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THE MICROORGANISMS COMMUNITIES IN THE SOIL UNDER THE CULTIVATION OF CARROT (*Daucus carota* L.)

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Abstract. The composition of populations of soil microorganisms depends on a number of factors, for example the species of intercrop ground cover plants. The purpose of the studies was to determine the quantitative and qualitative composition of the populations of soil microorganisms in the cultivation of carrot considering different manners of soil cultivation and with the use of oat, spring vetch and tansy phacelia as intercrop ground cover crops. The greatest population of total bacteria occurred in the soil where oat was the ground cover plants. The most of total fungi was observed in the control. The application of ground cover plants contributed to an increased number of antagonistic *Bacillus* spp., *Pseudomonas* spp., *Gliocladium* spp., *Penicillium* spp. and *Trichoderma* spp. The tillage system had no significant effect on the population of microorganisms in the soil.

Key words: oat, spring vetch, tansy phacelia, antagonistic bacteria and fungi

INTRODUCTION

The composition of populations of soil microorganisms depends on a number of factors, including the species of intercrop ground cover plants [Patkowska and Konopiński 2013]. These plants play an important role in protecting and preserving root vegetables [Patkowska and Konopiński 2011, Błażewicz-Woźniak and Wach 2012]. They can be introduced into the soil or remain on its surface in the form of mulch [Konopiński et al. 1999, 2001, Kęsik et al. 2000, Patkowska and Konopiński 2011].

White mustard, tansy phacelia, spring vetch, oat, rye, oil radish, buckwheat, sunflower and mixtures of pulses are used in mulching the soil [Borowy and Jelonkiewicz 1999, Głowacka 2010, Błażewicz-Woźniak and Konopiński 2011, Patkowska and Konopiński 2011, 2013]. Ground cover plants are used in the cultivation of various plants, including root vegetables such as carrot, parsley, red beet, root celery, parsnip as well as less known in Poland and more rarely cultivated high-inulin vegetables (scorzonera,

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salsify, root chicory) [Kęsik et al. 2000, Adamczewska-Sowińska 2004, Patkowska and Konopiński 2011, 2013]. Intercrop ground cover plants in conservation tillage affect the phytosanitary condition of the soil, especially owing to their root exudates [Pięta and Patkowska 2001, Pięta et al. 2003]. They also limit the occurrence of pathogens and nematodes. They properly modify the composition of soil microorganisms and can contribute to limited occurrence of pathogenic fungi [Patkowska and Konopiński 2011, 2013]. Thus, they improve the healthiness of root vegetables in this way increasing the size and quality of the yield [Adamczewska-Sowińska 2004, Patkowska and Konopiński 2011].

In view of the lack of information on the role of mulches in shaping of communities of soil microorganisms in the cultivation of *Daucus carota* L., the present studies were undertaken. Their purpose was to determine the quantitative and qualitative composition of the populations of soil microorganisms in the cultivation of this plant considering different manners of soil cultivation and with the use of oat, spring vetch and tansy phacelia as intercrop ground cover crops. Besides, the studies determined the occurrence of antagonistic bacteria and fungi on soil-borne plant pathogens.

MATERIAL AND METHODS

The field experiment was conducted in the years 2010–2012 at the Felin Experimental Station belonging to the University of Life Sciences in Lublin, 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 a depth of 5–6 cm of the plough layer of the field where carrot of 'Flakkee 2' cv. sown in the third 10 days of April was cultivated. The experiment took into consideration soil mulching with ground cover plants such as oat, spring vetch and tansy phacelia. Ground cover plants 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. The experiment used three systems of soil tillage, i.e. A) tillage before winter (ploughing) and spring tillage (a combined cultivator), B) tillage before winter (a grubber) and spring tillage (a combined cultivator), C) spring tillage (a combined cultivator). The soil from the field without ground cover plants cultivated in the conventional cultivation was the control.

In each study year the way of sampling the soil and making a laboratory microbiological analysis of it were in accordance with the method described by Patkowska [2012] and Martyniuk et al. [1991]. The total bacteria population was established on the medium Nutrient Agar. In the case of *Bacillus* spp. bacteria, the medium of Tryptic Soy Agar was used, while for *Pseudomonas* spp. the medium Pseudomonas Agar F was used. The total fungi population in each sample was determined on Martin's medium. The populations of bacteria and fungi colonies were calculated per 1g of the soil dry mass.

