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EFFECT OF FUNGICIDE APPLICATION ON WHEAT HEAD BLIGHT, OCCURRENCE OF *FUSARIUM* SPP. AND MYCOTOXIN PRODUCTION

ABSTRACT

The aim of the study was to determine if azoxystrobin and metconazole used for the control of wheat FHB at half, full, and quarter more the recommended dose rate may affect in differentiated way on the occurrence of *Fusarium* spp. and their ability to mycotoxin production in harvested grain, in wheat ears artificially inoculated with two DON-producing isolates of *F. culmorum*. Macroscopic evaluation showed high incidence of fusariosis. Plant health in the plots where the heads were artificially inoculated and fungicide was not applied was similar to the protected ones. Only increasing the dose metconazole resulted in a stronger reduction of fusariosis. The advantageous effect of azoxystrobin was not observed. Mycological analysis of harvested grain showed the presence of a number of *F. culmorum*, but from samples sprayed with metconazole it was isolated in smaller quantities. Also *F. avenaceum, F. graminearum, F. poae* and *F. tricinctum* were isolated. Molecular analysis showed the presence of *F. culmorum* in all samples of harvested grain. Also genes from *Tri* cluster were identified, involved in the synthesis of type-A and type-B trichothecenes - especially DON and 3Ac-DON. Chromatography revealed the presence of small quantities of mycotoxins. In all samples DON and 3Ac-DON were predominant. In general, *F. culmorum* isolate, which caused weaker symptoms of FHB and was less numerously isolated from grain that the other one, produced smaller amounts of mycotoxins. Samples protected with azoxystrobin contain the largest quantities of DON. Effect of different doses of fungicides on the number of mycotoxins was not clearly established.

Key words: Fusarium, mycotoxins, fungicides, azoxystrobin, metconazole

INTRODUCTION

Fusarium head blight (FHB) is one of the most significant problems affecting wheat (*Triticum aestivum* L.) due reduction of both crop yields and grain quality. It is associated with a decrease of grain number per ear, 1000-

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grain weight and grain weight per ear (Chelkowski *et al.* 2000). Occurrence of *Fusarium* species effects unfavorably on grain quality, especially in the case of mycotoxin contamination. Due to the ability of several *Fusarium* species to produce trichothecene such as deoxynivalenol (DON) and its derivatives, nivalenol (NIV), T-2 toxin and also zearalenone (ZEA) (D'Mello and MacDonald 1997, Magan *et al.* 2002, Schollenberger *et al.* 2006) the use of grain contaminated by these fungi is harmful to humans and livestock (Bottalico 1998, Hussein and Brasel 2001). In order to reduce *Fusarium* spp. fungicide treatment is applied. The literature data show that the fungicide applying does not guarantee getting rid of the pathogens and, sometimes, can increase the concentration of mycotoxins (Simpson *et al.* 2001, Magan *et al.* 2002, Pirgozliev *et al.* 2002). *Fusarium culmorum* is a common pathogen of wheat and other small cereal grains involved in the etiology of FHB (Parry *et al.* 1995). In addition, *F. culmorum* can produce various mycotoxins mentioned above (Bakan *et al.* 2001, Bottalico and Perrone 2002, Logrieco *et al.* 2003, Waalwijk *et al.* 2003).

The study started in 2009 were undertaken to determine (i) if fungicides used for the control of winter wheat head fusariosis at different dose rates may affect in differentiated way on the occurrence of *Fusarium* spp. and (ii) their ability to mycotoxin production in harvested grain, in wheat ears artificially inoculated with *F. culmorum*.

MATERIAL AND METHODS

Fungicide-treated grain (tebuconazole, Raxil, Bayer CropScience) of winter wheat was sown at experimental plots (1.3x6.0m) in Lisewo Malborskie (Żuławy Region, Pomorskie Province, Poland), where natural moisture conditions are advantageous for such studies. Heads of wheat on selected plots were treated with metconazole (Caramba 60 SL, BASF) and azoxystrobin (Amistar 250 SC, Syngenta Crop Protection) at half, full, and quarter more the manufacturer's recommended dose rate at the head emergence complete stage (ZGS-59; Zadoks *et al.* 1974). Then, at the stage of half of flowered florets (ZGS-65), heads were artificially inoculated with conidia of two different isolates of DON-producing *F. culmorum* (Fc24 and Fc32), originated from winter wheat (Table 1). Conidia of *F. culmorum* were produced from cultures grown on sucrose nutrient agar (SNA), incubated under darkness at 23°C for 20 days.

