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THE IMPACT OF DIFFERENT CONSERVATION SOIL TILLAGE AND NITROGEN FERTILIZATION ON WHEAT GRAIN INFECTION WITH *Fusarium* sp.

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SUMMARY

The effect of different conservation soil tillage (CST) treatments and different level of nitrogen fertilization on **Fusarium** ***wheat grain infection on two locations are presented in this paper. The research was conducted on winter wheat with different soil tillage treatments: conventional tillage to 30 cm depth, subsoiling to 35-40 cm depth, chiselling up to 25 cm, disk-harrowing to 10-15 cm and no-tillage, without any tillage treatments and three different nitrogen rates (N1 - amount reduced by 30% of recommended; N2 - according to standard recommendation and N3 - amount increased by 30% of recommended). The occurrence of*** **Fusarium** ***species was determined in all the tested variants of tillage and fertilization treatments. The effect of reduced fertilization (N1) on*** **Fusarium** ***sp. grain infection in all tillage treatments was statistically lower in comparison with other (N2 and N3) nitrogen treatments. The lowest percentage of wheat grains infected with*** **Fusarium** ***sp. was recorded in conventional tillage on location Magadenovac and no tillage treatments on location Cacinci.***

Key words: conservation soil tillage, nitrogen fertilization, wheat grain infection, Fusarium sp.

INTRODUCTION

Conservation soil tillage (CST) is one of the most prospect and prominent soil tillage technology which can prevail many negative effects arising from application of conventional crop production and especially conventional soil tillage technology (Jug et al., 2017) in different agroecological conditions and in expecting climate change conditions (Jug et al., 2018). Except positive effect of CST, and because of specific conditions of that technology (especially crop residue management), some negative events can appear in production of wheat causing diversification in wheat yields and yield components (Jug et al., 2011). Fusarium head blight (FHB) caused by the fungal plant pathogens from genus *Fusarium* is destructive disease of wheat in most wheat-growing regions around the world and in all soil tillage systems for wheat cropping. The disease causes yield loss, low test weights, low seed germination and contamination of grain with mycotoxins. Several species are capable of causing FHB. In Croatia the species most commonly encountered is *Fusarium graminearum*.

Agricultural practices are very important factors for the control of *Fusarium* survival (Leplat et al., 2013) since pathogen survive on plant residues left from the previous crop (Blandino et al., 2010) and weeds (Poštić et al., 2012). An appropriate crop rotation and tillage system can limit the risk of FHB development (Fernandez et al., 2008). Burying infested crop residues deeper in the soil is commonly used for *Fusarium* inoculum reduction. Many studies have evaluated the effects of nitrogen application rates on FHB (Pageau et al., 2008; Yoshida et al., 2008; Burgt et al., 2011). The research presented in this paper was set up to study the effect of different CST treatments and different level of nitrogen fertilization on *Fusarium* wheat grain infection on two locations.

MATERIAL AND METHODS

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Field experiment

Stationary field experiments were established at two experimental stations (two different soil types, Table 1) in eastern Croatia in 2009. An experiment on Stagnosols soil type was established at location Cacinci (Long. 17.86336 E, Lat.45.61316 N) and on Gleysols at location Magadenovac (Long. 18.70648 E, Lat.45.55555 N). Data for winter wheat (cv. Lucija), presented in this paper were collected in the years 2013-2014. The winter wheat was grown in five-year crop rotation with pre-crop maize. The research was conducted with five different soil tillage treatments (main factor, TT-tillage treatment), the size of each basic soil tillage plot was 600 m^2. Three different nitrogen rates (sub-factor N) was used, with basic nitrogen fertilization plot size of 195 m^2. The experiment was set up on RCBD design in three repetitions. The following five TT were applied: Conventional tillage (CT) based on autumn mouldboard ploughing to 30 cm depth and four CST treatment with different crop residue cover on soil surface (Table 2); Subsoiling (SS) to 35-40 cm depth, Chiselling (CH) up to 25 cm, Disk-harrowing (DH) to 10-15 cm and No-tillage (NT) without any tillage treatments. The crop residues cover was measured using Line-transect method (Morrison et al., 1993). Nitrogen fertilization treatment had three levels of applied nitrogen: N1 - amount reduced by 30% of recommended (80 kg ha^{-1} N + 110 kg ha^{-1} K_2O + 100 kg ha^{-1} P_2O_5), N2 - according to recommendation (115 kg ha^{-1} N + 110 kg ha^{-1} K_2O + 100 kg ha^{-1} P_2O_5), N3 - amount increased by 30% of recommended (150 kg ha^{-1} N + 110 kg ha^{-1} K_2O + 100 kg ha^{-1} P_2O_5). Except for the soil tillage and nitrogen fertilization, all the other technology measures such as: sowing, P and K fertilization, pests control, machinery and equipment used were identical in all the treatments.

