

## Effect of fungicides on *Fusarium* infection and production of deoxynivalenol in spring cereals

R. Semaškienė, A. Mankevičienė, Z. Dabkevičius and S. Supronienė

Department of Plant Pathology and Protection, Lithuanian Institute of Agriculture, Instituto 1, LT 58344 Akademija, Kedainiu distr., Lithuania; e-mail: roma@lzi.lt, audre@lzi.lt

**Abstract.** Field trials in spring wheat and spring barley were carried out over two years in Dotnuva, in the center of Lithuania. Different fungicidal spray programs were used in 2004–2005 to determine their efficacy in controlling *Fusarium* infection and toxin deoxynivalenol (DON) accumulation in the grain. Azoxystrobin alone, and in a tank mixture with tebuconazole, a commercial mixture of prothioconazole and tebuconazole were used in spring barley. Epoxiconazole commercial mixture with pyraclostrobin and fenpropimorph, and tebuconazole alone were used in spring wheat. Fungicides were used at booting and heading or flowering stages. Naturally contaminated freshly harvested grain was analyzed. The *Fusarium* fungi infection level in grain was very high in both experimental years: in 2004 the level was 38.5–50.0%, and in 2005, 45.0–70.8%. A lower percent of infected grains was found in spring wheat compared with spring barley. During 2004 there was identified 16.8–28.3% infection level; in 2005, 28.3–49.3%. Only in 2005 did fungicide treatments at heading or flowering slightly reduce the *Fusarium* infection. The level of mycotoxin DON detected in the grain samples was generally low and varied from 21 to 168  $\mu\text{g kg}^{-1}$ .

**Key words:** *Fusarium* infected grain, deoxynivalenol, fungicides, barley, wheat

### INTRODUCTION

*Fusarium* infection of cereal grains not only results in a reduction of crop yield, but also in lower grain quality, especially through the production of mycotoxins. *Fusarium* mycotoxins are known to be significant dangers to human and animal health. The types of mycotoxins produced depend on the species and strains of *Fusarium*. Deoxynivalenol (DON) is produced mainly by *Fusarium graminearum* and *F. culmorum* (Bottalico, 1998). Some effective foliar fungicides may be good for the control of *Fusarium* head blight, caused by toxigenic *Fusarium* spp. and non-toxigenic *Microdochium nivale*. Metconazole, prothioconazole and tebuconazole have been reported as the most effective fungicides for controlling *Fusarium* spp. and reducing the level of the main mycotoxins occurring in cereal grain (Kang et al., 2001; Suty-Heinze & Dutzmann, 2004).

This paper reports the results of the effect of fungicides differing in the mode of action and applied at different times on *Fusarium* infection and production of deoxynivalenol in spring barley and wheat.

## MATERIALS AND METHODS

During 2004–2005 at the Lithuanian Institute of Agriculture in Dotnuva, in central Lithuania, the field trials were carried out under natural infection conditions. Fungicides with different modes of action were applied to determine their efficacy in controlling *Fusarium* fungi and toxin deoxynivalenol (DON) accumulation in grain. Azoxystrobin alone and in a tank mixture with tebuconazole, a commercial mixture of prothioconazole and tebuconazole, were used in spring barley. Epoxiconazole commercial mixtures with pyraclostrobin and fenpropimorph, and tebuconazole alone were used in spring wheat. Tebuconazole and prothioconazole gave good control of the toxin-producing *Fusarium* fungi (Suty-Heinze & Dutzmann, 2004), therefore they were included in the trial design. The fungicides were used at booting (at BBCH 39-45 in spring barley and at BBCH 39-43 in spring wheat) as conventional treatment against leaf diseases. The fungicides at heading (at BBCH 55-59 in spring barley) or flowering (at BBCH 65 in spring wheat) were used for *Fusarium* fungi control because the most susceptible and economically important development stage of cereals for *Fusarium* infection is flowering or close to this stage (MCCALLUM & TEKAUZ, 1998; Xu, 2003).

