

CHANGES IN SOIL MICROBIAL COMMUNITIES AS A RESULT OF GROWING BRASSICACEAE CROPS

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Received: 24.09.2009

Abstract

The study was conducted in 2006 – 2008 at the Production and Experimental Station of the University of Warmia and Mazury in Olsztyn, located in Bałcyny (NE Poland). The objective of this study was to determine the microbial quality of soil after Brassicaceae grown as forecrops for winter wheat. A field experiment was established on grey-brown podsolic soil, and it involved the following forecrops: winter rapeseed, spring rapeseed, white mustard, Chinese mustard, and winter wheat as control. Soil samples for microbiological analyses were collected in the spring, before the sowing of forecrops, and in the autumn, after the harvest of Brassicaceae and ploughing-in crop residues. Bacterial and fungal communities isolated from soil sown with Brassicaceae as forecrops were generally more abundant and diverse. These communities exerted an inhibitory effect on the growth of soil pathogens. Forecrops with the greatest microbiological potential were white mustard and winter rapeseed.

Key words: forecrops, wheat, Brassicaceae, soil, fungi, bacteria

INTRODUCTION

Cereal growing in monoculture contributes to a substantial yield decrease (Kurowski, 2002), which may be caused by changes in the abundance and biological activity of soil microbes. The growing of a single crop leads to the selection of soil-dwelling microorganisms – some species are eliminated, while the populations of other species expand (Bis et al. 2005). Changes that promote the growth of soil pathogens are particularly undesirable. The presence of crops showing phytosanitary effect in crop rotation, including species of the family Brassicaceae, may contribute to a decrease in the counts of pathogenic microorganisms and to an increase in the abundance of saprotrophs exhibiting antagonistic activity against soil pathogens (Sturz and Bernier, 1989).

Soil-born microbes include species with antagonistic properties against phytopathogens, such as fungi of the genera *Trichoderma*, *Gliocladium* and *Penicillium* as well as bacteria of the genera *Pseudomonas* and *Azotobacter* (Pięta, 1998; Garbeva et al. 2004). The phytosanitary quality of soil may be evaluated on the base of the population size of soil-born microbial communities. Bacteria of the genus *Pseudomonas* form the most abundant and best investigated group of bacteria developing in the soil environment. *Pseudomonas* spp. can colonize the roots of crops and successfully compete with pathogens for space and nutrients. They also secrete various substances with antagonistic properties against cereal pathogens (Paul and Clark, 2000; Garbeva et al. 2004). Bacteria of the genus *Azotobacter* are also commonly found in the soil environment. They produce hormones stimulating plant growth and supply plants with nitrogen fixed with the enzyme nitrogenase (Paul and Clark, 2000).

The objective of this study was to determine the effect of selected species of the family Brassicaceae, grown as forecrops for winter wheat, on the quantitative and qualitative composition of soil-dwelling microbes.

MATERIALS AND METHODS

Field experiment. The study was conducted in 2006-2008. The experimental material comprised soil samples collected in an exact field experiment carried out at the Production and Experimental Station in Bałcyny near Ostróda, Province of Warmia and Mazury (N=53°35'49", E=19°51'20.3"). The experiment was established on typical medium-silty grey-brown podsolic soil developed from light loam, of quality class IIIa and good wheat complex. Brassicaceae plants were grown in accordance with the relevant cultivation and fertilization standards. Mineral NPK fertilizers were applied as follows (kg ha⁻¹): winter

rapeseed – 240:70:220, spring rapeseed and white mustard – 100:40:120, Chinese mustard –70:30:80. Crop protection chemicals were applied as recommended by the Institute of Plant Protection. Before sowing, rape seeds were dressed with Crusier OSR 322 FS (thiamethoxam + matalaxyl-M + fludioxinal), and mustard seeds with TC (thiram). The plantation was protected against pests with insecticides when the economic threshold of harmfulness was exceeded.