Laboratory analysis

Health analysis of wheat grains was done by deep freezing method. Wheat grains were washed under running water, disinfected for 30 sec. with 96% ethanol and washed in distilled water three times. For each sample 3x100 grains were analysed and average value of diseases incidence calculated for each sample in percentages. Petri dishes with moisture filter paper were kept in chamber for 24 h at 20°C and light regime 12 hours day/12 hours night, then 24 h in freezer at -18°C and finally 12 days in chamber on 20°C. The examination was performed after 14 days with stereo microscope (Olympus SZX9) and microscope (Olympus BX41). Grain infection with *Fusarium* was evaluated for each sample for 3x100 grains by determining mycelia development on grain surface with stereo microscope. If necessary, mycelia developed on wheat grain was transferred to potato dextrose agar (PDA) for further determination and growing pure fungal cultures (Vrandečić et al., 2013). Identification to genus level was done based on fungal morphological characteristics.
All collected data were statistically processed with SAS software (1999).

Experimental site conditions

According to Croatian Meteorological and hydrological service (DHMZ, Jug et al., 2017), (Table 3) the precipitation amounts from October 2013 to July 2014 (period when the experiment was conducted) was 662 mm (Cacinci) and 591 (Magadenovac). In May (heading and flowering period) the precipitations were 160 mm (Cacinci) and 140 (Magadenovac) whereas in June 64 (Cacinci) and 62 mm (Magadenovac).

Table 1. Soil chemical composition on the experimental sites.
Tablica 1. Kemijski sastav tla na eksperimentalnim površinama.

Experimental site *Pokusna površina*	pH (H_2O)	pH (KCl)	P_2O_5 (mg 100 g^{-1})	K_2O (mg 100 g^{-1})	Humus (%)	Hy* (cmol kg^{-1})
Cacinci	5.09	4.03	6.2	12.7	2.4	5.1
Magadenovac	5.29	4.27	17.2	22.7	1.3	4.4

* Soil hydrolytic acidity

Table 2. Crop residue cover on soil surface related to soil tillage treatment and experimental sites.
Tablica 2. Pokrivenost površine tla na različitim sustavima obrade tla i eksperimentalnim površinama.

Experimental site *Pokusna površina*	Crop residue soil cover (%) *Pokrivenost površine tla žetvenim ostatcima (%)*				
	CT	SS	CH	DH	NT
Cacinci	4	37	53	30	99
Magadenovac	5	46	57	31	99

Table 3. Average weather conditions in the experimental period (2013/2014) and long-term average (1984-2012) on both localities.
Tablica 3. Prosječne vremenske prilike tijekom razdoblja istraživanja (2013./2014.) i višegodišnje prosječne vremenske prilike (1984.-2012.) na obje lokacije istraživanja.

