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EFFECTS OF FERTILISERS ON WINTER WHEAT INFECTION CAUSED BY *FUSARIUM* SPECIES

ABSTRACT: Effects of fertilisers on the grain yield (t ha^{-1}) and the development of mycobiota, especially of the *Fusarium* species, in winter wheat kernels were observed. Fertilisers were applied in the following variants: T1 — 30 m^3 of slurry (pre-sowing treatment) and 25 m^3 of slurry (top-dressing), T2 — 22.500 kg of manure (pre-sowing treatment) and 220 kg of urea (top-dressing), T3 — 300 kg of urea (top-dressing) and T4 — the control without application of fertilisers.

The average wheat grain yield was the highest in the treatment T2 (6.9 t ha^{-1}), then in T3 (6.3 t ha^{-1}) and T1 (6.2 t ha^{-1}), while the lowest wheat grain yield (4.3 t ha^{-1}) was registered in the control (T4). Fungi of genera *Alternaria* (88.8—96.3%) and *Fusarium* (3.7—11.1%) were mainly isolated from all wheat kernel samples collected after harvest (T1—T4). Species of the genus *Dreschlera* were isolated in treatments T1 (0.3%) and T2 (0.2%). The species *Stemphylium botryosum* was isolated only in the treatment T1 (0.2%). *F. graminearum* (3.5—10.8%) was isolated from wheat kernels in all observed treatments, while *F. sporotrichioides* was isolated in treatments T1 (0.6%), T2 (0.5%) and T3 (0.3%). *F. poae* (0.3%) and *F. subglutinans* (0.2%) were isolated in the treatment T2, while *F. graminearum* was predominantly present in treatments T3 (10.8%), T1 (8.7%) and T2 (7.8%), and in the control T4 (3.5%). The obtained results point out that the application of urea affected the decrease in the intensity of *Alternaria* species infestation, but it also affected the increase of frequency of *Fusarium* species, especially *F. graminearum*, in comparison with the control and other treatments. Negative correlation was registered between the grain yield and the intensity of infestation of *Fusarium* species (collectively) and *F. graminearum* (individually).

KEY WORDS: N-fertilisers, slurry, manure, urea, wheat, yield, *Fusarium*, incidence

INTRODUCTION

Fusarium head blight (FHB) or scab, caused by *Fusarium graminearum* Schwabe (teleomorph: *Gibberella zeae* (Schw.) Petch) is one of the most devastating diseases of wheat, barley and other cereals worldwide. The disease reduces grain yield through a number of ways including floret sterility, poor

seed filling and reduced seed size (S u b e d i et al., 2007). The infection does not only reduce yield as a result of shrunken kernels, but also reduces milling and malting quality and contaminates kernels with mycotoxins. Mycotoxins are hazardous to animal and human health. Therefore, guidelines and legislation are already in place, or under consideration, in most countries to protect consumers and animal welfare. As fusarium mycotoxins are produced within the growing crop, it is important to understand how cropping practices affect mycotoxin contamination of kernels. The evidence is provided to show the importance of a choice of cultivar, crop rotation, soil cultivation, fertiliser and the chemical and biological control of insects, weeds and fungi.

Due to the development of FHB in Serbia in years favourable for such development, as it was in 2005, the wheat grain yield loss can vary from 1.8% to 38.3%, or it is, on the average, lower by 6.9%, depending on the reaction of wheat varieties to FHB and environmental conditions at the site of observations (L e v i ć et al., 2008).

Cropping practises can also influence susceptibility to the disease. The type and application rates of fertilisers, especially nitrogen (N), can affect the disease incidence and severity in some crops for some pathogens, although it is difficult to make generalisations (R e i d et al., 2001). The nutrients in both inorganic and organic fertilisers are able to cause the incidence and severity of biotic plant diseases, pest and weed populations and their impact on the crop. The majority of information on this aspect deals with the effect of nutrients via plant. Individual elements have different roles; in general, it can be stated that they change the losses caused by pests by influencing plant resistance, alter the plant growth and in this way they change the microclimate in the stand. The increased nutrition used to be prescribed as the first measure to control plant diseases. The most important aspect of this is an increase in the ability of a crop to compensate losses. The effect of individual nutrients is very complex — the severity of one plant disease can be decreased, while that of others can be increased (V e v e r k a et al., 2007).

As it is important to understand the effect of fertilisation on the presence of the casual organism of head blight of wheat, the objective of the present study was not only to observe the effects of fertilisers on the frequency of pathogenic fungi, especially *Fusarium* species in wheat kernels sampled after harvest, but also to record wheat grain yield under the same conditions of fertilising.

