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Control of *Fusarium* head blight and accumulation of deoxynivalenol in durum wheat grain, semolina and bran

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Summary. The effects of E.B.I. fungicides (bromuconazole, prochloraz and tebuconazole) on *Fusarium* head blight (FHB) and on the deoxynivalenol (DON) content in grain, semolina and bran were examined in three separate trials carried out in fields near Bologna (Italy) on susceptible durum wheat varieties artificially inoculated with *Fusarium graminearum* and *F. culmorum,* responsible for head blight. Bromuconazole, prochloraz and tebuconazole applied in the field reduced FHB incidence and severity significantly by 56 and 73% respectively, and the numbers of kernels infected with *F. graminearum* and *F. culmorum* by 66.6%. These fungicides also reduced the DON content in kernels, semolina and bran, compared to the non treated samples. The correlation (r) between DON and the incidence of *F. graminearum* and *F. culmorum*-infected kernels was 0.90 in the original sample.

Key words: FHB, E.B.I. fungicides, mycotoxin, cereals.

Introduction

In these last years a *Fusarium* head blight (FHB) outbreak has been reported in wheat growing areas of central and northern Italy. It was first reported at the beginning of the last century (Peglion, 1900), but since 1995, it has become a permanent presence in wheat growing areas, with the incidence and severity varying between wheat varieties, between years and between areas (Pancaldi *et al*., 1996, 1997; Balmas *et al*., 1998, 1999, 2000). Several species of *Fusarium* cause head blight; the most common in our country are *Fusarium graminearum* (Schwabe), *F. culmorum* (Smith) Sacc., *F. avenaceum* (Corda ex Fr.) Sacc. and *F. poae* (Peck) Wollenw. Another causal agent is *Microdochium nivale* (Fr.) Samuels & Hallet var. *majus* and var. *nivale,* synonym of *F. nivale* (Fr.) (Pancaldi *et al.,* 1996; Balmas *et al.,*1999; Corazza *et al*., 2001). Head blight causes partial or total premature ear necrosis or emptiness of the mature ears with stunted kernels and, when attacks are severe, a loss in production from 30 to 70% (Parry *et al*., 1995; Suty and Mauler-Machnik, 1995). FHB can also infect the grains, which become the principal means of diffusion of the disease (Pancaldi *et al*., 1997; Pancaldi and Torricelli, 1998, 1999). Toxigen-

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ic strains of *F. graminearum* and *F. culmorum* produce deoxynivalenol (DON), a mycotoxin that in mammals causes haemorrhagic and anorexic syndromes as well as neurotoxic and immunotoxic effects (Bottalico, 2002).

Concentrations of DON higher than 1 mg kg^{-1}, with peaks of 6 mg kg^{-1}, have been found in recent surveys of durum and bread wheat kernels from cultivations affected with FHB in several cereal areas of Emilia-Romagna and other Regions of Italy (Lops *et al*., 1998; Pascale *et al*., 2000, 2001a, 2001b). Control programmes to limit losses in yield and quality have been followed in several European areas, in the US and Canada, where the disease first appeared and has become widespread and severe. Such control programmes include the adoption of correct agronomic practices, utilization of healthy grains or grains treated with fungicides protecting them against *Fusarium* species, and the application of fungicides at the beginning of anthesis.

Fungicide treatment at anthesis was also found to reduce the DON content in wheat kernels (Jugnet, 1994; Capisano, 1995; Mesterhazy, 1996; Jennings *et al*., 2000; Simpson *et al*., 2001; Menniti *et al*., 2003).

Since little is known about the control of FHB in Italy, the purpose of the present work was to evaluate the effectiveness of some fungicides in limiting the incidence and severity of FHB induced by *F. graminearum* and *F. culmorum* on 3 durum wheat varieties grown in an area near Bologna, Emilia-Romagna, Italy. The study also examined whether the fungicide active ingredients lowered the levels of DON in harvested grains, bran and semolina.

Materials and methods

Fungal isolates

Isolates of *F. graminearum* (PDPVA 98) and *F. culmorum* (PDPVA 74), were maintained in potato dextrose agar (PDA) at 4°C and came from the collection of the Dipartimento di Protezione e Valorizzazione Agroalimentare (DIPROVAL), Bologna, Italy. The isolates were obtained in 2000 from durum wheat kernels of the cv. Simeto, which had showed a high FHB infection the year before (from 87 to 100% of infected heads). A sample of these kernels revealed a DON concentration of 7.65 mg kg^{-1}.