Among the microorganisms colonies isolated from the soil, in each studied year 300 isolates of *Bacillus* spp. and *Pseudomonas* spp. were tested from each as well as all

fungi isolates from the genera *Gliocladium*, *Penicillium* and *Trichoderma* with the aim of determining their antagonistic effect towards *Alternaria alternata*, *A. dauci*, *A. radicina*, *Fusarium culmorum*, *F. oxysporum*, *F. solani*, *Pythium irregulare*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*. The antagonistic effect of the bacteria was studied according to the method described by Martyniuk et al. [1991] and Patkowska and Konopiński [2013]. The scale of the antagonistic effect of bacteria comprised five degrees, i.e. 0º – no inhibition zone, 1º – inhibition zone of 1–2 mm, 2º – inhibition zone of 3–5 mm, 3º – inhibition zone of 6–10 mm, 4º – 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: 0º – no fungus growth inhibition, 1º – colony growth inhibited to 20%, 2º – colony growth inhibited to 50%, 3º – colony growth inhibited to 80%, 4º – 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.

Results concerning the population of microorganisms were statistically analyzed using variance analysis. The significance of differences between the means was studied using Tukey's confidence intervals. Statistical calculations were carried out using Statistica program, version 7.1.

RESULTS AND DISCUSSION

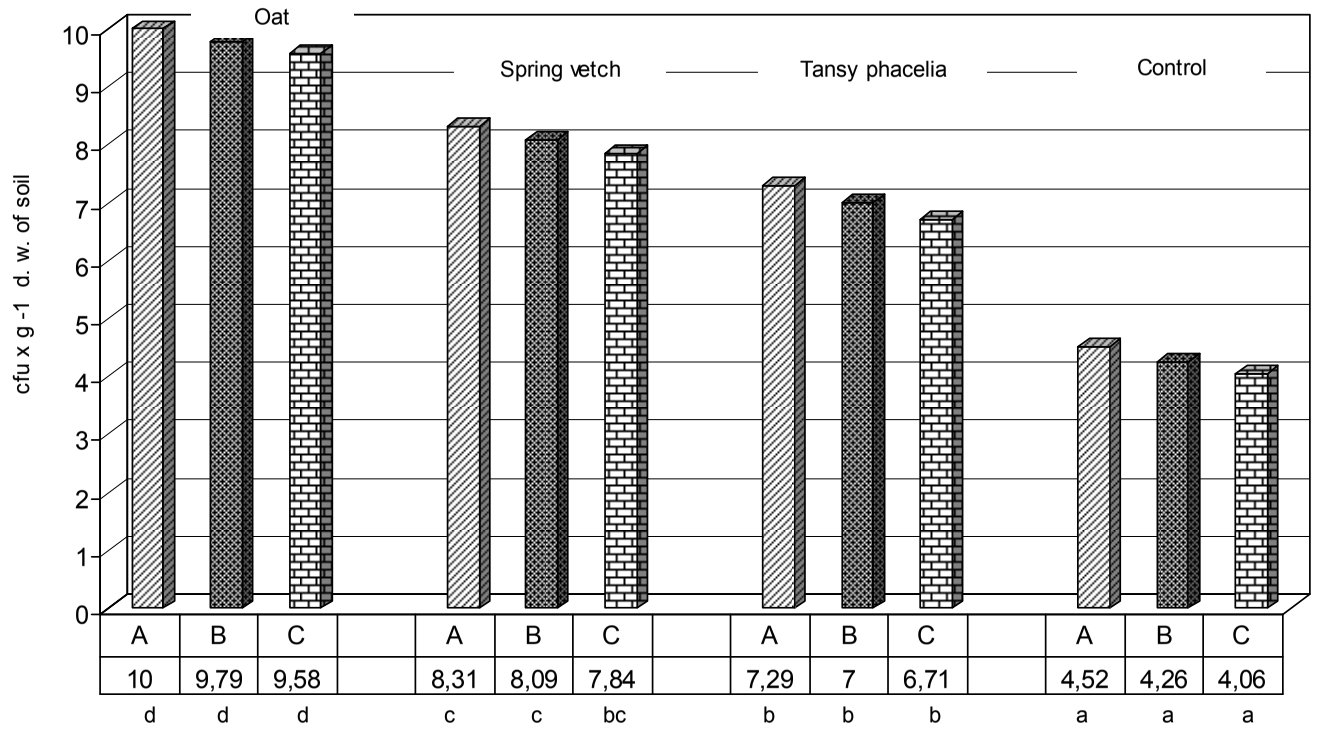
Varied quantitative and qualitative composition of soil microorganisms was found out on the basis of the laboratory mycological analysis of the soil samples from the plough layer of particular experimental combinations (tab. 1). The total population of bacteria in particular years of studies ranged from 3.55×10^6 to 10.98×10^6 cfu in 1 g of soil d.w. The greatest population of total bacteria occurred in 1 g of soil d.w. from the experimental combination where oat was the ground cover plant (on average from 9.58×10^6 to 10×10^6 cfu) (fig. 1). A lot of total bacteria were also obtained from the samples of soil when spring vetch constituted the mulch. The smallest population of bacteria colonies (on average, from 3 years of studies) was obtained in the control (from 4.06×10^6 to 4.52×10^6 cfu) (fig. 1). The system of cultivation had no significant effect on the population of bacteria in the soil. According to Pięta and Bełkot [2002], ground cover crops such as tansy phacelia, white mustard and winter wheat can stimulate the growth and development of bacteria in the soil.

