

## GROWTH AND SPORULATION OF *Septoria carvi* Syd. IN DIFFERENT CULTURE CONDITIONS

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**Abstract.** The quantity and quality of the herb material are reduced by pathogenic fungi. In the case of caraway this pathogen is *Septoria carvi*. In the vegetation periods the fungus occurs with varying intensity, which is related to the requirements of life. The present study found that the growth of fungal colonies is possible at the temperatures from 0 to 30°C, the optimum being from 20 to 25°C, and sporulation is possible at the temperatures from 10 to 30°C, with the optimum at 25°C. It was shown that the most useful medium for the isolation of *S. carvi* from caraway plants is malt medium and malt medium with decoction of the leaves of this plant. Pycnidia and conidia of the fungus, which are necessary to identify the species, are formed the fastest on the malt medium with decoction of the leaves or schizocarps of caraway and on PDA medium and it is these substrates that should be used for the cultivation of *S. carvi*.

**Key words:** culture medium, temperature, caraway, septoriosis

### INTRODUCTION

Within the fungi of the *Septoria* genus, species *Septoria cari* (Brezchn.), *S. umbelliferarum* Kalchbr. and *S. carvi* Syd. are considered to be the cause of death of the leaves and umbels of caraway cultivated in European countries [Sydow 1932, Pidopličko 1978, Ondfej 1983, Teterevnikova-Babayan 1987, Farr et al. 1995, Odstrčilova et al. 2002, Machowicz-Stefaniak and Zalewska, 2004, Mazur and Nawrocki 2004, Bedlan 2005]. The species *S. carvi* caused damage to about 50% of the plantation in Austria in 2005 year [Bedlan 2005]. Inhabitation of the aboveground and underground parts of caraway by various species of fungi was found in 2001–2005 in Lublin and Chełm regions. One of the fungi was *Septoria carvi*, which was considered as the primary and main cause of the dying leaves, petioles, stems, umbels and even schizocarps of this plant [Machowicz-Stefaniak et al. 2003, Machowicz-Stefaniak and Zalewska 2004, Zalewska 2008].

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Increased occurrence of *S. carvi* was recorded during the growing seasons of increased relative humidity and high temperature, which usually takes place at the beginning or the first half of June and it is combined with caraway flowering. The life requirements of fungi infested herbs are very different and therefore there are numerous species with varying intensity in different growing seasons. It seems *S. carvi* belongs to them. To find the optimal conditions for fungal development and be able to predict the periods of epidemic prevalence of the fungi such research should be conducted. In the available literature no information on the growth conditions of *S. carvi* was found, although they have been tested for other species of *Septoria* spp. [Richards 1951, Marcinowska 1977, Błaszkowski 1990]. Determination of the conditions for growth and development of *S. carvi*, especially the optimum and minimum temperatures for the mycelium and spores formation was considered as purposeful and necessary for understanding the epidemiology of the fungus. Moreover, the study of the growth and development of *S. carvi* on different culture mediums let us determine the most suitable medium for the isolation of the pathogen from the plant tissue and substrates to culture for diagnostic purposes.

## MATERIAL AND METHODS

The studies included six single spore sporulating isolates of *S. carvi*: K 1806, K 1813, K 1833, K 1860, K 1861 and K 1863 obtained from plants of caraway *Carum carvi* L. varieties Konczewicki in 2001–2010. These isolates (photo 1) were selected at



Photo 1. 14-days-old colonies of *S. carvi* on PDA medium (isolates: K 1806, K 1833 and K 1863) (photo E. Zalewska)

Fot. 1. 14-dniowe kolonie *S. carvi* na PDA (izolaty: K 1806, K 1833 i K 1863) (wyk. E. Zalewska)



Photo 2. Septoriosis of various organs of caraway (photo E. Zalewska)  
Fot. 2. Septorioza różnych organów kminku zwyczajnego (wyk. E. Zalewska)

random from our own culture collection, gathered during research on diseases of caraway grown in the vicinity of Lublin and Chełm [Machowicz-Stefaniak and Zalewska, 2004, 2008, Zalewska 2008]. *S. carvi* isolates were obtained from the aboveground parts of caraway plants, i.e. from the leaves, stems, umbels and schizocarps showing symptoms of septoriosis and etiological symptoms typical of the fungi form the *Septoria* genus (photo 2). Each of the isolates was cultured at the following temperatures: 0, 5, 10, 15, 20, 25, 30 and 35°C, on PDA medium in dispersed light. Moreover, the growth of the above mentioned isolates were investigated on the following mediums: PDA (potato-dextrose agar) and Czapek-Dox (Difco), malt agar (bioMerieux), oat agar [Boerema et al. 2004], malt agar with decoction of the caraway leaves (100 g leaves/dm<sup>3</sup> distilled water), malt agar with decoction of caraway schizocarps (20 g schizocarps/dm<sup>3</sup> of distilled water) at the temperature 25°C.

