Fungi colonizing various parts of parsnip *Pastinaca sativa* L.

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Summary

The occurrence and harmfulness of fungi towards *Pastinaca sativa* L. parsnip of White Gem cultivar were determined in 2005–2007. The mycological analysis of schizocarps with spots and without spots, seedlings and plants at the time of harvest was conducted every year. The fungi were identified on the basis of etiological symptoms visible on the infected parts of plants and on the basis of the results of mycological analysis. Parsnip schizocarps were colonized by various fungi species. Larger frequency of occurrence and biodiversity of fungi species on plants at the time of the harvest was found as compared to the seedlings. The fungus *Itersonilia pastinacae,* recognized as a cause of black cancer of parsnip in the regions where this plant is cultivated, was isolated for the first time in Poland. *Alternaria alternata, A. raphani, Fusarium oxysporum, F. equiseti, F. solani* and *Stemphylium botryosum* belonged to the species of fungi frequently isolated from various parts of this plant.

Key words: *parsnip, fungi, occurrence*

INTRODUCTION

Parsnip (*Pastinaca sativa* L.) is a vegetable and a medicinal plant. Roots and young leaves are used for consumption but the fruit of parsnip, i.e. *Fructus Pastinacae* is used in medicine. Commonly, this plant is recognized as a healthy plant. However, this argument is not confirmed by our study, and a lot of information about the occurrence of various factors causing diseases of parsnip is mentioned in the world literature [1].

In decayed tissues of parsnip roots the fungi *Phoma nebulosa*, *Phytophthora nicotiana* var. *parasitica* and *Rhizoctonia solani* were identified but lesions of the roots

during transport were caused by the fungus *Botrytis cinerea* [1]. Parsnip is recognized as a first host of *Phomopsis diachenii,* a very dangerous fungal pathogen for the *Apiaceae* plant [1-3]. The occurrences of the fungi were observed on the stems, the leaves and the fruits of parsnip. Disease symptoms on the leaves usually observed as spots were caused by the following fungi: *Septoria* sp., *Cercospora pastinacae* and *Altrenaria* sp. [1]. The occurrence of *Chaetomium globosum, Chaetomium murorum* and *Alternaria alternata* was recorded in the seeds of parsnip [1].

Cancer of hypocotyl and crown of the root is the most often occurring disease of parsnip in the Great Britain and USA [4, 5]. Three types of this disease are described. Black cancer – the first type of the disease is caused by *Itersonilia pastinacae,* second – black cancer is caused by *Phoma* sp. or by both together. The third type is orange-brown cancer of parsnip but the origin of this type of disease is unknown [4].

Because of the lack of mycological studies on parsnip and increasing agricultural significance of this plant the present research was carried out.

MATERIAL AND METHODS

The study material consisted of schizocarps of White Gem parsnip cultivars from RIJK ZWAAN Poland Ltd. and plants from plantation located in Sporniak (Lublin district). The area of cultivation was 0.5, 2.0 and 1.3 ha, respectively, in 2005, 2006 and 2007. The potatoes were the forecrop of this plant in the first year, triticale in the second and parsnip in the third year of cultivation. Adequate cultivation, organic and mineral fertilization and plant protection action were performed each year.

Mycological analysis of schizocarps

Each time two samples were studied, i.e. 100 schizocarps without spots on their surface and 100 schizocarps with spots on the surface. Schizocarps were superficially disinfected in a 10% solution of sodium hypochlorite for 1.5 min. and three times rinsed in sterile distilled water for 3 min. Then the schizocarps were placed on mineral agar medium in Petri dishes [6].

Estimation of healthiness and mycological analysis of plant

The observation was carried out twice, i.e. at the phase of six-week-old seedlings (second part of July) and at the time of harvest (mid-October). On the plantations the percentage of plants with disease symptoms was determined on 1 m^2 area, including four places. Plant samples of 20 seedlings or 10 mature plants were taken to the laboratory. Fungi were found on the basis of etiological symptoms occurring on plants and on the basis of mycological analysis conducted by the method of artificial cultures. The roots, the base of stems and the leaves were subject to separate analysis (100 items of three-millimeter pieces each time), in

the manner described in the case of seeds [6, 7]. The isolated fungi were identified on the medium used for cultivation (PDA) or on standard media [3, 8-12].

