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FUNGAL DISEASES

Colonization of winter wheat grain with *Fusarium* and *Alternaria* species and influence on pest control management

Radivoje Jevtić[1] · V. Župunski[1] · M. Lalošević[1] · S. Tančić Živanov[1]

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Abstract

The interactions among grain-colonizing species on wheat and their effects on incidence and severity of Fusarium head blight are usually neglected in studies. Although saprophytes can predominate over pathogenic species, studies related to the control of saprophytic mycoflora in wheat production are rare. Here we hypothesized that the infection level of *Fusarium* and contamination level of *Alternaria* spp. are significantly influenced by environmental factors and their interactions and investigated the relationship between *Fusarium* and *Alternaria* spp. under field conditions and estimate its effect on conventional wheat production, The most prevalent species associated with wheat grain were *Alternaria* spp. (80% in 2012 and 55% in 2013), and the second was *F. graminearum* (9% in 2012 and 38% in 2013). In general, varieties that are moderately resistant to *Fusarium* infection are less contaminated with *Alternaria* spp. compared to susceptible and moderately susceptible varieties. *F. graminearum* on *Alternaria* spp. had a strong antagonistic effect on moderately susceptible and susceptible varieties with $P < 0.001$ using Spearman's coefficient of correlation. An infection level of *F. graminearum* over 25% showed antagonistic activity against *Alternaria* spp. under field conditions. Using prothioconazole + tebuconazole as a chemical measure to prevent *F. graminearum* infection on susceptible varieties can be related to an increase in the contamination level of *Alternaria* spp., jeopardizing the effectiveness of seed health control measures.

Keywords Wheat · *Fusarium* · *Alternaria* · Interaction · Prothioconazole + tebuconazole

Introduction

The use of certified, healthy seed is one of the most important inputs for the production of plants. Fungal diseases can be a primary limiting factor for plant production, especially regarding seed health. Wheat grains are often contaminated before and after harvest with various fungal species causing yield loss and reducing grain quality. The reduction of grain quality is usually associated with reduced germination capacity and the production of harmful mycotoxins (Bălău et al. 2015). As a result, the European Commission defined maximum admissible levels for mycotoxins in cereals and derived products intended for human consumption (European Commission 2006a) and animal feed (European Commission 2006b). Fungal species belonging to the genera *Fusarium, Aspergillus, Penicillium*, and *Alternaria* are common contaminants of wheat grain capable of producing mycotoxins, but studies assessing the influence of their interactions on the level of wheat grain contamination have so far been rare.

Until now, many studies have been focused on the management of Fusarium head blight, including combined control strategies dependent on weather conditions, the timing of fungicide application, resistance of varieties, tillage practices, and crop rotation (Wegulo et al. 2011). These factors are also considered to be the most important for a reliable prognosis of Fusarium head blight incidence and mycotoxin accumulation, but the effects of other species on the initiation of infection and development of *Fusarium* spp. have usually been neglected (Müller et al. 2012).

In addition, the negative effects of grain colonization by *Alternaria* spp. are reported in many studies, but studies dealing with the control of *Alternaria* spp. are also rare. Perello and Larran (2013) reported that colonization of wheat grain by *Alternaria* spp. at a level of 80% can reduce germination from 20 to 60% depending on the variety tested. However, it was also reported that a contamination level of

✉ Radivoje Jevtić
radivoje.jevtic@ifvcns.ns.ac.rs

[1] Institute of Field and Vegetable Crops, Maksima Gorkog 30, Novi Sad 21000, Serbia

20% can reduce germination by up to 80% (Đisalov 2015). The importance of controlling *Alternaria* spp. cannot be understated, since *Alternaria* mycotoxins are reported to have many harmful effects, such as carcinogenicity, mutagenicity, disruption, or the inhibition of enzyme activity and photophosphorylation (Escrivá et al. 2017).

Little is known about the factors influencing the interaction between *Fusarium* and saprophytic species in field conditions. It is well documented in the literature that high humidity and species-specific mycotoxins accelerate infection and plant colonization by *Fusarium* and *Alternaria* spp. (Bateman 2005; Strandberg 1992), but the impact of competitive/antagonistic interactions between them has generally been neglected. In addition, antagonistic interactions between *Fusarium* and *Alternaria* spp. were primarily reported in experiments conducted in controlled conditions (Liggitt et al. 1997; Musyimi et al. 2012; Müller et al. 2012; Riungu et al. 2007; Saß et al. 2007), but the factors influencing their interaction in natural environments are yet to be resolved.

The infection level of *Fusarium* spp. and the contamination level of other mycobiota species that colonize the grain of winter wheat are significantly influenced by environmental conditions, varietal resistance, and tillage practice (Wegulo et al. 2011). In the present study, we hypothesized that the interaction among these factors can also influence the level of infection/contamination of wheat grain. We thus investigated how the relationship between *Fusarium* and *Alternaria* spp. colonizing wheat grain in the field affects control measures in conventional winter wheat production.

