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OCCURRENCE, HARMFULNEES AND MORPHOLOGICAL STRUCTURES OF *Colletotrichum gloeosporioides* (Penz.) Sacc. (*TELEOMORPH: Glomerella cingulata* (Stonem.) Spauld. et Schrenk)

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Abstract. The occurrence of plant diseases is determined by the interaction of three factors – pathogen, susceptible host plant and environment. *C. gloeosporioides* is a polyphagous species occurring in different geographical regions of the world. The present work shows results from 10 years of studies concerning the occurrence *C. gloeosporioides* on various crops including herbs (spices) and medicinal plants in South-East Poland. The presence of the pathogen causing plant anthracnose was detected on the above-ground parts of angelica *Archangelica officinalis* Hoffm, thyme *Thymus vulgaris* L., caraway *Carum carvi* L. and elder *Sambucus nigra* L. However, the epidemic occurrence of this fungus was recorded on ornamental lupine plants in the years 1999–2001 as well as on angelica plants and elder umbels in 2010, i.e. during the vegetative periods with temperatures above 20^{o}C and frequent rainfalls. In these conditions the intensive sporulation of pathogen during the necrotrophic phase of growth was the essential diagnostic factor. Mineral and malt extract culture media were convenient for the isolation of fungus while PDA-Difco medium for identification.

Key words: anthracnose, symptomathology, characteristics of fungus population, host plants

INTRODUCTION

Within fungi from genus *Colletotrichum*, family *Coelomycetes*, order *Melanconiales*, *Colletotrichum gloeosporioides* is the most widespread species on many host plants [Sutton 1980, Borecki 1990, Farr et al. 1995, Marcinkowska 2003]. Among other things *C. gloeosporioides* causes anthracnose of stone and berry fruits and is one of the pathogens causing apple bitter rot [Borecki 1990]. The fungus is known all over the world as a causal agent of field lupines crops anthracnose [Mills et al. 1992, Sutton 1992, Farr et

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al. 1995]. This disease did not appear in Europe until 1982 [Korneichuk 1998, Fiedorow et al. 2001] and in Poland until 1995 [Fiedorow et al. 2001]. It was recorded that within the Polish population of the fungus there are one of two vegetative compatibility groups commonly known around the world, i.e. VCG-2 [Pieczul and Rataj-Guranowska 2004]. Application of molecular techniques including RAPD, RFLP and the analysis of ITS region of DNA indicated a big homology of *C. gloeosporioides* isolates from the same host plant and country of origin [Mills et al. 1992, Medeiros et al. 2010].

There is little information about occurrence of anthracnose on ornamental lupine in literature. Disease was identified on ornamental lupines in some municipal gardens in Australia. The disease occurred commonly in 1996 on plants which probably were grown from seeds imported from Europe [Lindbeck et al. 1998]. In Poland this disease was observed for the first time in 1995 on individual plants of white ornamental lupine *Lupinus hartwegii* L. and marsh lupine *Lupinus polyphyllus* Lindl. Since that time anthracnose has been recorded with increasing intensity causing serious loss in the cultivation of ornamental lupine for seeds [Błaziak no press].

C. gloeosporioides is a pathogen of the aboveground parts of herbs which are the source of biologically active substances as well as mineral compositions [Zawiślak and Dzida 2010, Biesiada and Kuś 2010]. In Germany it was recognized as the causal agent of anthracnose, the most dangerous disease of St. John's Worth [Gärber and Schrenk 2001]. During rainy weather with temperatures to 28°C anthracnose caused by *C. gloeosporioides* may have epidemic character [Frencel et al. 1998, Gärber and Schrenk 2001]. The ability of C. *gloeosporioides* to affect plants is so big that it was used as a biological herbicide to kill the plants on waste lands [Yang and TeBeest 1992].

This paper present the results of many years' studies on the occurrence of *C. gloeosporioides* on different species of crops, especially ornamental lupine and various species of herb plants in South-East Poland and on macro- and microscopical features of the pathogen.

