

# ***In vitro* sensitivity of *Fusarium graminearum*, *F. avenaceum* and *F. verticillioides* to carbendazim, tebuconazole, flutriafol, metconazole and prochloraz**

Dario Ivić<sup>1</sup>, Zdravka Sever<sup>2</sup> and Biljana Kuzmanovska<sup>3</sup>

<sup>1</sup>*Institute for Plant Protection, Croatian Centre for Agriculture, Food and Rural Affairs, Svetosimunska cesta 25, 10000 Zagreb, Croatia (dario.ivic@hpcphs.hr)*

<sup>2</sup>*University of Zagreb, Faculty of Agriculture, Svetosimunska cesta 25, 10000 Zagreb, Croatia*

<sup>3</sup>*Ss. Cyril and Methodius University in Skopje, Faculty of Agricultural Sciences and Food, Aleksandar Makedonski bb, 1000 Skopje, Republic of Macedonia*

*Received: April 1, 2011  
Accepted: April 20, 2011*

## **SUMMARY**

Growth of 13 *F. graminearum* isolates, 6 *F. avenaceum* isolates and 6 *F. verticillioides* isolates was analysed on potato-dextrose agar amended with 0.1, 0.33, 1, 3.3 and 10 mg l<sup>-1</sup> of carbendazim, tebuconazole, flutriafol, metconazole, and prochloraz. Average concentration which reduced mycelial growth by 50% comparing it to control (EC<sub>50</sub>) was calculated for each isolate. Among fungicides tested, prochloraz was shown to be the most effective in growth inhibition of all three species, while flutriafol was proven to be the least effective. Metconazole was more efficient in comparison with carbendazim and tebuconazole. EC<sub>50</sub> values of all isolates on prochloraz were lower than 0.1 mg l<sup>-1</sup>, while on flutriafol they ranged between 1.66 and 8.51 mg l<sup>-1</sup> for 18 isolates, or were higher than 10 mg l<sup>-1</sup> for 7 isolates. EC<sub>50</sub> values on carbendazim were 0.39-1.41 mg l<sup>-1</sup> for *F. graminearum* isolates, 0.91-1.35 mg l<sup>-1</sup> for *F. avenaceum*, and 0.47-0.6 mg l<sup>-1</sup> for *F. verticillioides*. EC<sub>50</sub> values on tebuconazole were 0.85-2.57 mg l<sup>-1</sup> for *F. graminearum*, 0.85-1.58 mg l<sup>-1</sup> for *F. avenaceum* and 0.22-0.85 mg l<sup>-1</sup> for *F. verticillioides*, while on metconazole EC<sub>50</sub> values ranged between less than 0.1 mg l<sup>-1</sup> to 1.66, 0.56, and 0.17 mg l<sup>-1</sup> for *F. graminearum*, *F. avenaceum* and *F. verticillioides*, respectively. Average growth inhibitions of different *Fusarium* species and all *Fusarium* isolates together on different concentrations of fungicides tested were significantly different. Significant differences in growth were not determined among isolates of the same species on neither one of fungicides tested, indicating that no decreased sensitivity to the fungicides exists among isolates included in the study.

**Keywords:** *Fusarium*; Carbendazim; Tebuconazole; Flutriafol; Metconazole; Prochloraz

## INTRODUCTION

Fusarium head blight (FHB) is one of the most important diseases of wheat worldwide (McMullen et al., 1997). FHB epidemics are common in Croatia, as most of the wheat cultivars grown are susceptible to FHB, and wheat is frequently followed by maize in crop rotation. Continental climate with frequent spring rains further contribute to FHB epidemics, which are particularly severe in certain years. In such conditions, the use of fungicides is still one of the main strategies in FHB management in Croatia. The use of fungicides in control of FHB has become more intensively investigated during the last two decades, since studies have shown that chemical control of *Fusarium* diseases of wheat can contribute to the lower contamination of grain with mycotoxins, especially deoxynivalenol and zearalenone (Ellner, 1997; Mesterházy and Bartók, 1997; Matthies and Buchenauer, 2000; Pirgozliev et al., 2002; Menniti et al., 2003).

