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PATHOGENICITY OF *Phoma complanata* (Tode) Desm. TOWARDS ANGELICA (*Archangelica officinalis* Hoffm.)

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Abstract. The plants of angelica *Archangelica officinalis* may be colonized by various species of fungi including *Phoma* species. The paper concerns the fungus *Phoma complanata,* isolated from above-ground and underground parts of angelica in 2009–2011. Pathogenicity tests according to Koch's postulates were carried out on angelica plants using various methods of inoculation. Observations of the infection process were made using the scanning electron microscope (SEM). Three our own isoletes and reference isolate of *P. complanata* were used in the study. *P. complanata* was found to be a facultative pathogen of angelica, which enters to the plant through stomata and wounds without creating appressoria in the top part of the germination hyphae.

Key words: pathogenicity tests, methods of inoculation, scanning electron microscopy – SEM

INTRODUCTION

In the literature there are numerous reports about fungi that threaten angelica plants but there is little information on the species of the genus *Phoma sensu lato* [Farr et al. 1995, Mazur and Szczeponek 2005, Zalewska et al. 2013]. Those fungi commonly occur in different climatic zones on plants from various botanical groups [Farr et al. 1995, Marcinkowska 2012]. *Phoma herbarum* Westendrop was recognized as typical of the genera and the section of *Phoma* [Boerema et al. 2004]. *P. complanata* according to the current rules of taxonomy, belongs to the family *Didymellaceae*, which according to the old system included species of the section *Phoma*, *Peyronella*, *Heterospora* oraz *Paraphoma* [Avescamp et al. 2010, Marcinkowska 2012]. *P. complanata* was isolated from roots, stems, leaves and umbels of angelica (*Archangelica officinalis*) in 2009–2011 [Zalewska et al. 2013]. The isolation of the fungus was repeated in subsequent years and plants even those which did not show specific symptoms of disease. *P. complanata* was

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commonly transferred by the seeds of parsnip (*Pastinaca sativa*), parsley (*Petroselinum crispum*), and carrot (*Daucus carota*) and damaged petioles, leaves and roots of these plants [Boerema et al. 2004]. Farr et al. [1995] reported that this species occurred on angelica stems in the USA.

Taking into account the lack of information about potential harmfulness of this fungus towards angelica the pathogenicity tests according Koch's postulates were undertaken. The aim of the study was to recognize the symptoms of a disease caused by *P. complanata* and the infection process under the scanning electron microscope (SEM).

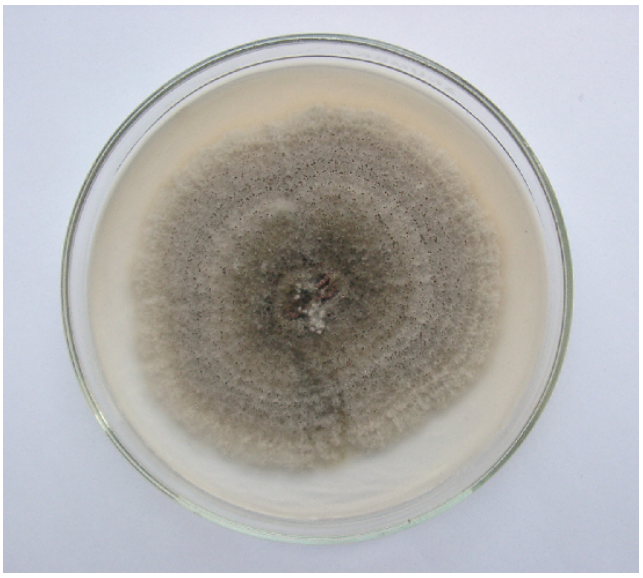
MATERIAL AND METHODS

The studies included:

