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PATHOGENICITY OF *Phomopsis diachenii* SACC. ISOLATES TO CARAWAY *Carum carvi* L. (*Apiaceae*)

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Abstract. *Phomopsis diachenii* belongs to the fungi recently detected on the herbs in the Polish climatic conditions. The purpose of this work was to study the fungus harmfulness to sowing material, seedlings, stems and leaves of caraway. The pathogenicity tests according to Koch's postulates were carried out for 2 and 4 *P. diachenii* isolates using post-culture liquids, water suspension of conidia and discs of fungus sporulating mycelium to inoculate the plants' parts. The studies were conducted both in the laboratory and a climatic chamber. *P. diachenii* caused disease symptoms in the form of necrosis on all plant organs tested. The ability for asymptomatic *P. diachenii* colonization of inoculated plants' parts was determined. It was found that injuries of the plant epidermal tissue significantly increased the efficiency of inoculation. The performed pathogenicity tests and the results from electron microscopy SEM observation showed that the tested isolates of *P. diachenii* were occasional pathogens of caraway.

Key words: methods of inoculation, symptomatology, infection phase, SEM

INTRODUCTION

Phomopsis diachenii is the fungus species occurring on herb plants from *Apiaceae* family [Saccardo 1915, Sutton 1980, Farr et al. 1995]. In the studies of these authors parsnip was given as the first host plant of *P. diachenii* because the picnidia of this fungus were found on the stems and fruits of this plant [Saccardo 1915]. This fungus was isolated first time from caraway in Germany in 1998 [Gabler and Ehrig 2000], where it was recognized as the dangerous pathogen of this plant. The fungus caused necrosis of the above ground parts of caraway, mainly umbels, but it was not found on the roots and stems [Gabler and Ehrig 1999, 2000, Kusterer et. al. 2002]. According to Gabler and Ehring [2000] infection occurs at the flowering stage and then the umbels becomes partly discolored. The infected parts of such umbels did not develop further and remained sterile. If umbels infection occurs at a later stage of development, the even

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unripe fruits which are being formed die. Infection of ripe fruit caused the reduction of schizocarps germination in 60–80% [Gabler and Ehrig 2000]. The infected schizocarps are also a source of primary infection of plants [Machowicz-Stefaniak and Zimowska 2000].

Infection can also occur at the base of the umbels, especially when they were injured by *Lygus* sp. [Gabler and Ehring 2000]. *P. diachenii* was recently also detected on caraway in the Czech Republik, Bulgaria and Lithuania [Gabler and Machowicz--Stefaniak 2004, Rodeva and Gabler 2004].

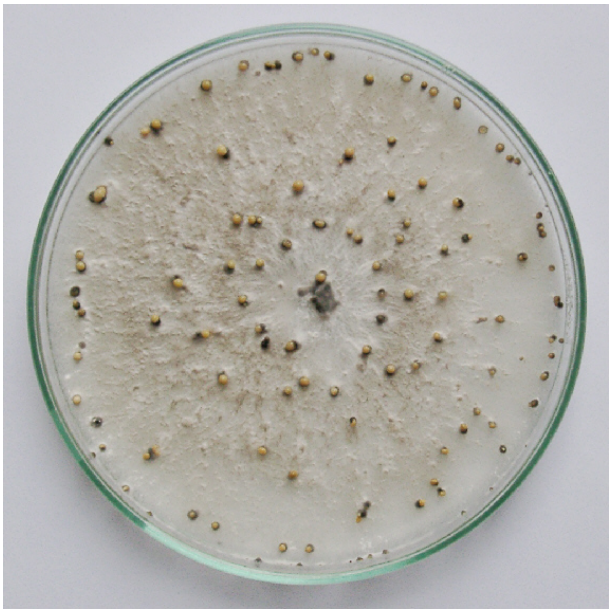
In Poland some isolates of the fungus were obtained from roots and the root crown of eight six-week-old seedlings in 2006 and in 2007 from the stems of two plants being in the second year of cultivation [Machowicz-Stefaniak 2009]. In the years 2008–2010 the isolation of *P. diachenii* was not successful. There were no specific symptoms on the plants from which the fungus was isolated. *P. diachenii* has not yet been isolated from caraway umbels in Poland, although the symptoms observed on umbels in 2005 were similar to those described by Gabler and Ehring [2000].

However, the thermophilic properties of *P. diachenii* [Gabler and Ehring 2000] also create the possibility of its more common occurrence on caraway in Poland, especially in hot and humid growing seasons. Therefore, pathogenicity tests were conducted to study the harmfulness of the fungus to seeds, seedlings, stems and leaves of caraway.