The exact field trial was performed in a randomized block design, in three replications, and it involved the following forecrops:

- winter rapeseed (*Brassica napus* var. *arvensis* f. *bienis*) cv. Californium;
- spring rapeseed (*Brassica napus* var. *arvensis* f. *annua*) cv. Hunter;
- white mustard (*Sinapis alba* L.) cv. Borowska;
- Chinese mustard (*Brassica juncea* L.) cv. Małopolska;
- winter wheat (*Triticum aestivum* L.) cv. Dorota – as control.

Soil sampling. Soil samples for mycological analysis were collected into sterile Petri dishes twice: in the spring, before the sowing of forecrops, and in the autumn, after the harvest of Brassicaceae and ploughing-in crop residues. Soil was sampled at three evenly distributed points in each plot, at a depth of 5-15 cm, under aseptic conditions. A bulk sample of three replicates was placed in each dish. Bacteria were isolated from soil samples collected in the autumn of 2007 and 2008.

Mycological analysis of soil. Fungi were isolated from soil at the laboratory as described by Mańk a (1974). 149 g of quartz sand was put into a 0.5 l conical flask which was sterilized in an autoclave for 25 min. 1 g of soil was added to cooled sand. The flask was shaken manually for 2 min. The mixture was transferred to Petri dishes with a measuring scoop (25 mg), and cool Martin-Johnson agar medium was poured into the dishes. After several days of incubation at 25°C, the colonies were inoculated onto PDA slants and were kept in a thermostat until microscopic identification based on sporulation.

Isolates of bacteria. Bacterial isolates were obtained from the soil in which winter wheat, winter rapeseed and white mustard had been grown. They are listed in Table 2. The activity of bacteria against pathogens was tested immediately after isolation.

Isolates of pathogens. The fungus *Rhizoctonia cerealis* was isolated from the stem bases of winter wheat showing symptoms of sharp eyespot. The species *Gaeumannomyces graminis* was isolated from the roots of winter wheat. The cultures were stored on PDA at 4°C.

Isolation of soil bacteria. Bacteria of the genera *Pseudomonas* and *Azotobacter* and chitin-degrading bacteria were isolated from the soil in which

winter wheat, winter rapeseed and white mustard had been grown. 10 g of soil was put into 250 ml flasks filled with 90 ml of sterile water. The samples were shaken for 30 min. in a shaker, type 358S (frequency 170/min, amplitude 8). Bacteria were isolated from soil by plating soil dilutions on selective media (Hidink et al. 2005; Wachowska et al. 2006).

Inhibitory effect of *Pseudomonas* bacteria. The antagonistic activity of 55 isolates of *Pseudomonas* bacteria against the causal agents of take-all diseases of cereals, *Gaeumannomyces graminis* and *Rhizoctonia cerealis*, was determined under laboratory conditions. 5-mm PDA disks overgrown with 10-day pathogenic cultures were placed on Petri dishes filled with PDA (Garbeva et al. 2004). The isolates of the tested bacteria were placed within 2 cm from the pathogen (Wachowska et al. 2003). Images of Petri dishes with fungal colonies were obtained with a digital camera (Nikon Coolpix 2500) and stored as data on a computer. The isolates of the genus *Pseudomonas* which caused changes in the shape of phytopathogen colonies were considered to be biologically active, at the coefficient of the colony's ellipticity below 0.79.

Statistical analysis of results. Data from bacteriological analyses were verified statistically using Statistica 8.0 software. Data on bacterial counts in soil were transformed according to the formula: $\log_{10}(x+1)$, and were subjected to an analysis of variance. The significance of differences between mean values was estimated by the Student-Newman-Keuls (SNK) test.

RESULTS

The microbiological analysis of soil revealed wide biodiversity of fungal and bacterial communities (Tables 1, 2). *Aureobasidium pullulans* (13.4%), fungi of the genera *Fusarium* (7.9%) and *Penicillium* (9.5%), as well as various species of the family Mucoraceae (25.8%), were isolated most frequently from soil before the experiment had been established. Fungal species potentially pathogenic to cereals and other crops accounted for 13.5% in this environment. A considerable number of antagonistic fungi, such as Mucoraceae, members of the genera *Penicillium*, *Trichoderma* and *Gliocladium*, were also isolated from this type of soil. They accounted for 42.8% of all analyzed colonies.

