Effect of cover crops on the microorganisms communities in the soil under scorzonera cultivation

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ABSTRACT

The purpose of the studies was to determine the microorganisms communities in the soil under scorzonera cultivation with the cover crops using. The greatest total CFU of bacteria occurred in the soil from the experimental combination where oat ploughed over in spring or autumn was the cover crops. The smallest total CFU of bacteria was obtained after ploughing over the mulch of tansy phacelia. The use of oats and spring vetch had a positive effect on the population of bacteria from the genera of *Bacillus* and *Pseudomonas*. The smallest CFU of fungi was obtained from the soil where oat constituted the mulch. *Alternaria alternata*, *Fusarium oxysporum*, *F. culmorum*, *Haematonectria haematococca* and *Thanatephorus cucumeris* were most frequently isolated from the soil. The most isolates of the enumerated fungi were obtained from the soil cultivated in a conventional cultivation and after ploughing over the mulch of tansy phacelia plants. The most antagonistic bacteria and fungi occurred in the soil after ploughing over the mulch of oat, while the least in the conventionally cultivated soil.

Keywords: oat; spring vetch; tansy phacelia; fungi, bacteria

Recently, the application of cover crops has played a big role, particularly in vegetable cultivation. They can be used as green manure, which – after being ploughed in – provide the organic mass and mineral elements into the soil. Depending on the species, cover crops exert different effects on the physicochemical properties of the soil, weed infestation, the growth of plants, their healthiness and yielding (Lithourgidis et al. 2011). Cover crops, left in the field for winter and used as mulch, have a very good effect on the soil environment (Jodaugiene et al. 2006, Patkowska and Konopiński 2011).

The greatest influence on the formation of microorganisms communities in the soil is exerted by plants through their roots exudates or hulling root cells (Steinkellner et al. 2008, Jaroszuk-Ściseł et al. 2009). Root exudates stimulate or inhibit the development of soil plant pathogens and they increase the population of antagonistic microorganisms.

The purpose of the studies was to determine the formation of microorganisms communities in the cultivation of scorzonera, considering different manners of soil cultivation and using oats, spring vetch and tansy phacelia as cover crops. Besides, the occurrence of microorganism antagonistic towards facultative soil-borne plant pathogens was established.

MATERIAL AND METHODS

Fieldwork. The field experiment was conducted in the years 2006–2008 at the Felin Experimental Station belonging to the University of Life Science in Lublin (Poland), on grey brown podzolic soil made of loess formations lying on chalk marls with the mechanical composition corresponding to silty medium loams. The object of the studies was the soil taken each year from the depth of 5–6 cm of the plough layer of the

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field where scorzonera (*Scorzonera hispanica* L.) of cv. Duplex sown in the first 10 days of May was cultivated for obtaining the roots. The experiment took into consideration soil mulching with cover crops such as oats, spring vetch and tansy phacelia. Cover crops were sown in the first half of August of each year preceding setting the experiment. Those plants formed an abundant yield of green matter before winter and it constituted a natural mulch on the surface of the land and that mulch was managed in two ways: (1) mixed with the soil as a result of pre-winter ploughing, or (2) mixed with the soil as a result of spring ploughing. The soil from the field without cover crops cultivated in the conventional cultivation was the control.

Laboratory analyses. In each study year the soil sampling and microbiological analysis were done in accordance with the method described by Martyniuk et al. (1991) and Patkowska (2005). With the aim of obtaining a total CFU of bacteria in the soil, PDA medium was used with an addition of a yeast extract. Tryptic Soy Agar was used to isolate *Bacillus* spp., whereas Pseudomonas Agar F was used for *Pseudomonas* spp. Martin's medium was used to determine the total CFU of fungi occurring in the soil. The populations of colonies and fungi were converted into 1 g of soil dry weight (DW).