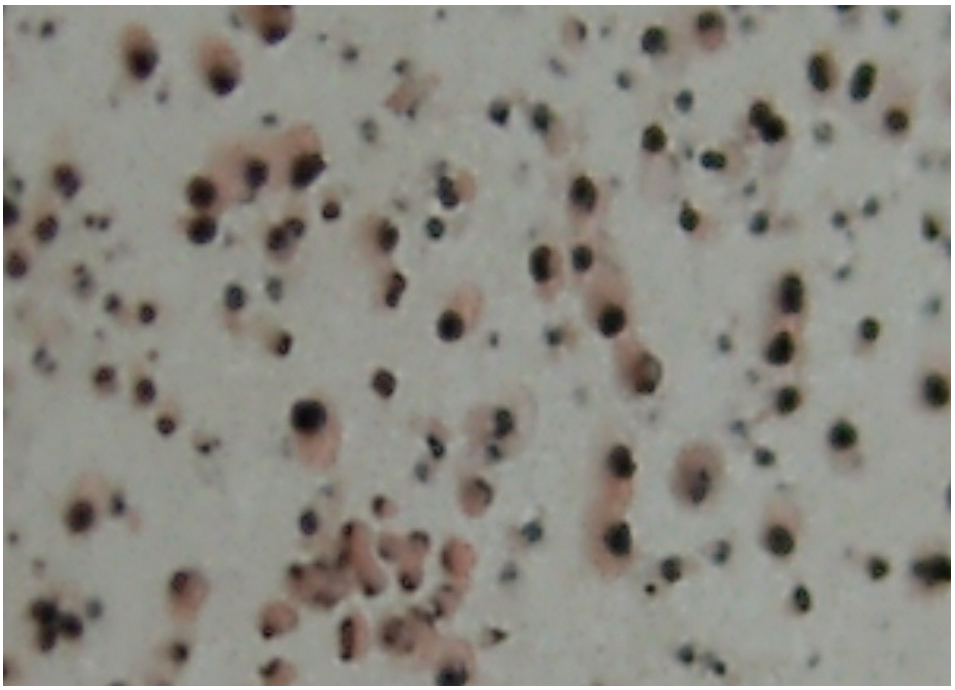
– samples of angelica (*Archangelica officinalis* Hoffm.) schizocarps obtained from Legutko company, leaves and stems of plants in the second year of cultivation from the collection of the Botanical Garden of the University of Maria Curie-Skłodowska in Lublin and from the authors' own collection;

– one-spore, cultures of *Phoma complanata* (Tode) Desm. (phots 1, 2) from the collection of the Department of Plant Pathology and Mycology of the University of Life Sciences in Lublin. These cultures were obtained from angelica leaves [Zalewska et al. 2013] and identified on standard media [Boerema et al. 2004], taking into account the up to date rules of taxonomy of fungi from *Phoma* genera [Marcinkowska 2012];

– reference isolate CAB 100311 was isolated from the stems of hogweed (*Heracleum spondyllium* L.) in the Netherlands. This isolate was obtained from Centraalbureau voor Schimmelcultures (CBS), Utrecht, Netherlands.



Phot. 1. Colony of *P. complanata* A 233 after 14 days of cultivation on malt medium (phot. E. Zalewska)



Phot. 2. Pycnidia and exudate of conidia of *P. complanata* A 233 on oat medium (phot. E. Zalewska)

The pathogenicity tests. Our own three randomly selected isolates: A103, A 233 and A 235 and reference isolate CAB 100311 were used in pathogenicity tests. The cultures of the fungus grew on the malt agar medium in Petri plates in a thermostat at the temperature 24ºC without access of light by two weeks.

The effect of *P. complanata* isolates on the possibility of germination of angelica schizocarps and on the healthiness of stems and leaves of this plant were studied in the laboratory using various techniques of inoculation.

The effect of post-culture liquids and water suspension of conidia on the schizocarps of angelica. In order to obtain the post-culture liquids containing the mycelium, spores and metabolites of *P. complanata*, the studied isolates were kept in 250 ml Erlenmeyer flasks on the liquid maltose medium at the temperature 24ºC, in a thermostat for 8 days [Mishra and Behr 1976]. The experiments were conducted in sterile moist chambers, i.e. Petri dishes of the 12 cm diameter. Five hundred well-formed and well-coloured schizocarps were selected from samples of the sowing material, i.e. 100 for each fungal isolate of the fungus and 100 for the control combination. The superficially disinfected schizocarps of angelica (90 sec. in 10% sodium hypochlorite) were placed in sterile moist-chambers and after then the post-culture liquid of each isolate was applied on them, using an automatic pipette VE-1000xr, in the amount of 3 ml to each Petri dish [Machowicz-Stefaniak et al. 2012]. The control combination consisted of schizocarps placed in sterile moist-chambers on the blotting paper soaked with 3 ml of the liquid malt medium.

