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Occurrence and Characterization of *Colletotrichum dematium* (Fr.) Grove

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Abstract

Colletotrichum dematium was isolated from caraway for the first time in Poland in 2005. Isolations of this fungus were repeated in 2006 and 2007. The cultures of fungus were obtained from superficially disinfected leaves, root necks, roots, stems and umbels. The isolates were identified on culture media: PDA and malt agar with addition of pieces of caraway stems and on the base of macro and microscopic structures. Studies on the biotic effect between *C. dematium* and other species of phyllosphere fungi of caraway showed that the majority of the studied species limited the growth and development of *C. dematium*, but the size of the limiting effect was different. The species from *Trichoderma* and *Gliocladium* genera were the most effective against *C. dematium*, causing degeneration and lysis of hyphae and precluded the formation of the pathogen's acervuli and conidia. *C. dematium* in dual culture with *E. purpurascens*, *A. radicina*, *S. sclerotiorum*, *B. cinerea* and *R. solani* produced an inhibition zone which indicated its capacity for antibiosis.

Key words: *Colletotrichum dematium*, biotic activity, caraway isolates, phylosphere fungi

Introduction

Fungi from the genus *Colletotrichum* occur in all climatic zones and cause diseases of various plant species, especially in hot and moderate climates. They can be polyphagic but some of them are pathogens of only one species of host plant (Frencel *et al.*, 1997; Sutton, 1980). Till the middle of the 20th century, over 1000 species of these fungi were described basing on the morphological structures of conidia, setoses and host plant (Von Arx, 1957). As a result of studying these fungi on artificial culture media in the laboratory, the number of species was reduced to 40 (Sutton, 1980). At present, the numerous existing species of the genus *Colletotrichum* are gathered in one common species on the basis of genetic diversity (Frencel *et al.*, 1997). The following species belong to the commonly occurring pathogenic species: *C. gloeosporioides* (Penz.) Sacc. (teleomorph: *Glomerella cingulata* (Stonem.) Spauld. *et* Schrenk), *C. lindemuthianum* (Sacc. *et* Magn.) Br. *et* Cav., *C. atramentarium* (Berk. *et* Br.) Taubenh., *C. acutatum* Simmonds, *C. lini* (Manns) Bolley, *C. orbiculare* (Berk. *et* Mont.) Arx and others (Sutton, 1980; Farr *et al.*, 1995; Frencel *et al.*, 1997; Gärber and Schenk, 2001).

C. dematium, the genus typical species, is a saprotroph and colonizes various plant species as a secondary pathogen and pathogenic strains of this fungus cause plant diseases (Von Arx, 1957; Sutton, 1980; Farr *et al.*, 1995). *C. dematium* f. *circinans* cause anthracnose of onion cultivated in various climatic regions in the world (Sutton, 1980). The occurrence of *C. dematium* was ascertained in India on various pea-plant cultivars and in South Africa on stems and pods of cowpea (Smith *et al.*, 1999; Shinde *et al.*, 2003). The species was isolated from dying plants of *Catharanthus roseus* (L.) G. Don in Florida in 1991 and positive results of artificial infection of plants were obtained (McMillan and Graves, 1996). *C. dematium* caused anthracnose of spinach on some farms in Australia and the harmfulness of this fungus for various spinach cultivars was confirmed by pathogenicity tests (Washington *et al.*, 2006). In Japan, the harmfulness of *C. dematium* for Japanese radish was confirmed by pathogenicity tests (Smith *et al.*, 1999). Anthracnose caused by *C. dematium* was discovered on various cultivars of strawberry in India, which fact was confirmed by pathogenicity tests (Singh *et al.*, 2003). *C. dematium* occurred on mulberry in Japan and on various species of plants from the *Amaryllidaceae* family (Yoshida and Shirata, 1999; Bonilla-Bernal *et al.*, 2003). In post-culture liquids of *C. dematium* f. sp. *epilobii*, the pathogenic species for willow herb (*Epilobium angustifolium* L.), the presence of

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secondary metabolites with phytotoxic and zootoxic abilities was found (Abou-Zaid *et al.*, 1997; Mendiratta *et al.*, 2005).

As a result of many-years' studies conducted on diseases of herb plants *C. dematium* was isolated for the first time on caraway in Poland in 2005. Therefore, attention to the occurrence of this fungus in the next years of studies was directed and macroscopic and microscopic features of isolates of the pathogen and the biotic effects between *C. dematium* and other species of phyllosphere fungi of caraway were studied.

Table I
Occurrence of *C. dematium* on aboveground organs of caraway (*Carum carvi* L.) in 2005–2007

Organs \ Years	Participation of izolates		
	2005	2006	2007
leavs	++	++	++
stems	++	+	+++
base of stems	+++	+	–
roots	+++	++	++
umbels	+++	++	+++

\+ – frequency of occurrence <5%
++ – frequency of occurrence from 5 to 10%
+++ – frequency of occurrence >10%

Material and Methods

The studied material consisted of isolates of *Colletotrichum dematium* (Fr.) Grove obtained from the organs of one-year-old and two-years-old plants of caraway cultivated in the Lublin region in the years 2005–2007 (Table I). The artificial culture method and malt agar medium were used for the isolation of this fungus (Machowicz-Stefaniak and Zalewska, 2008). One- spore cultures of 15 isolates of fungus: K117, K123, K425, K426, K510, K514, K612, K625, K626, K 628, K630, K631, K633, K651, K657 were chosen randomly from our professional collection. For identification, the isolates were cultured on malt agar medium with an addition 50 g/dm 3 of 2–3 mm pieces of caraway stems and on PDA medium (Difco), in a thermostat, at the temperature 24°C, in dark conditions for 14 days (Machowicz-Stefaniak, 2009). The character of cultures, the color of the averse and the reverse, the formation and morphology of fungus acevuli and conidia were studied at this time. To determine the structures mentioned above, the measurements of 150 acervuli (15 isolates per 10 acervuli) and 600 conidia (15 isolates per 40 spores) were made. Moreover, the size of setoses and appressoria of the studied isolates was determined. The photos of the above mentioned morphological elements were taken using light and scanning – SEM microscope. To identify the studied isolates, the descriptions of Von Arx (1957), Pidopličko (1977) and Sutton (1980) were used.