Fungal communities isolated from soil used for growing the investigated forecrops differed in both abundance and species composition (Fig. 1). The highest number of fungi characterized by the most diverse species composition were isolated from white mustard plots (664 isolates belonging to 87 species), and their lowest abundance was noted in winter rapeseed plots (556 isolates representing 70 species). The following

fungus species occurred in greatest abundance in soil in which Brassicaceae had been grown: members of the genera *Aureobasidium*, *Fusarium*, *Penicillium*, *Phoma* and *Spicaria*, and representatives of the family Mucoraceae (*Mortierella*, *Mucor*, *Rhizopus*). They accounted for more than 50% of all isolates (Table 1).

A. pullulans dominated in this environment. Fungal species considered pathogenic to crops were most abundant in soil used for growing white mustard (12.8%) and spring rapeseed (12.7%). The largest group among them was formed by members of the genus *Fusarium*, in particular *F. equiseti* and *F. solani*.

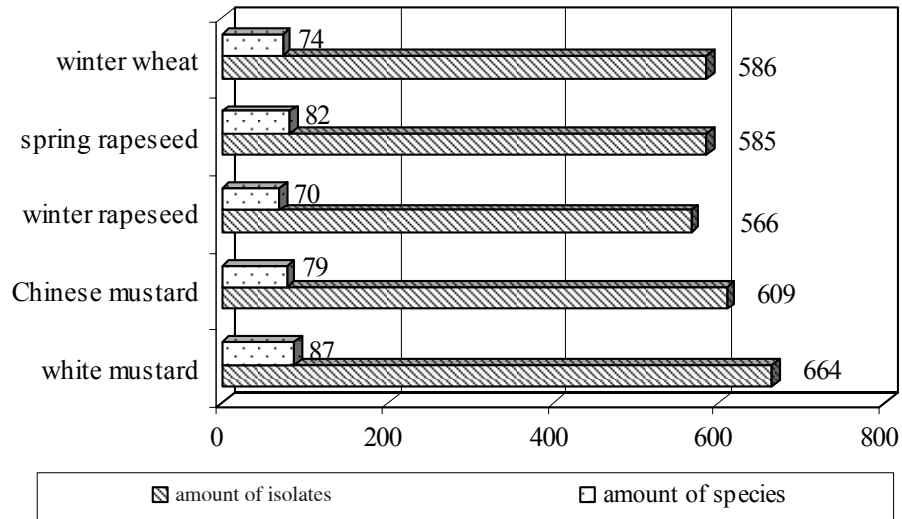


Fig. 1. Number of fungi isolated from soil after different forecrops in years 2006-2008

