ACTA MYCOLOGICA
Vol. 35 (2): 311–321
2000

The structure of fungal population from *Galega orientalis* root system formed as the result of fertilization

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Cwalina-Ambroziak B., Majchrzak B.: *The structure of fungal population from Galega orientalis root system formed as the result of fertilization.* Acta Mycol. 35 (2): 311–321, 2000.

The composition of the fungal population colonizing the rhizosphere, the rhizoplane and the roots of *Galega orientalis* Lam. plants was studied. The following factors had an influence on the quantity and the composition of the species: fertilization, the analysed root zone and the date of the completed analyses.

Key words: pathogenic fungi, saprophytic fungi, *Galega orientalis*, fertilization.

INTRODUCTION

In the case of perennial plant cultivation such as alfalfa and clover, quantitative and qualitative changes of the fungi colonizing plant soil environment occur. Finally fungal groups characteristic for the soil environment of a given plant are formed.

Both plant excretions (Gilbert 1969; Levis and Papavizas 1983) and the soil itself along with its cultivation and fertilization contribute to the formation of the soil fungal population. The following authors report on the influence of soil type and fertilization on species quantity and variability: Mikołajska and Majchrzak (1988), Mukhamed and Zubenko (1992), Deb and Bora (1996), Boven and Rovina (1999). Diversification of the fungal environment is a favourable phenomenon as it increases possible competition between saprophytic and pathogenic organisms of crops. Therefore, learning about this environment serves the increased crop health. Toth et al. (1995) report on the reduction of alfalfa infection by *Fusarium oxysporum* at the adequate nitrogen fertilization level.

The aim of the study was to determine the quantitative and qualitative composition of fungal population of root zones – the rhizosphere, the rhizoplane and the root of goat rue cultivated with and without fertilization.

MATERIALS AND METHODS

The investigation was carried out between 1997–1999 in the experimental field, of the Chair of Agricultural Chemistry of the University of Warmia and Mazury, located in Bałcyny on wheat good soil complex. The experiment was established with the application of random block method in four repetitions. Plant samples with (starter dose N-30 kg/ha – magnesium nitrate, P_2O_5 – 120 kg/ha – superphosphate, K_2 – 240 kg/ha – potassium salt) and without fertilization were the material for the experiment. During each vegetative period, the experiment included two periods: spring and the period of goat rue blooming. The collected fungi were isolated from the rhizosphere, the rhizoplane and the roots of goat rue in laboratory with the use of M a ń k a's method (1974).

RESULTS

The *Galega orientalis* cultivation environment in the studied period varied in quantity and quality. The type of cultivation, the root zone and the date of the examination modified the structure of the fungal population.

6664 fungi colonies were collected from the root system of *Galega orientalis* (Tables 1, 2 and 3). The total species number in the individual combinations slightly varied but some population quantities differed significantly. The largest number of isolated fungi was obtained in 1997, which was 21.7% more than in the poorest year of 1999. Pathogenic fungi were represented by the following species: *Botrytis cinerea*, *Fusarium avenaceum*, *F. culmorum*, *F. equiseti*, *F. oxysporum*, *F. solani*, as well as few species of *Rhizoctonia solani* and *Sclerotinia sclerotiorum*. The following were most frequently isolated saprotrophic fungi: *Mortierella alpina*, *M. isabelina*, *Mucor circinelloides*, *M. hiemalis*, *M. racemosus*, *Penicillium* spp., *Rhizopus moelleri*, *R. nigricans*, *Trichoderma aureoviride*, *T. hamatum*, *T. harzianum*, *T. Koningii* and *T. polysporum*, as well as few species *Gliocladium* genus: *G. catenulatum*, *G. fimbriatum* and *G. penicilloides*. The remaining fungi that were rarely isolated were represented by: *Aspergillus* spp., *Acremonium strictum*, *Aureobasidium pullulans*, *Cladosporium cladosporioides*, *Paecilomyces lilacinus*, *Scytalidium lignicola*, *Sporotrichum olivaceum*, *Zygorhynchus* spp.