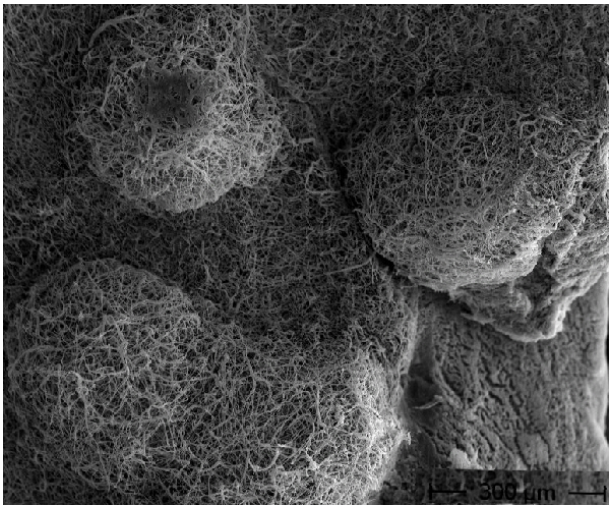
MATERIAL AND METHODS

The study material consisted of samples of caraway schizocarps Konczewicki cultivar obtained from CNOS in Poznań, the plants grown from them and one-spore cultures of *Phomopsis diachenii* Sacc. isolates from the collection of the Department of Phytopathology and Mycology, University of Life Sciences. Four randomly selected isolates of *P. diachenii*: K253, K651, K 657, K 658, which were obtained in earlier years from the aboveground organs of caraway were studied [Machowicz-Stefaniak 2009]. Fungal cultures chosen for pathogenicity tests were grown for two weeks on Czapek-Dox medium in Petri dishes with Czapek-Dox medium (photo 1, 2, 3) put in a thermostat, at the temperature 25°C without the access of light. The effect of post-culture liquids containing the mycelium, spores and metabolites and water suspension of *P. diachenii* conidia on the germination ability of caraway schizocarps and the effect of *P. diachenii* on stem fragments, leaves and on the healthiness of the seedlings were studied [Marcinkowska 1984, Machowicz-Stefaniak et. al. 2008, Zalewska 2010].

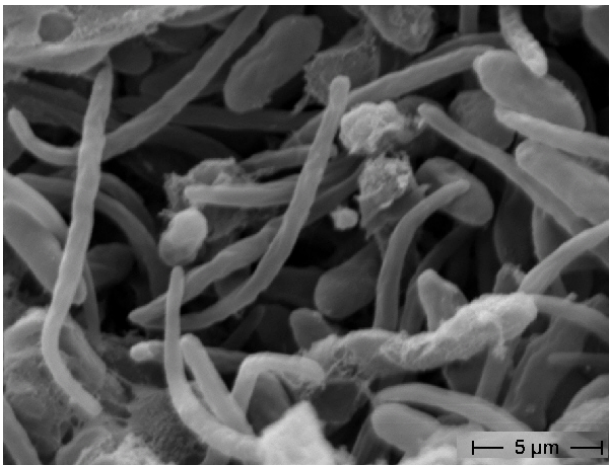
The effect of *P. diachenii* on caraway schizocarps. In order to obtain the post-culture liquids containing the mycelium, spores and metabolites of *P. diachenii*, the studied isolates of the fungus were kept in 250 ml Erlenmeyer flasks on a liquid medium Czapek-Dox Broth (Difco), at the temperature 25°C, in a thermostat for 8 days [Mishra and Behr 1976]. The experiment was conducted in sterile Petri dishes lined with several layers of lignin and a layer of filter paper. Five hundred well-formed and well-coloured schizocarps were selected from samples of the sowing material, i.e. 100 for each fungal isolate, and 100 for control combination. The superficially disinfected schizocarps of caraway (90 seconds in 10% sodium hypochlorite) were placed in sterile



Phot. 1. 14-days-old colony of *P. diachenii* with conidiomata and exudate of conidia on Czapek--Dox medium (photo E. Zalewska)
Fot. 1. 14-dniowa kolonia *P. diachenii*, konidiomy stromatyczne z widoczną na nich wydzieliną konidiów na pożywce Czapek-Dox (fot. E. Zalewska)



Phot. 2. Conidiomata of *P. diachenii* on Czapek-Dox medium, SEM (photo. M. Wróbel)
Fot. 2. Konidiomy stromatyczne *P. diachenii* na pożywce Czapek-Dox, SEM (fot. M. Wróbel)



Phot. 3. Conidia (α and β) of *P. diachenii*, SEM (photo M. Wróbel)
Fot. 3. Konidia (α and β) *P. diachenii*, SEM (fot. M. Wróbel)

moist chambers and post-culture liquids of the studied isolates were applied on them in the amount of 3 ml, using an automatic pipette VE-1000xr [Zalewska 2010]. For each combination, 100 schizocarps were used, i.e. five dishes per 20 schizocarps. The control combination consisted of schizocarps placed in a sterile moist chamber on blotting paper soaked with 3 ml of liquid medium Czapek-Dox Broth.

The studies on the influence of *P. diachenii* spores water suspension were also conducted in sterile moist chambers. Water suspension of spores density $3x10^6$ml was used in this and following tests. Before placing on Petri dishes the superficially disinfected schizocarps were singly soaked in a Czapek-Dox broth medium supplemented with agar in an amount of 3 g/l medium, and then in a suspension of spores. [Marcinkowska 1984, Zalewska 2010]. The control consisted of schizocarps covered with Czapek-Dox Broth medium with an addition of agar and immersed individually in sterile distilled water.

In both experiments the dishes with seeds were placed in a thermostat at 25°C, without the light access. Observations of germination were conducted after 3, 6 and 9 days. The number of schizocarps that showed and those that did not show germination as well as the number of germs with necrosis was determined. By the end of the experiment, all schizocarps and germs were submitted to reisolation according to Koch's postulates.