The studies on the effect of water suspension of *P. complanata* conidia on angelica schizocarps were also conducted in a sterile moist chamber, using 500 schizocarps. In the present and in the next studies the water suspension of conidia, density $3 \cdot 10^6 \cdot ml^{-1}$, prepared from two-week-old, sporulating cultures of each isolate of *P. complanata* was used. Superficially disinfected schizocarps were singly immersed in liquid malt medium with an addition of agar in the amount of 3 $g \cdot l^{-1}$ medium, and then in the suspension of spores. Schizocarps prepared in such a way were placed in moist-chambers [Marcinkowska 1984, Machowicz-Stefaniak et al. 2012]. The control samples consisted of schizocarps covered with the liquid malt medium with an addition of agar and immersed individually in sterile distilled water.

Five dishes with 20 schizocarps (total 100 schizocarps) were used for each isolate of the fungus and for control in each method of inoculation. Petri dishes with the sowing material were placed under conditions permitting germination of the inoculated schizocarps, i.e. in a thermostat at the temperature 24ºC without access to light. Observations of germination were conducted after 3, 6 and 9 days. The number of germinating and not germinating schizocarps as well as the number of germs with and without necrosis was determined. Till the end of the experiment all schizocarps and germs were submitted to reisolation according to Koch's postulates [Agrios 2005].

Inoculation of fragments of stems. Stem fragment of 3 cm length, superficially disinfected in the same manner as schizocarps and 3 mm discs of sporulating mycelium of the studied isolates were used for inoculation. Individual inocula of the fungus were placed on epiderma, uninjured and injured with a sterile needle, in the middle of each fragment, with the mycelium to the surface of the skin. The control consisted of stem fragments with injured and uninjured epidermal tissue on which discs of sterile malt agar medium were placed [Marcinkowska 1984, Machowicz-Stefaniak et al. 2012].

Inoculation with water suspension of conidia with an addition of 3% agar was also performed, in which fragments of stems were immersed for 3 minutes. After this time the inoculated fragments of stems were transferred to moist-chambers. The control consisted of stem fragments immersed in sterile distilled water with an addition of 3% agar for the same period. For each isolate and for the control 4 plates with 5 stem fragments in each plate (total 300 fragments) were prepared for each experiment. Plates with inoculated and with control stem fragments were kept in a thermostat at the temperature 24ºC without access of light.

Inoculation of leaves. The study was conducted in sterile moist-chambers with the diameter of 15 cm. Inocula, i.e. 3 mm discs of sporulating mycelium of the pathogen were used for inoculation. Inocula were placed on the epidermal surface, uninjured and injured with a sterile needle, in the middle of each leaf. Discs of sterile malt medium placed on the injured or not injured epidermal surface of the leaves constituted the control. Moreover, inoculation of superficially disinfected leaves immerse in water suspension of conidia with an addition of 3% agar, for three minutes, was also conducted. Control leaves were immersed for three minutes in sterile distilled water with an addition of 3% agar [Machowicz-Stefaniak et al. 2012].

The inoculated plant material was kept in sterile moist-chambers with the diameter of 15 cm, with 6 leaves for each isolate and for the control, i.e. totally 90 leaves. The formation and development of necrosis on the inoculated parts of plants were observed

after 3, 6 and 9 days in all tests. After 9 days infection of the stems and leaves was evaluated according to a 5 point scale and the average infection index was calculated using the formula $P = \sum [(n \times v) / 4 \times N] \times 100$, where P – infection index, n – number of inoculated organs in different degrees of infection, v – degree of infected organs (0–4), N – total number of inoculated organs and 4 – the highest degree of the scale [Golenia 1970]. After the experiments were finished, reisolation of the tested fungus isolates was performed on the malt agar medium.