Conidial suspensions ($10^{6}\cdot\times$ ml^{-1}) were obtained for each isolate by washing conidia from sporulating mycelium using sterile water. Inoculation was carried out by spraying the conidial suspension using a pressure atomizer at a rate of 300 $l\cdot\times$ ha^{-1}. Before inoculation plants were sprayed with water. To create conditions favorable for infection by the pathogens, fields

immediately after inoculation were covered with nonwoven, which was sprayed with water over the next five days and then removed.

Visual fusariosis assessment was made at early dough stage (ZGS-83) on 20 heads x 4 replications using a 0-6° scale - from 0 (healthy heads) to 6(> 50% of head surface infected). The level of infestation was transformed to a disease index (DI in %) according to Townsend`s and Heuberger`s formula (Wenzel 1948). The obtained data were analysed statistically by ANOVA and a Tukey test used to compare means.

At harvest all grain was collected separately from each plot. The mass of 1000 grain was weight. Mycological analysis of harvested grain was made on PDA (Difco, USA). Grain (four harvested 100 grain samples from each combination) was disinfected in 1% NaOCl for 2 minutes, washed three times in sterile water, dried on sterile blotting paper and put onto Petri dishes with medium. After 7 days of incubation in the dark at 23°C, the pieces of culture were placed in test tubes with PDA. After an appropriate time fungi were identified according to mycological keys.

A 14 g representative sample of grain from each plot was milled in coffee grinder and DNA was extracted from 10 g of the milled material. DNA was isolated according to f Edwards *et al.* (2001). PCR reactions were carried out with specific primers and amplification conditions described in publications. To identify *F. culmorum* Fc01F/Fc01R (Nicholson *et al.* 1998) were used, for the *Tri*5 gene: HATriF/ HATriR (Edwards *et al.* 2001); for DON chemotype determination: MinusTri7F/ MinusTri7F (Chandler *et al.* 2003); to identify DON derivatives (3Ac-DON and 15AC-DON) respectively: Tri303F/ Tri303R and Tri315F/Tri315R (Jennings *et al.* 2004); for genes specific for type-A and type-B trichothocene-producing species respectively: T4F1506/T4EndR2 and Tri4BF/Tri4BR (Nicholson *et al.* 2004). As controls *F. culmorum* – Fc, obtained from UMCS, Lublin, Poland (NCBI DQ453700, CBS 120098) and *F. langsethiae* - Fl, own isolate of our Department (GenBank, EU088404) were used. Depending on the purpose of analysis they were positive or negative control. Amplification products were separated by electrophoresis on 1.4% agarose gels with TBE running buffer and stained with ethidium bromide. A molecular marker of 100 bp (EURx, Poland) was used. The results were scanned into a computer imaging file with a gel documentation system (VILBER LOURMAT) equipped with a digital camera.

Grain samples from the field trial were analyzed for type-B trichothecenes: DON and derivatives (3Ac-DON, 15Ac-DON), NIV and FUS-X content by GC-MS in Department of Chemistry, in Poznan University of Life Sciences, Poland using gas chromatograph (Hewlett Packard 6890) coupled with mass detector (Hewlett Packard 5972 A) and equipped with a capillary column (HP-5MS, 0,25mm x 30 m) according to Perkowski *et al.* (2003).

RESULTS AND DISCUSSION

Table 1

Details of field experiment, mean head infection [DI in %], weight of 1000 grains [WTG in g] and mycotoxin concentration in harvested grain [mg/kg]