The effect of *P. diachenii* on fragments of caraway stems. The studies were conducted in sterile moist chambers, i.e. in Petri dishes with a diameter of 9 mm. Discs of the sporulating mycelium of the pathogen obtained from one-spore cultures of the studied isolates were used for inoculation. The surface of the two-year-old caraway stem fragments with the length of 5 cm were superficially disinfected in the same manner as the disinfection of schizocarps. Then the fungus inocula were placed on the uninjured and injured epidermal tissues with a sterile scalpel. The inocula were placed in the middle of each part of the stems, with the mycelium to the surface of the skin. For

each of the tested isolates and control combination five dishes per 5 parts of caraway stems were prepared. The control consisted of parts of the stems with uninjured and injured epidermal tissues on which the discs of sterile Czapek-Dox medium were placed. For inoculation of stem parts the water suspension of conidia with an addition of 3% agar were also used, in which fragments of stems were immersed for 3 minutes. After this time the stems were transferred to moist chambers – 5 dishes per 5 caraway stems for each isolate and control. The control consisted of stem parts immersed for the same period in sterile distilled water with an addition of 3% agar.

The effect of *P. diachenii* on caraway leaves. The study was conducted in sterile moist chambers. The water suspension of fungus conidia with 3% agar was used for inoculation, in which superficially disinfected leaves were immersed for 3 minutes, like in the previous tests. The control consisted of leaves immersed for 3 minutes in sterile water with 3% agar. Then the plant material was kept in sterile moist chambers – 5 Petri dishes with a diameter of 25 cm per 25 leaves for all five combinations.

The effect of *P. diachenii* on healthiness of seedlings. In the studies on the effect of *P. diachenii* on healthiness of seedlings inoculated through hypocotyl, two isolates of *P. diachenii*, i.e. K651 and K657 were used. The seedlings designated for the experiment were grown in a climatic chamber, in plastic pots with a diameter of 6 cm, filled with 3-times sterilized garden soil, in which superficially disinfected schizocarps of caraway were sown. The growing seedlings were kept at the temperature of 20–22°C, at full exposure during the day (14 h) and in darkness during the night (10 h), with 80–84% of relative humidity %. During the plants' growth the soil was wetted with sterile distilled water. Totally, 150 seedlings were used for pathogenicity tests, since each of 6 combination consisted of 25 seedlings (5 pots with 5 plants). The water suspension of the fungus conidia with an addition of 3% agar was used, as well as two methods of inoculation, i.e. applying a drop of infectious suspension size 0.001 ml, using a pipette VE-200xt, on uninjured and injured hypocotyl. A drop of sterile distilled water with an addition of 3% agar was applied on the injured and uninjured hypocotyl of control plants.

In all three infection tests the formation and development of necrosis on the inoculated parts of plants were observed after 3, 6 and 9 days. After the experiments were finished, reisolation of the tested isolates of the pathogen from all seedlings was performed on Czapek-Dox medium. In all experiments the results obtained during the last observation, as the mean values of five replicates, were analyzed statistically using Tukey's confidence intervals. In the case of caraway stems, the behavior of the fungus was observed after 12, 24, 36 and 48 hours since inoculation under an electron microscope SEM at the Central Laboratory of Agroecology University of Life Sciences in Lublin.

RESULTS

It was found that 86 to 90% caraway schizocarps did not germinate after being treated 9 days in *P. diachenii* post-culture liquids (tab. 1). The average number of schizocarps (mean of 5 replications) that did not germinate was from 17.2 to 18,

Table 1. Effect of *P. diachenii* post culture liquids on the germination of caraway (*Carum carvi* L.) schizocarps

Tabela 1. Oddziaływanie płynów pohodowlanych *P. diachenii* na kiełkowanie rozłupek kminku zwyczajnego (*Carum carvi* L.)

Combinations Kombinacje	Total (mean) number of tested schizocarps Ogólna (średnia) liczba testowanych rozłupek	Observation after 9 days Obserwacja po 9 dniach						Reisolation of *P. diachenii* Reizolacja *P. diachenii*
		number = % of not germinated schizocarps liczba = % nieskiełkowanych rozłupek		number = % of germinated schizocarps liczba = % rozłupek skiełkowanych				
				germs with necrosis kiełki z nekrozą		germs without necrosis kiełki bez nekrozy		
		total ogółem	mean średnio	total ogółem	mean średnio	total ogółem	mean średnio	
P. diachenii Isolate K253	100 (20)	90	18.0a	9	1.8ab	1	0.2b	99
Isolate K651	100 (20)	86	17.2a	14	2.8a	0	0.0b	100
Isolate K657	100 (20)	86	17.2a	8	1.6ab	6	1.2b	95
Ioslate K658	100 (20)	90	18.0a	10	2.0ab	0	0.0b	97
Control Kombinacja kontrolna	100 (20)	6	1.0b	0	0.0b	94	18.8a	0
		LSD (NIR) = 3.747		LSD (NIR) = 2.1074		LSD (NIR) = 2.5532		

Explanations – Objaśnienia:
Level of essentiality $p \leq 0.5$ – Poziom istotności $p \leq 0,5$
Values marked with the same letter do not differ significantly – Wartości oznaczone tą samą literą nie różnią się istotnie

Phot. 4. Schizocarps of caraway after inoculation with post-culture liquids of *P. diachenii* K 657 × 2 (a), control combination × 1.5 (b), (photo E. Zalewska)