To study the biotic effects of fungi, 3 isolates of *C. dematium*: K425, K426, K625 and 22 isolates of fungi species mentioned in Table III were taken. Those isolates were chosen randomly from our professional collection of fungi gathered in the years 2001–2008, as a result of a study on diseases of caraway (Machowicz-Stefaniak and Zalewska, 2004; 2008; Machowicz-Stefaniak, 2009).

Because of the lack of information concerning the biotic relation between *C. dematium* and other fungi, the maximum number of fungal species was taken for this study, irrespective of the frequency of their isolation from caraway (Machowicz-Stefaniak and Zalewska, 2004; 2008; Machowicz-Stefaniak, 2009). The species *Gliocladium catenulatum*, *G. fimbriatum*, *G. roseum* and *Trichoderma viride* were taken from other cultivated plants, because they were not isolated from caraway.

The study of biotic effects was carried out using the biotic series method on PDA (Difco) medium, which was elaborated for soil fungi community firstly (Mańka, 1974; Mańka, 1995). This method was adapted for fungi colonizing the phyllosphere of plants (Mańka, 1995; Machowicz-Stefaniak, 1998; Król and Machowicz-Stefaniak, 2008). The two-organism cultures consisting of *C. dematium* and one of the fungi representing the community component were studied on PDA medium in Petri dishes, according to the method by Mańka (1974) and Mańka (1995). The dishes with medium on which the mycelium of a single fungal species was placed constituted the control. For each combination, *i.e.* for *C. dematium* with a species of fungus representing the community component and control, 4 replications were made. The biotic effect of the fungi in dual cultures was evaluated after 12 days of common growth, but in the case of *Gliocladium* spp. after 24 days, at 23°C, in dispersion light, based on an eight-degree scale. One colony being surrounded by other species, the occurrence of the inhibition zone between them and the reduction of the colony size were taken into account while the IBEs were evaluated (Mańka, 1995). If the colony of *C. dematium* was overgrown by other species of fungi, the appearance of mycelium and conidia of the studied fungus were evaluated. The biotic effect of fungi representing the phyllosphere of caraway on *C. dematium* was estimated as an individual biotic effect (IBE). The size of IBE consisted of the arithmetic sum of values for surrounding of the colony, the inhibition zone and the reduction of colony size. The size of IBE indicates the effect of one isolate of the community species on pathogen growth (Mańka, 1974). Positive IBE indicates suppressive effect on pathogen growth, negative – indi-

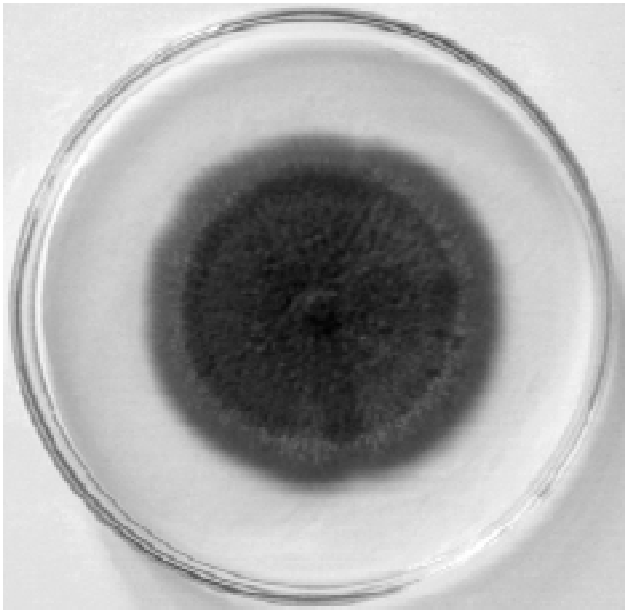

Fig. 1. 14-day-old colony of *C. dematium* isolate K 425 on the PDA medium, at 25°C (Photo E. Zalewska).

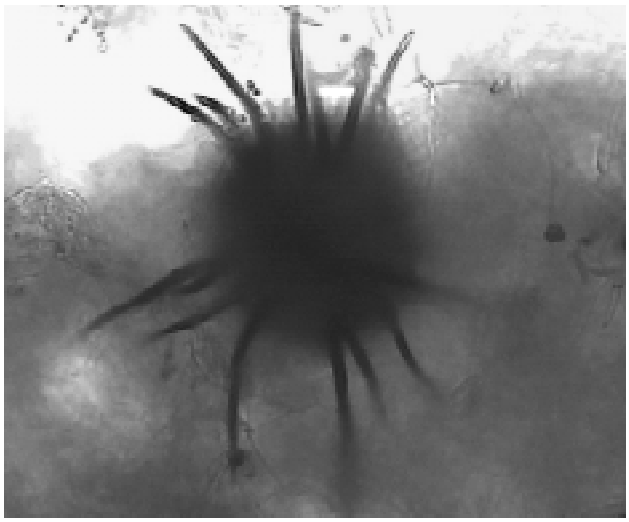

Fig. 2. Acervuli and setose of *C. dematium* in light photo microscope (Photo E. Zalewska).

cate slack of suppressive effect on pathogen growth, while the value of the effect may be „0" indicating neutral influence (Mańka, 1974; Mańka, 1995).

Results

The isolates of *C. dematium* were obtained for the first time in 2005 from the leaves, the neck of roots and the roots of 0.66% of caraway seedlings. The isolations of fungi repeated in 2006 and 2007, respectively from 1.2% and 2.16% of plants in the second year of cultivation. The cultures of fungus were obtained most often from the stems, the umbels and the roots with different frequency in different years (Table I). *C. dematium* was isolated from the parts of plants with nonspecific etiological and disease lesions, which can indicate the presence of the pathogen in the plant tissues. On the other hand, among the fungi isolated from plants on malt agar medium there were isolates coloring the medium violet and forming dark gray, velvet mycelium with numerous brown appressoria an the top and crossing hyphae, the organs characteristic of *Colletotrichum* genera. However, acervuli and spores of fungi were obtained scarcely on malt agar medium with an addition of pieces of caraway stems and on PDA medium.

