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THE OCCURRENCE AND BIOTIC ACTIVITY OF *Phomopsis diachenii* SACC.

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Abstract

Phomopsis diachenii was isolated from caraway cultivars Konczewicki, firstly in 2006 and next in 2007. Single cultures were obtained from the roots and the stem base of eight six-week-old seedlings and from the stems of two plants with symptoms of necrosis, in the second year of planting. This fungus was isolated from the plant parts superficially disinfected on malt agar medium with an addition 0.01% of streptomycin. The identification of the species was made on PDA medium. The biotic interactions between *P. diachenii* and *S. carvi* and other species of phyllosphere fungi of caraway were studied. Interactions among the fungi, i.e. between *P. diachenii* and one of the fungi representing the studied community, were examined using the biotic series method. The biotic effects of the fungi in dual cultures were evaluated after 10 and 20 days of common growth and were expressed as the individual biotic effect (IBE). It was shown that *P. diachenii* is a weak competitor because its growth was limited by numerous species of phyllosphere fungi. The obtained results indicated the dominance of biotic activity of *P. diachenii* over that of *S. carvi.* It is possible that *P. diachenii* has a greater ability to survive in the phyllosphere fungal community than *S. carvi,* causing septoriosis of caraway.

Key words: *Phomopsis diachenii,* occurrence, biotic activity, caraway

INTRODUCTION

Fungi from the genus *Phomopsis* are known as commonly occurring pathogens of plants from various botanical groups. Many species were identified both on spices and herbs (Ucker, 1988; Farr et al. 1995). This species includes *Phomopsis sclareae* occurring on sage, *P. subordinaria* was found on ribword plantain and *P. lavandulae* attacked lavender plants in various European countries (Uecker, 1988; Laine, 2003; Zimowska, 2008). *Phomopsis foeniculi* was observed on fennel in Italy, France and Germany (Mugnai and Anzidei, 1994; Kusterer et al. 2002) and *P. diachenii* (Saccardo, 1915; Sutton, 1980; Farr et al. 1995), belonging to the species, occurred on Apiaceae plants. According to the papers of the above mentioned authors, parsnip was recognized as the first host of *P. diachenii,* and it was not supposed that this species could infect other Apiaceae plants for a long time. In 1988 *P. diachenii* was first found on caraway (*Carum carvi* L.) in Germany (Gabler and Ehrig, 2000). The fungus was recognized as a dangerous pathogen of caraway umbels in various phases of their development. *P. diachenii* caused their browning, decay and total necrosis of the other parts of this plant, except the roots and the base of stems (Gabler and Ehrig, 2000). These authors confirmed high harmfulness of this fungus to caraway in pathogenicity tests (Gabler and Ehrig, 2000). In recent years *P. diachenii* has been found on caraway in the Czech Republic, Bulgaria and Lithuania (Gabler and Machowicz-Stefaniak, 2004; Rodeva and Gabler, 2004). During the studies on caraway diseases in Poland in 1998, the discussed pathogen was not recorded for a long time, although on the aboveground parts of plants various species of fungi were recorded and *Septoria carvi* was recognized as a more dangerous pathogen for umbels, schizocarps, stems and leaves (Machowicz-Stefaniak and Zalewska, 2008; Zalewska, 2008). Despite the fact that these two mentioned species have similar thermal requirements, i.e. the optimal temperature for growth and development of *P. diachenii* is $25^{o} – 28^{o}$C (Gabler and Ehrig, 2000) and $20^{o} – 25^{o}$C for *S. carvi* (Zalewska not published), and very hot vegetative periods occurred, *S. carvi* showed to be the main pathogen of caraway in Poland. The fact that in recent years several isolates of *P. diachenii* have been obtained from caraway suggests that the growth of this fungus could be limited by other fungi colonizing host plant tissues, and this fact was indicated earlier with respect to *S. carvi* (Machowicz-Stefaniak et al. 2008). It seems most probable that, within the consider-

able biodiversity of fungi colonizing the aboveground parts of caraway plants, there occurred antagonistic microorganisms, i.e. *Trichoderma* spp., *Gliocladium* spp., *Epicoccum* sp., and fast growing species from the genera *Alternaria, Fusarium, Botrytis, Colletotrichum* and *Rhizoctonia* (Farr et al. 1995; Machowicz-Stefaniak and Zalewska, 2004; 2008; Machowicz-Stefaniak et al. 2003).

The above mentioned data inspired us to study macroscopic and microscopic features of our native isolates of the pathogen and to study the biotic interactions between *P. diachenii* and *S. carvi* and other species of phyllosphere fungi of caraway.

MATERIALS AND METHODS

The material used for the study consisted of native isolates K 255, K 257 and K 561 of *P. diachenii* obtained as a result of a study on caraway disease in the years 2005-2007 as well as three isolates 72, 6 and Ondr. of this fungus obtained from BA f. Züchtungsforschung an Kulturpflanzen Inst. f. Resistenzforschung und Pathogendiagnostik in Aschersleben.