Galega orientalis cultivation with the application of fertilization produced 3% less isolated fungi in comparison to the cultivation without fertilization (Fig. 1b). Saprotrophic fungi of *Trichoderma* and *Gliocladium* occurred

Table 1

Fungi colonizing the rhizosphere, rhizoplane and roots of *Galega orientalis* (1997)

Fungi	I date observation						Summ	II date obsevation						Summ
	Fertilization			Without fertilization				Fertilization			Without fertilization			
	Rhizo-sphere	Rhizo-plane	Roots	Rhizo-sphere	Rhizo-plane	Roots		Rhizo-sphere	Rhizo-plane	Roots	Rhizo-sphere	plane	Roots	
Alternaria alternata (Fr.) Keissler		1	3	2	2		8	3	3		1	4		11
Aureobasidium pullulans (de Bary) Arnaud	5	3			3	5	16	2	2	3	2	1	4	14
Botrytis cinerea Pers.			4			7	11			5			9	14
Fusarium avenaceum (Fr.) Sacc.		2	4	1	3	1	11	2	3	2	1	4	3	15
Fusarium culmorum (W.G. Sm.) Sacc.		2	1		3	3	9		2	4		1	5	12
Fusarium equiseti (Corda) Sacc.		1	1		1	1	4			1	1			2
Fusarium oxysporum Schlecht.	4	3	3	2	4	7	23	3	5	4		4	7	23
Fusarium solani (Mart.) Sacc.		1	2		3	2	8	3	4	2	5	2	4	20
Gliocladium catenulatum Gilman et Abbott		2					2	2	2			1		5
Gliocladium fimbriatum Gilman et Abbott		1				1	2		1					1
Gliocladium penicillioides Corda				1			1							
Mortierella alpina Peyronel	2	1	1	4	1	3	12	7	2	2	1	5	4	21
Mortierella isabelina Qudemans	5	2	3	1	3	2	16	4	7	6	4	8	5	34
Mucor circinelloides van Tieghem	2	3	3	3	4		15	1	3	3	5	4		16
Mucor hiemalis Wehmer	2	1	2	4	2	3	14	1	3	2		2	1	9
Mucor racemosus Fres.	1	1			1		3	1	1					2
Penicillium sp.	4	3	3	7	3	4	24	10	12	5	16	20	9	72
Rhizoctonia solani Kuhn			3			5	8			5			7	12
Rhizopus nigricans Ehrenberg	3	3	5	6	3	2	22	4	3	5	3	4	6	25
Sclerotinia sclerotiorum (W.G. Sm.) Sacc.			2			4	6						1	1
Trichoderma aureoviride Rifai	2	2	1	1	1		7			1	1	2	3	7
Trichoderma hamatum (Bon.) Bain		2	4	2	2		10	2	2					4
Trichoderma harzianum Rifai		3	5				8	1	1					2
Trichoderma koningii Oudemans		2		2		2	6	2	2					4
Trichoderma polysporum (Link ex Pers.) Rifai		1	1				2	1						1
Yeast – like fungi	340			381			721	493			575			1068
Other fungi	17	14	7	11	25	13	87	11	20	20	24	23	18	116
Total	387	54	58	428	64	65	1056	553	78	70	639	85	86	1511

Other fungi: *Acremonium strictum* (W. Gams), *Cladosporium cladosporioides* (Fres.) de Vries, *Paecilomyces lilacinus* (Thom) Samson, *Phoma exiqua* Desm., *P. herbarum* Westend., *Scytalidium lignicola* (Pesante), *Sporotrichum olivaceum* Fries, *Zygorhynchus* spp.

