ACTA MYCOLOGICA
Vol. 43 (2): 181–191
2008

Fungi isolated from the rhizosphere of spring cruciferous plants

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Majchrzak B., Okorski A., Chodorowski B.: *Fungi isolated from the rhizosphere of spring cruciferous plant*s. Acta Mycol. 43 (2): 181–191, 2008.

Fungal communities isolated from the rhizosphere of spring cruciferous plants were analysed in the study. It was found that the rhizosphere of crucifers was colonized primarily by fungi of the order *Mucorales* and of the genus *Fusarium*. Members of the genus *Fusarium* dominated in the rhizoplane. The roots of cruciferous plants secrete glucosinolates – secondary metabolites known for their antifungal properties, thus affecting the communities of soil-dwelling fungi.

Key words: rhizosphere, cruciferous plants, fungi, *Fusarium*

INTRODUCTION

The soil provides habitat for both phytopathogenic and saprotrophic microorganisms, including bacteria, actinomycetes and fungi (Patkowska 1998). The rhizosphere, i.e. the zone that surrounds the roots of plants, plays a particularly important role due to its specific biological properties. It is teeming with a wide variety of microbes (Morgan et al. 2005), which can be divided into plant growth-promoting rhizobacteria (PGPR), deleterious rhizosphere microorganisms (DRMO) and neutral microorganisms – having no impact on plant growth (Sturz, Christie 2003). Under natural conditions, in undisturbed soil, the groups of beneficial and harmful microorganisms remain in the state of dynamic equilibrium. PGPR contribute to yield increment, usually resulting from higher nutrient availability and suppression of the growth and activity of deleterious microorganisms. DRMO compete with PGPR for food, thus negatively affecting crop development (Kurek, Kobus 1990). Due to their antagonist potential, soil microorganisms are able to colonize suitable niches. The antagonistic mechanisms include antibiosis, competition and mycoparasitism (hyperparasitism) (Sturz, Christie 2003). Many pathogens develop in the after-harvest residues of forecrops, so the type of forecrop may have a significant influence on the

yield of successive crops (Bojarczuk, Bojarczuk 1988). Crucifers and legumes play a positive role in crop rotation because they improve the chemical, physical and biological properties of the soil (Majchrzak et al. 2002). Members of the family *Brassicaceae* are among the best forecrops, because they leave in the soil large amounts of after-harvest residues rich in glucosinolates and other secondary metabolites (Oleszek 1997). Moreover, they exert a positive effect on the health of successive crops (Majchrzak et al. 2004; Majchrzak et al. 2005).

The objective of this study was to determine the species composition of fungi isolated from the rhizosphere of selected cruciferous plants.

MATERIALS AND METHODS

The study was conducted during the years 1999-2001 at the Production-Experimental Station in Bałcyny near Ostróda (NE Poland), on the experimental plots of the Department of Plant Production, University of Warmia and Mazury in Olsztyn. The experiment was established on gray-brown podsolic soil developed from light silty clay, of quality class III a, of good wheat complex (1999 and 2000) or very good rye complex (2001). Crucifers were grown after spring wheat in 1999 and 2000, and after winter rapeseed in 2001. Mineral fertilizers (NPK) were applied at the following rates: 60-100 : 40-60 : 60-100 kg•ha^{-1}, as recommended by the Institute of Soil Science and Plant Cultivation. The field trial was performed in a randomized block design, in three replications. The experimental factors were as follows:

factor I – cruciferous plants

- spring oilseed rape (*Brassica napus* f. *annua*) – cv. Margo,
- white mustard (*Sinapis alba*) – cv. Heter,
- Chinese mustard (*Brassica juncea* var. *sareptana*) – cv. Małopolska,
- radish (*Raphanus sativus* var. *oleifera*) – cv. Pegletta,
- false flax (*Camelina sativa*) – cv. Borowska,
- Spanish colewort (*Crambe abyssinica*)- cv. Borowski

factor II – years of the study

No fungicides were applied. Fungi were isolated from the rhizosphere, rhizoplane and roots of crops as described by Mańka (1974). The quantitative and qualitative composition of fungal communities was determined at full blooming (BBCH 65-69).

