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GROWTH AND DEVELOPMENT OF *Phomopsis diachenii* Sacc. IN DIFFERENT CULTURE CONDITIONS

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Abstract. The knowledge of life requirements of phytopathogenic fungi allows to provide the periods of their mass occurrence on plant plantations and determine the optimal conditions for their isolation and culture on artificial media. Areas of occurrence of *P. diachenii* on caraway plants indicate the thermophilic nature of the fungus although there is a lack of information about research on this topic. In the present study the growth and development of one-spore cultures of six isolates of *P. diachenii* at the temperature: 0°C, 4°C, 10°C, 16°C, 22°C, 28°C, 33°C and 38°C on Czapek-Dox medium and also the growth and development of the same isolates in eight agar media at the temperature 25°C were examined. It was shown that the optimum temperature for the growth and sporulation of most isolates of *P. diachenii* and for the secretion of mature conidia from conidioma ranged 22°C to 28°C. Based on the nature of growth and development of *P. diachenii* on the examined media, Czapek-Dox and mineral ones as slightly acidic, and possibly malt extract should be recommend for isolation of the fungus from the plant tissue. PDA and Czapek-Dox media were considered the most suitable for diagnostic purposes due to the formation of characteristic macroscopic and microscopic features on these substrates. These substrates with fragments of carnation leaves should be recommended for the stimulation of fungal sporulation.

Key words: temperature, culture media, growth of colonies, formation of conidioma

INTRODUCTION

Growth, development and metabolic activity of fungi are heavily modified by external environmental factors, which include, among others, temperature, pH, moisture and nutrients [Udayanga et al. 2011]. Because the living conditions of fungi are diverse, even among the species within the same genus, hence they are present on plants in different geographical regions of the world [Farr et al. 1995]. Also, the possibility of the

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development of numerous species of fungi under specific conditions causes that they are present in variable intensity in different periods of plant vegetation [Machowicz-Stefaniak 2009, Zalewska 2012]. Species of *Phomopsis* (*Ascomycota, Diaporthales*) belong to such fungi [Uecker 1988, Marcinkowska 2003, King 2007]. *Phomopsis viticola*, whose growth and development are the most intense at 24°C to 28°C, commonly inhabits grapevine canes growing under field conditions in warm climates [Cavanni et al. 1987, Mármureanu et al. 1990, Król 2007]. In moderate climate of Poland this fungus intensively developed on grapevine canes cultivated under cover [Machowicz-Stefaniak 1993, Machowicz-Stefaniak and Kuropatwa 1993], while in the field conditions it was found sporadically and usually it inhabited canes only latently [Król 2006a, b]. Only in recent years, during hot and humid periods of vegetation, the presence of the fungus in grapevine canes significantly increased [Król 2007].

P. diachenii is a dangerous pathogen of caraway in central Germany, the occurrence of the fungus was also noted in the Czech Republic and Bulgaria [Rodeva and Gabler 2004], and in the years 2006–2007 it appeared on caraway in the south-eastern part of Poland [Machowicz-Stefaniak 2009]. In order to know the epidemiology of the fungus the studies was carried out on its growth and development at different temperatures and various culture media.

MATERIAL AND METHODS

The studies included six single spore isolates of *Phomopsis diachenii:* K 251, K 253, K 255, K 651, K 657, K 658. Isolates of the fungus were obtained in 2006–2007 years as a result of many yeares` studies on diseases of caraway cultivated in the vicinity of Lublin. Isolates of *P. diachenii* were obtained from various organs of plants using artificial culture method described earlier by Machowicz-Stefaniak and Zalewska [2004, 2008] and Machowicz-Stefaniak [2009]. The culture of mother colonies for the study was carried out for two weeks in Petri dishes on Czapek-Dox medium, in thermostat, at the temperature 25°C, without access to light (photo 1a, b, c, d).

The growth and development of fungus was studied at the following temperatures: 0°C, 4°C, 10°C, 16°C, 22°C, 28°C, 33°C, 38°C, on Czapek-Dox medium.