The native isolates of the fungus were obtained during the isolation of the fungi from various parts of caraway cultivated in the Lublin region. The fungi were isolated from the plant parts superficially disinfected in a 10% solution of sodium hypochlorite for 1.5 minute on malt agar medium with 0.01% addition of streptomycin (Gabler and Ehrig, 2000; Machowicz-Stefaniak and Zalewska, 2004; 2008). One spore cultures of *P. diachenii* were grown on PDA medium, which is recognized as adequate for growing a lot of microscopic fungi species (Sutton, 1980). The fungus cultures were studied for 20 days of growing on culture medium at a temperature 25°C in dark conditions. The character of the cultures, the shape, size and dimension of pycnidia and conidia of *P. diachenii* were determined on the basis of 180 pycnidia (60 per three isolates) and 300 conidia α and 300 conidia β (100 per three isolates). To identify the species, light and scanning electron microscopy (SEM) was used. The character and microscopic features of the native isolates were compared with the foreign cultures of *P. diachenii.* Based on the description of Sutton (1980) and Gabler and Ehrig (2000), the identification of the studied isolates was made.

To study the biotic interactions of caraway phyllosphere fungi (Machowicz-Stefaniak et al. 2008), two isolates of *P. diachenii,* i.e. 72 and K 561, as well as isolates of the species mentioned in Tab. 2 were taken. Because of the lack of information about the biotic relationships between *P. diachenii* and other species, the maximum number of fungal species was taken for this study, irrespective of the frequency of their isolations from caraway plants (Machowicz-Stefaniak and Zalewska, 2004; Machowicz-Stefaniak et al. 2008). The species *Gliocladium catenulatum, G. roseum* and *T. viride* taken into consideration originated from other cultivated plant species, because they were not isolated from caraway in the years of the study (Machowicz-Stefaniak, 1998; Machowicz-Stefaniak and Zalewska, 2000). The interactions among the fungi, i.e. between *P. diachenii* and one of the fungi representing the studied community, were studied using the biotic series method, according to Mańka (1974), Mańka and Mańka (1992) and Mańka (1995), on PDA (Difco) medium, solidified in Petri dishes. Dishes with the mycelium of single fungal species constituted the control. For each experimental combination, i.e. *P. diachenii* and one of the fungus species representing the community and the control, four replications (dishes) were made. The biotic effects of the fungi in dual cultures were evaluated after 10 and 20 days of common growth, using an eight-degree scale.

The evaluation of biotic effects of the fungi was the arithmetic sum of three points: the first one – the surrounding of the pathogen colony by other fungi species, the second one – the occurrence of an inhibition zone between the colonies, and the third – the reduction of the size of one of the fungus colony (Mańka, 1995). If the colony of *P. diachenii* was overgrown by other fungi species, the appearance of the mycelium and conidia of the studied fungus was evaluated. The biotic effect of the fungi community representing the caraway phyllosphere and *P. diachenii* was expressed as an individual biotic effect (IBE) (Mańka, 1974). The positive value of IBE indicates the suppressive effect on pathogen growth, the negative value of IBE indicates the absence of the suppressive effect on pathogen growth, while the effect may be "0" as well, which indicates a neutral influence (Mańka, 1974; Mańka, 1995).

RESULTS

The native isolates of *P. diachenii* were isolated from caraway plants cv. Konczewicki. These cultures were obtained from the roots and the base of stems of eight six-week-old seedlings in 2006 and from the stems of two plants in 2007 in the second year of planting. The studied pathogen was not isolated in 2005. The fungus was obtained from caraway organs with lesions of nonspecific shape and size. Moreover, *P. diachenii* has not been isolated from leaves, umbels and schizocarps of caraway so far.

The diameter of twelve-day-old colonies on PDA medium ranged from 65.0 to 80.0 mm and after 20

Table 1
The size of pycnidia and conidia of *P. diachenii.*

Author	Size of pycnidia (μm)		Size of conidia (μm)			
	Plant material	PDA medium	Plant material		PDA medium	
			α–conidia	β–conidia	α–conidia	β–conidia
Individual measurements	–	300 – 864	–	–	9.25 – 20.35 x 2.5 – 4.6	24.83 – 32.47 x 1.5
Saccardo 1915	150 – 180	–	8 – 10 x 3	–	–	–
Pidopličko 1978	150 – 180	–	8 – 10 x 3	–	–	–
Sutton 1980	400	–	7.5 – 16.5 x 2.5 – 4.5	15 – 20	–	–
Gabler and Ehrig 2000	–	80 – 350	–	–	(6) 8 – 18 x 2.5 – 4.5 (5)	10 – 22 x 0.5 – 1.5

Table 2
The biotic effect of fungi isolated from caraway (*Carum carvi* L.) on *Phomopsis diachenii.*