RESULTS

In the studied samples of the sown material schizocarps with symptoms of disease were found. These schizocarps – as opposed to healthy ones - had pale beige colour and dark gray necrotic spots ranged from 1.5 to 3.0 mm in diameter, with a brown hallo (fig. 1). Single or accumulated in aggregates fruit bodies occurred on their surface, but no spores were observed inside them. The percentage of diseased schizocarps ranged from 2% to 5% in the years of study.

In total, 170 fungi isolates were obtained from the analyzed schizocarps with spots, and 81 fungi isolates from schizocarps without spots (tab. 1). The fungi from *Alternaria, Cladosporium, Epicoccum, Fusarium, Phoma, Pleospora* genera and *Stemphylium botryosum* colonized them (fig. 2). The greatest percentage among the isolated fungi, i.e. 28.9%, 22.3% and 17.1%, respectively, was found for *Stemphylium botryosum, Phoma exigua* var. *exigua* and *Alternaria alternata* (tab. 1).

Table 1.

Fungi isolated from parsnip schizocarps *Pastinaca sativa* L. in 2005–2007

species of fungi	number of isolates						total number (%) of isolates
	schizocarps with spots			schizocarps without spots			
	2005	2006	2007	2005	2006	2007	
Alternaria alternata Keissler	10	23	2	6	2	-	43 (17.1)
Alternaria radicina Meier, Drechsler et Eddy	-	12	-	-	3	-	15 (5.9)
Cladosporium cladosporioides (Fres.) de Vries	-	3	-	-	1	-	4 (1.6)
Epicoccum purpurascens Ehrenberg	5	3	-	-	-	-	8 (3.2)
Fusarium equiseti (Corda) Sacc.	-	2	-	-	-	-	2 (0.8)
Phoma exigua Desm. var. *exigua*	4	-	21	1	-	30	56 (22.3)
Pleospora tarda Simmons	17	-	-	7	2	-	26 (10.3)
Saccharomyces spp.	1	-	12	-	-	12	25 (9.9)
Stemphylium botryosum Wallr.	-	44	11	-	5	12	72 (28.9)
total	37	87	46	14	12	54	251 (100)
			170			81	251 (100)

The observation conducted on six-week-old seedlings of parsnip showed the presence of small necrosis on the hypocotyls (fig. 3). The growth of these plants was inhibited and chlorosis of two drawer leaves was observed. The percentage of seedlings with symptoms of disease was from 12.8 to 30.0%, from 14.8 to 16.6% and from 4.65 to 5.8 %, respectively, in particular years.

Brown necrotic spots with irregular shape and size ranging from 3.0 to 5.0 mm occurred on the drawer leaves of parsnip rosette at the harvest time (fig. 4). On the leaves of some plants those spots were large, carmine-brown, covering whole

surface of the leaves between veins. On the roots, especially on their top parts, brown or brown-black spots with a hallo occurred. The size of single spots did not exceed 5.0–7.0 mm in diameter, but spots grouped together covered from ¼ to 1/3 of the root surface (fig. 5). The necrotic lesions were visible on the root surface. When rotting extended down, they reached 2–3 mm inside the parenchyma. Distinct softness of diseased tissues of roots was not observed. The percentage of plants with symptoms of disease at the harvest time ranged from 1.5 to 5.5%, from 11.2 to 15.8% and from 15.0 to 20.0%, respectively, in the study years.

Using the accepted method of isolation, 289 cultures of fungi belonging to 17 genera were isolated from seedlings (tab. 2). Most of cultures of fungi from the leaves and hypocotyls and the fewest from roots were obtained in particular years (tab. 2). *Alternaria alternata* was isolated from all parts of seedlings, most often from leaves. The cultures of this fungus made 28.3% of all isolates obtained from the seedlings, 21.4% made cultures of *Fusarium oxysporum,* 15.5% *Stemphylium botryosum* and 9.3% *F. equiseti* (tab. 2). The last species of fungi, among them *Acremonium* spp., *Cladosporium* sp., *Cylindrocarpon* spp., *Itersonilia* sp., *Penicillium* sp., *Phoma* sp. and *Trichoderma* spp., were obtained with lesser frequency (fig. 6).