Moreover, the studies on the effect of culture medium on the growth and development of fungus used the following solidified culture media: malt agar (bioMerieux), Czapek-Dox (Difco), Czapek-Dox with decoction of caraway leaves (100 g leaves/dm^3 distilled water), Czapek-Dox with fragments of carnations leaves (a few per dish), mineral agar, oat agar (40 g of oat flakes/dm^3 distilled water), PDA (Difco), PDA fragments of carnations leaves (a few per dish) [Castillo-Pando at all. 1997, Boerema 2004, Król 2005].

The studies were carried out on solidified agar media in Petri dishes, which were inoculated with the inoculum of the tested fungus [Zalewska 2012]. The inoculation material consisted of 3-mm disc excised from 14-day old mother colonies of *P. diachenii.* For replicates (single dish as replication) were used for each isolate, temperature and culture medium. The observations of linear growth of the studied isolates colonies were carried out during the period of 14 days while the formation of morphological structures was determined until the 40th day of the culture. The macroscopic feature of the fungus,

the presence of the fungus conidiomata and conidia were observed in slashes under a light microscope with intervals of 2–3 days intervals. The obtained data on linear growth of 14-days-old cultures were subjected to the statistical analysis using SAS programme of variance analysis and Tukey`a confidence intervals.

RESULTS

The large variations in the growth of colonies of *P. diachenii* isolates cultured at different temperatures was shown (tab. 1, 2, photo 2). Inocula of the fungus on Czapek--Dox medium at the temperature of 38°C did not show any the growth until the 14th day of the culture (tab. 1, 2, photo 2). The colonies of the fungus started to growth after moving the Petri dishes to the room temperature. The formation of conidiomata was observed, whereas on the 40th day of the culture deformed β spores, but not α spores were produced. At the temperature of 33°C the diameter of 14-days-old colonies was significantly higher than at the temperatures 38°C, 0°C and 4°C, and significantly lower than the diameter of colonies growing at the temperatures of 10°C, 16°C, 22°C and 28°C (tab. 1). The diameter of 14-days-old colonies at temperature 10°C and 16°C, with the exception of isolates K 657 and K 658, were significantly lower than at the temperatures of 22°C and 28°C (tab. 1). The diameter of 14-days-old colonies of all isolates of *P. diachenii* was the biggest, i.e. their size was 90 mm at the temperatures of 22°C and 28°C (tab. 1). At these temperatures the colonies form abundant, loose or floccose, concentric zones air mycelium. The colour of mycelium was white, white-grey, while in some isolates it was olive with a light olive reverse (photo 2 e, f). The colonies cultured at the temperatures of 10°C and 16°C had a similarly appearance (photo 2 c, d). On the other hand, small colonies of the fungus formed at the temperature of 33°C were compact, like felt and their diameter ranged from 22.62 to 26.75 mm. The colour of these colonies was golden brown, olive-brown and they had a brown reverse (photo 2g). At the temperatures of 0°C and 4°C the growth of colonies was minimal, the colonies formed only singly, loose, hyaline hyphae of the aerial mycelium with a hyaline reverse. Microscope studies show rather strong deformation of the hyphae at the temperatures of 33°C, 0°C and 4°C, whereas at 33°C the formation of chlamydospores was observed (photo 3). Moreover, conidimata and spores was not formed on these hyphae until the 40th day of the cultivation (tab. 2). All isolates at the temperature of 10°C and isolates K 255 and K 658 at 16°C did not form conidiomata and conidia until the 40th day of cultivation. The other four isolates formed sinlgle conidiomata without conidia at the temperature 16°C (tab. 2). At the temperatures of 22°C and 28°C, depending on the isolate, conidiomata were formed after 8, 10, 12 or 14 days of cultivation (tab. 2). However, at 28°C the production of conidia was observed only after 30 or 36 days, and isolate K 251 did not form spores at all until the 40th day of the cultivation. At the temperature of 22°C four isolates of the fungus formed conidia already 12, 14 or 30 day of the cultivation, but isolates K 253 and K 658 did not produced conidia at all (tab. 2). Sporulating isolates of *P. diachenii* massively formed α and β conidia in the form of big, dense and crème droplets of exudate which covered the conidiomata.