The one-spore-cultures of this fungus chosen for the subsequent studies were cultivated on the above mentioned two artificial media for 14 days with their size ranging from 3.5 to 5.5 cm. The colonies were loose and slightly fluffy in the beginning but later the hyphae became more and more thickened and formed a compact surface. The colour of the mycelium was gray or dark gray. The reverse of the colonies was pink to violet on PDA medium with a visible diffusion of the dye to the culture medium (Fig. 1). On the malt agar medium the reverse was beige-yellow. At the beginning of day 6 of cultivation, acervuli were formed on PDA medium and after 10 days on malt agar medium with an addition of caraway stems pieces. Numerous acervuli were observed after 14 days of the cultivation, and covered the entire surface of the colony but sometimes they were formed in sectors. Acervuli were slightly immersed in the medium, almost black, lenticular, flat or pulvinate with sufficiently visible and high rends. The diameter of acervuli was 495.44×371.48 µm (Table II). All around the ostiole and on the surface of acervuli numerous setoses were formed. (Fig. 2, 3). The setoses were dark brown or almost black, generally septate (Fig. 2), unpliant, smooth, tapered to the apices, their size ranging from 36.05 to 202.99 µm in length and at the

Table II
Size (µm) of morphological structures of *C. dematium* on PDA medium (mean for 15 isolates)

Author	acervuli (µm)	setose (µm)	conidia (µm)	appressoria
Own measurements	495.44×371.48	36.05–202.99×3.83–5.74	17.19–24.83×3.82–5.72	7.66–15.28×5.74–13.37
Sutton 1980			19.5–24×2–2.5 (–3.5)	8–14.5×6.5–8
Pidopličko 1977	250	150×4	25×5	
Von Arx 1957		100–600	18–30×3–4.5	

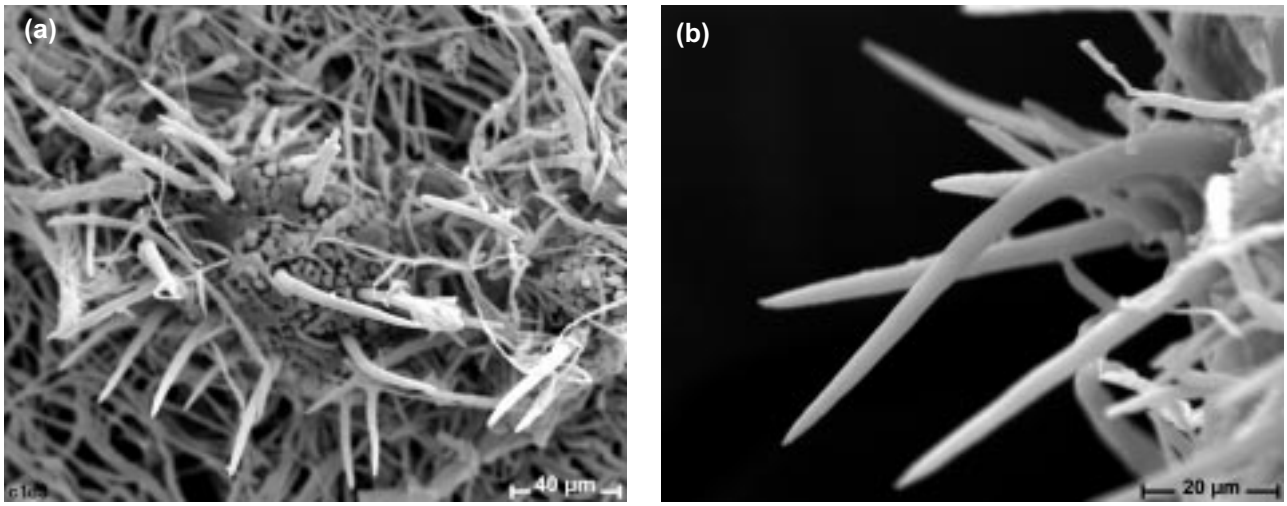

Fig. 3. Acervuli and setose of *C. dematium* (a), setose (b) in SEM (Photo M. Wróbel).

base from 3.83 to 5.74 µm in width (Table II). Thick, almost black, shining drops resulting from the large number of conidia were emerged from the mature acervuli. The conidia were hyaline, aseptate, smooth, falcate or fusiform, and they had acute apices (Fig. 4). A visible gutate breaking the light was observed in the middle of the conidia. The size of the studied isolates conidia was 17.19–24.83 × 3.2–5.72 µm on PDA, and 18.5–26.74 × 2.88–3.7 µm on malt agar with an addition of caraway stem pieces (Table II). At the end of hyphae or in the middle of them abundant appressoria occurred. Unlike the vegetative hyphae, they were brown or almost black, irregular in shape, single or gathered and their size was 7.66–15.28×5.74–13.37 µm (Fig. 5). The formation of sclerotia was not observed in the studied isolates.

Among the 22 tested species of phyllosphere fungi, the majority, *i.e.* 18 species limited the growth and development of two isolates of *Colletotrichum dematium* – K425 and K426, and 17 species of isolate K625,

Table III
Biotic effect of fungi isolated from caraway (*Carum carvi* L.) on *Colletotrichum dematium*

Fungal isolates	Individual biotic effect – IBE after 12 days *C. dematium* isolates		
	K 425	K 426	K 625
Alternaria alternata (Fr.) Keissler (K 461)	– 1	– 1	0
Alternaria radicina Meier, Drechsler et Eddy (K1723)	+ 1	+ 1	+ 1
Botrytis cinerea Pers. (K 1777)	+ 4	+ 3	+ 4
Cladosporium cladosporioides (Fres.) de Vries (K 518)	– 2	– 2	– 2
Epicoccum purpurascens Ehrenberg (K 1696)	+ 1	+ 1	– 2
Fusarium avenaceum (Fr.) Sacc. (K 56)	+ 4	+ 4	+ 4
Fusarium culmorum (W.G.Smith) Sacc. (K 284)	+ 5	+ 5	+ 4
Fusarium equiseti (Corda) Sacc. (K304)	+ 5	+ 4	+ 4
Fusarium oxysporum Schlecht (K 271)	+ 5	+ 5	+ 5
Fusarium sporotrichioides Sherb (K 465)	+ 7	+ 6	+ 7
Phoma exigua Desm. var *exigua* (K 1503)	+ 3	+ 3	+ 3
Phomopsis diachenii Sacc. (K 255)	+ 6	+ 6	+ 5
Rhizoctonia solani Kühn (K 1561)	+ 5	+ 5	+ 5
Septoria carvi Syd. (K 1833)	– 6	– 6	– 6
Sclerotinia sclerotiorum (Lib.) de Barry (K 2313)	+ 5	+ 5	+ 5
Stemphylium botryosum Wallv. (K 296)	– 4	– 3	– 3
Gliocladium catenulatum Gilman et Abbott (L 4940)	+ 2	+ 2	+ 2
Gliocladium fimbriatum Gilman et Abbott (W 76)	+ 2	+ 2	+ 2
Gliocladium roseum Bainier (L 830)	+ 2	+ 3	+ 3
Trichoderma harzianum Rifai (K 428)	+ 8	+ 8	+ 8
Trichioderma koningii Oud. (K 437)	+ 7	+ 7	+ 8
Trichoderma viride Pers. et Gray (W 1222)	+ 7	+ 7	+ 7