RESULTS

A total of 2 929 fungal colonies belonging to 99 species and non-spore forming fungi were isolated from the rhizosphere of spring cruciferous plants over the three-year experimental period (Tab.1). Members of the order *Mucorales* dominated among them (48.96% of all colonies). Representatives of the genus *Rhizopus* were isolated most frequently (15.94%). Fungi of the genus *Penicillium* were also abundant (12.43%). Antagonistic species, including the order *Mucorales* and the genera *Gliocladium, Penicillium* and *Trichoderma,* accounted for 66.58% of all isolates. The proportion of pathogens in the rhizosphere was 11.33%, and the predominant role was played by species of the genus *Fusarium* (8.77%), primarily *F. solani*, *F. oxysporum* and *F. equiseti*.

The most diverse fungal community, composed of 637 colonies representing 44 species, was isolated from the rhizosphere of spring rape (Tab. 1). *Mucorales* (46.10%), *Fusarium* (16.5%), *Penicillium* (7.85%) and *Gliocladium* (6.12%) dominated among them. The fewest fungi (339 isolates belonging to 36 species) were detected in the rhizosphere of false flax. The most abundant among them were: *Mucorales* – 57.8%, *Acremonium* – 9.44% and *Fusarium* – 6.49%. Fungi of the genus *Fusarium* were isolated least frequently from the rhizosphere of Spanish colewort (3.81%). The genus *Penicillium* (270 isolates – 46.80%) dominated in the rhizosphere of this crop. The rhizospheres of white mustard, Spanish colewort and radish were characterized by the lowest species diversity (35 fungal species each).

A total of 568 fungal colonies belonging to 80 species and non-spore forming fungi were isolated from the rhizoplane of spring cruciferous plants during the experimental period (Tab. 2). The most diverse fungal community, comprising 134 colonies representing 34 species, was isolated from the rhizosphere of spring rape. Members of the genera *Fusarium* (32.84%) and *Acremonium* (21.64%) as well as of the order *Mucorales* (5.69%) dominated among them. The fewest fungi were isolated from the rhizoplane of Spanish colewort (73 isolates). This community, composed of only 24 species, was found to be the least diverse. The rhizoplane of this crop was mostly colonized by *Gliocladium* spp. (21.90%), *Aspergillus fumigatus* (15.10%) and *Penicillium* spp. (15.10%). Representatives of the genus *Fusarium* constituted the least numerous group in the rhizoplane of Spanish colewort (6 isolates – 8.22%).

DISCUSSION

Research results show that plants of the genus *Brassicaceae* grown as forecrops or ploughed in as green manure have a beneficial effect on the health of field crops (Majtahedi et al. 1991). The roots of crucifers secrete glucosinolates, which affect the soil microflora and help to control the occurrence of phytopathogens (Bones, Rossiter 1996; Kierkegaard, Sarwar 1998). Decomposition of the tissues of *Brassicaceae* as well as the production of glucosinolates followed by their hydrolysis lead to the formation of isothiocyanates (ITCs) – volatile substances considered to be biofumigants (Sarwar et al. 1998). According to Snapp et al. (2007) and Charron and Sam (1999), growing plants of the genus *Brassicaceae* as forecrops and leaving their remainders in the field inhibits the growth of such soil pathogens as *Rhizoctonia solani* and *Pythium ultimum*. Marwar and Lodha (2002) demonstrated that plants of the family *Brassicaceae* limited the occurrence of *Fusarium oxysporum* f. sp *cumini*.

In the present study fungi of the genus *Fusarium* were not abundant in the rhizosphere of crucifers. Their population was considerably greater in the rhizoplane. The soil environment of particular cruciferous plants was colonized by members of this genus to a different degree. *Fusarium* colonies were isolated most frequently from the rhizosphere and rhizoplane of spring oilseed rape, and least frequently from the rhizosphere and rhizoplane of Spanish colewort and false flax. Ishimoto et al. (2000) reported that fungi of the genus *Fusarium* isolated from the roots of crucifers showed high tolerance to glucosinolates, which may suggest that they acquired resistance to this group of substances through adaptation.