Table 1. Effect of temperature on the size of 14-days-old colonies of *Phomopsis diacheniii* on Czapek-Dox medium
Tabela 1. Wpływ temperatury na wielkość 14-dniowych kolonii *Phomopsis diachenii* na pożywce Czapek-Dox

Temperature Temperatura Isolate Izolat	The diameter of studies isolates 14-days-old colonies (mm) Średnica 14-dniowych kolonii badanych izolatów (mm)								
	38°C	33°C	28°C	22°C	16°C	10°C	4°C	0°C	NIR
K 251	0 aD	23.87 aC	90 aA	90 aA	64.5 bB	64.5 aB	7.0 aD	3.5 cD	9.0029
K 253	0 aE	24.25 aD	90 aA	90 aA	77.25 abB	70.0 aC	3.0 bE	4.25 abcE	6.4268
K 255	0 aF	22.62 aD	90 aA	90 aA	78.0 abB	65.5 aC	3.0 bEF	4.5 abE	3.5189
K 651	0 aF	26.75 aD	90 aA	90 aA	83.87 aB	72.0 aC	3.0 bE	5.0 aE	3.3011
K657	0 aD	22.75 aD	90 aA	90 aA	80.87 aAB	72.5 aC	3.25 bE	4.0 cbE	9.2779
K 658	0 aD	24.75 aD	90 aA	90 aA	82.5 aA	70.5 aB	4.25 bE	4.0 cbE	7.9345
Mean – Średnia	0 F	24.16 D	90 A	90 A	77.83 B	69.16 C	3.91 EF	4.2 E	4.0849
NIR (LSD)	0	4.1496	0	0	16.249	8.7034	1.9995	0.8784	

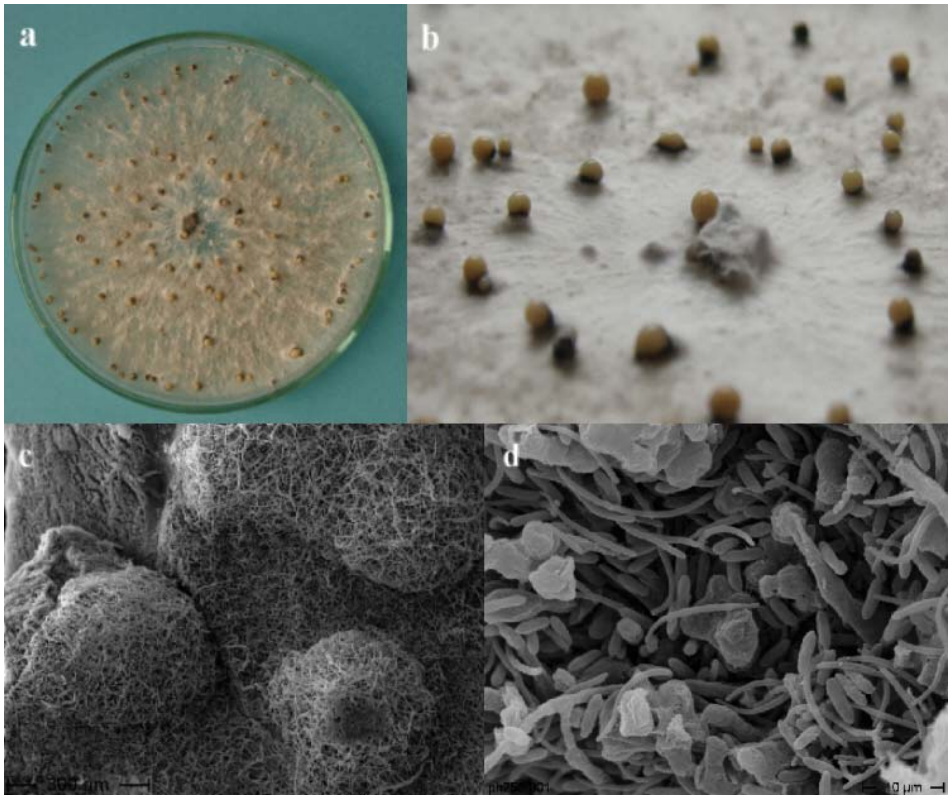
Differences between size of colonies depending on temperature for given isolate – capital letters
Różnice pomiędzy wielkością kolonii danego izolatu w różnych warunkach termicznych – duże litery
Differences between size of studies isolates colonies at a given temperature – small letters
Różnice pomiędzy wielkością kolonii badanych izolatów w danej temperaturze – małe litery
Values not marked do not differ significantly
Wartości nieoznaczone nie różnią się istotnie

