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MICROBIAL COMMUNITIES IN THE RHIZOSPHERE SOIL OF SOYBEAN CULTIVATED AFTER TANSY PHACELIA, WINTER WHEAT, WHITE MUSTARD, RYE, AGRIMONY AND SOYBEAN AS PREVIOUS CROPS

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Abstract: The object of the studies conducted in the years 2000–2002 on a field of 3 years' monoculture of soybean was rhizosphere soil of soybean cultivated after tansy phacelia, winter wheat, white mustard, rye, agrimony and soybean as previous crops. The purpose of the studies was to determine the effect of cultivating the above listed previous crop plants on the formation of microorganism communities in the rhizosphere soil of soybean. The lowest total number of fungal colonies was found in the rhizosphere soil of soybean cultivated after rye and winter wheat (21.09×10^3 and 22.58×10^3 c. f. u., respectively), while the highest number was found in soil after soybean (36.95×10^3 c. f. u.). The highest total number of bacteria was found in 1 g of dry weight of the rhizosphere soil of soybean cultivated after agrimony, and the lowest after soybean (5.80×10^6 and 4.09×10^6 c. f. u., respectively). The largest proportion of pathogenic fungi was characteristic of the rhizosphere soil of soybean cultivated after soybean, and the smallest – of the rhizosphere soil of soybean after agrimony as a previous crop. The dominating species among pathogenic fungi in all experimental objects was *Fusarium oxysporum*. The rhizosphere soil of soybean cultivated after soybean was the poorest in saprophytic fungi (35.2% of all isolations). On the other hand, the highest number of saprophytes, including antagonistic ones, was found in the rhizosphere soil of soybean after agrimony and winter wheat.

Key words: rhizosphere soil, previous crops, tansy phacelia, winter wheat, white mustard, rye, agrimony, soybean

INTRODUCTION

Plants – through their root exudates and crop residues – can affect the qualitative and quantitative composition of microorganisms and their biological activity in the soil (Funck-Jensen and Hockenhull 1984). Sugars, free aminoacids, vitamins, metal

ions, organic acids and enzymes exuded by roots of a particular plant species can stimulate or inhibit the growth and development of pathogenic organisms as well as saprophytic ones (Angus et al. 1994; Martyniuk et al. 1991; Pięta 1994). Organic matter introduced into the soil in the form of crop residues or green matter also contributes to an increased biological effect of the soil environment (Hoitink and Boehm 1999; Myśków 1989). The organic matter of plants, stimulating the development of organisms antagonistic towards phytopathogens, causes the improvement of phytosanitary condition of the soil (Curl 1982; Łacicowa 1979; Patkowska 1998; Smolińska 2000; Solarska 1995).

The purpose of the studies was to determine the effect of cultivation of previous crop plants (tansy phacelia, winter wheat, white mustard, rye, agrimony and soybean) on the formation of microorganism communities in the rhizosphere soil of soybean.

MATERIAL AND METHODS

The studies were conducted in the years 2000–2002 on a field of a 3 years' monoculture at the Experimental Station at Czesławice near Nałęczów.

The object of the studies was the rhizosphere soil of soybean at anthesis cultivated after such previous crop plants as tansy phacelia (*Phacelia tanacetifolia* Bantham) 'Stala', winter wheat (*Triticum aestivum* L.) 'Oda', white mustard (*Sinapis alba* L.) 'Arwis', rye (*Secale cereale* L.) 'Arant', agrimony (*Brassica campestris* L.) 'Brachina' and soybean [*Glycine max* (L.) Merrill] 'Polan'.

Seeds and kernels of particular species of previous crop plants were sown at the beginning of September on four plots (four repetitions) of the area of 2.25 m^2 each. Green matter of plants was ploughed in at the beginning of November. Soybean was cultivated as the main plant and as a previous crop. The reason for such cultivation was the fact that when soybean was harvested, plants were removed from the plot. In order to supplement the green matter of this plant, it was also sown as a previous crop.