Fot. 4. Rozłupki kminku zwyczajnego po inokulacji przy użyciu płynów pohodowlanych *P. diachenii* K 657 × 2 (a), kombinacja kontrolna × 1.5 (b), (fot. E. Zalewska)

depending on the isolate and those data differed significantly from the data of the control combination (tab. 1). The percentage of germinated schizocarps was only from 10 to 14. The average number of germs with necrosis was from 1.6 to 2.8 depending on the isolate and these values, with exception isolate K 651 did not differ significantly in comparison with control combination (tab. 1). On the other hand, the average number germs without necrosis was from 0.0 to 1.2 and these values were significantly lower compared to the value in the control combination (tab. 1). It was found that the majority of schizocarps which germinated had necrosis of the germs on the 1/3 to 2/3 of their length (photo 4). The cultures of *P. diachenii* with the macroscopic and microscopic features corresponding to those used for the production of post-culture liquids were isolated from all the decayed germs and not germininated schizocarps (tab. 1). Moreover, on the surface of both the dead germs and schizocarps mycelium and numerous conidiomata of *P. diachenii* developed with secretions of α and β conidia. In the control combination no germ necrosis of caraway or their colonization by *P. diachenii* was observed.

Table 2. Effect of *P. diachenii* water suspension of conidia on germination of caraway (*Carum carvi* L.) schizocarps

Tabela 2. Oddziaływanie wodnej zawiesiny zarodników konidialnych *P. diachenii* na kiełkowanie rozłupek kminku zwyczajnego (*Carum carvi* L.)

Combinations Kombinacje	Total (mean) number of tested schizocarps Ogólna (średnia) liczba testowanych rozłupek	Observation after 9 days Obserwacja po 9 dniach						Reisolation of *P. diachenii* Reizolacja *P. diachenii*
		number = % of not germinated schizocarps liczba = % nies-kiełkowanych rozłupek		number = % of germinated schizocarps liczba = % rozłupek skiełkowanych				
				germs with necrosis kiełki z nekrozą		germs without necrosis kiełki bez nekrozy		
		total ogółem	mean średnio	total ogółem	mean średnio	total ogółem	mean średnio	
P. diachenii								
Isolate K253	100(20)	96	19.2a	4	0.8bc	0	0.0b	100
Isolate K651	100(20)	91	18.2a	8	1.6abc	1	0.2b	100
Isolate K657	100(20)	83	16.6a	17	3.4a	0	0.0b	96
Isolate K658	100(20)	83	16.6a	15	3.0ab	2	0.4b	76
Control Kombinacja kontrolna	100(20)	6	1.2b	0	0.0c	94	18.8a	0
		LSD (NIR) = 3.075		LSD (NIR) = 2.5811		LSD (NIR) = 1.3907		

Explanations – Objaśnienia:
Level of essentiality $p \leq 0.5$ – Poziom istotności $p \leq 0,5$
Values marked with the same letter do not differ significantly – Wartości oznaczone tą samą literą nie różnią się istotnie

Nine days after the application of conidia water suspension of *P. diachenii* the percentage of not germinated schizocarps ranged from 83 to 96 depending on the isolate but in control combination there were only 6 (tab. 2). The average number of not

germinated schizocarps in five repetitions varied depending on the isolate from 16.6 to 19.2 while the control was 1.2. These values differed significantly among themselves (tab. 2). The percentage of germinated schizocarps after inoculation with *P. diachenii* was only from 4 to 17, while in the control combination 94% of germinated schizocarps was observed (tab. 2). In combinations with *P. diachenii* the necrosis of germs occurred on 1/4 to 3/4 of their length. The average number of schizocarps with necrosis ranged from 0.8 to 3.4. These values differed from the control value, which was 0 (tab. 2). The reisolation showed the presence of *P. diachenii* in the majority of inoculated schizocarps. Schizocarps in the control combination were not inhabited by *P. diachenii* (tab. 2).

Table 3. Results of inoculation of not wounded caraway (*Carum carvi* L.) stem fragments
Tabela 3. Wyniki inokulacji nieranionych fragmentów łodyg kminku zwyczajnego (*Carum carvi* L.)

Combinations Kombinacje	Total (mean) number of tested stems fragments Ogólna (średnia) liczba testowanych fragmentów	Observation after 9 days Obserwacja po 9 dniach				Reisolation of *p. diachenii* Reizolacja *p. diachenii*
		stems with necrosis łodygi z nekrozą		stems without necrosis łodygi bez nekrozy		
		total ogółem	mean średnio	total ogółem	mean średnio	
P. diachenii						
Isolate k253	25 (5)	5	1.0 ab	20	4.0 bc	20
Isolate k651	25 (5)	8	1.6 a	17	3.4 c	23
Isolate k657	25 (5)	6	1.2 ab	19	3.8 bc	21
Isolate k658	25 (5)	3	0.6 bc	22	4.4 ab	19
Control Kombinacja kontrolna	25 (5)	0	0.0 c	25	5.0 a	0
		LSD (NIR) = 0.757		LSD (NIR) = 0.757		

Explanations – Objaśnienia:
Level of essentiality $p \leq 0.5$ – Poziom istotności $p \leq 0,5$
Values marked with the same letter do not differ significantly – Wartości oznaczone tą samą literą nie różnią się istotnie

In the experiment with inoculation of uninjured parts of the stems with discs of the sporulating mycelium the number of infections ranged from 3 to 8 depending on isolates while in the control combination it was 0 (tab. 3). The mean values of five replicates differed significantly from the control value in the case of isolates K 253, K 651 and K 657 (tab. 3). On the inoculated stem fragments necrosis appeared after 6 days. Those were the long (0.8–1.0 cm) spots, starting from the site of infection, which after 9 days covered a large area of the inoculated stems (photo 5). *Phomopsis diachenii* was isolated from all such stems as well as from numerous fragments without necrosis (tab. 3).

