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The effect of cover crops on the fungal and bacterial communities in the soil under carrot cultivation

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ABSTRACT

The purpose of the study was to establish quantitative and qualitative composition of soil-borne microorganisms in the cultivation of carrot. The experiment considered rye, white mustard, buckwheat and sunflower as cover crops and three systems of cultivation. The population of bacteria and fungi having an antagonistic effect towards selected fungi pathogenic to carrot was determined. The greatest total population of bacteria as well as *Bacillus* spp. and *Pseudomonas* spp. was observed in the soil when rye was the cover crop. The greatest quantity of fungi was found in the control, a slightly smaller amount – after using sunflower, buckwheat and white mustard. The system of tillage had no effect on the communities of the investigated microorganisms. Rye and white mustard had the most positive effect on the quantity of antagonistic *Bacillus* spp., *Pseudomonas* spp., *Clonostachys* spp., *Myrothecium* spp., *Penicillium* spp. and *Trichoderma* spp. Regardless of the tillage system, the smallest quantity of antagonistic microorganisms occurred in the conventional cultivation of carrot.

Keywords: *Secale cereale*; *Sinapis alba*; *Fagopyrum esculentum*; *Helianthus*; soil environment

The occurrence of microorganisms in soil depends, among others, on the species of the cultivated cover crops (Patkowska and Konopiński 2013a, 2014, Patkowska and Błażewicz-Woźniak 2014). Moreover, those plants protect soil from negative external factors, they enrich it with organic substances and mineral nutrients in addition to protecting it from evaporation and surface runoff. As green fertilizers and living mulches ploughed over before winter, they fertilize the soil, thus increasing the plants' yielding (Kołota and Adamczewska-Sowińska 2013). Mulching the soil with cover crops decreases the field weed infestation since some of them contain allelopathic compounds inhibiting the germination of weed seeds and limiting their further growth (Głowacka 2010). These plants produce phytoncides which affect soil microorganisms and limit the occurrence of pathogens.

Oats, rye, common vetch and tansy phacelia as cover crops used in the cultivation of vegetables stimulate the development of antagonistic bacteria *Pseudomonas* spp. and *Bacillus* spp. as well as saprotrophic fungi, which have an antagonistic effect on, for example, *Trichoderma* spp. (Patkowska and Konopiński 2013a, 2014, Patkowska and Błażewicz-Woźniak 2014). They have a positive effect on healthiness of root vegetables by considerably decreasing the infection of roots of the seedlings and later older plants by *Alternaria alternata*, *Fusarium oxysporum*, *F. culmorum*, *Thanatephorus cucumeris*, *Sclerotinia sclerotiorum* (Patkowska and Konopiński 2011, 2013b). Therefore, they can also be used to protect carrot from alternariosis, fusariosis, rhisoctoniosis and *Sclerotinia sclerotiorum*.

The purpose of the studies was to determine the effect of rye, white mustard, buckwheat and

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sunflower as cover crops on the occurrence of bacteria and fungi in the soil under carrot cultivation.

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, district of Lublin (22°56'E, 51°23'N, Central Eastern Poland, 200 m a.s.l.), 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 sampled each year from a depth of 5–6 cm of the plough layer of the field where carrot (*Daucus carota* L.) cv. Flakkee 2 was cultivated. The experiment took into consideration the cover crops such as rye, white mustard, buckwheat and sunflower. The experiment used three systems of soil tillage, i.e. (A) tillage before winter (ploughing) and spring tillage (combined cultivator); (B) tillage before winter (grubber) and spring tillage (combined cultivator); (C) spring tillage (combined cultivator). The soil from the field without cover crops cultivated was the control.

Microbiological analysis was made according to the method described by Czaban et al. (2007). The soil was sampled from each experimental combination from four randomly chosen places. The total population of bacteria was marked on the Nutrient agar. In the case of bacteria from genus *Bacillus*, Tryptic soy agar was used, whereas *Pseudomonas* agar F was used for *Pseudomonas* spp. For isolation of *Bacillus* spp. soil dilutions were heated for 20 min at 80°C. Martin's medium was used to establish fungi number. After the incubation, the number of bacteria and fungi was converted into CFU/g of soil DW (colony forming units/g dry weight (DW) of soil).