Rhizosphere soil, obtained from a thin layer of soil directly adhering to the surface of soybean roots dug out in each experimental variant, was placed into sterile Petri dishes.

The rhizosphere soil from the same experimental variant was mixed under sterile conditions in the laboratory. Then, 10 g of each so-prepared samples were placed in a flask (250 ml) with 90 ml of sterile distilled water. It was mixed in a shaker for 30 minutes. Dilutions of 10^{-2} to 10^{-7} were prepared of each soil suspension.

The microbiological analysis of the rhizosphere soil samples was performed according to the method described by Martyniuk et al. (1991) and Pięta and Bełkot (2002).

The total number of bacteria in 1 g of dry weight of soil was determined using the dilutions of 10^{-5}, 10^{-6} and 10^{-7} and Nutrient agar medium.

In order to determine the number of bacteria from the genus *Bacillus* in 1 g of dry weight of the soil, the dilutions of 10^{-4}, 10^{-5}, 10^{-6} and nutrient medium Tryptic soy agar were used, while the dilutions of 10^{-2}, 10^{-3} and 10^{-4} and nutrient medium Pseudomonas agar F were used to determine the number of colonies of *Pseudomonas* spp.

The total number of fungal colonies in 1 g of dry weight of soil was determined on Matrin's (1950) nutrient medium using the dilutions of 10^{-2}, 10^{-3} and 10^{-4}.

The colonies of bacteria and fungi grown on particular media were counted, while in the case of fungi they were transferred into slants of maltose medium. The fungal colonies were assigned to the species using proper keys and monographic papers (Barnett 1960; DeVries 1952; Domsch and Gams 1970; Ellis 1976; Gilman 1957; Nelson et al. 1983; Ramirez 1982; Raper et al. 1968; Rifai et al. 1969; Sałata and Rudnicka-Jezierska 1979).

The results concerning the number of bacteria and fungi were analyzed statistically and significant differences were determined by means of Tukey's confidence intervals (Oktaba 1987).

RESULTS

It was found on the basis of microbiological analysis of the rhizosphere soil of soybean plants that 1 g of dry weight soil contained on average from 4.09×10^6 to 5.80×10^6 c. f. u. (Tab. 1). The lowest total number of bacteria was found in the rhizosphere soil of soybean growing after soybean as a previous crop, while the highest number was observed in the rhizosphere soil of soybean after agrimony (Tab. 1).

Table 1. The number of bacteria and fungi isolated from rhizosphere soil of soybean plants at anthesis from particular experimental variant

Experimental variant	Total number of bacteria (mln·g^{-1} d. w. of soil)	Number of *Bacillus* spp. (mln·g^{-1} d. w. of soil)	Number of *Pseudomonas* spp. (mln·g^{-1} d. w. of soil)	Total number of fungi (thous. ·g^{-1} d. w. of soil)
soybean rhizosphere after tansy phacelia	5.46 b	0.80 a	1.65 c	31.73 b
soybean rhizosphere after winter wheat	4.32 a	1.78 b	1.38 bc	22.58 a
soybean rhizosphere after white mustard	5.52 b	2.62 c	1.05 ab	27.97 ab
soybean rhizosphere after rye	4.62 a	1.60 b	1.09 ab	21.09 a
soybean rhizosphere after agrimony	5.80 b	2.88 c	0.75 a	33.07 b
soybean rhizosphere after soybean	4.09 a	1.30 ab	1.11 ab	36.95 b

Means followed by the same letter are not significantly different at p = 0.05

The microbiological analysis showed that the highest number of *Bacillus* spp. c. f. u. were present in the rhizosphere soil of soybean cultivated after agrimony (2.88×10^6 c. f. u., on average). The lowest number of c. f. u. of this genus was observed in the rhizosphere soil of soybean growing after tansy phacelia (0.80×10^6 c. f. u., on average) (Tab. 1).