In selected experiments, statistically possible to analyse, the obtained results from the last observation were statistically analysed as a means of replication, using two-factor variance analysis (Anova) according to SAS program [Snedecor and Cochran 1982].

Scanning electron microscope SEM. The preliminary step of the infection process was observed under a scanning electron microscope (SEM) in the Central Agroecological Laboratory at the University of Life Sciences in Lublin. Fragments of stems 3–5 mm long, taken from the stems earlier inoculated with the suspension of conidia, which were then called the preparation for the microscopic studies were used in the study. These fragments, according to the procedure described by Kulik [1988] and Zimowska [2012], were fixed in 4% glutaraldehyde for 3 hours at room temperature, and then for 24 h at the temperature of 5ºC. After that time, the specimens were placed in 1% cacodylate buffer for 2 h at room temperature. Next the specimens were dehydrated in series of acetone at the concentrations of 30, 50, 70, 95 and 100%, for 15 minutes in each concentration. The further step in the procedure was drying the specimens in critical point, in liquid CO_2 using the apparatus Bal-Tec CPD 030 Critical Point Dryer. Then the specimens were gold sputter-coated. Observations of samples were carried out after 6, 16, 24, 36 and 48 hours from inoculation. Documentation was performed in a microscope Vega 2, Tescan.

RESULTS

Effect of isolates on *Archangelica officinalis* shizocarps. The experiment results using post-culture liquids of *P. complanata* showed that after nine days from inoculation 82–100% schizocarps did not germinate (tab. 1). The mean number, ranging from 16.4 to 20.0, from five replications of schizocarps that did not germinate was significantly higher than in the control combination (tab. 1). The surface of such shizocarps showed the symptoms of extensive necrosis. The percentage of germs with necrosis ranged from 12 to 18 and the mean number of five replicates of such sprouts, depending on the isolate, from 2.4 to 3.6 (tab. 1). The latter values were significantly higher than the control value (tab. 1). The necrosis of the germs subjected to the influence of post-culture liquids occurred at 2/3 of their length. Such germs died after 6 to 9 days. The results of experiment did not obtain healthy germs. *P. complanata,* with the macroscopic and microscopic features corresponding to those of the cultures used in the production of post-culture liquids, was isolated from the decayed germs and schizocarps that did not germinate (tab. 1). In addition, the surface of the decayed schizocarps and germs developed the mycelium of *P. complanata* and numerous pycnidia with the drops of conidial exudate. The control remained symptomless.

Table 1. Effect of post culture liquids of *P. complanata* isolates on germination of angelica schizocarps

Isolates of *P. complanata*	Total (mean) number of tested schizocarps	Observation after 9 days						Reisolation of *P. complanata*
		percent of not germinated schizocarps		percent of germinated schizocarps				
				germs with necrosis		germs without necrosis		
		total	mean	total	mean	total	mean	
A 103	100 (20)	86	17.2 c	14	2.8 b	0	0.0 b	96
A 233	100 (20)	82	16.4 d	18	3.6 a	0	0.0 b	95
A 235	100 (20)	88	17.6 b	12	2.4 b	0	0.0 b	94
CBS 100311	100 (20)	100	20.0 a	0	0.0 c	0	0.0 b	100
Control	100 (20)	9	1.8 e	0	0.0 c	91	18.2 a	0
		LSD = 0.3989		LSD = 0.4983		LSD = 0.1128		

Explanations:
Level of essentiality $p \leq 0.5$
Values marked with the same letter do not differ significantly

Table 2. Effect of conidial water suspension of *P. complanata* isolates on germination of angelica schizocarps

