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THE IMPACT OF TILLAGE AND FERTILIZATION ON SOYBEAN GRAIN INFECTION WITH FUNGI

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ABSTRACT

The effects of tillage system and fertilization practice on soybean grain infection with fungi were examined during three year period (2008, 2009 and 2010). Stationary research of reduced soil tillage was conducted, on marsh gley (eugley) hydromeliorated soil type in eastern Croatia. Ten fungal genera were determined: *Penicillium*, *Cladosporium*, *Aspergillus*, *Alternaria*, *Fusarium*, *Peronospora*, *Cercospora*, *Diaporthe*, *Colletotrichum* and *Curvularia*. The percentage of fungi from genus *Diaporthe/Phomopsis* in 2010 was 15.06 while in 2008 and 2009 these percentages were significantly lower, 0.89 and 0.18 respectively. The number of *Diaporthe/Phomopsis* species was significantly higher with reduced tillage and no-tillage compared to conventional tillage and percentage of infected grains increased with the amount of fertilizer (nitrogen). The most important factors affecting the infection level were amount and timing of precipitation and temperature. In those circumstances some soybean pathogens could appear in higher percentage in reduced tillage. In year 2008 with usual amount of precipitation and in extremely dry and hot 2009 there was no significant differences between reduced and conventional tillage and amount of fertilizer in soybean grain infection with important fungal pathogens.

Key words: reduced tillage, no-tillage, soybean, seed-borne fungi.

INTRODUCTION

Soil tillage is one of the most important components of soil management, but in the same time has a great influence on intensity of plant diseases. According to different soil tillage approaches primarily intensity and frequency, different amount of plant residue remains on the soil surface (Jug et al., 2011) which, through interactions with other agro-ecological components, has various effects on pests, diseases and weeds (Jordan and Hutcheon, 2003). Extensive research related with plant pathogens and no-tillage cultivation has been conducted on numerous crops (Supronienė et al., 2012; Perez-Brandan et al., 2012; dos Reis et al., 2012). Since many plant pathogens can survive on plant debris (Poštić et al., 2012) the plough has traditionally been used to incorporate crop residues. On the other hand, good soil quality, which is enhanced with no-till, increases the level of microbial activity, nutrient cycling, microbial diversity and enhance natural disease suppression (Perez-Brandan at al., 2012). Research of Almeida et al. (2001) found out that crop residues had been connected to disease, but it is necessary to also consider other parameters involved in the disease epidemiology. *Fusarium* sp. and *Macrophomina phaseolina* (Tassi) Goid. showed an increase in survival when infected tissue was buried, while *Colletotrichum truncatum* (Schwein.) Andrus & W.D. Moore, *Cercospora kikuchii* (T. Matsumoto & Tomoy.) M.W. Gardner and *Phomopsis* sp. showed a decrease in survival, which suggest that soilborne pathogens were not affected as severely as the airborne pathogens. Soil texture has also important influence on debris and pathogens survival; there was a greater frequency of *Phytophthora sojae* Kaufm. & Gerd. in conservation tillage than in conventional tillage (silt loam and loam soils) and population densities of *Heterodera glycines* Ichinohe. were less in no-till fields compare to tilled fields (silty clay loam and clay soils) (Workneh et al., 1999).

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According to our knowledge there is only one scientific paper about influence of reduced tillage on soybean seed quality including seed borne microflora (Bowman et al., 1986). In Croatia, there was no research evidence on pathogen infection in soybean grains under different soil tillage system because the basic tillage practice in soybean production has been the conventional approach (Stipešević et al., 2009). Last 10 years tillage practice in soybean production is distinctively changing to direction of more adapting of different reduced soil tillage approaches (Jug et al., 2010). More than 40 fungal species, bacteria and viruses can be isolated from soybean grains and 15 of them are economically important (Kulik and Sinclair, 1999). The aim of this research was to evaluate influence of year, tillage and fertilization on soybean grains disease incidence.