From the 70s of the 20th century, carbendazim was commonly used in management of FHB. This benzimidazole fungicide was proven to be effective in laboratory studies and in practice, and it remained as one of the standards in control of wheat diseases caused by *Fusarium* (Delp, 1987). However, during the 80ies, demethylation inhibitors (DMI fungicides) have become the most commonly used fungicides in agriculture. DMI fungicides were shown to be extremely broad-spectrum chemicals, similar like benzimidazoles, and were proven to be effective in FHB control. Today, DMI fungicides still represent the largest group of fungicidal compounds on the market. In products registered to be used on cereals today, they are often combined with strobilurins. Beside the use of carbendazim, tebuconazole, flutriafol, prochloraz and metconazole as foliar sprays on wheat and barley, all of these fungicides are also used in seed treatments, for control of seed-borne *Fusarium* and Fusarium seedling blight.

According to several studies, the most common *Fusarium* species on wheat in Croatia are *F. graminearum*, *F. avenaceum* and *F. verticillioides* (Ćosić and Vrandečić, 2003; Ćosić et al., 2004). Among these species, *F. graminearum* is the main causal agent of FHB (McMullen et al., 1997; Lević, 2008). *F. avenaceum* is regarded as a saprotroph on cereals by some authors (Summerell et al., 2003), but several studies confirmed the pathogenicity of this species on wheat (Jenkinson and Parry, 1994; Kang et al., 2005). *F. verticillioides* is an important pathogen of maize, but it does not cause

FHB (Leslie and Summerell, 2006). Besides differing in their pathogenicity on wheat, *F. graminearum*, *F. avenaceum* and *F. verticillioides* are phylogenetically distinct, and have different life cycles and toxigenic profile (Leslie and Summerell, 2006; Lević, 2008).

This laboratory study was conducted to evaluate the sensitivity of *F. graminearum*, *F. avenaceum* and *F. verticillioides* to carbendazim, tebuconazole, flutriafol, metconazole, and prochloraz, by mycelial growth inhibition method (Wong and Wilcox, 2002; Wong and Midland, 2007).

## MATERIAL AND METHODS

### Isolation and identification of fungal strains

*Fusarium* strains used in the study were isolated from wheat grain collected in 2006 from the fields where epidemics of FHB were recorded. Grain was not surface-sterilised and was incubated on moist blotter for 7 to 10 days on 22°C and 12/12 h photoperiod. Colonies developed on grain were examined with stereomicroscope and microscope. Single-spore isolates were obtained from sporulating *Fusarium* colonies using procedure described by Leslie and Summerell (2006). Non-sporulating colonies resembling *F. graminearum* were transferred to water agar (WA), from which isolates were obtained using the hyphal tip method (Leslie and Summerell, 2006).

A concept described by Summerell et al. (2003) was used in identification of the isolates. *F. avenaceum* was identified according to the morphology on potato-dextrose agar (PDA) and carnation leaf agar (CLA), using the descriptions of Lević (2008) and Leslie and Summerell (2006). Six *F. avenaceum* isolates (F85A, FC6, FC7, F32, F35 and FJA) were used in this study. *F. graminearum* was also identified according to the morphology on PDA and CLA, but several isolates did not produce perithecia on carnation leaves. To avoid the misidentification with *Fusarium pseudograminearum*, such isolates were grown on carrot agar (CA) in conditions described by Leslie and Summerell (2006). Isolates producing perithecia on CA were determined as *F. graminearum*, and 13 isolates (FA5, FA6, FA10, FA11, FA12, F50A, F54A, F29B, F44B, FP5, F16, F27 and F4III) were used in this study. Eight isolates identified as *F. verticillioides* according to the morphology on PDA and CLA, were further analysed by PCR using primer pairs VER1/VER2, as

described by Mulè et al. (2004). Briefly, DNA from isolates was extracted using DNeasy Plant Mini Kit® (Qiagen Inc., USA), and approximately 4 ng of fungal DNA was used in 50 µl reaction mixtures. The content of chemicals in reaction mixtures, PCR conditions and electrophoresis were the same as described by Mulè et al. (2004). Seven of the eight isolates were confirmed by PCR as *F. verticillioides*, and six of them (FA21, F52A, F25, F6III, F8III and SRPII/6) were used in this study.