Isolates of fungi	Individual biotic effect – IBE			
	after 10 days		after 20 days	
	isolates of *P. diachenii*		isolates of *P. diachenii*	
	72	K561	72	K561
Alternaria alternata (Fr.) Keissler (K 461)	0	0	+5	+4
Alternaria radicina Meier, Drechsler et Eddy (K 1723)	0	0	+3	+4
Botrytis cinerea Pers. (K 1777)	+8	+8	+8	+8
Cladosporium cladosporioides (Fres.) de Vries (K 518)	0	0	+3	+2
Colletotrichum dematitum (Pers. ex Fr.) Grove (K 425)	-6	-6	-7	-7
Colletotrichum gloeosporioides (Penz.) Sacc. (K 1818)	+3	+3	+6	+6
Epicoccum purpurascens Ehrenberg (K 1696)	+3	+3	+7	+7
Fusarium avenaceum (Fr.) Sacc. (K 456)	+2	+2	+6	+6
Fusarium culmorum (W.G.Smith) Sacc. (K 284)	+2	+3	+5	+5
Fusarium equiseti (Corda) Sacc. (K 304)	+3	+3	+6	+6
Fusarium oxysporum Schlecht (K 271)	+2	+2	+4	+5
Fusarium sporotrichioides Sherb (K 465)	+4	+4	+5	+6
Phoma exigua Desm. var. *exigua (*K 1503)	+1	0	+6	+4
Rhizoctonia solani Kühn (K 1561)	+6	+6	+8	+8
Septoria carvi Syd. (K 1833) (K 860)	-2 -3	-2 -3	-4 -6	-4 -6
Gliocladium catenulatum Gilman et Abbott (L 4940)	+2	+2	+8	+8
Gliocladium roseum Bainier (L 830)	+2	+2	+8	+8
Trichoderma harzianum Rifai (K 428)	+8	+8	+8	+8
Trichoderma koningii Oud. (K 437)	+8	+8	+8	+8
Trichoderma viride Pers. ex Gray (W 1222)	+8	+8	+8	+8

The situation in dual fungi culture was estimated on a 0-8 point scale

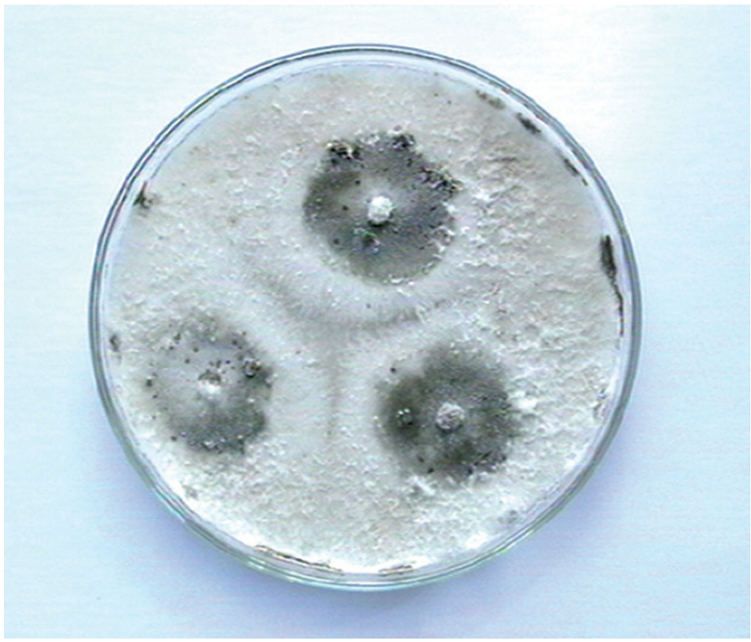

Fig. 1. 20-day-old colonies of *P. diachenii* K 255 with pycnidia on PDA. Photo: E. Zalewska.