Plot no	Combination	Fungicide applied, dose rate, *F. culmorum* isolate used for inoculation	Head infection	WTG	DON	3Ac-DON	15Ac-DON	NIV
1	A½, Fc24	azoxystrobin ½, Fc 24	71.9	35.6	0.235	0.008	0.000	0.007
2	A1, Fc24	azoxystrobin 1, Fc 24	72.3	36.8	0.577	0.011	0.001	0.006
3	A1¼,Fc24	azoxystrobin 1 ¼, Fc 24	77.1	35.6	0.198	0.006	0.000	0.000
4	C, Fc24	Control: no fungicide, Fc 24	77.1	40.2	0.251	0.005	0.000	0.000
5	M½, Fc24	metconazole ½, Fc 24	76.5	38.2	0.414	0.014	0.001	0.003
6	M1, Fc24	metconazole 1, Fc 24	71.5	41.0	0.242	0.006	0.000	0.005
7	M1¼, Fc24	metconazole 1 ¼, Fc 24	59.4	35.4	0.269	0.007	0.000	0.005
8	C 0-1	Control 0-1: no fungicide, no Fc	6.7	38.2	0.044	0.001	0.000	0.002
9	A½, Fc32	azoxystrobin ½, Fc 32	79.6	38.6	0.706	0.026	0.001	0.008
10	A1, Fc32	azoxystrobin 1, Fc 32	77.7	40.6	0.436	0.015	0.001	0.005
11	A1¼, Fc32	azoxystrobin 1 ¼, Fc 32	81.5	34.8	0.611	0.022	0.001	0.003
12	C, Fc32	Control: no fungicide, Fc 32	70.4	35.0	0.399	0.015	0.001	0.003
13	M½, Fc32	metconazole ½, Fc 32	78.5	33.6	0.402	0.017	0.000	0.003
14	M1, Fc32	metconazole 1, Fc 32	79.6	32.4	0.456	0.018	0.000	0.003
15	M1¼, Fc32	metconazole 1 ¼, Fc 32	73.5	33.6	0.387	0.013	0.000	0.003
16	C 0-2	Control 0-2: no fungicide, no Fc	9.8	36.0	0.126	0.002	0.000	0.003

Macroscopic evaluation showed high incidence of head fusariosis - Disease Index was over 70%. The level of disease at the plots where the heads were artificially inoculated and fungicides were not applied was similar to the protected ones. Mean DI for plots with azoxystrobin was 76.7 while for metconazol 73.1. Only increasing the dose of metconazole compared to the recommended rate resulted in a significantly stronger reduction of fusariosis (DI=59.4) (Table 1). It is consistent with the results of Pirgozliev *et al.* (2002) and Edwards *et al.* (2001) who noted that metconazole demonstrated high activity against *F. culmorum*, reducing significantly the severity of FHB. However our study did not revealed clear effect of this preparation. Similarly, Simpson *et al.* (2001), studying different fungicides including azoxystrobin, showed that none of the fungicide treatments gave a significant reduction of the visual symptoms. Here, significantly the lowest infection was noted at Control 0 (DI=8.2). The advantageous effect of azoxystrobin on head health was not observed here. However, Cromey *et al.* (2001), Siranidou and Buchenauer (2001) and Pirgozliev *et al.* (2002)

showed that applications of azoxystrobin reduced FHB severity, while Simpson *et al.* (2001) and Edwards *et al.* (2001) noticed that treatment with azoxystrobin did not control *F. culmorum*. The effect of azoxystrobin on growth of *Fusarium* species was very low also in vitro test (Müllenborn *et al.* 2008, not published our own studies 2009).
Weight of the thousand grains was variable and ranged from 32.4 g (M1, Fc32) to 41.0 g (M1, Fc24). There was no clear relation between this parameter and the severity of head fusariosis (Table 1).

Table 2

Grain infection with fungi [%]

Fungus/Plot number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
F.avenaceum	1	0	0	0	0	2	2	2	1	0	0	1	4	0	0	2
F.culmorum	80	69	71	68	64	59	58	25	90	80	92	84	79	88	67	23
F.equiseti	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
F.graminearum	0	0	0	0	0	0	1	1	0	3	0	2	0	0	1	1
F.poae	2	1	2	1	4	1	1	9	1	0	0	2	0	0	1	11
F.solani	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
F.sporotrichioides	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
F.tricinctum	1	1	1	2	0	1	0	4	4	0	0	1	2	0	0	3
A.alternata	11	21	25	16	22	29	25	44	14	15	5	6	13	11	15	42
Ar. phaeospermum	0	1	0	0	0	1	0	3	0	1	0	2	0	0	0	3
B.cinerea	0	0	0	1	2	0	1	2	0	1	1	0	0	0	0	1
C.herbarum	0	1	2	0	0	0	0	2	0	1	3	0	0	0	1	2
E.nigrum	6	10	4	13	10	11	9	12	5	4	7	7	7	5	15	20
Penicillium spp.	0	0	0	5	0	0	1	2	0	0	0	0	1	2	0	0
Tr. viride	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Non sporulating fungi	0	0	0	1	1	0	0	0	0	0	0	1	0	0	1	1
Total	101	104	105	108	103	104	98	107	115	105	108	106	106	106	101	111