Inoculum preparation

Disks 0.5 mm in diameter from 7-day-old colonies of each isolate on PDA were immersed in 2 l flasks (3 disks per flask) each containing 1600 ml of V8 juice liquid medium (V8 juice 200 ml + $CaCO_3$ 3 g, brought to 1 l with distilled water). After 3 weeks of incubation at 24°C with 12h day, the content of each flask was filtered through two layers of cheesecloth to obtain a concentrated conidial suspension, which was then measured with a Thomas camera under a light-microscope (LM).

Experimental design

Three separate trials were carried out on the susceptible durum wheat (*Triticum durum* Desf.) cv. Bracco, Duilio and Simeto in fields near Bologna (Italy). The experimental design was a randomised block with 4 replications (plots). All the plots were grown according to normal agronomic practices and each plot had an area of 11.7 m^2 (1.8×6.5 m).

The fungicides tested on the wheat cultivars were bromuconazole at 250 g ha^{-1} (Granit®), prochloraz at 585 g ha^{-1} (Sportak®45 EW) and tebuconazole at 250 g ha^{-1} (Horizon®). These fungicides are registered for FHB control in Italy. Untreated controls for each variety were also assessed. The fungicides were applied at the beginning of anthesis, Zadoks growth stage GS 60-61 (Zadoks *et al.,* 1974) using a motorised pump (Solo 422, Sindehfingen, Germany). Delivery pressure of this pump at the nozzles (Teej 8003 VS) was 4 atm and the volume of water delivered 600 l ha^{-1}.

Both treated and untreated plots were artificially inoculated with a mixture of *F. graminearum* (3.5×10^4 conidia ml^{-1}) and *F. culmorum* (2.5×10^4 conidia ml^{-1}). The difference in concentration was due to the fact that *F. culmorum* produced fewer conidia. Each plot, within the 24 hours following fungicide treatment, was inoculated with 400 ml of spore suspension with the relative humidity above 75% and no wind.

The trial plots were mechanically harvested (GS 90–92) and after threshing, a homogenous 2 kg sample of grain was taken from each replicate (plot) and bulked to provide an 8 kg sample per treatment (original sample, OS).

Head infection determination

Product activity in the field was evaluated at

the milk stage (GS 77) by examining 100 heads randomly collected from each plot. The percentage of infected heads (incidence) and the infected area of the heads (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, l0, 25, 50, 75, and 90% area infected) and by applying the following formula: Σ (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.

Mycological analysis of ears and kernels infected with FHB

Inoculated untreated samples of all cultivars were collected from each experimental plot to detect and quantify the different *Fusarium* species. Ten ears with different levels of FHB were taken from each plot, and from each ear 10 pieces of glume, rachis, sub-glume and palea were also collected. The pieces were washed in sterile water, disinfected in a 1% sodium hypochlorite solution for 2 minutes, rinsed twice in sterile water to eliminate any hypochlorite residue, dried on sterile filter paper, and placed in Petri dishes containing water-agar. Plates were incubated in the dark at 22°C±1. After 7 days the grown mycelium was placed in Petri plates containing PDA with the addition of 0.5 g l^{-1} of pentachloronitrobenzene (PCNB) to slow down the growth of other fungi, and 0.05 g l^{-1} of streptomycin sulphate. Plates were incubated in the dark at 22°C±1 for 5 days, after which they were placed under near-ultraviolet (NUV) light (12 h light and 12 h dark) for a further l0 days to favour sporulation. The *Fusarium* species and *M. nivale* were identified according to Nelson and Burgess (Nelson *et al.,* 1983; Burgess *et al.,* 1988) and the isolation frequency (number of isolates of the species/total number of isolates) of each variety, was calculated.

The incidence of grain infected with *F. graminearum* and *F. culmorum* was calculated on 100 kernels per treatment from a 100 g sub-sample of the OS. Grains were rinsed with sterile water, disinfected in a 2% sodium hypochlorite solution for 2 minutes, and treated following the methodology adopted for the ears.

Scanning electron microscopy (SEM) examination was performed on kernels from the OS that had been incubated on sterile filter paper until the *Fusarium* mycelium was visible, and on the mycelium produced, which was then transferred to PDA plates and incubated until the conidia were produced (Pisi and Filippini, 1994).

Diagnostic and qualitative PCR

A PCR reaction was performed to confirm the data obtained *in vitro*. *F. avenaceum, F. culmorum, F. graminearum, F. poae* and *M. nivale* isolated from ears (inoculated untreated samples), and *F. culmorum* and *F. graminearum* isolated from kernels of OS were maintained in purity and identified by molecular means.