MATERIAL AND METHODS

During three years (2008, 2009 and 2010) stationary research of reduced soil tillage was conducted for soybean, at marsh gley (eugley) hydromeliorated soil type in eastern Croatia (Brestovac site; 45°37′ N and 18°42′ E, elevation 83 m). Research was conducted with four soil tillage treatments (main factor, TT), with size of the basic soil tillage plot of 540 m^2, and three nitrogen fertilization treatments (sub-factor N), with basic fertilization plot size of 165 m^2, set up in split-plot design in four repetitions. The experiment was conducted on the same homogeneous field at the same location with winter wheat as forecrop in each experimental year. Chemical properties of investigated soil (in ploughing layer 0-30 cm) were as follows: $pH_{(H_2O)}$ = 5.61; $pH_{(KCl)}$ = 4.52; OM = 2 .13; P = 86.0 mg kg^{-1} and K = 242.3 mg kg^{-1} (determined by the Egner-Riehm Domingo AL-method) and Hy = 8.79 mm $100g^{-1}$. The following four TT were applied in continuation: 1) Conventional tillage (CT) based on autumn mouldboard ploughing on 30 cm depth; 2) Multiple disk harrowing (MD) to a depth of 15 cm and 10 cm depth; 3) Chiselling (CH) to a depth of 25 cm and No-tillage (NT) without any tillage treatments. Nitrogen fertilization treatment had three levels of applied nitrogen: N1=35, N2=70 and N3=110 kg N ha^{-1}. Fertilization with phosphorus and potassium was uniform for all tillage treatments in all investigated years (150 kg ha^{-1} P and 90 kg ha^{-1} K as basic dressing). No-till grain drill John Deer 750A was used for all TT at a depth of 3-5 cm and inter-row spacing at 33 cm. In all experimental years, soybean cultivar Podravka 95 (maturity group 0-I) was sown in optimal terms.

Weather conditions (primarily precipitation and temperatures) had significant aberrations during 2009 (extremely dry season) and 2010 (extremely humid), whereas 2008 season was moderately humid according to long term mean (45 year) (Table 1).

Table 1. Total precipitation (mm) and temperature (°C) in winter season (October through April) and in the growing season (May through October) at Brestovac site during 2008, 2009 and 2010

Season	Precipitation (mm)				Temperature (°C)			
	2008	2009	2010	45-yr average	2008	2009	2010	45-yr average
Winter season	416	243	531	316	6.0	5.3	5.9	5.3
May	55	44	197	58	18.4	18.9	16.5	16.5
June	73	118	167	88	21.9	19.7	19.8	19.7
July	73	17	34	68	22.2	23.2	21.5	21.2
August	38	35	60	54	22.4	22.9	21.0	20.9
September	67	12	148	55	15.8	19.1	16.7	16.4
October	82	50	63	50	10.6	13.0	11.4	11.3
Growing season	388	276	669	373	18.6	19.5	17.8	17.6

Health analysis of soybean grains was done by wet chamber method. Soybean grains were washed under running water to remove mechanical dirt, disinfected for 30 sec. with 96% ethanol and washed in distilled water three times. For each sample 4x100 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 on 24°C and light regime 12 hours day/12 hours night. Examination was performed after 7 days with stereo microscope (Olympus SZX9) and microscope (Olympus BX41). Grain infection with *Peronospora manshurica* (Naumov) Syd. was evaluated for each sample for 4 x 100 grains by determining incrustation on grain surface with stereo microscope. Mycelia developed on soybean grain were transferred to potato dexstroze agar (PDA) for further determination and for growing pure fungal cultures. Identification to genus/species level was done based on morphological characteristics of fungi. Data was statistically processed with SAS software (1999).