Phot. 5. Pieces of caraway stems after 9 days of inoculation with *P. diachenii* by not wounded epidermal tissues (a), control combination (b), (photo E. Zalewska)

Fot. 5. Fragmenty łodyg kminku zwyczajnego po 9 dniach od inokulacji *P. diachenii* przez nieuszkodzoną skórkę (a), kombinacja kontrolna (b), (fot. E. Zalewska)

Phot. 6. Pieces of caraway stems after 3 days since inoculation with sporulating mycelium disc of *P. diachenii* by wounded epidermal tissues (a), after 9 days (b) and control combination (c), (photo E. Zalewska)

Fig. 6. Fragmenty łodyg kminku zwyczajnego po 3 dniach od inokulacji krążkami zarodnikującej grzybni *P. diachenii* przez zranioną skórkę (a), po 9 dniach (b) i kombinacja kontrolna (c), (fot. E. Zalewska)

After inoculation of the injured parts of stems with discs of the sporulating mycelium the first symptoms of necrosis were found after 3 days. Those were spots with the length from 0.4 to 0.6 cm, which enlarged with time and after 6 days their length ranged from 1 to 2 cm while after 9 days they covered the whole surface of the stems (photo 6). After 9 days the number of stems with necrosis ranged from 22 to 24 but in the control combinations it was 0. The mean values of five repetitions were significantly different compared to the control combination (tab. 4). *P. diachenii* was reisolated from all inoculated stem parts and from a few without disease symptoms (tab. 4).

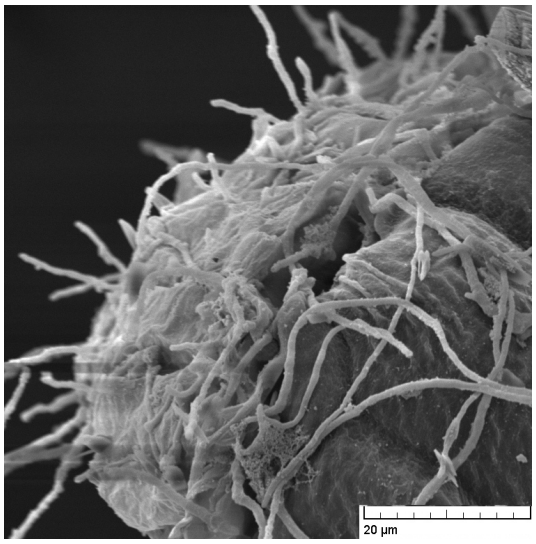
Table 4. Results of inoculation of wounded caraway (*Carum carvi* L.) stem fragments
Tabela 4. Wyniki inokulacji zranionych fragmentów łodyg kminku zwyczajnego (*Carum carvi* L.)

Combinations Kombinacje	Total (mean) number of tested stems fragments Ogólna (średnia) liczba testowanych fragmentów	Observation after 9 days Obserwacja po 9 dniach				Reisolatoin of *P. diachenii* Reizolacja *P. diachenii*
		stems with necrosis łodygi z nekrozą		stems without necrosis łodygi bez nekrozy		
		total ogółem	mean średnio	total ogółem	mean średnio	
P. diachenii						
Isolate K253	25 (5)	24	4.8a	1	0.2b	24
Isolate K651	25 (5)	22	4.4a	3	0.6b	25
Isolate K657	25 (5)	24	4.8a	1	0.2b	25
Isolate K658	25 (5)	23	4.6a	2	0.4b	23
Control Kombinacja kontrolna	25 (5)	0	0.0b	25	5.0a	0
		LSD (NIR) = 1.0014		LSD (NIR) = 1.0014		

Explanations – Objaśnienia:
Level of essentiality $p \leq 0.5$ – Poziom istotności $p \leq 0,5$
Values marked with the same letter do not differ significantly – Wartości oznaczone tą samą literą nie różnią się istotnie

When stem parts were inoculated by immersing stem parts in water suspension of the fungus conidia, the disease symptoms already occurred after 3 days. Those were necrotic spots of the size from 0.5 to 3.0 mm in length, which ranged from 4.0 to 5.5 after 6 days and from 7.5 to 8.0 mm after 9 days (photo 7). In addition, necrosis of the

Phot. 7. Pieces of caraway stems after inoculation with water suspension of *P. diachenii* conidia after 3 days (a), 6 days (b), 9 days (c) and control combination (d), (photo E. Zalewska)
Fot. 7. Fragmenty łodyg kminku zwyczajnego po inokulacji wodną zawiesiną konidiów *P. diachenii* po 3 dniach (a), 6 dniach (b), 9 dniach (c) oraz kombinacja kontrolna (d) (fot. E. Zalewska)