MATERIALS AND METHODS

The trial wheat field was set up in 2007 on chernozem soil at the Institute for Animal Husbandry, Belgrade, Serbia. The winter wheat variety Pobeda was sown in a field that had previously been sown with maize. The experiment was set up according to the randomised complete block design with three replications. The fertiliser was applied prior to sowing in autumn 2007 and in top-dressing in spring 2008. The following four treatments were observed:

T1 — 30 m^3 of pig slurry, in the pre-sowing treatment and 25 m^3 of pig slurry in top-dressing;

T2 — 22.500 kg sheep and dairy manure, in the pre-sowing treatment and 220 kg of urea in top-dressing;

T3 — 300 kg of urea in top-dressing;

T4 — control without application of fertilisers.

The wheat grain yield at 10.7% moisture and the incidence of *Fusarium* species in wheat kernels at three fertiliser rates with a different N source, beside the control (without fertiliser) plots, were investigated. A total of 2400 wheat kernels, 600 kernels per a treatment, were observed in regard to the presence of pathogenic fungi species, especially of the genus *Fusarium*. After a superficial disinfection in sodium hypochlorite, wheat kernels were placed on a 2% agar surface, 10 kernels per Petri dish, and incubated for 7—10 days at temperature of 25°C. According to the methods developed by Burgess et al. (1994) and Watanabe et al. (1994), fungi genera were determined with a special focus on the determination of species of the *Fusarium* genus. The wheat grain yield (t ha^{-1}) was determined at harvest.

RESULTS AND DISCUSSION

The highest wheat grain yield (6.9 t ha^{-1}) was recorded when manure and urea were applied in the pre-sowing treatment and top-dressing, respectively, and then when plants were treated with urea in top-dressing (6.3 t ha^{-1}) or when slurry was applied as a split application (pre-sowing treatment and top-dressing) (6.2 t ha^{-1}). The grain yield obtained in the control (without fertilisers) amounted to 4.3 t ha^{-1}, which is statistically significantly lower than the yields obtained in treatments with fertilisers (Table 1).

Tab. 1 — Wheat grain yield (t ha^{-1}) in treatments with different fertilisers (T1—T4)

Treatments[a]	Yield (t ha^{-1})
T1	6.2**
T2	6.9**
T3	6.3**
T4	4.3**
$LSD_{0.05}$	1.165
$LSD_{0.01}$	1.765

[a] T1 = 30 m^3 and 25 m^3 of pig slurry ha^{-1}, pre-sowing treatment and top-dressing, respectively; T2 = 22.500 kg cattle manure and 220 kg urea ha^{-1}, pre-sowing treatment and top-dressing, respectively; T3 = 300 kg urea ha^{-1}, top-dressing; T4 = no fertilisers applied.

The microbiological analysis of kernels collected in the wheat plots treated with different fertilisers showed a high presence of *Alternaria* (88.8—96.3%) and a relative by high presence of *Fusarium* (3.7—11.1%) in all investigated treatments (T1—T4). The presence of *Fusarium* spp. was three-fold higher in all observed treatments than in the control treatment. The low presence of *Dreschelera* spp. in the treatments T1 (0.3%) and T2 (0.2%) and *Stemphylium botryosum* in the treatment T1 (0.2%) were determined (Table 2).

Tab. 2 — Frequency (%) of fungal species in wheat kernels in all investigated treatments (T1—T4)

Fungal species	Treatments[a]			
	T1	T2	T3	T4
Alternaria spp.	90.2	91.0	88.8	96.3
Dreschlera spp.	0.3	0.2	—	—
Fusarium spp.	9.3	8.8	11.1	3.7
Stemphylium botryosum	0.2	—	—	—

[a] T1 = 30 m^3 and 25 m^3 of pig slurry ha^{-1}, pre-sowing treatment and top-dressing, respectively; T2 = 22.500 kg cattle manure and 220 kg urea ha^{-1}, pre-sowing treatment and top-dressing, respectively; T3 = 300 kg urea ha^{-1}, top-dressing; T4 = no fertilisers applied.

The following four species of the genus *Fusarium* were identified: *F. graminearum* Schwabe, *F. sporotrichioides* Sherbakoff, *F. poae* (Peck) Wollenweber and *F. subglutinans* (Wollenweber & Reinking) Nelson, Toussoun & Marasas. Of the four identified *Fusarium* species, *F. graminearum* was the most frequent in all the investigated treatments (3.5—10.8%), followed by *F. sporotrichioides* in the treatments T1 (0.6%), T2 (0.5%), and T3 (0.3%) and *F. poae* (0.3%) and *F. subglutinans* (0.2%) in the treatment T2. *F. graminearum* was mainly present in the treatment T3 (10.8%), followed by T1 (8.7%) and T2 (7.8%) and its lowest presence was detected in the treatment T4 (3.5%) (Table 3).