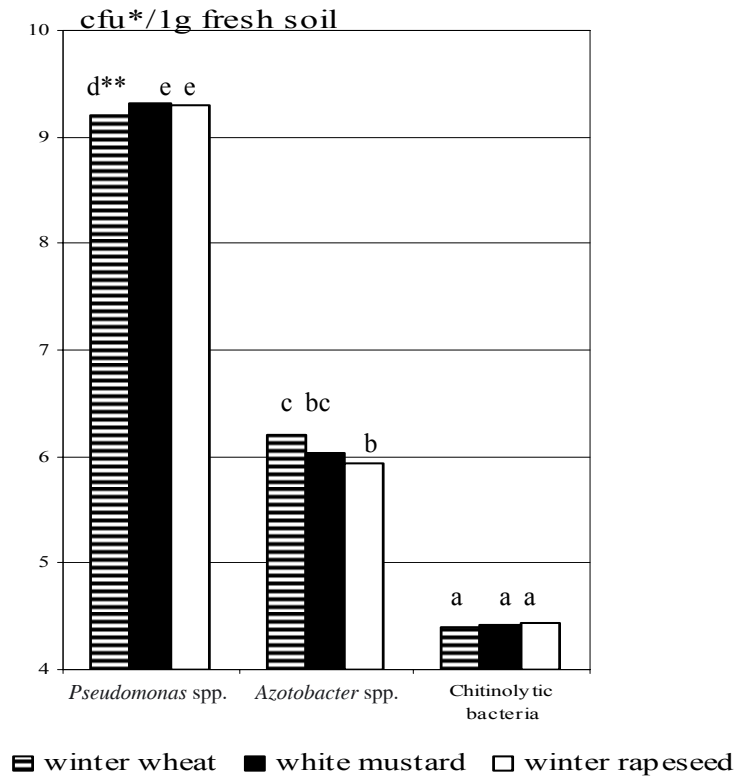


Fig. 2. Number of bacteria isolated from soil after different forecrops

* colony forming units

** mean values followed by the same letters are not significantly different according to SNK test (p=0.01)

Fungi showing antagonistic activity against crop pathogens, such as *Penicillium*, *Trichoderma* and *Gliocladium*, were isolated primarily from white mustard (36.2%) and winter rapeseed (35.2%) plots. 7.5% of pathogenic fungi and 29.0% of fungi with antagonistic properties were obtained from wheat plots.

Pseudomonas bacteria occurred in greatest abundance, compared with the remaining bacterial groups, regardless of the forecrop (Fig. 2). 94.7×10^6 colony-forming units (cfu) were obtained per gram of soil on average (Fig. 2). A community of diazotrophs of the genus *Azotobacter* developed relatively dynamically (0.15×10^6 cfu per gram of soil on average). A community of chitinolytic bacteria was found to be least abundant (1.41×10^3 cfu per gram of soil on average).

The communities of *Pseudomonas* bacteria isolated from soil used for growing white mustard and winter rapeseed were more abundant (by 29.7% and 34.4%, respectively) than the communities of these bacteria obtained from winter wheat plots (Fig. 2). The differences were statistically significant. The inhibito-

ry activity of isolates of *Pseudomonas* bacteria against the causal agents of take-all diseases of cereals, *Gaeumannomyces graminis* and *Rhizoctonia cerealis*, varied widely (Table 2). *G. graminis* showed a stronger response to the presence of bacteria on PDA than *R. cerealis*. The majority of isolates obtained from soil sown with winter rapeseed and white mustard as forecrops (54.0% and 88.0%, respectively) substantially inhibited the growth of *G. graminis*. 64.0% of isolates from white mustard plots also exhibited an antagonistic effect on *R. cerealis*. The isolates obtained from soil used for growing wheat were characterized by low inhibitory activity against the tested phytopathogens. Only two of them (13.0% of the total number) considerably limited the growth of *G. graminis*, and one inhibited the development of *R. cerealis*.

A community of diazotrophs was significantly more abundant in winter wheat plots than in white mustard plots (Fig. 2). No statistically significant differences were noted between the communities of chitinolytic bacteria dwelling in soil in which different forecrops had been grown.

Table 1
Fungi isolated from soil after different forecrops in years 2006 -2008

Species of fungi	Before sowing %	Brassicaceae %				\bar{x} %	Winter wheat %
		White mustard	Chinese mustard	Winter rapeseed	Spring rapeseed		
<i>Acremonium</i> spp.	1.99	6.17	5.91	6.71	5.47	6.06	3.41
<i>Acrostalagmus cinnabarinus</i> Corda	1.00	1.81	2.30	0.71	0.51	1.36	0.85
<i>Alternaria alternata</i> (Fr.) Keissler		5.42	2.96	1.41	2.39	3.14	3.07
<i>Arachniotus</i> sp.	0.50						
<i>Arthrinium sphaerospermum</i> Fuckel		1.96	0.49	1.24	0.68	1.11	1.88
<i>Ascochyta pisi</i> Lib.	1.49						
<i>Ascochyta</i> sp.		0.30				0.08	
<i>Aspergillus amstelodami</i> (Mangin) Thom et Church		0.75	0.33	1.94	1.03	0.99	0.34
<i>Aspergillus flavus</i> Link		0.45		0.71		0.29	
<i>Aspergillus nidulans</i> (Eidam) Winter		0.45	0.66			0.29	0.34
<i>Aspergillus niger</i> van Teighem							0.34
<i>Aspergillus ochraceous</i> Wilhelm				0.18		0.04	
<i>Aspergillus restrictus</i> Smith			0.16			0.04	
<i>Aspergillus sydowi</i> (Bainier et Sartory) Thom et Church	0.50						
<i>Aspergillus ustus</i> (Bainier) Thom et Chuch					0.17	0.04	
<i>Aspergillus</i> sp.	1.99						
<i>Aureobasidium bolleyi</i> (Sprague) von Arx	1.00	0.45	0.16	1.59	0.34	0.62	
<i>Aureobasidium pullulans</i> (de Bary) Arnaud	13.43	6.93	13.30	16.78	14.36	12.62	17.58
<i>Bipolaris sorokiniana</i> (Sacc.) Shoem.	2.49	0.15				0.04	