Mycelium, from 7-day-old colonies grown on PDA, was used to inoculate 50 ml of potato dextrose broth (PDB). Cultures were incubated at room temperature on an orbital shaker for 4–7 days in the dark. The harvested mycelium was filtered through Whatman no. 1 filter paper, then reduced to powder in a mortar with liquid nitrogen. DNA was extracted from the mycelium with the method described by Nicholson and Parry (1996) using 20 ml of hexadecylmetrimethyl-ammonium bromide (CTAB) buffer (CTAB 8 g, sarkosyl 10 g, sorbitol 25 g, NaCl 47 g, EDTA 8 g, polyvinylpolypyrollidone [PVPP] 10 g in 1 l water) at 65°C for 2 h, then an equal volume of chlorophorm was added and the mixture centrifuged at 3000 *g* for 15 minutes.

The acqueous phase was subsequently removed, an equal volume of cold isopropanol added and the mixture centrifuged. The resulting pellet was washed in 70% ethanol and dissolved in 300 μl TE buffer (10 mM Tris HCl, pH 8.0, 0.1 mM EDTA). PCR reactions were carried out in a volume of 50 μl containing 10–20 ng fungal DNA, 100 μM of dATP, dCTP, dGTP and dTTP, 10 nM of forward and reverse primers, 1 unit of Taq polimerase (Roche, Nutley, NJ, USA) in 5 μl buffer II and 3 μl $MgCl_2$ of Gene Amp 10× (Applera, Forster City, CA, USA) with a final concentration of 10 mM Tris-HCl, 50 mM KCl and 1.5 mM $MgCl_2$. The primers used are indicated in Table 1. The amplification process was carried out in a GeneAmp 9700 thermocycler (Applera), with initial denaturation of 7 min at 94°C, then 40 cycles with specific programmes for each primer pair, followed by 72°C for 7 min.

Electrophoresis was done in 2% NuSive (Cam-

Table 1. Primers and programmes used in the PCR reactions.

Primer	Primer sequence	Programme	Pathogen
$OPT18F_{470}$	GATGCCAGACCAAGACGAAG	94°C×30 s, 55°C×30 s, 72°C×40 s	*Fusarium culmorum*
$OPT18R_{470}$	GATGCCAGACGCACTAAGAT		
Fg16F	CTCCGGATATGTTGCGTCAA	95°C×30 s, 62°C×30 s, 72°C×40 s	*F. graminearum*
Fg16R	GGTAGGTATCCGACATGGCAA		
AF	CAAGCATTGTCGCCACTCTC	95°C×30 s, 60°C×30 s, 72°C×40 s	*F. avenaceum*
AR	GGTAGGTATCCGACATGGCAA		
Fp82F	CAAGCAAACAGGCTCTTCACC	95°C×30 s, 62°C×30 s, 72°C×40 s	*F. poae*
Fp82R	TGTTCCACCTCAGTGACAGGTT		
Y13NF	ACCAGCCGATTTGTGGTTATG	95°C×30 s, 61°C×30 s, 72°C×40 s	*Microdochium nivale* var. *nivale*
Y13NR	GGTCACGAGGCAGAGTTCG		
Mnm2F	TGCAACGTGCCAGAAGCT	95°C×30 s, 61°C×30 s, 72°C×40 s	*M. nivale* var. *majus*
Mnm2R	AATCGGCGCTGTCTACTAAAAGC		

brex, Rockland, ME, USA) agarose gel in 1× TAE buffer for 1.5 h at 100 volt with a 100 bp ladder (Cambrex). Gels were stained with Gel Star (Cambrex) and the images collected and elaborated with the BioDoc-it System of UVP (UVP Inc., Upland, CA, USA).

DON analysis

The concentration of DON was determined for each treatment and cultivar in kernels of the OS, in the clean sample (CS) and in the bran and semolina obtained from the CS.

Kernels of the OS were from a subsample of 500 g; kernels of the CS from a subsample of 500 g obtained by sieving the kernels of the OS with a 2×20 mm mesh sive; and the bran and the semolina fractions were obtained from 500 g of the whole wheat of the CS tempered to 17% moisture content for 19 hours and milled using a labor mill, 4 grinder (Bona, Monza, MI, Italy).

DON determination was carried out by HPLC on kernels, semolina and bran samples, after isolation, purification and concentration as described by Cahill *et al*. (1999).

Finely ground aliquots (25 g) of each sample were added to 5 g of polyethylene glycol (PEG 8000) and extracted with 100 ml distilled water by blending. Extracts were filtered through filter paper (Whatman No. 4) and a 1.5 μm glass microfiber filter (Whatman GF/A). An aliquot of 2 ml of filtered extract was cleaned up by a DON Test™ immunoaffinity column (Vicam Inc., Watertown, MA, USA). DON was identified and quantified by a reversed-phase HPLC system (model Jasco system LC-1500, Carpi, MO, Italy) with a diode array UV detector (model MD 1510, Jasco) set to 220 nm and using an RP Pinnacle ODS column (250×4.6 mm, 5 μm). The mobile phase consisted of a mixture of acetonitrile: 250 μl of acetonitrile:water (10:90, v:v) eluted at a flow rate of 1.0 ml min^{-1}. Appropriate dilutions of sample extracts before loading on the immunoaffinity columns were necessary to avoid saturation of the DON-antibody binding sites. The detection limit of the method was 0.05 mg kg^{-1}.