Mycological analysis of harvested grain (Table 2) showed that artificial inoculation led to a greater predominance of *F. culmorum* over the others species. *Fusarium culmorum* was less numerously isolated from grain inoculated with Fc 24 (mean % of infected grain - 67) compared to Fc32 (83%). This species was isolated in smaller quantities from samples sprayed with metconazole (mean % of infected grain - 69.2) compared with azoxystrobin (80.3%). This coincides with results of fusariosis evaluation of head and inhibitory effect of metconazole (Pirgozliev *et al.* 2002). *Fusarium avenaceum, F. graminearum* and *F. poae* were found not numerously and in not all samples. According Parry *et al.* (1995), Nicholson *et al.* (2004) they are often associated with FHB in

cooler regions, such as northern Europe. *Fusarium equiseti* and *F. sporotrichioides* isolated occasionally are the common species present in the temperate regions of northwest Europe (Chelkowski *et al.* 1989, Langseth *et al.* 1999). *Fusarium poae* was isolated mainly from Control 0, where inoculation with *F. culmorum* was not applied. Among saprotrophic species, commonly isolated from wheat kernels, *A. alternata* (Kosiak *et al.* 2004, Zhang *et al.* 2007) and *E. nigrum* (Grabarkiewicz-Szczesna *et al.* 1989) were noted most frequently. Negative correlation was observed between them and *F. culmorum,* especially in the case of azoxystrobin. Azoxystrobin was shown to be effective against saprotrophic fungi found on the wheat heads (Bertelsen *et al.* 1999) while being less effective against *F. culmorum* (Faure and Declercq 1999). Liggit *et al.* (1997) suggest that the application of fungicides which have significant activity against saprotrophic species, may lead to greater colonization of wheat ears by the *F. culmorum*, due to the removal of antagonistic saprotrophs.

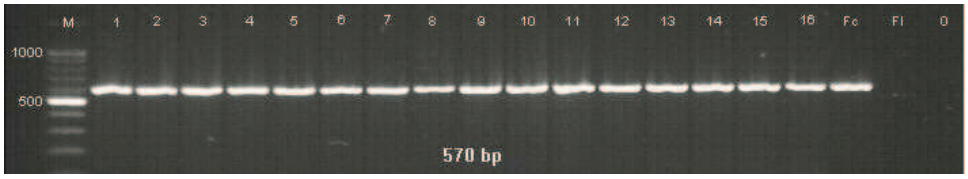

Fig. 1. Agarose gel of PCR assay with primers Fc01F/Fc01R. Marker 100bp (M), samples from plots (1-16), positive control (Fc), negative control (Fl), blank control (0)

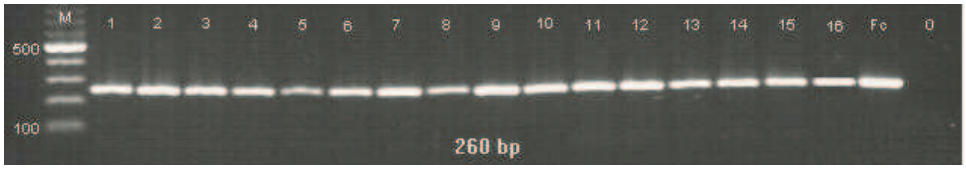

Fig. 2. Agarose gel of PCR assay with primers HATriF/ HATriR. Marker 100bp (M), samples from plots (1-16), positive control (Fc), blank control (0)

Fig. 3. Agarose gel of PCR assay with primers T4F1506/T4EndR2. Marker 100bp (M), samples from plots (1-16), negative control (Fc), positive control (Fl), blank control (0)

Molecular analysis (SCAR-PCR) of harvested grain showed incidence of *F. culmorum* and *Tri*5 gene in all samples (Fig. 1, 2). This allowed the assumption that all samples may contain toxins, because *Tri*5 gene is present in all known species of *Fusarium* are able to produce trichothecenes (Nicholson *et al.* 2004).