	X	XI	XII	I	II	III	IV	V	VI	VII	$\sum/X$
Average long-term and months precipitations (mm) *Prosječna mjesečna i godišnja količina oborina (mm)*											
Cacinci											
1984-2012	70.3	73.8	64.9	55.7	41.4	53.7	62.6	75.1	95.0	69.4	661.9
2013/2014	41.9	101.7	1.1	40.4	64.5	48.7	98.2	159.7	64.0	79.3	699.5
Magadenovac											
1984-2012	61.3	64.9	55.8	49.7	39.4	46.3	57.3	67.7	85.5	62.6	590.5
2013/2014	28.6	66.9	0.5	51.8	75.8	24.9	69.1	139.2	62.4	82.0	601.2
Average month and year air temperature (°C) *Prosječna mjesečna i godišnja temperatura zraka (°C)*											
Cacinci											
1984-2012	11.4	6.0	1.7	0.6	1.8	6.3	11.6	16.3	19.7	21.8	9.7
2013/2014	13.6	7.6	2.7	4.3	5.5	9.6	12.8	15.4	20.3	21.8	11.4
Magadenovac											
1984-2012	11.6	5.9	1.3	0.3	1.8	6.5	12.0	17.0	20.3	22.3	9.9
2013/2014	13.3	7.6	1.8	3.6	5.2	9.8	13.2	15.7	20.4	22.0	11.3

(Source: Jug et al., 2017)

RESULTS AND DISCUSSION

The presence of *Fusarium* species was determined in all tested treatments of soil tillage and fertilization. On Magadenovac, lower level of nitrogen (N1) reduced the grain infection with *Fusarium* species in comparison with other nitrogen treatments in three different tillage systems (CT, DH and NT) (Table 4). The highest percentage of wheat grains infected with *Fusarium* sp. 14.00% was increased by 30% (N3) on subsoiling treatment (SS) and N application. Although, the percentage of the infection by *Fusarium* on SS and CH tillage treatment was higher at N1 than at N2, these differences were not statistically significant. On location Cacinci (Table 4) effect of reduced fertilization (N1) on *Fusarium* sp. grain infection in all types of soil tillage was statistically lower in comparison with other (N2 and N3) nitrogen treatments.

Influence of nitrogen fertilization and tillage system on FHB incidence should not be monitored separately from all other factors (weather conditions, agrotechnical measures, genotype, amount of inoculum of plant pathogen, aggressivity and pathogenicity of *Fusarium* spp.) which influence the plant disease incidence. Due to that fact there are different opinions on influence of fertilization and tillage on *Fusarium* incidence.

Table 4. Effect of nitrogen fertilization on *Fusarium* sp. wheat grain infection (%) on different soil tillage treatments on both localities.

Tablica 4. Utjecaj gnojidbe dušikom na infekciju zrna kukuruza vrstama Fusarium *(%) na različitim sustavima obrade tla na oba lokaliteta.*

Soil tillage treatment *Sustav obrade tla*	N1	N2	N3	LSD 0.05
	Nitrogen level fertilization *Razina gnojidbe dušikom*			
Magadenovac				
CT	1.33 c	3.00 b	9.33 a	0.75
SS	7.00 b	6.33 b	14.00 a	2.40
CH	9.33 a	8.68 a	9.00 a	3.77
DH	4.68 b	8.00 a	10.33 a	2.76
NT	5.33 b	6.33 a	7.00 a	1.51
Cacinci				
CT	2.66 b	9.00 a	9.33 a	2.44
SS	3.00 b	9.33 a	10.33 a	1.51
CH	7.00 b	9.00 a	10.67 a	3.16
DH	6.33 b	7.00 a	10.33 a	4.59
NT	3.00 b	6.67 a	9.00 a	3.16

Conventional tillage (CT), Subsoiling (SS), Chiseling (CH), Disk-harrowing (DH), No-tillage (NT), Nitrogen fertilization: N1 - amount reduced by 30% according to recommendation, N2 - according to recommendation, N3 - amount increased by 30% according to recommendation

a,b,c - different letters mark statistically significant difference according to Duncan's Multiple Range Test at the level $P \leq 0.05$

Results of our fertilization influence research of in different soil tillage systems on Magadenovac and Cacinci locations showed that increased nitrogen fertilization resulted in higher infection of grains with *Fusarium* sp. This statement is supported by the results of Agrios (1997) where high concentration of nitrogen increases plant disease susceptibility. High concentration of nitrogen in soil supported incidence of wheat FHB (Ivashenko and Nazarovskaya, 1990; Lemmens et al. 2004; Milev et al. 2008; Suproniene et al., 2012). Burgt et al. (2011) after two years of the experiment concluded that increasing N content is not recommended due to favouring wheat *Fusarium* infection. In contrary, Fauzi and Paulitz (1994) and Yoshida et al. (2008) claimed that nitrogen amount did not influence infection of wheat with *Fusarium* sp.