Materials and methods

Field trial

The results of this study originated from the investigation related to the development of the DONcast Europe prediction model, which was supported by Bayer Crop Science, and intended for use by farmers to predict deoxynivalenol (DON) accumulation. The trials were conducted during 2012 and 2013, in which fungicide spray treatment with tebuconazole + prothioconazole (Prosaro) and a nonsprayed check were included. Fungicide efficacy trials were set up at seven localities over Serbia (Kikinda, Kragujevac, Leskovac, Pančevo, Pirot, Požarevac, and Zrenjanin) on four leading Serbian wheat varieties (Simonida, Zvezdana, Pobeda, and Rapsodija) showing different levels of resistance to kernel infection with *Fusarium* spp. Those varieties were released by the Institute of Field and Vegetable Crops, Novi Sad, Serbia. The choice of varieties for this study was based on long-term observations of resistance/susceptibility of kernels to infection by *Fusarium* spp.

Field trials were established using the instructions of the Guidelines for the DONcast Assessment provided by Bayer Crop Science. Briefly, field trials were set up using naturally occurring inoculum on farmer's fields in seven localities. The untreated area per farmer's field was defined as 200–300 m^2. Fungicide was applied in the treated plots at the end of the heading stage until the flowering stage (BBCH: 59–65) at the recommended dose rate of 1.0 l/ha. Standard cultivation practices were applied during the growing season.

Disease assessment

At harvest, 200 wheat heads were randomly collected from nonsprayed check plots and from treated plots, according to the Guidelines for the DONcast Assessment provided by Bayer Crop Science. After shelling, four replicates of 100 seeds were randomly chosen from each plot to identify the fungal species.

The agar plate incubation method, recommended by the International Seed Testing Association (ISTA 2018), was used to examine infection of the crop seeds. The seeds were surface-sterilized (1.0% NaOCl for 1 min) and rinsed twice with sterile distilled water. Water agar amended with streptomycin sulfate was used to isolate the fungi. Preliminary seed analysis was performed 7 days after incubation by microscopic observation of the emerging fungal colonies. In addition, the fungi were grown on PDA and V8 agar (HiMedia, Einhausen, Germany) to analyze morphological characters and identify them using the keys of Barnett and Hunter (1972) and Leslie and Summerell (2006).

The prevalence (infection/contamination frequency [IF]) of *F. graminearum* and *Alternaria* spp. was estimated in relation to the infected/contaminated samples and calculated as IF (%) = (Number of samples in which a species occurred/Total number of samples) × 100. The infection level of *F. graminearum* was estimated as the percentage of *Fusarium*-damaged kernels (FDK): FDK (%) = (Number of kernels in which *F. graminearum* occurred/Total number of kernels) × 100. The contamination level of *Alternaria* spp. was estimated compared to the *Alternaria*-contaminated kernels (ACK) and calculated as follows: ACK (%) = (Number of kernels in which *Alternaria* spp. occurred/Total number of kernels) × 100.

The FDK and ACK were calculated for each year, variety, and locality to examine the influence of abiotic and biotic factors on the level of infection and contamination and determine the relationship between *Fusarium* and *Alternaria* spp. under field conditions. The response of the varieties to kernel infection with *F. graminearum* was determined on the basis of the average disease assessment from all localities in 2013 using the scale reported by Lenc et al. (2015): 0, immune; 1–8%, resistant; 9–11%, moderately resistant;

12–20%, moderately susceptible; 21–50%, susceptible; > 50%, very susceptible.

Statistical methods

The factors influencing the infection and contamination level of *Fusarium* and *Alternaria* spp. on wheat grain were analyzed using a multivariate regression model (stepwise regression), partial least square regression (PLS), and polynomial regression. The year, locality, variety and treatments were considered to be categorical variables, while climatic elements (temperature, relative humidity, and total rainfall), with biotic factors, were introduced as continuous variables. Data were transformed using $\sqrt{x+0.5}$. The correlation between levels of infection/contamination of different fungal species was estimated using Spearman's coefficient of correlation. The entire analysis was performed using Minitab 17 (trial version; Minitab, State College, PA, USA).