RESULTS

During three year research on influence of different TT, fertilization and year on mycopopulation of soybean grain, the following ten fungal genera were determined: *Penicillium*, *Cladosporium*, *Aspergillus*, *Alternaria*, *Fusarium*, *Peronospora*, *Cercospora*, *Diaporthe*, *Colletotrichum* and *Curvularia*.

Presence of fungal genera depending on the treatment is presented in Table 2 and 3. Fungi from genera *Curvularia* and *Cercospora* (species *C. kikuchii*) were not statistically analysed due to sporadic incidence. Presence of bacteria is also presented in Tables 2 and 3, but without determination to genus/species level.

Table 2. Percentage (%) of soybean grains infection depending of the year of research, tillage treatment and nitrogen fertilization

	Pen.	*Clad.*	*Asper.*	*D/P*	*Coll.*	*Alter.*	*Fus.*	*P. man.*	Bact.
Year (Y)									
2008	1.62 b	32.87 b	0.50 b	0.89 b	0.54 b	9.50 a	1.02 b	0.87 b	1.62 b
2009	9.14 a	27.54 c	3.79 a	0.18 b	1.14 a	0.35 c	0.16 c	1.37 a	1.35 b
2010	0.06 c	36.37 a	0.00 b	15.06 a	1.54 a	1.31 b	2.21 a	1.27 ab	4.20 a
Tillage treatment (TT)									
CT	2.94 bc	35.27 a	1,44 a	3.88 b	1.22 a	2.91 c	1.41 a	0.72 b	1.80 b
MD	2.83 c	28.30 b	1,41 a	5.47 a	1.14 a	4.33 a	1.16 ab	1.39 a	3.22 a
CH	4.91 a	31.25 b	1,33 a	6.27 a	0.97 a	3.50 bc	1.28 ab	0.80 b	2.83 a
NT	3.75 b	34.64 a	1,52 a	5.89 a	0.97 a	4.14 ab	0.72 b	1.77 a	1.72 b
Fertilization (N)									
N1	3.43 a	31.47 a	1.41 a	4.75 b	0.96 a	2.66 c	0.91 a	1.25 a	2.14 a
N2	3.39 a	32.62 a	1.35 a	5.08 b	1.06 a	3.79 b	1.22 a	1.12 a	2.37 a
N3	4.00 a	33.00 a	1.52 a	6.31 a	1.20 a	4.70 a	1.29 a	1.14 a	2.66 a

The means in a column followed by the same letter are not significantly different at $P \leq 0.05$.
Pen. – *Penicillium* sp., Asper. – *Aspergillus* sp., *D/P* – *Diaporthe/Phomopsis* complex, *Coll.* – *Colletotrichum* sp., *Alter.* – *Alternaria* sp., *Fus.* – *Fusarium* sp., *P.man.* – *Peronospora manshurica*, Bact. – Bacterium.
Tillage treatments: CT – Conventional tillage; MD – Multiple disk harrowing; CH – Chiselling; NT – No-tillage.

Regardless of the year, tillage and fertilization, majority of fungi were from *Cladosporium* genus. The highest percentage of soybean grains infected with *Cladosporium* sp. was in 2010 (36.37%), regardless of TT (percentage of infection for all three years). Between CT and NT there was no significant influence on *Cladosporium* infection level. Isolation frequency of fungi from *Diaporthe/Phomopsis* complex, genus *Fusarium*, species *P. manshurica* and bacteria were statistically higher in 2010.

Table 3. Percentage (%) of infected soybean grains in 2010 depending on tillage treatment and nitrogen fertilization