Statistical analysis

Data were processed with analysis of variance (ANOVA) at P=0.05 to determine statistically significant differences between means (Tukey's test). Percentage data were arsine transformed prior to statistical analysis to normalize and homogenize the variance.

Results

Head infection determination

Head blight incidence on the inoculated controls of the cv. Duilio, Bracco and Simeto was very high (from 84.8 to 100%) and so was disease severity (from 36.3 to 54.4%) (Table 2). All fungicides (bromuconazole, prochloraz and tebuconazole) significantly reduced disease incidence by 53 to 65%, and disease severity by 68 to 77% compared with the control. There were no significant differences in efficacy among the fungicides.

Table 2. Effects of fungicides on FHB on *F. graminearum* (F.g.) and *F. culmorum* (F.c.) infected kernels and on deoxynivalenol (DON) content in durum wheat cultivars.

Treatment	FHB				F.g. + F.c. infected		DON (mg kg^{-1})							
							OS[e]				CS[f]			
	I (%)[a]	EI (%)[b]	DS (%)[c]	EDS (%)[d]	I (%)[a]	EI (%)[b]	Kernels		Kernels		Semolina		Bran	
Cv. Bracco														
Bromuconazole	34.1 a[g]	65	13.9 a	74	2.5 a	79	0.283	(72)[h]	0.279	(63)[h]	0.280	(41)[h]	0.090	(90)[h]
Procloraz	37.8 a	61	16.8 a	69	5.3 b	56	0.401	(60)	0.296	(61)	0.242	(49)	0.191	(79)
Tebuconazole	36.4 a	63	14.0 a	74	4.8 ab	60	0.258	(74)	0.240	(68)	0.387	(18)	0.157	(82)
Untreated control	97.5 b		54.4 b		12.0 c		1.010		0.758		0.474		0.894	
Cv. Duilio														
Bromuconazole	36.4 a	57	9.2 a	75	3.0 ab	65	0.117	(81)	0.098	(80)	0.196	(37)	0.160	(71)
Procloraz	34.0 a	60	11.5 a	68	2.0 a	76	0.065	(90)	0.088	(82)	0.124	(60)	0.104	(81)
Tebuconazole	39.6 a	53	8.6 a	76	4.8 b	44	0.236	(62)	0.253	(49)	0.292	(6)	0.181	(67)
Untreated control	84.8 b		36.3 b		8.5 c		0.625		0.500		0.312		0.546	
Cv. Simeto														
Bromuconazole	45.8 a	54	11.3 a	74	3.5 a	67	0.341	(67)	0.381	(57)	0.460	(26)	0.256	(50)
Procloraz	44.8 a	55	10.1 a	76	4.0 a	62	0.376	(64)	0.370	(58)	0.561	(10)	0.308	(40)
Tebuconazole	44.1 a	56	9.8 a	77	3.3 a	69	0.316	(70)	0.372	(58)	0.396	(37)	0.255	(50)
Untreated control	100.0 b		42.7 b		10.5 b		1.040		0.886		0.625		0.512	

[a] Incidence of infected heads or kernels (%).
[b] Efficacy of fungicides on infected heads or kernels (%).
[c] Disease severity: area of infected head (%).
[d] Efficacy of fungicide on disease severity (%).
[e] OS, original sample.
[f] CS, clean sample.
[g] Means followed by the same letter in each column are not significantly different at P=0.05 according to Tukey's test.
[h] In parenthesis reduction of DON referred to the untreated control (%).

Percentage of kernels infected with *F. graminearum* and *F. culmorum* (OS)

The percentage of kernels infected with *F. graminearum* and *F. culmorum* was significantly reduced, compared with the untreated kernels by 65 to 79% when applying bromuconazole, by 56 to 76%, with prochloraz, and by 44 to 69% with tebuconazole (Table 2). The percentage of kernels infected with *F. graminearum* and *F. culmorum* among untreated samples of the 3 cultivars varied from 8.5 to 12%, and the percentage of infected kernels treated with the three fungicides varied from 2 to 5.3% (Table 2).

Detection of fungal species in ears

The mycological analysis (Table 3) showed that the symptoms produced in inoculated untreated ears were mainly caused by species of *Fusarium* and only partly by *M. nivale*.

