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FUNGI THREATENING SCORZONERA (*Scorzonera hispanica* L.) CULTIVATION USING PLANT MULCHES

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Abstract. *Scorzonera hispanica* is a rich source of inulin – a glycoside which has a positive effect on human and animal organisms. The paper presents studies on fungi threatening the cultivation of scorzonera. Soil mulching with intercrop cover crops such as oats, tansy phacelia and spring vetch had a positive effect on the population and healthiness of the seedlings and the roots of the studied plant. The following fungi were most frequently isolated from the infected underground organs of scorzonera: *Alternaria alternata, A. scorzonerae, Botrytis cinerea, Cylindrocarpon didymum, Fusarium culmorum, F. oxysporum, F. solani, Rhizoctonia solani, Phytophthora* sp. and *Sclerotinia sclerotiorum.* Among the applied mulching plants, oats proved to be the most effective in inhibiting the occurrence of the enumerated fungi species.

Key words: cover crops, oats, spring vetch, tansy phacelia, soil-borne phytopathogens

INTRODUCTION

Scorzonera hispanica L. is a little known plants which is rarely cultivated. It produces an edible taproot, which resembles asparagus in taste. It contains a lot of vitamins (B_1, B_2, C), carbohydrates, mineral salts (calcium, magnesium, sodium, potassium, phosphorus, iron), polyphenolic acids and glycosides [Dolota and Dąbrowska 2004a, b, Wierzbicka 2000]. It is a rich source of inulin – a glycoside which – used as dietary fibre – may have a positive effect on the digestive system, blood circulation, liver, kidneys and inhibit cancerous processes [Koo-HyunNa et al. 2003]. Besides, it has a positive influence on *Bifidobacterium* sp. and *Lactobacillus* sp. [Koo-HyunNa et al. 2003]. Dietary, taste and pro-health values of scorzonera create a need to get to know this plant better, to spread it cultivation and protect it from plant pathogens.

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The main purpose of cultivating root plants is to obtain a high, root yield of good quality [Kęsik et al. 2000a, b, Błażewicz-Woźniak et al. 2001, Błażewicz-Woźniak 2003, Patkowska and Konopiński 2011]. The quality of the yield is determined not only by the chemical composition or morphological features [Błażewicz-Woźniak 2003] but healthiness as well [Patkowska and Konopiński 2011]. Soil-borne phytopathogens exert a big influence on the healthiness of roots. The system of cultivation has a great effect on the formation of soil-borne microorganisms communities, including plant pathogens [Pięta and Kęsik 2007, Patkowska and Konopiński 2008, 2011]. The so-called conservation system of cultivation, besides having a pro-ecological effect, also affects the phytosanitary state of the soil [Wyland et al. 1996, Kęsik et al. 2000b, 2006, Pięta and Kęsik 2007, Patkowska and Konopiński 2008]. Intercrop cover plants, which can be introduced into the soil or remain on its surface in the form of mulch, are used in this kind of cultivation [Boumann et al. 2000, Erenstein 2002, Sainju et al. 2002]. Patkowska and Konopiński [2008, 2011] found out that oats, spring vetch and tansy phacelia as cover crops used for soil mulching reduce the quantitative composition of soil-borne plant pathogens in the cultivation of high-inulin root vegetables.

The literature provides only scarce information on the occurrence of diseases of *Scorzonera hispanica.* As reported by Loerakker [1984], scorzonera cultivation may be threatened by *Altenaria scorzonera*e (Aderhold). Patkowska and Konopiński [2008], on the other hand, found out high harmfulness of soil-borne fungi (*A. alternata*, *F. culmorum*, *F. oxysporum*, *F. solani*, *R. solani*) towards the seedlings of the plant under discussion.

The purpose of the present studies was to establish the species composition of fungi infecting the underground parts of scorzonera cultivated with the use of oats, tansy phacelia and spring vetch as plant mulches.

MATERIAL AND METHODS

A field experiment was conducted in the years 2006–2007 at the Experimental Station of Felin, being a part of the University of Life Sciences in Lublin (51°14'N 22°34'E). It was carried out on grey brown podzolic soil formed from loess formations occurring on chalky clays, with the mechanical composition corresponding to silty medium loam. The object of the studies were scorzonera plants 'Duplex' cv., which were sown in the first 10 days of May. The experiment took into consideration soil mulching with cover crops such as oats (*Avena sativa* L.), tansy phacelia (*Phacelia tanacetifolia* B.) and spring vetch (*Vicia sativa* L.). Cover crops were sown in the first half of August of each year preceding the establishment of the experiment. Prior to winter those plants produced an abundant yield of green matter, which constituted a natural mulch on the surface of the ploughland which was mixed with the soil as a result of pre-winter ploughing. The control was a conventional cultivation of scorzonera, i.e. without any cover crops. The experiment was set up in a split-plots scheme in four replications, and the area of each plot was 15 m^2.