Results

Diversity of mycobiota associated with winter wheat grain

At least 11 fungal species were isolated from wheat seeds in 2012 and 12 in 2013. The overall mycobiota associated with winter wheat grain indicated that *Alternaria* spp. were prevalent with frequencies of 80% in 2012 and 55% in 2013. Among the *Fusarium* spp., *F. graminearum* was dominant with frequencies of 9% in 2012 and 38% in 2013. The number of replicates associated with the specified values of infection/contamination levels of *F. graminearum* and *Alternaria* spp. for all varieties and localities from the untreated and treated plots are presented as individual value plots (Fig. 1). The average contamination levels of *Alternaria* spp. in the untreated plots were 36.7% in 2012 and 28.8% in 2013 and ranged from 0 to 100% in both treated and untreated plots (Fig. 1). The average infection levels of *F. graminearum* in the untreated plots were 3.85% in 2012 and 19.9% in 2013 (Fig. 1). A low average infection level of *F. graminearum* of 3.85% in untreated plots made 2012 less suitable for additional study of the relationship between *Fusarium* and *Alternaria* spp.

Factors influencing the infection level of *F. graminearum* and contamination level of *Alternaria* spp.

A stepwise regression model showed a significant influence of the locality, variety, and fungicide treatment on the FDK and ACK ($P < 0.001$; α level of 0.05). The influence of the year was shown to be significant ($P < 0.001$; α level of 0.05) only for the FDK but not ACK ($P = 0.143$). Since α to enter and α to remove the influencing factors in the stepwise regression were set to be 0.15, the influence of the year on ACK was not neglected. A more prominent influence of the year on the infection level of *F. graminearum* than on the contamination level of *Alternaria* spp. was also shown in Fig. 1. In addition, the infection level of *F. graminearum* was significantly associated with a contamination level of *Alternaria* spp. ($P < 0.001$) and vice versa ($P = 0.004$).

To determine the relationship between climatic elements and FDK, which differed significantly in 2012 and 2013 at α level of 0.05, PLS regression was conducted. PLS regression analysis included both years as categorical variables together with the climatic elements of May and June as continuous variables (Fig. 2a). The climatic elements in May and June were reported to be the most important for the initiation of *Fusarium* infections and disease development in the literature (Wegulo et al. 2011). The absolute values of standardized coefficients for all climatic elements in May and June except for total rainfall in June were approximately similar. The total rainfall in June had higher fluctuations in the

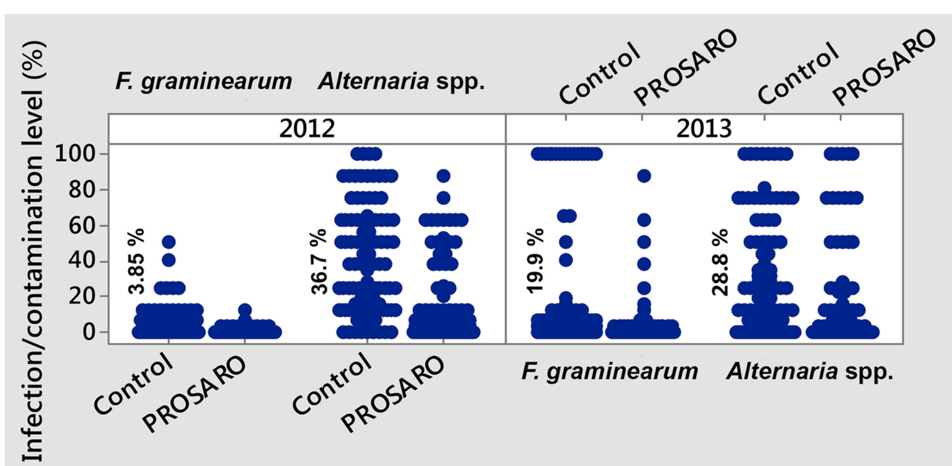

Fig. 1 Individual value plot of infection/contamination level of *Fusarium graminearum* and *Alternaria* spp. in treated and untreated plots in 2012 and 2013. Data points show individual assessments of the infection/contamination levels of *F. graminearum* and *Alternaria* spp. in treated and untreated plots. Values in bold are the means of infection/contamination levels