MATERIAL AND METHODS

The objects of investigations consisted of plants from two plantations of perennial lupine – *Lupinus polyphyllus* Lindl., the species which is a mixture of varieties grown for seeds in Boniewo (51.10810°N, 22.94582°E) and in several home gardens near Lublin (51.25016°N, 22.56719°E) in 1999–2005; plants of thyme (*Thymus vulgaris* L.) and lemon balm (*Melissa officinalis* L.) grown in several plantations in Fajsławice (51.09672°N, 22.96052°E) in 1998–2001, plants of caraway (*Carum carvi* L.) from the experimental cultivar plot and home gardens in Motycz (51.2408°N, 22.37972°E) near Lublin in 2001–2010. In 2008–2010 the plants of angelica (*Archangelica officinalis* Hoffm.) and corymbs of elder (*Sambucus nigra* L.) growing in Motycz near Lublin were considered in the study too. Observations on the occurrence of anthracnose were conducted during extensive studies on the species diversity of fungi inhabiting various organs of the above mentioned crops. The occurrence of the disease was determined twice during the growing season. The percentage of plants showing symptoms of disease and the etiological sings, typical of anthracnose, were evaluated. Appropriately

selected samples of plants were used for the isolation of *C. gloeosporioides* with artificial cultures method using mineral and malt agar culture medium simultaneously [Machowicz-Stefaniak et al. 2002a, 2002b, Machowicz-Stefaniak and Zalewska 2008]. In the case of ornamental lupine and the studied four species of herbs the samples of the sowing material and the material harvested on the plantation were subjected to mycological analysis. Three millimeter fragments of plant and seeds prepared from superficially disinfected parts of plants or schizocarps were placed on the solidified culture media in Petri dishes [Machowicz-Stefaniak and Zimowska 2000, Machowicz-Stefaniak and Zalewska 2008]. Representative one-spore cultures of fungi were grown on PDA (Difco) at the temperature of 20^{o}C. Macroscopic and microscopic photo documentation of colonies and morphological structures of numerous isolates of the fungus, as well as measurements of acervuli, conidia of *Colletotrichum gloeosporioides* and ascospores and perithecia of *Glomerella cingulata,* if the fungus formed there were successively made. The measurements were the basis for identifying the species in these studies. For diagnostic purposes, 150 acervuli (10 per isolate) and 600 conidia (40 per isolate) obtained from ornamental lupine and grown on PDA medium were measured. The above mentioned morphological structures were measured for 10 isolates obtained from angelica and elder and for 5 isolates from lemon balm and thyme too. Measurements of perythecia and ascospores were made from the two mentioned isolates from lemon balm, which generated teleomorph. Basing on scientific description of Von Arx [1957], Pidopličko [1977], Sutton [1980, 1992] and Marcinkowska [2003], the obtained isolates were indicated.

RESULTS

C. gloeosporioides commonly occurred on plants of ornamental lupine in 1999. The disease was evident at the beginning of flowering and pods of string ties in more than 60% of the plants. Small, dark brown spots with a distinct border, typical of anthracnose were formed on the leaves. These spots increased with time. Spots occurred along the edges of the leaves, causing unilateral growth inhibition and leaf deformation (phot. 1a). The signs of the disease occurred on the stems, especially on their lower parts, and they were not well visible, because the well developed leaves on the top part of stem covered them. A few of oval, black-brown, spots from 1cm to 4 cm in length were formed on one stem (phot. 1b). The surface of necrosis was tense firstly but with time it sank. The most visible signs of anthracnose occurred on pods. They were overclouded spots gray to gray-black, initially on a small area of the pods, then in the middle and on the whole surface of pods (phot. 1c). In the infested pods seeds did not form or they were scarce, deformed and overgrown by the gray mycelium (phot. 1d). On the surface of the infected plant parts there were signs in the form of etiological dense, confluent, pink drops which emerged from acervuli. The secretion, which covered all surface of necroses with a clammy, pink layer 1.0 to 2.0 thick, there were huge quantities of conidia typical of *C. gloeosporioides* (phot. 1). The spores produced at that time on the plants were viable, well-developed, filled with uniformly fine-grained cytoplasm. In wet seasons, with sustained temperatures above 20°C, which took place in 1999, the disease on ornamen-

Phot. 1. Symptoms of ornamental lupins anthracnose caused by *C. gloeosporioides*
Fot. 1. Objawy antraknozy łubinu ozdobnego powodowanej przez *C. Gloeosporioides*

Table 1. Occurrence of *C. gloeosporioides* on aboveground organs of plants in 1998–2010 years
Tabela 1. Występowanie *C. gloeosporioides* na nadziemnych częściach roślin w latach 1998–2010