Among the microorganisms colonies isolated from the soil, in each studied year 500 isolates of *Bacillus* spp. and *Pseudomonas* spp. were tested from each as well as all fungi isolates from the genera of *Gliocladium*, *Clonostachys*, *Myrothecium*, *Penicillium* and *Trichoderma* with the aim of establishing their antagonistic effect towards *Alternaria alternata*, *Fusarium culmorum*, *F. oxysporum*, *Haematonectria haematococca*, *Pythium irregulare* and *Thanatephorus cucumeris* (isolated from the infected scorzonera plants). The antagonistic effect of the studied bacteria was established according to the method described by Martyniuk et al. (1991). The scale of the antagonistic effect of bacteria comprised five degrees, i.e. 0° – no inhibition zone; 1° – inhibition zone of 1–2 mm; 2° – inhibition zone of 3–5 mm; 3° – inhibition zone of 6–10 mm; 4° – inhibition zone of over 10 mm. In order to fully determine the effect of bacteria on the pathogenic fungus, the studies also used the degrees of growth inhibition of plant pathogens as provided by Pięta and Kęsik (2007). It comprised the following: 0° – no fungus growth inhibition; 1° – colony growth inhibited to 20%; 2° – colony growth inhibited to 50%; 3° – colony growth inhibited to 80%; 4° – colony growth inhibited to 100%. The method provided by Mańka and Mańka (1992) was used to determine the antagonistic effect of the studied fungi towards the enumerated plant pathogens.

Statistical analysis. Results concerning the populations of microorganisms were statistically analyzed and the significance of differences was established on the basis of the Tukey's confidence intervals ($P < 0.05$). Statistical calculations were carried out using Statistica program, version 7.1.

RESULTS AND DISCUSSION

The total population of bacteria in particular years of studies ranged from 1.90×10^6 to 7.12×10^6 CFU/g of soil DW (Table 1). Statistically significantly the most population of total bacteria occurred in the soil from the experimental combination where oat ploughed over in spring or autumn was the cover crops (on average 6.16×10^6 and 5.86×10^6 CFU/g, respectively). As reported by Jezierska-Tys et al. (2012), cereals can stimulate the growth and development of bacteria in the soil. Statistically significantly the least population of bacteria colonies was obtained after ploughing over the mulch of tansy phacelia plants in spring or autumn. The total population of *Bacillus* spp. in particular years of studies ranged from 0.80×10^6 to 3.74×10^6 CFU/g, and the total population of *Pseudomonas* spp. ranged from 0.11×10^6 to 1.55×10^6 CFU/g. Statistically significantly the most CFU of *Bacillus* spp. and *Pseudomonas* spp. were observed in the soil after ploughing over the mulch of oat in spring or autumn (3.56×10^6 and 1.02×10^6, and 3.13×10^6 and 0.94×10^6 CFU/g, respectively). The system of cultivation had no significant effect on the population of those bacteria in the soil. Similar results concerning *Bacillus* spp. and *Pseudomonas* spp. were obtained by Pięta and Kęsik (2007), who used rye for soil mulching in the cultivation of onion.

The total CFU of fungi in the analyzed combination of the experiment ranged from 15.56×10^3 to 86.22×10^3 CFU/g of soil DW (Table 1). Statistically significantly the most total CFU of fungi was observed in 1 g DW of the control soil, i.e. cultivated without any cover plant (on average 78.95×10^3 CFU/g) and the soil from the combination where the mulch of tansy phacelia was ploughed over in autumn (on average 65.73 ×