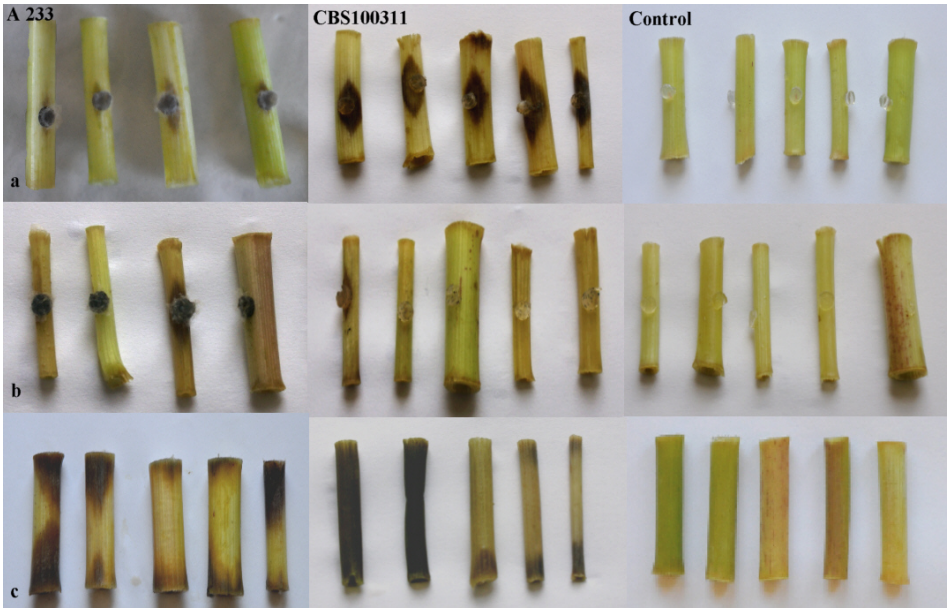
Isolates of *P. complanata*	Total (mean) number of tested schizocarps	Observation after 9 days						Reisolation of *P. complanata*
		percent of not germinated schizocarps		percent of germinated schizocarps				
				germs with necrosis		germs without necrosis		
		total	mean	total	mean	total	mean	
A 103	100 (20)	74	14.8 c	26	5.2 a	0	0,0	92
A 233	100 (20)	83	16.6 b	17	3.4 b	0	0,0	97
A 235	100 (20)	75	15.0 c	25	5.0 a	0	0,0	92
CBS 100311	100 (20)	100	20.0 a	0	0.0 c	0	0.0	100
Control	100 (20)	18	3.6 d	0	0,0 c	82	16.4	0
		LSD = 0.5863		LSD = 0.5917		LSD = 0.3290		

Explanations:
Level of essentiality $p \leq 0.5$
Values marked with the same letter do not differ significantly

In the experiment with the conidial suspension of *P. complanata* it was found that after 9 days the percent of not germinated schizocarps, depending on the isolate, was from 74 to 100 and in the control combination 18 (tab. 2). The mean number from five replications of the schizocarps which did not germinate ranged from 14.8 to 20. These values were significantly higher than the control value amounting to 3.6 (tab. 2). The

percentage of germinated schizocarps after their inoculation with *P. complanata* ranged from 17 to 26, and the average number of the germs with necrosis from 3.4 to 5.2. Necrosis occurring in all germs reached up to ¾ of their length. The control remained symptomless (tab. 2). The schizocarps treated with post-culture liquids and a conidial suspension of *P. complanata* of the reference isolate CBS 100311 did not germinate at all. The percentage of the schizocarps which did not germinate after the application of domestic isolates of *P. complanata* was significantly lower than with the reference isolate (tabs 1, 2). *P. complanata* was reisolated from most of the inoculated shizocarps, while in the control combination the schizocarps were not colonized by *P. complanata* (tabs 1, 2).

Inoculation of stem fragments. All studied isolates of *P. complanata* caused symptoms in the form of small, rounded, lenticular increasing dark necrotic spots (phots 3a, b). The most effective method of inoculation proved to be when colonized plugs were placed on the injured epidermis (tab. 3). After 9 days, the highest values of the infection index of the stems ranging from 98.75% to 97.5%, respectively, resulted for isolates A 235 and A 233. These values were significantly higher than those after the application of isolate A 103 and the reference isolate (tab. 4). After 9 days since inoculation with colonized plugs placed on non-injured epidermis, isolates A 103, CBS 100311 caused significantly higher values of the infection index of the stems than isolates A 235 and A 233 (tab. 5).