In each year of the experiment the number and healthiness of scorzonera seedlings were determined. 10 seedlings with disease symptoms were taken from particular expe-

rimental combinations (phot. 1) with the aim of making a laboratory mycological analysis of the infected roots. Besides, after the harvest (the second 10-days' period of October) a mycological analysis was conducted on 10 randomly chosen roots with necrotic symptoms from each experimental combination (phot. 2). The mycological analysis was carried out according to the method described by Patkowska and Konopiński [2011] for salsify roots. This analysis made it possible to determine the quantitative and qualitative composition of fungi infecting the underground organs of scorzonera. The infected parts of plants were rinsed for 30 min under running tap water, after which they were disinfected in 0.1% sodium hypochlorite. The plant material disinfected on the surface was rinsed three times in sterile distilled water, 3 min in each. 3-milimetre fragments were made from so prepared plant material and 10 of them were put on each of the Petri dishes on solidified mineral medium with the following composition: 38 g saccharose, 0.7 g NH_4NO_3, 0.3 g KH_2PO_4, 0.3 g $MgSO_4 \times 7H_2O$, 20 g agar and trace quantities of $FeCl_3 \times 6\ H_2O$, $ZnSO_4 \times 7\ H_2O$, $CuSO_4 \times 7\ H_2O$ and $MnSO_4 \times 5\ H_2O$. The whole was filled up with distilled water up to 1000 ml and was sterilized in an autoclave for 20 min at the temperature of 121°C under the pressure of 1 atmosphere. 100 fragments of infected seedling roots and plants after the harvest were examined for each of the experimental combinations.

The Petri dishes together with the plant material on them were incubated in a thermostat for 7 days at the temperature of 23°C. Fungi colonies grown from the infected plant fragments were taken to test tubes for slants with maltose medium (a ready-made product by bio Merieux company). The isolated fungi were marked for the species after earlier preparing one-spore cultures using the method of multiple dilutions [Raiłło 1950]. The fungi of *Fusarium* genus were determined on PDA and selected agar medium SNA by Leslie and Summerell [2006]. The malt and Czapek-Dox media were used for the fungi of *Penicillium* spp. [Ramirez 1982]. The other fungi were marked on the malt medium using the corresponding keys and monographic papers [De Vries 1952, Barnett 1960, Booth 1971, Domsch and Gams 1970, Ellis 1976, Gillman 1957, Raper et al. 1968, Rifai 1969, Sałata and Rudnicka-Jezierska 1979, Marcinkowska 2003].

Results concerning the emergencies and healthiness of scorzonera seedlings were analyzed statistically, and the significance of differences was established on the basis of Tukey's confidence intervals [Oktaba 1987]. Statistical calculations were carried out using Statistica program, version 7.1.

RESULTS AND DISCUSSION

Good emergencies of plants were observed in the objects with the cultivation of tansy phacelia and spring vecht. The number of plants in this combinations were, on average, 44.7 and 42 per m^{-2} (tab. 1). The smallest density of plants (31.6 per m^{-2}) was found in objects where the oats biomass was covered with the soil.

Observations found out seedlings of inhibited growth and development on the plots of particular experimental combinations. After digging out the roots, brown necrotic spots were visible (phot. 1). Disease symptoms in the form of rot or dry necrosis were also observed on the roots after the harvest of scorzonera (phot. 2).

Table 1. Field stand and healthiness of scorzonera seedlings

Experimental combination	Field stand per 1 m^2			Percentage of diseased seedlings (%)		
	2006	2007	mean	2006	2007	mean
Oats mulch	42.0 b*	21.2 a	31.6 a	10.5 a	12.4 a	11.4 a
Tansy phacelia mulch	40.2 b	49.2 c	44.7 b	25.0 c	26.3 b	25.6 c
Spring vetch mulch	52.2 c	31.8 b	42.0 b	18.6 b	20.2 b	19.4 b
Conventional cultivation (control)	31.0 a	47.6 c	39.3 b	38.4 d	34.2 c	36.3 d

* Means in columns followed by the same letters are not significantly different at $P \leq 0.05$

