.05$ according to Fisher's PLSD test. [6]Cv. inoculated by spraying a spore suspension of *F. graminearum* (4.5×10^5 conidia ml^{-1}) and *F. culmorum* (2.5×10^4 conidia ml^{-1}). [7]Cv. naturally infected.

in cvs Duilio and Simeto as untreated controls was high (100%) and was not reduced by bromuconazole, tebuconazole, prochloraz or epoxiconazole application. However, these fungicides reduced severity of head blight symptoms by over 50% (Table 2).

Percentage of Fusarium *infected kernels*

In 1998–99, in cv. Duilio, the incidence of *Fusarium* infected kernels was reduced by tebuconazole and bromuconazole by 48% and 44%, respectively, compared to the untreated control, while epoxiconazole reduced infection by 32%. The percentage of kernels infected with *F. graminearum* and *F. culmorum* was reduced by tebuconazole and epoxiconazole by 57% and 54%, respectively. No significant reduction was shown by bromuconazole. In cv. Simeto, tebuconazole, epoxiconazole and bromuconazole reduced the percentage of *Fusarium* spp. infected kernels by 38%, 34% and 28%, respectively, while the percentage of kernels infected with *F. graminearum* and *F. culmorum* was reduced by tebuconazole and epoxiconazole by 45% and 55%, respectively. In both cultivars, kresoxim-methyl did not effectively reduce the disease (Table 1).

In 1999–2000, prochloraz gave the best results in cv. Duilio, since it reduced the incidence of *Fusarium* spp. infected kernels by 42.3% and lowered *F. graminearum* and *F. culmorum* infections by 50%. Tebuconazole, prochloraz and bromuconazole, in cv. Simeto, reduced *F. graminearum* and *F. culmorum* infections by 39.3%, 48.5% and 54.5%, respectively, while epoxiconazole was less effective (24.2%) (Table 2).

Chemical analysis of DON in kernels

In 1998–99, the mean concentration of DON in cv. Duilio samples treated with tebuconazole was 0.38 mg kg^{-1} and in the untreated control

1.23 mg kg^{-1}. In cv. Simeto, the presence of DON, in samples treated with tebuconazole, was 0.30 mg kg^{-1} compared to 0.57 mg kg^{-1} in the control.

In 1999–2000, DON concentration in cvs Duilio and Simeto as untreated controls was 7.52 and 7.65 mg kg^{-1}, respectively. Tebuconazole, bromuconazole and prochloraz reduced DON concentration in cv. Duilio by 49.2%, 67.2% and 60.4%, respectively, and in cv. Simeto by 25.1%, 12.3% and 16.1%, respectively. Epoxiconazole was ineffective in both cultivars and especially in cv. Simeto where DON concentration was 8.96 mg kg^{-1}, significantly higher than in the untreated control (Table 2).

Naturally infected trials

Head infection determination
In cvs Bracco and Lloyd as untreated controls, the incidence of *Fusarium* infection was 96.4% and 94.2% respectively and disease severity 32.7% and 28.5%, respectively. Fungicide treatments (tebuconazole, epoxiconazole, prochloraz and bromuconazole) reduced FHB incidence by over 54% and disease severity by over 81% (Table 2).

Percentage of Fusarium *infected kernels*
In cvs Bracco and Lloyd, prochloraz reduced the incidence of *Fusarium* spp. infected kernels by 69.2% and 62.8%, respectively, compared to 26% and 43% of the untreated controls. Prochloraz, tebuconazole, epoxiconazole and bromuconazole all reduced *F. graminearum* and *F. culmorum* infections of kernels (Table 2).

Chemical analysis of DON in kernels
DON concentrations in cvs Bracco and Lloyd as untreated controls were 1.12 and 2.75 mg kg^{-1}, respectively. In cv. Bracco, applications of prochloraz and bromuconazole reduced DON concentration by 100%, whereas tebuconazole and epoxiconazole reduced DON by 59% and 44%, respectively. In cv. Lloyd, all the fungicide treatments reduced DON concentration (Table 2). The correlation (r) between DON and incidence of *F. graminearum* and *F. culmorum* infected kernels was 0.89, while in *Fusarium* spp. infected kernels it was 0.56.

Thousand grain weight
In 1999–2000, samples of all four cultivars treated with fungicides showed an insignificant increase in thousand grain weight (TGW) compared to the untreated controls (Table 2).

Discussion

The infection pressure in the 1999–2000 trials was higher than that recorded in the 1998–99 trials. This is probably due to favourable FHB epidemic conditions and to the susceptible wheat cultivars used in this study. Our data suggest that one application of tebuconazole, epoxiconazole, bromuconazole or prochloraz reduced the incidence and severity of FHB, when infection pressure was low to medium, and the main pathogens were *F. graminearum* and *F. culmorum*. These results are in agreement with those of Capisano (1995) and Mesterhazy (1996). Furthermore the same fungicides, in the presence of high infection pressure were able to reduce the severity of head blight symptoms and not the incidence of *Fusarium* infection. Kresoxim-methyl, a strobilurin, showed less effectiveness in reducing the incidence and severity of FHB disease. Caron (2000) and Thomas (2000) also reported that strobilurins do not control *F. graminearum* and *F. culmorum*. The incidence of *F. graminearum* and *F. culmorum* infected kernels was controlled by tebuconazole, epoxiconazole, prochloraz and bromuconazole treatments, while application of kresoxim-methyl was ineffective.

In cvs Duilio and Simeto, in both years, the amount of DON in inoculated kernels was high (average of 4.2 mg kg^{-1}). Tebuconazole, prochloraz and bromuconazole, applied in the field, reduced the production of DON mycotoxin by an average of 43%, while epoxiconazole was ineffective. In cv. Simeto, DON concentration increased after an application of epoxiconazole, despite there being no significant increase of *F. graminearum* and *F. culmorum*. This mycotoxin increase was also noted in field trials by other authors (Milus and Parsons, 1994). Application of epoxiconazole may therefore represent a risk, potentially high, of DON contamination in grain.

In our present study, naturally infected cvs Bracco and Lloyd had a DON concentration exceeding the maximum level of contamination allowed in the USA. It also emerged that a single application of either prochloraz, bromuconazole or tebuconazole at the manufacturer's recommended dose rate at the beginning of the anthesis stage effectively reduced DON concentration in grain below the permissible maximum limit (Fehrmann and Ahrens, 1984;

Mauler-Machnik and Suty, 1997; Matthies and Buchenauer, 2000; Homdork et al., 2000). Moreover DON concentration in grains is not always correlated with the incidence of *Fusarium* infected kernels, but in our trials, in accordance with Matthies and Buchenauer (2000), we observed significant positive linear correlations between *Fusarium* infections and the presence of DON. Other researchers, however, (Arseniuk et al., 1999; Bai et al., 2001) have observed that the accumulation of DON can be independent of *Fusarium* infection.

From the survey carried out, it has emerged that toxigenic *Fusarium* species which produced DON in concentrations higher than 1 mg kg^{-1}, are present in Italy. Therefore, future research should concentrate on reducing DON in grain to a safe concentration for human and animal consumption.

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