Phot. 3. Pices of angelica stems 3 days after inoculation with *P. complanata* when: a – injured epidermal tissue were inoculated, b – not injured epidermal tissues were inoculated, c – conidial water suspension was used for inoculation (phot. E. Zalewska)

Table. 3. Pathogenicity of *P. complanata* isolates to stems and leaves of angelica using various methods of inoculation (mean for 4 isolates)

Inoculation methods	Infection index (%) after 9 days	
	stems	leaves
Colonized plugs placed at injured epidermis of stems and leaves	93.56 a	74.08 a
Untreated	0 c	0 c
Colonized plugs placed at not injured epidermis of stems and leaves	58.25 b	52.25 b
Untreated	0 c	0 c
Stems and leaves soaked in conidial suspension (1×10^6 conidia/ml)	77.81 ab	93.33 a
Untreated	0 c	0 c
	LSD = 32.3669	LSD = 19.97

Infection index evaluated on the basis of scale: 0º – lack of disease symptoms; 1º – slight of necrosis visible only around of the inoculation points; 2º – 25 to 50% surface of inoculated organs showed disease symptoms; 3º – 51 to 75% surface of inoculated organs showed disease symptoms; 4º – 76 to 100% surface of inoculated organs showed disease symptoms

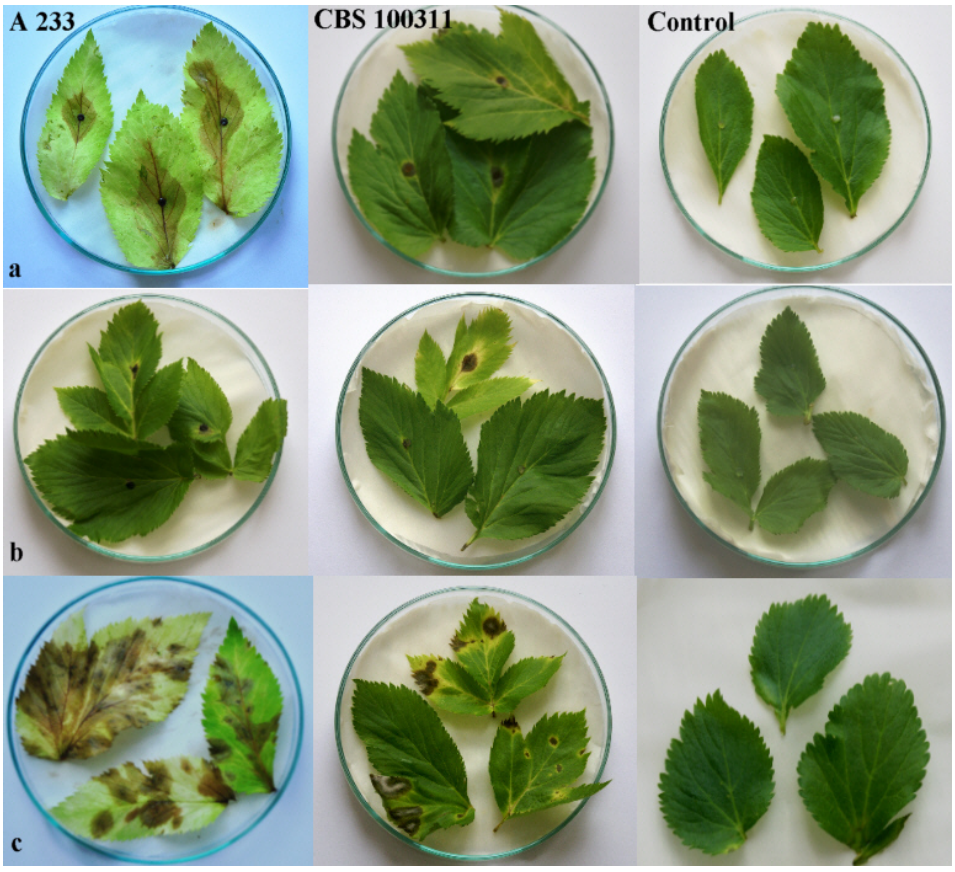
The highest values of the infection index ranging from 85 to 100% in the method involving inoculation by water conidial suspension were obtained, respectively, for isolates CBS 100311 and A 233 (tab. 6). After 3 days on the stems inoculated with this method, necrosis occurred at the ends of the stems (phot. 3c). After 6 days, necrosis occurred on all parts of the stems, and after nine days, pycnidia of the pathogen were observed. *P. complanata* cultures were reisolated from all inoculated fragments of the stems, for all methods (tabs 4, 5, 6).