The population of *Pseudomonas* spp. was represented in the largest quantity in the rhizosphere soil of soybean cultivated after tansy phacelia, while the lowest after agrimony (1.65×10^6 and 0.75×10^6 c. f. u., respectively) (Tab. 1).

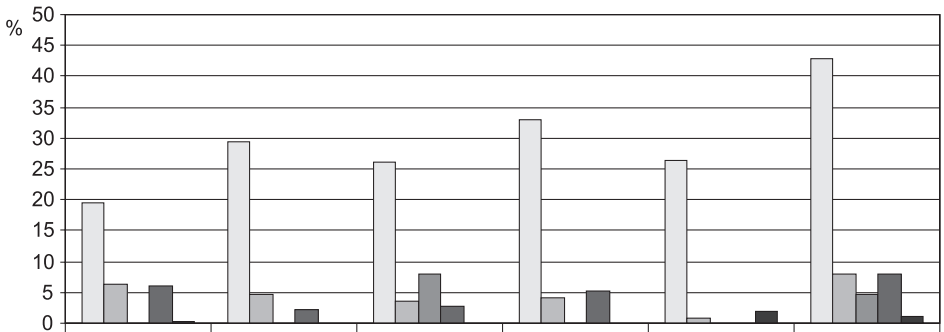
The rhizosphere soil of soybean cultivated after rye and winter wheat contained 21.09×10^3 to 22.58×10^3 fungal c. f. u., respectively. The highest number of fungal c.f.u. was found in 1 g of dry weight of the rhizosphere soil of soybean grown after soybean (36.95×10^3 c. f. u.) (Tab. 1).

Mycological analysis of the rhizosphere soil of soybean provided 1746 fungal colonies (Tab. 2). The highest proportion of fungi pathogenic towards soybean was found in the rhizosphere soil of soybean cultivated after soybean as a previous crop (64.8% of all isolations) (Fig. 1). On the other hand, the lowest proportion of those fungi was found in the rhizosphere soil of soybean cultivated after agrimony (29.3% of total isolations) (Fig. 1). *Fusarium oxysporum* was the most frequently isolated species from the studied samples of rhizosphere soil of soybean (Tab. 2, Fig. 1). The proportion of this species within the isolated fungi was the highest and ranged from 19.4% to 42.9% of all isolates. The lowest number of *F.oxysporum* colonies was observed in the rhizosphere soil of soybean cultivated after tansy phacelia and winter wheat, while the highest was found in the soil after soybean cultivated as a previous crop (Fig. 1).

Fusarium genus was also represented by the species *F. solani*. This fungus was most frequently isolated from the rhizosphere soil of soybean cultivated on the plots where soybean was also a previous crop (8% of all fungi) (Fig. 1).

Table 2. Total number of fungi isolated from rhizosphere soil of soybean plants at anthesis from particular experimental variant

Fungal species	Number of isolates in experimental variant rhizosphere soil after cultivation of:						Total
	tansy phacelia	winter wheat	white mustard	rye	agrimony	soybean	
Acremonium murorum (Corda) W. Gams						2	2
Acremonium roseum (Oud.) W. Gams						1	1
Acremonium strictum W. Gams	5			8	13		26
Alternaria alternata (Fr.) Keissler	11		4	1	4		20
Epicoccum purpurascens Ehr. ex Schl.		3					3
Fusarium equiseti (Corda) Sacc.	2	5	7	12	5	3	34
Fusarium oxysporum Schl.	58	69	78	71	86	160	522
Fusarium solani (Mart.) Sacc.	19	11	11	9	3	30	83
Fusarium sporotrichioides Sherb.		2					2
Gliocladium catenulatum Gilman et Abbott	26	28	44	22	44	32	196
Gliocladium roseum (Link) Bainier	10	9	10	5	9		43
Humicola fuscoatra Traaen		2				35	37
Mucor hiemalis Wehmer	14	6	12	4	12	22	70
Mucor racemosus Fres.	2		2	1	6		11
Myrothecium roridum Tode ex Fries	49	31	3		17		100
Myrothecium verrucaria Ditmar ex Fr.	13				8		21
Penicillium frequentans Westling			2		5	1	8
Penicillium janthinellum Biourge	18	13	21	20	15		87
Penicillium nigricans (Bain.) Thom	13	8	23	19	34		97
Penicillium verrucosum Dierckx var. *verrucosum* Samson. Stolk et Hadlok	17	7	26	14	34	28	126
Phomopsis sojae Lehman			24			18	42
Pythium irregulare Buisman	18	5	8	11		30	72
Rhizoctonia solani Kühn	1				6	4	11
Trichoderma harzianum Rifai	10	9	8	2	4		33
Trichoderma koningii Oud.	4	18	10	8	10	6	56
Trichoderma viride Pers. ex S. F. Gray	9	8	7	9	9	1	43
Total	299	234	300	216	324	373	1746