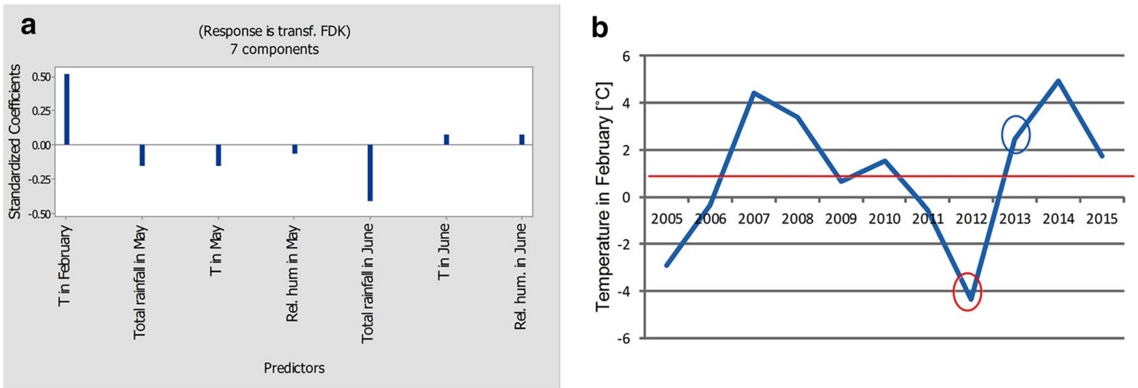

Fig. 2 **a** Standardized coefficients of climatic elements influencing FDK in 2012 and 2013; and **b** 10-year February temperature average in Serbia. *T* average temperature, *Rel. hum*. relative humidity; circles in **b** emphasize *T* in February in the years examined

years examined, but these were still favorable for *Fusarium* infection. Minimal total rainfall of 28.4 mm in May was observed in 2013, while the maximal total rainfall in May of 174.3 mm was observed in 2012. The total rainfall in June also varied from 8.3 to 120.2 mm when considering both years. The influence of winter temperature was also checked using PLS analysis, since hydrometeorological data indicated that the average temperature (T) in February 2012 in the examined localities (−4.37 °C) was below the 10-year average temperature in Serbia (0.99 °C). In 2013, it was above the 10-year temperature average (Fig. 2b). Since the PLS analysis showed that the T in February had the greatest absolute value of the standardized coefficient compared with those of the climatic elements in May and June, those results indicated that winter climatic elements should be factored into the studies concerning *F. graminearum* control management.

To determine the influence of variety on FDK and ACK, we classified the varieties into different categories based on the average FDK from all localities in 2013 using the scale of Lenc et al. (2015). The Pobeda variety was considered to be moderately resistant to *F. graminearum* (average level of infection equals 9%); the Simonida and Zvezdana varieties were moderately susceptible (average level of infection equals 17%), and the Rapsodija variety was susceptible (average level of infection equals 36%). It was evident in both years that the varieties susceptible and moderately susceptible to *F. graminearum* were more prone than the moderately resistant variety to be contaminated with *Alternaria* spp. The average contamination level of *Alternaria* spp. on the moderately resistant variety Pobeda in the untreated plots was 22.4% in 2012 and 15.2% in 2013. The contamination level of *Alternaria* spp. on the susceptible variety of Rapsodija and moderately susceptible varieties of Zvezdana and Simonida in the untreated plots exceeded 30% in 2012 and 19.6% in 2013 (Fig. 3).

In 2013, *Alternaria* spp. predominated over *F. graminearum* in Kikinda, Kragujevac, Pirot, and Zrenjanin,

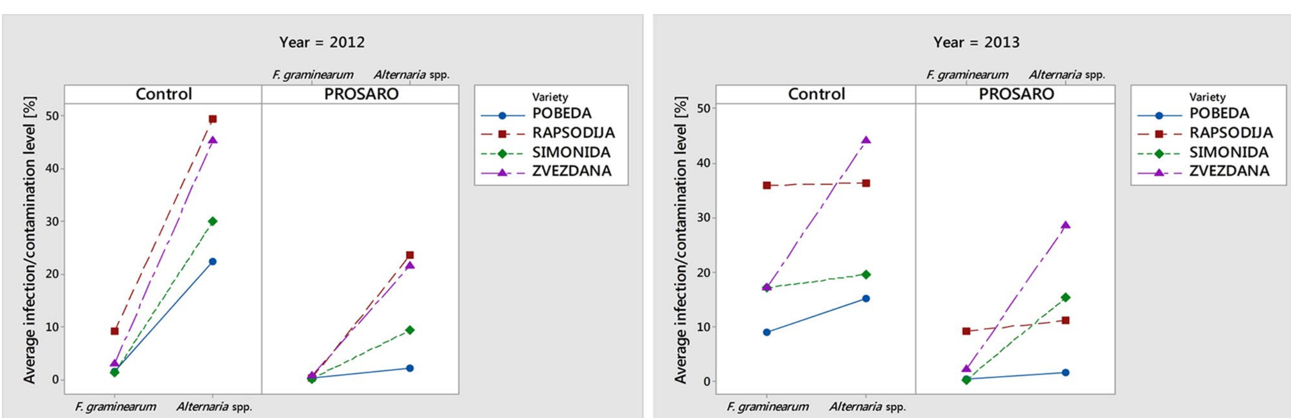

Fig. 3 Average infection/contamination level of *Fusarium graminearum* and *Alternaria* spp. in untreated and treated plots in 2012 and 2013. Variety Rapsodija is susceptible to *F. graminearum*, varieties Simonida and Zvezdana are moderately susceptible, and variety Pobeda is moderately resistant

exceeding the average contamination level of 60% on the varieties susceptible and moderately susceptible to *F. graminearum* (Fig. 4a–d). However, in Pančevo, Požarevac and Leskovac, *F. graminearum* predominated over *Alternaria* spp. on specified varieties in untreated plots (Fig. 5a–c). In Leskovac, the pressure of the pathogen was so high that the average infection level of *F. graminearum* on moderately susceptible varieties reached 100% (Fig. 5c).