The use of oat and spring vetch as ground cover plants in the cultivation of carrot had a positive effect on the population of bacteria from the genera *Bacillus* and *Pseudomonas* in the soil. The total population of *Bacillus* spp. in particular years of studies ranged from 1.31×10^6 to 7.52×10^6 cfu, and the total population of *Pseudomonas* spp. ranged from 0.08×10^6 to 3.25×10^6 cfu in 1 g of soil d.w. (tab. 1). The greatest number of colonies of *Bacillus* spp. (on average from 6.13×10^6 to 6.31×10^6 cfu) and *Pseudomonas* spp. (on average from 2.69×10^6 to 2.91×10^6 cfu) were observed in the soil after the mulch of oat (fig. 2). Those bacteria genera had small populations in the control (*Bacillus* spp. – on average from 1.97×10^6 to 2.37×10^6 cfu; *Pseudomonas* spp. –

Table 1. Number of bacteria and fungi isolated from soil in individual experiments in 2010–2012

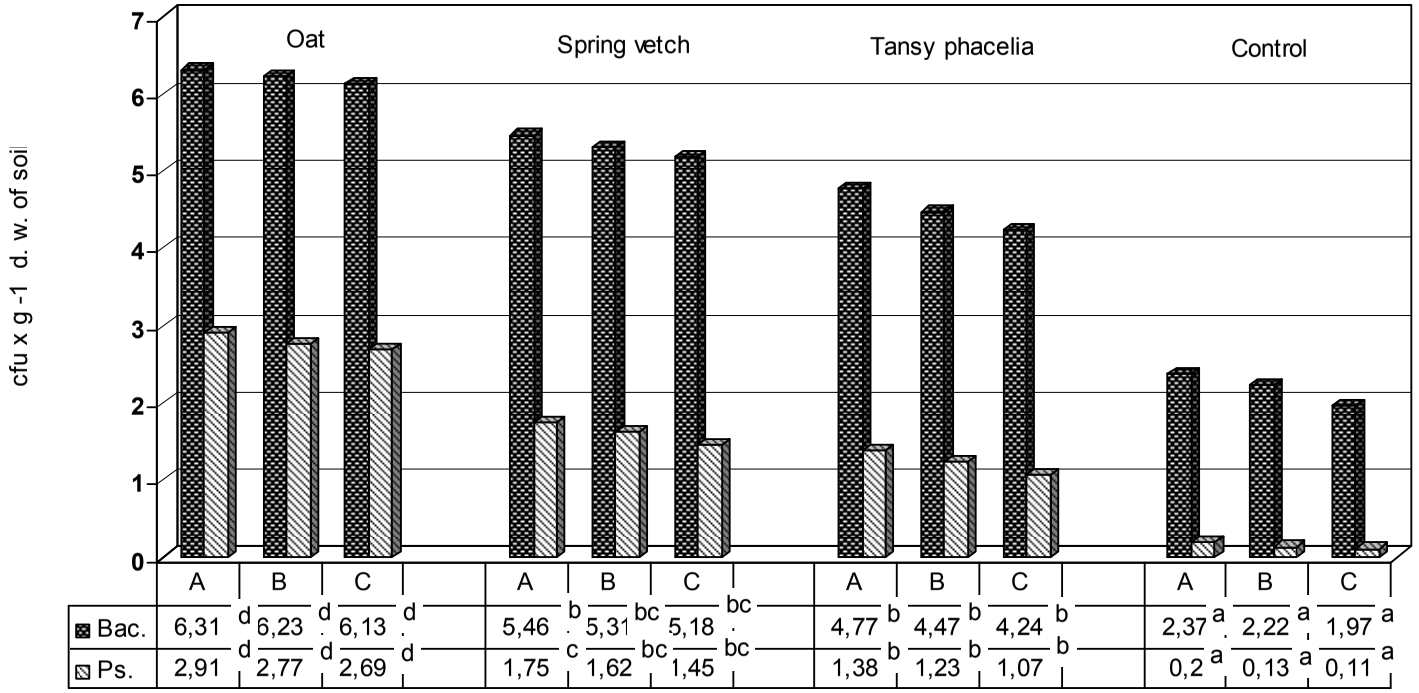
Experimental combination		Total number cfu of bacteria (mln·g^{-1} d.w. of soil)			Number cfu of *Bacillus* spp. (mln·g^{-1} d.w. of soil)			Number cfu of *Pseudomonas* spp. (mln·g^{-1} d.w. of soil)			Total number cfu of fungi (thous.·g^{-1} d.w. of soil)		
Cover crop	soil cultivation	2010	2011	2012	2010	2011	2012	2010	2011	2012	2010	2011	2012
Oat	A	9.84^{e*}	10.98^{d}	9.17^{c}	6.25^{d}	7.52^{d}	5.16^{d}	2.35^{d}	3.14^{d}	3.25^{d}	27.38^{a}	40.04^{a}	36.55^{a}
	B	9.62^{c}	10.64^{d}	9.10^{c}	6.18^{d}	7.40^{d}	5.10^{d}	2.10^{d}	3.10^{d}	3.10^{d}	30.15^{ab}	44.27^{a}	40.00^{b}