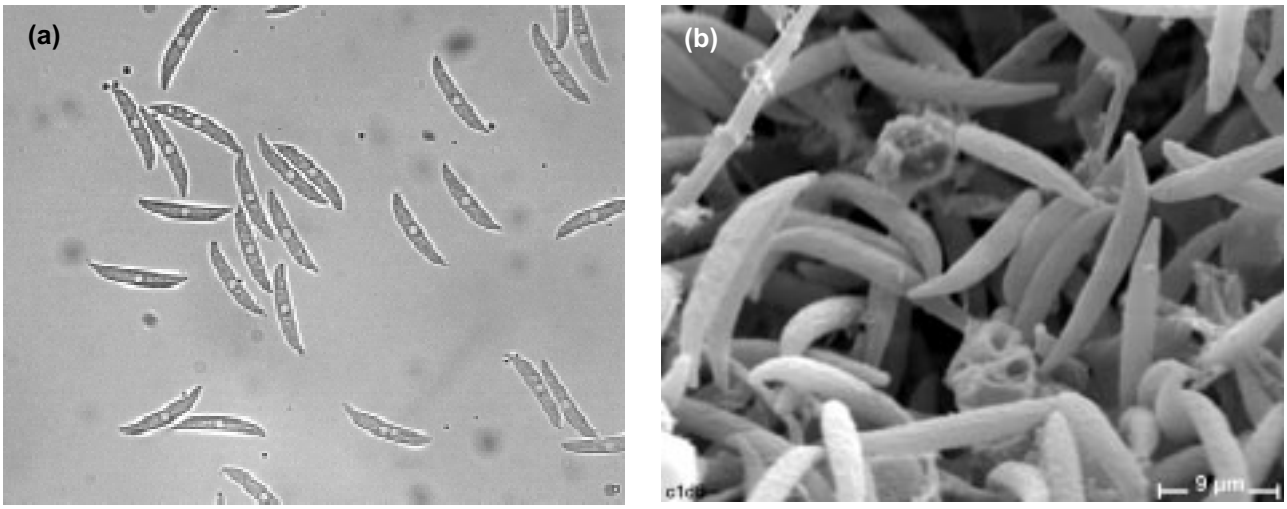

Fig. 4. Conidia of *C. dematium* in light photo microscope (magnification × 500) (a) (Photo E. Zalewska), SEM (b) (Photo M. Wróbel).

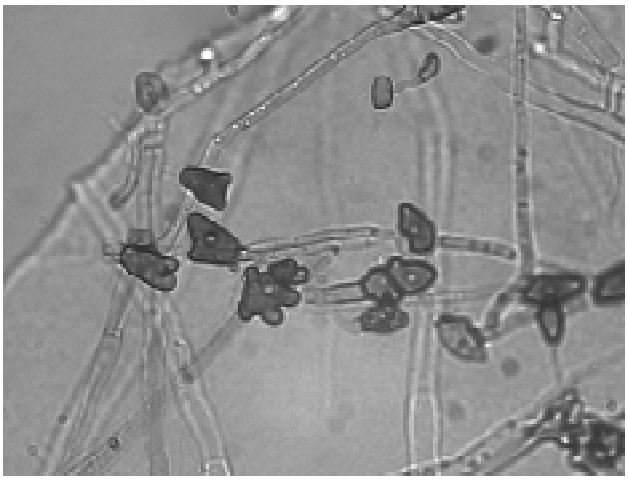

Fig. 5. Appressoria of *C. dematium* on PDA medium (magnification × 500) (Photo E. Zalewska).

which indicates the positive individual biotic effects observed after 12 days of dual growth (Table III).

The species of fungi from *Trichoderma* genus limited the growth of the studied isolates of *C. dematium* to the highest degree because their individual biotic effect was +8 for *T. harzianum* and +7 for *T. viride* and *T. koningii* (Table III, Fig. 6). The studied species from *Trichoderma* genus caused lyses and degeneration of *C. dematium* hyphae (Fig. 7). The observation showed that the colonies of each studied *Trichoderma* species totally overgrew the inoculums of *C. dematium* and made the growth and sporulation of the pathogen impossible. In the slides of 12-days-old colonies of *C. dematium* and *T. harzianum* and of *C. dematium* with *T. viride* the concentration and disintegration of cytoplasm in *C. dematium* hyphae were observed. Moreover, after 12 days of dual growth of the pathogen with *T. koningii* deformed hyphae of *C. dematium* with a big concentration of melanine were observed (Fig. 7).

The fungi from the genera *Gliocladium* slightly limited the growth of *C. dematium* in the first day of common growth. *G. catenulatum* and *G. fimbriatum* grew on 1/3 and *G. roseum* on 1 of the surface of the pathogen's colony after 12 days of dual growth (Fig. 8). In the slides chains of miss-shapen, dark hyphae of *C. dematium* and numerous hyphae of the pathogen being subject to lysis were observed (Fig. 9). Moreover, it was noticed that after 18 days of dual growth micoparasitic fungi practically overgrew the whole surface of the pathogen, *i.e.* 7/8 of the pathogen's colony and after 24 days the whole surface of *C. dematium* colony was overgrown by them causing total lyses of the pathogen»s hyphae.