In the current experiment the rhizosphere of the *Cruciferae* was colonized by numerous representatives of the order *Mucorales*, dominated by members of the

Table 1
Fungi isolated from rhizosphere spring cruciferous plants

Fungal species	Plants																		Sum	%
	Oilseed rape			White mustard			Chinese mustard			Oilseed rape			False flax			Spanish colewort				
	1*	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3		
Acremonium breve (Sukap. et Thirum)W. Gams		33	2																35	1.19
Acremonium charticola (Lindau) W. Gams	21			3	4		3						11	6					48	1.64
Acremonium chrysogenum (Sukap.&Thirum)W. Gams																2			2	0.07
Acremonium curvulum W. Gams		16			6									6					28	0.96
Acremonium kiliense Gruetz									15			8							23	0.79
Acremonium larvarum (Petch) W. Gams									14										14	0.48
Acremonium minutisporum (Sukap. et Thirum)W. Gams														6					6	0.20
Acremonium ochraceum (Onions et Barron) W. Gams													1						1	0.03
Acremonium potroni Vuill			1													4			5	0.17
Acremonium psammosporum W. Gams	4						2						2			11			19	0.65
Acremonium sclerotigenum (F. et R. Moreau ex Valenta) W. Gams								1											1	0.03
Acremonium strictum W. Gams																		3	3	0.10
Acremonium sp.	1																		1	0.03
Alternaria alternata (Fries) Keissler	1				4				1			27		5	3			1	42	1.43
Arthrinium sphaerospermum Fuckel									1	3									4	0.14
Aspergillus repens de Bary												1							1	0.03
Aureobasidium bolleyi Sprague						2				2		1							5	0.17
Aureobasidium pullulans de Bary					14									7	7			3	31	1.06
Botryodiplodia sp.									1										1	0.03
Botrytis cinerea Persoon	3											1				4			8	0.27
Cephalosporium atrum de Bary			5																5	0.17
Cercospora spp.															5				5	0.17
Cladosporium cladosporoides (Fres.) de Vries			18	10	6		2		8	10		15				5			74	2.53
Cladosporium herbarum Link ex FR.				2			1					11	2						16	0.55
Colletotrichum spp.											5								5	0.17
Coniothyrium spp.	1			5			3						4						13	0.44
Cylindrocarpon destructans (Zins.) Scholten			4	6		2	2			1									15	0.51
Endothia spp.			3																3	0.10
Epicoccum purpurascens Ehrenb. ex Schlecht									1										1	0.03
Epicoccum spp.										1									1	0.03
Fusarium avenaceum Corda ex Fr.		1		3						2			1			9			16	0.55
Fusarium chlamydosporum (Wollenweber et Reinking)			2							2									4	0.14
Fusarium culmorum (W.G. Smith) Sacc.		1					1												2	0.07

Fungal species	Plants																		Sum	%
	Oilseed rape			White mustard			Chinese mustard			Oilseed rape			False flax			Spanish colewort				
	1*	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3		
Fusarium dimerum (Penzig)											7			4					11	0.38
Fusarium equiseti (Corda) Sacc.		5	10	4	3	1				12	5			2	1			3	46	1.57
Fusarium nivale (Fr.) Ces.			1						13			1				1			16	0.55
Fusarium oxysporum(Schlecht.)	13	29	2	7	2	8	1		12	4	1			4	9	2			94	3.21
Fusarium sambucinum Fuck.																		3	3	0.10
Fusarium solani (Mart.) Sacc.	18	1	2	17	1	2	1		4	7			1					4	58	1.98
Fusarium solani var. *coeruleum* Thum									2										2	0.07
Fusarium tabacinum (Beyma) W. Gams					4														4	0.14
Fusarium sp.		1																	1	0.03
Gliocladium catenulatum Gilman et Abbott		1					2			10		2				11			26	0.89
Gliocladium fimbriatum Gilman et Abbott			4													5			9	0.31
Gliocladium penicilloides Corda	11	22		12	7					2	1					4			59	2.01
Gliocladium salmonicolor Raillo				1															1	0.03
Gliomastix cerealis (Kart.) Dickinson	1																		1	0.03
Gliomastix murorum (Corda) Hughes							1												1	0.03
Humicola fusco-atra Traaen	5			2						3			11			1		6	28	0.96
Humicola brevis Gilman et Abbott				5									4					5	14	0.48
Humicola nigrescens Omvik			1	2					3			1	1						8	0.27
Kickxella alabastrina Coemans													1						1	0.03
Monocilium mucidum W. Gams																		2	2	0.07
Monodictis levis (Wiltsh.) Hughes			2	2			2												6	0.20
Mortierella alliacea Linn		1																	1	0.03
Mortierella elongata Linnemann														2					2	0.07
Mortierella gemmifera Ellis								1											1	0.03
Mortierella gracilis Linnemann		7																	7	0.24
Mortierella marburgensis Linnemann					2													2	4	0.14
Mortierella alpina Peyrond		13			7									10					30	1.02
Mortierella vinacea Dixon -Stewart																		1	1	0.03
Mortierella spp.		30									4	1		1			96		132	4.51
Mucor circinelloides van Tieghem																		2	2	0.07
Mucor hiemalis Wehmer		86	36	7	72	14	2	253		11	115	11		15	5		30	18	675	23.05
Mucor microsporus Namyslowskii					6														6	0.20
Mucor mucedo (Linne) Brefeld				3													2	5	10	0.34
Mucor piriformis Fischer								37											37	1.26
Mucor pusillus Lindt								23			5								28	0.96
Mucor racemosus Fresenius		8			7				5		11								31	1.06
Paecillomyces variabilis Barron									1										1	0.03