Table 2.

Fungi isolated from seedlings of parsnip *Pastinaca sativa* L. in 2005–2007

species of fungi	number of isolates			total number (%) of isolates
	leaves	hypocotyls	roots	
Acremonium roseum (Oud.) W. Gams	–	10	3	13 (4.4)
Acremonium strictum W. Gams spec. nov.	–	1	–	1 (0.3)
Alternaria alternata Keissler	66	13	3	82 (28.3)
Alternaria radicina Meier, Drechsler et Eddy	14	6	–	20 (6.9)
Cladosporium cladosporioides (Fres.) de Vries	–	1	–	1 (0.3)
Cylindrocarpon ianthothele Wollenw.	–	–	1	1 (0.3)
Cylindrocarpon obtusisporum (Cooke et Harkness)	–	–	5	5 (1.7)
Fusarium equiseti (Corda) Sacc.	10	17	–	27 (9.3)
Fusarium oxysporum Schlecht. emend. Snyd et Hans	1	41	20	62 (21.4)
Fusarium poae (Peck) Wollenw.	–	2	–	2 (0.6)
Itersonilia pastinacae sp. nov.	–	4	–	4 (1.3)
Penicillium verruculosum Peyronel	–	2	1	3 (1.0)
Phoma exigua Desm. var. *exigua*	–	7	1	8 (2.7)
Saccharomyces spp.	–	1	6	7 (2.4)
Stemphylium botryosum Wallr.	41	4	–	45 (15.5)
Trichoderma harzianum Rifai	–	2	–	2 (0.6)
Trichoderma koningii Oud.	–	–	6	6 (2.0)
total	132	111	46	289 (100)

In total, 586 cultures of fungi classified to 22 species were isolated from plants at the harvest time (tab. 3). *Alternaria raphani, Fusarium equiseti, F. solani* and *Stemphylim botryosum* belonged to fungi colonizing all studied parts of plants, but species

F. oxysporum, A. raphani, A. alternata, F. solani and *Rhizoctonia solani* had the largest percentage among the isolated cultures (tab. 3). The percentage of mentioned fungi was 17.5, 17.2, 10.1, 10.1 and 6.6%, respectively (tab. 3). The fungi from genera *Alternaria, Stemphylium* and *Itersonilia* were obtained mainly from the leaves, but *Fusarium* spp., *Rhizoctonia* sp., *Cylindrocarpon* sp. and *Phoma exigua* from the base of stems and the roots (tab. 3). In autumn of 2006, on the top part of parsnip leaf geometrical spots with a sharp-shaped point were observed. On the other hand, on the bottom parts of these spots white-gray coating composed of conidiophores and conidia of *Plasmopara umbelliferarum,* causing downy mildew occurred (fig. 7). The infected leaves became yellowish and necrotic at the end. The appearance of downy mildew in 2006 was connected with temporary high temperature and frequent rainfalls, which exceeded the long-term means for September and October. In general, in 2007 at the time of harvest on the leaves and petals of plants the symptoms of powdery mildew caused by *Erysiphe heraclei* DC. Ex St.-Am. (fig. 8) were observed. At that time the temperature increased periodically and reached 24^{0}C with very scarce rainfalls.

Table 3.