The cultures were carried out on solidified agar media on Petri dishes, which were inoculated with the inoculum of the tested fungus. The inoculation material comprised 3-mm disc excised from 14-days-old mother colonies of *S. carvi* isolates growing in dispersed light at the temperature of 25°C. For each isolate and for each combination of experiment 4 replicates were used.

The observations of the linear growth of the studied isolates colonies were carried out during a period of 14 days and after that time the obtained data were subjected to statistical analysis using variance analysis and Tukey's confidence intervals. Spores and chlamydospores formation was determined to 40 day of studies. Measurements of conidia (100 conidia for each isolate) formed on all culture media tested at the temperature of 25°C were made for the purpose of a comparison.

## RESULTS

The conducted studies showed large variations in the growth of colonies of *S. carvi* isolates cultured at different temperatures (tab. 1). There was no growth of the aerial mycelium on any of the isolates at 35°C; however, it remained only on the mother inoculum of the fungus. The most intensive growth of colonies of all the tested isolates was found at the temperatures of 20 and 25°C, whereas at 10, 15 and 30°C the growth of colonies of all tested isolates was moderate and at 0 and at 5°C minimal (tab. 1).

The statistical analysis of the obtained results showed that the size of 14-day colonies of *S. carvi* growing at the temperature of 10, 15 and 30°C, in most cases did not differ significantly (tab. 1). Moreover, there were no significant differences between the

Table 1. Effect of temperature on the size of 14-days-old colonies of *S. carvi* on PDA medium  
Tabela 1. Wpływ temperatury na wielkość 14-dniowych kolonii *S. carvi* na pożywce PDA

Isolate Izolat	The diameter of studies isolates 14-days-old colonies (mm) Średnica 14-dniowych kolonii badanych izolatów (mm)								
	Temperature – Temperatura								
	35°C	30°C	25°C	20°C	15°C	10°C	5°C	0°C	NIR
K 1806	3.0 <sup>G</sup> <sub>a</sub>	25.0 <sup>C</sup> <sub>a</sub>	38.12 <sup>B</sup> <sub>ab</sub>	28.75 <sup>B</sup> <sub>d</sub>	19.5 <sup>D</sup> <sub>a</sub>	15.5 <sup>E</sup> <sub>d</sub>	6.0 <sup>F</sup> <sub>b</sub>	5.0 <sup>FG</sup> <sub>a</sub>	2.3631
K 1813	3.0 <sup>E</sup> <sub>a</sub>	19.0 <sup>B</sup> <sub>c</sub>	36.0 <sup>A</sup> <sub>a</sub>	37.0 <sup>B</sup> <sub>bc</sub>	19.25 <sup>B</sup> <sub>a</sub>	16.0 <sup>C</sup> <sub>cd</sub>	6.0 <sup>D</sup> <sub>b</sub>	5.75 <sup>D</sup> <sub>a</sub>	2.64
K 1833	3.0 <sup>E</sup> <sub>a</sub>	21.50 <sup>B</sup> <sub>b</sub>	36.5 <sup>A</sup> <sub>b</sub>	37.5 <sup>B</sup> <sub>bc</sub>	21.0 <sup>B</sup> <sub>a</sub>	17.5 <sup>C</sup> <sub>bc</sub>	5.0 <sup>D</sup> <sub>b</sub>	4.87 <sup>D</sup> <sub>a</sub>	1.806
K 1860	3.0 <sup>D</sup> <sub>a</sub>	24.75 <sup>B</sup> <sub>a</sub>	41.0 <sup>A</sup> <sub>a</sub>	41.25 <sup>A</sup> <sub>a</sub>	20.75 <sup>C</sup> <sub>a</sub>	19.0 <sup>C</sup> <sub>ab</sub>	6.0 <sup>D</sup> <sub>b</sub>	5.62 <sup>D</sup> <sub>a</sub>	3.6854
K 1861	3.0 <sup>E</sup> <sub>a</sub>	21.00 <sup>B</sup> <sub>bc</sub>	35.5 <sup>A</sup> <sub>b</sub>	34.25 <sup>A</sup> <sub>c</sub>	19.75 <sup>C</sup> <sub>a</sub>	18.0 <sup>C</sup> <sub>ab</sub>	7.5 <sup>D</sup> <sub>a</sub>	5.75 <sup>D</sup> <sub>a</sub>	2.4492
K 1863	3.0 <sup>E</sup> <sub>a</sub>	20.25 <sup>B</sup> <sub>bc</sub>	37.0 <sup>A</sup> <sub>ab</sub>	38.25 <sup>A</sup> <sub>ab</sub>	19.12 <sup>B</sup> <sub>a</sub>	19.25 <sup>B</sup> <sub>a</sub>	8.0 <sup>C</sup> <sub>a</sub>	5.87 <sup>D</sup> <sub>a</sub>	1.4039
LSD NIR	0.0	2.2782	4.469	3.3499	2.0811	1.5668	1.1844	1.1314	

Differences depending on temperature for given isolate – capital letters

Różnice pomiędzy wielkością kolonii danego izolatu w różnych warunkach termicznych – duże litery

Differences between isolates 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

sizes of colonies of isolates growing at 20 and 25°C, with the exception of isolate K 1806, whose size of 14-day-old colony at 20°C was significantly lower than at 25°C (tab. 1). Statistically, it was also shown that the size of colonies of all isolates growing at 20 and 25°C was significantly higher than the size of the colonies of the same isolates cultured in the other conditions of temperature (tab. 1, photo 3).