Also genes from *Tri* cluster were identified, involved in the synthesis of type-A and B trichothecenes - especially deoxynivalenol and its derivatives. Products (bands) characteristic for type-A trichotecenes were very weak and identified in not all samples, unlike the products specific to type-B trichothecenes (Fig 3, 4). This follows from the fact that in the samples were identified very few fungi that are able to produce A-type trichotecenes, which include *F. poa*e, *F. equiseti* and *F. sporotrichioides* (Chelkowski *et al.* 1989, Morrison *et al.* 2002, Halstensen *et al.* 2006). Among the identified species predominated *F. culmorum*, known producer of deoxynivalenol (Okubara *et al.* 2002, Bottalico and Perrone 2002), which, according to Pestka (2007), is usually isolated mycotoxin from the trichothecenes. Genes responsible for producing DON and 3-Ac DON were detected in all samples (Fig 5, 6). Genes encoding 15Ac-DON were also identified in all samples, but band intensity was very weak (Fig. 7), suggesting a small number of copies of these genes. This was confirmed during the GC-MS, where a very small amount of this derivative was extracted.

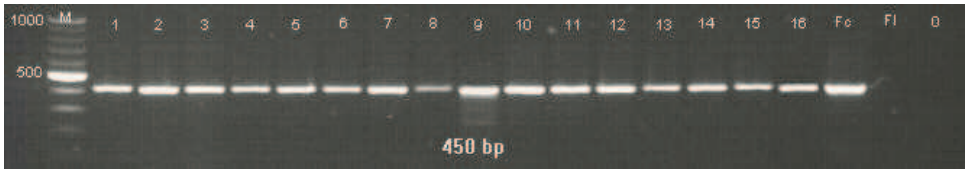

Fig. 4. Agarose gel of PCR assay with primers Tri4BF/Tri4BR. Marker 100bp (M), samples from plots (1-16), positive control (Fc), negative control (Fl), blank control (0)

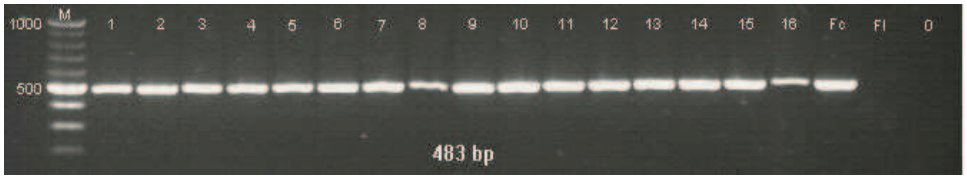

Fig. 5. Agarose gel of PCR assay with primers MinusTri7F/ MinusTri7F. Marker 100bp (M), samples from plots (1-16), positive control (Fc), negative control (Fl), blank control (0)

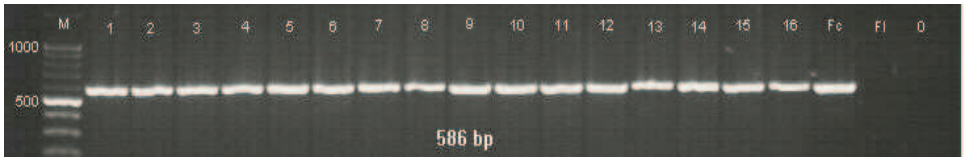

Fig. 6. Agarose gel of PCR assay with primers Tri303F/ Tri303R. Marker 100bp (M), samples from plots (1-16), positive control (Fc), negative control (Fl), blank control (0)

Chromatographic analysis (GC-MS) confirmed molecular results and revealed the incidence of trichothecenes and predominance of DON and 3Ac-DON in all samples (Table 1) This is consistent with the results of Arseniuk and Góral (2005) and Perkowski *et al.* (1995) who found that in Europe, 3AC-DON clearly prevails. There

were differences in mycotoxin production among samples inoculated with different isolates of *F. culmorum* and treated with the same fungicides. In the case of isolate Fc32 applying of half and quarter more the recommended dose of azoxystrobin increased the content of DON compared with the control, while in the case Fc24 effect was the opposite. In general, Fc 32, which caused stronger symptoms of FHB and was more numerously isolated from grain that Fc24, produced higher amounts of mycotoxins.