Table 2

Fungi colonizing the rhizosphere, rhizoplane and roots of goats rue (1998)

Fungi	I date of observation						Summ	II date of vegetation						Summ
	Fertilization			Without fertilization				Fertilization			Without fertilization			
	Rhizo-sphere	Rhizo-plane	Roots	Rhizo-sphere	Rhizo-plane	Roots		Rhizo-sphere	Rhizo-plane	Roots	Rhizo-sphere	plane	Roots	
Alternaria alternata (Fr.) Keissler		1			4		5					1		1
Aureobasidium pullulans (de Bary) Arnaud	18			5			23							
Botrytis cinerea Pers.			5			2	7	3		20	9		5	30
Fusarium avenaceum (Fr.) Sacc.	1				1	1	3							
Fusarium culmorum (W.G. Sm.) Sacc.		1	1	1	3	2	2			1			2	3
Fusarium oxysporum Schlecht.	6	4	3	10	2	7	32	1	8			9	3	21
Fusarium solani (Mart.) Sacc.		3					3		1	2			2	5
Gliocladium catenulatum Gilman et Abbott										1				1
Gliocladium fimbriatum Gilman et Abbott	2	1					3						1	1
Mortierella alpina Peyronel		2	3	2	3	4	14		5	4		3	7	19
Mortierella isabelina Qudemans		11	4		7	5	27	5	11	5		6	9	36
Mucor circinelloides van Tieghem		2			2		4							
Mucor hiemalis Wehmer	2	4			3		9		1			2		3
Penicillium sp.	3	5		10	2	3	23	7	1	3	11	3	12	37
Rhizoctonia solani Kuhn										4			5	9
Rhizopus nigricans Ehrenberg		5			6	4	15		12	4		21	4	41
Sclerotinia sclerotiorum (W.G. Sm.) Sacc.						2	2							
Trichoderma aureoviride Rifai		3	1				4		2					
Trichoderma hamatum (Bon.) Bain	2	8	4	3	5	6	28		6	3	1	3	7	20
Trichoderma harzianum Rifai	4	4	2	1	1	2	14		2	1	1	4	4	12
Trichoderma koningii Oudemans	1	2	1		4	2	10		4	4	2	7	5	22
Trichoderma polysporum (Link ex Pers.) Rifai			1			2	3		5					5
Yeast – like fungi	300			249			549	475			456			931
Other fungi	11	3	5	7	15	6	47	19		6	18	3	9	55
Total	350	59	30	288	57	48	832	510	58	58	491	62	75	1254

Other fungi: *Chaetomium* sp., *Cladosporium cladosporioides* (Fres.) de Vries, *C. herbarum* (Pers.) Link ex S. F. Gray, *Paecilomyces lilacinus* (Thom) Samson, *Scytalidium lignicola* (Pesante), *Sporotrichum olivaceum* Fries, *Zygorhynchus* spp.

Table 3

Fungi colonizing the rhizosphere, rhizoplane and roots of goats rue (1999)