Phot. 8. Hyphae of *P. diachenii* gathered on around the cut stem of caraway (photo M. Wróbel)
Fot. 8. Strzępki *P. diachenii* skupiające się wokół przeciętej łodygi kminku zwyczajnego (fot. M. Wróbel)

Tabele 5. Inoculation of caraway (*Carum carvi* L.) stems fragments by soaking in water suspension of *P. diachenii* conidia
Tabela 5. Inokulacja fragmentów łodyg kminku zwyczajnego (*Carum carvi* L.) przez moczenie w wodnej zawiesinie zarodników *P. diachenii*

Combinations Kombinacje	Number of inoculated fragments of stems Liczba inokulowanych fragmentów łodyg	Observation after 9 days Obserwacja po 9 dniach				Reisolation of *P. diachenii* Reizolacja *P. diachenii*
		stems with necrosis łodygi z nekrozą		stems without necrosis łodygi bez nekrozy		
		total ogółem	mean średnio	total ogółem	mean średnio	
P. diachenii Isolate K253	25 (5)	19	3.8 a	6	1.2 b	23
Isolate K651	25 (5)	21	4.2 a	4	0.8 b	25
Isolate K657	25 (5)	20	4.0 a	5	1.0 b	25
Isolate K658	25 (5)	17	3.4 a	8	1.6 b	24
Control Kombinacja kontrolna	25 (5)	0	0.0 b	25	5.0 a	0
		LSD (NIR) = 0.9272		LSD (NIR) = 0.9272		

Explanations – Objaśnienia:
Level of essentiality $p \leq 0.5$ – Poziom istotności $p \leq 0,5$
Values marked with the same letter do not differ significantly – Wartości oznaczone tą samą literą nie różnią się istotnie

stems appeared at the ends of the stem fragments, i.e. in places where they were cut (photo 7b, c) and in the microscopic image SEM a massive amount of *P. diachenii* hyphae grown inside the stems tissue was seen (photo 8). After 9 days the number of stems with necrosis ranged from 17 to 21, depending on the isolate of the fungus, and the average value of 5 replications differed significantly in compared to the control (tab. 5). *P. diachenii* was reisolated from all parts of the stems with necrosis and numerous parts with no visible disease symptoms (tab. 5).

Three days after inoculation of caraway leaves in water suspension of *P. diachenii* conidia, small, brown spots of 0.5 mm (photo 9a) developed at their tips. After 6 days, the necrosis progressed from the tips of the leaves towards to the base (photo 9b, c) and after 9 days the whole leaf surface was brown and necrosis moved to the stem from the leaves (photo 9c, d). Conidioma of *P. diachenii* with α and β conidia formed on the dead leaves. Moreover, *P. diachenii* was reisolated from the leaves with symptoms of necrosis and without such symptoms (tab. 6).

Phot. 9. Leaves of caraway after inoculation with *P. diachenii* conidia water suspension after 3 days (a), 6 days (b), 9 days (c, d, e) and control combination (f), (photo. E. Zalewska)

Fot. 9. Liście kminku zwyczajnego po inokulacji wodną zawiesiną konidiów *P. diachenii* po 3 dniach (a), 6 dniach (b), 9 dniach (c, d, e) oraz kombinacja kontrolna (f) (fot. E. Zalewska)

Table 6. Inoculation of caraway (*Carum carvi* L.) leaves with *P. diachenii* conidia water suspension

Tabela 6. Inokulacja liści kminku zwyczajnego (*Carum carvi* L.) wodną zawiesiną zarodników *P. diachenii*

Combinations Kombinacje	Number of infected leaves Liczba inokulowanych listków	Observation after 9 days Obserwacja po 9 dniach				Reisolation of *P. diachenii* Reizolacja *P. diachenii*
		leaves with necrosis listki z nekrozą		leaves without necrosis listki bez nekrozy		
		total ogółem	mean średnio	total ogółem	mean średnio	
P. diachenii						
Isolate K253	100 (25)	82	20.5 a	18	4.5 b	88
Isolate K651	100 (25)	86	21.5 a	14	3.5 b	96
Isolate K657	100 (25)	78	19.5 a	22	5.5 b	80
Isolate K658	100 (25)	80	20.0 a	20	5.0 b	81
Control Kombinacja kontrola	100 (25)	12	3.0 b	88	22.0 a	0
		LSD (NIR) = 3.139		LSD (NIR) = 3.139		

Explanations – Objaśnienia:
Level of essentiality $p \leq 0.5$ – Poziom istotności $p \leq 0,5$
Values marked with the same letter do not differ significantly – Wartości oznaczone tą samą literą nie różnią się istotnie

Table 7. Inoculation of not wounded caraway (*Carum carvi* L.) seedling hypocotyl with water suspension of *P. diachenii* conidia

Tabela 7. Inokulacja siewek kminku zwyczajnego (*Carum carvi* L.) wodną zawiesiną zarodników *P. diachenii* przez niezraniony hypokotyl

Combinations Kombinacje	Number of inoculated seedlings Liczba inokulowanych siewek		Observation after 9 days Obserwacja po 9 dniach				Reisolation of *P. diachenii* Reizolacja *P. diachenii*
			seedlings with necrosis siewki z nekrozą		seedlings without necrosis siewki bez nekrozy		
	total ogółem	on average średnio	total ogółem	on average średnio	total ogółem	on average średnio	
P. diachenii							
Isolate K651	25	5	21	4.2 b	4	0.8 b	20
Isolate K657	25	5	25	5.0 b	0	0.0 a	21
Control Kombinacja kontrolna	25	5	0	0.0 a	25	5.0 c	0
			LSD (NIR) = 0.849143		LSD (NIR) = 0.453886		