Fungi isolated from various parts of parsnip *Pastinaca sativa* L. in 2005–2007

species of fungi	number of isolates			total number (%) of isolates
	leaves	bBase of stems	roots	
Alternaria alternata Keissler	50	9	–	59 (10.1)
Alternaria radicina Meier, Drechsler et Eddy	–	2	–	2 (0.3)
Alternaria raphani Groves et Skolko	96	4	1	101 (17.2)
Cladosporium cladosporioides (Fres.) de Vries	5	–	–	5 (0.8)
Cylindrocarpon didymum (Hartig) Wollenw.	–	–	16	16 (2.7)
Cylindrocarpon ianthothele Wollenw.	–	3	20	23 (3.9)
Cylindrocarpon obtusisporum (Cooke et Harkness)	–	1	7	8 (1.3)
Epicaccum purpurascens Ehrenberg	–	6	2	8 (1.3)
Fusarium avenaceum (Fr.) Sacc.	–	9	–	9 (1.5)
Fusarium equiseti (Corda) Sacc.	1	6	8	15 (2.5)
Fusarium nivale (Fr.) Ces.	–	–	4	4 (0.6)
Fusarium oxysporum Schlecht. emend. Snyd et Hans	–	56	47	103 (17.5)
Fusarium solani (Mart.) Appel et Wollenw. Emend. Snyd. et Hans	3	38	18	59 (10.1)
Fusarium sporotrichioides Sherb.	–	33	1	34 (5.8)
Humicola fuscoatra Traaen	–	–	1	1 (0.2)
Itersonilia pastinacae sp. nov.	14	–	2	16 (2.7)
Penicillium decumbens Thom	–	1	–	1 (0.2)
Penicillium verruculosum Peyronel	–	6	5	11 (1.8)
Phoma exigua Desm. var. *exigua*	–	3	13	16 (2.7)
Rhizoctonia solani Kühn	–	13	26	39 (6.6)
Stemphylium botryosum Wallr.	22	6	2	30 (5.1)
Trichoderma koningii Oud.	–	24	2	26 (4.4)
total	191	220	175	586 (100)

Figure 1. Schizocarps of parsnip with spots – (a) , without spots – (b) (photo 1–8 E. Zalewska)

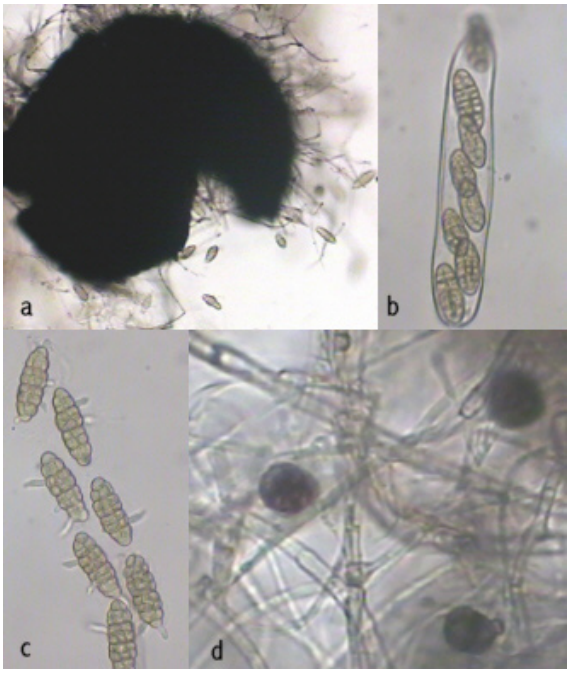

Figure 2. *Pleospora tarda*: a) fruit body x 125, b) sacs of spores x 250, c) germinate ascospores x 300, d) anamorph *Stemphylium botryosum* – conidia x 500

Figure 3. Necrosis of parsnip hypocotyls

Figure 4. Leaf from which various fungi were isolated

Figure 5. Root of parsnip from which *Itersonilia pastinacae* was isolated

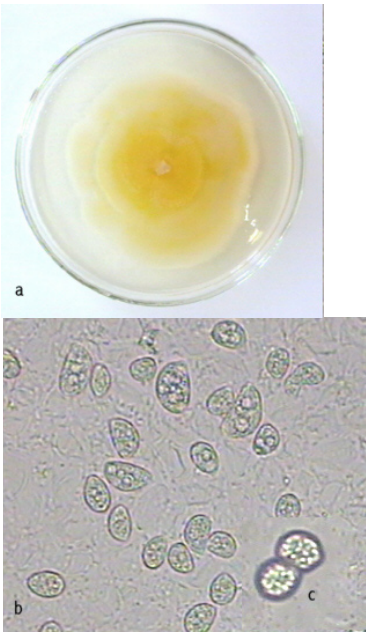

Figure 6. a) Four-week-old colony of *Itersonilia pastinacae* on PDA medium, b) ballistospores, c) chlamydospores