Multivariate regression analysis also revealed that the influence of the interactions between individual predictors on *F. graminearum* infection level was significant, with the exception of the year × treatment interaction (data not shown). A lack of significance in the year × treatment interaction indicated stability of the fungicide efficacy in controlling *F. graminearum*. In contrast, the fungicide efficacy in controlling *Alternaria* spp. was not as stable as it was for *F. graminearum*, since the year × treatment interaction was recognized to be an influencing factor with a *P* value (0.105), which was less than alpha to remove the value (0.15). Those results indicated that other factors besides fungicide

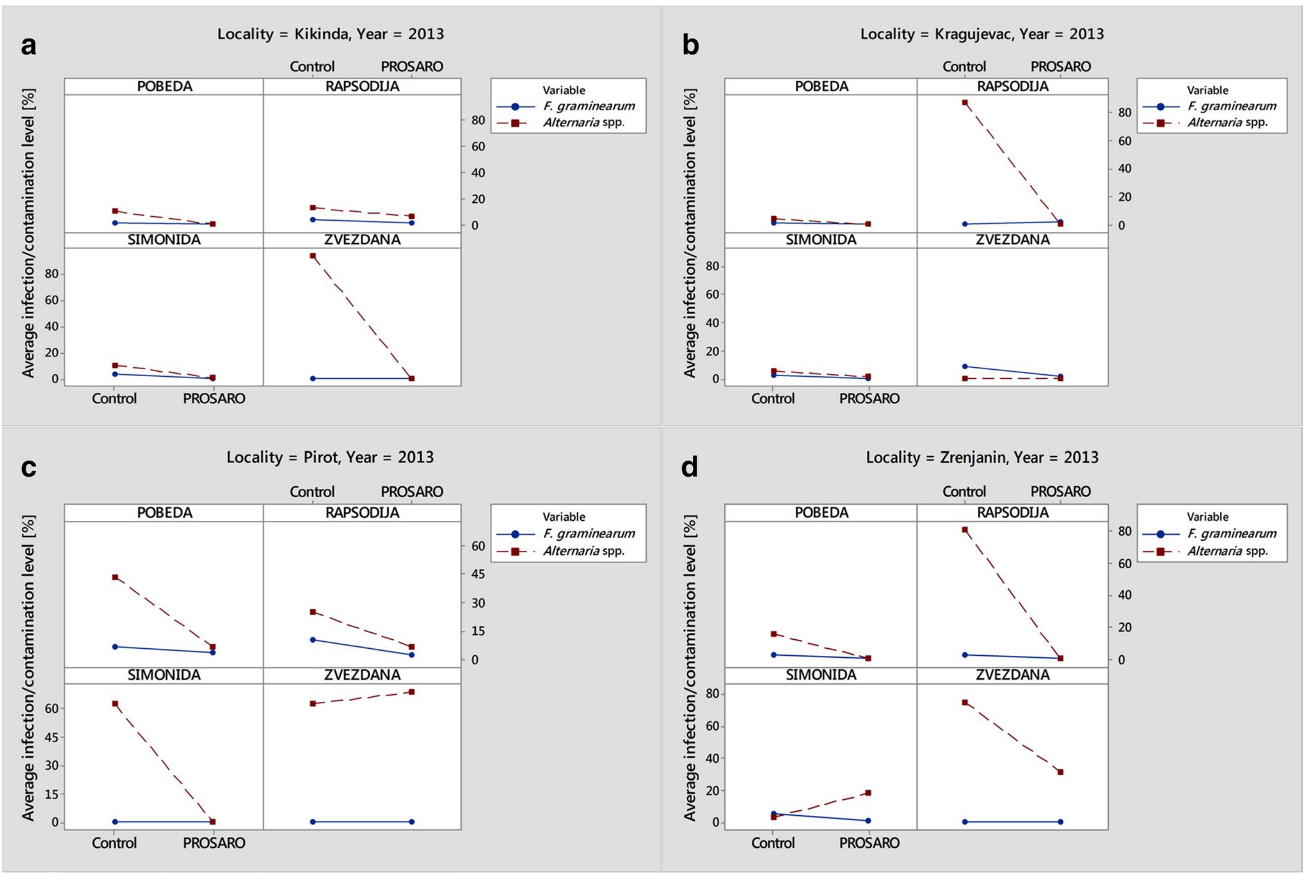

Fig. 4 Interaction between *Fusarium graminearum* and *Alternaria* spp. in fields in localities with *Alternaria* spp. predominant in 2013