Table 1
Fungi isolated from rhizosphere spring cruciferous plants – cont.

Fungal species	Plants																		Sum	%
	Oilseed rape			White mustard			Chinese mustard			Oilseed rape			False flax			Spanish colewort				
	1*	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3		
Penicillium nigricans (Bain.) Thom						2							1						3	0.10
Penicillium spp.		35	15		12	5	1		16			2	1	4		1	230	39	361	12.33
Periconia funerea (Ces.) Mason et Ellis									2										2	0.07
Phoma eurypena Sacc.			9												6				15	0.51
Phoma fimeti (Brun)										9			2						11	0.38
Phoma glomerata (Corda) Wollenweber et Hochapfel	2																		2	0.07
Phoma herbarum Westend.							2												2	0.07
Rhizoctonia solani Kühn		8																	8	0.27
Rhizoctonia spp.								1			1								2	0.07
Rhizopus nigricans Ehrenberg											1			6			2		9	0.31
Rhizopus spp.			113			71			39			39		5	152			39	458	15.64
Sclerotinia sclerotiorum (Lib.) de Bary											15								15	0.51
Scopulariopsis acremonium (Delacr.) Vuill.				2									1						3	0.10
Scopulariopsis brevicaulis (Sacc.) Bain											13								13	0.44
Scytalidium lignicola Pesante				4														3	7	0.24
Spicaria carnosa Miller.Giddens et Foster																1			1	0.03
Spicaria divaricata (Than.) Gilman&Abbott										7									7	0.24
Spicaria griseola Saccardo													9						9	0.31
Spicaria simplicissima Oudemans													1						1	0.03
Spicaria violacea Abbott					41				32										73	2.49
Sporotrichum carnis Brooks et Hansford							1						1						2	0.07
Sporotrichum chlorinum Link							1												1	0.03
Sporotrichum olivaceum Fries													2						2	0.07
Torula herbarum (Pers.) Link ex Fr.															5				5	0.17
Trichoderma aureoviride Rifai	10								5									2	17	0.58
Trichoderma hamatum (Bon.) Bain	6			2	5		1								1			1	16	0.55
Trichoderma harzianum Rifai			1						10								8		19	0.65
Trichoderma polysporum (Link et Pers.) Rifai			5																5	0.17
Verticillum cellulosae Daszewska				3															3	0.10
Non-sporulating fungi	1	1	4	4					5	1		5		5		6			32	1.09
Sum	98	299	240	106	203	107	29	339	190	87	189	126	57	88	194	67	368	142	2929	100.0
Sum of plant	637			416			558			402			339			577				