	tansy phacelia	winter wheat	white mustard	rye	agrimony	soybean
F.o.	19.4	29.5	26	3.9	26.5	42.9
F.s.	6.4	4.7	3.7	4.2	0.9	8
P.s.	0	0	8	0	0	4.8
P.i.	6	2.1	2.7	5.1	0	8
R.s.	0.3	0	0	0	1.9	1.1
Total	32.1	36.3	40.4	42.2	29.3	64.8

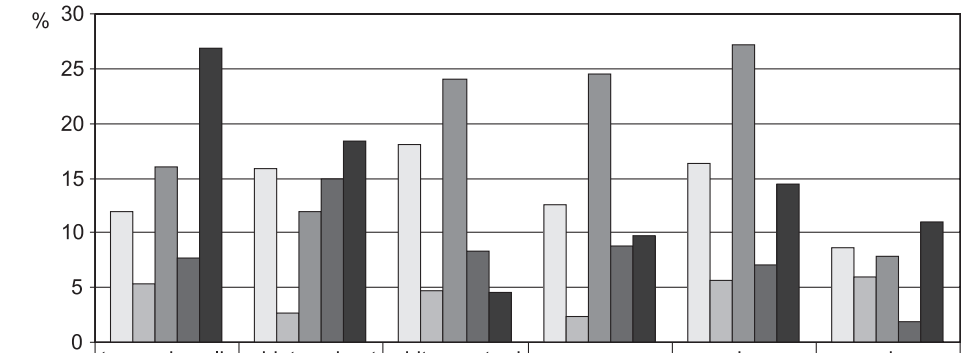
Fig. 1. Participation of pathogenic fungi isolated from soybean rhizosphere soil after cultivation of particular previous crop plants
F.o. – *Fusarium oxysporum*; F.s. – *F. solani*; P.s. – *Phomopsis sojae;* P.i. – *Pythium irregulare; R.s.* – *Rhizoctonia solani*

The species *P. irregulare* was obtained from all the examined samples of the rhizosphere soil of soybean, with the exception of soil taken from the experimental variant where agrimony was a previous crop. The highest proportion of this species (8%) was characteristic of the rhizosphere soil of soybean cultivated after soybean (Tab. 2, Fig. 1).

Other fungi pathogenic towards soybean e. g. *Phomopsis sojae* and *Rhizoctonia solani* were also obtained from the studied soils but in lowest numbers and not in each year of the studies.

The species of saprophytic fungi mainly belonging to the genera of *Gliocladium, Trichoderma, Penicillium* and *Mucor* were also isolated from the rhizosphere soil of soybean from particular experimental variants (Tab. 2, Fig. 2). The highest number of saprophytic fungi was obtained from the rhizosphere soil of soybean cultivated on the plots after agrimony (70.8% of total isolations), while the lowest number came from the soil of experimental variants where soybean was an previous crop (35.2% of total isolations) (Fig. 2).