In the untreated samples of all cultivars, the main infective agents, with a high frequency of isolation, were *F. graminearum*, from 46.3 to 56.1% and *F. culmorum*, from 38.2 to 45.2%, while *F. poae* and *F. avenaceum* on all cultivars, and *M. nivale* in cv. Bracco and Duilio, were less frequent.

SEM confirmed the presence of mycelium and conidia of *F. graminearum* in kernels of the OS (Fig. 1 and 2).

Chemical analysis of DON in kernels of the OS and in kernels, semolina and bran of the CS

In the untreated kernels of all cultivars, in the OS, DON concentration was always higher than that of the untreated kernels in the CS (Table 2). In all cultivars, DON concentration in both the OS (0.625–1.04 mg kg^{-1}) and the CS (0.500–0.886 mg kg^{-1}) was higher than in the OS and CS treated

Table 3. Fungi (%) isolated from inoculated untreated wheat ears.

Wheat cultivar	Fungus				
	F. avenaceum	*F. culmorum*	*F. graminearum*	*F. poae*	*M. nivale*
Bracco	1.3	38.2	56.1	2.8	1.6
Duilio	1.8	45.2	46.3	5.3	1.4
Simeto	2.3	43.2	48.6	5.9	0.0

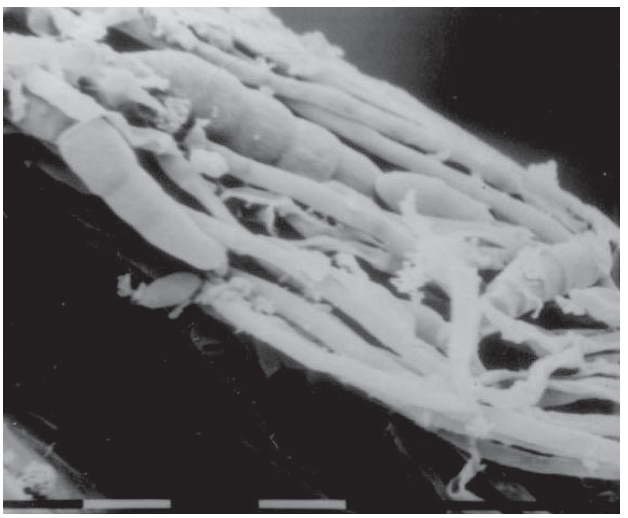

Fig 1. SEM micrograph of conidia and mycelium of *F. graminearum* on a kernel of the original sample. Bar = 10μm

Fig. 2. SEM micrograph of conidia of *F. graminearum* on potato dextrose agar. Bar = 10μm

with bromuconazole (0.117–0.341 mg kg^{-1} in OS; 0.098–0.381 mg kg^{-1} in the CS), prochloraz (0.065–0.401 mg kg^{-1} in OS; 0.088–0.370 mg kg^{-1} in the CS) and tebuconazole (0.236–0.316 mg kg^{-1} in the OS; 0.240–0.372 mg kg^{-1} in the CS).

The quantity of DON in the bran samples (0.512–0.894 mg kg^{-1}) of the untreated cv. Bracco, Duilio and Simeto was higher than that in the semolina samples (0.312–0.474 mg kg^{-1}).

The quantity of DON in bran and semolina samples treated with the three fungicides was always lower than that in the untreated samples. The correlation (r) between DON concentration and incidence of *F. graminearum* and *F. culmorum* in infected kernels was 0.90 in the OS (Fig. 3).

Conclusions

One application of bromuconazole, prochloraz or tebuconazole on wheat at the beginning of anthesis (GS 60-61) reduced the incidence and severity of FHB in all cultivars when the infective pressure came mostly from the main two pathogens *F. graminearum* and *F. culmorum*. These results were in accordance with those of Capisano (1995), Mesterhazy (1996), Matthies and Buchenauer (2000) and Menniti *et al*. (2003). Also the percentage of kernels infected with the two fungi was reduced. The low levels of DON in the CS may have been caused by sieving, which eliminated a large proportion of *Fusarium* infected kernels. This result was in accordance with Seittz (1986) and Dexter (1997).