Table 1. Number of bacteria and fungi isolated from soil in the years 2006–2008

Experimental combination	Total CFU of bacteria (mln/g DW of soil)				CFU of *Bacillus* spp. (mln/g DW of soil)				CFU of *Pseudomonas* spp. (mln/g DW of soil)				Total CFU of fungi (thous./g DW of soil)			
	2006	2007	2008	mean	2006	2007	2008	mean	2006	2007	2008	mean	2006	2007	2008	mean
1	6.90^{c}	5.66^{e}	5.91^{b}	6.16^{d}	3.74^{e}	3.53^{d}	3.43^{bc}	3.56^{c}	1.28^{d}	0.45^{c}	1.33^{c}	1.02^{c}	15.56^{a}	32.74^{a}	34.31^{a}	27.54^{a}
2	6.49^{c}	5.57^{e}	5.52^{b}	5.86^{d}	3.03^{d}	3.03^{d}	3.32^{bc}	3.13^{c}	1.10^{c}	0.42^{c}	1.29^{c}	0.94^{bc}	23.28^{b}	34.30^{a}	37.26^{a}	31.61^{a}
3	5.31^{b}	4.28^{d}	4.65^{a}	4.74^{c}	1.83^{c}	2.42^{c}	3.11^{b}	2.45^{b}	1.13^{c}	0.38^{c}	1.16^{c}	0.89^{bc}	25.00^{b}	46.25^{b}	52.65^{b}	41.30^{b}
4	5.28^{b}	4.15^{cd}	4.52^{a}	4.65^{c}	1.46^{bc}	2.14^{bc}	3.03^{b}	2.21^{b}	0.74^{b}	0.35^{c}	1.12^{bc}	0.74^{b}	46.50^{c}	48.55^{b}	53.05^{b}	54.03^{c}
5	4.01^{a}	3.44^{bc}	4.11^{a}	3.85^{ab}	1.70^{b}	1.44^{a}	1.94^{a}	1.69^{a}	0.79^{b}	0.25^{b}	0.62^{ab}	0.55^{a}	53.12^{c}	63.19^{c}	58.87^{b}	58.39^{cd}
6	3.92^{a}	2.72^{b}	4.02^{a}	3.55^{a}	0.82^{a}	1.78^{ab}	1.71^{a}	1.44^{a}	0.65^{a}	0.14^{a}	0.54^{a}	0.44^{a}	65.00^{d}	66.88^{c}	65.30^{c}	65.73^{d}
7	3.63^{a}	1.90^{a}	7.12^{c}	4.22^{bc}	0.80^{a}	1.28^{a}	3.66^{c}	1.91^{ab}	0.63^{a}	0.11^{a}	1.55^{c}	0.76^{b}	83.31^{e}	86.22^{d}	67.32^{c}	78.95^{c}

Means in columns followed by the same letter do not differ significantly at $P \leq 0.05$. 1 – oats mulch + spring ploughing; 2 – oats mulch + pre-winter ploughing; 3 – spring vetch mulch + spring ploughing; 4 – spring vetch mulch + pre-winter ploughing; 5 – tansy phacelia mulch + spring ploughing; 6 – tansy phacelia mulch + pre-winter ploughing; 7 – conventional cultivation

10^3 CFU/g). Statistically significantly the least total CFU of fungi was obtained in the combination where oat was the mulch (on average 27.54 × 10^3 and 31.61 × 10^3 CFU/g, depending on the cultivation system). The way of managing the oat mulch had no significant effect on the population of fungi in the soil. The use of oat, spring vetch and tansy phacelia in the cultivation of salsify limited the development of the population of soil fungi (Patkowska and Konopiński 2011).

Among the fungi considered to be pathogenic, different species from the genera of *Alternaria*, *Fusarium*, *Giberella*, *Haematonectria*, *Pythium* and *Thanatephorus* were isolated (Table 2). The most frequently isolated species were *Alternaria alternata*, *Fusarium oxysporum*, *F. culmorum*, *Haematonectria haematococca* and *Thanatephorus cucumeris*. The largest number of isolates of the enumerated fungi was isolated from the conventionally cultivated soil (control) and from the soil after ploughing over the mulch of tansy phacelia plants. As reported by Patkowska and Błażewicz-Woźniak (2013), cover crops used in vegetable cultivation not only decrease weed infestation or reduce the costs of production but they can also considerably decrease the activity of plant pathogens in the soil. They influence on the fungi communities through their root exudates or products of decomposition of aftercrop residue (Badri and Vivanco 2009, Jaroszuk-Ściseł et al. 2009). Oat, common vetch and tansy phacelia limited the infection of salsify by such soil-borne fungi as *Alternaria alternata*, *Fusarium oxysporum*, *F. culmorum*, *Haematonectria haematococca*, *Pythium irregulare*, *Thanatephorus cucumeris* (Patkowska and Konopiński 2011). Zahid et al. (2012) report that a similar role can be played by fennel cultivated as a cover plant.