	C	9.15^{c}	10.55^{d}	9.04^{c}	6.09^{d}	7.25^{d}	5.04^{d}	2.03^{d}	3.00^{d}	3.04^{d}	36.17^{b}	48.58^{a}	42.26^{b}
Spring vetch	A	8.10^{bc}	9.16^{c}	7.66^{bc}	5.22^{c}	6.84^{cd}	4.32^{c}	1.20^{c}	1.92^{c}	2.12^{c}	44.50^{c}	62.47^{b}	53.17^{c}
	B	7.95^{bc}	8.88^{bc}	7.43^{bc}	5.15^{bc}	6.62^{cd}	4.15^{bc}	1.18^{bc}	1.66^{c}	2.03^{c}	46.27^{c}	65.60^{b}	56.25^{c}
	C	7.66^{bc}	8.70^{bc}	7.15^{b}	5.03^{b}	6.43^{c}	4.08^{bc}	1.05^{bc}	1.45^{bc}	1.84^{bc}	49.83^{c}	69.61^{b}	59.44^{c}
Tansy phacelia	A	6.88^{b}	8.06^{b}	6.94^{b}	4.12^{b}	6.22^{c}	3.97^{b}	1.02^{bc}	1.32^{b}	1.80^{bc}	56.14^{cd}	70.61^{bc}	52.48^{c}
	B	6.45^{b}	7.90^{b}	6.66^{b}	4.05^{b}	5.81^{b}	3.55^{b}	0.98^{b}	1.15^{b}	1.55^{b}	59.25^{d}	72.50^{bc}	55.61^{c}
	C	6.16^{b}	7.59^{b}	6.37^{b}	3.94^{b}	5.59^{b}	3.20^{b}	0.84^{b}	1.05^{b}	1.32^{b}	62.18^{e}	74.35^{c}	58.80^{c}
Conventional cultivation (control)	A	3.96^{a}	4.90^{a}	4.69^{a}	2.02^{a}	3.47^{a}	1.63^{a}	0.16^{a}	0.20^{a}	0.24^{a}	90.15^{f}	94.62^{d}	84.25^{d}
	B	3.71^{a}	4.65^{a}	4.42^{a}	1.90^{a}	3.28^{a}	1.49^{a}	0.10^{a}	0.18^{a}	0.12^{a}	90.52^{f}	98.11^{d}	86.67^{d}
	C	3.55^{a}	4.27^{a}	4.35^{a}	1.55^{a}	3.06^{a}	1.31^{a}	0.08^{a}	0.16^{a}	0.09^{a}	96.46^{f}	99.78^{d}	88.34^{d}

* Means in columns followed by the same letter do not differ significantly at $p \leq 0.05$
A – tillage before winter (ploughing) and spring tillage (a combined cultivator)
B – tillage before winter (a grubber) and spring tillage (a combined cultivator)
C – spring tillage (a combined cultivator)