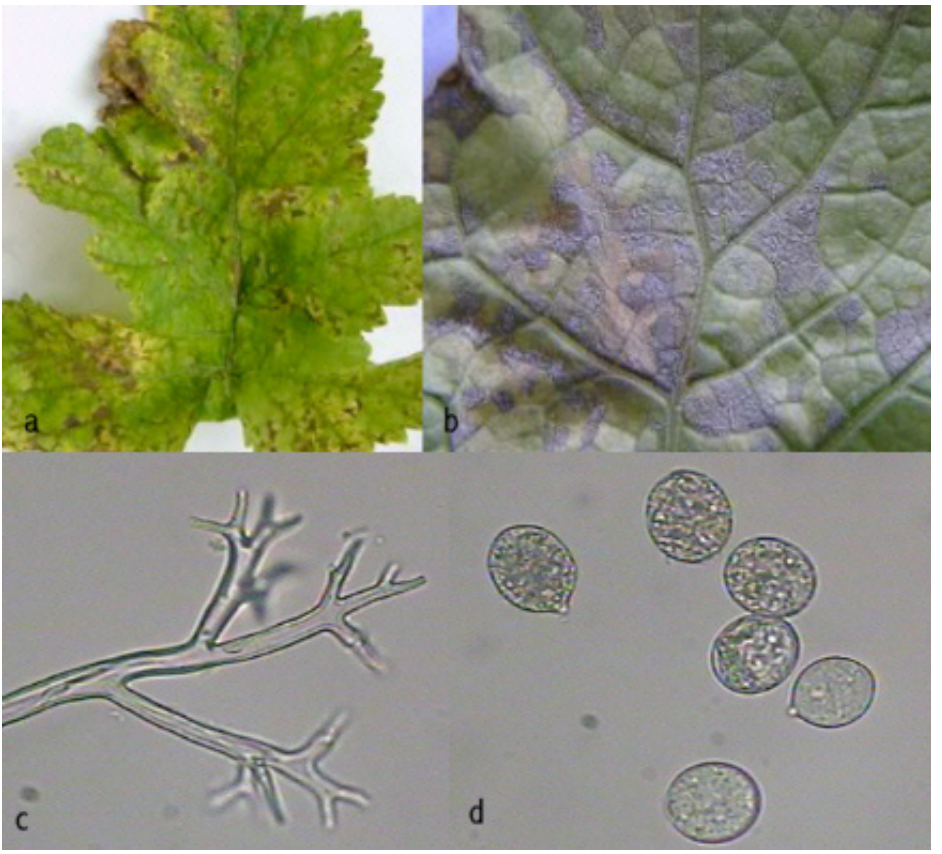

Figure 7. Downy mildew on parsnip *Plasmopara umbelliferarum* – a) necrosis on a top part of leaf, b) etiological stamp on dower part of leaf, c) conidiophores x 250, d) conidia of the fungus x 750

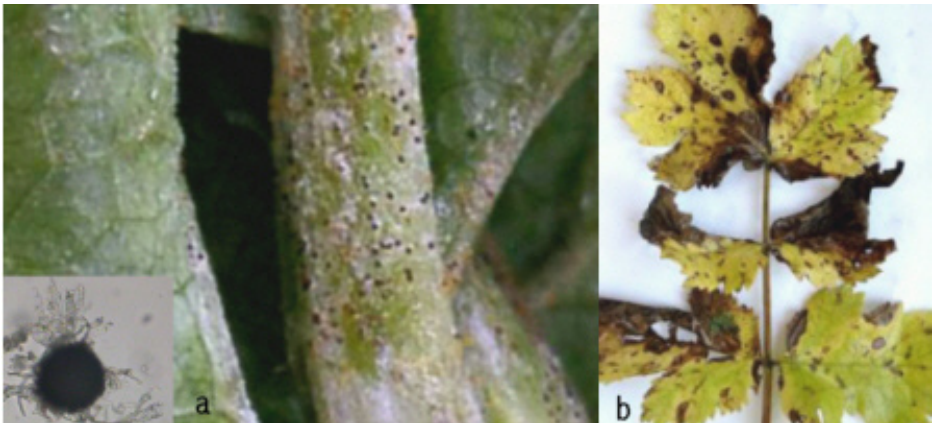

Figure 8. Powdery mildew of parsnip *Erysiphe heraclei* – a) mycelium and cleistothecia of fungus, b) necrosis of leaf after pathogen`s infection