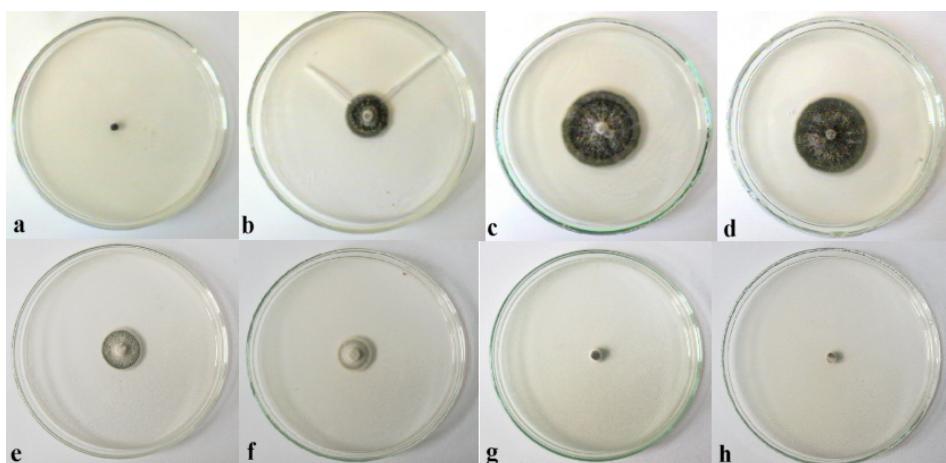


Photo 3. 14-days-old colonies of *S. carvi* K 1833 on PDA medium at the temperature: 35°C – a, 30°C – b, 25°C – c, 20°C – d, 15°C – e, 10°C – f, 5°C – g and 0°C – h (photo E. Zalewska)

Fot. 3. 14-dniowe kolonie *S. carvi* K 1833 na PDA w temperaturze: 35°C – a, 30°C – b, 25°C – c, 20°C – d, 15°C – e, 10°C – f, 5°C – g i 0°C – h (wyk. E. Zalewska)

On the basis of macro- and microscope studies it was found that the studied isolates of *S. carvi* growing at the temperatures of 0, 5 and 35°C did not produce pycnidia and conidia until the 14<sup>th</sup> day of the cultivation. Only isolates K 1806 and K1813 growing at the temperature 5°C during 34 days produced a few pycnidia, but inside of these pycnidia no spores were observed until 40<sup>th</sup> day (tab. 2). In the mycelium of isolates growing at the temperature 30°C numerous chlamydospores were formed. The colony of these isolates had an unusual appearance, the mycelium was tight, compact with a corrugated surface. The strongly tight mycelium caused shrinking and cracking of the culture medium with time (photo 3b). Isolates growing at the temperatures from 10 to 25°C formed a characteristically flat and rather compact air mycelium (photo. 3c, d, e, f). The formation of pycnidia was observed from 6<sup>th</sup> day of the culture on the surface of the colonies of the tested isolates growing at the temperature from 20 to 30°C, and from 10<sup>th</sup> day of the culture on the surface of colonies growing at 10 and 15°C. The formation of conidia was observed between 8<sup>th</sup> and 20<sup>th</sup> day of the culture by all tested isolates growing at the temperature from 15 to 30°C (tab. 2). However, the production of conidia was most intense at 25°C, because creamy exudate of conidia flowed from the inside of pycnidia after 8 or 10 days of the culture (tab. 2).

Table 2. Effect of temperature on the production of pycnidia and conidia by *S. carvi* on PDA medium  
 Tabela 2. Oddziaływanie temperatury na tworzenie pikniów i zarodników przez *S. carvi* na pożywce PDA

Isolate Izolat	35°C	30°C	25°C	Temperature – Temperatura				
				20°C	15°C	10°C	5°C	0°C
K 1806	pycnidia and conidia – absent	pycnidia and conidia – after 6 days	pycnidia and conidia – after 6 days	pycnidia and conidia – after 10 days	pycnidia – after 10 days	pycnidia – after 10 days	pycnidia – after 34 days	pycnidia and conidia – absent
K 1813	pycnidia and conidia – absent	pycnidia and conidia – after 10 days	pycnidia and conidia – after 6 days	pycnidia and conidia – after 8 days	pycnidia and conidia – after 8 days	pycnidia – after 10 days	pycnidia – after 34 days	pycnidia and conidia – absent
K 1833	pycnidia and conidia – absent	pycnidia and conidia – absent	pycnidia and conidia – after 6 days	pycnidia and conidia – after 6 days	pycnidia – after 6 days	pycnidia – after 10 days	pycnidia – after 14 days	pycnidia and conidia – absent
K 1860	pycnidia and conidia – absent	pycnidia – after 6 days	pycnidia and conidia – after 6 days	chlamydospores – after 6 days	chlamydospores – after 6 days	pycnidia – after 10 days	pycnidia – after 12 days	pycnidia and conidia – absent
K 1861	pycnidia and conidia – absent	pycnidia – after 8 days	pycnidia and conidia – after 8 days	pycnidia and conidia – after 8 days	pycnidia and conidia – after 8 days	pycnidia – after 12 days	pycnidia and conidia – after 20 days	pycnidia and conidia – absent
K 1863	pycnidia and conidia – absent	pycnidia – after 10 days	pycnidia – after 6 days	pycnidia and conidia – after 14 days	pycnidia and conidia – after 14 days	pycnidia – after 10 days	pycnidia and conidia – absent	pycnidia and conidia – absent