The growth of *C. dematium* colony was strongly limited by *P. diachenii* as its IBE was +6 and in the case of isolate K625 IBE was +5 (Table III). Among fungi of the *Fusarium* genus the growth of the pathogen was limited more strongly by *F. sporotrichioides* species and its IBE was +7 (Table III). On the other hand, *F. avenaceum*, *F. culmorum*, *F. equiseti*, *F. oxysporum*, *Rhizoctonia solanii*, *Sclerotinia sclerotiorum* and *Botrytis cinerea* slightly limited the growth of *C. dematium* colony in comparison to *F. sporotrichioides* (Table III). Moreover, it was observed that *C. dematium* in dual growth with *S. sclerotiorum*, *B. cinerea* and *R. solani* formed an inhibition zone (Fig. 10).

The fungi *Alternaria radicina* and *Epicoccum purpurascens* inhibited the growth of *C. dematium* colonies in a small degree and their IBEs were +1. However, isolate K625 *C. dematium* limited the growth of *Epicoccum purpurascens*, which was shown by the negative IBE value (Table III). Moreover, the fungus *C. dematium* limited the growth of *Septoria carvi* giving IBE-6, *Cladosporium cladosporioides*-2, *Alternaria alternata*-1, *Epicoccum purpurascens* and *Stemphylium botryosum* from – 3 to – 4 (Table III).

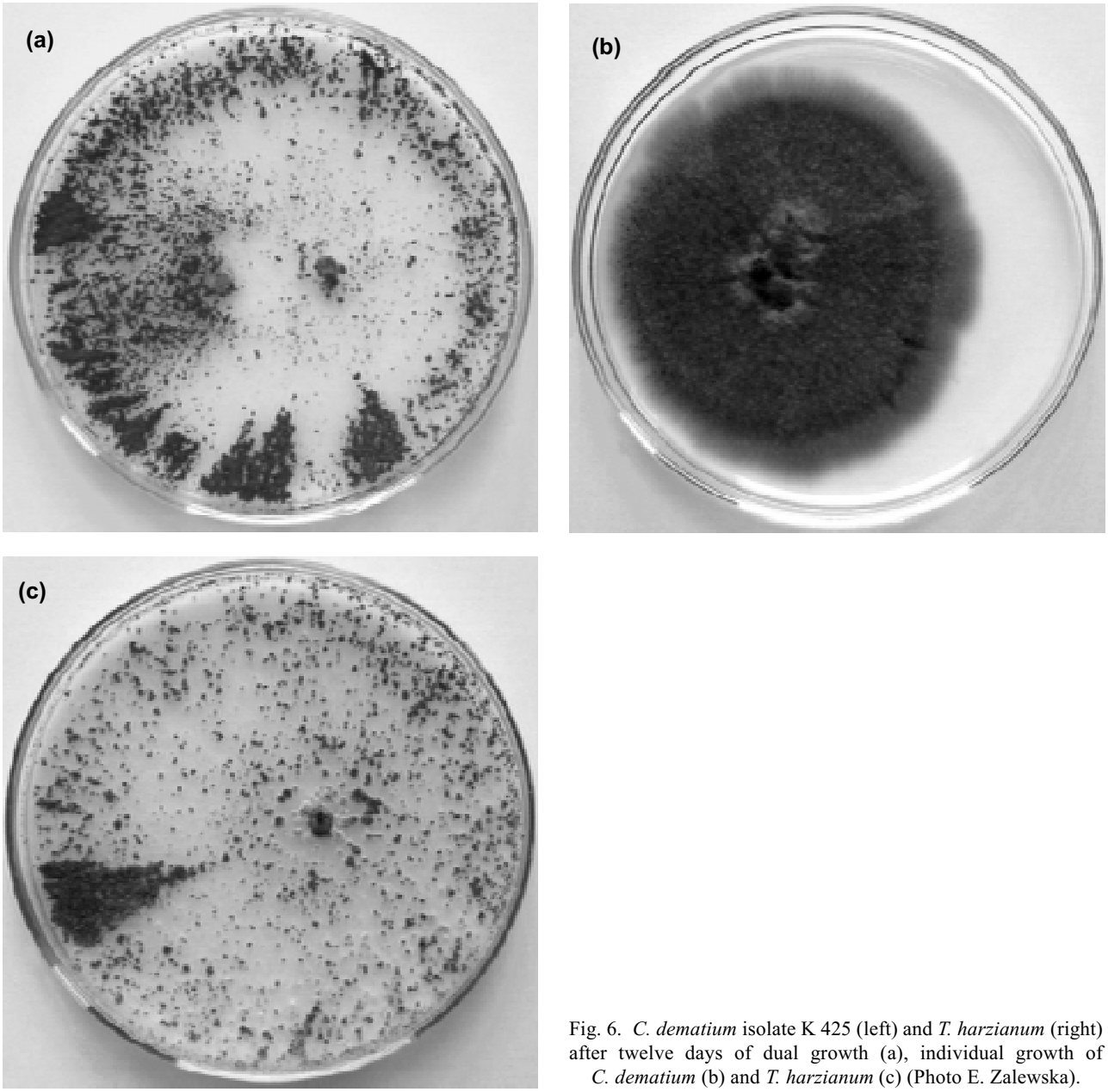

Fig. 6. *C. dematium* isolate K 425 (left) and *T. harzianum* (right) after twelve days of dual growth (a), individual growth of *C. dematium* (b) and *T. harzianum* (c) (Photo E. Zalewska).