DISCUSSION

Results of presented studies showed that microbiological contamination of the sowing material was small. Especially, well-shaped schizocarps of parsnip free from lesions were colonized by fungi more rarely than the schizocarps with spots on their surface, which corresponded with results of earlier studies [13, 14]. Taking into account the healthiness of shoots, the schizocarps with disease symptoms on their surface should not occur in the sowing material [13]. Treating the schizocarps with fungicides – before sowing – had a big significance because this intervention eliminates contaminated microorganisms and protects infection by factors from the soil [14]. The fact of frequent colonization of parsnip schizocarps by pathogenic fungi, *Stemphylium botryosum* and their teleomorph *Pleospora tarda* [15] and *Phoma exigua* var. *exigua* [16] should be taken into account. The isolation of the above-mentioned fungi from the schizocarps without spots and with spots suggests that the fungi can colonize the sowing material without any symptoms.

The studies on the healthiness of six-week-old seedlings of parsnip and plants at the time of the harvest showed that all parts of this plant were colonized by fungi, but the largest number of species colonized the hypocotyl. A large frequency of occurrence and biodiversity of species was indicated on plants at the time of the harvest as compared to six-week-old seedlings. That fact results from a moderate effect of the environment on pathogens and plants. Numerous fungi species isolated from plants were not isolated from schizocarps in the present studies. This fact indicated that these fungi penetrate into the seedlings from the surrounding environment.

The achievement of present studies is the isolation of *Itersonilia pastinacae* from necrotic spots on the base of stems, the leaves and roots of a few parsnip plants at the time of harvest. This fungus was recognized as a cause of black cancer of parsnip in the regions of cultivation [4, 5]. Moreover, the creation of chlamydospores in studied cultures of the fungus suggests that obtained isolates of fungus are pathogenic to parsnip [4]. There is no information in available literature on the occurrence of this pathogen on parsnip in Poland. Hence, attention should be directed in the following years of studies to their harmfulness and intensity of occurrence on the leaves, the hypocotyl and the roots of this plant. Similarly, the obtained cultures of *Phoma* sp. could threaten parsnip, which is reported by Chanonn [4].

The species of *Alternaria alternata, A. raphani, F. oxysporum, F. equiseti* and *F. solani* belong to the fungi occurring on all parts of parsnip.

Frequent isolation of fungi of *Fusarium* genera and *Rhizoctonia solani* from the base of stems and the roots of plants suggests that the species penetrated the plant from the soil environment. These soil fungi live as saprotrophs and could be a threat to the underground parts of plants or for the parts which contact the soil [12]. From among the obtained species of fungi *F. equiseti* was recognized as a cause of caraway roots necrosis [17], and *F. oxysporum* f. sp. *cumini* and *F. oxysporum* f. sp. *corianderii* as a cause of caraway and coriander vascular wilt of plants [18, 19].

The ascertained presence of *Plasmopara umbelliferarum* on the leaves is probably connected with temperature reduced to 15^0C in autumn and periodical rainfalls, which is favorable to the development of this fungus [20]. The presence of *Erisiphe heraclei* on parsnip was possible during the autumn warming up, because the high temperature favored the germination of these fungi conidia [21].