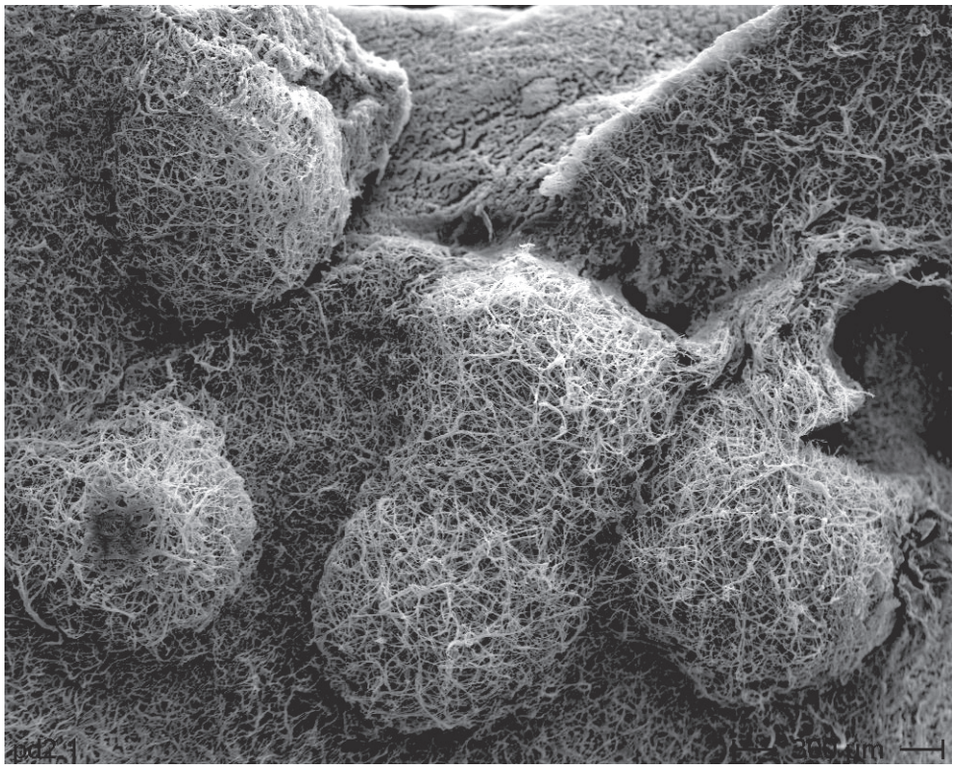

Fig. 2. Pycnidia of *P. diachenii* from PDA, SEM. Photo: M. Wróbel.

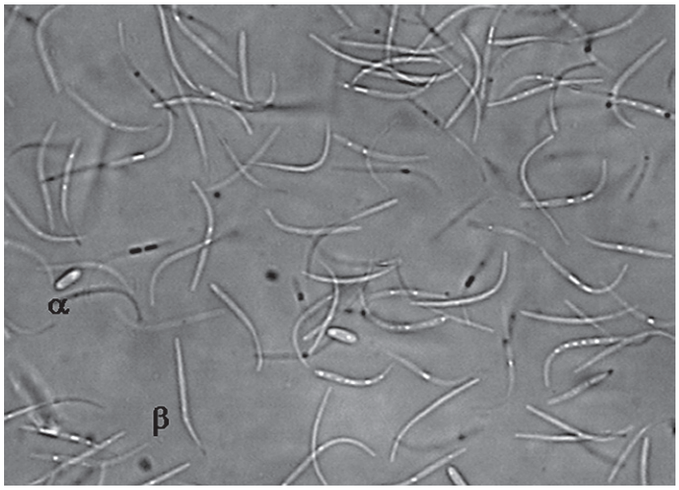

Fig. 3. Conidia (α, β) of *P. diachenii* on PDA. Photo: E. Zalewska.

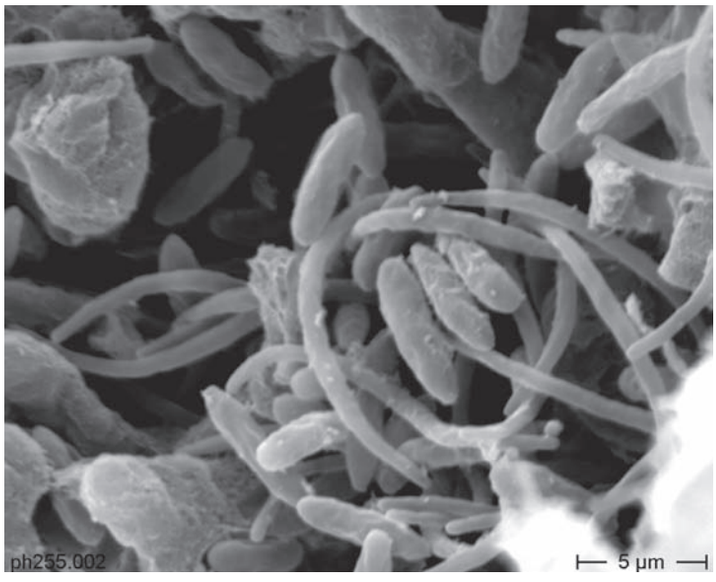

Fig. 4. Conidia (α, β) of *P. diachenii,* SEM. Photo: M. Wróbel.