Fungi	I date of observation						Summ	II date of obsevation						Summ
	Fertilization			Without fertilization				Fertilization			Without fertilization			
	Rhizo-sphere	Rhizo-plane	Roots	Rhizo-sphere	Rhizo-plane	Roots		Rhizo-sphere	Rhizo-plane	Roots	Rhizo-sphere	plane	Roots	
Alternaria alternata (Fr.) Keissler			2	2			4	1		1		2		4
Aureobasidium pullulans (de Bary) Arnaud	10			4	3		17	2			3	1		6
Botrytis cinerea Pers.			3			5	8			7			6	13
Fusarium avenaceum (Fr.) Sacc.		1		1		1	3	1	1			2		5
Fusarium culmorum (W.G. Sm.) Sacc.		1			2	1	4	1	1			1	2	5
Fusarium equiseti (Corda) Sacc.					2		2					2		
Fusarium oxysporum Schlecht.			2	1	1	1	5		1	3	3		3	10
Fusarium solani (Mart.) Sacc.				1			1			2		2		4
Gliocladium catenulatum Gilman et Abbott		1				2	3	2	1			1	1	5
Gliocladium fimbriatum Gilman et Abbott	2	2	1		3	2	10		2			1	1	3	7
Gliocladium penicillioides Corda	1				1		2					1			1
Mortierella alpina Peyronel	4	5	1	4	5	2	21		2	3	2	8	7	22
Mortierella isabelina Qudemans		2	2	2	3	1	10		2	4	2	5	4	17
Mucor circinelloides van Tieghem		1		1			2			1	1			2
Mucor hiemalis Wehmer	1	2	2	2	2	2	11		2	2	3	2		9
Penicillium sp.	5	3	4	8	3	2	25	17	2	5	9	10	4	47
Rhizoctonia solani Kuhn			1			2	3			1			6	7
Rhizopus nigricans Ehrenberg	4	5	1	1	5	5	21	9	11	2	6	1	4	33
Sclerotinia sclerotiorum (Lib.) de Bary			1				1			2				2
Trichoderma aureoviride Rifai	2	5	5	2		4	18	2	5	4	6	3	4	24
Trichoderma hamatum (Bon.) Bain	2	4	1	2	1	4	14	1	6	1	1		3	12
Trichoderma harzianum Rifai	3	2	3	2	6		16		3	2	2	4	6	17
Trichoderma koningii Oudemans						2	2		1		1			2
Trichoderma polysporum (Link ex Pers.) Rifai	2		3		3	2	10		5		3	2		10
Yeast – like fungi	250			223			473	433			486			919
Other fungi	13	16	5	21	13	8	76	12	11	13	11	12	7	66
Total	299	50	37	277	53	45	761	481	56	53	542	58	60	1250

Other fungi: *Acremonium strictum* (W. Gams), *Arthrinium sphaerospermum* Fuckel, *Cylindrocarpon radicicola* Wollenweber, *Monodictis glauca* Cooke et Harkn.) Hughes, *Paecilomyces lilacinus* (Thom) Samson, *Phialophora* spp., *Sporotrichum olivaceum* Fries, *Zygorhynchus* spp.