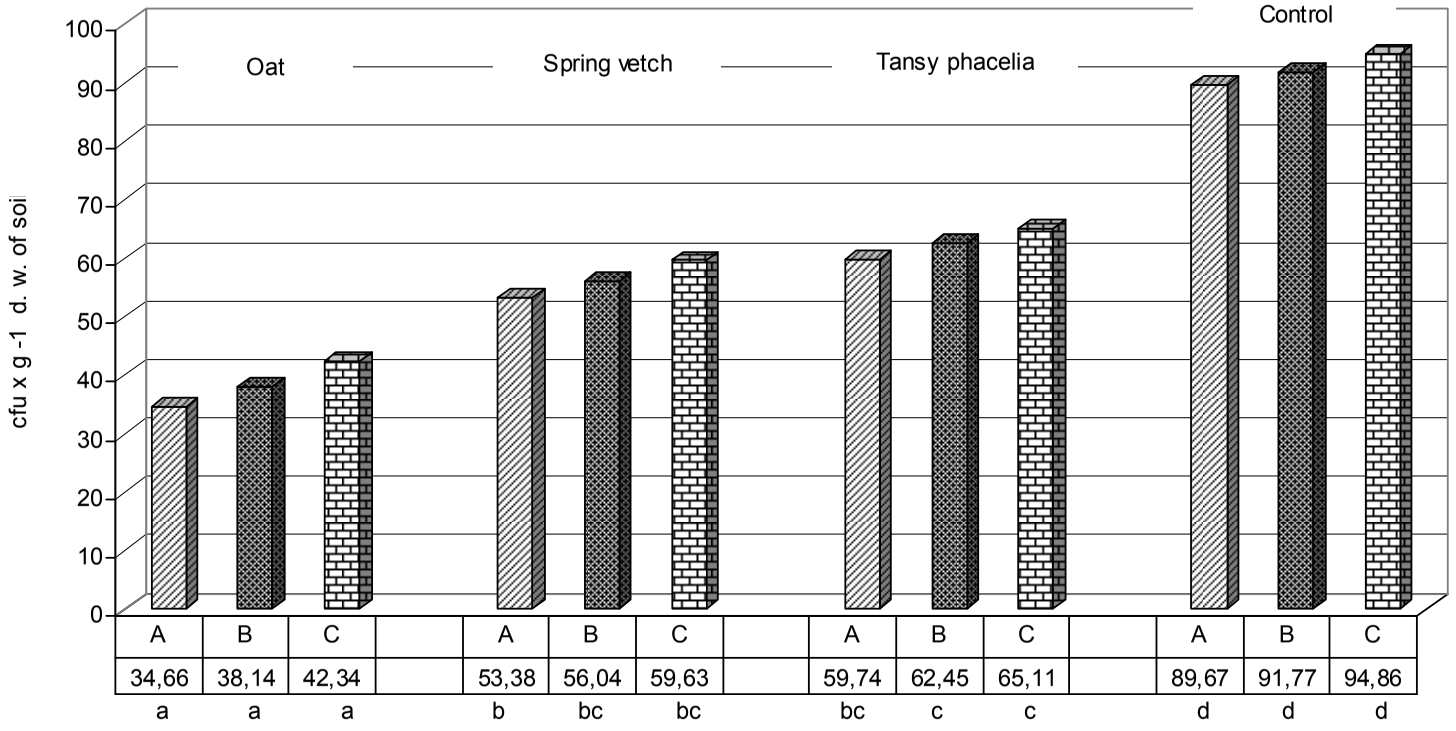
A – tillage before winter (ploughing) and spring tillage (a combined cultivator), B – tillage before winter (a grubber) and spring tillage (a combined cultivator), C – spring tillage (a combined cultivator), * means differ significantly ($p < 0.05$), if they are not marked with the same letter

Fig. 1. Total number of bacteria isolated from the soil in individual experimental combinations (means from the years 2010–2012 in mln · g^{-1} d.w. of soil)