Table 2. Effect of temperature on the production of conidiomata and conidia by *P. diachenii* on Czapek-Dox medium
Tabela 2. Oddziaływanie temperatury na tworzenie konidiom i zarodników przez *P. diachenii* na pożywce Czapek-Dox

Isolate Izolat	Temperature – Temperatura							
	38°C	33°C	28°C	22°C	16°C	10°C	4°C	0°C
K 251	Lack of the growth of the fungus colony to 14th day of observation	Dark, olive-brown mycelium, lack of conidiomata and conidia, deformation of hyphae, chlamydospores present	Abundant mycelium, 12th day formation of conidiomata, lack of conidia	Abundant mycelium, 12th day formation of conidiomata, 30th day numerous α and β conidia	Delicate, white, floccose mycelium, 30th day formation of conidiomata, lack of conidia to 40th day of culture	Delicate, white, floccose mycelium, lack of conidiomata and conidia to 40th day of culture	Thin, delicate, hyaline hyphae long from 6 to 7 mm, lack of conidiomata and conidia to 40th day of culture	Thin, delicate, hyaline, very short hyphae, lack of conidiomata and conidia to 40th day of culture
K 253			Abundant mycelium, 10th day formation of conidiomata and β conidia, 30th day exudate of α and β conidia	Abundant mycelium, 14th day formation of conidiomata, lack of conidia to 40th day of culture	Delicate, white, floccose mycelium, 30th day formation of conidiomata, lack of conidia to 40th day of culture			
K 255			Abundant mycelium, 10th day formation of conidiomata, 36th day numerous α and β conidia	Abundant mycelium, 8th day formation of conidiomata, 12th day numerous α and β conidia	Delicate, white, floccose mycelium, 30th day formation of conidiomata, lack of conidia to 40th day of culture			
K 651			Abundant mycelium, 10th day formation of conidiomata, 36th day numerous α and β conidia	Abundant mycelium, 10th day formation of conidiomata, 14th day numerous α and β conidia	Delicate, white, floccose mycelium, 30th day formation of conidiomata, lack of conidia to 40th day of culture			
K657			Abundant mycelium, 10th day formation of conidiomata, 36th day numerous α and β conidia	Abundant mycelium, 10th day formation of conidiomata, 12th day numerous α and β conidia	Delicate, white, floccose mycelium, 30th day formation of conidiomata, lack of conidia to 40th day of culture			
K 658			Abundant mycelium, 8th day formation of conidiomata, 36th day numerous β conidia, lack of α conidia	Abundant mycelium, 8th day formation of conidiomata, lack of conidia to 40th day of culture	Delicate, white, floccose mycelium, 30th day formation of conidiomata, lack of conidia to 40 day of culture			

Table 3. Effect of culture media on formation of conidiomata and conidia by *P. diachenii*
Tabela 3. Oddziaływanie podłoży hodowlanych na tworzenie konidiom i zarodników przez *P. diachenii*

