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Fumonisin B_1 and zearalenone contamination of wheat in Croatia and influence of fungicide treatments

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Résumé de l'article

Un essai a été effectué en Croatie afin de déterminer la présence de fumonisine B_1 et de zéaralenone, des mycotoxines de *Fusarium*, dans des grains de blé et d'évaluer l'efficacité de neuf fongicides à réduire la gravité de la brûlure de l'épi causée par le fusarium ainsi que l'accumulation de fumonisine B_1 et de zéaralenone dans les grains de blé. La fumonisine B_1 et la zéaralenone ont été détectées dans tous les échantillons de grains, avec des concentrations moyennes variant entre 182,0 et 446,6 µg kg^{-1} (fumonisine B_1) et entre 2,59 et 5,33 µg kg^{-1} (zéaralenone). Aucune différence significative n'a été trouvée entre les différents traitements fongicides quant à la teneur en fumonisine B_1 et en zéaralenone dans les grains de blé. Aucune corrélation positive n'a été obtenue entre la gravité de la brûlure de l'épi causée par le fusarium et la teneur en fumonisine B_1 ou en zéaralenone dans les grains de blé, ou encore entre l'efficacité des fongicides et la teneur en fumonisine B_1 ou en zéaralenone dans les grains de blé. Sous des conditions sévères de maladie, l'efficacité des fongicides se situait entre 85,7 % (tébuconazole + triadiméfon) et 72,1 % (carbendazime).

Fumonisin B_1 and zearalenone contamination of wheat in Croatia and influence of fungicide treatments

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In Croatia, a trial was conducted to determine the presence of the *Fusarium* mycotoxins fumonisin B_1 and zearalenone in wheat kernels and to evaluate the efficacy of nine fungicides on Fusarium head blight severity as well as fumonisin B_1 and zearalenone accumulation in wheat grain. Fumonisin B_1 and zearalenone were detected in all grain samples in mean concentrations ranging from 182.0 to 446.6 µg kg^{-1} (fumonisin B_1) and from 2.59 to 5.33 µg kg^{-1} (zearalenone). No significant differences were found among fumonisin B_1 and zearalenone content in wheat grain for the different fungicide treatments. No correlation was revealed between Fusarium head blight severity and fumonisin B_1 or zearalenone content in wheat grain, nor between fungicide efficacy and fumonisin B_1 or zearalenone content in wheat grain. Under conditions of high disease pressure, efficacy of the fungicides was between 85.7% (tebuconazole + triadimefon) and 72.1% (carbendazim).

Keywords: Fumonisin B_1, fungicides, Fusarium head blight, zearalenone.

[Contamination du blé par la fumonisine B_1 et la zéaralenone en Croatie et effet des traitements fongicides]

Un essai a été effectué en Croatie afin de déterminer la présence de fumonisine B_1 et de zéaralenone, des mycotoxines de *Fusarium*, dans des grains de blé et d'évaluer l'efficacité de neuf fongicides à réduire la gravité de la brûlure de l'épi causée par le fusarium ainsi que l'accumulation de fumonisine B_1 et de zéaralenone dans les grains de blé. La fumonisine B_1 et la zéaralenone ont été détectées dans tous les échantillons de grains, avec des concentrations moyennes variant entre 182,0 et 446,6 µg kg^{-1} (fumonisine B_1) et entre 2,59 et 5,33 µg kg^{-1} (zéaralenone). Aucune différence significative n'a été trouvée entre les différents traitements fongicides quant à la teneur en fumonisine B_1 et en zéaralenone dans les grains de blé. Aucune corrélation positive n'a été obtenue entre la gravité de la brûlure de l'épi causée par le fusarium et la teneur en fumonisine B_1 ou en zéaralenone dans les grains de blé, ou encore entre l'efficacité des fongicides et la teneur en fumonisine B_1 ou en zéaralenone dans les grains de blé. Sous des conditions sévères de maladie, l'efficacité des fongicides se situait entre 85,7 % (tébuconazole + triadiméfon) et 72,1 % (carbendazime).