The samples of spring barley and wheat grain for the mycological assays were taken from each plot. Freshly harvested grains were analyzed according to the methods described by ISTA (2003) and Mathur & Kongsdal (2003). The sub-samples of grains were surface-sterilised for 5 minutes in 1% NaOCl solution, then rinsed three times in sterile distilled water and dried before plating. The surface-sterilised grains were plated on Petri dishes with Potato Dextrose Agar (PDA) and incubated at 26±2°C. The infection level of grain was evaluated in percent (0 – all grain healthy, 100% - all grain infected). Microscopic studies of *Fusarium* fungi were carried out after 7–8 days (Nelson et. al, 1983; Mathur & Kongsdal, 2003). Spring barley and wheat grain samples were stored at -18°C prior to examination for mycotoxins. The level of mycotoxin DON in grain was determined by the ELISA method. Neogen diagnostic tests were used for the estimation of the mycotoxin. Multiskan MS was used for the reading of immunoenzymic micro strips. Treatment means were separated by least significant difference (LSD) at the 0.05 probability level (Clewer & Scarisbrinck, 2001).

## RESULTS AND DISCUSSION

The *Fusarium* infection level and DON content for spring barley and spring wheat grown in fungicide-treated and untreated plots are shown in Tables 1 and 2. The infection level of *Fusarium* fungi in the grain of both cereals was high. Barley grain infection was higher compared with wheat during both experimental years. It is known that the risk of infection in barley is higher during optimum weather conditions, because *Fusarium* infection of barley can take place over a two-week span following heading (MCCALLUM & TEKAUZ, 1998), while wheat plants are most susceptible to the *Fusarium* fungi during flowering, which is a relatively short phase of the growth (Xu, 2003). The lowest *Fusarium* infection level was detected in barley and wheat grain grown without fungicides during 2004, however, significant differences between untreated and those treated with fungicides using a different mode of action at different times were not established. The *Fusarium* infection level in the grain of both

cereals during 2005 was higher compared with that in 2004. The commercial mixture of prothioconazole and tebuconazole reduced the *Fusarium* infection of spring barley grain, while azoxystrobin alone or in tank mixture with tebuconazole did not exert any effect on the *Fusarium* infection. Simpson et al. (2001) reported that tebuconazole selectively controlled *F. culmorum* and *F. avenaceum* and reduced levels of DON, but showed little control of *M. nivale*. Suty-Heinze & Dutzmann (2004) reported that prothioconazole has a high activity potential against both *F. graminearum* and *M. nivale*. Application of azoxystrobin, however, selectively controlled *M. nivale* and allowed greater colonization by toxigenic *Fusarium* species (Simpson et al., 2001). In 2005, application times of these fungicides did not show significant differences in the infection level of spring barley grain by *Fusarium*. By contrast, in 2005, the *Fusarium* infection in spring wheat grain was lower after conventional sprays at booting (BBCH 39-43) and tebuconazole application at flowering, compared with untreated and conventional treatments with fungicides only at BBCH 39-43. Significant reduction of *Fusarium* infection in spring wheat grain was established after application of epoxiconazole+pyraclostrobin at BBCH 39-43 and tebuconazole at BBCH 65 compared with untreated and conventional spraying with epoxiconazole+pyraclostrobin at BBCH 39-43.

**Table 1.** The effect of fungicides differing in the mode of action applied at different growth stages on *Fusarium* infection level and DON content in spring barley grain.

Fungicide	Rate of active ingredients g ha <sup>-1</sup>	Fungicide application time (BBCH)	<i>Fusarium</i> infected grain (%)		DON content µg kg <sup>-1</sup>	
			2004	2005	2004	2005
Untreated	-	-	38.5ab	61.8abc	57	138
Prothioconazole + tebuconazole	125+125	55-59	38.5ab	45.0a	-	-
Azoxystrobin	200	55-59	50.0b	60.8abc	-	-
Azoxystrobin + tebuconazole	100 +125	55-59	47.3ab	65.8bc	68	108
Prothioconazole + tebuconazole	125+125	39-45	39.8ab	46.5a	-	-
Azoxystrobin	200	39-45	42.8ab	70.8c	63	150

Means by the same letter do not differ significantly ( $P < 0.05$ )

**Table 2.** The effect of fungicides differing in the mode of action applied at different growth stages on *Fusarium* infection level and DON content in spring wheat grain.