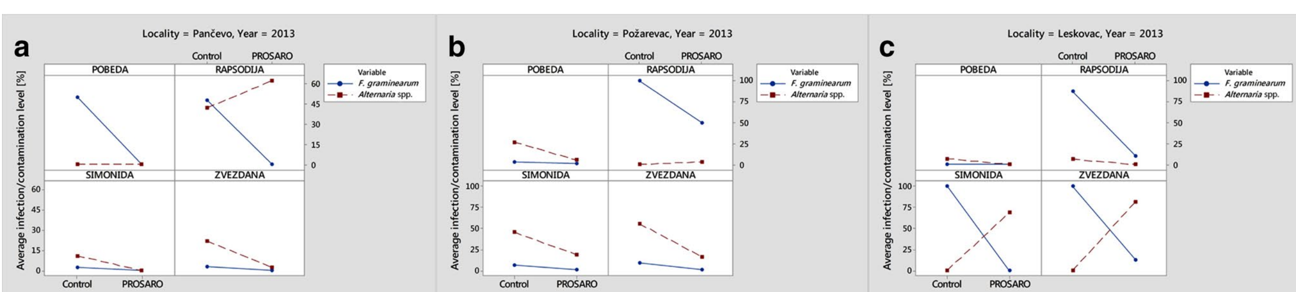

Fig. 5 Interaction between *Fusarium graminearum* and *Alternaria* spp. in fields in localities with *F. graminearum* predominant in 2013

treatment could influence the contamination level of *Alternaria* spp. in the treated plots. Further analysis showed that the interaction between *F. graminearum* and *Alternaria* spp. or escaping the fungicidal effect by *Alternaria* spp can be one of those factors influencing variability in the efficacy of controlling the *Alternaria* spp. contamination of wheat grain.

Interaction between *F. graminearum* and *Alternaria* spp. in the field and effect on pest control management

Since the infection level of *F. graminearum* was significantly associated with the contamination level of *Alternaria* spp. ($P<0.001$) and vice versa ($P=0.004$), the nature of their interaction in the field was analyzed in more detail. The analyses were related to 2013, since the relationship between two species or genera can be studied only if the environmental factors are conductive for both of them. The correlation between the infection level of *F. graminearum* and the contamination level of *Alternaria* spp. was determined using Spearman's correlation coefficient, and a high negative correlation was confirmed on the moderately susceptible variety Zvezdana ($r=-0.694$, $P<0.001$) and the susceptible variety Rapsodija ($r=-0.651$, $P<0.001$). Spearman's correlation coefficient was significantly moderately negative for the moderately susceptible variety Simonida ($r=-0.397$, $P=0.036$). Also, there was no significant correlation for the moderately resistant variety Pobeda ($r=0.335$, $P=0.085$).

Because the level of susceptibility or resistance of the varieties highly influenced the occurrence of *F. graminearum* and *Alternaria* spp., we further analyzed their interactions using data related to the susceptible variety Rapsodija showing a high negative correlation ($P<0.001$) between *F. graminearum* and *Alternaria* spp. In the polynomial regression analysis to investigate the best fit of the data to a regression model, all replicates of infection and contamination levels in 2013 were analyzed. The distribution of data best fit the linear regression model ($P<0.001$) in contrast to the quadratic ($P=0.376$) and cubic regression ($P=0.177$) models, indicating that the high infection levels of *F. graminearum* were related to the low contamination levels of *Alternaria* spp. (Fig. 6a). In addition, *F. graminearum* infection level over 25% (a transformed value greater than 5.5) resulted in suppression of the contamination level of *Alternaria* spp. If not suppressed, the average contamination level of *Alternaria* spp. could exceed 80% on susceptible and moderately susceptible varieties (Figs. 4, 5).

The infection level limit when *F. graminearum* is antagonistic to *Alternaria* spp. was not investigated in detail using the moderately susceptible varieties of Simonida and Zvezdana, since the range of the infection level of *F. graminearum* was narrower in those varieties than in the susceptible variety of Rapsodija. The assessed infection levels of *F. graminearum* in the moderately susceptible varieties did not range between 12.5 and 100%, which made them less suitable for the finite analysis of the relationship between *F. graminearum* and *Alternaria* spp. (Fig. 6b). However, the negative coefficients of correlation and descending regression lines shown in Fig. 6b indicated an antagonistic relationship between *F. graminearum* and *Alternaria* spp. on moderately susceptible varieties. Notably, a high infection level of 100% on the moderately resistant variety Pobeda did not change the magnitude of the slope of the linear regression line. In contrast, high infection levels of 100% on moderately susceptible varieties contributed to the magnitude of the slope of the regression line, making them negative. Those results indicated that the high infection levels in Leskovac should be factored in and not considered exceptional,