REFERENCES

1. Farr DF, Bills GF, Chamuris GP, Rossman AY. Fungi on plants products in the United States. St. Paul, Minnesota 1995.
2. Saccardo PA. Notae mycologicae. Annals Mycol 1915; 13:115-38.
3. Sutton BC. The *Coelomycetes.* Fungi Imperfecti with Pycnidia, Acervuli and Stroma. Kew 1980.
4. Channon AG. Studies on parsnip caker. I. The causes of diseases. Ann Appl Biol 1963; 51:1-15.
5. Wilkinson RE. Parsnip canker is caused by *Itersonilia* sp. Phytopathol 1952; 42:23.
6. Machowicz-Stefaniak Z, Zimowska B, Zalewska E. The occurrence and pathogenicity of *Phoma exigua* Desm. var. *exigua* for selected species of herb. Acta Agrobot 2008; 61, 2:157-166.
7. Machowicz-Stefaniak Z, Zalewska E. Biodiversity of fungi colonizing various organs of caraway (*Carum carvi* L.). Electron J Pol Agric Univ, ser. Horticultura 11 (1), # 21, http://www.ejpau.media.pl/volume11/issue1/art-21.html, 2008.
8. Booth C. The genus *Fusarium.* Com Myc Inst, Kew, England 1971.
9. Rifai MA. A revision of the genus *Trichoderma.* Kew Surrey, England 1969.
10. Ellis MB. *Dermatiaceous, Hyphomycetes.* Com Myc Inst. Kew Surrey, England 1971.
11. Ramirez ZC. Manual of atlas of the Penicillia. Oxford 1982.
12. Nelson PE, Toussoun TA, Marasas WFO. *Fusarium* species – An illustrated manual for identification. The Pensylw St Univ Press, University Park of London 1983.
13. Łacicowa B, Kiecana I, Pięta D. Mikoflora materiału siewnego roślin ozdobnych. Cz. I. Mikoflora materiału siewnego cynii (*Zinnia elegans* L.) i groszku pachnącego (*Lathyrus odoratus* L.). Pr Inst Sadown Kwiac ser. B 1991; 16:109-116.
14. Machowicz-Stefaniak Z., Zimowska B. Fungi transporting by sowing seed material of herbs. Acta Agrobot 2000; 53 (2):25-38.
15. Marcinkowska J. Oznaczanie rodzajów grzybów w fitopatologii roślin. Fundacja Rozwój SGGW, Warszawa 2003.
16. Boerema GH, Gruyter J de, Noordeloos ME, Hamers MEC. *Phoma* identification manual. Differentiation of specific and intra-specific taxa in culture. CABI Publishing 2004.
17. Reuveni R. *Fusarium equiseti* – A new cause of cumin spice plant wilt in Israel. Plant Dis 1982: 66:498-9.
18. Srivastava US. Effect of interaction of factors on wilt coriander caused by *Fusarium oxysporum* Schlecht. ex Fr. f. sp. *corianderi* Kulkarni, Nikam and Joshi. Indian J Agric Sci 1972; 42 (7):618-621.
19. Pappas AC, Elena K. Occurrence of *Fusarium oxysporum* f. sp. *cumini* in the Island of Chios, Greece, J Phytopathology 1997; 145:271-2.
20. Kochman J, Majewski T. Grzyby (*Mycota)* tom IV Glonowce (*Phycomycetes*) Wroślikowe (*Peronosporales*). Flora Polska, Rośliny Zarodnikowe Polski i Ziem Ościennych. PWN, Warszawa 1970.
21. Sałata B. Grzyby (*Mycota*) tom XV Workowce (*Ascomycetes*) Mączniakowe (*Erysiphales*). Flora Polska, Rośliny Zarodnikowe Polski i Ziem Ościennych. PWN, Warszawa-Kraków 1985.

GRZYBY WYSTĘPUJĄCE NA RÓŻNYCH ORGANACH PASTERNAKU ZWYCZAJNEGO *PASTINACA SATIVA* L.

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S t r e s z c z e n i e

W latach 2005–2007 określano występowanie i szkodliwość grzybów dla pasternaku zwyczajnego *Pastinaca sativa* L., odmiany White Gem. Co roku wykonywano analizę mikologiczną rozłupek z plamami i bez plam oraz siewek i roślin w okresie zbioru. Grzyby określano na podstawie oznak etiologicznych występujących na porażonych częściach roślin oraz na podstawie wyników analizy mikologicznej. Rozłupki były zasiedlane przez różne gatunki grzybów. Stwierdzono większą częstotliwość występowania i większą bioróżnorodność grzybów na roślinach w okresie zbioru niż w fazie siewek. Po raz pierwszy w Polsce wyizolowano grzyb *Itersonilia pastinacae*, uznawany za przyczynę powstawania czarnego raka pasternaku w rejonach, w których uprawia się tę roślinę. Do gatunków często izolowanych z różnych części pasternaku należały *Alternaria alternata, A. raphani, F. oxysporum, F. equiseti F. solani* and *S. botryosum.*

Słowa kluczowe: *pasternak zwyczajny, grzyby, występowanie*