The studies of the effect of culture medium on the growth and sporulation of *S. carvi* at the temperature of 25°C showed that the diameter of 14-day-old colonies of the studied isolates ranged between from 22.0 to 45.25 mm according to the studied culture medium (tab. 3, photo 4). The statistical analysis showed that the size of the colonies of most isolates growing on various culture media differed significantly (tab. 3). The diameter of the colonies of most isolates of *S. carvi* growing on PDA and malt agar with decoction of caraway leaves was significantly higher than on the other culture media (tab. 3). The statistical analysis also showed that the diameter of colony isolates growing on the same culture medium was significantly different (tab. 3). On malt agar medium with decoction of caraway leaves, the size of the colonies of the studied isolates did not differ significantly with the exception of isolate K 1806 (tab. 3). On PDA medium, the diameter of the colonies of isolates K 1813, K 1833 and K 1860 was significantly higher than the other three isolates (tab. 3).

Table 3. Effect of culture medium on the size of 14-days-old colonies of *S. carvi*  
Tabela 3. Oddziaływanie podłoża hodowlanego na wielkość 14-dniowych kolonii *S. carvi*

Isolate Izolat	The diameter of studies isolates 14-days-old colonies (mm) Średnica 14-dniowych kolonii badanych izolatów (mm)							LSD NIR	
	Medium – Pożywka								
	PDA	Czapek- Dox	Oat agar Owsiana	Malt agar Maltozowa	Malt agar with decoction of caraway leaves Maltozowa z odwarem z liści	Malt agar with decoction of caraway schizocarps Maltozowa z odwarem z rozłupkiem kminku			
K 1806	37.5 <sub>b</sub> <sup>A</sup>	27.75 <sub>c</sub> <sup>D</sup>	34.0 <sub>cd</sub> <sup>B</sup>	31.25 <sub>b</sub> <sup>BC</sup>	29.75 <sub>b</sub> <sup>CD</sup>	23.0 <sub>c</sub> <sup>E</sup>	3.4834		
K 1813	42.75 <sub>a</sub> <sup>A</sup>	32.0 <sub>bc</sub> <sup>D</sup>	32.62 <sub>d</sub> <sup>CD</sup>	36.0 <sub>a</sub> <sup>BC</sup>	37.25 <sub>a</sub> <sup>B</sup>	24.0 <sub>b</sub> <sup>E</sup>	3.3993		
K 1833	42.0 <sub>a</sub> <sup>A</sup>	32.25 <sub>abc</sub> <sup>C</sup>	37.0 <sub>ab</sub> <sup>B</sup>	28.0 <sub>c</sub> <sup>D</sup>	40.0 <sub>a</sub> <sup>A</sup>	23.0 <sub>c</sub> <sup>E</sup>	2.1678		
K 1860	45.25 <sub>a</sub> <sup>A</sup>	33.5 <sub>ab</sub> <sup>C</sup>	38.75 <sub>a</sub> <sup>B</sup>	36.0 <sub>a</sub> <sup>BC</sup>	38.0 <sub>a</sub> <sup>B</sup>	26.75 <sub>a</sub> <sup>D</sup>	4.3436		
K 1861	34.0 <sub>b</sub> <sup>A</sup>	37.0 <sub>a</sub> <sup>A</sup>	36.5 <sub>abc</sub> <sup>A</sup>	22.0 <sub>d</sub> <sup>B</sup>	36.5 <sub>a</sub> <sup>A</sup>	23.0 <sub>c</sub> <sup>B</sup>	3.6697		
K 1863	35.0 <sub>b</sub> <sup>AB</sup>	33.0 <sub>ab</sub> <sup>B</sup>	35.5 <sub>bc</sub> <sup>AB</sup>	24.5 <sub>d</sub> <sup>C</sup>	37.0 <sub>a</sub> <sup>A</sup>	23.0 <sub>c</sub> <sup>C</sup>	3.8195		
LSD NIR	3.8378	4.8110	2.8246	3.1670	4.3838	0.4587			

Differences depending on culture medium for given isolate – capital letters

Różnice pomiędzy wielkością kolonii danego izolatu na badanych podłożach hodowlanych – duże litery