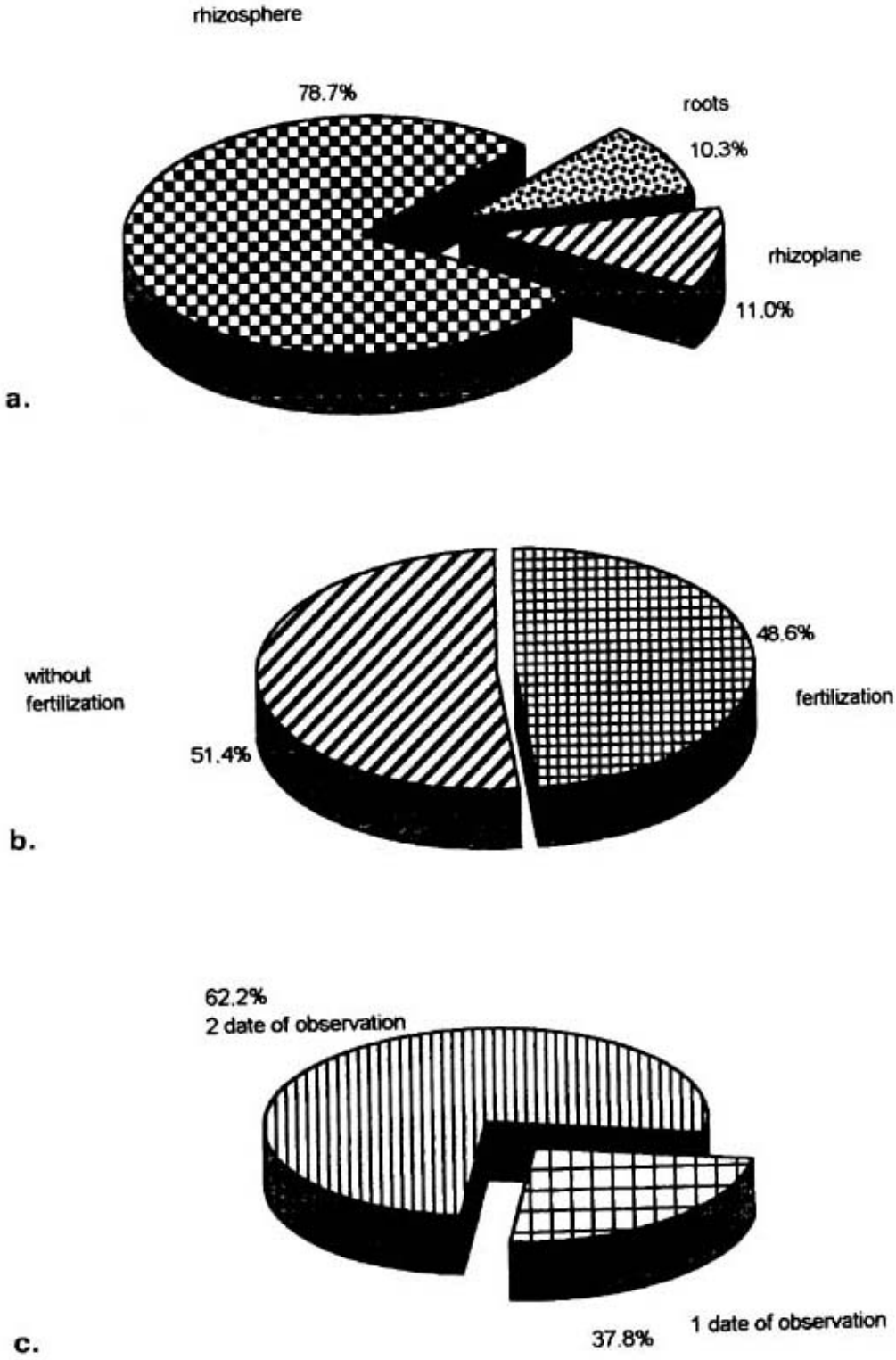

Fig. 1. Percentage of fungi isolated from goat rue in dependent: a – root zone; b – kind of fertilization; c – date of observation

more frequently in this combination. However, larger number of pathogenic fungi of *Fusarium* was found for the combination without fertilization. The presence of these fungi was reported during the whole vegetative period – in spring and during blooming for both combinations.

The goat rue rhizosphere was the most wealthy populated root zone by fungi – 78.7% of the total number of colony (Fig. 1a). The species selected from the goat rue rhizoplane and root constituted 11.0% and 10.3% of the total colony, respectively. Yeast-like fungi appeared to be common in the rhizosphere (89% – Fig. 2). Among other fungi the following genera are important: *Penicillium, Trichoderma.* The share of *Fusarium* was small (1%).