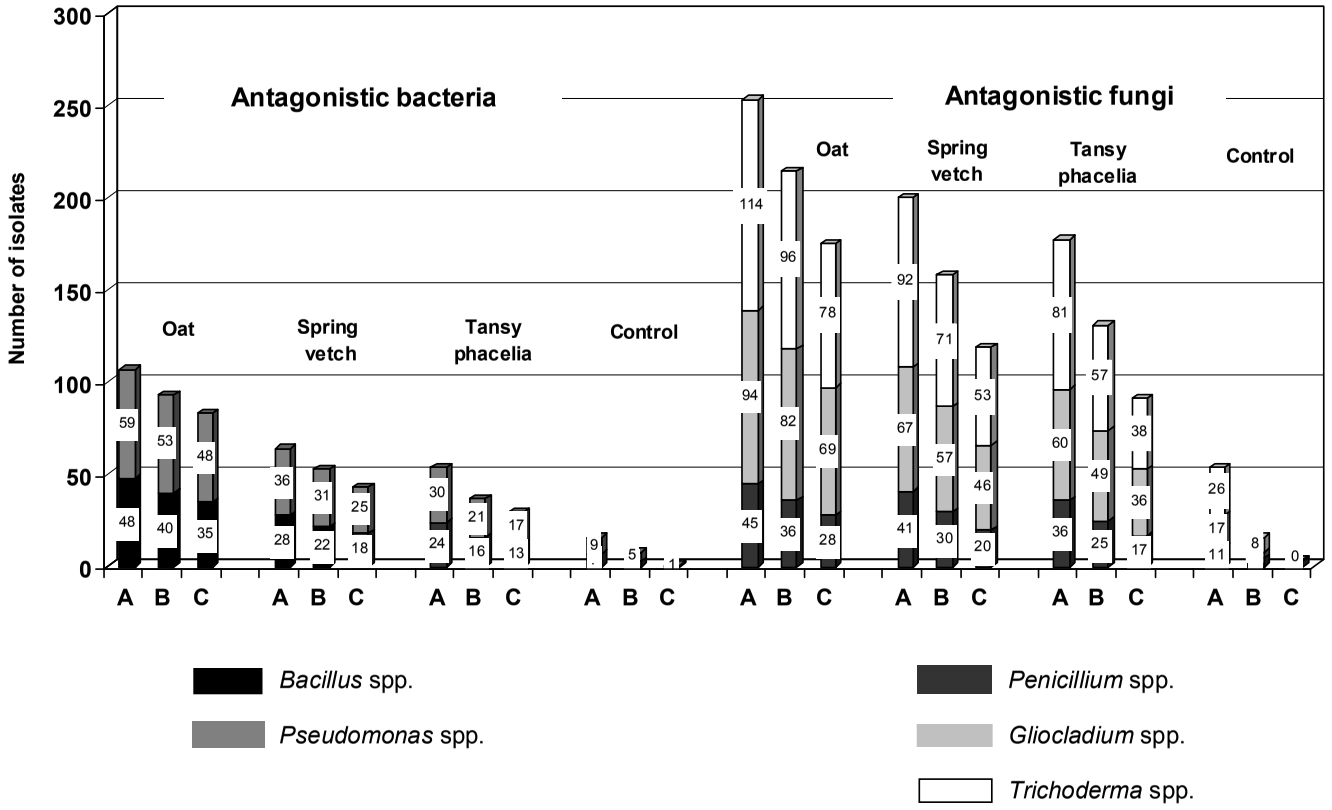
Bac. – *Bacillus* spp., Ps. – *Pseudomonas* spp.
A – tillage before winter (ploughing) and spring tillage (a combined cultivator), B – tillage before winter (a grubber) and spring tillage (a combined cultivator), C – spring tillage (a combined cultivator), * means differ significantly ($p < 0.05$) if they are not marked with the same letter

Fig. 2. Total number of *Bacillus* spp. and *Pseudomonas* spp. isolated from the soil in individual experimental combinations (means from the years 2010–2012 in mln · g^{-1} d.w. of soil)



A – tillage before winter (ploughing) and spring tillage (a combined cultivator), B – tillage before winter (a grubber) and spring tillage (a combined cultivator), C – spring tillage (a combined cultivator), * means differ significantly ($p < 0.05$) if they are not marked with the same letter

Fig. 3. Total number of fungi isolated from the soil in individual experimental combinations (means from the years 2010–2012 in thous. · g^{-1} d.w. of soil)



A – tillage before winter (ploughing) and spring tillage (a combined cultivator); B – tillage before winter (a grubber) and spring tillage (a combined cultivator); C – spring tillage (a combined cultivator)

Fig. 4. Number of antagonistic bacteria and fungi towards pathogenic fungi in individual experimental combinations (sum from the years 2010–2012)

Table 2. Fungi frequently isolated from the soil in individual experimental combinations (sum 2010–2012)