The average amount of DON was close to the maximum levels recommended in Austria by the Ministry of Health and the Austrian Institute for Agriculture: 0.5–0.75 mg kg^{-1} on wheat for human consumption, and were lower than the maximum levels set in Russia by the Ministry of Health: 1 mg kg^{-1} on durum wheat. In the US the maximum

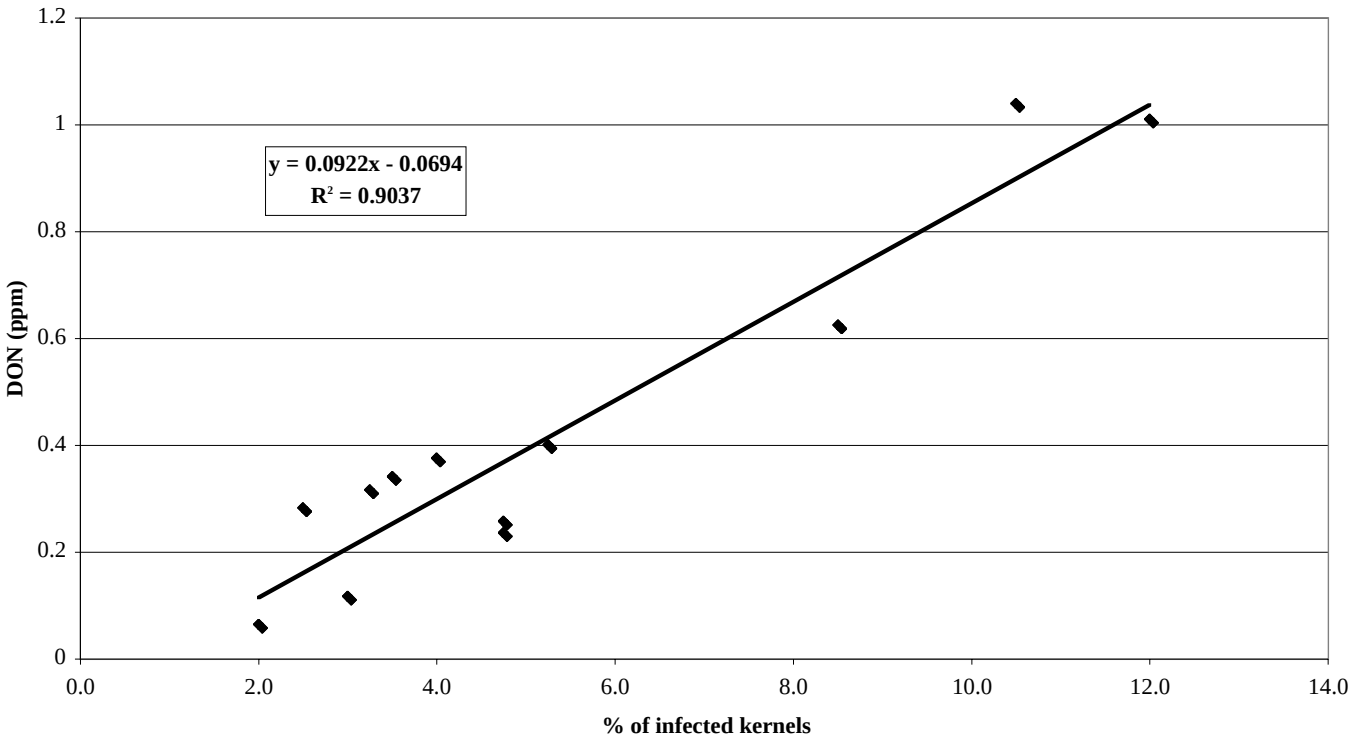

Fig. 3. Correlation between the percentage of kernels of OS infected by *F. graminearum* and *F. culmorun* and contamination by DON.

limits, set by the Food and Drug Administration, are 1 mg kg^{-1} in products derived from wheat for human consumption and 5–10 mg kg^{-1} in cereals for animal feed. In Canada the limits are set by the Health Protection Branch as 2–1 mg kg^{-1} in uncleared bread clean wheat and in wheat for newborn babies. In Italy legal limits for DON in wheat and/or cereals or derived products are not fixed yet and so no comparison can be made.

Field treatment with bromuconazole, prochloraz and tebuconazole reduced DON production in the OS by a minimum of 60% to a maximum of 90%, with an average of 70%.

A reduction of DON in kernels brought about by fungicides applied at anthesis is also reported by Mesterhazy (1996), Jennings *et al*. (2000), Simpson *et al*. (2001) and Menniti *et al*. (2003). Bran samples of the untreated cultivars had higher DON levels than semolina samples. A similar result was reported by Scott *et al*. (1983), Trigo-Stockli *et al*. (1996), and Romani *et al*. (2004). DON levels on semolina and bran were always lower in fungicide treated than in untreated samples.