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 *Clonostachys*, *Myrothecium*, *Penicillium* and *Trichoderma* with the aim of determining their antagonistic effect towards *Alternaria alternata*, *A. dauci*, *A. radicina*, *Fusarium culmorum*, *F. oxysporum*, *Fusarium solani*, *Sclerotinia sclerotiorum* and *Thanatephorus cucumeris*. These fungi were isolated from the infected carrot plants. The antagonistic effect of the bacteria was studied according to the method described by Martyniuk et al. (1991) and Patkowska and Konopiński (2013a). 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 populations of microorganisms were statistically analysed 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 the Statistica program, version 6.0 (StatSoft, Krakow, Poland).

RESULTS AND DISCUSSION

The mean total population of bacteria in particular years of studies was on average from 4.06×10^6 to 9.40×10^6 CFU/g of soil DW (Table 1). The mean population of *Bacillus* spp. ranged from 1.97×10^6 to 6.15×10^6 CFU/g, while the mean population of *Pseudomonas* spp. ranged from 0.11×10^6 to 2.49×10^6 CFU/g. The largest population of those bacteria was found in the soil environment of carrot when rye was used as a cover crop and it differed in a statistically significant way from the populations in the other experimental treatments. The population of bacteria in the soil was smaller when white mustard, buckwheat and sunflower were as cover crops. The total population of fungi ranged from 46.55×10^3 to 94.86×10^3 CFU/g, on average. The largest population of fungi was observed in the control, while it was a little smaller after using sunflower, buckwheat and white mustard as cover crops. Similar relations in the composition of soil-borne microorganisms were observed after mulching the soil with oats and common vetch in the cultivation of carrot (Patkowska and Błażewicz-Woźniak 2014), scorzonera (Patkowska and Konopiński 2013a) and onion (Pięta and Kęsik 2007). The present studies showed that after using rye, the fungi population was twice as low as in the control and it differed in a statistically significant way from the population in the other experimental treatments. The system of tillage had no effect on the communities of the examined microorganisms.

During the three years of studies, 5032 isolates of fungi belonging to 28 species were isolated (Table 2). The most frequent ones included *Fusarium oxysporum*, *F. culmorum*, *Thanatephorus cucumeris*,