### Fungicides used, media preparation and inoculation

Active ingredients of fungicides in the study were obtained by using the commercial products Bavistin FL (carbendazim, BASF®, Germany), Impact (flutriafol, Cheminova®, Denmark), Folicur EW 250 (tebuconazole, Bayer CropScience®, Germany), Sportak 45 EC (prochloraz, Bayer CropScience®, Germany), and Caramba (metconazole, BASF®, Germany). Stock solutions of each fungicide were prepared in sterile water, after which aliquots of stock solutions were added to PDA cooled to approximately 50°C. All fungicides were added in PDA in concentrations of 10 mg l<sup>-1</sup>, 3.3 mg l<sup>-1</sup>, 1 mg l<sup>-1</sup>, 0.33 mg l<sup>-1</sup> and 0.1 mg l<sup>-1</sup>.

Prior to inoculation on PDA with fungicides, *Fusarium* isolates were grown on WA for several days. Mycelial discs 10 mm in diameter were cut off from the WA colonies and placed in the centre of PDA amended with fungicides, and control PDA plates without fungicide. Assay was performed in two replicates, in Petri dishes with 10 cm diameter. Plates were incubated at 22°C in darkness, and growth of isolates was measured in mm at the underside of the colonies after 3 and 7 days. For each isolate, mean values from two replicates were used in data analysis.

### Assay on fungal growth and data analysis

Relative inhibition of growth (%) was calculated for each isolate, fungicide and concentration by using the growth data values measured after 7 days on control plates and plates amended with fungicides. Concentration of fungicides which reduced mycelial growth of isolates by 50% (EC<sub>50</sub>) was calculated by regressing relative growth inhibition values (dependent data, y-value on regression plot) against the log<sub>10</sub>-transformed fungicide concentrations (independent data, x-value on regression plot). Linear trendline was generated on regression plots, and

log<sub>10</sub>EC<sub>50</sub> was determined by appointing log<sub>10</sub> interception of a linear trendline corresponding to relative growth inhibition value of 50%. EC<sub>50</sub> values were calculated as an antilog<sub>10</sub> of log<sub>10</sub>EC<sub>50</sub> values.

In order to further compare the effectiveness of fungicides included in the study, relative growth inhibition of *Fusarium* species on each fungicide and concentration was analysed using analysis of variance (ANOVA). Means were separated using Duncan's New Multiple Range Test (P=0.05). ANOVA was also used to determine the eventual significant differences in sensitivity to the fungicides among isolates of the same species. In this analysis, growth inhibition of all isolates of the same species (*F. graminearum*, *F. avenaceum* or *F. verticillioides*) was compared on each fungicide separately, with all concentrations included. Prior to each ANOVA, relative growth inhibition values were transformed using the arcsin transformation. SAS® 9.1 software was used for all data analysis.

## RESULTS

*Fusarium* isolates included in the study showed different reaction to different fungicides (Tables 1 and 2). Prochloraz showed the highest inhibition of growth of three *Fusarium* species investigated, with EC<sub>50</sub> values lower than 0.1 mg l<sup>-1</sup> in all isolates (Table 1). Beside prochloraz, EC<sub>50</sub> values lower than 0.1 mg l<sup>-1</sup> was recorded only on metoconazole, for two isolates of *F. graminearum*, one isolate of *F. avenaceum*, and five out of the six *F. verticillioides* isolates. EC<sub>50</sub> values recorded on carbendazim and tebuconazole were higher than on metoconazole, except in cases of *F. graminearum* FA10 and F44B isolates on tebuconazole and *F. graminearum* FA10, F44B, F4III and FP5 isolates on carbendazim. EC<sub>50</sub> values on carbendazim ranged from 0.39 mg l<sup>-1</sup> to 1.41 mg l<sup>-1</sup>, while on tebuconazole a range between 0.22 mg l<sup>-1</sup> and 2.57 mg l<sup>-1</sup> was recorded. Flutriafol showed the lowest growth inhibition of all *Fusarium* species and isolates tested, with EC<sub>50</sub> values higher than 10 mg l<sup>-1</sup> recorded in seven isolates (four *F. graminearum* and three *F. avenaceum*). For other isolates, EC<sub>50</sub> values on flutriafol were in all cases higher than on other fungicides included in the study, ranging from 1.66 mg l<sup>-1</sup> to 8.51 mg l<sup>-1</sup>. Generally, isolates of *F. verticillioides* were shown to be the most sensitive to fungicides tested. Isolates of *F. avenaceum* were showed to be generally less sensitive to carbendazim, tebuconazole and flutriafol than most of the isolates of *F. graminearum*.