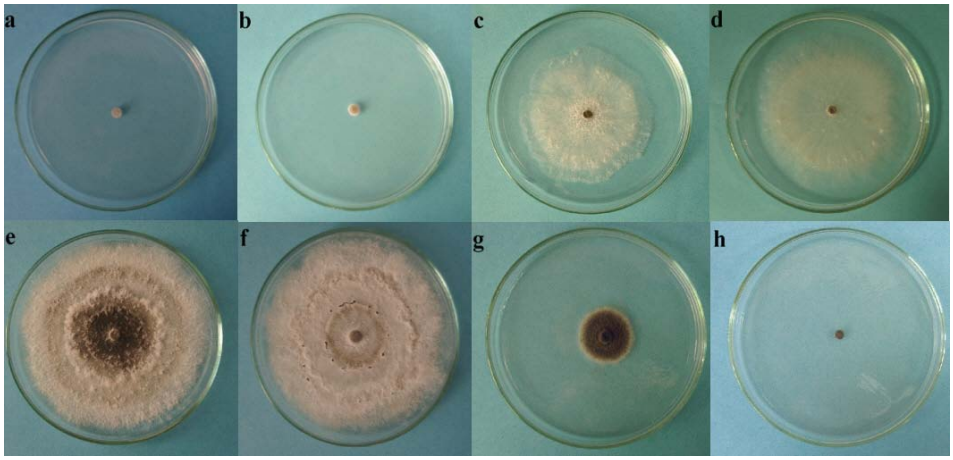
Isolate Izolat	Medium – Pożywka							
	PDA	Czapek-Dox	mineral agar mineralna	malt agar maltozowa	oat agar owsiana	Czapek-Dox with decoction of caraway leaves Czapek-Dox z wyciągiem z liści kminku	Czapek-Dox with fragments of carnations leaves Czapek-Dox z fragmentami liści goździka	PDA with fragments of carnations leaves PDA z fragmentami liści goździka
K 251	14th day conidiomata 25th day α and β conidia 30th day exudate of conidia	10th day conidiomata, 30th day β conidia	14th day conidiomata, lack of conidia	14th day conidiomata, 19th day α and β conidia	Abundant, white and floccose mycelium, lack of conidiomata and conidia	Lack of conidiomata and conidia	10th day conidiomata, 14th day α and β conidia 20th day exudate of conidia	8th day conidiomata, 10th day exudate of α conidia, 40th day sporadically β conidia
K 253	14th day conidiomata 19th day numerous numerous α and sporadically β conidia, 19th day exudate of conidia	10th day conidiomata, 12th day numerous α and β conidia, 30th day exudate of conidia	10th day conidiomata, 14th day α and β conidia, 20th day exudate of conidia	14th day conidiomata and α conidia, 20th day exudate of α and β conidia	Abundant, white and floccose mycelium, 12th day conidiomata, lack of conidia to 40th day of cultivation	14th day conidiomata and numerous α conidia, 20th day exudate of α conidia, sporadically β conidia, 30th day numerous β conidia	30th day conidiomata on leaves of carnation and α conidia, 40th exudate of α and β	12th day conidiomata, lack of conidia
K 255	14th day conidiomata 30th day sporadically β conidia, lack of exudate	12th day conidiomata, 30th day α and β conidia	8th day conidiomata, 14th day α and β conidia	14th day conidiomata 30th day α and β conidia, exudate of β conidia and sporadiclly α conidia	Abundant, white and floccose mycelium, lack of conidiomata and conidia	10th day conidiomata, lack of conidia	14th day conidiomata and α conidia on leaves of carnation, 40th day exudate of α and β conidia	12th day conidiomata and α conidia
K 651	10th day conidiomata 19th day α and β conidia 20th exudate of conidia	10th day conidiomata, 14th day α and β conidia	8th day conidiomata, 14th day α and β spores, 20th day exudate of conidia	10th day conidiomata, 19th day α and β conidia, 20th day exudate of conidia	Abundant white and floccose mycelium, 14th day conidiomata, 30th day singly α and β conidia	10th day conidiomata, 14th day α and β conidia, 20th exudate of conidia	10th day conidiomata, 14th day numerous α and β conidia, 20th day exudate of conidia	10th day conidiomata and numerous α conidia, 14th day exudate of α conidia, 40th day sporadically β conidia
K657	8th day conidiomata 10th day α and β conidia 20th day exudate of conidia	10th day conidiomata, 40th day α and β conidia	8th day conidiomata, 19th day numerous β conidia, 30th day exudate of α and β conidia	12th day conidiomata, 19th day α and β conidia, 20th day exudate of conidia	Abundant white and floccose mycelium, 10th day conidiomata, and singly α conidia	10th day conidiomata, lack of conidia	30th day conidiomata on leaves of carnation and exudate of α and β conidia	12th day conidiomata on leaves of carnations, numerous α conidia, 14th day exudate of α and β conidia
K 658	10th day conidiomata 25th day numerous α conidia, 30th day exudate of α and β conidia	10th day conidiomata, 40th day α and β conidia	8th day conidiomata, lack of conidia	10th day conidiomata, 19th day α and β conidia, 20th day exudate of cinidia	Abundant, white and floccose mycelium, 14th day conidiomata, lack of conidia to 40th day of cultivation	10th day conidiomata, 20th day α and β conidia	8th day conidiomata on leaves of carnation, 10th day numerous α and β conidia, 14th day exudate of conidia	8th day conidiomata on leaves of carnation, numerous α conidia, 10th day exudate of α conidia, sporadically β conidia