Table 1 cont.

Species of fungi	Before sowing %	Brassicaceae %				\bar{x} %	Winter wheat %
		White mustard	Chinese mustard	Winter rapeseed	Spring rapeseed		
<i>Botryotrichum piluliferum</i> Sacc. et Marchal		0.15				0.04	
<i>Botryodiplodia</i> sp.					0.34	0.08	
<i>Botrytis cinerea</i> Pers. ex Pers.		0.30	0.33	0.00	0.17	0.21	0.34
<i>Chaetomium</i> spp.	2.99	1.66	2.13	1.41	2.56	1.94	4.61
<i>Chloridium virescens</i> (Pers. ex Fr.) W. Gams et Hol.-Jech.	0.50						
<i>Cladosporium cladosporioides</i> (Fresen.) de Vries	1.00	0.90	3.12	3.36	3.59	2.68	3.24
<i>Coniothyrium</i> sp.	2.49	1.36	0.82	3.53	3.59	2.27	1.37
<i>Coniella</i> sp.		0.15				0.04	
<i>Coremiopsis spicarioides</i> Kamyschko		0.45	0.49	0.53	1.03	0.62	0.51
<i>Cylindrocarpon</i> spp.		1.05	0.33	0.53	0.17	0.54	0.17
<i>Dendryphion</i> sp.		0.30	1.15		0.17	0.41	0.34
<i>Dimargaris</i> spp.	8.96	0.15	0.33	0.88	0.17	0.37	0.34
<i>Endothia</i> sp.	0.50	0.45	2.13			0.66	0.17
<i>Fusarium aquaeductuum</i> (Radlk. et Rabenh.) Lagerh.				0.53	0.85	0.33	
<i>Fusarium chlamydosporum</i> Wollenw. et Reinking		0.15				0.04	
<i>Fusarium coeruleum</i> (Libert) ex Sacc.	1.00						
<i>Fusarium culmorum</i> (W.G. Smith) Sacc.		0.15	0.33			0.12	
<i>Fusarium dimerum</i> (Penzing) Raillo		0.30	0.82	0.53	1.03	0.66	0.51
<i>Fusarium equiseti</i> (Corda) Sacc.	1.00	3.92	2.79	2.12	3.76	3.18	2.90
<i>Fusarium merismoides</i> Corda	1.49	0.75	0.49	0.35	1.37	0.74	0.34
<i>Fusarium oxysporum</i> Schlecht.	1.00	0.60	1.31	0.88	2.39	1.28	0.51
<i>Fusarium poae</i> (Peck) Wollen.		0.15				0.04	
<i>Fusarium sambucinum</i> Fuckel	0.50						
<i>Fusarium sacchari</i> (Butler) W. Gams							0.34
<i>Fusarium solani</i> Mart.) Appel et Wollenw.	1.49	2.41	1.64	1.06	0.34	1.40	1.88
<i>Fusarium tabacinum</i> (Beyma) W. Gams	1.00	1.05	0.82		0.51	0.62	
<i>Fusarium tricinctum</i> (Corda) Sacc.			0.16			0.04	
<i>Fusarium verticillioides</i> (Sacc.) Nirenberg	0.50						
<i>Fusarium</i> sp.			0.16		0.34	0.12	
<i>Gaeumannomyces graminis</i> (Sacc.) Arx et Olivier		0.75	0.16	0.18	0.68	0.45	
<i>Gliocladium</i> spp.	2.49	1.36	3.28	1.59	3.93	2.52	1.19
<i>Gliomastix</i> spp.	1.00	2.86	1.31	0.18	1.37	1.49	1.71
<i>Gonytrichum macrocladum</i> (Sacc.) Hughes				0.18	0.17	0.08	
<i>Graphium</i> sp.		0.45				0.12	
<i>Gymnoascus subumbrinus</i> Shmith et Ramsbottom			0.16			0.04	0.85
<i>Humicola</i> spp.	3.48	2.26	0.16	2.12	0.68	1.32	0.17