**Table 1.** EC<sub>50</sub> values (mg l<sup>-1</sup>) of *Fusarium graminearum*, *F. avenaceum* and *F. verticillioides* isolates grown on potato-dextrose media amended with carbendazim, tebuconazole, flutriafol, metconazole and prochloraz

Species	Isolate	EC <sub>50</sub> , mg l <sup>-1</sup>				
		Carbendazim	Tebuconazole	Flutriafol	Metconazole	Prochloraz
<i>F. graminearum</i>	FA10	0.47	1.12	3.89	1.12	<0.1
	F44B	1.41	1.35	>10	1.66	<0.1
	FA5	0.60	1.45	>10	0.16	<0.1
	F4III	0.83	2.57	>10	1	<0.1
	F27	0.74	1	4.37	<0.1	<0.1
	FA11	0.41	1.05	8.51	0.35	<0.1
	F29B	0.72	1.62	>10	0.19	<0.1
	F54A	0.91	0.93	5.13	0.16	<0.1
	FA6	0.54	0.95	3.63	0.16	<0.1
	FP5	0.39	0.85	5.37	0.63	<0.1
	F50A	0.52	1.17	10	<0.1	<0.1
	F16	1	0.91	4.17	0.23	<0.1
FA12	0.40	1.70	4.47	0.38	<0.1	
<i>F. avenaceum</i>	FC6	0.91	1.17	5.25	<0.1	<0.1
	F32	1.35	1.05	>10	0.26	<0.1
	F35	1.26	1.23	>10	0.56	<0.1
	FC7	1.35	1.58	>10	0.19	<0.1
	FJAB	1.17	0.95	7.08	0.13	<0.1
	F85A	1.23	0.85	7.08	0.56	<0.1
<i>F. verticillioides</i>	F52A	0.54	0.29	2.40	<0.1	<0.1
	FA21	0.54	0.43	1.82	<0.1	<0.1
	F25	0.48	0.85	2.88	0.17	<0.1
	F8III	0.60	0.22	1.66	<0.1	<0.1
	F6III	0.55	0.60	2.24	<0.1	<0.1
	SRP 6	0.47	0.40	2	<0.1	<0.1

**Table 2.** Growth inhibition (%) of *Fusarium graminearum*, *F. avenaceum* and *F. verticillioides* on different concentrations of carbendazim, tebuconazole, flutriafol, metconazole and prochloraz

Fungicide	Concentration, mg l <sup>-1</sup>				
	0,1	0,33	1	3,3	10
	Inhibition of growth, %				
	<i>F. graminearum</i>				
Carbendazim	0.0 c <sup>1</sup>	21.0 c	83.1 a	99.1 a	100 a
Tebuconazole	0.0 c	23.0 c	50.1 b	71.2 b	87.8 b
Flutriafol	0.0 c	0.0 d	3.5 c	39.0 c	65.6 c
Metconazole	30.3 b	52.8 b	70.4 a	79.3 b	100 a
Prochloraz	70.1 a	79.1 a	88.7 a	98.9 a	100 a
	<i>F. avenaceum</i>				
Carbendazim	0.0 c	0.0 d	31.6 c	92.0 a	100 a
Tebuconazole	0.7 c	35.5 c	46.2 b	73.3 b	84.3 b
Flutriafol	0.0 c	0.0 d	19.5 c	40.8 c	54.5 c
Metconazole	32.0 b	55.8 b	70.6 a	84.5 b	90.3 b
Prochloraz	62.3 a	75.5 a	86.6 a	97.6 a	100 a
	<i>F. verticillioides</i>				
Carbendazim	0.0 c	31.0 b	96.8 a	100 a	100 a
Tebuconazole	11.6 b	44.8 b	83.2 b	91.3 a	97.0 a
Flutriafol	0.0 c	0.8 c	30.6 c	64.5 b	81.5 b
Metconazole	69.6 a	81.0 a	92.3 a	99.3 a	100 a
Prochloraz	80.3 a	89.8 a	96.2 a	99.3 a	100 a
	All species				
Carbendazim	0.0 c	12.1 b	74.9 a	98.4 a	100.0 a
Tebuconazole	2.0 c	34.1 b	60.8 a	79.6 b	90.6 b
Flutriafol	0.0 c	0.1 c	16.5 b	48.4 c	68.2 c
Metconazole	43.9 b	63.9 a	78.8 a	89.8 ab	99.9 a
Prochloraz	71.2 a	81.9 a	91.0 a	98.7 a	98.9 a