Differences between isolates on given culture medium – small letters

Różnice pomiędzy wielkością kolonii badanych izolatów na danym podłożu hodowlanym – małe litery

Values not marked do not differ significantly

Wartości nieoznaczone nie różnią się istotnie

The conducted studies showed large differentiation in macroscopic and microscopic features of *S. carvi* isolates growing on different culture mediums (photo 4). Colonies of *S. carvi* had gray or olive gray, flat and powder air mycelium and a metallic reverse side on PDA medium. The colony of isolate K 1860 colored the culture medium pink (photo 4a). On this studied culture medium, some isolates formed the first pycnidia as early as

6<sup>th</sup> day of the culture, and between 10<sup>th</sup> and 14<sup>th</sup> days the formation of conidia and chlamydospores of the fungus were found (tab. 4, photo 5).

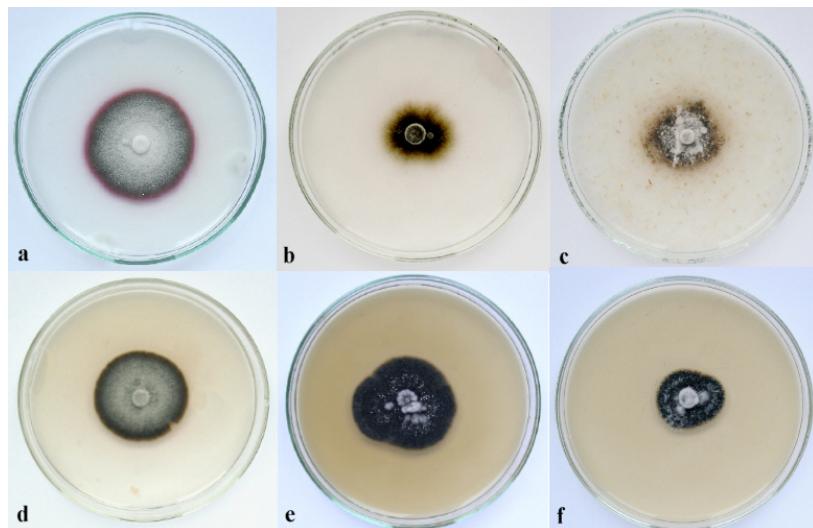


Photo 4. 14-days-old colonies of *S. carvi* K 1860 on medium: PDA – a, Czapek-Dox – b, oat agar – c, malt agar – d, malt agar with decoction of caraway leaves – e and malt agar with decoction of caraway schizocarps – f (photo. E. Zalewska)  
 Fot. 4. 14-dniowe kolonie *S. carvi* K 1860 na pożywkach: PDA – a, Czapek-Dox – b, owsianej – c, maltozowej – d, maltozowej z odwarem z liści kminku – e i maltozowej z odwarem z rozłupkiem kminku – f (wyk. E. Zalewska)

On Czapek-Dox medium the colonies were of olive colour, and the aerial mycelium was poor, dark and dense (photo 4b). The colonies had olive, dim reverse side. Pycnidia were formed only by isolates K 1806 and K 1833, but spores only by K 1806 (tab. 4). The other isolates did not form pycnidia and conidia until 40<sup>th</sup> day of the observation (tab. 4). Deformed hyphae of aerial mycelium and chlamydospores were formed by the majority of isolates on this medium (photo 6).

On oat agar, the colonies of the studied isolates were white and with time they became slightly grey with a delicate floccose aerial mycelium (photo 4c). The reverse of the colony was dark olive. The formation of a single pycnidia was observed just after 6<sup>th</sup> day of the cultivation on this medium and after 10 days cream exudate of conidia flowed from the pycnidia.

On the malt medium and malt medium with decoction of the leaves and schizocarps of caraway the colonies of *S. carvi* had a gray or black air mycelium of compact structure, and a reverse side (photo 4d, e, f). From the above mentioned mediums, the formation of pycnidia was observed the earliest, i.e. after 4<sup>th</sup> day of cultivation on the malt medium with decoction of leaves and on the malt medium with decoction of schizocarps of caraway (tab. 4). The pycnidia were small on the malt medium, and the formation of conidia was found only between 10<sup>th</sup> and 14<sup>th</sup> days of the cultivation (tab. 4). Large – up

Table 4. Effect of culture medium on pycnidia and conidia production by *S. carvi*  
 Tabela 4. Oddziaływanie podłoży hodowlanych na tworzenie pikniów i konidiów *S. carvi*