Table. 4. Effect of infection of *P. complanata* isolates on occurrence of angelica leave and stem necrosis when injured epidermis was inoculated

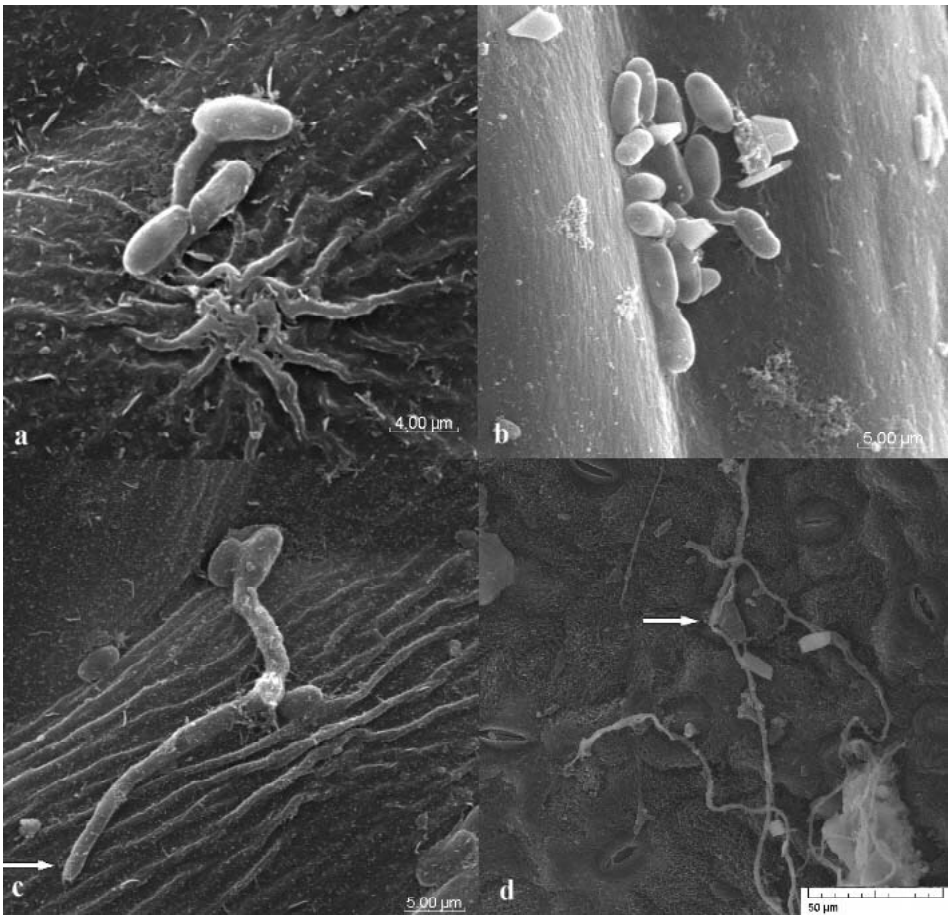
Isolate	Infection index after 9 days		Reisolation	
	stems	leaves	stems	leaves
A 103	85 c	95.83 a	100	100
A 233	97.5 a	75 b	100	100
A235	98.75 a	62.5 c	100	100
CBS 100311	93 b	63 c	100	100
Control	0 d	0 d	0	0
LSD	4.3609	3.077		