Table 2
The values of the pathogen colony's ellipticity coefficient

Isolates	Winter rapeseed		Isolates	White mustard		Isolates	Winter wheat	
	<i>R. cerealis</i>	<i>G. graminis</i>		<i>R. cerealis</i>	<i>G. graminis</i>		<i>R. cerealis</i>	<i>G. graminis</i>
B2	0.98	0.66	B148	1.00	0.98	B134	1.00	0.99
B3	1.01	0.93	B23	1.01	1.03	B136	1.00	1.02
B13	0.99	1.00	J25	0.66	0.71	B137	1.00	1.10
B14	0.97	1.00	J37	0.74	0.65	B138	1.04	1.00
B124	1.00	0.93	J24	0.73	0.72	B140	1.01	0.95
B126	1.00	1.05	J29	0.89	0.69	B141	1.02	0.80
B127	0.97	0.68	J64	0.80	0.79	B143	1.00	0.98
B128	0.97	0.75	J33	0.75	0.62	B144	1.01	1.00
B129	1.00	0.97	J51	0.70	0.59	B145	1.01	0.99
B130	1.00	0.87	J28	0.80	0.83	B146	0.99	0.91
B132	1.00	0.92	J34	0.69	0.55	B15	1.01	1.00
B133	1.02	0.99	J18	0.70	0.57	J113	0.86	0.61
J135	0.72	0.68	J32	0.66	0.64	J92	0.75	0.90
J143	0.74	0.61	J74	0.67	0.78	J98	0.91	0.62
J144	0.91	0.68	J66	0.92	0.54			
J127	1.00	0.66	J31	0.74	0.75			
J146	0.80	0.57	J57	0.79	0.75			
J134	0.72	0.58						
J140	1.00	0.71						
J161	0.81	0.79						
J117	0.82	0.63						
J151	0.95	0.85						
J118	1.00	0.90						
J150	0.86	0.71						

B2 ... J98 – code of cultures` symbol

DISCUSSION

Fungal communities dwelling in soil sown with Brassicaceae were found to be greatly diverse in regard of both abundance and species composition. They included pathogenic fungi as well as many genera of bacteria and fungi with antagonistic properties. According to Sturz and Bernier (1989), alternate growing of rapeseed or mustard and grain crops contributes to a reduction in the population size of pathogenic fungi such as *F. culmorum*, *Microdochium nivale*, *G. graminis* and *Cochliobolus sativus*.

The highest number of fungi with the most diverse species composition were isolated from white mustard plots, while their lowest abundance was noted in winter rapeseed plots. Bialy et al. (1990) demonstrated that species of the genera *Brassica* and *Sinapis* grown as forecrops or intercrops may reduce agrophage populations due to their allelochemical activity. Glucosinolates and the products of their breakdown, isothiocyanates, contained in the tissues of these plants vary in their activity towards soil microbes (Sarwar and Kirkegaard, 1998; Majchrzak et al. 2001). Most probably, these compounds inhibit