*1 - 1999 2 - 2000 3 - 2001

Table 2
Fungi isolated from rhizoplane of spring cruciferous plants

Fungal species	Plants																		Sum	%
	Oilseed rape			White mustard			Chinese mustard			Oilseed rape			False flax			Spanish colewort				
	1*	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3		
Acremonium breve (Sukap.&Thirum) W. Gams		8																	8	1.41
Acremonium charticola (Lindau) W. Gams	2						1				2		2	1					8	1.41
Acremonium chrysogenum (Sukap. et Thirum) W. Gams	1			1															2	0.35
Acremonium curvulum W. Gams		7																	7	1.23
Acremonium incoloratum (Sukap. et Thirum) W. Gams	1																		1	0.18
Acremonium kiliense Gruetz	1		1												3				5	0.88
Acremonium larvarum (Petch) W. Gams																		1	1	0.18
Acremonium minutisporum (Sukap. et Thirum) W. Gams													1					1	2	0.35
Acremonium potroni Vuill			2			1									2				5	0.88
Acremonium psammosporum W. Gams	1		1																2	0.35
Acremonium strictum W. Gams		1		1															2	0.35
Acremonium spp.	3			1											1	1	3		9	1.58
Acrospeira mirabilis Berk. et Br.									2										2	0.35
Alternaria alternata (Fries) Keissler		1	1			1		9		1		2				2			17	2.99
Aspergillus fumigatus Fresenius									1									11	12	2.11
Aspergillus funiculosus (G. Smith)																1			1	0.18
Aspergillus repens de Bary																		1	1	0.18
Ascochyta spp.			1			2													3	0.53
Aureobasidium bolleyi (Sprague)				1	1	1			1			1			3			1	9	1.58
Aureobasidium pullulans de Bary				1		6	1	3	1		1	1		2	2			1	19	3.35
Botryotrichum piluliferum Saccardo et Marchal		1																	1	0.18
Botrytis cinerea Persoon						1						2							3	0.53
Cladosporium cladosporoides (Fres.) de Vries	1		2	1	1				4	4	1	1			6	1			22	3.87
Cladosporium herbarum (Persoon) Link				1			1					3			1				6	1.06
Coniothyrium spp.			1			1													2	0.35
Cylindrocarpon destructans (Zins.) Scholten			2	1			1		1	1								1	7	1.23
Endothia sp.	1																		1	0.18
Epicoccum sp.	1																		1	0.18
Fusarium avenaceum (Corda ex Fr.)	1	1											1			1			4	0.70
Fusarium chlamydosporum (Wollenweber et Reinking)					2			1							1			1	5	0.88
Fusarium culmorum (W.G. Smith) Sacc.		1			1			1						1			1		5	0.88
Fusarium equiseti (Corda) Sacc.		6	1		3						1	3			1				15	2.64

Table 2
Fungi isolated from rhizoplane of spring cruciferous plants

Fungal species	Plants																		Sum	%
	Oilseed rape			White mustard			Chinese mustard			Oilseed rape			False flax			Spanish colewort				
	1*	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3		
Fusarium fusarioides (Frag. Cif.)														1					1	0.18
Fusarium oxysporum (Schlecht.)	3		1	1			1	3	2					3	5			2	21	3.70
Fusarium solani (Mart.) Sacc.	1	15	1	4	3	4			2		2	2		3	2			1	40	7.04
Fusarium solani var. *coeruleum* Thum	1							7					1						9	1.58
Fusarium tabacinum (Beyma) W. Gams et Abbott			1							1									2	0.35
Gliocladium catenulatum Gilman et Abbott		8								1		1			1	1	2		14	2.46
Gliocladium fimbriatum Gilman & Abbott								1									13		14	2.46
Gliocladium penicilloides Corda				2	1				2				1						6	1.06
Gliomastix murorum (Corda) Hughes			1																1	0.18
Humicola fusco-atra Traaen	1	4	2			2		3	1			2			1				16	2.82
Humicola brevis Gilman et Abbott						1				1									2	0.35
Humicola nigrescens Omvik												2			1				3	0.53
Kickxella alabastrina Coemans						1													1	0.18
Microdochium nivale (Fr.) Ces.		11							1			2							14	2.46
Monocilium arctiicola (W. Gams)									1										1	0.18
Mortierella alpina Peyrond	1	1														1			3	0.53
Mortierella arcuata Wolf												1							1	0.18
Mortierella isabelina Oudemans		1																	1	0.18
Mortierella vinacea Dixon -Stewart			1																1	0.18
Mucor circinelloides van Tieghem								11											11	1.94
Mucor hiemalis Wehmer		3		2	7			30	2	2		1			3	1	1		52	9.15
Mucor mucedo (Linne) Brefeld											2					1			3	0.53
Mucor piriformis Fischer								2											2	0.35
Mucor racemosus Fresenius				1					2								1		4	0.70
Paeciliomyces variotii Bainier									1										1	0.18
Paecilomyces niveus Stolk et Samson												2							2	0.35
Penicillium nigricans (Bain.) Thom	1																		1	0.18
Penicillium spp.	2	8	7		2	2		7	4				1		4	1	8	2	48	8.45
Phoma chrysantemicola Hollos																1			1	0.18
Phoma eurypena Sacc.			2	2			1												5	0.88
Phoma glomerata (Corda) Wollenweber et Hochapfel						2													2	0.35
Phoma herbarum Westend.															1				1	0.18