Fungicide	Rate of active ingredients g ha <sup>-1</sup>	Fungicide application time (BBCH)	<i>Fusarium</i> infected grain (%)		DON content µg kg <sup>-1</sup>	
			2004	2005	2004	2005
Untreated			16.8a	46.0bc	21	108
Epoxiconazole+fenpropimorph; tebuconazole	63+187.5 250	39-43 65	28.3abc	35.8abc	-	-
Epoxiconazole+pyraclostrobin; tebuconazole	37.5+99.8 250	39-43 65	23.3a	28.3a	168	122
Epoxiconazole+fenpropimorph	63 +187.5	39-43	26.0abc	44.3abc	-	-
Epoxiconazole+pyraclostrobin	37.5+99.8	39-43	24.0abc	49.3c	166	141

Means by the same letter do not differ significantly ( $P < 0.05$ )

The DON content in freshly harvested grain of spring barley and wheat was very low in both experimental years. According to EU legislation, the maximum level of DON in unprocessed cereals is 1250 µg kg<sup>-1</sup> (EC) No 856/2005). In spring barley grain the differences in DON content level between untreated and treated with fungicides were not detected. Untreated spring wheat grain was less contaminated with DON than that treated with fungicides. Higher differences of DON content between the wheat grain from fungicide-sprayed plots and untreated were established in 2004.

## CONCLUSIONS

The *Fusarium* infection level in spring barley and wheat grain was high during 2004-2005. The treatments performed at heading or flowering slightly reduced *Fusarium* infection only in 2005. The level of *Fusarium* infection was influenced by the crop and year also. *Fusarium* toxin DON was found in freshly harvested barley and wheat grain at very low levels in both experimental years.

ACKNOWLEDGEMENTS. The study was supported by the Lithuanian State Science and Studies Foundation. Program: “Regularities of mycotoxins accumulation in food and development of preventive safety means system”. Registration No. C-03003.

## REFERENCES

- Bottalico, A. 1998. *Fusarium* diseases of cereals: Species complex and related mycotoxin profiles in Europe. *Journal of Plant Pathology* **80**, 85–103.
- Clewer, A. G. & Scarisbrick, D. H. 2001. *Practical statistics and experimental design for plant and crop science*. John Wiley and Sons, LTD, Chichester, 332 pp.
- International Rules for Seed Testing (ISTA). 2003. *Seed Health Testing Methods*. International Seed Testing Association, Bassersdorf, Switzerland.
- Kang, Z., Huang, L., Buchenauer, H. 2001. Ultrastructural and cytochemical studies of effects of the fungicide metconazole on *Fusarium culmorum* in vitro. *Journal of Plant Diseases and Protection* **108**, 419–432.
- Mathur, S. B. & Kongsdal, O. 2003. *Common laboratory seed health testing methods for detecting fungi*. ISTA, Copenhagen, Denmark, 425 pp.
- McCallum, B. D., Tekauz, A. 1998. Inoculation methods for *Fusarium* head blight in barley. *Canadian Journal Plant Pathology* **20**, 125–126.
- Nelson, P. E., Tousson, T. A. & Marasas, W. F. O. 1983. *Fusarium Species: an Illustrated Manual for Identification*. The Pennsylvania State University Press, Pennsylvania, 193 pp.
- 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. *European Journal of Plant Pathology* **107**, 421–431.
- Suty-Heinze, A. & Dutzmann, S. 2004. *Fusarium* head blight: an additional strength of Prothioconazole. *Pflanzenschutz-Nachrichten Bayer* **57**, 265–282.
- XU, X. 2003. Effects of environmental conditions on the development of *Fusarium* ear blight. *European Journal of Plant Pathology* **109**, 683–689.