Mots clés : Brûlure de l'épi causée par le fusarium, fongicides, fumonisine B_1, zéaralenone.

Fusarium head blight (FHB) is one of the most important diseases of wheat in many areas of the world. Besides causing yield loss, FHB often results in the accumulation of *Fusarium*-specific mycotoxins in wheat grain (Bottalico and Perrone 2002). Cultivation of susceptible wheat cultivars, the lack of FHB forecasting schemes implemented in practice and high disease pressure in regions of Croatia where wheat is grown in rotation with maize lead to frequent yield losses and increase the risk of mycotoxin accumulation in wheat grain, especially in years or conditions conducive to FHB development. Most of the *Fusarium* species reported to occur in wheat grain in Europe are potential producers of various mycotoxins, and their mycotoxigenic activity on wheat has been the object of numerous studies, with *F. graminearum* Schwabe and *F. culmorum* (W.G. Smith) Saccardo and their products deoxynivalenol (DON) and zearalenone (ZEN) being the most intensively studied. Limited data exist on the mycotoxigenic activity of fumonisin-producing *Fusarium* species in wheat (Marin *et al.* 1999) and wheat grain contamination with fumonisins. The most important producers of fumonisins are *F. verticillioides* (Saccardo) Nirenberg, *F. proliferatum* (Matsushima) Nirenberg, and *F. nygamai* Burgess & Trimboli (Leslie and Summerell 2006).

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The presence of *F. verticillioides* and *F. proliferatum* in wheat kernels implies that fumonisins could be present on wheat, among the other mycotoxins reported so far. Bottalico and Perrone (2002) suggested that the detection of fumonisin B_1 (FB_1) in wheat should be expected and pointed out that the relatively frequent occurrence of *F. verticillioides* and *F. proliferatum* in wheat in Europe could indicate the spread of these fumonisin-producing fungi in small-grain cereals. Presuming that the fungicides used for FHB management are active against all *Fusarium* species, they could be effective against the accumulation of various *Fusarium* mycotoxins by reducing fungal presence and activity in wheat spikes. Studies in which the relationship between fungicide treatments and mycotoxin accumulation in wheat has been investigated have shown that a positive correlation is not always present between fungicide efficacy, FHB severity and mycotoxin accumulation in wheat grain (Gareis and Ceynova 1994; Martin and Johnston 1982; Milus and Parsons 1994). The aim of the present study was to determine the presence of FB_1 and ZEN in wheat grain in Croatia and to determine the relationship between fungicide effectiveness and FB_1 and ZEN contamination of wheat kernels. This experiment also presented an opportunity to evaluate field performance of new products registered for FHB control in Croatia.

A field trial was conducted in 2006 near Kutjevo in northeastern Croatia (45°23′ N, 17°47′ E). Plots were arranged according to a randomized complete block design with four replicates, and the trial was exposed to natural FHB infections. All fungicides were applied twice, with the first application at GS 53 (1/4 of head emerged) and the second one at GS 60 (beginning of flowering) (Zadoks *et al.* 1974), using the rates recommended by the manufacturers diluted in 500 L ha^{-1} of water (Table 1). FHB severity assessment was done at GS 75 (medium milk) (Zadoks *et al.* 1974). It was assessed visually using a scale of 0 to 5 (0 = no symptoms visible; 1 = 1 to 5% of symptomatic spikelets; 2 = 6 to 10% of symptomatic spikelets; 3 = 11 to 25% of symptomatic spikelets; 4 = 26 to 50% of symptomatic spikelets; 5 = more than 50% of symptomatic spikelets). Both FHB severity and product efficacy were expressed as percentage.