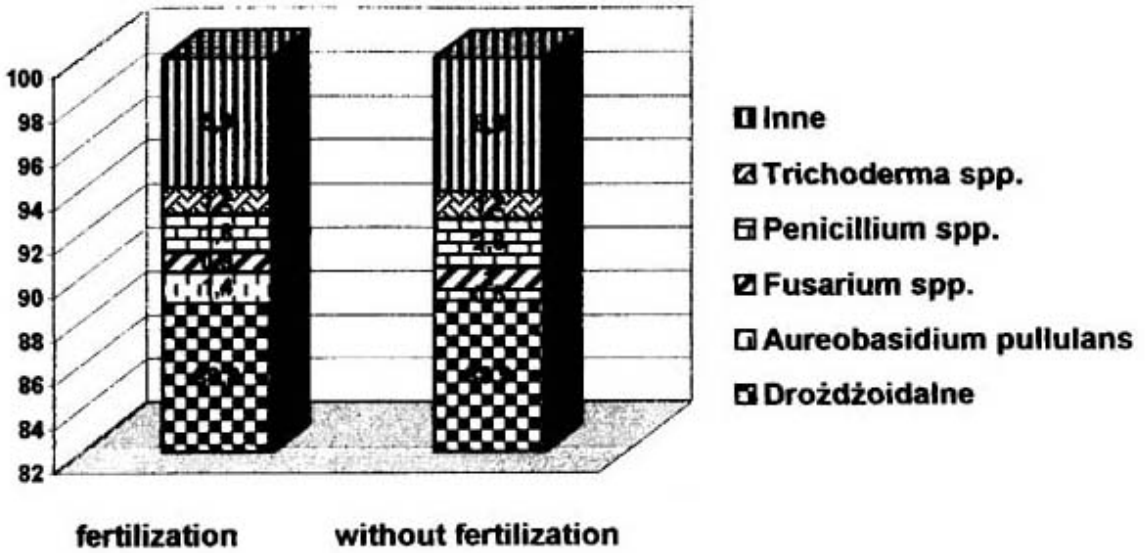

Fig. 2. Percentage of fungi isolated from rhizosphere

The rhizoplane was widely represented by fungi from *Mucorales* order (*Rhizopus, Penicillium* i *Mucor*) – 32% for each combination (Fig. 3). *Trichoderma* were also frequently isolated. Large numbers of *Fusarium* constituted 12.7% and 13.5% of the total colony respectively for the combination with and without fertilization.

The fungal population isolated from goat rue roots was different. Pathogenic fungi constituted 32% and 37% of the total colony for combination with and without fertilization, respectively (Fig. 4). Species such as: *Fusarium oxysporum, F. culmorum* and *Botrytis cinerea* were isolated most frequently. Other pathogens represented by *Sclerotinia sclerotiorum* and *Rhizoctonia solani* were rare. Saprotrophic fungi also colonized roots widely. Species belonging to the *Trichoderma* and fewer to *Mortierella, Penicillium, Rhizopus* and *Gliocladium* were important among the pathogen antagonists.