	Pen.	*Clad.*	*Asper*	*D/P*	*Coll.*	*Alter.*	*Fus.*	*P. man.*	Bact.
Tillage treatment (TT)									
CT	0.16 a	39.83 a	0.00 a	10.83 b	1.33 a	0.83 a	2.58 a	1.25 a	2.83 b
MD	0.00 a	33.66 b	0.00 a	15.25 a	1.50 a	1.91 a	2.41 a	1.58 a	5.88 a
CH	0.08 a	32.41 b	0.00 a	17.08 a	1.50 a	1.66 a	2.57 a	1.26 a	4.81 a
NT	0.00 a	40.83 a	0.00 a	17.10 a	1.83 a	1.33 a	1.25 a	1.00 a	3.16 b
Fertilization (N)									
N1	0.18 a	37.00 a	0.00 a	12.2 b	1.37 a	0.75 c	1.56 b	1.13 a	3.37 b
N2	0.00 a	36.37 a	0.00 a	13.8 b	1.75 a	2.00 b	2.25ab	1.31 a	4.31 a
N3	0.00 a	36.68 a	0.00 a	18.12 a	1.50 a	4.25 a	2.81a	1.37 a	4.93 a

The means in a column followed by the same letter are not significantly different at P≤0.05.
Pen. – *Penicillium* sp., *Asper*. – *Aspergillus* sp., *D/P* – *Diaporthe/Phomopsis* complex, *Coll.* – *Colletotrichum* sp., *Alter.* – *Alternaria* sp., *Fus.* – *Fusarium* sp., *P.man.* – *Peronospora manshurica*, Bact. – Bacterium.
Tillage treatments: CT – Conventional tillage; MD – Multiple disk harrowing; CH – Chiselling; NT – No-tillage.

It is important to emphasize that percentage of grains infected with *Diaporthe/Phomopsis* species in 2010 was 15.06% while in 2008 and 2009 these percentages were significantly lower, 0.89 and 0.18, respectively. The number of grains infected with *Diaporthe/Phomopsis* species was significantly higher with reduced TT's and NT compared to CT and percentage of infected grains increased with the amount of fertilizer (nitrogen).

In 2008 and 2009 there were no significant differences between trial variances (data not presented). The common *pathogens* associated with *soybean anthracnose* are the fungi from genus *Colletotrichum*. The percentage of non-germinated infected grains in all years and trial variances was low and there were no differences depending on tillage and fertilization. Percentage of grains infected with *Fusarium* sp. ranged from 0.16% in 2009 to 2.21% in 2010. In 2010, there were no statistical differences in the occurrence of *Fusarium* sp. regarding to the TT. Regardless of the year, the only differences due to tillage treatments were noted between NT and CT.

Obtained yields were in accordance to weather conditions during the period of research (Table 4).

Table 4. Soybean grain yield (t ha^{-1}) on different soil tillage treatment at Brestovac site from 2008 to 2010

Tillage treatment (TT)		Soybean yield, t ha^{-1} (Y)			Average 2008-2010 (O)
		2008	2009	2010	
CT		3.80 a	2.50 a	4.19 a	3.14 a
MD		3.60 b	2.28 b	4.04 a	3.04 ab
CH		3.60 b	2.30 b	4.08 ab	3.06 ab
NT		3.40 c	2.41 ab	3.89 b	2.91 b
Average (Y)		3.60 B	2.37 C	4.05 A	3.04
LSD 0.05	TT-O				0.18
	TT	0.19			
	Y	0.34			

The means in a column followed by the same lowercase letter are not significantly different at P≤0.05.
The means in a row with the same uppercase letter are not significantly different at P≤0.05.
Tillage treatments: CT – Conventional tillage; MD – Multiple disk harrowing; CH – Chiselling; NT – No-tillage.

DISCUSSION

Frequency of fungal isolation from soybean grains depends on several factors: plant organ (Baird et al., 2001), genotype (Ploper et al., 1992; Freitas et al., 2002), growing stage (Holland and Abney, 1988; Baird et al., 2001), location, maturity group and year (quantity of precipitation/ temperature) (Villarroel et al., 2004).

Fungi from genus *Cladosporium* were determined in the highest percentage during three year research, regardless of year, tillage and fertilization, but they are considered as soybean saprophites and are frequently isolated from soybean grains. Till now their pathogenicity was not confirmed (Kulik and Sinclair, 1999).