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

Experimental treatment		Total CFU of bacteria (10^6/g DW of soil)				CFU of *Bacillus* spp. (10^6/g DW of soil)				CFU of *Pseudomonas* spp. (10^6/g DW of soil)				Total CFU of fungi (10^3/g DW of soil)			
Cover crop	soil cultivation	2010	2011	2012	mean	2010	2011	2012	mean	2010	2011	2012	mean	2010	2011	2012	mean
Rye	A	9.12^{d}	10.06^{e}	9.03^{c}	9.40^{d}	6.08^{d}	7.24^{d}	5.12^{d}	6.15^{c}	2.02^{g}	2.81^{g}	2.65^{e}	2.49^{e}	38.11^{a}	51.49^{a}	44.06^{a}	46.55^{a}
	B	8.71^{d}	9.68^{e}	8.55^{c}	8.98^{d}	6.02^{d}	7.09^{d}	4.87^{d}	5.99^{c}	1.90^{g}	2.42^{f}	2.31^{e}	2.21^{e}	40.24^{a}	54.67^{a}	47.20^{a}	47.37^{a}
	C	8.53^{d}	9.55^{e}	8.26^{c}	8.78^{d}	5.54^{d}	6.90^{cd}	4.60^{d}	5.68^{c}	1.24^{f}	2.08^{f}	2.18^{e}	1.83^{e}	42.66^{a}	56.32^{a}	50.64^{a}	49.87^{a}
White mustard	A	5.80^{c}	6.84^{d}	6.23^{bc}	6.29^{c}	3.18^{bc}	5.16^{c}	3.00^{c}	3.78^{b}	0.76^{e}	0.91^{ef}	1.20^{d}	0.96^{c}	66.51^{b}	76.32^{b}	62.55^{b}	68.46^{b}
	B	5.63^{c}	6.51^{d}	5.98^{b}	6.04^{c}	3.10^{bc}	4.73^{b}	2.86^{bc}	3.56^{b}	0.55^{d}	0.73^{e}	1.11^{d}	0.80^{c}	68.24^{b}	80.04^{b}	66.34^{b}	71.54^{b}
	C	5.37^{c}	6.38^{cd}	5.71^{b}	5.82^{c}	2.86^{b}	4.54^{b}	2.33^{b}	3.24^{b}	0.42^{c}	0.56^{d}	1.03^{d}	0.67^{c}	71.00^{b}	82.17^{b}	69.70^{b}	74.29^{b}
Buck-wheat	A	4.88^{bc}	5.99^{b}	5.54^{b}	5.47^{bc}	2.76^{b}	4.32^{b}	2.28^{b}	3.12^{b}	0.40^{c}	0.56^{d}	0.96^{d}	1.00^{d}	78.10^{c}	85.44^{b}	70.45^{b}	77.99^{b}
	B	4.53^{b}	5.71^{b}	5.20^{b}	5.15^{bc}	2.53^{b}	3.91^{bc}	2.09^{b}	2.84^{ab}	0.35^{bc}	0.38^{bc}	0.67^{c}	0.47^{b}	80.05^{c}	86.63^{b}	73.26^{b}	79.98^{bc}
	C	4.42^{b}	5.46^{b}	5.08^{b}	4.99^{b}	2.31^{ab}	3.70^{bc}	2.00^{b}	2.67^{ab}	0.31^{b}	0.26^{b}	0.38^{b}	0.32^{b}	81.14^{c}	87.14^{b}	75.80^{bc}	81.36^{bc}
Sunflower	A	4.30^{b}	5.50^{b}	5.32^{b}	5.04^{bc}	2.30^{ab}	4.28^{b}	2.14^{b}	2.91^{b}	0.30^{b}	0.43^{c}	0.85^{cd}	0.53^{bc}	82.60^{c}	88.27^{bc}	77.14^{bc}	82.67^{bc}
	B	4.26^{b}	5.31^{b}	5.18^{b}	4.92^{a}	2.24^{ab}	3.99^{bc}	1.95^{ab}	2.73^{ab}	0.24^{ab}	0.31^{b}	0.56^{b}	0.37^{b}	85.45^{cd}	90.40^{bc}	80.05^{c}	85.30^{bc}
	C	4.15^{ab}	5.22^{ab}	5.00^{b}	4.79^{a}	2.11^{ab}	3.75^{a}	1.77^{ab}	2.54^{ab}	0.18^{a}	0.25^{b}	0.32^{b}	0.25^{a}	89.03^{d}	92.55^{bc}	82.33^{c}	87.97^{bc}
Conventional cultivation (control)	A	3.96^{a}	4.90	4.69^{ab}	4.52^{a}	2.02^{a}	3.47^{a}	1.63^{a}	2.37^{a}	0.16^{a}	0.20^{a}	0.24^{ab}	0.20^{a}	90.15^{d}	94.62^{c}	84.25^{c}	89.67^{c}
	B	3.71^{a}	4.65^{a}	4.42^{a}	4.26^{a}	1.90^{a}	3.28^{a}	1.49^{a}	2.22^{a}	0.10^{a}	0.18^{a}	0.12^{a}	0.13^{a}	90.52^{d}	98.11^{c}	86.67^{c}	91.76^{c}
	C	3.55^{a}	4.27^{a}	4.35^{a}	4.06^{a}	1.55^{a}	3.06^{a}	1.31^{a}	1.97^{a}	0.08^{a}	0.16^{a}	0.09^{a}	0.11^{a}	96.46^{d}	99.78^{c}	88.34^{c}	94.86c

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; (combined cultivator); B – tillage before winter (grubber) and spring tillage (combined cultivator); C – spring tillage (combined cultivator); DW – dry weight; CFU – colony forming units

Fusarium solani, *Sclerotinia sclerotiorum* and *Alternaria dauci.* The dominating saprotrophic fungi were *Clonostachys rosea*, *Trichoderma* spp. (mainly *T. koningii* and *T. viride*), *Myrothecium verrucaria*, *Epicoccum nigrum* and *Rhizopus stolonifer*. Rye and white mustard showed the most positive effect on the quantitative and qualitative composition of fungi. The smallest population of fungi considered being pathogenic and the highest population of fungi with antagonistic properties were isolated from those experimental treatments. Buckwheat and sunflower as cover crops also showed a protective effect in the cultivation of carrot as in those treatments of the experiment the population of soil-borne fungi was smaller than in the control. When white mustard, buckwheat and sunflower were the cover crops, the soil cultivation before winter (ploughing) and spring tillage (combined cultivator) – system A – proved the most effective. The population of isolated fungi in this system was the smallest. When rye was the cover crop, spring tillage of the soil (combined cultivator) – system C – had the most positive effect. As reported by Kołota and Adamczewska-Sowińska (2013), mulches limit the occurrence and development of phytopathogens in the cultivation of different vegetable species. Moreover, studies conducted by Patkowska and Konopiński (2011, 2013b) confirmed a positive effect of oats and common vetch on the communities of soil-borne fungi in the cultivation of salsify and root chicory.