Fungus species	Number of isolates / Experimental combination												Total
	Oat			spring vetch			tansy phacelia			control			
	A	B	C	A	B	C	A	B	C	A	B	C	
Alternaria alternata (Fr.) Keissler	–	1	4	2	6	8	4	8	9	14	18	24	98
Alternaria chartarum Preuss	–	–	–	–	–	–	–	–	1	2	3	5	11
Alternaria dauci (Kühn) Groves & Skolko	3	5	8	7	10	13	8	12	15	20	28	36	165
Alternaria radicina Meier, Drechsler & Eddy	–	–	–	–	–	–	1	1	2	9	13	16	42
Cladosporium cladosporioides (Fres) de Vries	–	–	–	1	–	1	–	2	6	11	17	24	62
Cylindrocarpon didymum (Harting) Wollenw.	–	–	–	–	–	–	–	2	6	7	12	16	43
Epicoccum purpurascens Ehrenb. & Schleht.	–	–	1	1	2	5	2	5	10	15	21	27	89
Fusarium avenaceum (Corda ex Fr.) Sacc.	1	2	4	3	5	8	5	7	11	16	20	27	109
Fusarium culmorum (W.G.Sm.) Sacc.	5	6	11	10	12	16	13	16	20	31	40	48	228
Fusarium oxysporum Schl.	9	12	17	13	17	25	16	21	30	49	58	71	338
Fusarium solani (Mart.) Sacc.	–	1	3	6	11	13	10	14	19	29	40	48	194
Gliocladium catenulatum Gilman et Abbott	40	36	30	25	22	18	21	19	14	6	1	–	232
Gliocladium fimbriatum Gilman et Abbott	30	26	22	23	19	15	22	18	13	7	2	1	198
Gliocladium roseum (Link) Bainier	24	20	17	19	16	13	17	12	9	4	–	–	151
Mucor hiemalis Wehmer	–	–	–	–	–	–	–	2	4	7	10	15	38
Mucor racemosus Fresenius	–	–	2	–	2	2	2	2	6	4	9	17	46
Penicillium canescens Scopp.	6	5	3	5	4	3	5	4	2	2	–	–	39
Penicillium chrysogenum Thom	8	7	5	8	6	5	7	5	2	2	1	1	57
Penicillium meleagrinum Biourge	–	–	–	–	–	–	–	–	3	–	–	–	3
Penicillium verrucosum Dierckx var. *cyclopium* (West.) Samson, Stolk et Hadlok	16	13	11	14	11	7	12	9	6	4	3	2	108
Penicillium verrucosum Dierckx var. *verrucosum* Samson, Stolk et Hadlok	15	11	9	14	9	5	12	7	4	3	1	–	90
Phoma exigua Desm.	–	–	–	–	1	2	2	2	4	7	8	10	36
Pythium irregulare Buisman	–	–	1	–	1	5	2	5	11	16	23	31	95
Rhizoctonia solani Kühn	4	7	11	7	12	16	10	17	22	24	35	48	213
Rhizopus nigricans Ehrenberg	–	–	1	–	–	2	–	1	5	5	8	14	36
Sclerotinia sclerotiorum (Lib.) de Bary	–	–	–	–	–	–	1	4	7	21	33	41	107
Trichoderma aureoviride Rifai	15	11	9	14	8	7	12	7	5	4	–	–	92
Trichoderma hamatum (Bon.) Bain.	8	6	5	7	5	3	6	4	1	1	–	–	46
Trichoderma harzianum Rifai	15	14	12	12	10	9	10	8	6	3	2	–	101
Trichoderma koningii Oud.	37	31	24	30	23	16	26	18	13	6	3	–	227
Trichoderma viride Pers. ex. S.F. Gray	39	34	28	29	25	18	27	20	13	12	3	–	248
Total	275	248	238	250	237	235	253	252	279	341	412	522	3542

A – tillage before winter (ploughing) and spring tillage (a combined cultivator); B – tillage before winter (a grubber) and spring tillage (a combined cultivator); C – spring tillage (a combined cultivator)

on average from 0.11×10^6 to 0.2×10^6 cfu) (fig. 2). The system of cultivation rather had no significant effect on the population of those bacteria in the soil. According to Patkowska and Konopiński [2013], leaving a mulch of oat and tansy phacelia on the soil surface had a positive effect on the development of *Pseudomonas* spp. in the soil from under the cultivation of scorzonera. Similar results on *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 population of fungi in the analyzed combination of the experiment ranged from 27.38×10^3 to 99.78×10^3 cfu (tab. 1). The total number of fungi was highest in 1 g d.w. of the control soil, i.e. cultivated without any ground cover plants (on average, from 89.67×10^3 to 94.86×10^3 cfu) (fig. 3). Much fewer colonies of fungi as compared to the control occurred in the soil sampled from the experimental combination where oat, tancy phacelia or spring vetch were the ground cover plants. The way of managing the mulch had no significant effect on the population of fungi in the soil. As reported by Pięta and Kęsik [2007], the use of spring vetch or spring rye in the conservation tillage of onion also had a positive effect on the biological activity of the soil through decreasing the population of soil-borne fungi. The use of oat, spring vetch and tansy phacelia in the cultivation of scorzonera limited the development of the population of soil fungi [Patkowska and Konopiński 2013].