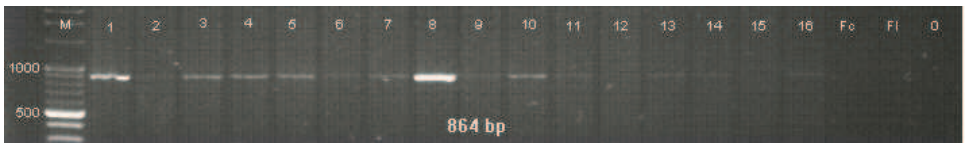

Fig. 7. Agarose gel of PCR assay with primers Tri315F/Tri315R. Marker 100bp (M), samples from plots (1-16), negative controls (Fc and Fl), blank control (0)

Also Menniti *et al.* (2003) and Haidukowski *et al.* (2004) noted positive correlation of disease severity with DON level. Samples protected with azoxystrobin contained more of DON (mean concentration – 0.46 mg/kg) than samples protected with metconazole (0.36), controls inoculated with *F. culmorum* (0.33) and control 0 (0.08). Literature data, concerned this issue, are confused. Pirgozliev *et al.* (2003) noted that applications of azoxystrobin might have a direct effect on DON production, through imparting a stress factor on *F. culmorum*, inducing the pathogen to produce more mycotoxin. Simpson *et al.* (2001) also associated applications of azoxystrobin with increased DON concentrations in harvested wheat grain. Opposite result were observed by Siranidou and Buchenauer (2001). They showed that applications of azoxystrobin reduced FHB severity and that DON concentration was similar to that in unsprayed controls. Pirgozliev *et al.* (2002) showed that azoxystrobin reduced DON compared to unsprayed controls, but, as in this study, its effectiveness was less than that of metconazole. Also Blandino and Reyneri (2009) have shown that triazoles, which include metconazole, in contrast to the strobilurin (azoxystrobin with the head), significantly reduces the amount of DON in wheat grain. Different 15Ac-DON and NIV were found in trace amounts (Table 1) and FUS-X was not identified. Effect of different doses of fungicides on the number of mycotoxins was not clearly established. The use of fungicides, in most cases did not result in reduction of DON in comparison to the non treated samples inoculated with *F. culmorum*. It was noted, however, some trends: decreasing doses, in some cases (e.g. A½, Fc32), increases the amount of mycotoxins produced. The mechanisms by which the fungicides stimulate toxin production in *Fusarium* spp. are not known. It may be assumed that in the presence of sub-lethal concentrations of certain fungicides the fungal strains respond to this stress by increased production of secondary metabolites including mycotoxins, as a possible mechanism (Ramirez *et al.* 2004)

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REFERENCES

Arseniuk E., Góral T., 2005. Mikotoksyny fuzaryjne w ziarnie zbóż i kukurydzy/ *Fusarium* mycotoxins in cereal grain and maize (in Polish). Hodowla Roślin i Nasiennictwo 3: 27–33.

Bakan B., Pinson L., Cahagnier B., Melcion D., Semon E.R., Richard-Molard D., 2001. Toxigenic potential of *Fusarium culmorum* strains isolate from French wheat. Food Addit Contam 18: 998–1003.

Bertelsen J.R., de-Neergaard E., Smedegaard-Petersem V., 1999. Reasons for improved yield when using azoxystrobin in winter wheat. In: 16th Danish Plant Protection Conference. Crop protection in organic farming, Pests and Diseases, Tjele, Denmark.

Blandino M., Reyneri A., 2009. Effect of fungicide and foliar fertilizer application to winter wheat at anthesis on flag leaf senescence, grain yield, flour bread-making quality and DON contamination. Eur J Agron 30: 275–282.

Bottalico A., 1998. *Fusarium* diseases of cereals: species complex and related mycotoxin profiles, in Europe. J Plant Pathol 80: 85–103.