Phot. 1. *Phomopsis diachenii*, isolate K 251: a – 24-days-old colony on Czapek-Dox, b – exudate of conidia on conidiomata, c – conidiomata SEM and d – α and β conidia SEM (Photo 1a, b – E. Zalewska, Photo 1c, d – M. Wróbel)

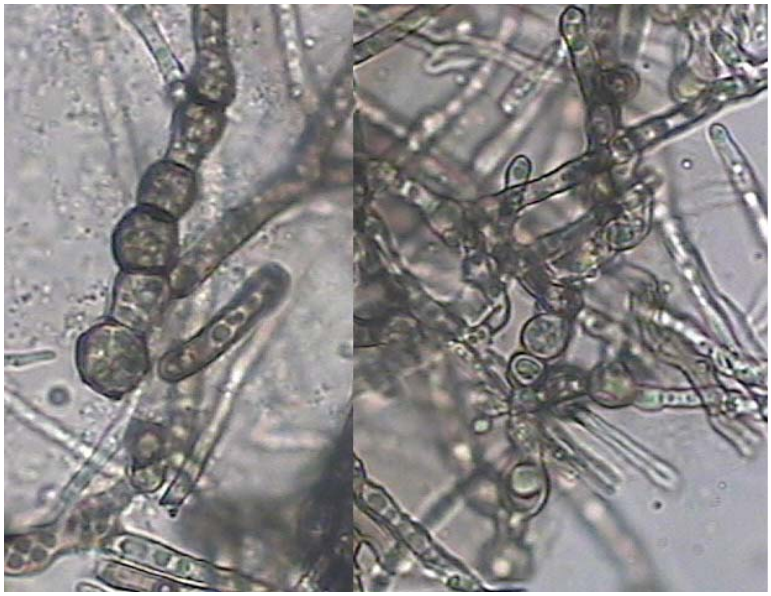
Fot. 1. *Phomopsis diachenii*, izolat K 251: a – 24-dniowa kolonia na Czapek-Dox, b – wydzielina zarodników na konidiomach, c – konidiomy SEM i d – zarodniki α i β SEM (Foto 1a, b – E. Zalewska, Foto 1c, d – M. Wróbel)

The experiment on the growth of isolates of *P. diachenii* on different culture media shows that the diameter of the 14-days-old colonies of all isolates does not differ significantly. The colonies reached their maximum size, i.e. 90 mm (photo 4). Colonies on the studied media had loose structure and a repent mycelium, but only on oat agar their growth was abundant, and the mycelium grew to 5 mm above the medium surface and had the floccose structure. The colour of the aerial mycelium was white-crème or grey to grey-olive. On the other hand, the mycelium was white on oat agar (tab. 3, photo 4). Numerous conidiomata were formed, depending on the isolates, the earliest, i.e. from 8th to the 14th days of the cultivation on medium: Czapek-Dox, PDA and mineral agar and after 10–20 days on malt agar medium (tab. 3). On oat agar only single conidiomata were formed by four isolates after 10–14 days of cultivation (tab. 3). The production of conidia by isolates of *P. diachenii* started the earliest, i.e. after 10–30 days of cultivation