Plants – Rośliny	Frequency of occurrence *C. gloeosporioides* on plants – Częstotliwość występowania *C. gloeosporioides* na roślinach												
	years – lata												
	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
Lupinus polyphyllus		++++	+++	+++	++	++	++	+					
Thymus vulgaris	+	+	+	+									
Melissa officinalis	+	+	+	+									
Carum carvi				+	+	+	+	+	+	+	+	+	+
Archangelica officinalis											+	+	+++
Sambucus nigra											++	++	++++

+ frequency of occurrence < 5% – m częstotliwość występowania < 5%
++ frequency of occurrence from 5 to 10% – częstotliwość występowania od 5 do 10%
+++ frequency of occurrence from 10 to 40% – częstotliwość występowania od 10 do 40%
++++ frequency of occurrence > 40% – częstotliwość występowania > 40%

tal lupine was epidemic and caused a complete destruction of the crop (tab. 1). In 2000–2001, the disease was observed on all aboveground organs of plants 10–30%, while in 2002–2004 the disease was observed mainly on the leaves and pods of 5–8% of the plants and in 2005 the disease was observed mainly on the pods of 1–3% of ornamental plant lupine.

Phot. 2. Anthracnose on the leaves of angelica
Fot. 2. Antraknoza na liściach arcydzięgla litwora

Phot. 3. *C. gloeosporioides* on the fruits of elder
Fot. 3. *C. gloeosporioides* na owocach bzu czarnego

During the study period *C. gloeosporioides* belonged to the species rarely isolated from the thyme plant. In 1998 and 1999 single isolates of this fungus were obtained from the leaves of several plants in second year of cultivation, and in 2001 from the roots, shoots and leaves of several plants in the first year of cultivation. This fungus was also isolated from the lower parts of the stems of several one-year-old plants of lemon balm and from the leaves of two plants in the second year of cultivation. *C. gloeosporioides* was isolated from the plants without typical symptoms of anthracnose. Similarly in the years 2001–2010 the cultures of this fungus were obtained from the leaves and stems of individual plants of caraway, but in these plants as well as in the plants of thyme and lemon balm, there were no specific symptoms of anthracnose.

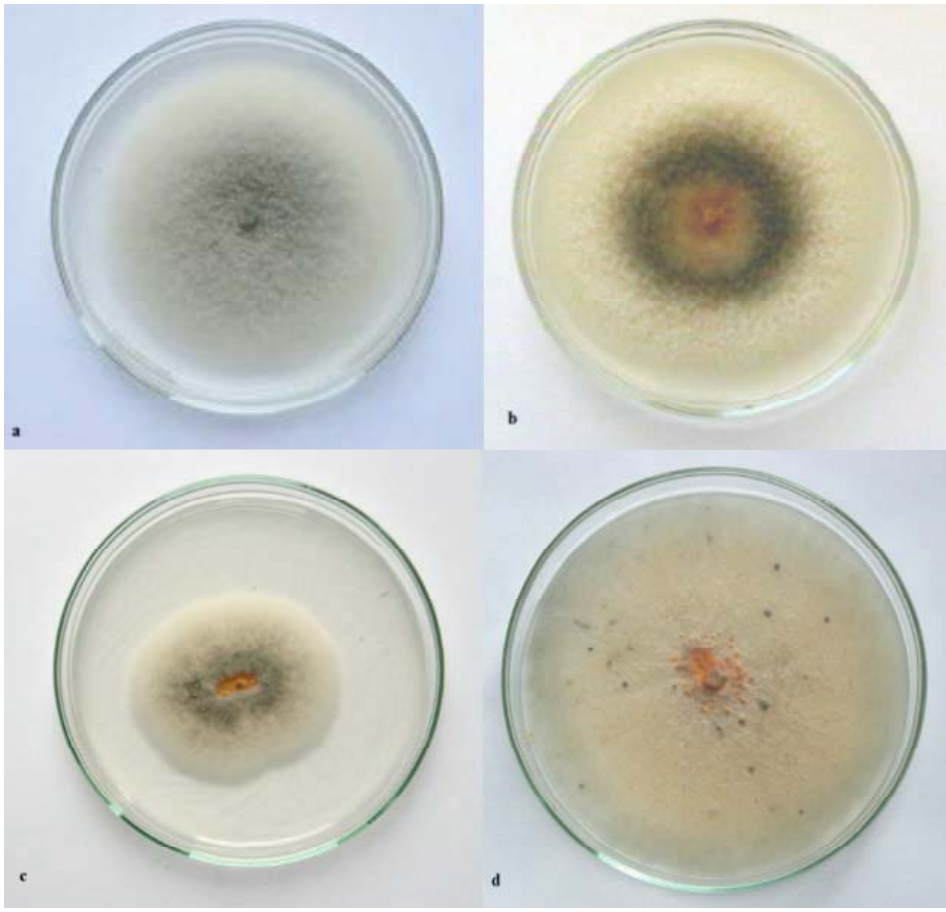
In 2008 and 2009 a few isolates of *C. gloeosporioides* were obtained from the leaves of one-year-old plants of angelica; however the presence of the fungus was observed only on 1.5 to 3.0 percent of plants. On the other hand, in 2010, two years after overwintering of plants the symptoms of anthracnose occurred on 20% of the plants and at the early stage of the flowering period, which at the beginning of July accounted for 40% of the plants. Anthracnose of angelica plants occurred on the stems, leaves, umbels and schizocarps. The most characteristic symptoms, i.e. necrotic, roundish spots with a brown edge and a dipping surface, occurred on the stems and leaves (phot. 2). The surface of the infected organs had peach color caused by exudates emerging from the acervuli with the mass of conidial spores. The disease spread very rapidly on the leaves. Within a few days, the leaves were infected in 60–80%, they dried, curled and wrinkled, especially during the hot and wet weather in July 2010.