Nitrogen fertilization with N amount reduced by 30% in conventional tillage (CT) on location Magadenovac resulted in the lowest number of infected grains (1.33%) (Table 5). For nitrogen fertilization according to recommendation, significant statistical difference was determined between conventional tillage (CT) with the lowest number of infected grains (3%) and other investigated treatments. Nitrogen fertilization increased by 30% caused *Fusarium* incidence between 7% (NT) and 14% (SS) of the infected grains. Infection level with *Fusarium* sp. in the reduced tillage system (SS, CH, DH and NT) was significantly higher than on CT in N1 and N2 nitrogen treatments.

Statistical difference in different tillage system was determined on location Cacinci only in nitrogen fertilization with N amount reduced by 30% (Table 5). The lowest percentage of wheat grains infected with *Fusarium* sp. was recorded on no-tillage (NT) at nitrogen fertilization according to recommendation and at nitrogen fertilization increased by 30%.

Table 5. Effect of soil tillage on *Fusarium* sp. maize grain infection (%) on different nitrogen fertilization treatments on both localities.

Tablica 5. Utjecaj obrade tla na infekciju zrna kukuruza vrstama Fusarium *(%) na različitim tretmanima gnojidbe dušikom na oba lokaliteta.*

Nitrogen level fertilization *Razina gnojidbe dušikom*	CT	SS	CH	DH	NT	LSD 0.05
	Soil tillage treatment *Sustav obrade tla*					
Magadenovac						
N1	1.33 d	7.00 b	9.33 a	4.68 c	5.33 c	0.66
N2	3.00 c	6.30 b	8.67 a	8.00 ab	6.33 b	1.40
N3	9.33 bc	14.00 a	9.00 bc	10.33 b	7.00 c	2.33
Cacinci						

N1	2.66 b	3.00 b	7.00 a	6.33 a	3.00 b	2.29
N2	9.00 a	9.33 a	9.00 a	7.00 a	6.67 a	3.01
N3	9.33 a	10.33 a	10.67 a	10.33 a	9.00 a	2.78

Conventional tillage (CT), Subsoiling (SS), Chiseling (CH), Disk-harrowing (DH), No-tillage (NT), Nitrogen fertilization: N1 - amount reduced by 30% according to recommendation, N2 - according to recommendation, N3 - amount increased by 30% according to recommendation

a,b,c,d - different letters mark statistically significant difference according to Duncan's Multiple Range Test at the level P≤ 0.05

Soil tillage had influence on *Fusarium* sp. incidence of wheat grain while infection percentage with *Fusarium* sp. was higher on the reduced tillage with N1 and N2 nitrogen level. Vrandečić et al. (2013), determined higher number of grains infected with *Fusarium* sp. in no-tillage and reduced tillage systems in the two-year research (2008 and 2009) of influence of soil tillage and fertilization on wheat grain infection with fungi. Tillage systems had significant influence on FHB frequency, but no effect on infection with other fungal genera. However, nitrogen treatments did not show significant influence on *Fusarium* population. Incorporation of infected plant residues, which are one of the main sources of *Fusarium* infection, reduced inoculum amount (Leplat et al., 2013) deep into the ground. It can be expected that in conventional tillage infection percentage is lower than in reduced tillage. Hofgaard et al. (2016) also determined that soil tillage and incorporation of plant residue reduced inoculum amount of *Fusarium* sp.

According to Lori et al. (2009) no-tillage system can result in higher *Fusarium* incidence, but optimal climate conditions for disease incidence are probably more important than tillage and fertilization systems. Soil tillage treatments had no significant influence on *Fusarium* infection level on location Cacinci probably due to higher precipitation during the vegetation period (Table 3). Conventional tillage recorded lower infection percentage than conservation tillage due to a higher amount of harvested residues on Magadenovac location.