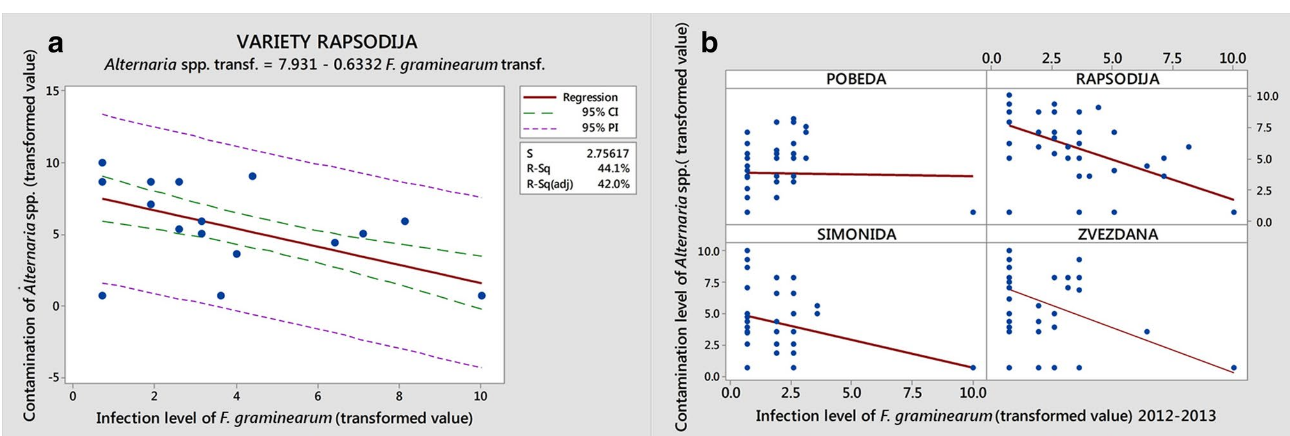

Fig. 6 Linear regression of infection level of *Fusarium graminearum* and contamination level of *Alternaria* spp. **a** Interaction between *F. graminearum* and *Alternaria* spp. on Rapsodija in 2013; and **b** interaction between *F. graminearum* and *Alternaria* spp. in untreated plots in 2012–2013

since they reflect the level of susceptibility of moderately susceptible varieties under high pressure of the pathogen.

In general, a significant influence of the fungicide treatments on FDK and ACK was shown ($P < 0.001$), indicating good potential of prothioconazole + tebuconazole to control *Fusarium* and *Alternaria* spp. (Fig. 3). However, in Leskovac, the reduction of the *F. graminearum* infection level with prothioconazole + tebuconazole on the moderately susceptible varieties Zvezdana and Simonida resulted in an increase in the contamination level with *Alternaria* spp. up to 100% (Fig. 5c). In addition, in Pančevo, the reduction of the infection level with *F. graminearum* resulted in an increase in contamination with *Alternaria* spp. to over 60% in the susceptible variety Rapsodija (Fig. 5a). These results indicated that other factors besides fungicide treatment could influence the contamination level of *Alternaria* spp. in the treated plots. In contrast, the decreased average infection level of *F. graminearum* from 87.5 to 10% on treated plots in Leskovac on the variety Rapsodija did not result in an increase in the average contamination level of *Alternaria* spp. (Fig. 5c). The same was observed on treated plots in Pančevo on the variety Pobeda where a decrease in the average infection level with *F. graminearum* did not result in an increase in the average contamination level with *Alternaria* spp. (Fig. 5a). The efficacy of tebuconazole + prothioconazole in reducing the ACK in the moderately resistant Pobeda variety was more prominent than it was in the moderately susceptible and susceptible varieties (Fig. 3).

Discussion

Grain quality is the major precondition for efficient plant production. Until now, much effort has been used to develop effective control strategies against Fusarium head blight, that causes major yield and quality reductions. However, little is known about the factors influencing the interactions between *Fusarium* and saprophytic species that could also influence the efficacy of fungicide treatments and jeopardize grain quality. In this study, the most prevalent species associated with wheat grain was an *Alternaria* spp.; *F. graminearum* ranked second. The dominance of *F. graminearum* among the *Fusarium* spp. in Serbia was also confirmed by Lević et al. (2012), but they did not analyze factors that influenced the dominance of one species over the other. In the present study, we demonstrated that climatic elements provided preconditions for kernel infection with *F. graminearum*, but the significant impact of the interaction between the year, locality, and variety emphasized the complexity of the crop pathosystem. In addition, there was a significant association between *F. graminearum* and *Alternaria* spp., confirming the initial hypothesis that the level of infection of the pathogenic species and of contamination by the saprophytic species depends on their interactions.