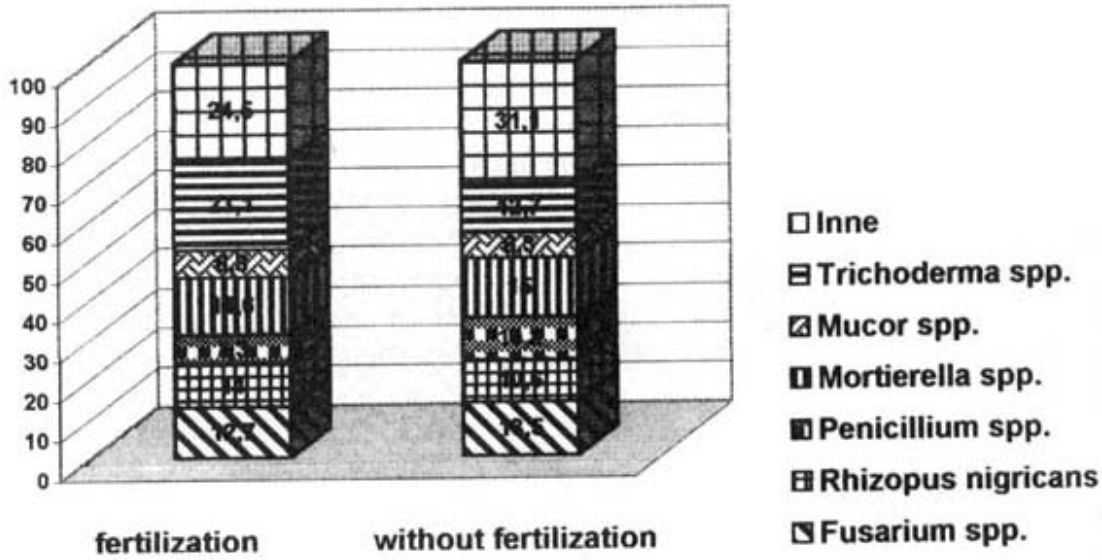

Fig. 3. Percentage of fungi isolated from rhizoplane

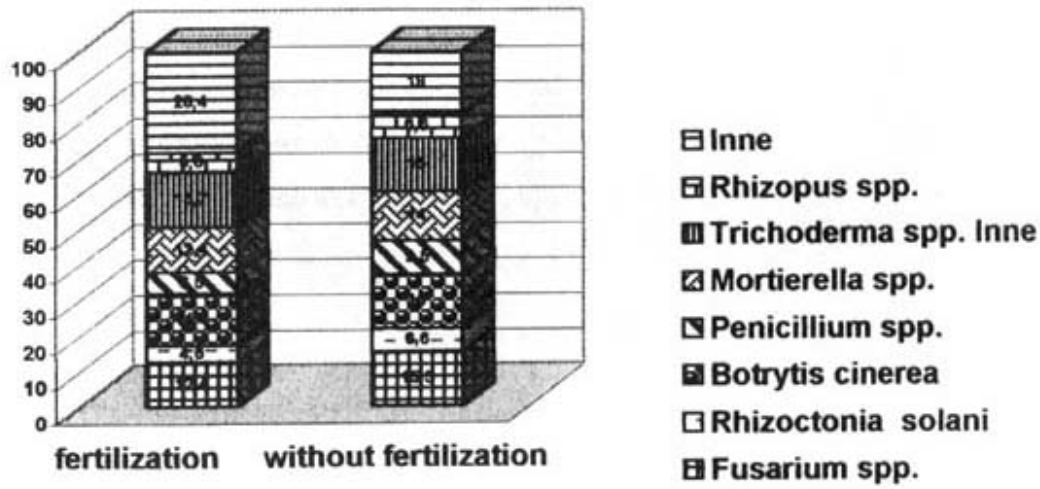

Fig. 4. Percentage of fungi isolated from roots

An analysis of subsequent years of the experiment found that differences in the quantity of the selected pathogens and their possible competitors from the goat rue rhizoplane and root. The largest number of fungi from *Fusarium* was obtained in the first year of the experiment. For the subsequent years a decrease in this fungus population was reported. At the same time in the subsequent years, the number of the saprotrophic fungi colonies from *Trichoderma* and *Mortierella* genera increased.

The fungal population structure also depended on the date of plant sampling. The number of isolated fungi from the second observation period, during *Galega orientalis* blooming, was larger and constituted 62.2% of the total colony (Fig. 1 c).

DISCUSSION

Fungi from *Mucor*, *Penicillium*, *Rhizopus* and *Trichoderma* genera appeared to be common inhabitants of the cultivation environment of the *Galega orientalis* during the three years of the experiment. Gibert (1969) and Lewis and Papavizas (1983) claim that root excretions of plants stimulate the development of the saprotrophic fungi in soil. Potential threat to *Galega orientalis* might come from pathogenic fungi such *Fusarium* genus, most frequently represented by species such as *F. culmorum* i *F. oxysporum* and *Botrytis cinerea*. They were most often isolated from roots, however the former occurred in all the analysed root zones. Dorenda (1986) reports on an important role of fungi from *Fusarium* genus in the cultivation environment of the papilionaceous plants. Other pathogen species such *Rhizoctonia solani* and *Sclerotinia sclerotiorum* were rare.

Rhizosphere was the most widely colonized by fungi and constituted 80% of the total number of the isolated fungi. It agrees with the report of Mańka et al. (1993), who regard the rhizosphere fungal population as most widely inhabited. Yeast-like fungi appeared to be dominant in the rhizosphere during the experiment analyses. Soil rich in nitrogen, such as the soil used for *Galega orientalis* cultivation, stimulate their development. Species from *Penicillium* and *Trichoderma* were rare.