During the vegetation period in 2010 the intensity of *C. gloeosporioides* was recorded on the umbels of elder. Symptoms of anthracnose occurred on about 80–90% of elder fruit. They were pale or willow and then darkening spots around the spiracles at various locations on berries (phot. 3a). The surface of spots with numerous acervuli sank gradually. Infected, diseased and deformed fruit were covered with a thick layer of fungal conidial spores (phot. 3b).

It was observed that conidia of *C. gloeosporioides* were produced very intensively on the infected plant parts and they were viable during prolonged periods of high temperatures and frequent rainfall, which occurred in the summers of 1999, 2001, 2009 and 2010.

In the years of increased presence of anthracnose, *C. gloeosporioides* was isolated commonly, i.e. from 98% of the studied lupine seeds with spots on their surface and from 21.5% of the studied seeds without spots as well as from 38% of schizocarps of angelica, while the fungus infested between 0.5 to 1.0% of the other herbs schizocarps.

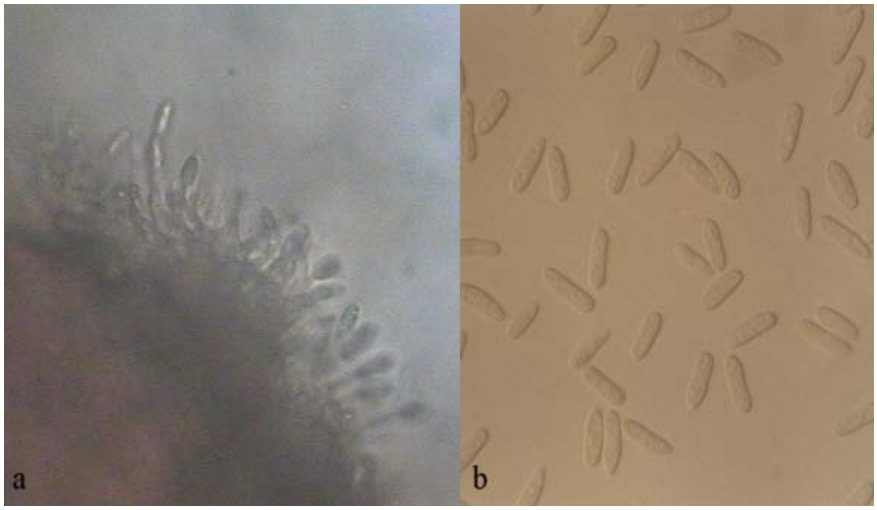
On the two agar culture media applied for isolation of *C. gloeosporioides,* a similar number of pathogen cultures was obtained. However, the effect of isolation of fungi from ornamental lupines was the best on the mineral medium on which the obtained colonies were free from bacteria. In the case of other species of herbs and fruit of elder, the isolation of *C. gloeosporioides* from the part of the plant was the best on mineral and malt medium. PDA medium was the most suitable for culture of representative isolates and identification of *C. gloeosporioides*. The growth of *C. gloeosporioides* colonies on this culture medium was intensive. The diameter of one-week-old colony, depending on the isolate, was from 7.5 to 9.0 cm. The aerial mycelium, initially white,