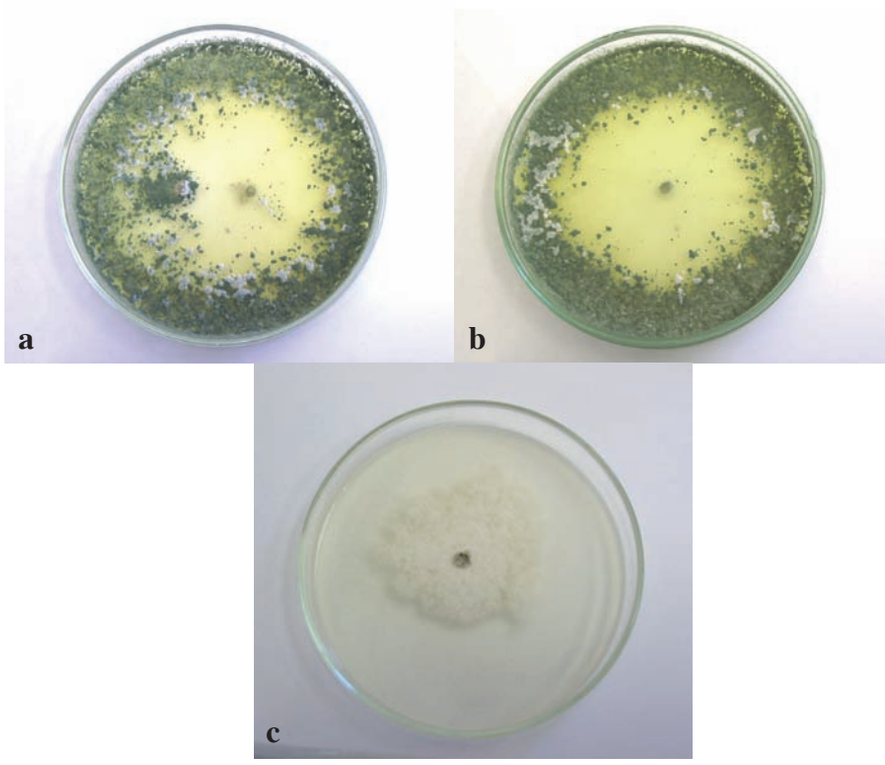

Fig. 5. Dual growth of *P. diachenii* (left) and *Trichoderma koningii* (right) after ten days – a, individual growth of *T. koningii* – b and *P. diachenii* – c. Photo: E. Zalewska.

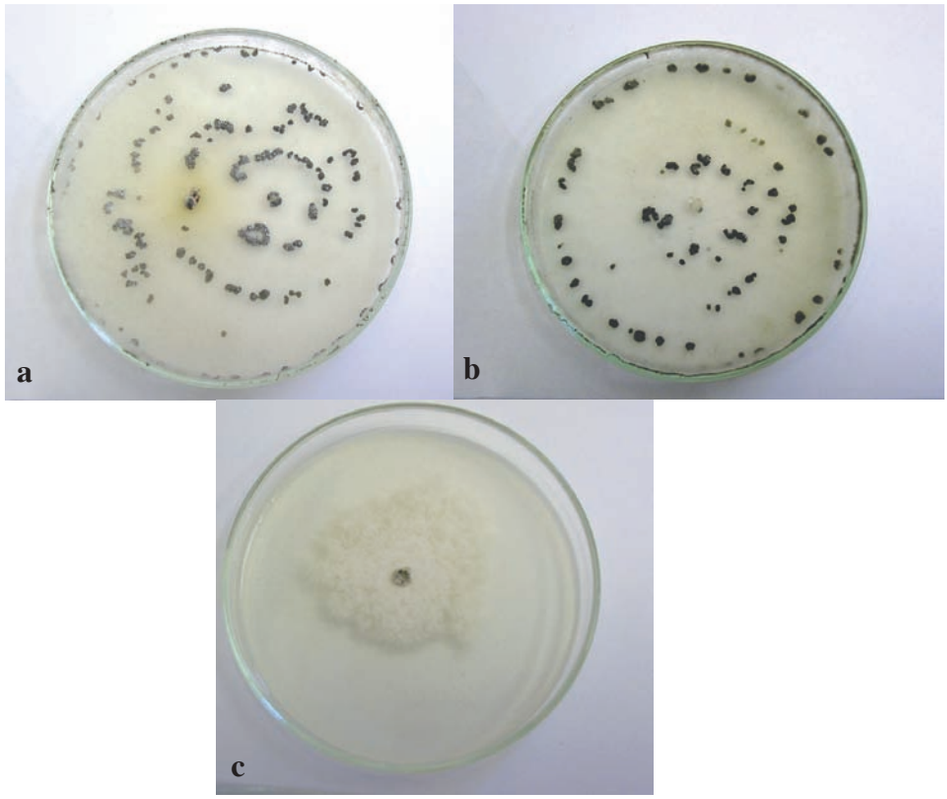

Fig. 6. Dual growth of *P. diachenii* (left) and *Botrytis cinerea* (right) after ten days – a, individual growth of *B. cinerea* – b and *P. diachenii* – c. Photo: E. Zalewska.