Fungal species	Plants																		Sum	%
	Oilseed rape			White mustard			Chinese mustard			Oilseed rape			False flax			Spanish colewort				
	1*	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3		
Pyrenophora semeniperda (Brittlebank et Adam)				1					1				1		1				4	0.70
Rhizoctonia solani Kühn			1																1	0.18
Rhizoctonia spp.			2																2	0.35
Rhizopus spp.		1							3		15	6		5	4			2	36	6.34
Scopulariopsis brevicaulis (Sacc.) Bain															1				1	0.18
Scopulariopsis brumpti Salvanet-Duval																		1	1	0.18
Scytalidium lignicola Pesante															1				1	0.18
Spicaria elegans Corda									3							2			5	0.88
Spicaria simplicissima Oudemans	1						1												2	0.35
Spicaria violacea Abbott					3				6		2							2	13	2.29
Sporotrichum carnis Brooks et Hansford					1														1	0.18
Torula herbarum (Pers.) Link ex Fr.															1				1	0.18
Trichoderma aureoviride Rifai								1	1							1			3	0.53
Trichoderma hamatum (Bon.) Bain						1		1	3		2								7	1.23
Trichoderma harzianum Rifai												1							1	0.18
Trichoderma polysporum (Link et Pers.) Rifai					1														1	0.18
Non-sporulating fungi			1	1					1		1	6		1	4	1			16	2.82
spring cruciferous plants	24	78	32	22	26	26	7	80	46	11	29	39	8	17	50	16	29	28	568	100.0
Sum of plant	134			74			133			79			75			73				

*1 - 1999 2 - 2000 3 - 2001

genus *Rhizopus*. Ishimoto et al. (2000) confirmed the predominant role of the genus *Rhizopus* in the rhizosphere of cruciferous plants. According to these authors, fungi of the genus *Rhizopus* showed significantly higher tolerance for glucosinolates than fungi of the genus *Fusarium.*

CONCLUSIONS

1. The largest and the most diverse fungal community was isolated from the soil environment of spring oilseed rape.

2. The fungal populations that colonized the rhizosphere and rhizoplane of Spanish colewort and false flax were found to be the smallest.

3. Members of the order *Mucorales* dominated in the soil environment of cruciferous plants.

4. Fungi of the genus *Fusarium* were isolated least frequently from the soil environment of Spanish colewort and most frequently from the soil environment of spring oilseed rape.

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Grzyby ryzosferowe jarych roślin kapustnych

Streszczenie

W badaniach poddano analizie zbiorowiska grzybów ryzosferowych jarych roślin kapustnych. Ryzosferę roślin kapustnych zasiedlały przede wszystkim grzyby z rzędu *Mucorales* i rodzaju *Fusarium*. W ryzoplanie roślin znacznie częściej występowali przedstawiciele rodzaju *Fusarium*. Korzenie rośliny kapustnych wydzielają do gleby glukozynolany, wtórne metabolity o właściwościach antygrzybowych, w ten sposób być może wpływają na zbiorowiska grzybów zasiedlających środowisko glebowe roślin.