Isolate Izolat	Medium – Pożywka				
		PDA	Czapek-Dox	Oat agar Owsiana	Malt agar Maltozowa
K 1806	pycnidia – after 6 days conidia and chlamydospores – after 10 days	pycnidia – after 6 days conidia and chlamydospores – after 10 days	pycnidia – after 6 days conidia – after 10 days spores discharge – after 14 days	pycnidia – after 6 days conidia and chlamydospores – after 10 days	pycnidia and conidia – after 6 days conidia – after 10 days spores discharge and chlamydospores – after 10 days
K 1813	pycnidia and chlamydospores – after 10 days	pycnidia and conidia – absent	pycnidia and conidia – chlamydospores after 10 days	pycnidia and conidia – spores discharge – after 10 days	pycnidia and conidia – after 6 days conidia – after 14 days chlamydospores – after 10 days
K 1833	pycnidia and conidia – chlamydospores – after 10 days	pycnidia and chlamydospores – after 10 days	pycnidia and conidia – chlamydospores – after 10 days	pycnidia and conidia – after 12 days	pycnidia and conidia – after 6 days conidia – absent
K 1860	pycnidia and conidia – absent	pycnidia and conidia – absent	pycnidia and conidia – chlamydospores – after 10 days	pycnidia and conidia – spores discharge – after 12 days	pycnidia and conidia – after 6 days conidia – after 14 days chlamydospores – after 10 days
K 1861	pycnidia and conidia – conidia – after 10 days	pycnidia and conidia – chlamydospores – after 10 days	pycnidia and conidia – conidia – after 10 days	pycnidia and conidia – after 10 days conidia – after 14 days	pycnidia and conidia – after 6 days conidia – after 10 days spores discharge – after 10 days
K 1863	pycnidia – after 8 days conidia – after 10 days	pycnidia and conidia – absent	pycnidia and conidia – chlamydospores – after 10 days	pycnidia and conidia – spores discharge – after 10 days	pycnidia and conidia – after 6 days conidia – after 8 days spores discharge – after 10 days

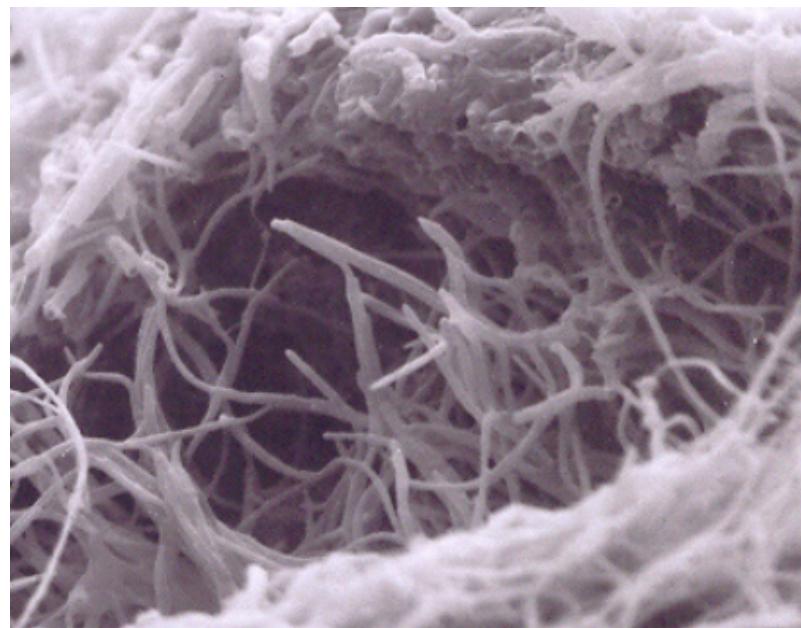


Photo 5. Ostiole of pycnidium and conidia of *S. carvi* K 1833 SEM (photo. M. Wróbel)  
Fot. 5. Ujście piknidium i konidia *S. carvi* K 1833 SEM (wyk. M. Wróbel)

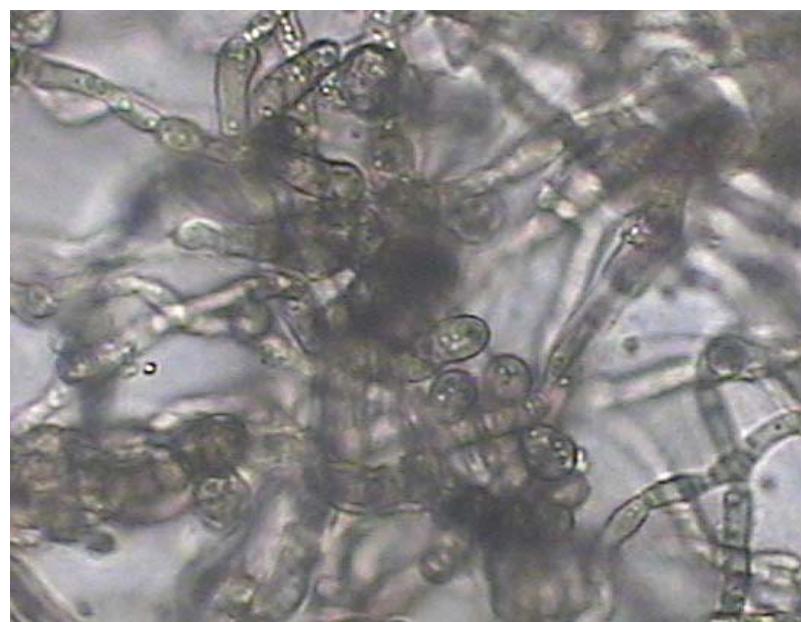


Photo 6. Chlamydospores of *S. carvi* K 1860 on Czapek-Dox  $\times 500$  (photo. E. Zalewska)  
Fot. 6. Chlamydospory *S. carvi* K 1860 na pożywce Czapek-Dox  $\times 500$  (wyk. E. Zalewska)

