ABSTRACT: In this study, the time of infestation by fungi from genus *Alternaria* spp. and *Fusarium* spp. was investigated in different stages of wheat maturity (milk, waxy, and technological maturity); the effects of different fungicides on the yield, technological properties, and content of mycotoxin DON were studied also.

The results showed that *Alternaria* spp. attacked spike and kernel in flowering and end-flowering stage, as it was already known for *Fusarium* species. Fungicide treatment increases the yield up to 20%, test weight by 3.7%, and thousand-kernel weight up to 19.1%. High content of mycotoxin DON, above tolerable limits, was detected only in the treatment with fungicide Caramba and in untreated control.

KEY WORD: chemical treatment, *Fusarium*, *Alternaria*, technological quality, DON

INTRODUCTION

In difference to the 70’s and 80’s of the last century, when *Fusarium* species dominated on wheat kernels, diseases caused by *Alternaria* spp. are of recent widespread. In 2004, *Alternaria* spp. and some other fungi caused extremely low technological quality of wheat kernel (Balaž, 2010).

Fungi from genus *Fusarium* have high economic significance causing decrease of yield and grain quality. In Serbia, agro-climatic conditions favor development of *Fusarium graminearum* (Schwabe) which is the main causal agent of wheat head blight (Balaž et al., 2006; 2010). According to Bagi (1999) in years with average precipitation level, disease incidence of wheat head blight is about 5%. Beside *F. graminearum*, some other *Fusarium* species as well

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as fungi from genera *Alternaria, Aspergillus, Penicillium* etc., colonize wheat kernel inducing significant losses.

As previously described, fungal contamination causes significant yield decrease, but the losses are even greater because of mycotoxins produced by these fungi. Cereals contaminated by *Fusarium* spp. and other mycotoxin producers, are not only a risk to human and animal health, but also influence reduced technological properties (Prainge et al., 2005; Havlova et al., 2006; Suchowilska et al., 2007, 2010).

Haidukowski et al. (2005) consider that fungicide treatment during or immediately after wheat flowering stage can significantly reduce *Fusarium* head blight infestations and control wheat leaf diseases as well.

The aim of this research was to investigate the time of head infection by *Alternaria* spp. and *Fusarium* spp. and chemical control possibilities against pathogens, as well as their influence on technological wheat quality.

**MATERIAL AND METHODS**

The experiment was conducted in 2010 on the location of Bački Petrovac using wheat variety Renesansa.

During vegetation period standard agrotechnical measures were applied. Chemical treatments were applied in flowering stage using TwenJet 11004 nozzle. In the research were used following fungicides: Osiris 2l/ha (metconazole+epoxiconazole), Prosaro 1 l/ha (tebuconazole+prothioconazole), Champion 1.2 l/ha (epoxiconazole +boscalid), Caramba 1.5 l/ha (metconazole), Bumper 1l/ha (propiconazole), Zamir 1l/ha (prochloraz+tebuconazole). Plots of each treated wheat variety were established randomly in four replications with untreated control. Each parcel was 5 m².

Wheat kernel mycobiota was detereminated by phytopathological isolations in milk stage, waxy, and technological maturity. *Fusarium* head blight severity was evaluated calculating the number of infected spikes/m² during early milk stage of wheat. Immediately after harvest, in purpose of mycotoxin and quality parameters analysis and mycobiota determination, representative seed samples were taken.

Thousand-kernel weight was determined by Perten SK CS 4100 (Kernel Hardness Tester, Perten Instruments, Reno, Nevada, USA). The hectoliter weight was measured by dusting the procedure and equipment Schopper scale. Level of fungal infection was determined by Petri dish test according to Pitt and Hocking (1985). Determination of fungi to the level of genus was performed based on morphology and cultural characteristics.

Protein content was determined according to AOAC approved methods 992.23. Gluten content was determined according to ICC standard No 106/1. Mycotoxin DON was determined from both treated and untreated wheat cultivars by ELISA (enzyme-linked immunosorbent assay) method. Screening method for analysis was done using Neogen Veratox® testing kits with limits of detection 0.25 mg/kg for DON (342 Veratox DON-a 5/5).
All data were processed using Statistica 10 software (StatSoft Inc., Tulsa, Oklahoma). Statistically significant differences between means were detected by Tukey's test after analysis of variance (ANOVA).

RESULTS

During last two decades, in agricultural practice in Serbia, fungicide application in wheat protection has been reduced and focused on good agrotechnical measures and fertilization. Fungicides are used only in the high yield targeted production. Since *F. graminearum* is dominated pathogen, *F. culmorum* appears only in very humid years (Bagić, 1999). Beside *Fusarium* spp., *Alternaria* spp. occurs very frequently nowadays (Balaž et al., 2010).

The results showed that *Alternaria* spp. infected spike and kernel in flowering and end-flowering stage such as fungi from *Fusarium* genera. That explains positive effect of chemical treatments on wheat in flowering stage. Chemical treatments were applied in flowering stage using TwenJet 11004 nozzle that enables better ear coverage with fungicides, which is in correlation with Lehoczyk-Kršjak et al. (2008).

Effects of fungicide treatments had high significance in all stages, i.e. less percentage of infestations by fungi in respect to the control plot. Infection was positively correlated with yield decrease.

On the control plot yield was 6.132 t/ha, that is 19.7% less than yield achieved in the treatment with fungicide Osiris of 7.635 t/ha (Table 1).