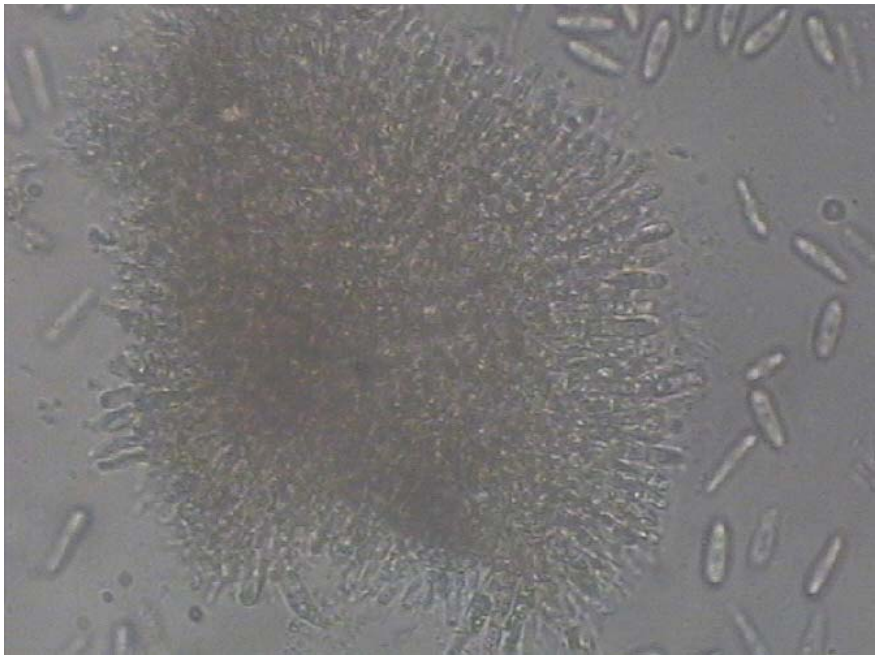
Phot. 4. Eight-day-old colonies of *C. gloeosporioides* on PDA K 177 from caraway – a, T 765 from thyme – b, B 7 from elder – c and A 290 from angelica – d

Fot. 4. 8-dniowe kolonie *C. gloeosporioides* na PDA K 177 z kminku zwyczajnego – a, T 765 z tymianku właściwego – b, B 7 z bzu czarnego – c i A 290 z arcydzięgla litwora – d

became dark-gray with time (phots. 4 a, b, c). White or only light gray color of the mycelium was present in the isolates from angelica (phot. 4d). The structure of the mycelium was the most relaxed and relatively flat or fluffy. In isolates from angelica the aerial mycelium was sparse, low and attached to the culture medium (phot. 4d). Acervuli appeared in most of the isolates on the fourth day of the culture. In the following days they were formed intensively on the whole surface of the colony or in wide circles, they were embedded in the mycelium and culture medium. Acervuli were relatively flat or pulvinate, with diameters ranging from 133.3 to 378.2 (tab. 2, phots. 5a, 6). On the culture mediums the formation and appearance of the setae from the acervuli of fungus were not observed. Elongated and hyaline conidial handles grew in a more or less compact layer of mycelium on the lower part of acervuli. Quite large, sticky, salmon-colored



Phot. 5. Acervuli – a (magnification × 500) and conidia (magnification × 640) *C. gloeosporioides* Ł 181 isolated from ornamental lupins
Fot. 5. Acerwulus – a (pow. × 500) i konidia – b (pow. × 640) *C. gloeosporioides* Ł181 z łubinu ozdobnego