Various *Fusarium* sp. can infect different soybean plant parts. *Fusarium* wilt and necrosis of root and lower stem is an important type of disease. Other very important disease is sudden death syndrome (SDS) caused by different *Fusarium* species, since distinct species cause SDS in North and South America. On soybean *Fusarium* sp. are known mainly as pathogens causing root diseases, but they can also infect grains, especially when pods are in contact with infected soil and in that case grains can be infected up to 50% (Kulik and Sinclair, 1999). We also isolated *Fusarium* sp. from soybean grains, but, generally, their percentage was low. In humid tropical regions percentage of infected seed could be up to 36%, but in moderate climate percentage of infection is mostly below 10% (McGee, 1992). In research of Medić-Pap et al. (2007), in majority of samples, percentage of infected seed was 1-3%. In our research in 2010 there was no significant influence of applied tillage methods on the occurrence of *Fusarium* sp. In the same year, higher percentage of infection level was in trial variants with higher amounts of fertilizer (Table 3). It is well known that supplementary nitrogen increased the incidence of *Fusarium* infection (Lemens et al., 2004; Supronienė et al., 2012).

Regardless of the year, differences in percentage of infected grains were noted between NT and CT considering only the tillage system. Supronienė et al. (2012) found out that tillage systems had no significant influence on *Fusarium* infection level of wheat grains.

The one of most important pathogenic fungi for soybean grains are fungi from genera *Diaporthe/Phomopsis*. According to new findings this complex includes 4 species (Santos et al., 2011). *Diaporthe longicolla* (Hobbs) J.M. Santos, Vrandečić & A.J.L. Phillips (Vidić et al., 1995; Vrandečić et al., 2006) is the most pathogenic to soybean grains. In our samples the dominant species that caused soybean grain rot was *Diaporthe caulivora* (Athow & Caldwell) J.M. Santos, Vrandečić & A.J.L. Phillips. In Villaorroel et al. (2004) research, the dominant species (more than 75% isolates) on soybean pods and grains of conventional and transgenic cultivars was also *D. caulivora*, followed by *Diaporhte sojae* (Cooke & Ellis) Sacc. (former *Diaporthe phaseolorum* var. *sojae* (Lehman) Wehm), and less than 1% *D. longicolla*. Higher percentage of infected grains with fungi from *Diaporthe/Phomopsis* complex in 2010 can be explained by higher amount of precipitation and optimal temperatures for pathogen development during August and September. Warm and wet weather between phenophases R7 (beginning of ripening) and R8 (full ripening), significantly influence the infection of grains with *Diaporthe/Phomopsis* species and disease spread (Hepperly and Sinclair, 1980; McGee, 1986).

D. caulivora was more frequently isolated from stem and petioles of soybean grown in no-tillage system, which means that tillage practice can reduce frequency of infection with this species (Rothrock et al., 1985). It is well known that plant debris are the main source of inoculum for infection with fungi of genera *Diaporthe*/*Phomopsis,* therefore their destruction has great practical importance and is one of the most important measures in control of these fungi and some other fungal species that appear on soybean. Soybean plant debris, especially in no-tillage system, can significantly increase level of soybean diseases and yield. Fungi that cause diseases of wheat and maize can survive on soybean debris which can result in increase of inoculum density (Baird et al., 1997). Reduced tillage and higher amount of fertilizer primarily influence root and green parts infection and, secondarily, grain infection. According to three years research of Bowman et al. (1986) reduced tillage and no tillage did not influence the quality of soybean grains (grain weight, germination, vigour and mycoflora).

CONCLUSIONS

In our research of soybean grain mycopopulation depending on the year, tillage and fertilization, the most important factors affecting the infection level were amount and timing of precipitation and temperature. In years with large amount of precipitation during the time of grain filling and maturation we can expect high percentage of infected soybean grains. In those circumstances some soybean pathogens could appear in higher percentage in reduced tillage.

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