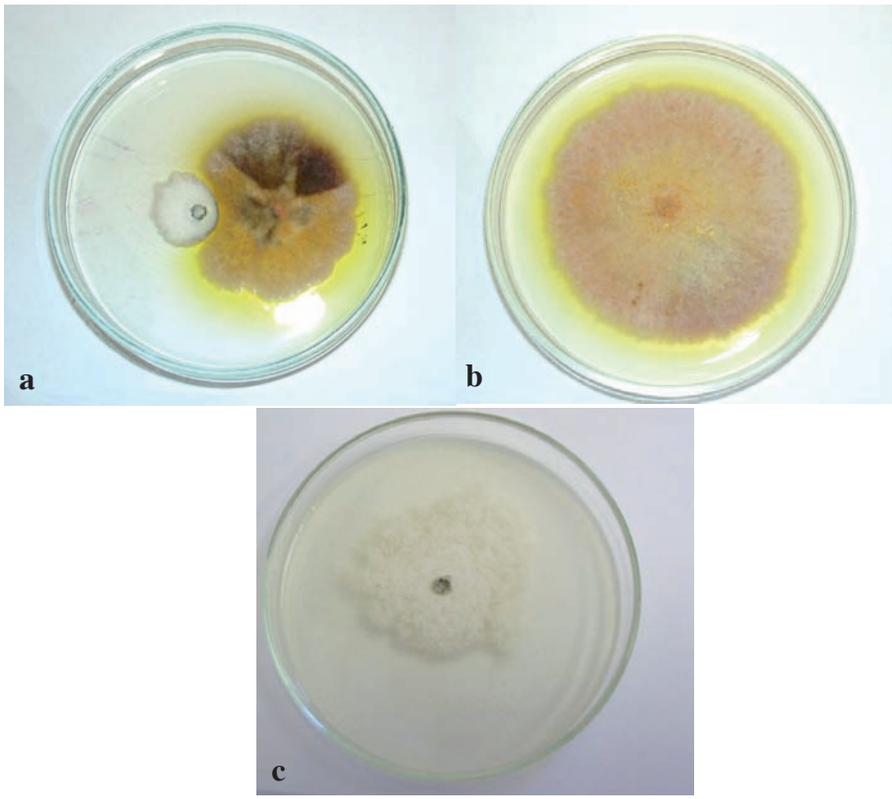

Fig. 7. Dual growth of *P. diachenii* (left) and *Epicoccum purpurascens* (right) after ten days – a, individual growth of *E. purpurascens* – b and *P. diachenii* – c. Photo: E. Zalewska.

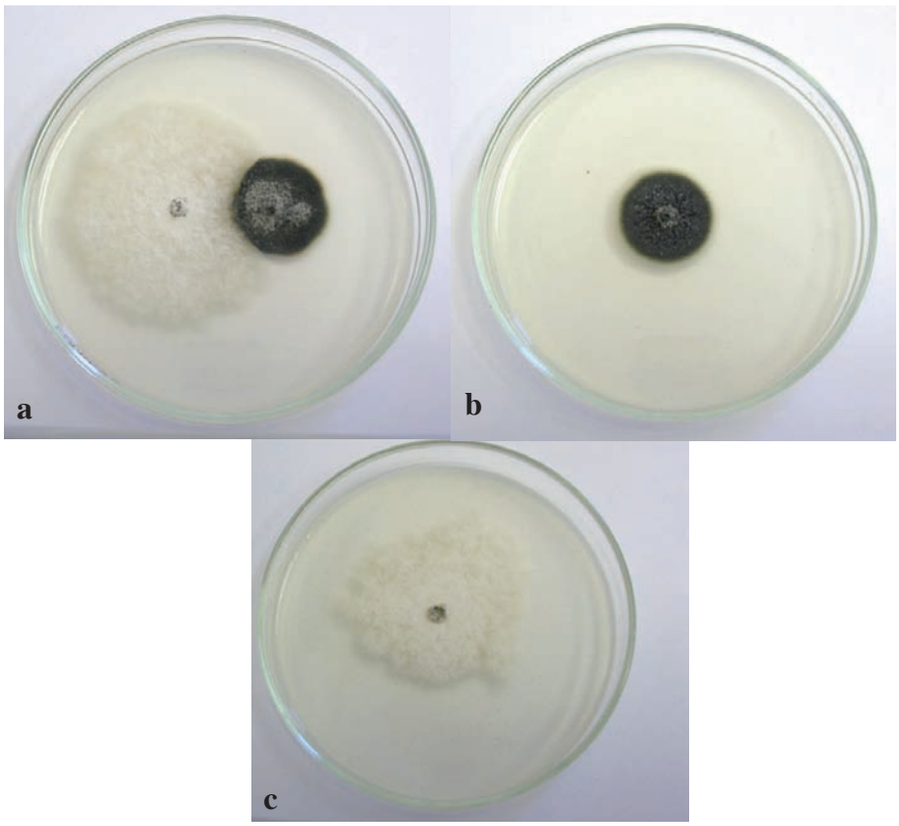

Fig. 8. Dual growth of *P. diachenii* (left) and *Septoria carvi* K 1833 (right) after ten days – a, individual growth of *S. carvi* – b and *P. diachenii* – c. Photo: E. Zalewska.