Finally, the fungicides containing as active ingredients bromuconazole, prochloraz and tebuconazole, applied at the beginning of blossoming (10% of visible anthers), reduced the incidence and severity of FHB and at the same time lowered the percentage of infected kernels and the DON content in kernels. These data are relevant since they were obtained in spite of a severe infection, induced artificially by virulent and toxigenic *F. graminearum* and *F. culmorum* isolates. When fungicides are necessary, they should always be integrated with other measures: using FHB-tolerant or resistant varieties, adopting proper agronomic practices that minimise the spread of primary inoculum (avoiding rotation with other cereals, working the soil without turning, minimizing soil work, not sowing on unbroken soil, avoiding high-nitrogen fertilization, etc.) and using healthy grain, or grain treated with fungicides effective against *Fusarium* spp.These measures, besides reducing FHB, as recently reported by Simpson *et al*. (2001), Ioos and Faure (2001) and Menniti *et al*. (2003), will also lower the DON con-

tent in kernels, since there is a correlation between the two.

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Literature cited

Balmas V., M. Maccaroni, A. Santori and L. Corazza, 1999. Fusariosi della spiga di cereali autunno-vernini: indagine epidemiologica e valutazione della resistenza varietale di frumento duro nell'Italia centrale. *Informatore Fitopatologico* 49(12), 37–42.

Balmas V., A. Santori and L. Corazza, 1998. Fusariosi della spiga di frumento duro. Speciale grano duro. *L'Informatore Agrario* 36, 53–55.

Balmas V., S. Vitale, A. Marcello and L. Corazza, 2000. Fusariosi della spiga. Supplemento *L'Informatore Agrario* 35, 27–29.

Bottalico A., 2002. Funghi tossigeni e micotossine: aspetti generali. *Informatore Fitopatologico* 52(12), 10–16.

Burgess L.W., C.M. Liddell and B.A. Summerell, 1988. *Laboratory Manual for* Fusarium *Research*. 2nd Edition. Department of Plant Pathology and Agricultural Entomology, University of Sydney, Sydney, Australia, 156 pp.

Cahill L.M., S.C. Kruger, B.T. McAlice and C.S. Ramsey, 1999. Quantification of deoxynivalenol in wheat using an immunoaffinity column and liquid chromatography. *Journal of Chromatography* 859, 23–28.

Capisano C., 1995. Fusarioses de l'épi: Pourquoi la lutte reste difficile. *Cultivar* 384, 28–30.

Corazza L., V. Balmas, A. Santori, L. Vitale Sluongo and M. Maccaroni, 2001. Head blight and foot rot of wheat in Italy. In: *Proceedings of the Conference 'Sustainable Systems of Cereal Crop Protection against Fungal Diseases as the Way of Reduction of Toxin Occurence in Food Webs'*, 2–6 July 2001, Kromeriz, Czech Republic, 25–36.

Dexter J.E., R.M. Clear and K.R. Preston, 1996. *Fusarium* head blight: effect on the milling and baking of some Canadian wheats. *Cereal Chemistry* 73(6), 695–701.

Ioos R. and A. Faure, 2001. Cereal head blight disease, mycotoxins and fungicide treatments. *Phytoma-La défense des Végétaux* 539, 56–63.

Jennings P., J. Turner and A. P. Nicholson, 2000. Overview of Fusarium ear blight in the UK–effect of fungicide treatment on disease control and mycotoxin production. *The Brighton Crop Protection Conference 2000 - Pests and Diseases* Vol. 2, 707–712.

Jugnet M.P., 1994. Fusarioses: vers des traitements mieux ciblés. *Cultivar* 356, 43–45.

Lops R., M. Pascale, D. Pancaldi and A. Visconti, 1998. Infezioni fungine e presenza di deossinivalenolo in cariossidi di frumento prodotte in diverse regioni italiane. *Informatore Fitopatologico* 48(4), 60–66.

Matthies A. and H. Buchenauer, 2000. Effect of tebuconazole (Folicur) and prochloraz (Sportak) treatments on *Fusarium* head scab development, yield and deoxynivalenol (DON) content in grains of wheat following artificial inoculation with *Fusarium culmorum*. *Journal of Plant Disease and Protection* 107, 33–52.

Menniti A.M., D. Pancaldi, M. Maccaferri and L. Casalini, 2003. Effect of fungicides on *Fusarium* head blight and deoxynivalenol content in durum wheat grain. *European Journal of Plant Pathology* 109, 109–115.

Mesterhazy A., 1996. Fungicide control of Fusarium Scab and impact on toxin contamination. Fusarium Head Scab: global status and future prospects. *Proceedings of Workshop CIMMYT*, 13–17 October 1996, EL Batan, Mexico, 120–124.

Nelson P.E., T.A. Toussoun and W.F.O. Marasas, 1983. Fusarium *species*. *An illustrated manual for identification.* Pennsylvania State University Press, University Park, PA, USA, 193 pp.