Table 5. The size(μm) of *S. carvi* conidia on the culture mediums (means for 6 isolates)Tabela 5. Wymiary konidiów (μm) *S. carvi* na badanych podłożach hodowlanych (średnie dla 6 izolatów)

Author Autor	Plant material Materiał roślinny	Culture medium – Podłoże hodowlane					
		PDA	Czapek-Dox	Oat medium Owsiana	Malt agar Malto- zowa	Malt agar with decoction from caraway schizocarps Maltozowa z odwarem z rozłupkiem kminku zwyczajnego	Malt agar with decoction from caraway leaves Maltozowa z odwarem z liści kminku zwyczajnego
Own measurements Pomiary własne		19.0–51.8 × 1.85–3.7	19.1–57.35 × 1.85–3.7	13.37–20.35 × 1.85	19.1–89.77 × 0.9–3.82	19.1–76.4 × 0.9	24.83–78.31 × 0.9
Zalewska 2008		19.0–51.8 × 1.85–3.7	20.35–57.35 × 1.85–3.7	-	-	-	-
Sydow 1932		18.0–45.0 × 0.8–1.3	-	-	-	-	-
Pidopličko 1978		2.0–10.6 × 1.5–2.0	-	-	-	-	-
Bedlan 2005		10.0–18.0 × 0.8–1.0 (12.5 × 1.0)	-	-	-	-	-

to 1.0 mm in diameter, black and shiny pycnidia gathered in aggregates on all the colony surface were observed on the malt medium with decoction of the leaves and decoction of schizocarps of caraway, and the formation of aggregates were observed after 8 days of the cultivation (photo 4e). Moreover, all the tested isolates formed numerous chlamydospores on the two above mentioned mediums.

The measurements of *S. carvi* conidia showed their large differentiation in size on the studied culture mediums. The conidia were simple, hyaline, tapering slightly towards the their base with slightly visual granules and pseudosepta. The conidia were short, and their size was 13.37–20.35 × 1.85 μm on Czapek-Dox medium. The size of spores of the studied isolates ranged from 19.1–57.35 × 1.85–3.7 μm on PDA, from 19.1–89.77 × 0.9–3.82 μm on oat agar medium, from 19.1–76.4 × 0.9 μm on malt medium, from 22.92–95.5 × 0.9 μm on malt medium with a decoction of the leaves and from 24.38–78.31 × 0.9 μm on malt medium with a decoction of caraway schizocarps.

## DISCUSSION

The conducted studies showed that the culture conditions significantly influenced the appearance of colonies, sporulation time, and even the size of the conidia of *Septo-*

*ria carvi*. Similar relationships were observed for *Monilia coryli*, *Topospora myrtilli*, *Phomopsis viticola* and other fungi [Zalewska 2001, Szmagara and Zalewska 2008, Kuropatwa 1993]. Macroscopic and microscopic features of fungi are often associated with the temperature and the composition of the culture medium. The present study showed that the optimal temperature for the growth and sporulation of the studied isolates of *S. carvi* was 25°C. The character and rate of growth of *S. carvi* colonies as well as the abundance of sporulation and the rate of spore release of pycnidia confirmed that. The obtained data correspond with the previous results of studies conducted by Marcinkowska [1977] and Błaszkowski [1990] in relation to *S. lycopersici* and *S. nodorum*, since the authors recognized the temperatures 22–25°C and 24°C, respectively, as appropriate for the linear growth and sporulation of the above mentioned fungi. Basing on the current studies, 0–5°C was recognized as a minimum temperature for the growth of *S. carvi* and 30°C as the maximum. These values are similar to the results found by Marcinkowska [1977]. Perhaps the ability of the mycelium of *S. carvi* to a marginal increase at a low temperature indicates the possibility of survival of the pathogen on the lower parts of the wintering caraway. This assumption is confirmed by frequent isolation of the fungus from the two-year-old plants after overwintering [Machowicz-Stefaniak and Zalewska 2004, 2008].