In this study, a strong antagonistic effect of *F. graminearum* on *Alternaria* spp. was determined on susceptible and moderately susceptible varieties using Spearman's coefficient of correlation. Under field conditions, plants infected with pathogens would be expected to be more prone to saprophytic colonization, but in this study, a significantly high negative correlation ($P < 0.001$) between *F. graminearum* and *Alternaria* spp. was identified. Many factors can influence the prevalence of one species over the other. Saß et al. (2007) reported the influence of mycotoxins on competitive interactions. Liggitt et al. (1997) and Müller et al. (2012) reported that in vitro, consecutively inoculated isolates of either *Alternaria* or *Fusarium* strain would show weaker growth compared with the first inoculated strain. Liggitt et al. (1997) also reported that fungicide treatment could promote Fusarium head blight occurrence if saprophytic fungi with antagonistic activity are reduced.

In our experimental conditions, it was not possible to determine whether *F. graminearum* colonized wheat kernels before *Alternaria* spp. or vice versa. However, it was possible to examine whether the level of kernel infection or contamination influenced the prevalence of one species over the other. In this study, when the infection of kernels with *F. graminearum* exceeded the level of 25%, it reduced the contamination level of *Alternaria* spp. Similarly, Kosiak et al. (2004) reported that an infection level of *F. graminearum* of 10% had an effect on *Alternaria* spp. after an analysis of *Alternaria* and *Fusarium* frequencies in Norwegian grains of reduced quality. However, after an examination of Danish malt barley seeds, Andersen et al. (1996) reported that a decrease in *Alternaria* contamination started when the *Fusarium* infection levels reached 60%. This infection level limit for *Fusarium* spp. was much higher than the infection level limits for *F. graminearum* reported in our study or by Kosiak et al. (2004). The difference can be attributed to the lack of *F. graminearum* in the Danish malt barley samples, indicating that *F. graminearum* may have a stronger impact on competitor fungi than other *Fusarium* spp. (Kosiak et al. 2004). Since different strains of the same species can act differently in the presence of the same competitor strains depending on mycotoxin production (Müller et al. 2012), the mechanisms related to the antagonistic relationship between *Fusarium* and *Alternaria* spp. in the field need to be elucidated.

In addition, this study showed that the contamination level of the *Alternaria* species was related to the level of resistance of the host varieties to *F. graminearum*. The average contamination level of the variety Pobeda with *Alternaria* spp. did not exceed 22.4% if both years were considered. Alternatively, the average contamination level of

Alternaria spp. on susceptible and moderately susceptible varieties was broader: 30–49% in 2012 and 19–44% in 2013.

Finally, we determined that fungicide treatment can be related to the increase in the contamination level of *Alternaria* spp. if the infection level of *F. graminearum* with antagonistic activity was reduced and if *Alternaria* spp. managed to avoid any fungicidal effect. The application of prothioconazole + tebuconazole resulted in a decrease in the infection level of *F. graminearum* and an increase in the contamination level of *Alternaria* spp. when applied to the moderately susceptible Simonida and Zvezdana varieties in Leskovac and the susceptible variety Rapsodija in Pančevo. Higher levels of *Alternaria* spp. contamination over 75% in the treated plots in Leskovac and Pončevo are probably due to an avoidance of the fungicidal effects by *Alternaria* spp., but it should be noted that at the same time, the contamination levels for *Alternaria* spp. in the untreated plots were below the contamination level in the treated plots. In Požarevac, the infection level for *F. graminearum* in variety Rapsodija in the plots treated decreased from 100 to 50%, but an infection level of 50% was high enough for *F. graminearum* to have an antagonistic effect on *Alternaria* spp. As a result, it is not clear whether the antagonistic effect of *F. graminearum* or some other environmental factor influenced the low contamination level of *Alternaria* spp. in Požarevac. Finally, the efficacy of prothioconazole + tebuconazole in reducing *Alternaria* spp. was better for moderately resistant varieties than on susceptible varieties.

In general, our results support those of other studies related to the ability of triazoles to inhibit in vitro mycelial growth of saprophytic fungi (Müllenborn et al. 2008; Nopsa 2010), but in the field in the present study, there were exceptions particularly for varieties susceptible to *F. graminearum* infection. The results of our study indicated that the effectiveness of the control measures is complicated and that more attention should be focused on the interaction between *Fusarium* and *Alternaria* spp. to assure sustainable wheat production.

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Compliance with ethical standards

Conflict of interest R. Jevtić, V. Župunski, M. Lalošević, S. Tančić Živanov declare no conflicts of interest.

Research involving human and/or animal participants This article does not contain any studies by any of the authors using human or animal participants.

Informed consent Not applicable.

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