Among saprotrophic fungi, different species of *Gliocladium* spp., *Clonostachys* spp., *Myrothecium* spp., *Penicillium* spp., *Trichoderma* spp., *Acremonium* spp., *Gliomastix* spp., *Cladosporium* spp., *Mucor* spp. and *Rhizopus* spp. were isolated. The most *Gliocladium* spp., *Clonostachys* spp., *Myrothecium* spp. and *Trichoderma* spp. were isolated from the soil when oats or spring vetch were the cover crops, whereas the least in the case of the conventional cultivation (Table 2). According to Patkowska and Konopiński (2011), cereals as well as green manure and their straw stimulate the development of *Trichoderma* spp.

Laboratory tests established the number of antagonistic bacteria and fungi occurring in soil samples from particular experimental combinations (Figure 1). The most antagonistic *Bacillus* spp. and *Pseudomonas* spp. occurred in the soil after ploughing over the mulch of oats (respectively, 43 and 64 isolates), while the least in the conventional cultivation of scorzonera, i.e. without a cover crops (respectively, 6 and 17 isolates). The most antagonistic *Gliocladium* spp., *Penicillium* spp. and *Trichoderma* spp. were observed after managing the oat mulch (in both cultivation systems). The smallest number of antagonists was obtained from the conventional cultivation of scorzonera.

Table 2. Fungi frequently isolated from the soil in experimental combinations (sum 2006–2008)

Fungus species	Number of isolates/experimental combination							
	1	2	3	4	5	6	7	total
Acremonium rutilum W. Gams	7	6	5	–	4	11	9	42
Alternaria alternata (Fr.) Keissler	5	6	12	13	16	19	29	100
Cladosporium cladosporioides (Fres) de Vries	18	2	5	–	2	5	15	47
Clonostachys rosea f. *catenulata* (Gilman et Abbott) Schroers	6	8	6	–	1	8	–	29
Clonostachys rosea (Link) Schroers, Samuels, Seifert et W. Gams	6	5	5	3	2	2	–	23
Chaetomium piluliferum Daniels	1	–	2	–	–	–	9	12
Epicoccum nigrum Link	3	1	3	–	3	10	2	22
Fusarium culmorum (W.G.Sm.) Sacc.	5	1	11	12	8	17	26	80
Fusarium oxysporum Schl.	9	13	19	23	22	33	62	181
Giberella intricans Wollenw.	–	–	–	2	1	4	11	18
Gliomastix murorum (Corda) S. Hughes var. *murorum*	1	–	–	–	–	2	–	3
Haematonectria haematococca (Berk. et Broome) S. Rossman	–	–	5	7	5	13	23	53
Humicola fuscoatra Traaen	1	–	2	12	2	5	6	28
Mucor hiemalis Wehmer	1	3	2	4	2	3	–	15
Mucor racemosus Fresenius	2	3	5	10	14	10	20	64
Myrothecium verrucaria (Alb. et Schwein) Ditmar	13	10	–	2	5	1	–	31
Papulaspora irregularis Hotson	–	–	–	–	15	2	–	17
Penicillium aurantiogriseum Dierckx	9	10	10	3	3	5	–	40
Penicillium chermesinum Biourge	1	2	–	–	3	9	–	15
Penicillium chrysogenum Thom	5	7	8	8	3	2	6	39
Penicillium expansum Link ex S. F. Gray	4	4	1	–	–	7	–	16
Penicillium janczewskii Zaleski	8	6	4	2	3	4	2	29
Penicillium janthinellum Biourge	6	2	1	–	3	–	–	12
Penicillium lividum Westling	6	4	3	–	2	–	–	15
Penicillium verrucosum Dierckx var. *verrucosum* Samson, Stolk et Hadlok	17	15	15	7	7	18	9	88
Pythium irregulare Buisman	–	2	7	–	1	4	10	24
Rhizopus stolonifer (Ehrenb.) Vuill.	5	4	7	7	16	16	28	83
Thanatephorus cucumeris (A.B. Frank) Donk	2	2	4	5	5	9	13	40
Trichoderma aureoviride Rifai	17	16	9	6	2	4	2	56
Trichoderma harzianum Rifai	11	12	5	3	6	8	–	45
Trichoderma koningii Oud.	14	14	8	9	8	4	4	61
Trichoderma pseudokoningii Rifai	8	5	2	4	5	1	3	28
Trichoderma piluliferum Webster et Rifai	5	6	5	2	–	4	3	25
Trichoderma viride Pers. ex. S.F. Gray	21	16	12	15	9	8	6	87
Total	217	185	183	159	178	248	298	1468