Studies on the effect of type of culture medium on growth and sporulation of *S. carvi*, at the temperature of 25°C showed, that the PDA, malt medium and malt medium with a decoction of caraway leaves are the most appropriate mediums for the growth of the studied isolates colonies. The growth of the fungus colony was most intense on these mediums. The malt medium with a decoction of caraway schizocarps and oat agar medium, despite a favorable effect on the spores, was considered as less suitable for the cultivation of *S. carvi* for diagnostic purposes, due to the unusual growth and appearance of fungus colony. The great usefulness of glucose-potato mediums for the growth of various species of fungi was indicated by many authors [Błaszkowski 1990, Kwaśna et al. 1991, Gabler and Ehrig 2000, Zalewska 2001, Machowicz-Stefaniak and Zalewska 2004, 2008, Machowicz-Stefaniak et al. 2008, Szmagara and Zalewska 2008]. Moreover, glucose-potato mediums are used to identify many other fungi [Nelson et al. 1983, Zalewska and Machowicz-Stefaniak 2000, Zalewska 2001, Marcinkowska 2003], on this medium the fungi produce all the macro- and microscopic features specific for the species.

Malt medium with a decoction of caraway leaves and with a decoction of caraway schizocarps, PDA, oat agar medium and malt medium were recognized as the most suitable for the sporulation of *S. carvi*. These culture mediums in combination with the temperature 25°C favored the extremely rapid sporulation and secretion of conidia exudate from pycnidia. Czapek-Dox medium proved to be unfavorable for the production of pycnidia and conidia of *S. carvi*. Mediums with an aqueous decoction of the host plant appear to be both proper and suitable not only for the cultivation and sporulation of fungi, but also to isolate them from the plant material [Zimowska 2002]. Current results of studies are consistent with the results of Marcinkowska [1977], who considered the tomato medium containing an extract from the leaves and roots of this plant as the most suitable for the growth and production rates of pycnidia and conidia of *S. lycopersici*. The knowledge of the mediums for the production of abundant sporulation by

the fungus has great importance in studies on the diagnostic and pathogenicity of the species, which need a large number of infectious material [Marcinkowska 2003].

Results of the present study confirm the existence of significant discrepancies in the dimensions of conidia produced directly on the plants and the culture media, as noted earlier by Zalewska [2008]. Similarly, the length of the spores of *S. carvi* from plant material reported by Sydow [1932], Pidopličko [1978] and Bedlan [2005] differ from the length of the conidia from the culture media used in the present study. Therefore, for diagnostic purposes, studies should take into account the appearance of colonies and microscopic features of the fungus not only from plants but mainly from the respective culture media.

It seems that the population of *S. carvi* from caraway is differentiated morphologically as indicated by the ability of some isolates to color the glucose-potato substrate pink and by the varied rate of sporulation of isolates. This phenomenon also occurs in other species of fungi genera like *Phoma*, *Fusarium*, and *Seimatosporium*. This demonstrates the secretion of secondary metabolites by fungi that are involved in the pathogenesis of necrosis and decay of plant tissues [Kwaśna et al. 1991, Boerema et al. 2004, Zimowska and Machowicz-Stefaniak 2005]. However, these problems, particularly in relation to the species of the genus *Septoria* is not exactly known and require further investigation.

On the basis of certain current thermal requirements, *S. carvi* should be regarded as a thermophilic species, which explains its epidemic occurrence in hot and humid growing seasons [Zalewska and Machowicz-Stefaniak 2000, Machowicz-Stefaniak and Zalewska 2008].

## CONCLUSIONS

1. Culture conditions significantly modify the macroscopic and microscopic features of *S. carvi*.
2. The growth of *S. carvi* colonies is possible at the temperatures from 0 to 30°C, and sporulation from 10 to 30°C. The temperature of 25°C is optimal both for the growth of fungus colony and sporulation.
3. It is suggested that standard media optimal for the growth and sporulation of the fungus should be introduced for the identification of *S. carvi*. PDA and malt medium with a decoction of leaves of caraway, combined with the culture temperature of 25°C can be recommended for such a medium.
4. The determined thermal requirements of *S. carvi* and previous observations of plants' healthiness on caraway plantations indicate that the massive presence of caraway septoriose in Poland is possible in hot and humid growing seasons.

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## **WZROST I ZARODNIKOWANIE *Septoria carvi* Syd. W RÓŻNYCH WARUNKACH HODOWLI**

**Streszczenie.** Wielkość i jakość surowca zielarskiego obniżają grzyby patogeniczne. W przypadku kminku zwyczajnego takim patogenem jest *Septoria carvi*. W okresach wegetacji grzyb występuje ze zmiennym nasileniem, co jest związane z jego wymaganiami życiowymi. W obecnych badaniach ustalono, że wzrost kolonii grzyba jest możliwy w temperaturze od 0 do 30°C, przy optimum od 20 do 25°C, a zarodnikowanie w temperaturze od 10 do 30°C, przy optimum w 25°C. Wykazano, że najbardziej przydatnym podłożem do izolacji *S. carvi* z roślin kminku zwyczajnego jest pożywka maltozowa oraz pożywka maltozowa z odwarem z liści tej rośliny. Piknidia i zarodniki konidialne grzyba, niezbędne do identyfikacji gatunku, tworzą się najszybciej na pożywkach maltozowej z odwarem z liści lub z rozlupek kminku zwyczajnego oraz na pożywce PDA i podłoża te należy stosować do hodowli *S. carvi*.

**Slowa kluczowe:** podłoża, temperatura, kminek, septorioza

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