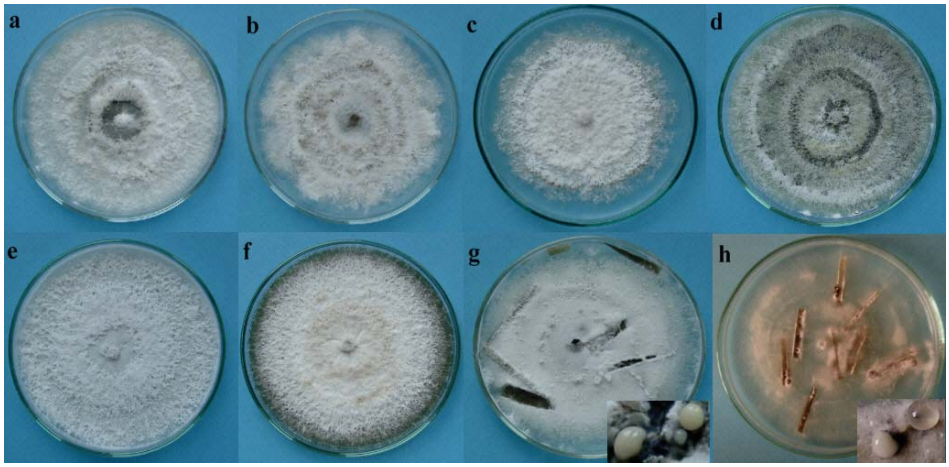
Phot. 2. 14-day-old colonies of *P. diachenii*, isolate K 251 on Czapek-Dox at temperature: a – 0°C, b – 4°C, c – 10°C, d – 16°C, e – 22°C, f – 28°C, g – 33°C and h – 38°C (Photo E. Zalewska)

Fot. 2. 4-dniowe kolonie *P. diachenii* izolat K 251 na pożywce Czapek-Dox w różnej temperaturze: a – 0°C, b – 4°C, c – 10°C, d – 16°C, e – 22°C, f – 28°C, g – 33°C i h – 38°C (Fot. E. Zalewska)



Phot. 3. Chlamydospores of *P. diachenii* at temperature 33°C on Czapek-Dox (magnification × 500), (Photo E. Zalewska)

Fot. 3. Chlamydospory *P. diachenii* w temperaturze 33°C na Czapek-Dox (pow. × 500), (Fot. E. Zalewska)



Phot. 4. 14-days-old colonies of *P. diachenii* isolate K 251 growing on mediums: a – PDA, b – Czapek-Dox, c – mineral agar, d – malt agar, e – oat agar, f – Czapek-Dox with decoction of caraway leaves, g – Czapek-Dox with fragments of carnation leaves and h – PDA with fragments of carnation leaves (Photo E. Zalewska)

Fot. 4. 14-dniowe kolonie *P. diachenii* izolat K 251 wzrastające na pożywce: a – PDA, b – Czapek-Dox, c – mineralnej, d – maltozowej, e – owsianej, f – Czapek-Dox z wyciągiem z liści kminku zwyczajnego, g – Czapek-Dox z fragmentami liści goździka i h – PDA z fragmentami liści goździka (Fot. E. Zalewska)

on Czapek-Dox and PDA, and after 14–30 days by the other isolates growing on mineral agar and malt agar medium (tab. 3). On oat agar medium only weak sporulation was noted by two isolates, i.e. K 651 and K 657, whereas the other isolates did not produced any conidia (tab. 3). The most intensive production of conidiomta and conidia was noted on Czapek-Dox and PDA medium with fragments of carnation leaves, whereas on Czapek-Dox medium with a decoction of caraway leaves the sporulation was not so intensive.

DISCUSSION

The obtained results and statistical analysis showed that for the majority of the pathogen isolates the temperatures ranging from 22°C to 28°C is optimal for the growth, sporulation and the rate of conidia release of conidiomata. Such findings allow to assume that the warm vegetation periods combined with high relative humidity of the air have a positive effect on sporulation of *P. diachenii*, the infection of plants and on the occurrence of disease on caraway. This is consistent with the results of research conducted by other authors [Gabler and Ehrig 2000, Machowicz-Stefaniak and Zalewska 2004, 2008]. In the present study it was shown, that *P. diachenii* has the ability to keep the vital mycelium in thermal conditions unfavourable for the growth, i.e. from 0°C to

4°C and from 33°C to 38°C. This indicates the possibility of *P. diachenii* to survive and occur on plants in hot as well as moderate climates. It is probably possible due to formation of chlamydospores and β spores, which was indicated earlier for the other species of *Phomosis* genera [Król 2005, 2007, Zimowska 2010].