days of cultivation from 85.0 to 90.0 mm. The structures, especially fourteen- or twenty-day-old colonies, were rather compact, frequently zoned, with white-gray colour (Fig. 1). The reverse of the colonies was brown-gray to brown-black. Pycnidia of the fungus were formed after 10 or 14 days of culturing. The pycnidia were produced singly or in aggregates and they were immersed in the mycelium (Fig. 2). The size of pycnidia was differentiated and ranged from 300 to 864 μm (Tab. 1). Their walls were formed of a few thin layers of intergrown hyphae, which was visible under an electronic microscope (Fig. 2). In the studied isolates, the presence of conidia was noticed after 14 days of culturing at a temperature of 25°C. The fungus formed oval α conidia, with one end slightly reduced and their size ranging from 9.25 to 20.35 μm in length and from 2.5 to 4.6 μm in width (Tab. 1, Figs 3, 4). In two conidia extremes, 2-3 guttulate conidia were observed. Conidia β occurred in greater numbers in the older, i.e. 18-20-day-old colonies. They were straight or filiform to hamate and their size ranged from 24.83 to 32.47-μm in length and 1.5 μm in width (Tab. 1). The mature conidia emerged from pycnidia as big, thick and dull drops covering the ostiole of pycnidia. The macroscopic and microscopic features of the studied native isolates were similar to the features of the colonies of the foreign isolates.

Among the 20 tested fungi species, the majority, i.e. 15, limited the growth of the pathogen after 10 days of dual growth, and in the case of 18 species of fungi after 20 days of dual growth (Tab. 2).

The fungi species from the genus *Trichoderma*, i.e. *T. harzianum, T. viride* and *T. koningii,* totally limited the growth of both the studied isolates of *P. diachenii* after 10 and 20 days of dual growth with their IBE + 8 (Tab. 2, Fig. 5). The fungi from the genus *Gliocladium*, i.e. *G. catenulatum* and *G. roseum*, slightly limited the growth of *P. diachenii* after 10 days of dual growth because IBE was + 2 (Tab. 2). Just after 20 days of dual growth, *G. catenulatum* grew over the colony surface of the studied isolates and its IBE increased to +8 (Tab. 2). It was recognized that *Trichoderma* spp. and *Gliocladium* spp. caused total lysis of *P. diachenii* hyphae and pycnidia at the primary phase of their formation, and they made the production of conidia impossible.

Alternaria alternata, A. radicina and *Cladosporium cladosporioides* showed a neutral effect on *P. diachenii* after 10 days of dual growth with their IBE 0 (Tab. 2). Moreover, the above-mentioned fungi, after 20 days of dual growth, started to limit the growth of *P. diachenii* and their IBE was +5 and +3 for isolate 72 and +4 and +3 for isolate K 561, respectively (Tab. 2).

The highest inhibitory effect on *P. diachenii* was caused by *Botrytis cinerea*, because both after 10 and 20 days of dual growth its IBE was +8 (Tab. 2, Fig. 6). *Rhizoctonia solani* limited the growth of two studied isolates after 10 days of dual growth and its IBE was +6, and + 8 after 20 days. On the other hand, on the surface of the *P. diachenii* inocula in dual culture with these fungi (*B. cinerea* and *R. solani*), single pycnidia and alive well-formed conidia α and β were formed. The growth of *P. diachenii* was limited by *Colletotrichum gloeosporioides* too, as its IBE was +2, and after 20 days of dual growth its inhibitory effect increased three times, as its IBE was +6 (Tab. 2). Between the colonies of *C. gloeosporioides* and *P. diachenii,* a small inhibition zone was formed. Similarly, during the dual culture of *Epicoccum purascens* (Fig. 7) or *Fusarium avenaceum* and *P. diachenii*, the inhibition zones were observed. The IBE of these fungi was +3 and +2 after 10 days and +7 and +6 after 20 days of dual growth, respectively (Tab. 2). The other species of fungi from the genera *Fusarium* limited the growth of the colonies of the studied isolates of *P. diachenii* in the scale from +2 to +4 after 10 days and from +4 to +6 after 20 days of dual growth (Tab. 2).

Phoma exigua limited the growth of ten-day-old colonies of isolate 72 of *P. diachenii* to the least extent, as the value of IBE for the fungus was +1, but for isolate K 561 of *P. diachenii* the effect of this fungus was neutral. After 20 days of dual growth, the biotic effect of *P. exigua* on *P. diachenii* changed and the value of IBE was +6 and +4, respectively for isolates 72 and K 561. During the dual culture of *P. diachenii* with fungi species partly limiting its growth, in the middle or in the margin of the pathogen colony numerous pycnidia with α and β conidia were observed. This fact indicates the big expansive character of this fungus.

Among the 20 tested fungi species, *Septoria carvi* and *Colletotrichum dematium* did not limit the growth of the colony of the *P. diachenii* isolates. Their IBE was negative both after 10 days (- 2 and –6) and after 20 days of dual growth (-4 and –7). Under these growth conditions, the studied isolates of *P. diachenii* partly limited the growth of *S. carvi* and *C. dematium* (Tab. 2, Fig. 8). During the dual culture of *P. diachenii* and *S. carvi*, on the surface of the colonies of each of the species pycnidia and conidia were formed, but the number of *P. diachenii* pycnidia was larger.