Bottalico A., Perrone G., 2002. Toxigenic *Fusarium* species and mycotoxins associated with head blight in small cereals in Europe. Eur. J. Plant Pathol. 108: 611–624.

Chandler E.A., Simpson D.R., Thomsett M.A., Nicholson P., 2003. Development of PCR assays to *Tri*7 and *Tr*13 trichothecene biosynthetic genes, and characterization of chemotypes of *Fusarium graminearum, F. culmorum* and *F. cerealis*. Physiol Mol Plant P 62: 355-367.

Chelkowski J., Manka M., Kwasna H., Visconti A., Golinski P., 1989. *Fusarium sporotrichioides* Sherb., *Fusarium tricinctum* (Corda) Sacc. and *Fusarium poae* (Peck) Wollenw – cultural characteristics, toxigenicity and pathogenicity towards cereals. J Phytopathol 24: 155–161.

Chelkowski J., Wishniewska H., Adamski T., Golinski P., Kaczmarek Z., Kostecki M., Perkowski J., Surma M., 2000. Effect of *Fusarium culmorum* head blight on mycotoxin accumulation and yield traits in barley doubled haploids. J Phytopathol 148: 541–545.

Cromey M.G., Lauren D.R., Parkes R.A., Sinclair K.I., Shorter S.C., Wallace A.R., 2001. Control of *Fusarium* head blight of wheat with fungicides. Aust J Plant Pathol 31: 301–308.

D'Mello J.P.F., MacDonald A.M.C., 1997. Mycotoxins. Anim Feed Sci Tech 69,155–66.

Edwards S.G., Pirgozliev S.R., Hare M.C., Jenkinson P., 2001. Quantification of trichothecene-producing *Fusarium* species in harvested grain by competitive PCR to determine efficacies of fungicides against *Fusarium* head blight of winter wheat. Appl Environ Microb 67: 1575–1580.

Faure A., Declercq J., 1999. Wheat head blight disease – visible efficacy and grain analysis. Phytoma – La défense des végétaux 517: 12–15.

Grabarkiewicz-Szczesna J., Chelkowski J., Zajkowski P., 1989. Natural occurrence of *Alternaria* mycotoxins in the grain and chaff of cereals. Mycotoxin Res 5: 77–80.

Haidukowski M., Pascale M., Perrone G., Pancaldi D., Campagna C., Visconti A., 2005. Effect of fungicides on the development of *Fusarium* head blight, yield and deoxynivalenol accumulation in wheat inoculated under field conditions with *Fusarium graminearum* and *Fusarium culmorum*. J Sci Food Agric 85:191–198.

Halstensen A. S., Nordby K. C., Klemsdal S. S., Elen O., Clasen P. E., Eduard W., 2006. Toxigenic *Fusarium* spp. as determinants of trichothecene mycotoxins in settled grain dust. J Occup Environ Hyg 3: 651–659.

Hussein S.H., Brasel J.M., 2001. Toxicity, metabolism, and impact of mycotoxins on humans and animals. Toxicology 167: 101–134.

Jennings P., Coates M.E., Turner J.A., Chandler E.A., Nicholson P, 2004. Determination of deoxynivalenol and nivalenol chemotypes of *Fusarium culmorum* isolates from England and Wales by PCR assay. Plant Pathol 53: 182–190.

Kosiak B., Torp M., Skjerve E., Andersen B., 2004. *Alternaria* and *Fusarium* in Norwegian grains of reduced quality - a matched pair sample study. Int J Food Microbiol 93: 51– 62.

Langseth W., Bernhoft A., Rundberget T., Kosiak B., Gareis M., 1999. Mycotoxin production and cytotoxicity of *Fusarium* strains isolated from Norwegian cereals. Mycopathologia 144: 103–113.

Liggitt J., Jenkinson P., Parry D.W., 1997. The role of saprophytic microflora in the development of *Fusarium* ear blight of winter wheat caused by *Fusarium culmorum*. Crop Prot 16: 679–685.

Logrieco A., Bottalico A., Mulé G., Moretti A., Perrone G., 2003. Epidemiology of toxigenic fungi and their associated mycotoxins for some Mediterranean crops. Eur. J. Plant Pathol 109: 645–667.