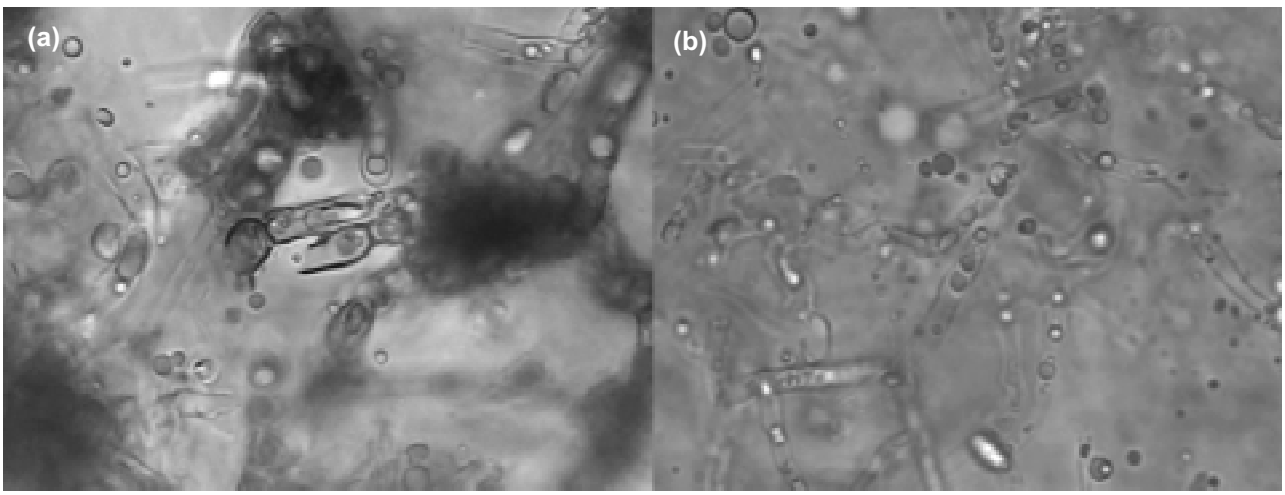

Fig. 7. Degeneration of *C. dematium* hyphae isolate K 425 caused by *T. harzianum* (magnification x 500) (Photo E. Zalewska).

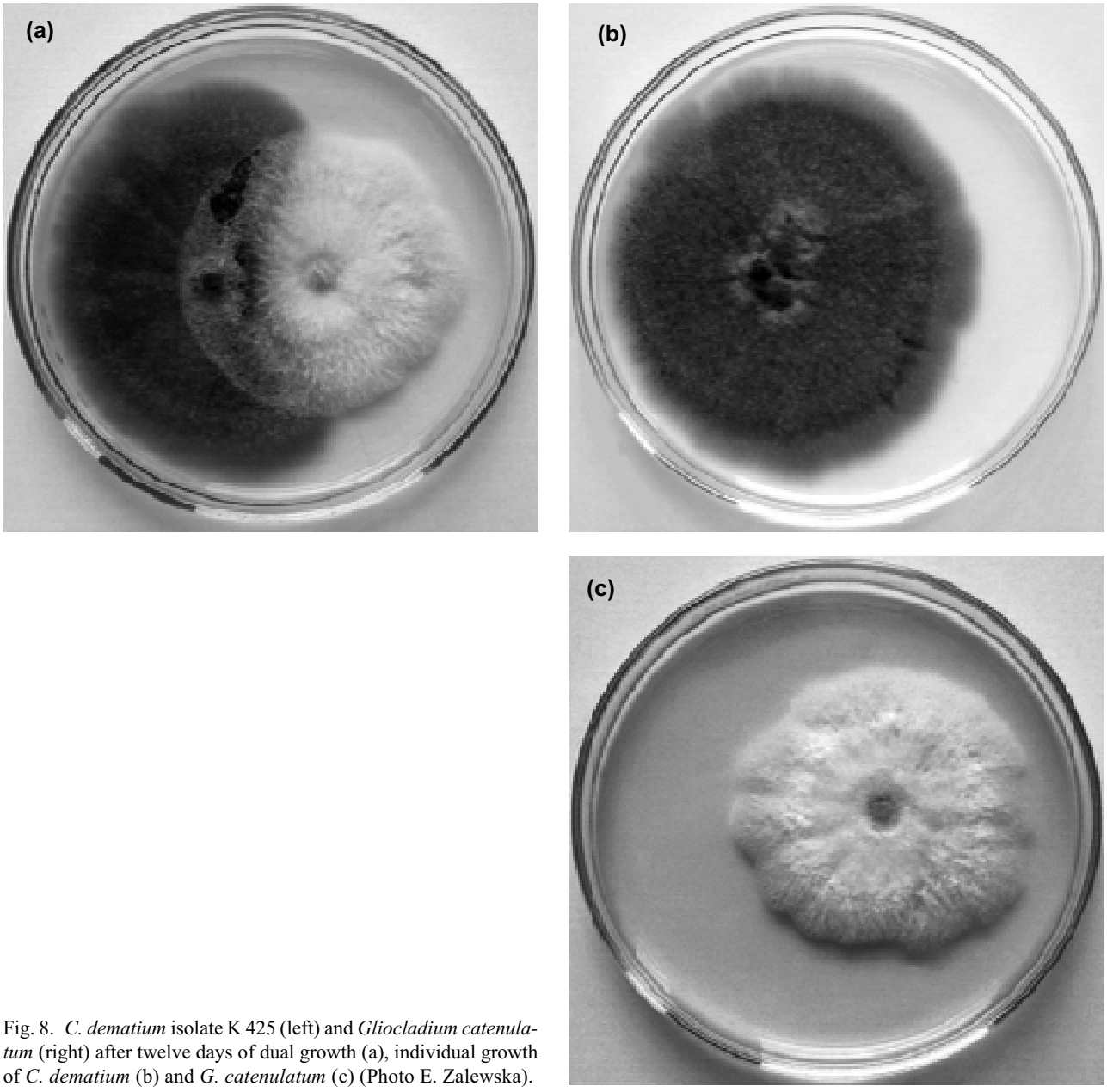

Fig. 8. *C. dematium* isolate K 425 (left) and *Gliocladium catenulatum* (right) after twelve days of dual growth (a), individual growth of *C. dematium* (b) and *G. catenulatum* (c) (Photo E. Zalewska).