Laboratory tests showed that rye and white mustard as cover crops had the most positive effect on the population of antagonistic bacteria (*Bacillus* spp. and *Pseudomonas* spp.) and fungi (*Clonostachys* spp., *Myrothecium* spp., *Penicillium* spp. and *Trichoderma* spp.) (Figure 1). After buckwheat and sunflower were used as the cover crops, a smaller population of microorganisms antagonistic towards the studied pathogenic fungi was observed

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

Fungus species	Number of isolates/experimental combination															Total
	rye			white mustard			buckwheat			sunflower			control			
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	
Alternaria alternata (Fr.) Keissler	–	2	6	6	10	12	9	11	15	10	13	17	14	18	24	**167**
Ulocladium chartarum Preuss) E.G. Simmons	–	–	–	–	1	2	1	2	3	1	2	4	2	3	5	**26**
Alternaria dauci (J.G. Kühn) J.G.Groves & Skolko	3	7	10	10	13	17	14	18	23	16	22	26	20	28	36	**263**
Alternaria radicina Meier, Drechsler & E.D. Eddy	–	–	–	2	2	5	6	7	9	8	10	10	9	13	16	**97**
Cladosporium cladosporioides (Fresen.) G.A. de Vries	–	–	–	2	6	9	4	8	15	8	13	17	11	17	24	**134**
Clonostachys rosea (Link) Schroers, Samuels, Seifert & W. Gams	54	48	38	38	28	19	30	23	14	27	18	11	10	1	–	**363**
Cylindrocarpon didymum (Harting) Wollenw.	–	–	–	2	5	8	4	7	10	5	8	12	7	12	16	**96**
Epicoccum nigrum Link	–	–	2	3	8	13	7	12	18	10	13	21	15	21	27	**170**
Fusarium culmorum (Wm.G.Sm.) Sacc.	6	9	13	15	21	26	19	26	32	24	30	39	31	40	48	**379**
Fusarium oxysporum E.F. Sm. & Swingle	11	15	19	23	31	39	29	42	49	36	47	53	49	58	71	**572**
Gibberella avenacea R.J. Cook	2	3	6	6	9	14	8	11	16	10	14	19	16	20	27	**181**
Fusarium solani (Mart.) Sacc.	1	5	8	14	20	28	19	27	34	22	33	39	29	40	48	**367**
Mucor hiemalis Wehmer	–	–	–	2	4	6	3	5	8	5	7	11	7	10	15	**83**
Mucor racemosus Bull.	–	–	1	–	–	8	2	4	12	3	7	14	4	9	17	**81**
Myrothecium verrucaria (Alb. et Schwein) Ditmar	28	23	19	20	15	10	16	12	8	16	10	5	7	2	1	**192**
Penicillium aurantiogriseum Dierckx	16	11	9	11	7	4	10	6	3	7	4	2	4	3	2	**99**
Penicillium canescens Scopp.	6	5	4	5	3	2	4	3	1	4	3	–	2	–	–	**42**
Penicillium chrysogenum Thom	8	6	4	6	7	6	6	6	1	4	3	2	2	1	1	**63**
Penicillium verrucosum Dierckx var. *verrucosum* Samson, Stolk et Hadlok	15	11	7	12	6	3	10	5	2	7	3	1	3	1	–	**86**
Rhizopus stolonifer (Ehrenb.) Vuill.	–	–	3	7	14	27	12	20	34	17	25	39	28	39	55	**320**
Sclerotinia sclerotiorum (Lib.) de Bary	–	–	–	1	5	11	7	12	18	11	20	29	21	33	41	**209**
Thanatephorus cucumeris (A.B. Frank) Donk	5	9	14	13	21	28	16	23	34	20	29	40	24	35	48	**359**
Trichoderma aureoviride Rifai	15	9	8	10	5	3	9	4	2	6	3	1	4	–	–	**79**
Trichoderma hamatum (Bonord.) Bainier	8	5	4	6	3	1	5	2	1	4	–	–	1	–	–	**40**
Trichoderma harzianum Rifai	14	13	12	9	7	5	8	6	4	8	6	3	3	2	–	**100**
Trichoderma koningii Oud.	35	29	22	25	16	12	19	13	8	17	12	8	6	3	–	**225**
Trichoderma viride Pers.	36	30	23	25	17	11	21	14	10	19	12	6	12	3	–	**239**
Total	**267**	**240**	**232**	**273**	**284**	**329**	**298**	**329**	**384**	**325**	**367**	**429**	**341**	**412**	**522**	**5032**

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

as compared to the use of rye and white mustard. Regardless of the system of tillage, the smallest population of antagonistic bacteria and fungi was observed in the conventional cultivation of carrot. The activity of soil-borne pathogens can be limited by the root exudates of phytosanitary plants which promote the development of antagonistic microorganisms (Patkowska 2009, Li et al. 2013).