The proportion of the infected seedlings of scorzonera varied depending on the species of the mulching plant. It ranged from 10.5% to 38.4% (tab. 1). In each year of studies the smallest number of infected seedlings was observed after the mulch of oats, while slightly more after mulching the soil with spring vetch. The highest proportion of infected seedlings of scorzonera was found out in the conventional cultivation (34.2% and 38.4%, depending on the year of studies) (tab. 1). The cultivation system, i.e. the spring or pre-winter plough had no significant effect on the proportion of infected seedlings of chicory. As reported by Loerakker [1984], the emergences and the yield of scorzonera were considerably lowered as a result of infection by *Alternaria scorzonerae.*

Phot. 1. The necrosis of 4-week-old scorzonera seedlings growing in the conventional cultivation (photo E. Patkowska)

Phot. 2. The necrosis of scorzonera roots growing in the conventional cultivation (photo E. Patkowska)

From the infected seedlings of scorzonera were obtained 486 colonies of different species of fungi (tab. 2). In each experimental combination, slightly more fungi isolates were isolated from the roots as compared to the root heads (hypocotyle). The fewest fungi (83 isolates) were obtained from the infected seedlings of scorzonera cultivated after oats mulch, and slightly more after mulching the soil with spring vetch (96 isolates) or tansy phacelia (137 isolates). The greatest amount of fungi were obtained from scorzonera seedlings cultivated conventionally, i.e. without cover crops (170 isolates) (tab. 2). Independently of the species of the mulching plant, the fungi were obtained from the examined organs of scorzonera seedlings, namely *Alternaria* spp., *Cylindrocarpon didymum, Fusarium* spp., *Rhizoctonia solani* and *Phytophthora* sp. The genus of *Alternaria* was represented by the species of *Alternaria alternata* and *A. scorzonerae*, and their total proportion was 4.7% and 8.0% (tab. 2). The genus of *Fusarium* included the species of *Fusarium culmorum* (6.0% totally), *F. oxysporum* (17.5% totally) and *F. solani* (6.2% totally). The proportion of *Cylindrocarpon didymium, Rhizoctonia solani* and *Phytophthora* sp. among all the fungi isolated from the infected seedlings of scorzonera constituted, respectively, 2.9%, 9.0% and 8.2% (tab. 2). From the examined

Table 2. Fungi isolated from diseased seedlings of scorzonera (sum from the years 2006–2007)

Fungus species	Experimental combination – Number of isolates										total (%)
	1		2		3		4		total		
	a	b	a	b	a	b	a	b	a	b	
Acremonium roseum (Oud.) W. Gams	1	–	2	–	–	–	3	2	6	2	8 (1.6)
Alternaria alternata (Fr.) Keissler	–	2	3	4	1	2	5	6	9	14	23 (4.7)
Alternaria scorzonerae (Aderhold) comb. nov.	2	3	5	6	4	4	7	8	18	21	39 (8.0
Cladosporium cladosporioides (Fres) de Vries	–	1	–	3	–	3	–	5	–	12	12 (2.5)
Cylindrocarpon didymum (Harting) Wollenw.	2	3	2	2	–	–	3	2	7	7	14 (2.9)
Epicoccum purpurascens Ehr. ex. Schl.	–	–	1	1	1	–	2	1	4	2	6 (1.3)
Fusarium culmorum (W.G.Sm.) Sacc.	2	1	5	4	3	2	7	5	17	12	29 (6.0)
Fusarium oxysporum Schl.	9	5	13	11	10	5	17	15	49	36	85 (17.5)
Fusarium solani (Mart.) Sacc.	2	–	5	4	3	2	8	6	18	12	30 (6.2)
Mucor mucedo Fresenius	–	–	2	–	1	1	3	2	6	3	9 (1.8)
Penicillium janczewskii Zaleski	2	2	5	3	4	3	2	2	13	10	23 (4.7)
Penicillium verrucosum Dierckx var. *cyclopium* (West.) Samson, Stolk et Hadlok	3	2	6	2	3	3	4	5	16	12	28 (5.8)
Phytophthora sp.	4	2	6	6	5	3	8	6	23	17	40 (8.2)
Rhizoctonia solani Kühn	3	2	8	6	4	4	10	7	25	19	44 (9.0)
Rhizopus nigricans Ehrenberg	1	1	6	5	3	3	8	5	18	14	32 (6.6)
Trichoderma koningii Oud.	10	4	5	1	8	2	3	–	26	7	33 (6.8)
Trichoderma viride Pers. ex. S.F. Gray	8	6	3	2	5	4	2	1	18	13	31 (6.4)
Total	49	34	77	60	55	41	92	78	273	213	486(100.0)
Total	83		137		96		170		486		