Saprotrophic fungi from *Mortierella*, *Mucor*, *Rhizopus* and *Trichoderma* genera were quite frequently isolated from the goat rue rhizoplane and roots. The large population of fungi from *Trichoderma* genus is advantageous. In the references (Howell 1987; Lewis and Papavizas 1987; Łacicowa 1988) these fungi are recognised as the source of factors limiting the occurrence of pathogens from *Fusarium* genus, mainly *F. oxysporum*, *Verticillium*, *Phoma* and *Rhizoctonia solani*.

In our experiments fewer fungi colonies were obtained from the *Galega orientalis* cultivation with fertilization than without fertilization. This phenomenon is demonstrated in the paper (Deb and Dutta 1992), whose authors found the reduction of fungal population in soybean rhizosphere was the result of adequate mineral fertilization. The fertilization treatment also had a positive effect on the fungal population structure in soybean cultivation. Fungi development was not monodirectional. The *Galega orientalis* cultivation with fertilization favoured the development of saprotrophic fungi, mainly from *Trichoderma* genus. Deb and Bora (1996) report the following fungi composition from pea rhizosphere formed as the result of the NPK fertilization: *Aspergillus flavus*, *Curvularia lunata*, *Rhizopus nigricans*, *Fusarium oxysporum*, *Trichoderma lignorum*, *Penicillium lilacinum*, *Chaetomium* spp.. Pathogenic fungi, represented mainly by *Fusarium* genus occurred more commonly in the combination without fertilization.

Analysing the subsequent years of the experiment, it is clear that the number of the isolated fungi from *Fusarium* genus decreased. The smallest quantity was obtained for the third year of the experiment when compared to the largest number obtained in the first vegetative period. This fact is supported in the publication of K o w a l i k (1997). Significantly lower number of the isolated fungi colonies from *Fusarium* genus resulted from competitive action of fungi from *Trichoderma* and *Mortierella* genera, which at the same time were isolated in a larger amount than in previous vegetative periods.

CONCLUSIONS

1. The analysed *Galega orientalis* root zones featured varied quantitative and qualitative fungal composition. The rhizosphere was most widely inhabited by fungi and yeast-like fungi were dominant.
2. Pathogenic fungi from *Fusarium* and *Botrytis cinerea* genera formed the majority among the colonies isolated from the *Galega orientalis* roots. Saprotrophic fungi from *Mortierella*, *Mucor*, *Rhizopus* and *Trichoderma* genera were commonly isolated from the rhizoplane and the roots.
3. *Galega orientalis* cultivation in the combination with fertilization favoured the development of antagonistic fungi from *Trichoderma* and *Gliocladium* genera.

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Struktura zbiorowiska grzybów spod *Galega orientalis* ukształtowanego pod wpływem nawożenia

Streszczenie

Badania prowadzono na obiekcie doświadczalnym Katedry Chemii Rolnej zlokalizowanym w Bałcynach k. Olsztyna. W testach fitopatologicznych uwzględniono rutwicę wschodnią uprawianą w dwóch kombinacjach; bez nawożenia i z nawożeniem. W laboratorium określano skład ilościowy i jakościowy grzybów zasiedlających ryzosferę, ryzoplanę i korzenie rutwicy.

Środowisko uprawne rutwicy wschodniej było bardzo urozmaicone. Spośród grzybów patogenicznych dominowały gatunki z rodzaju *Fusarium* i *Botrytis cinerea*. Szeroko rozpowszechnionymi w środowisku uprawnym rutwicy okazały się grzyby saprofityczne, reprezentowane przez gatunki z rodzaju *Mortierella*, *Mucor*, *Rhizopus* i *Trichoderma*.

Spośród badanych stref ryzosfera była najbardziej zasiedlona przez grzyby.

Spod uprawy rutwicy w kombinacji z nawożeniem uzyskano o 3% izolatów mniej niż z kombinacji bez nawożenia. Duży udział miały grzyby saprofityczne z rodzajów *Trichoderma* i *Gliocladium*.