Environment is the most important factor influencing *Fusarium* disease incidence (Fernandez et al. 2005; Bíliková and Hudec, 2013). However, in years with average or high levels of disease, farming system can affect disease severity. Since *Fusarium* is highly adaptable to different, often extreme, environment conditions wheat head infection favours wet conditions in flowering, i.e. in May and June. In our research the amount of rain in May and June favoured *Fusarium* infections. Temperatures in May were little lower compared to long-term average, i.e. the period was determined to be colder and June was marked as warmer than year average (DHMZ data, Jug et al., 2017).

Some of *Fusarium* species produce mycotoxins. However, according to Baliukoniene et al. (2011) in no-tillage there is no significant mycotoxin production. Trichothecene mycotoxin type B - deoxynivalenol (DON) could be regarded as an indicator of the overall mycotoxin contamination (Lacko-Bartosova et al., 2017). Tillage systems had no significant influence on *Fusarium* infection level, but they had indirect effect on mycotoxin content depending on years (Suproniene et al., 2012). During the 4-year experiment, nitrogen fertilization had no significant effect on deoxynivlenol (DON) content of barley (Pageau et al., 2008). The organic crop rotation and conventional crop rotation does not influence DON content in winter wheat grain (Vanova et al., 2008). CST system is considered to be cause of epidemic FHB infection in Central America (Dill-Macky and Jones, 2000), due to production system where maize is produced in rotation with wheat.

There is no simple solution for *Fusarium* incidence on wheat, but knowledge about agroecological conditions and cultural practice which influence infection and disease development in some particular area are the key for potential risk estimate of disease incidence and development of effective strategy against this pathogen. From the agroecological point of view a reduced tillage has several advantages (increased microbiological activity of soil, increased biological diversity as fungi, bacteria, nematodes, earthworms, etc.) and if disease incidence in some areas is not significant this soil tillage should be implemented. Biological diversity is the key of soil health preservation. Implementation of reduced tillage provides maintenance of soil health being more important than disease incidence at lower intensity. Holistic approach to crop systems and plant health management may provide the solution to disease problems.

CONCLUSION

Fusarium head blight on cereals occurs every year all around the world and there is no simple solution to reduce it. Different strategies of plant protection should be applied including agrotechnical measures (crop rotation, soil tillage system, nitrogen fertilization). In this research the presence of *Fusarium* species was determined in all the tested tillage and fertilization treatments. The effect of reduced nitrogen fertilization on *Fusarium* sp. grain infection in all tillage treatments was statistically lower in comparison with other nitrogen treatments. Implementation of conservation soil tillage provides biological diversity and soil health being more important than disease incidence at lower intensity.

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UTJECAJ RAZLIČITIH SUSTAVA KONZERVACIJSKE OBRADE TLA I GNOJIDBE DUŠIKOM NA INFEKCIJU ZRNA PŠENICE VRSTAMA *Fusarium*

SAŽETAK

U radu je prikazan utjecaj različitih konzervacijskih sustava obrade tla i različitih razina gnojidbe dušikom na zarazu zrna pšenice vrstama* Fusarium*. Pokus je postavljen na dvije lokacije s pet sustava obrade tla: konvencionalna obrada s oranjem do 30 cm, podrivanje na dubinu 35-40 cm, rahljenje do 25 cm, tanjuranje na 10-15 cm i te direktna sjetva bez ikakve obrade tla, kao i s tri razine gnojidbe dušikom: N1- umanjena za 30% u odnosu na preporuku; N2 - prema preporuci i N3 - uvećana za 30% u odnosu na preporuku. Pojava zrna zaraženih vrstama* Fusarium *utvrđena je u svim istraživanih tretmana. Postotak zaraženih zrna vrstama* Fusarium *bio je statistički značajno niži na tretmanu gnojidbe N1 u odnosu na varijante gnojidbe N2 i N3. Najniži postotak zaraženih zrna pšenice zabilježen je kod konvencionalne obrade tla u Magadenovcu i no tillage tretmana u Čačincima.

Ključne riječi: konzervacijska obrada tla, gnojidba dušikom, infekcija zrna pšenice,* Fusarium *sp.

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