ited the growth of fungi of the genus *Fusarium* in this study. In addition, winter rapeseed and white mustard, considered to be good forecrops, were found to exert a selective effect on the communities of *Pseudomonas* bacteria. The abundance of these bacteria in soil was limited by, among others, the root secretions of forecrops (Bis et al. 2005). Considerably more colonies of *Pseudomonas* bacteria were isolated from soil used for growing winter wheat, winter rapeseed and white mustard, in comparison with the rhizosphere of oat and maize (Garbeva et al. 2004). The majority of the tested isolates obtained from soil in which Brassicaceae had been grown showed inhibitory activity against the causal agents of take-all diseases of cereals. The percentage of *Pseudomonas* isolates from the rhizosphere of white mustard, active against the causal agent of take-all, was particularly high, reaching 88%. Garbeva et al. (2004) evaluated the activity of *Pseudomonas* bacteria against *Rhizoctonia solani* and found that on average 17.4% of isolates exhibited an inhibitory effect. In the present experiment, 6% to 64% of bacterial isolates showed inhibitory activity against *R. cerealis*, depending on the forecrop. Slightly higher abundance of beneficial bacteria, protecting plants against pathogens, was observed in winter rapeseed and white mustard plots. This is probably why soil sown with Brassicaceae is characterized by high protective potential with respect to successive crops.

According to Schippers et al. (1990), crop protection against pathogens is possible due to hydrogen cyanide produced by selected *Pseudomonas* isolates. Bacteria living around plant roots may produce the antibiotic 2,4-diacetyl phloroglucinol which decreases the incidence of take-all (Hiddink et al. 2005). Another mechanism of bacterial action against pathogenic fungi involves the breakdown of chitin, the main component of the cell wall of fungi. The process is catalyzed by chitinolytic enzymes which release acetylglucosamine monomers from chitin (Paul and Clark, 2000).

It seems that *Aureobasidium pullulans* – the predominant species among the tested eukaryotic microorganisms – also showed antagonistic activity against soil pathogens. The fungus is commonly found in the phyllosphere of various plants across many climate zones (Domsch and Gams, 1969). As reported by the cited authors, this species is considered to be non-pathogenic, but it may cause root necrosis in some plants. The antagonistic activity of *A. pullulans* results primarily from its antibiotic properties (Rai and Singh, 1980). As demonstrated by Łacicowa et al. (1997), this fungus may act as a natural biological barrier protecting crops against the infestation by pathogenic microbes.

Members of the genus *Fusarium* were also

present in the investigated soil, in the abundance of 5.5% to 10.6%. These fungi occur commonly in the natural environment. They colonize soil as well as the aboveground and underground parts of plants, causing infections collectively referred to as fusariosis (Kwaśna et al. 1991; Majchrzak et al. 2008).

Acknowledgments

This study was partially financed by research funds for the years 2007-2010 under a research project N31003132/1670 of the Ministry of Science and Higher Education in Poland.

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Zmiany mikrobiologiczne w środowisku glebowym pod wpływem uprawy roślin kapustnych (Brassicaceae)

Streszczenie

Badania prowadzono w latach 2006-2008 w Zakładzie Produkcyjno-Doświadczalnym Uniwersytetu Warmińsko Mazurskiego w Bałcynach. Celem badań było określenie jakości mikrobiologicznej środowiska glebowego jako stanowiska dla pszenicy ozimej uprawianej po roślinach kapustnych. Doświadczenie polowe założono na glebie płowej uwzględniając następujące rośliny przedplonowe: rzepak ozimy, rzepak jary, gorczycę białą, gorczycę sarepską, pszenicę ozimą jako próbę kontrolną. Wiosną przed siewem roślin przedplonowych oraz jesienią po zbiorze roślin kapustnych i przyoraniu resztek pozbiorowych pobierano próby gleby i wykonywano analizy mikrobiologiczne. Zbiorowiska bakterii i grzybów pochodzące z gleby po roślinach kapustnych były na ogół liczniejsze i bardziej różnorodne. Charakteryzowały się także większym mikrobiologicznym potencjałem inhibycyjnego oddziaływania na patogeny glebowe. Najlepszymi przedplonami pod względem mikrobiologicznym okazały się gorczyca biała i rzepak ozimy.