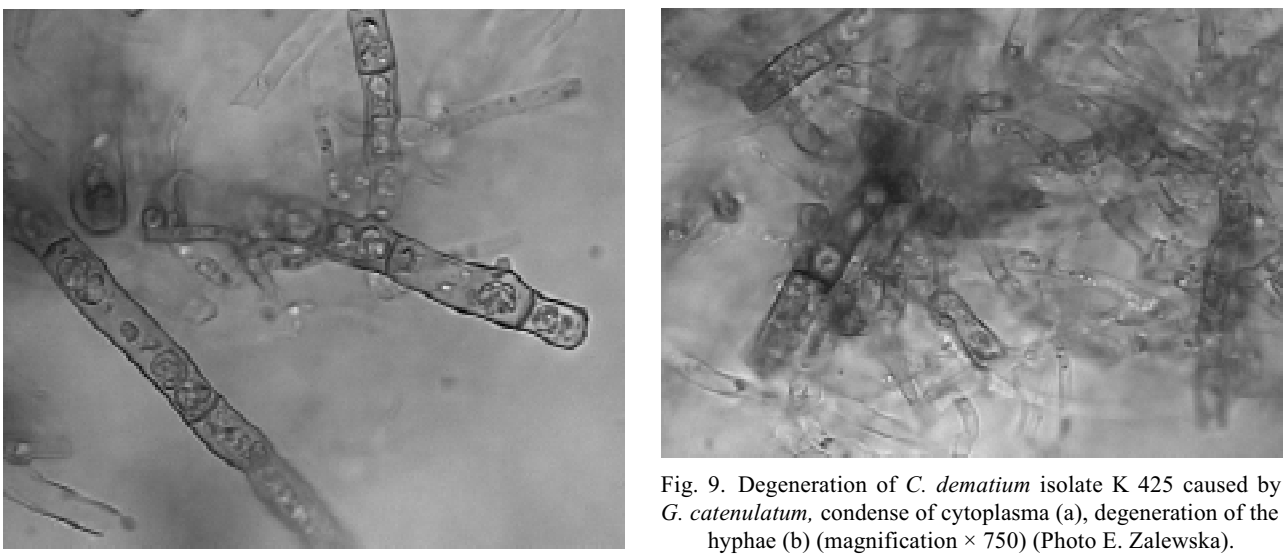

Fig. 9. Degeneration of *C. dematium* isolate K 425 caused by *G. catenulatum,* condense of cytoplasma (a), degeneration of the hyphae (b) (magnification × 750) (Photo E. Zalewska).

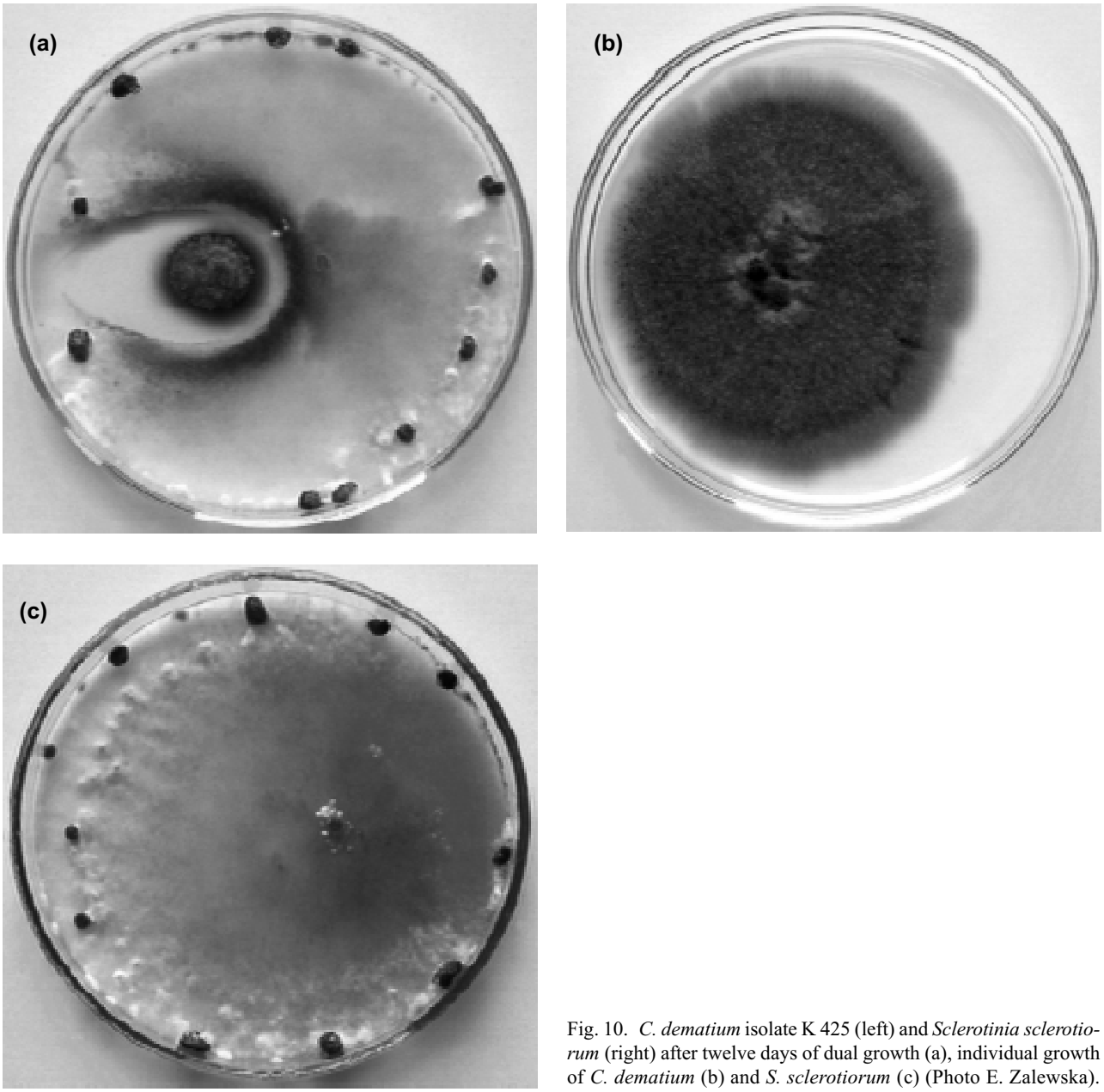

Fig. 10. *C. dematium* isolate K 425 (left) and *Sclerotinia sclerotiorum* (right) after twelve days of dual growth (a), individual growth of *C. dematium* (b) and *S. sclerotiorum* (c) (Photo E. Zalewska).