Studies on the effect of the type of the culture medium on the growth and sporulation of the fungus showed that the oat agar was the medium where the growth of mycelium was most intense. However, on the basis of the obtained results, it is not possible to recommend this medium as optimal for the culture of *P. diachenii*, because the fungus does not produce spores on this medium or the sporulation is scare. Moreover, the results suggest that the most appropriate medium for the growth and sporulation of *P. diachenii* are Czapek-Dox and PDA media and then mineral and malt agar media. The great usefulness of glucose-potato medium for the growth of various species of fungi was indicated by many authors [Gabler and Ehrig 2000, Machowicz-Stefaniak and Zalewska 2004, 2008, Zalewska 2012]. Due to the slow sporulation of *Phomopsis* spp. isolates, the possibility of stimulating sporulation with the proper culture medium is very important [Castillo-Pando et all. 1997, Król 2005]. In the present study these substrates were Czapek-Dox and PDA with an addition of carnations leaves. These culture media should be recommended for the stimulation of *P. diachenii*. Similarly, in the case of *P. viticola* isolates a very effective sporulation was obtained on PDA with an addition of carnation leaves [Castillo-Pando et all. 1997, Król 2005].

The obtained result of studies suggest that Czapek-Dox and mineral agar medium as slightly acid pH and possibly the malt agar medium should be recommended for isolation of the pathogen from plant tissues. Thanks to south pH, the colonies of fungi growing from fragments of the plant material are not contaminated by other microorganisms. On the other hand, PDA and Czapek-Dox mediums should be recognized as the most appropriate for the cultivation of *P. diachenii* for diagnostic purposes because the fungus forms characteristic macroscopic features of the colony on these culture media. Additionally, these culture media with an addition of carnation leaves should be recommended for the stimulation of sporulation. Conidimata and conidia α and β are produced very early on these media. Moreover, the morphological diversity of single isolate growing on different culture media and within isolates growing on the same medium indicates the necessity to introduce standard media, considering Czapek-Dox and PDA as such, for the diagnose of one-spore cultures of *Phomopsis* spp.

CONCLUSIONS

1. Composition of culture medium and cultivation temperature modify the macroscopic features of the fungus.

2. The optimum temperature for the growth and sporulation of *P. diachenii* ranges from 22°C to 28°C.

3. Czapek-Dox and PDA media are the most appropriate for the cultivation of *P. diachenii* for diagnostic purposes.

4. Czapek-Dox and PDA medium with an addition of carnation leaves should be recommended for the stimulation of *P. diachenii* sporulation.

5. *P. diachenii* can be a dangerous pathogen of caraway in hot and wet vegetation periods.

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WZROST I ROZWÓJ *Phomopsis diachenii* Sacc. W RÓŻNYCH WARUNKACH HODOWLI

Streszczenie: Znajomość wymagań życiowych fitopatogennych grzybów pozwala przewidzieć okresy ich masowego występowania na plantacjach roślin, ustalić optymalne warunki ich izolacji i hodowli na sztucznych podłożach. Rejony występowania *P. diachenii* na roślinach kminku zwyczajnego wskazują na ciepłolubny charakter grzyba, aczkolwiek brak jest informacji o badaniach na ten temat. W niniejszej pracy przebadano wzrost i rozwój jednozarodnikowych kultur sześciu izolatów *P. diachenii,* w temperaturze: 0°C, 4°C, 10°C, 16°C, 22°C, 28°C, 33°C, 38°C, na pożywce Czapek-Dox oraz wzrost i rozwój tych samych izolatów na ośmiu pożywkach agarowych w temperaturze 25°C. Wykazano, że optymalną temperaturą do wzrostu i zarodnikowania większości izolatów *P. diachenii* oraz wypływu dojrzałych zarodników z konidiomy jest temperatura 22°C do 28°C. Na podstawie charakteru wzrostu i rozwoju *P. diachenii* na testowanych podłożach należy polecać do izolacji grzyba z tkanek roślinnych pożywki Czapek-Dox i mineralną jako lekko kwaśne i ewentualnie maltozową. Do hodowli kultur *P. diachenii* w celach diagnostycznych za najodpowiedniejsze uznano PDA i Czapek-Dox, ze względu na wytwarzanie na tych podłożach charakterystycznych cech makroskopowych i mikroskopowych. Natomiast podłoża te z fragmentami liści goździka należy polecać do stymulacji zarodnikowania grzyba.

Słowa kluczowe: temperatura, podłoża hodowlane, wzrost kolonii, tworzenie konidiom, zarodnikowanie

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