Explanations – Objaśnienia:
Level of essentiality $p \leq 0.5$ – Poziom istotności $p \leq 0,5$
Values marked with the same letter do not differ significantly – Wartości oznaczone tą samą literą nie różnią się istotnie

Phot. 10. Caraway plants after inoculation with *P. diachenii* isolate K 651 by wounded epidermal tissues of hypocotyl (a, b), control combination (c, d), (photo. E. Zalewska)
Fot. 10. Rośliny kminku zwyczajnego po inokulacji izolatem K 651 *P. diachenii* przez zranioną skórkę hypokotylu (a, b), kombinacja kontrolna (c, d), (fot. E. Zalewska)

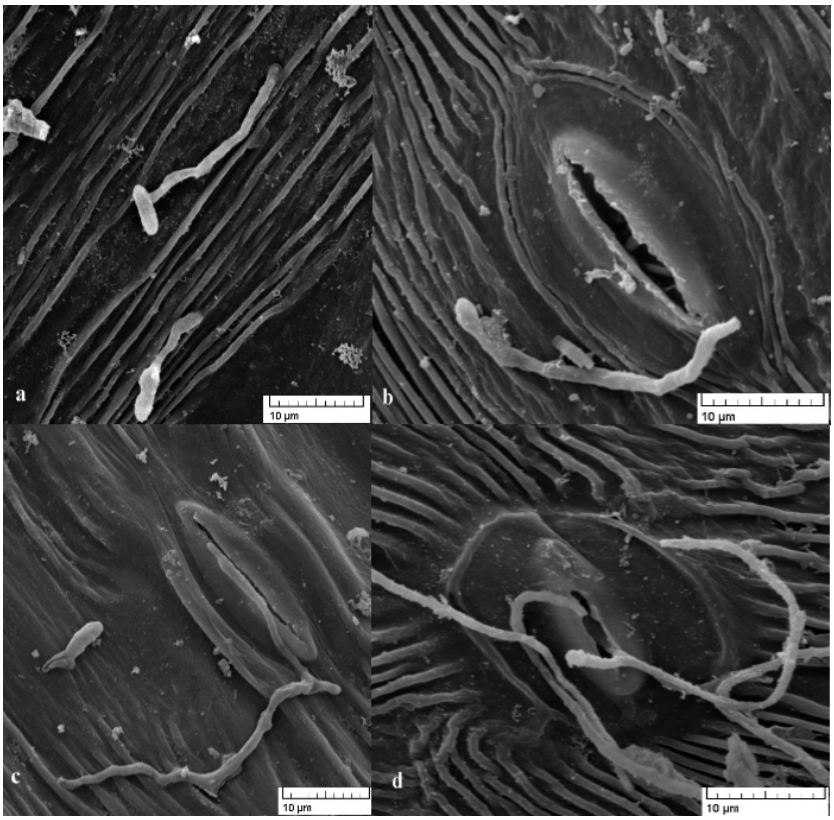
As a results of the seedlings inoculation through the uninjured epidermal tissue, after 6 days from inoculation with K 657 isolate the necrosis of hypocotyl occurred only on a few plants. After 9 days the plant growth was inhibited in growth compared to the control combination. Moreover, the necrosis of 25 seedlings infected with K 657 isolate as well as 21 seedling infected by K651 isolate had necrosis of the hypocotyl (tab. 7.) In the control combination the number of seedlings without necrosis was 25 and there were no seedlings with necrosis (tab. 7, photo 10). The mean values of inoculated seedlings with necrosis were significantly different comparing to the values of control combination (tab. 7).

As a result of inoculation of injured epidermal tissues the seedlings necrosis of the hypocotyl occurred on the length of 0.5 mm. Moreover, yellowing and drying of cotyledons and the first leaves was noted. Necrosis of the hypocotyl enlarged and after 6 days its length was 3 mm. Plant growth inhibition subsequent drying of the leaves and lack of formation of young leaves were also observed. Nine days after inoculation 25 seedlings showed necrosis and among them were dead seedlings (tab. 8, photo 10). In the control combination there were 0 seedlings with necrosis and 25 without necrosis (tab. 8). *P. diachenii* was reisolated from all inoculated seedlings.