According to the results obtained by Stanković et al. (2007), mycobiota of fungi of genera *Alternaria* and *Fusarium* are highly frequent in wheat kernels and *F. graminearum* has the highest frequency. According to data of Lemmens et al. (2004) and Heier et al. (2005) nitrogen fertilisation significantly increased the FHB severity and amounts of mycotoxin deoxynivalenol (DON) in winter wheat.

Tab. 3 — Frequency (%) of *Fusarium* species in wheat kernels in all investigated treatments (T1—T4)

Fusarium spp.	Treatments[a]			
	T1	T2	T3	T4
F. graminearum	8.7	7.8	10.8	3.5
F. poae	—	0.3	—	—
F. sporotrichioides	0,6	0.5	0.3	—
F. subglutinans	—	0.2	—	0.2

[a] T1 = 30 m^3 and 25 m^3 of pig slurry ha^{-1}, pre-sowing treatment and top-dressing, respectively; T2 = 22.500 kg cattle manure and 220 kg urea ha^{-1}, pre-sowing treatment and top-dressing, respectively; T3 = 300 kg urea ha^{-1}, top-dressing; T4 = no fertilisers applied.

This study shows that the application of nitrogen fertiliser (urea) also significantly affected the increase of the intensity of incidence of the *Fusarium* species, particularly of *F. graminearum*.

CONCLUSIONS

The application of nitrogen organic (slurry and manure) and mineral fertilisers (urea), individually or in a combination, affected the following:

— the increase of the wheat grain yield in treatments with fertilisers from 2.0 (T3) to 2.6 t ha^{-1} (T2) in relation to the control (T4);

— the intensities of infestation of species of the genus *Fusarium* in treatments with urea (T3), which were higher by 7.4, 2.4 and 1.8% than in treatments T4, T2 and T1, respectively;

— the intensity of infestation of species of the genus *Alternaria* in the treatment T3 (urea), which was lower by 7.5, 2.2 and 1.4% than in treatments T4 (control), T2 (manure + urea) and T1 (slurry, split application), respectively;

— the intensity of infestation of *F. graminearum* in the treatment T3 (urea), which was higher by 7.3, 3.0 and 2.1% u than in treatments T4, T2 and T1, respectively.

Obtained results indicate a negative correlation between the grain yield and the intensity of infestation of species of the genus *Fusarium* (collectively) and *F. graminearum* (individually).

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УТИЦАЈ ЂУБРИВА НА ИНФЕКЦИЈУ ЗРНА ОЗИМЕ ПШЕНИЦЕ *FUSARIUM* ВРСТАМА

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Резиме

У раду је проучаван утицај ђубрива на принос зрна (t ha^{-1}) и развој микобиоте зрна озиме пшенице, посебно врста рода *Fusarium*. Ђубрива су примењена у следећим варијантама: Т1 — 30 m^3 (предсетвено) и 25 m^3 осоке (у прихрањивању), Т2 — 22.500 kg стајњака (предсетвено) и 220 kg уреe (у прихрањивању), Т3 — 300 kg уреe (у прихрањивању) и Т4 — контрола без примене ђубрива.

Примена азотних органских (осока и стајњак) и минералних ђубрива (уреа), појединачно или у комбинацији, утицала је на:

— повећање приноса зрна пшенице у третманима с ђубривима од 2.0 (Т3) до 2.6 t ha^{-1} (Т2) у односу на контролу (Т4);

— већи интензитет напада врсте рода *Fusarium* у третману са уреом (Т3) за 7.4, 2.4 и 1.8% у поређењу с третманима Т4, Т2 и Т1;

— мањи интензитет напада врсте рода *Alternaria* у Т3 третману (уреа) за 7.5, 2.2 и 1.4% у односу на третмане Т4 (контрола), Т2 (стајњак + уреа) и Т1 (осока, двократна примена);

— већи интензитет напада *F. graminearum* у третману са уреом (Т3) за 7.3, 3.0 и 2.1% у поређењу с третманима Т4, Т2 и Т1.

Добијени резултати указују на негативну корелацију између приноса зрна и интензитета напада врста рода *Fusarium* (збирно) и *F. graminearum* (појединачно).