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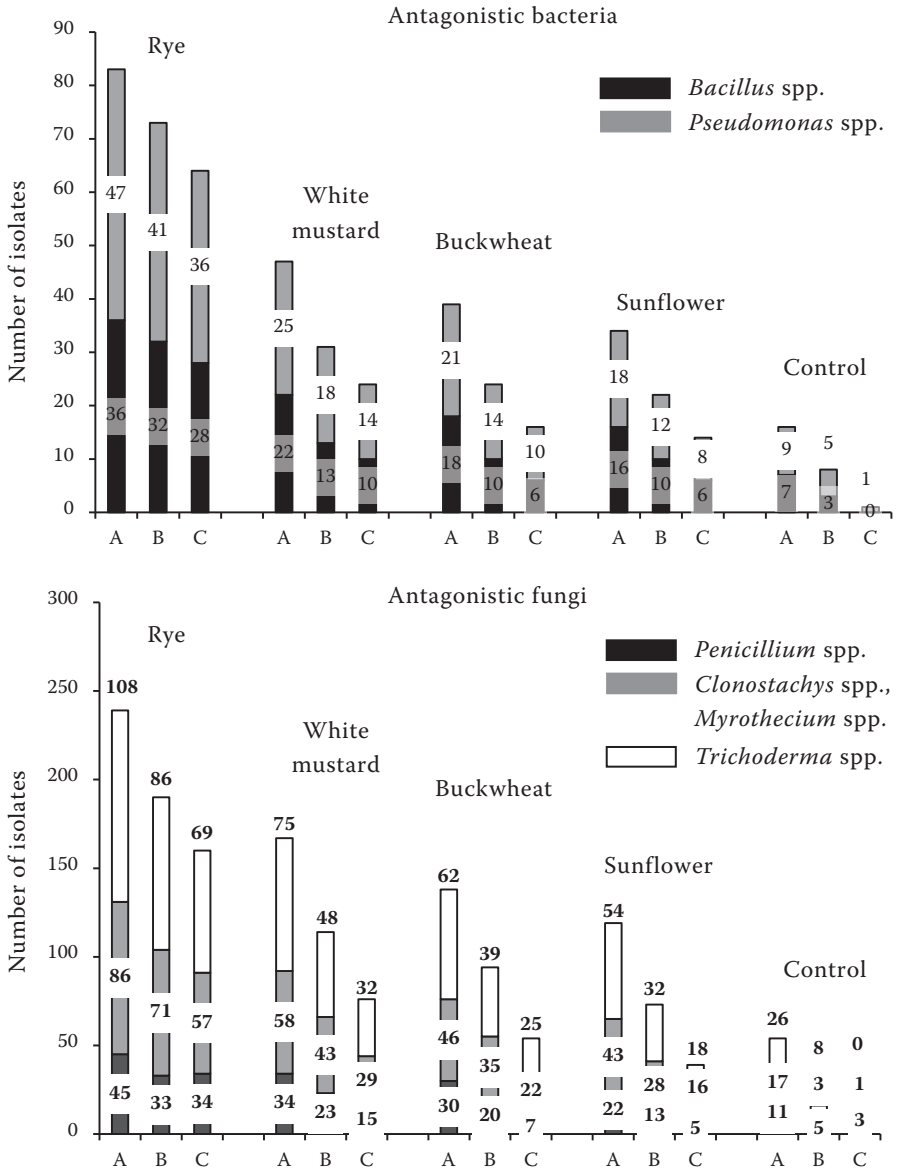

Figure 1. Number of antagonistic bacteria and fungi towards pathogenic fungi in individual experimental treatments (sum from 2010–2012). A – tillage before winter (ploughing) and spring tillage (combined cultivator); B – tillage before winter (grubber) and spring tillage (combined cultivator); C – spring tillage (combined cultivator)

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