Grain samples from the fungicide efficacy trial were collected after harvest and stored at -20 °C prior to ZEN and FB_1 contamination analysis, which was performed using HPLC with fluorescence detection. For ZEN analysis, the samples were cleaned up using C-18 solid phase extraction columns (Varian, Harbor City, CA, USA). ZEN bound to the column was eluted with 4 mL of methanol and evaporated to dryness. Before injection into the HPLC system, samples were

Table 1. Fusarium head blight (FHB) severity, efficacy of fungicides, fumonisin B_1 (FB_1) and zearalenone (ZEN) contents in wheat grain

Product	Active ingredient	Dose 07 May (g a.i. ha^{-1})	Dose 22 May (g a.i. ha^{-1})	FHB severity (%)	Efficacy of fungicides[a] (%)[a]	FB1 content (µg kg^{-1})	ZEN content (µg kg^{-1})
Folicur BT 225 EC®	Tebuconazole + triadimefon	100 80	125 100	7.6 a[b] (0.8)	85.7	182.0 a[c] (31.3)	4.10 a[c] (1.67)
Prosaro 421 SC®	Prothioconazole + tebuconazole	75 75	125 125	9.3 ab (1.3)	82.5	446.6 a (123.3)	2.94 a (0.80)
Artea 330 EC®	Propiconazole + cyproconazole	125 40	125 40	10.5 ab (0.6)	80.2	248.1 a (35.9)	2.86 a (0.49)
Duett®	Epoxiconazole + carbendazim	125 125	125 125	11.5 ab (1.8)	78.2	256.7 a (39.1)	5.30 a (2.46)
Sphere 267.5 EC®	Trifloxystrobin + cyproconazole	**112** **48**	**150** **64**	11.7 abc (1.2)	78.0	356.8 a (111.4)	3.43 a (0.51)
Opera®	Pyraclostrobin + epoxiconazole	100 38	100 38	11.8 abc (1.0)	77.8	372.6 a (91.5)	2.64 a (0.55)
Fandango®	Fluoxastrobin + prothioconazole	60 60	100 100	12.7 abc (2.7)	76.1	365.6 a (90.4)	5.33 a (2.11)
Controlan®	Epoxiconazole + kresoxim-methyl	100 100	100 100	13.9 abc (1.5)	73.7	293.3 a (63.3)	2.33 a (0.24)
Sphere 267.5 EC®	Trifloxystrobin + cyproconazole	**112** **48**	**112** **48**	14.2 bc (1.2)	73.2	311.1 a (82.4)	3.79 a (0.71)
Bavistin FL®	Carbendazim	250	150	14.8 bc (1.6)	72.1	335.7 a (109.7)	2.59 a (0.82)
Untreated				53.0 d (9.3)		220.7 a (26.1)	4.21 a (1.04)
Mean						308.1 (24.0)	3.59 (0.36)

[a] Product efficacy was calculated using Abbot's formula where effectiveness (%) = (FHB severity in control - FHB severity in treatment) X 100/FHB severity in control.

[b] Means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's Multiple Range Test. Values in parentheses refer to the standard error of the mean.

[c] Means followed by the same letter are not significantly different ($P = 0.05$) according to ANOVA. Values in parentheses refer to the standard error of the mean.