DISCUSSION

The macroscopic and microscopic features of the colonies, the morphology of pycnidia and conidia obtained in the present study, in comparison with the papers by Sutton (1980) and Gabler and Ehrig (2000), provided the basis to recognize the studied fungi species as *Phomopsis diachenii.*

It is the first piece of information about the occurrence of this pathogen on caraway cultivated in Poland. The fact that *P. diachenii* isolates were obtained from caraway plants has increased the number of fungi species described as colonizing this plant in Poland.

The studies of the biotic effect of the fungi indicate that *P. diachenii* is a weak competitor because its growth was limited by numerous species of phyllosphere fungi. This species may be eliminated from its ecological niche by other fast-growing fungi species. The results obtained in the present study indicate the dominance of biotic activity of *P. diachenii* over that of *S. carvi.* It is possible that *P. diachenii* has a greater possibility to survive in the phyllosphere fungal community than slow-growing species, i.e. *S. carvi* (Machowicz-Stefaniak et al. 2008). It seems that, when the two above-mentioned species colonize a plant, *P. diachenii* will dominate. It is difficult to explain clearly why in geographical regions where there are similar climatic conditions the main pathogens of caraway were not the same fungi species (Gabler and Machowicz-Stefaniak, 2003). Actually, it seems that *P. diachenii* is not domesticated on caraway cultivated in Polish conditions. On the other hand, in the presence of this fungus on caraway, the quantitative occurrence of the pathogen should be taken into consideration. The presence of numerous species of antagonistic fungi to *P. diachenii* on caraway suggests that the isolation of the fungus from plant tissues on artificial media is possible at the initial stage of the disease, which was indicated earlier with respect to *S. carvi* (Machowicz-Stefaniak et. al. 2008).

Among the studied fungi, only *Trichoderma* spp, *Gliocladium* spp. and *Epicoccum purpurascens* were recognized as positive antagonistic fungi to *P. diachenii.* The high antagonistic activity of *Trichoderma* spp. result from their high competitive abilities, antibiosis as well as the ability to parasite (Fokkema, 1995). The antagonistic effect of *Gliocladium* spp. with respect to *P. diachenii* was slow, because the antagonistic activity of these fungi results (unlike that of *Trichodema*) only from antibiosis and the ability to parasite (Fokkema, 1995; Machowicz-Stefaniak et al. 2008). Besides, *Epicoccum purpurascens* had possibilities to produce siderophores and toxic metabolites (Frederick et al. 1981; Mallea et al. 1991).

All the other fungi species studied, including toxigenic species such as *Fusarium* spp. and *Alternata* spp., could not be recognized as positive antagonistic fungi because they are dangerous pathogens of many various cultivated plant (Mańka, 1974; Gabler and Ehrig, 2000; Kusterer et al. 2002; Machowicz-Stefaniak and Zalewska, 2000; 2004; 2008).

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Występowanie i biotyczna aktywność *Phomopsis diachenii* Sacc.

Streszczenie

Phomopsis diachenii wyizolowano po raz pierwszy w Polsce z roślin kminku zwyczajnego, odmiany Konczewicki w 2006 roku, a następnie w 2007 r. Pojedyncze kultury otrzymano z korzeni i szyjki korzeniowej ośmiu 6-tygodniowych siewek oraz z łodyg dwu roślin w drugim roku uprawy z objawami nekrozy. Grzyb wyizolowano z odkażonych powierzchniowo części roślin na pożywce maltozowej z dodatkiem 0,01% streptomycyny. Identyfikację do gatunku prowadzono na pożywce PDA. Przebadano również biotyczne oddziaływanie pomiędzy *P. diachenii* i *Septoria carvi* oraz innymi gatunkami grzybów fylosferowych z kminku zwyczajnego. Na pożywce PDA w szalkach Petriego zakładano hodowle dwuorganizmowe, tj. składające się z *P. diachenii* i jednego z grzybów reprezentujących badane zbiorowisko. Biotyczne oddziaływanie grzybów w kulturach dwuorganizmowych oceniono po 10 i 20 dniach wspólnego wzrostu i wyrażono jednostkowym efektem biotycznym IBE. Wykazano, że *P. diachenii* posiada niewielkie właściwości konkurencyjne. Jego wzrost ograniczają liczne gatunki szybko rosnących grzybów fylosferowych. Uzyskane wyniki wskazały na przewagę właściwości biotycznych *P. diachenii* nad *Septoria carvi.* Można wnioskować, że *P. diachenii* ma większe możliwości utrzymania się w zbiorowiskach grzybów fyllosferowych niż *S. carvi,* powodujący septoriozę kminku.