Results achieved in this work are in accordance to previous researches, which showed that yield is increased by fungicides treatment quantitatively such as qualitatively (Balaž et al., 2006).

| Tab.1 – Infestation of *Alternaria* spp. and *Fusarium* spp. in different seed development stages of wheat with different fungicide treatments |
|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| **Fungicides**               | **SEED DEVELOPMENT STAGES**                  | **YIELD** |
|                              | **Milk stage**                                     | **Waxy maturity**                                | **Technological maturity**          | **t/ha** |
| **Osiris**                   | 0 a                                                 | 0 a                                               | 0 a                                               | 0.5 a                                  | 0 a                                  | 1.2 a                                  | 7.635 c |
| **Prosaro**                  | 10.1 b                                              | 5.3 b                                             | 0.8 a                                             | 0.3 a                                  | 2 a                                  | 7.582 c                                 |
| **Caramba**                  | 15.3 c                                              | 5.2 b                                             | 2.8 ab                                            | 14.9 b                                 | 4.6 b                                 | 7.254 bc                                 |
| **Champion**                 | 10.2 b                                              | 8.5 bc                                            | 13.4 c                                            | 6.2 b                                  | 12.7 b                                | 7.3 b                                  | 6.972 b |
| **Bumper**                   | 13.8 be                                             | 10.6c                                            | 12.9 c                                            | 11.2 c                                 | 14.1 b                                | 12.6 c                                 | 6.725 b |
| **Zamir**                    | 22 d                                                | 15.8 d                                           | 21.7 d                                            | 17.3 d                                 | 24.1 c                                | 16.6 c                                 | 6.725 b |
| **CONTROL**                  | 36.4 e                                               | 26.7 e                                           | 34.8 e                                            | 25.6 e                                 | 38.4 d                                | 28.7 d                                 | 6.132 a |

Mean values in the same column followed by different letters of the same case are significantly different (*P* < 0.05)
In the case of 1000 kernel weight and hectoliter weight, fungicide treated cultivars had significant higher values compared to untreated control on the level of p<0.05 (Table 2).

Previously described fungi are of great importance due to toxin production, particularly DON produced by *Fusarium* spp., which was examined in many researches focused on resistance of genotypes to *Fusarium* head blight, fungicide efficacy, and mycotoxin accumulation (Simpson et al., 2001, Pirgozliev et al., 2002, Andersen and Thrane, 2006).

Deoxynivalenol is mycotoxin produced by *F. graminearum* and *F. culmorum*. Although it is considered the most frequent contaminant of wheat kernel infested by *Fusarium* head blight, it should not neglect other toxin producers harmful for animal and human health.

According to Simpson et al. (2001) and Haidukowski et al. (2005) fungicides treatments with triazoles, very effectively control *Fusarium* head blight and content of DON and combination of triazoles (tebuconazole + protioconazole) is even more efficient stated by Hauser Hahn et al. (2008).

<table>
<thead>
<tr>
<th>Fungicides</th>
<th>Test weight (kg/hl)</th>
<th>1000 kernel weight /sm</th>
<th>Protein content (%/d.m.)</th>
<th>Gluten content (%)</th>
<th>DON mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zamir</td>
<td>79.7 b</td>
<td>38.9 e</td>
<td>12.13 b</td>
<td>24.12 b</td>
<td>&lt;0.25 a</td>
</tr>
<tr>
<td>Champion</td>
<td>79.5 b</td>
<td>33.4 c</td>
<td>12.42 bc</td>
<td>25.14 bc</td>
<td>&lt;0.25 a</td>
</tr>
<tr>
<td>Osiris</td>
<td>79.7 b</td>
<td>34.7 d</td>
<td>12.23 b</td>
<td>24.50 b</td>
<td>&lt;0.25 a</td>
</tr>
<tr>
<td>Prosaro</td>
<td>79.7 b</td>
<td>34.4 d</td>
<td>14.4 d</td>
<td>30.47 d</td>
<td>&lt;0.25 a</td>
</tr>
<tr>
<td>Pumper</td>
<td>79.7 b</td>
<td>32.7 b</td>
<td>12.06 b</td>
<td>23.92 b</td>
<td>&lt;0.25 a</td>
</tr>
<tr>
<td>Caramba</td>
<td>80.1 b</td>
<td>33.7 c</td>
<td>12.9 c</td>
<td>26.60 c</td>
<td>0.77 b</td>
</tr>
<tr>
<td>CONTROL</td>
<td>77.2 a</td>
<td>31.5 a</td>
<td>11.45 a</td>
<td>22.01 a</td>
<td>1.30 c</td>
</tr>
</tbody>
</table>

Mean values in the same column followed by different letters of the same case are significantly different (\(P<0.05\))

Mycotoxin DON was present above detection limit in two cases wheat sample treated with fungicide Caramba (0.77 mg/kg) and untreated control (1.3 mg/kg). This research shows that chemical treatments in flowering stage can effectively suppress the occurrence of all contaminate products of fungi (Table 2).

Chemical composition of kernel, regarding protein and gluten, also indicates the positive effect of wheat protection. Higher content of protein and gluten in wheat treated with fungicide can be explained by better conditions for the synthesis of protein compared to untreated control. The highest protein content was remarked in the treatment with Prosaro, in amount of 14.4%, which is a highly significant difference compared to untreated control 11.45% (Table 2).
CONCLUSION

Results of this work showed that fungus from genus *Alternaria* genera attacked head in flowering and end-flowering phase respectively.

Fungicides Osiris and Prosaro applied with Twen Jet 11004 nozzle provided efficient protection against the most common wheat diseases.

Efficient protection of wheat from *Alternaria* spp. and *Fusarium graminearum* contribute to high technological kernel quality and high yield.

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AOAC approved methods 992.23


ICC standard No 106/1