Pancaldi D., F. Casulli, G. Grazzi and F. Grifoni, 1997. Indagine sulla fusariosi della spiga del frumento duro in Emilia Romagna. *Informatore Fitopatologico* 47(10), 43–48.

Pancaldi D., G. Grazzi and I. Alberti, 1996. La fusariosi della spiga di frumento in Emilia Romagna nel 1995. *Informatore Fitopatologico* 46(4), 16–20.

Pancaldi D. and R. Torricelli, 1998. Presenza di specie di *Fusarium* su cariossidi di frumento tenero in Emilia-Romagna nel triennio 1994–1996. *Informatore Fitopatologico* 48(12), 46–50.

Pancaldi D. and R. Torricelli, 1999. Presenza di specie di *Fusarium* su cariossidi di frumento duro in Emilia-Romagna nel triennio 1994–1996. *Informatore Fitopatologico* 49(3), 42–46.

Parry D.W., R.A. Bayles and R.H. Priestley, 1984. Resistance of winter wheat varieties to ear blight (*Fusarium culmorum*). *Journal National Institute Agriculture Botany* 16, 465–468.

Parry D.W., P. Jenkinson and L. McLeod, 1995. *Fusarium* ear blight (scab) in small grain cereals - a review. *Plant Pathology* 44, 207–238.

Pascale M., A. Bottalico, D. Pancaldi, G. Perrone and A. Visconti, 2001a. Occurrence of deoxynivalenol in cereals from experimental fields in various Italian regions. *European Commission activities: Human Potential Programme–High-level Scientific Conference-Sustainable Systems of Cereal Crop Protection against Fungal Diseases as the Way of Reduction of Toxin Occurrence in Food Webs-Healthy Cereals,* 2–6 July 2001, Kromeriz, Czech Republic,101–105.

Pascale M., D. Pancaldi, A. De Girolamo and A. Visconti, 2000. Indagine sulla presenza di deossinivalenolo in cereali prodotti in alcune aree del nord Italia nel 1998. *Informatore Fitopatologico* 42(10), 68–73.

Pascale M., D. Pancaldi, A. Visconti, G. Perrone and A. Bottalico, 2001b. Fusarium ear blight, deoxynivalenol and toxigenic Fusarium species in selected wheat culti-

vars assayed all over Italy, in 2000. *Proceedings 11th Congress of the Mediterranean Phytopathological Union and 3rd Congress of the Sociedade Portuguesa de Fitopatologia,* 17–20 September 2001, Evora, Portugal, 123–125.

Peglion V., 1900. Sulla cosidetta "Golpe bianca" del frumento. *Bollettino Notiziario Agrario* 20, 1–7.

Pisi A. and G. Filippini, 1994. La microscopia elettronica a scansione in micologia. *Micologia Italiana* 1, 17–26.

Romani S., G.G. Pinnavaia, D. Pancaldi and M. Dalla Rosa, 2004. Studio di alcune caratteristiche qualitative e della presenza di micotossine in grano duro (*Triticum durum*) infetto da *Fusarium* spp. *Atti 6° Convegno Italiano di Scienze degli Alimenti*, 18–19 Settembre 2003, Cernobbio (CO), Italy (in press).

Scott P.M., S.R. Kanhere, P.Y Lau., J.E. Dexter and R. Greenhalgh, 1983. Effects of experimental flour milling and breadbaking on retention of deoxynivalenol (vomitoxin) in hard red spring wheat. *Cereal Chemistry* 60, 421–424.

Seitz L.M., W.D. Eustace, H.E. Mohr, M.D. Shogren and W.T. Yamazaki, 1986. Cleaning, milling, and baking tests with hard red winter wheat containing deoxynivalenol. *Cereal Chemistry* 63, 146–150.

Simpson D.R., G.E. Weston, J.A. Turner, P. Jennings and P. Nicholson, 2001. Differential control of head blight pathogens of wheat by fungicides and consequences for mycotoxin contamination of grain. *European Journal of Plant Pathology* 107, 421–431.

Suty A. and A. Mauler-Machnik, 1995. Les fusarioses de l'épi du blé: activité du tébuconazole sur la forme sexuée d'un des principaux pathogènes. *Phytoma-La Defénse des Végétaux* 476, 16–18.

Trigo-Stockli D.M., C.W. Deyoe, R.F. Satumbaga and J.R. Pedersen, 1996. Distribution of Deoxynivalenol and Zearalenone in milled fractions of wheat. *Cereal Chemistry* 73(3), 388–391.

Zadoks J.C., T.T. Chang and C.F. Konzak, 1974. A decimal code for the growth stages of cereals. *Weed Research* 14, 415–442.

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