Penicillium spp., represented by 4 species, namely *P. frequentans, P. janthinellum, P. nigricans, P. verrusocum* var. *verrusocum,* were the most frequently isolated (Tab. 2). The proportion of *Penicillium* spp. ranged from 7.8% to 27.2% of all isolations (respectively, from the rhizosphere soil of soybean after soybean and after agrimony) (Fig. 2).



	tansy phacelia	whinter wheat	white mustard	rye	agrimony	soybean
G.spp.	12	15.8	18	12.5	16.3	8.6
M.spp.	5.4	2.6	4.7	2.3	5.6	5.9
P.spp.	16	12	24	24.5	27.2	7.8
T.spp.	7.7	14.9	8.3	8.8	7.1	1.9
another	26.8	18.4	4.6	9.7	14.5	11
Total			59.7		70.8	35.2

Fig. 2. Participation of saprophytic fungi isolated from soybean rhizosphere soil after cultivation of particular previous crop plants

G. spp. – *Gliocladium* spp.; M. spp . – *Mucor* spp.; P. spp . – *Penicillium* spp.; T. spp – *Trichoderma* spp.; other – other saprophytic fungi

The genus *Gliocladium*, represented by *G. catenulatum* and *G. roseum,* occurred in the highest number in the rhizosphere of soybean cultivated after white mustard (18% of total isolations) (Fig. 2).

On the other hand, the species from the genus *Trichoderma* such as *T. harzianum, T. koningii* and *T. viride* were represented most numerously in the rhizosphere soil of soybean cultivated after winter wheat (14.9% of total isolations) (Fig. 2).

Saprophytic fungi were also represented by the genera *Acremonium, Mucor* and *Myrothecium* as well as the species *Epiccocum purpurascens* and *Humicola fuscoatra* (Tab. 2).

DISCUSSION

The studies showed that the composition of microbial communities in the rhisosphere soil of soybean at anthesis cultivated after different previous crops varied in respect of both quality and quantity.

The rhizosphere soil of soybean after winter wheat and rye contained the lowest number of fungal colonies and numerous bacterial colonies. Saprophytic fungi dominated over the pathogenic ones in both experimental variants. It seems that a high content of aminoacids, especially alkaline and aromatic ones, in root exudates of winter wheat as well as products of decay of the green matter of this plant create favourable nutrient conditions for the development of saprophytic microorganisms (Patkowska 2000; Pięta 1999). On the other hand, according to

Solarska (1996), the cultivation of rye had a positive effect on the development of saprophytic fungi, including those that have the antagonistic effect on phytopathogens. A significant influence of antagonistic populations of *Trichoderma* spp. in the soil after rye cultivation was also found by Pięta et al. (2002). A high amount of cellulose in the green matter of winter wheat could have contributed to the growth and development of fungal populations from the genera *Gliocladium* and *Trichoderma* (Papavizas 1985). The cellulolitic and chitinolitic properties of those fungi and their abilities of antagonistic effect towards the others, especially pathogenic, representatives of *Mycobionta* can significantly affect the reduction of the population of soybean phytopathogens (Łacicowa and Pięta 1985a; b; 1989; Papavizas 1985).

The total number of fungal colonies in the rhizosphere soil of soybean after tansy phacelia, white mustard and agrimony was lower than the number of colonies in the soil after soybean, while the total number of bacterial colonies was significantly higher. The proportion of pathogenic fungi in the rhizosphere soil of soybean cultivated after the above mentioned previous crop plants was much lower than after soybean as a previous crop.

The studies by Paszkowski and Dwornikiewicz (1997) showed that ploughing over white mustard stimulated the growth and development of antagonistic microorganisms, mainly *Bacillus* spp. Results of the present studies showed that the rhizosphere soil of soybean after the cultivation of white mustard contained relatively numerous colonies of *Bacillus* spp.

Numerous colonies of *Trichoderma* spp. were obtained from the rhizosphere soil of soybean after agrimony. According to Smolińska (2000), the compounds liberated from decomposed residues of *Brassicaceae* plants (sources of glucosinolanes such as isothiocyanates, sulphur, epitionitril, carbon disulphide, dimethyl sulphide) can have an inhibiting effect on microorganisms living in the soil. Nevertheless, the species from the genus *Trichoderma* are distinguished by a considerable tolerance towards toxic compounds (Papavizas 1985; Smith and Kirkegaard 2002), which was confirmed by their presence in the rhizosphere soil of soybean cultivated after *Brassica campestris*.