1 – oats mulch; 2 – tansy phacelia mulch; 3 – spring vetch mulch; 4 – conventional cultivation (control)
a – root; b – head of root

Table 3. Fungi isolated from diseased roots of scorzonera after harvest (sum from the years 2006–2007)

Fungus species	Experimental combination – Number of isolates										total (%)
	1		2		3		4		total		
	a	b	a	b	a	b	a	b	a	b	
Alternaria alternata (Fr.) Keissler	2	2	5	5	4	3	8	2	19	12	31 (3.3)
Alternaria scorzonerae (Aderhold) comb. nov.	4	3	8	6	4	4	10	9	26	22	48 (5.2)
Botrytis cinerea Pers.	-	-	-	4	-	2	-	6	-	12	12 (1.3)
Cladosporium cladosporioides (Fres) de Vries	2	1	3	2	2	2	4	3	11	8	19 (2.1)
Cylindrocarpon didymum (Harting) Wollenw.	4	3	5	5	5	3	7	6	21	17	38 (4.1)
Epicoccum purpurascens Ehr. ex. Schl.	1	-	3	2	2	-	4	2	10	4	14 (1.5)
Fusarium culmorum (W.G.Sm.) Sacc.	3	3	7	5	5	4	8	6	23	18	41 (4.4)
Fusarium equiseti (Corda) Sacc.	-	1	2	3	2	2	4	3	8	9	17 (1.8)
Fusarium oxysporum Schl.	11	8	14	13	12	10	19	18	56	49	105 (11.4)
Fusarium solani (Mart.) Sacc.	4	2	6	3	6	4	10	10	26	19	45 (4.9)
Humicola grisea Domsch	2	1	3	2	-	-	5	-	10	3	13 (1.4)
Mucor hiemalis Wehmer	-	-	3	1	2	-	5	4	10	5	15 (1.6)
Mucor mucedo Fresenius	-	1	5	2	3	2	7	3	15	8	23 (2.5)
Penicillium janczewskii Zaleski	3	1	9	8	5	4	15	11	32	24	56 (6.1)
Penicillium janthinellum Biourge	2	2	8	5	6	6	12	10	28	23	51 (5.5)
Penicillium verrucosum Dierckx var. *cyclopium* (West.) Samson, Stolk et Hadlok	-	2	6	5	4	4	11	9	21	20	41 (4.4)
Penicillium verrucosum Dierckx var. *verrucosum* Samson, Stolk et Hadlok	4	4	9	7	6	5	13	14	32	30	62 (6.7)
Phytophthora sp.	5	5	9	8	7	6	11	10	32	29	61 (6.6)
Rhizopus nigricans Ehrenberg	3	1	7	4	5	3	6	3	21	11	32 (3.5)
Rhizoctonia solani Kühn	6	8	10	7	6	10	15	11	37	36	73 (7.9)
Sclerotinia sclerotiorum Lib. de Bary	3	2	10	11	7	5	12	15	32	33	65 (7.0)
Trichoderma harzianum Rifai	5	3	1	1	3	3	-	2	9	9	18 (1.9)
Trichoderma koningii Oud.	7	5	3	1	5	2	1	-	16	8	24 (2.6)
Trichoderma viride Pers. ex. S.F. Gray	6	4	2	2	3	2	1	1	12	9	21 (2.3)
Total	77	62	138	112	104	86	188	158	507	418	925 (100.0)
Total	139		250		190		346		925		

1 – oats mulch; 2 – tansy phacelia mulch; 3 – spring vetch mulch; 4 – conventional cultivation (control)
a – root; b – head of root

seedlings of scorzonera were isolated *Acremonium roseum, Cladosporium cladosporioides, Epicoccum purpurascens, Mucor mucedo, Penicillium janczewskii, Penicillium verrucosum* var. *cyclopium, Rhizopus nigricans, Trichoderma koningii* and *T. viride* (tab. 2). Only a little more of those fungi were obtained from the seedlings of scorzonera cultivated conventionally as compared to the cultivation with the use of oats, tansy phacelia or spring vetch as mulch. Studies conducted by Patkowska and Konopiński [2008] pointed to considerable pathogenicity of soil-borne fungi towards scorzonera seedlings. The most harmful proved to be the isolates of *P. irregulare* and *F. oxysporum* [Patkowska and Konopiński 2008]. Besides, as reported by Tylkowska and Van der Bulk [2001] and Bralewski et al. [2004], soil-borne fungi constitute a big danger towards a number of root plant species, including scorzonera. The pathogenic character of *F. oxysporum, Pythium irregulare* and *R. solani* towards parsley seedlings was found out by Nawrocki [2005] on the basis of field and glasshouse studies.