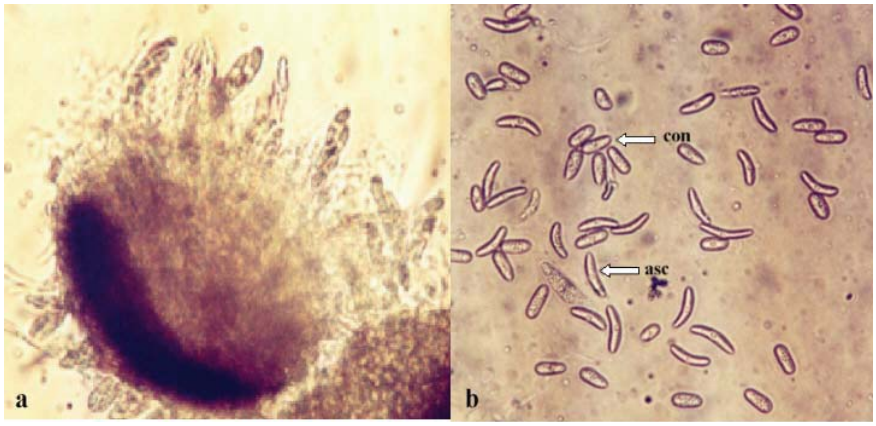


Phot. 6. Acervuli and conidia (magnification × 500) of *C. gloeosporioides* B 7 obtained from elder
Fot. 6. Acerwulus i konidia (pow. × 500) *C. gloeosporioides* B 7 z bzu czarnego

Table 2. The size (µm) of *C. gloeosporioides* morphological structures
Tabela 2. Wymiary (µm) struktur morfologicznych *C. gloeosporioides*

Author – Autor	Length of conidia Długość konidiów µm	Width of conidia Szerokość konidiów µm	Diameter of acervuli Średnica acerwulusów µm
Own studies from PDA Badania własne z PDA:			
Lupinus polyphyllus	7.66 – 19.1	3.5 – 5.73	146.12 – 296.8
Carum carvi	11.1 – 20.35	3.7 – 6.4	148.98 – 319.06
Archangelica officinalis	12.95 – 20.35	3.82 – 5.73	155.82 – 378.2
Melissa officinalis	12.95 – 20.35	3.7 – 5.55	133.56 – 304.22
Thymus vulgaris	11.66 – 19.1	3.7 – 6.4	140.98 – 326
Sambucus nigra	12.95 – 21.1	3.82 – 5.73	133.56 – 248.74
Sutton 1980[x)]	9 – 24	3.0 – 4.5	[xx)]
Pidopličko 1977[x)]	10 –28	3.5 – 7	[xx)]
Von Arx 1957[x)]	12 – 19	4 – 6	[xx)]
Filoda 2004	11 – 20	4 – 6	120 – 300
Borecki 1990[x)]	12 – 16	4 – 6	[xx)]
Pieczul and Rataj-Guranowska 2004 from PDA – z pożywki PDA	6 – 20	3 – 6	[xx)]

[x)] The size from diseased plants – Wymiary z zakażonych roślin
[xx)] Lack information in literature – Brak informacji w literaturze



Phot. 7. Perythecium with asci with ascospores (magnification × 320) – a and ascospores–asc. of *Glomerella cingulata* and conidia – con. of *C. gloeosporioides* (magnification × 640) obtained from lemon balm – b
Fot. 7. Perytecjum z workami i askosporami (pow. × 320) – a i askospory – asc. *Glomerella cingulata* oraz konidia – con. (pow. × 640) *C. gloeosporioides* uzyskane z melisy lekarskiej – b

drops that did surface merging into a sticky smear and clammy colony emerged from the mature, i.e. a few days' old acervuli (phot. 4d). In the drops of exudates there were numerous large conidial spores of pathogens. The conidia of the studied isolates were one-celled, hyaline, long-ellipsoid, with rounded one or both ends (phots. 5b, 6). The length of conidia on PDA medium ranged from 11.1 to 23.35, and the width from 3.5 to 6.4 (tab. 2). In the 20-day-old cultures of two isolates grown from lemon balm, perytehecium of the perfect stage with their bags and ascospores appeared in addition to the conidial stage. Ascospores were unicellular tubular, elongated, slightly curved, colorless with a characteristic shining drop of fat in the middle of their surface (phots. 7a, b). The size of ascospores ranged from 12.0 to 12.22 μm in length and from 3.0 to 5.0 μm in width.