<sup>1</sup> - values were arcsin transformed before analysis. Means within column followed by the same letter do not differ significantly (P=0.05)

Comparing the average growth inhibition of all *Fusarium* isolates on different concentrations of fungicides, prochloraz inhibited the growth of isolates for even 71.2%, metconazole for about 44%, while carbendazim, tebuconazole and flutriafol did not inhibited the growth of isolates on 0.1 mg/l concentration (Table 2) Prochloraz and metconazole significantly higher inhibited the growth of isolates on all the other concentrations in comparison with carbendazim, tebuconazole and flutriafol, while inhibition on flutriafol was in all cases significantly lower than inhibition on carbendazim or tebuconazole. Growth inhibition on carbendazim and tebuconazole was not significantly different on concentrations of 0.33 mg l<sup>-1</sup> and 1 mg l<sup>-1</sup>, but the inhibition on carbendazim was significantly higher comparing it to tebuconazole on concentrations of 3.3 mg l<sup>-1</sup> and 10 mg l<sup>-1</sup>. Significant differences in sensitivity to fungicides were also determined

for each *Fusarium* species analysed separately. Although data varied depending on concentration, prochloraz generally showed the highest effectiveness in growth inhibition of all three species, which was in several cases significantly higher compared to other fungicides. In many cases, flutriafol was shown to be significantly less effective than other fungicides included in the study.

There were no significant differences in growth among different isolates of the same *Fusarium* species on neither one of the fungicides tested (data not shown).

## DISCUSSION

In this study, prochloraz was the fungicide which showed the best effect in inhibition of growth of *F. graminearum*, *F. avenaceum* and *F. verticillioides*, while

flutriafol showed relatively poor effect comparing it to other fungicides. The results of this study are somewhat different from the results of similar *in vitro* studies, but also different if compared to the efficacy trials conducted in the field. In experiments of Jones (2005), neither one out of 50 *F. graminearum* isolates did not grow on agar with 10 mg l<sup>-1</sup> of tebuconazole. In this study, even 12 out of 13 *F. graminearum* isolates still grew on 10 mg l<sup>-1</sup> of tebuconazole in media. Matthies et al. (1999) reported over 90% inhibition of *F. graminearum* mycelial mass growth on 1 mg l<sup>-1</sup> of tebuconazole, and about 40% inhibition of mycelial mass growth on 1 mg l<sup>-1</sup> of carbendazim. In this study, the mean growth inhibition of *F. graminearum* isolates on 1 mg l<sup>-1</sup> of tebuconazole was 50%, while it was 83% on the same concentration of carbendazim. However, the results of this study determined for fungicide tebuconazole were relatively similar to results of Müllenborn et al. (2008). In a similar experiment, ED<sub>50</sub> values recorded for different *Fusarium* isolates on tebuconazole were from 0.24 mg l<sup>-1</sup> to 6.5 mg l<sup>-1</sup> (Müllenborn et al., 2008). In this study, ED<sub>50</sub> values on tebuconazole ranged from 0.22 mg l<sup>-1</sup> to 2.57 mg l<sup>-1</sup>.