The species composition of fungi isolated from the soil of particular experimental combinations varied (tab. 2). Among the fungi considered to be pathogenic, species from the genera *Alternaria*, *Fusarium*, *Phoma*, *Pythium*, *Rhizoctonia* and *Sclerotinia* were isolated. *Fusarium oxysporum* proved to be the dominating one (total of 338 isolates). The highest number of isolates of the enumerated fungi were isolated from the conventionally cultivated soil and after the mulch of tansy phacelia plants (tab. 2). Ground cover plants, which are used, for example, in the cultivation of vegetables significantly limit the activity of pathogens in the soil [Lemańczyk and Sadowski 2002, Mazur and Nawrocki 2007, Nawrocki and Mazur 2011a]. They influence the fungi communities through root exudates or products of the decomposition of crop residues [Pięta and Patkowska 2001, Gregory 2006]. Vitamins, metal ions, organic acids, phenolic acids, and especially sugars (glucose) and aminoacids found in the root exudates considerably modify the composition of the communities of soil microorganisms [Koo et al. 2005, Tamás et al. 2005, Bais et al. 2006, Steinkellner et al. 2007, 2008]. Acidic amino acids (aspartic acid and glutamic acid) have a positive effect on the growth and development of pathogenic soil-borne fungi such as *Fusarium culmorum*, *F. oxysporum*, *F. solani*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*. Aromatic amino acids (tyrosine, phenylalanine) and basic amino acids (lysine, histidine, arginine) inhibit the growth of pathogenic fungi [Pięta and Patkowska 2001]. Therefore, they have fungistatic and bacteriostatic properties [Hirsch et al. 2003, Walker et al. 2003, Badri and Vivanco 2009]. Hence, the proper choice of plants used in crop rotation and intended for green manure has a modifying effect on the populations of bacteria and fungi occurring in the soil environment.

The application of oat, spring vetch and tansy phacelia as ground cover plants contributed to an increased number of antagonistic microorganisms as compared to the cultivation without any ground cover plants (fig. 4). The test showed that most antago-

nistic *Bacillus* spp. and *Pseudomonas* spp. occurred in the soil after the mulch of oat (total of 123 and 160 isolates, respectively), while the least in the conventional cultivation of carrot (total of 15 and 1 isolates, respectively). Most antagonistic *Gliocladium* spp., *Penicillium* spp. and *Trichoderma* spp. were observed after managing the oat and spring vetch mulch (in allcultivation systems) (fig. 4).

Antagonistic fungi occurring in the soil play a positive role in the cultivation of root vegetables since they protect, for example, carrot from infection by *Alternaria alternata*, *A. dauci*, *A. radicina* [Mazur and Nawrocki 2007, Nawrocki and Mazur 2011a, 2011b]; scorzonera from *A. scorzonerae*, *R. solani*, *S. sclerotiorum*, *Phytophthora* spp. [Patkowska and Konopiński 2008a]; root chicory from *Alternaria cichori*, *Phytophthora* sp., *F. culmorum*, *F. solani*, *Phoma cichoracearum*, *Ramularia cichorii*, *Sclerotium rolfsii*, *Thielaviopsis basicola*, *Verticillium daliae* [Patkowska and Konopiński 2008b], while salsify from *A. alternata, F. culmorum, F. oxysporum, R. solani* and *S. sclerotiorum* [Patkowska and Konopiński 2011]. According to Pięta et al. [2003], Pięta and Kęsik [2007] and Patkowska [2012], antagonistic saprophytic microorganisms occurring in the soil, especially in the root zone, protect plant roots from infection by pathogenic fungi and bacteria, which is especially important in the cultivation of root vegetables.

CONCLUSIONS

1. Using a mulch of oat and spring vetch has the best effect on the communities of soil microorganisms in the cultivation of carrot.
2. Oat, spring vetch and tansy phacelia used as ground cover crops increased population of antagonistic microorganisms.
3. The tillage before winter (ploughing) and spring tillage (a combined cultivator), tillage before winter (a grubber) and spring tillage (a combined cultivator), and spring tillage (a combined cultivator) had no significant effect on the population of bacteria and fungi in the soil.

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ZBIOROWISKA MIKROORGANIZMÓW W GLEBIE SPOD UPRAWY MARCHWI (*Daucus carota* L.)

Streszczenie. Skład populacji mikroorganizmów glebowych zależy od wielu czynników, m.in. od gatunku międzyplonowych roślin okrywowych. Celem badań było określenie składu ilościowego i jakościowego populacji drobnoustrojów glebowych w uprawie marchwi z uwzględnieniem różnego sposobu uprawy roli oraz wykorzystaniem owsa, wyki siewnej i facelii błękitnej jako międzyplonowych roślin okrywowych. Najwięcej bakterii ogółem wystąpiło w glebie, gdzie rośliną okrywową był owies. Najwięcej grzybów ogółem stwierdzono w kontroli. Zastosowanie roślin okrywowych przyczyniło się do zwiększenia liczby antagonistycznych *Bacillus* spp., *Pseudomonas* spp., *Gliocladium* spp., *Penicillium* spp. i *Trichoderma* spp. System uprawy roli nie miał istotnego wpływu na liczebność mikroorganizmów w glebie.

Słowa kluczowe: owies, wyka siewna, facelia błękitna, antagonistyczne bakterie i grzyby

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