redissolved in a methanol/water solution (80/20 v/v). The mobile phase for HPLC consisted of a methanol/water solution (80/20 v/v). The flow rate of the mobile phase was set to 0.5 mL min^{-1}. Guard column LiChrospher RP-18 (Merck, Darmstadt, Germany) 4 x 4 mm and reversed-phase analytical column LiChrospher RP-18 (Merck, Darmstadt, Germany) 250 x 4 mm with 5 µm particles were used. Wavelengths of the detector were set at 274 nm for excitation and 440 nm for emission. Standard ZEN solutions were obtained by dissolution and dilution of the standard (Sigma Chemical Co., St. Louis, MO, USA) with methanol. The standard calibration curve was linear (R^2 = 0.999) and the detection limit for ZEN was 0.39 µg kg^{-1}. For FB_1 analysis, the samples were cleaned up using the immunoaffinity column (Vicam, Watertown, MA, USA). FB_1 bound to the column was eluted with 1.5 ml of methanol and evaporated to dryness. Before injection into the HPLC system, samples were redissolved in methanol and derivatized with 100 µL of o-phthaldehyde (OPA) solution (40 mg o-phthaldehyde dissolved in 1.0 mL of methanol and added to 5 mL of 0.1 M sodium tetraborate and 50 µL of mercaptoethanol). The mobile phase for HPLC consisted of methanol and 0.1 M NaH_2PO_4 (68/32 v/v) adjusted to pH 3.35 with o-H_3PO_4. The flow rate of the mobile phase was set to 0.8 mL min^{-1}. Guard column LiChrospher RP-18 (Merck, Darmstadt, Germany) 4 x 4 mm and reversed-phase analytical column LiChrospher RP-18 (Merck, Darmstadt, Germany) 125 x 4 mm with 5 µm particles were used. Wavelength of the detector was set at 336 nm for excitation and 440 nm for emission. Standard FB_1 solutions were obtained by dissolution and dilution of the standard (Sigma Chemical Co., St. Louis, MO, USA) with an acetonitrile/water solution (1/1 v/v). The standard calibration curve was linear (R^2 = 0.997) and the detection limit for FB_1 was 10 µg kg^{-1}.

ZEN and FB_1 content in grain samples from different fungicide treatments were determined by analysis of variance at P = 0.05 with the ANOVA procedure of SAS 9.1 (SAS Institute, Cary, NC, USA). Linear correlations were generated and Pearson correlation coefficients were calculated with the CORR procedure of SAS 9.1 using untransformed data of ZEN content, FB_1 content, fungicide efficacy and FHB severity from all replicates.

FB_1 and ZEN contents were determined in all wheat grain samples (Table 1). Mean FB_1 and ZEN contents in all tested samples were 308.1 µg kg^{-1} and 3.59 µg kg^{-1}, respectively. Mean FB_1 content ranged from 446.6 µg kg^{-1} in samples treated with prothioconazole + tebuconazole (Prosaro 421 SC®) to 182.0 µg kg^{-1} in samples treated with tebuconazole + triadimefon (Folicur BT 225 EC®). Mean ZEN content ranged from 5.33 µg kg^{-1} in samples treated with fluoxastrobin + prothioconazole (Fandango®) to 2.33 µg kg^{-1} in samples treated with epoxiconazole + kresoxim-methyl (Controlan®). Mean FB_1 and ZEN contents in untreated control samples were 220.7 µg kg^{-1} and 4.21 µg kg^{-1}, respectively.

The detection of FB_1 in wheat grain samples confirmed the hypothesis of Bottalico and Perrone (2002) that these mycotoxins are likely to occur in wheat. Marin *et al.* (1999) hypothesized that the low incidence of fumonisin-producing *Fusarium* spp. in wheat grain could be the reason for the absence of fumonisins in wheat. Research on *Fusarium* spp. populations in wheat grain in Croatia revealed the common presence of *F. verticillioides* in kernels (Čosic *et al.* 2004). Among 846 isolates of *Fusarium* isolated from wheat kernels in eastern Croatia from 1996 to 2002, 166 (20%) were even identified as *F. verticillioides*; only *F. graminearum* (53%) was found in higher percentage (Čosic *et al.* 2004). Considering the obvious common presence of fumonisin-producing *F. verticillioides* in wheat grain in Croatia, the presence of FB_1 in the samples analyzed was not unexpected, although such results are in contrast with the study of Marin *et al.* (1999). These authors reported that two isolates of *F. verticillioides* and two of *F. proliferatum* produced minimal quantities of FB_1 in irradiated wheat grain (maximum amount of 3.5 µg g^{-1}) compared with irradiated maize grain (maximum amount of 2019.2 µg g^{-1}). This would mean that wheat grain is an unsuitable substrate for FB_1 production, which was not confirmed by the results of the present study.