The rhizosphere soil of soybean cultivated on the plots where soybean grew as a previous crop contained the highest number of fungal colonies and the lowest number of bacterial colonies. This soil was characterized by the largest proportion of pathogenic fungal colonies, especially *F. oxysporum*. Accumulation of microorganisms pathogenic towards soybean can be explained by repeated cultivation of this plant on the same field. Such a technology causes a decrease of soil fertility and a disturbs microbiological balance through excessive development of microorganisms pathogenic towards plants. Root exudates and the green matter of soybean contain considerable quantities of aminoacids, especially the acidic ones, which favour the development of pathogenic microorganisms (Funck-Jensen and Hockenhull 1984; Patkowska 2000; Pięta 1988). Such conditions probably inhibited the development of *Trichoderma* spp., which was found in very small numbers in the soil after soybean.

The composition of microorganism populations obtained from the rhizosphere soil after the cultivation of particular previous crops was varied. It can be, therefore,

stated that the green fertilizer from tansy phacelia, winter wheat, white mustard, rye and agrimony had a modifying effect on the formation of those communities.

CONCLUSIONS

1. The proportion of particular populations of saprophytic microorganisms as compared with those pathogenic towards soybean was more positive in the rhizosphere soil of soybean cultivated after tansy phacelia, winter wheat, white mustard, rye and agrimony than in the case of the rhizosphere soil of soybean cultivated after soybean as a previous crop.
2. Monoculture of soybean without cover crops ploughed under in the autumn caused the increase of pathogens' propagules in soil while the populations of saprotrophic microorganisms decreased.

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POLISH SUMMARY

ZBIOROWISKA MIKROORGANIZMÓW W GLEBIE RYZOSFEROWEJ SOI UPRAWIANEJ PO FACELII BŁĘKITNEJ, PSZENICY OZIMEJ, GORCZYCY BIAŁEJ, ŻYCIE, RZEPIKU I SOI JAKO PRZEDLONACH

Przedmiotem badań przeprowadzonych w latach 2000–2002 na polu trzyletniej monokultury soi była gleba ryzosferowa soi uprawianej po facelii błękitnej, pszenicy ozimej, gorczycy białej, życie, rzepiku i soi jako przedplonach. Celem badań było określenie wpływu uprawy wyżej wymienionych roślin przedplonowych na kształtowanie się zbiorowisk mikroorganizmów w glebie ryzosferowej soi. Najmniejszą ogólną liczbą jednostek tworzących kolonie grzybów charakteryzowała się gleba ryzosferowa soi uprawianej po życie i pszenicy ozimej (odpowiednio $21{,}09 \times 10^3$ i $22{,}58 \times 10^3$ jednostek tworzących kolonie), a największą po soi ($36{,}95 \times 10^3$ j. t. k. grzybów). Najwięcej ogółem bakterii znajdowało się w 1 g s. m. gleby ryzosferowej soi uprawianej po rzepiku, a najmniej po soi (odpowiednio $5{,}80 \times 10^6$ i $4{,}09 \times 10^6$ j. t. k.). Największym udziałem grzybów chorobotwórczych charakteryzowała się gleba ryzosferowa soi uprawianej po soi, a najmniejszym gleba ryzosferowa soi po rzepiku jako przedplonie. Wśród grzybów chorobotwórczych we wszystkich kombinacjach doświadczenia dominował gatunek *Fusarium oxysporum*. Gleba ryzosferowa soi uprawianej po soi była najuboższa w grzyby saprotroficzne (35,2% wszystkich wyosobnień). Natomiast najwięcej saprotrofów, w tym i antagonistycznych, zanotowano w glebie ryzosferowej soi po rzepiku i pszenicy ozimej.