In Croatia, for the control of FHB in wheat, prochloraz-based fungicides are used at dose rate of 450 g a.i.<sup>-1</sup> per ha, tebuconazole at 125 to 250 g a.i.<sup>-1</sup> per ha, and carbendazim at 125 to 180 g a.i.<sup>-1</sup> per ha. Considering the results of this study, where prochloraz was the most effective in reducing *Fusarium* growth, it might be concluded that prochloraz would be the most efficient in the field, while tebuconazole and carbendazim would be more or less of equal effectiveness. However, several field trials recorded higher efficacy of tebuconazole compared to carbendazim and prochloraz. In the study of Ellner (1997), tebuconazole has shown to be more effective than prochloraz in control of FHB caused by *Fusarium culmorum* in field conditions. Similar results were recorded in other field study from Germany, where tebuconazole was also proven to be more effective than prochloraz (Matthies and Buchenauer, 2000). Tebuconazole reduced FHB severity by 56% and 43%, depending on the time of application, whereas prochloraz reduced disease severity for 41% and 22% (Matthies and Buchenauer, 2000). In the study of Siranidou and Buchenauer (2001), tebuconazole and metconazole were effective in control of FHB, while prochloraz and benzimidazole benomyl were not. In trials of Cromey et al. (2001), tebuconazole reduced FHB for 41%, while carbendazim reduced FHB for only 29% comparing it to control. Tebuconazole showed the best efficacy on FHB in trials of Mesterhazy and Bartok

(1997), where a percentage of *Fusarium*-infected seed on variants treated with tebuconazole was 12%, while it reached even 42% on variants treated with carbendazim. Tebuconazole was also more effective than prochloraz in trials conducted in Croatia (Ivic et al., 2009). In trials conducted on four wheat cultivars in Italia, tebuconazole was more effective in control of FHB on two cultivars, while prochloraz showed better efficacy in other two cultivars (Menniti et al., 2003).

Cultivar response, temperature, persistence of fungicides on plant organs, sensitivity of fungal spores to fungicides, curative effects, or dynamics and extent of translocation of different systemic fungicidal compounds are only some of the features which condition the performance and efficacy of fungicides in field conditions (Jones, 2000; Simpson et al., 2001; Pirgozliev et al., 2002). Such characteristics can be especially important in control of a certain plant disease, and especially of FHB. This is why the effect of a fungicide *in vitro* may not reflect the efficacy of a product in practical conditions. Beside this, it must be mentioned that the results of many field efficacy trials remain unpublished, and that available data from several recent studies published in journals cannot give a comprehensive picture of a fungicide performance on a certain plant disease.

The response of different *Fusarium* species to the fungicides tested in this study varied, which was expected. Reaction of a fungal strain or an isolate to the certain fungicidal compound is a phenotypic characteristic which is always variable in populations of plant pathogenic fungi, and this is proven in numerous other laboratory studies. In already mentioned study of Müllenborn et al. (2008), different ED<sub>50</sub> values were recorded for seven different *Fusarium* species grown on media with prothioconazole, tebuconazole, azoxystrobin and fluoxastrobin. Differences in reaction of different *Fusarium* species to the fungicides *in vitro* was also recorded in the study of Allen et al. (2004), where *Fusarium solani* was inhibited by 60% on difenconazole amended media, while *Fusarium circinatum*, *F. oxysporum* and *F. proliferatum* were inhibited by 90%. Indirect evidence for different sensitivity of different *Fusarium* species to a certain fungicide are shown in studies of Pirgozliev et al. (2002) and Simpson et al. (2001). FHB severity on variants artificially inoculated with *F. culmorum* and treated with metconazole was higher than on variants inoculated with *F. graminearum* and treated with the same fungicide (Pirgozliev et al., 2002). Tebuconazole significantly

reduced the amount of *F. graminearum* and *F. culmorum*, but not of *F. avenaceum*, in conditions of natural and artificial infections of wheat heads (Simpson et al., 2001).

In this study, no differences between isolates of the same species were recorded, which means that no decreased sensitivity or resistance was noted among isolates tested. Considering the common agricultural practice of wheat cultivation in Croatia and in Europe in general, it can be concluded that the risk of resistance of *Fusarium* species causing FHB is very low. In Croatia, fungicides are applied on wheat one to three times during the vegetation, products with two different fungicidal compounds are commonly used today, and wheat is almost never cultivated in monoculture.