On the other hand, ZEN is a toxin frequently found in wheat in European countries (Bottalico and Perrone 2002). The mean ZEN content found in the present study (3.59 µg kg^{-1}) was higher than those found in maize samples in Croatia, which ranged from 0.2 to 1.2 µg kg^{-1} (Mitak *et al.* 2005) and from 0.62 to 3.22 µg kg^{-1} (Domijan *et al.* 2005). ZEN concentrations detected in the present study are generally low compared with those found in some other European countries (De Nijis *et al.* 1996; Lepschy-von Gleissenthal *et al.* 1989; Perkowski *et al.* 1990; Vrabcheva *et al.* 1996).

No significant differences between treatments were found among FB_1 and ZEN levels in the grain samples analyzed. No significant correlation was found between FB_1 content in wheat grain and the efficacy of the fungicides tested or between FB_1 content in wheat grain and FHB severity. Correlation coefficients were 0.056 and -0.097, respectively. Similarly, no correlation was found between ZEN content in grain and fungicide efficacy or between ZEN content and FHB severity, with correlation coefficients of 0.172 and -0.215, respectively. One of the possible reasons for the lack of correlation between fungicide efficacy and FB_1 or ZEN content in wheat grain in the present study could be the period during which mycotoxin-producing *Fusarium* spp. colonize wheat grain. If such colonization occurs at late growth stages, from the hard dough to the end of ripening, it is likely that fungicides applied earlier lose their effect on fungi that develop on spikes and grain late in the growing season. Regardless of such hypothesis, studies have shown that the interaction between mycotoxin production, the environment and fungicidal activity of active ingredients is complex (D'Mello *et al.* 1998; Pirgozliev *et al.* 2003). Most of these studies have focused on DON, and it is obvious that all three cases can occur. Fungicides can reduce, increase, or have no effect on mycotoxin content in wheat grain, and it is hard to predict their effect on mycotoxin production (Gareis and Ceynova 1994; Martin and Johnston 1982; Menniti *et al.* 2003; Milus and Parsons 1994; Pirgozliev *et al.* 2002; Simpson *et al.* 2001). It should be emphasized that the present study included only

one year and location, and more research is needed to make hypothetical conclusions.

The efficacy of all products tested on FHB severity was higher than 70% (Table 1). The tebuconazole + triadimefon mixture (Folicur BT 225 EC®) was the most efficient one with 85.7% efficacy, followed by prothioconazole + tebuconazole (Prosaro 421 SC®) with 82.5% efficacy, and propiconazole + cyproconazole (Artea 330 EC®) with 80.2% efficacy, while carbendazim (Bavistin SL®) was the least efficient product (72.1%). FHB severity ranged from 7.6% on plots treated with the tebuconazole + triadimefon mixture to 53.0% on the untreated control (Table 1). Results from the present study confirm the fact that even after two fungicide treatments during the growing season, some damage resulting from FHB is likely to occur in conditions conductive to FHB development. Fungicide efficacy in this study was higher than 70%, which can be considered a good result in conditions of high disease pressure. Jones (2000) reported that complete control of FHB with less than six to eight fungicide treatments cannot be expected. All fungicides evaluated in the present study, except carbendazim, had one or both active ingredients from the triazole group, so their effectiveness is not unexpected. Various triazole fungicides are effective in FHB management (Hutcheon and Jordan 1990; Menniti *et al.* 2003; Mesterházy and Bartók 1997; Pirgozliev *et al.* 2002; Simpson *et al.* 2001). The same has been proven for carbendazim in field trials (Ellner 1997; Mesterházy and Bartók 1997). In the matter of efficacy, results from the present study are in accordance with other field studies in which tebuconazole-containing fungicides showed the highest efficacy for controlling FHB among all products tested (Cromey *et al.* 2001; Ellner 1997; Matthies and Buchenauer 2000; Mesterházy and Bartók 1997; Simpson *et al.* 2001).

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