Magan N., Hope R., Colleate A., Baxter E.S., 2002. Relationship between growth and mycotoxin production by *Fusarium* species, biocides and environment. Eur J Plant Pathol 108: 685–690.

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

Morrison E., Rundberget T., Kosiak B., Aastviet A.H., Bernhoft A., 2002. Cytotoxicity of trichothecenes and fusarochromanone produced by *Fusarium equiseti* strains isolated from Norwegian cereals. Mycopathologia 153: 49–56.

Müllenborn C., Steiner U., Ludwig M., Oerke E-C., 2008, Effect of fungicides on the complex of *Fusarium* species and saprophytic fungi colonizing wheat kernels. Eur J Plant Pathol 120: 157–166.

Nicholson P., Simpson D. R., Weston G., Rezanoor H. N., Lees A. K., Parry D., Joyce D., 1998. Detection and quantification of *Fusarium culmorum* and *Fusarium graminearum* in cereals using PCR assays. Physiol Mol Plant P 53: 17–37.

Nicholson P., Simpson D.R., Wilson A.H., Chandler E., Thomsett M., 2004. Detection and differentiation of trichothecene and enniatin-producing *Fusarium* species on small-grain cereals. Eur J Plant Pathol 110: 503–514.

Okubara P.A., Blechl A.E., McCormick S.P., Alexander N.J., Dill-Macky R., Hohn T.M., 2002. Engineering deoxynivalenol metabolism in wheat through the expression of a fungal trichothecene acetyltransferase gene. Theor Appl Genet 106: 74–83.

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

Perkowski J., Miedaner T., Geiger H.H., Muller H.M., Chelkowski J. 1995. Occurrence of deoxynivalenol (DON), 3-Acetyl-DON, zearalenone, and ergosterol in winter rye inoculated with *Fusarium culmorum*. Cereal Chem 72 (2): 205–209.

Perkowski J., Kiecana I., Kaczmarek Z., 2003. Natural occurrence and distribution of *Fusarium* toxins in naturally contaminated 15 barley cultivars. Eur J Plant Pathol 109: 331–339.

Pestka J.J., 2007. Deoxynivalenol: Toxicity, mechanisms and animal health risks. Anim Feed Sci Tech 137: 283–298.

Pirgozliev S.R., Edwards S.G., Hare M.C., Jenkinson P., 2002. Effect of dose rate of azoxystrobin and metconazole on the development of *Fusarium* head blight and the accumulation of deoxynivalenol (DON) in wheat grain. Eur J Plant Pathol 108: 469–478.

Pirgozliev S.R., Edwards S.G., Hare M.C., Jenkinson P., 2003. Strategies for the control of *Fusarium* head blight in cereals. Eur J Plant Pathol 109: 731–742.

Ramirez M.L., Chulze S., Magan N., 2004. Impact of environmental factors and fungicides on growth and deoxinivalenol production by *Fusarium graminearum* isolates from Argentinian wheat. Crop Prot 23: 117–125

Schollenberger M., Muller H-M, Rufle M., Suchy S., Plank S., Drochner W., 2006. Natural occurrence of 16 *Fusarium* toxins in grains and feedstuffs of plant origin from Germany. Mycopathologia 161: 43–52

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

Siranidou E., Buchenauer H., 2001. Chemical control of *Fusarium* head blight on wheat. J Plant Dis Protect 108: 231–243.

Waalwijk C., Kastelein P., De Vries I., Kerényi Z., Van der Lee T, Hessenlink T., Kohl J., Kema G., 2003. Major changes in *Fusarium* spp. in wheat in the Netherlands. Eur. J. Plant Pathol 109: 743–754.

Wenzel H., 1948. Zur Erfassung des Schadenausmasses in Pflanzenschutzversuchen. Pflanzenschutz-Ber. 15: 81–84.

Zadoks J.C., Chang T.T., Konzak C.F., 1974. A decimal code for the growth stages of cereals. Weed Res 14: 415–421.

Zhang Q., Yang S., Dong Z., Pei L., Ding L., Song A., GongY., Liu G., Xue L., 2007. Determination of grain contamined by *Alternaria alternata* and exposure of its toxins for residents in the high incident area of esophageal cancer. Life Science Journal 4 (4): 25–28.