The results of this study have shown significant differences in sensitivity of *Fusarium* species to carbendazim and four DMI fungicides commonly used in management of FHB. Beside this, differences in reaction to the fungicides were recorded among three distinct and economically important *Fusarium* species, *F. graminearum*, *F. avenaceum* and *F. verticillioides*. This study can be regarded as a supplement to the fungicide efficacy trials conducted in the field. Beside this, data from this study can be useful in defining the strategy for integrated management of FHB, where chemical control is still one of the basic measures implemented in most of the wheat-growing areas in the world.

## REFERENCES

- Allen, T.W., Enebak, S.A. and Carey, W.A.*: Evaluation of fungicides for control of species of *Fusarium* on longleaf pine seed. *Crop Protection*, 23: 979-982, 2004.
- Cromey, M.G., Lauren, D.R., Parkes, R.A., Sinclair, K.I., Shorter, S.C. and Wallace, A.R.*: Control of *Fusarium* head blight of wheat with fungicides. *Australasian Plant Pathology* 31: 301-308, 2001.
- Delp, C.J.*: Benzimidazole and related fungicides. In: *Modern selective fungicides - properties, applications, mechanisms of action* (Horst Lyr, ed.). Longman Scientific & Technical, Harlow, UK, 1987, pp. 233-244
- Ćosić, J., Vrandečić, K. i Svitlica, B.*: *Fusarium* vrste izolirane s pšenice i kukuruza u istočnoj Hrvatskoj. *Poljoprivreda*, 1: 5-9, 2004.
- Ćosić, J. i Vrandečić, K.*: Fuzarijske bolesti pšenice. *Glasilo biljne zaštite*, 5: 284-288, 2003.
- Ellner, F.M.*: Influence of fungicide treatment on deoxynivalenol content in winter wheat artificially infected with *Fusarium culmorum*. *Cereal Research Communications*, 3/2: 735-737, 1997.
- Ivić, D., Domijan, A.-M., Peraica, M. and Cvjetković, B.*: Fumonisin B<sub>1</sub> and zearalenone contamination of wheat in Croatia and influence of fungicide treatments. *Phytoprotection*, 90: 31-34, 2009.
- Jenkinson, P. and Parry, D.W.*: Isolation of *Fusarium* species from common broad-leaved weeds and pathogenicity to winter wheat. *Mycological Research*, 98: 776-780, 1994.
- Jones, R.K.*: Assessments of *Fusarium* head blight of wheat and barley in response to fungicide treatment. *Plant Disease*, 9: 1021-1031, 2000.
- Kang, Z., Irmgard, Z. and Buchenauer, H.*: Infection of wheat spikes by *Fusarium avenaceum* and alterations of cell wall components in the infected tissue. *European Journal of Plant Pathology*, 11: 19-28, 2005
- Leslie, J.F. and Summerell, B.A.*: *The Fusarium Laboratory Manual*. Blackwell Publishing, Ames, USA, 2006, pp. 388.
- Lević, J.T.*: Vrste roda *Fusarium* u oblasti poljoprivrede, veterinarske i humane medicine. Institut za kukuruz „Zemun Polje“, Beograd, Srbija, 2008, pp. 1-1230.
- Matthies, A. and Buchenauer, H.*: 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 Diseases and Protection*, 107: 33-52, 2000.
- Matthies, A., Walker, F. and Buchenauer, H.*: Interference of selected fungicides, plant growth retardants as well as piperonyl butoxide and 1-aminobenzotriazole in trichothecene production of *Fusarium graminearum* (strain 4528) *in vitro*. *Journal of Plant Diseases and Protection*, 106: 198-212, 1999.
- McMullen, M., Jones, R. and Gallenberg, D.*: Scab of wheat and barley: a re-emerging disease of devastating impact. *Plant Disease*, 81: 1340-1348, 1997.
- Menniti, A.M., Pancaldi, D., Maccaferri, M. and Casalini, L.*: Effect of fungicides on *Fusarium* head blight and deoxynivalenol content in durum wheat grain. *European Journal of Plant Pathology*, 109: 109-115, 2003.
- Mesterházy, A. and Bartók, T.*: Effect of chemical control on FHB and toxin contamination of wheat. *Cereal Research Communications*, 3/2: 781-783, 1997.
- Müllernborn, C., Steiner, U., Ludwig, M. and Oerke, E.-C.*: Effect of fungicides on the complex of *Fusarium* species and saprophytic fungi colonizing wheat kernels. *European Journal of Plant Pathology*, 120: 157-166, 2008.