DISCUSSION

The occurrence of *C. gloeosporioides* on numerous host plants species in various climatic zones and its high pathogenicity contributed to recognizing this fungus as one of the most dangerous pathogenic species causing plants anthracnose [Lenné 1992, Farr et al. 1995, Gärber and Schrenk 2001, Medeiros et al. 2010]. The authors' own observations and literature data indicated that the fungus is present in the populations of fyllosphaere microorganisms; however, its intensity and harmfulness are limited by weather conditions. Temperature from 20°C to 28°C and high humidity promote sporulation of the fungus on affected parts of plants, germination of conidia and widespread secondary infections [Frencel et al. 1998]. These conditions surely contributed to the epidemic occurrence of anthracnose on ornamental lupine, angelica and elder in some years of the study. In case of the other herb plant species studied from family *Apiaceae* it seems that this fungus is still not widespread within herb cultivations in South-East Poland. However, the fact of its detection should suggest some preventive treatments because it may be transmitted onto the new generations of plants with the seeds material and the infected crop residue [Fiedorow et al. 2001]. Besides, a great ability of *C. gloeosporioides* isolates to affect and inhabit the plants results from the capacity of this fungus of producing pectate lyase, the enzyme that degrades cell walls during the necrotrophic phase of pathogen infection. Additionally, it was indicated that pectate lyase production was encoded by gene *pal*B detected in *C. gloeosporioides* isolates [Medeiros et al. 2010]. Furthermore, a genetic homology, even in 100%, between the fungus isolates from the same plant species was noticed, which suggests nutritive specialization of *C. gloeosporioides* in relation to the host [Medeiros et al. 2010]. A possible relationship between the morphological features of isolates from particular host plants and their genetic characterization require explanations. In the present study morphological variability of colonies *C. gloeosporioides* from angelica was observed in comparison with isolates from the other plant species but especially from ornamental lupine and elder fruit. However, the morphology of the conidia, their similar dimensions independently of the host plant and not diverging from those described by other authors, together with the manner of conidia exudation resulted in including the studied isolates within genus *C. gloeosporioides* [Sutton 1980, 1992, Fiedorow et al. 2001, Filoda 2004].

Mycological analysis conducted during the years of studies indicated rare occurrence of the fungus teleomorph (*Glomerella cingulata*), which suggests small variability within the pathogen population [Mills et. al. 1992]. Among the culture media used in the present study the mineral and malt extract ones should be recommended for isolation of the pathogen and potato-dextrose medium for its identification. In spite of numerous species of host plants for *C. gloeosporioides* [Farr et al. 1995, Fiedorow et al. 2001, Jeske 2006, Medeiros et al. 2010] the results of our studies broaden the list of this pathogen host plants in the Polish conditions with an exception of angelica [Mazur and Szczeponek 2005].

CONCLUSIONS

1. The studies result in broadening the list of *C. gloeosporioides* host plants.
2. Occurrence intensity and harmfulness of the pathogen are limited by atmospheric conditions.
3. Fungus isolates obtained from different host plants show similar morphological features.
4. PDA is the most adequate medium for *C. gloeosporioides* identification.

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WYSTĘPOWANIE, SZKODLIWOŚĆ I CHARAKTERYSTYKA MORFOLOGICZNA *Colletotrichum gloeosporioides* (Penz.) Sacc. (TELEOMORFA: *Glomerella cingulata* (Stonem.) Spauld. et Schrenk)

Streszczenie. Występowanie chorób roślin jest uwarunkowane oddziaływaniem na siebie trzech czynników – patogenu, wrażliwej rośliny żywicielskiej i warunków środowiska. *C. gloeosporioides* jest gatunkiem polifagicznym, występującym w różnych rejonach geograficznych świata. W pracy przedstawiono wyniki z 10 lat badań nad występowaniem *C. gloeosporioides* na roślinach uprawnych, w tym przyprawowo-leczniczych w południo-

wo-wschodniej Polsce. Obecność patogenu, powodującego antraknozę roślin wykryto na nadziemnych częściach łubinu ozdobnego *Lupinus polyphyllus* Lindl., arcydzięgla litwora *Archangelica officinalis* Hoffm., tymianku zwyczajnego *Thymus vulgaris* L., kminku zwyczajnego *Carum carvi* L. i bzu czarnego *Sambucus nigra* L. Jednakże epidemiczne występowanie grzyba stwierdzono na roślinach łubinu ozdobnego w latach 1999–2001, arcydzięgla litwora i na baldachogronach bzu czarnego w 2010 r., tj. w okresach wegetacji z temperaturą powyżej 20^{o}C i z częstymi opadami deszczu. W takich warunkach istotnym elementem diagnostycznym było intensywne zarodnikowanie patogenu w fazie nekrotroficznego rozwoju. Do pozyskiwania izolatów grzyba z roślin żywicielskich stosowano z dobrym skutkiem pożywki mineralną i maltozową, a do identyfikacji PDA-Difco.

Słowa kluczowe: antraknozy, symptomatologia, charakterystyka populacji, *Colletotrichum gloeosporioides*

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