Table 8. Results of inoculation of wounded caraway (*Carum carvi* L.) seedling hypocotyl with water suspension of *P. diachenii* conidia

Tabela 8. Wyniki inokulacji siewek kminku zwyczajnego (*Carum carvi* L.) wodną zawiesiną zarodników *P. diachenii* przez zraniony hypokotyl

Combinations Kombinacje	Number of inoculated seedlings Liczba inokulowanych siewek	Observation after 9 days Obserwacja po 9 dniach		Reisolation of *P. diachenii* Reizolacja *P. diachenii*
		seedlings with necrosis siewki z nekrozą	seedlings without necrosis siewki bez nekrozy	
P. diachenii				
Isolate K651	25	25	0	25
Isolate K 657	25	25	0	25
Control Kombinacja kontrola	25	0	25	0



Phot. 11. Staged of infection of caraway stems inoculated with *P. diachenii:* after 12 (a), 24 (b), 36 (c) and 48 hours (d), SEM (photo. M. Wróbel)

Fot. 11. Etapy infekcji łodyg kminku zwyczajnego inokulowanych *P. diachenii*: po 12 (a), 24 (b), 36 (c) i po 48 godz. (d) , SEM (fot. M. Wróbel)

Observations under electron microscope SEM showed that 12 hours after inoculation, the length of conidial germ tube of *P. diachenii* ranged from 7 to 14 µm (photo 11a). After 24 hrs germ tubes were directed towards the stomata creating a slight thickening in the top part, i.e. appressorium (photo 11b). After 36 hours a thin infective hyphae grew from the top part of the swelling of the germ tube. The formation of such a structure was observed along the stoma hole (photo 11c). After 48 hours, the infectious hyphae were inside the plant stoma (photo 11d).

DISCUSSION

Results of pathogenicity tests suggest large harmfulness of *Phomopsis diachenii* towards caraway. They make it possible to recognize this fungus as a potential pathogen of this plant. It turned out that *P. diachenii* causes disease symptoms on all plant organs tested, i.e. schizocarps, germs, fragments of stems, the leaves and the seedlings during their growth. The studies showed that the isolates tested under the conditions adopted in this experiment can inhibit or even prevent germination of schizocarps and cause necrosis, dying and necrosis of seedling stems and leaves.

The obtained positive results of pathogenicity tests are similar to the results of pathogenicity tests conducted on caraway in Germany by Gabler and Ehrig [1999].

All four tested isolates of *P. diachenii,* proved to be pathogenic towards the studied herbal plant. Inoculation methods can be considered while determining the pathogenicity of fungi for schizocarps, stems, leaves and seedlings of herbal plants. However, the fastest and the most harmful effects could be observed after application of post-culture liquids of the pathogen. This clearly indicates the presence of phytotoxic compounds in fungal metabolites, like in many other numerous phytopathogens [Chełkowski 1985, Kiecana and Kocyłak 1999, Kiecana et al. 2002]. For this reason, this method should be considered particularly useful for testing large quantities of the plant material, especially the seeds. The results suggest that artificial tissue injury facilitated the penetration of *P. diachenii* to plant tissues, increasing clearly the efficiency of inoculation. This explains the easy penetration of *P. diachenii* into caraway plants damaged by *Lygus* sp [Gabler and Ehrig, 2000]. Results of electron microscopy studies confirmed conclusively the ability of *P. diachenii* to infect plants through natural openings as observed via stomata. The other *Phomopsis* spp. infected plants in similarly way [Gabler and Ehrig 2000, Zimowska 2010]. The positive results of inoculation through the injured epidermal tissue confirmed by reisolation of the fungus indicate that the tested isolates of *P. diachenii* are characterized by facultative parasitism. The present reisolation of *P. diachenii* from caraway organs, which despite the inoculation showed no symptoms of the disease, indicates that *P. diachenii* was able to colonize plants in a latent way. A similar phenomenon was found for other species of fungi of genus *Phomopsis* [Król 2006]. Under the conditions conducive to the occurrence of the fungus the latter can prove dangerous for the growth and quality of caraway plant yield in Poland. This is more likely because *P. diachenii* occurs on herbal plants in Poland since the pathogen isolates were obtained in 2010 from stalk umbels of angelica grown near Lublin [Zalewska not published].

CONCLUSIONS

1. *P. diachenii* should be considered as an occasional pathogen of caraway.
2. Pathogen penetration may be able to the stomata of inoculated plants.
3. Tissue injuries play a significant role as ways of infection for *P. diachenii.*
4. *P. diachenii* infects schizocarps, germs, fragments of stems, leaves, seedlings while the symptomatic features are necrosis and the formed conidioma.

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PATOGENICZNOŚĆ IZOLATÓW *Phomopsis diachenii* SACC. DLA KMINKU ZWYCZAJNEGO *Carum carvi* L. (*Apiaceae*)

Streszczenie. *Phomopsis diachenii* należy do grzybów niedawno wykrytych na roślinach zielarskich w warunkach klimatycznych Polski. Celem pracy było rozpoznanie szkodliwości grzyba dla materiału siewnego, siewek, łodyg i liści kminku zwyczajnego. Testy infekcyjne, zgodnie z regułami Kocha, przeprowadzono dla 2 i 4 izolatów *P. diachenii*, stosując do inokulacji organów roślinnych: płyny pohodowlane, wodną zawiesinę konidiów i krążki pożywki z zarodnikującą grzybnią. Badania prowadzono w laboratorium oraz w fitotronie. *P. diachenii* wywoływał objawy chorobowe w postaci nekroz na wszystkich testowanych organach roślin. Wykazano zdolność *P. diachenii* do bezobjawowego zasiedlania inokulowanych części roślinnych. Zranienia tkanki okrywającej podnosiły wyraźnie efektywność inokulacji. Wykonane testy patogeniczności oraz wyniki spod mikroskopu elektronowego SEM wskazały, że testowane izolaty *P. diachenii* należą do okolicznościowych patogenów kminku zwyczajnego.

Słowa kluczowe: metody inokulacji, symptomatologia, fazy infekcji SEM

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