**Mulè, G., Susca, A., Stea, G. and Moretti, A.:** A species-specific PCR assay based on the calmodulin partial gene for identification of *Fusarium verticillioides*, *F. proliferatum* and *F. subglutinans*. European Journal of Plant Pathology, 110: 495-502, 2004.

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

**Simpson, D.R., Weston, G.E., Turner, J.A., Jennings, P. and Nicholson, P.:** 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, 2001.

**Siranidou, E. and Buchenauer, H.:** Chemical control of fusarium head blight on wheat. Journal of Plant Diseases and Protection, 108: 231-243, 2001.

**Summerell, B.A., Salleh, B. and Leslie, J.F.:** An utilitarian approach to *Fusarium* identification. Plant Disease, 87: 117-128, 2003.

**Wong, F.P. and Midland, S.L.:** Sensitivity distributions of California populations of *Colletotrichum cereale* to the DMI fungicides propiconazole, myclobutanil, tebuconazole, and triadimefon. Plant Disease, 91: 1547-1555, 2007.

**Wong, F.P. and Wilcox, W.F.:** Sensitivity to azoxystrobin among isolates of *Uncinula necator*: Baseline distribution and relationship to myclobutanil sensitivity. Plant Disease, 86: 394-404, 2002.

---

## *In vitro* osetljivost vrsta *Fusarium graminearum*, *F. avenaceum* i *F. verticillioides* na karbendazim, tebukonazol, flutriafol, metkonazol i prohloraz

### REZIME

U istraživanju je ispitan rast 13 izolata *Fusarium graminearum*, 6 izolata *F. avenaceum* i 6 izolata *F. verticillioides* na krompir-dekstroznoj podlozi s dodatkom 0,1, 0,33, 1, 3,3 i 10 mg/l karbendazima, tebukonazola, flutriafola, metkonazola i prohloraza. Za svaki izolat izračunata je srednja efektivna koncentracija ( $EC_{50}$ ), pri kojoj je prosečni rast izolata bio inhibiran za 50% u odnosu na kontrolu. Prohloraz je bio najučinkovitiji u inhibiciji rasta sve tri vrste, dok je flutriafol pokazao najmanju učinkovitost. Metkonazol je pokazao višu učinkovitost u poređenju s karbendazimom i tebukonazolom.  $EC_{50}$  vrednosti svih izolata na prohlorazu bile su manje od 0,1 mg/l, dok su na flutriafolu varirale između 1,66 i 8,51 mg/l za 18 izolata, ili bile veće od 10 mg/l za sedam izolata.  $EC_{50}$  vrednosti na karbendazimu bile su 0,39-1,41 mg/l za izolate *F. graminearum*, 0,91-1,35 mg/l za *F. avenaceum*, te 0,47-0,6 mg/l za *F. verticillioides*. Na tebukonazolu  $EC_{50}$  vrednosti bile su 0,85-2,57 mg/l za *F. graminearum*, 0,85-1,58 mg/l za *F. avenaceum* i 0,22-0,85 mg/l za *F. verticillioides*, dok su na metkonazolu utvrđene  $EC_{50}$  vrednosti između manjih od 0,1 do 1,66, 0,56 i 0,17 mg/l za *F. graminearum*, *F. avenaceum* i *F. verticillioides*. Prosečne inhibicije rasta različitih *Fusarium* vrsta i svih *Fusarium* izolata ukupno na različitim koncentracijama različitih fungicida značajno su se razlikovale. Nisu utvrđene značajne razlike u rastu između izolata unutar pojedinih *Fusarium* vrsta na niti jednom od ispitanih fungicida, što pokazuje da ne postoji smanjena osetljivost na fungicide kod izolata uključenih u istraživanje.

**Ključne reči:** *Fusarium*; karbendazim; tebukonazol; flutriafol; metkonazol; prohloraz