1 – oats mulch + spring ploughing; 2 – oats mulch + pre-winter ploughing; 3 – spring vetch mulch + spring ploughing; 4 – spring vetch mulch + pre-winter ploughing; 5 – tansy phacelia mulch + spring ploughing; 6 – tansy phacelia mulch + pre-winter ploughing; 7 – conventional cultivation

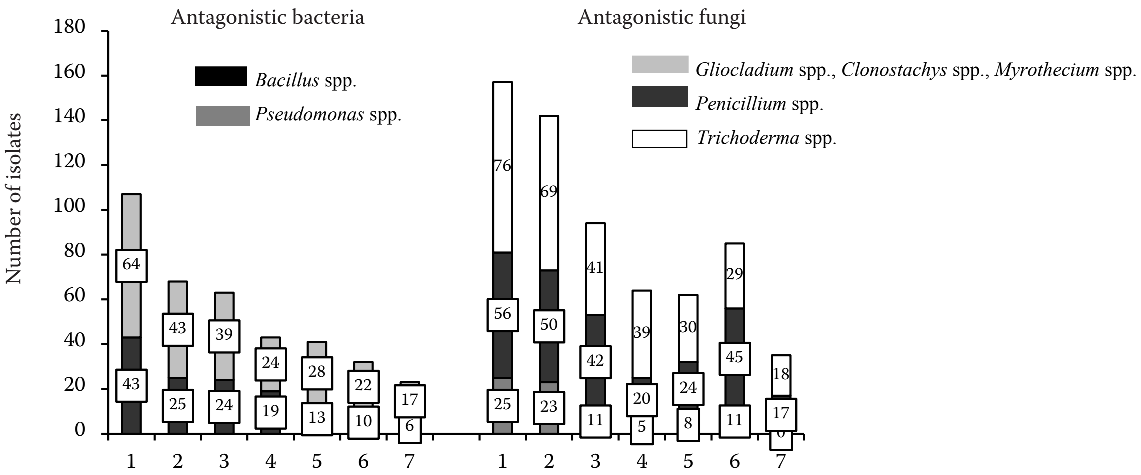

Figure 1. Number of antagonistic bacteria and fungi towards pathogenic fungi in individual experimental combinations (sum from the years 2006–2008). 1 – oats mulch + spring ploughing; 2 – oats mulch + pre-winter ploughing; 3 – spring vetch mulch + spring ploughing; 4 – spring vetch mulch + pre-winter ploughing; 5 – tansy phacelia mulch + spring ploughing; 6 – tansy phacelia mulch + pre-winter ploughing; 7 – conventional cultivation

The application of spring vetch or tansy phacelia as cover crops also contributed to an increased number of antagonistic microorganisms as compared to the cultivation without any cover crops.

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