The mycological analysis conducted after the harvest of scorzonera found out that more fungi were also obtained from the roots as compared to the root heads. Totally, after the harvest, 925 isolates of fungi belonging to 14 genera were obtained from the roots of *Scorzonera hispanica* (tab. 3). The smallest total number of fungi (139 isolates) was isolated from the roots of scorzonera cultivated after oats mulch, and slightly more after mulching the soil with spring vetch (190 isolates) and tansy phacelia (250 isolates). The most fungi (346 isolates) were obtained from the roots of scorzonera cultivated conventionally (tab. 3). After the harvest, from scorzonera roots were isolated: *Alternaria alternata, A. scorzonerae, Botrytis cinerea, Fusarium culmorum, F. oxysporum, F. solani, Rhizoctonia solani, Phytophthora* sp. and *Sclerotinia sclerotiorum.* A little more the enumerated fungi were isolated from the infected roots of scorzonera cultivated conventionally or after tancy phacelia as a cover crops, while the least after mulching the soil with oats. Moreover, from scorzonera roots were isolated *Cladosporium cladosporioides, Epicoccum purpurascens, Humicola grisea, Rhizopus nigricans*, *Mucor* spp., *Penicillium* spp. and *Trichoderma* spp. (tab. 3). The cultivation of scorzonera – according to Loerakker [1984] – may be threatened by *Alternaria* spp., especially the species of *Alternaria scorzonerae.* On the other hand, as reported by Santos et al. [2000] and Ben-Noon et al. [2001], the species of *Alternaria dauci* poses a threat towards other root plants, especially carrot.

The present studies pointed to the negative effect of cover crops on the growth and development of plant pathogens, this decreasing the infection of scorzonera roots by soil-borne fungi. The plants used for mulching, especially oats, inhibited the infection of underground organs of S*corzonera hispanica*, e.g. by *Alternaria* spp., *Penicillium* spp. and *Fusarium* spp. These fungi have the ability to produce mycotoxins, which are very harmful towards homoithermic organisms [Labuda and Tancinova 2006, Desjardins and Proctor 2007]. That is the reason why such methods of *Scorzonera hispanica* cultivation should be used that will limit the occurrence of plant pathogens and the infection of plants and food with harmful metabolites of fungi.

CONCLUSIONS

1. Soil mulching with intercrop cover crops had a positive effect on the population and healthiness of the seedlings and the roots of scorzonera.

2. *Alternaria alternata, A. scorzonerae, Botrytis cinerea, Cylindrocarpon didymum, Fusarium culmorum, F. oxysporum, F. solani, Rhizoctonia solani, Phytophthora* sp. and *Sclerotinia sclerotiorum* proved to be the most harmful towards the studied underground parts of scorzonera.

3. Oats proved to be the most effective in inhibiting the occurrence of the pathogenic fungi for scorzonera.

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GRZYBY ZAGRAŻAJĄCE UPRAWIE SKORZONERY (*Scorzonera hispanica* L.) Z ZASTOSOWANIEM MULCZÓW ROŚLINNYCH

Streszczenie. *Scorzonera hispanica* jest bogatym źródłem inuliny – glikozydu, który ma pozytywny wpływ na organizm człowieka i zwierząt. Praca przedstawia badania dotyczące grzybów zagrażających uprawie skorzonery. Mulczowanie gleby międzyplonowymi roślinami okrywowymi, takimi jak owies, facelia i wyka siewna, wpłynęło korzystnie na liczebność i zdrowotność siewek oraz korzeni badanej rośliny. Z porażonych organów podziemnych skorzonery najczęściej izolowano takie grzyby jak: *Alternaria alternata, A. scorzonerae*, *Botrytis cinerea, Cylindrocarpon didymum*, *Fusarium culmorum, F. oxysporum, F. solani, Rhizoctonia solani*, *Phytophthora* sp. i *Sclerotinia sclerotiorum.* Spośród zastosowanych roślin mulczujących owies okazał się najskuteczniejszy w ograniczeniu występowania wymienionych gatunków grzybów.

Słowa kluczowe: rośliny okrywowe, owies, wyka siewna, facelia błękitna, fitopatogeny przeżywające w glebie

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