Discussion

Including the studied isolates in the species *Colletotrichum dematium* was possible on the basis of macroscopic and microscopic features of their colony and on the basis of morphology and size of acervuli and conidia. The above-mentioned features were compared to those shown by Von Arx (1957), Pidopličko (1977) and Sutton (1980). Small differences, especially in the size (length and weight) of conidia are a consequence of differentiation of culture medium on which the fungus grows. The dependence of the growth and development of numerous fungi species from the culture conditions and from the composition of culture medium was indicated by a lot of authors (Uecker 1988, Sutton 1980). The straight growth of studied isolates as well as the ability to produce the acervuli and conidia just after a few days old colonies on PDA medium suggested that medium is favorable to the culture and identification of *C. dematium*. The production of a numerous number of acervuli by *C. dematium* on PDA was indicated earlier by Azad *et al.* (2005).

The presence of a numerous and high setoses occurring all around the ostiole of acervuli and many brown appressoria should be recognized as a favorable and characteristic micromorphological feature causing the identification of the studied fungus easier, which was indicated earlier by Von Arx (1957). The latter organs, *i.e.* appressoria could be significant in pathogenesis because they could attach hyphae of the pathogen to the surface of plants (Von Arx, 1957). The

high tinctorial power of the culture medium characteristic of all the studied isolated of *C. dematium* points to the possibility of this fungus producing secondary metabolites.

The detection of *C. dematium* in Poland for the first time on caraway plants increased the number of host plant to this fungus. The present studies showed that a lot of caraway phyllosphere fungi limited the growth of *C. dematium* and the positive values of IBE indicate it, but the size of limiting possibilities was different. Among the fungi inhibiting the growth of *C. dematium* there were the species from the genera *Trichoderma* and *Gliocladium*. These fungi are known for their antagonistic influence against pathogenic fungi (Fokkema, 1993; Machowicz-Stefaniak, 1998; Król and Machowicz-Stefaniak, 2008). *T. harzianum*, *T. koningii* and *T. viride* should be recognized as the most effective antagonists for *C. dematium*, which was indicated by complete overgrowth and destruction of the pathogen colonies by these antagonists just after a few days of dual growth. Similarly, these special high antagonistic abilities of *Trichoderma* spp., were indicated earlier for other pathogens of caraway like *Septoria carvi* and *Phomopsis diachenii* (Machowicz-Stefaniak *et al.*, 2008; Machowicz-Stefaniak, 2009). It is probably possible thanks to the emission of constitutional enzymes and those produced as an effect of contact with the pathogen, as well as the ability to form toxic metabolites and the ability to mycoparasite (Fokkema, 1993). Thanks to these abilities, *Trichoderma* spp. are used in the production of biopreparates (Cohen *et al.*, 1996). A significantly slower antagonistic effect of *Gliocladium* spp., (unlike that of *Trichderma* spp.) towards *C. dematium* and other pathogenic fungi results from antibiosis and the ability to mycoparasite in the lack of competitive abilities (Fokkema, 1993; Machowicz-Stefaniak *et al.*, 2008; Machowicz-Stefaniak, 2009). Therefore, full antagonistic activity of *Gliocladium* spp. to *C. dematium* was not shown until 20 days *in vitro*. On the other hand, recently *Epicoccum purpurascens* have been recognized as a fungus strongly inhibiting the growth of different microorganisms thanks to the possibilities to produce siderophores, flavipine and Epicorazine B (Frederick *et al.*, 1981; Mallea *et al.*, 1991; Fokkema, 1993) In the present studies the fungus showed only slight possibilities of inhibiting the growth of *C. dematium*. On the other hand, the production of inhibition zone by one of the pathogen's studied isolates in a dual culture with *E. purpurascens* confirmed the ability of *C. dematium* to produce secondary metabolites, which were detected in ethyl-acetyl extract of culture liquids pathogenically forms of *C. dematium* (Abou-Zaid *et al.*, 1997, Mendirata *et al.*, 2005).

The inhibiting effect of *C. dematium* isolates to *A. alternata*, *C. cladosporioides*, *S. carvi* and *S. botryosum* also seems to confirm the ability of *C. dematium* for antibiose. Fast-growing phytopathogenical fungi possessing a big enzymatic ability and used in the present studies, *i.e. S. sclerotiorum*, *B. cinerea* and *R. solani* only partly limited the growth of *C. dematium* because the last mentioned species produced an inhibition zone during the common growth with each of the above mentioned species. The inhibition zone formed by *C. dematium* was not observed in dual growth with the species of genera *Fusarium*. The last mentioned species and especially *F. sporotrichioides* suppressed the colonies of *C. dematium* from the culture medium. It was probably possible thanks to the big power of their growth and the ability to produce secondary metabolites (Kiecana and Perkowski, 1998).

Taking into account the inhibiting effect of the studied phyllosphere fungi towards *C. dematium*, it seems that the species of *Trichoderma* and *Gliocladium* genera could be recognized as positive antagonistic fungi. In perspective, these species may be used in biological control of *C. dematium*. The other studied species of fungi, despite only partly limiting *C. dematium* growth, are dangerous pathogens of many cultivated plant and their occurrence in culture plant's phyllosphere is undesirable.

Comparing the results of the present studies with the results of similar studies conducted for other pathogens of caraway like *Septoria carvi* (Machowicz-Stefaniak *et al.*, 2008) and *Phomopsis diachenii* (Machowicz-Stefaniak, 2009) it is possible to notice that they have numerous antagonists among phyllosphere fungi. That is why their isolation from plant tissues on artificial media may be difficult. Among the three above-mentioned pathogenic fungi *S. carvi* showed the worst antagonistic activity (Machowicz-Stefaniak *et al.*, 2008). It seems that isolates of *P. diachenii* have better competitive abilities than *C. dematium* (Machowicz-Stefaniak, 2009). On the other hand, *C. dematium* have high abilities for antibiosis. On the basis of the present results and the data in literature we can suggest the need to study the secondary metabolites of fungi not only in the aspect of their phytotoxicy and zootoxicy but also in the direction of knowing about their mechanisms of antibiosis. It is interesting if it results from secretion of antibiotic, lytic enzymes or from excessive acidification or alkalization of the medium (Fokkema, 1993).

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