Table. 5. Effect of infection of *P. complanata* isolates on occurrence of angelica leave and stem necrosis when not – injured epidermis was inoculated

Isolate	Infection index after 9 days		Reisolation	
	stems	leaves	stems	leaves
A 103	85 a	46 b	100	100
A 233	37.5 c	50 b	100	100
A235	32.5 c	50 b	100	100
CBS 100311	78 b	63 a	100	100
Control	0 d	0 c	0	0
LSD	6.7582	9.5075		



Phot. 4. Angelica leaves 3 days after inoculation with *P. complanata* when: a – injured epidermal tissue were inoculated, b – not injured epidermal tissues were inoculated, c – conidial water suspension was used for inoculation (phot. E. Zalewska)



Phot. 5. Scaning electron micrographs of *P. complanata* on angelica stems. (a, b) – view of germinated pycniospores 16 h after inoculation, (c) – 24 h after inoculation at the end of the germ tube non-adhesive structure was seen (arrow), (d) – unbranched hyphae entered through the stomatal opening after 36 h after inoculation (arrow). (phot. M. Wróbel)

Effect of *P. complanata* on the leaves of angelica. All studied isolates of *P. complanata* caused symptoms in the form of necrotic spots on the inoculated leaves of angelica. 3 days after inoculation necrosis from 4 to 7 mm long, around the infection site were observed when the method involving colonized plugs was used. After the same time on the leaves soaked in conidial suspension necrosis ranged from 3 to 10 mm (phot. 4 a, b, c). After the next three days the necrotic spots occurred on 50% of the leaf surface, and disintegration of tissue was observed in the place of the necrosis. After 9 days necrosis covered from 50 to 100% of the area of the leaf blade. The values of the infection index ranged, respectively, from 62.5 to 95.83% in case of inoculation through the injured epidermis, from 46 to 63% through the non-injured epidermis and from 83.33

to 100% in case of inoculation with the conidial suspension (tabs 4, 5, 6). After this time, further disintegration of the inoculated leaf tissue was observed. *P. complanata* cultures were reisolated from all inoculated organs of plants in all methods (tabs 4, 5, 6).

Table. 6. Effect of infection of *P. complanata* isolates on occurrence of angelica leave and stem necrosis when conidial suspension was used for inoculation

Isolate	Infection index after 9 days		Reisolation	
	stems	leaves	stems	leaves
A 103	62.5 c	100 a	100	100
A 233	85 b	100 a	100	100
A235	63.75 c	83.33 b	100	100
CBS 100311	100 a	92 b	100	100
Control	0 d	0 c	0	0
LSD	7.0408	10.1979		

Scanning electron microscope. Six hours after inoculation, conidia of *P. complanata* were visible on the surface of angelica stems. After 16 hours single conidia formed germ tubes with the length not exceeding 6 µm (phot. 5a). This process occurred in the furrows of angelica stems where there was a greater density of conidia than on the surface of the ribs (phot. 5a, b). Twenty four hours after inoculation most conidia formed germ tubes. The surface of the cuticle in contact with the germ tube showed no change; moreover, at the end of the germ tube a non-adhesive structure was seen (phot. 5c). After 36 hours, it was observed at unbranched hyphae entered through the stomatal opening (phot. 5d). After 48 hours, especially in the furrows of the stems, a grid of aerial hyphae of *P. complanata* was visible.

DISSCUSION

The studies of pathogenicity showed that all tested isolates of *P. complanata* caused infections of the inoculated organs of angelica. This is testified to by high values of infection indexes and fulfillment of Koch's postulates. Of all methods of inoculation, the most effective proved to be the one consisting in placing plugs of colonized agar on the injured tissue of the stems and leaves, and the method considering soaking of the fragments in a conidial suspension. These results are consistent with the information from the literature, according to which the enumerated inoculation methods also proved the most effective for other facultative pathogens such as *Phoma exigua* var. *exigua* [Marcinkowska 1984], *Phoma lingam* [Sock and Hoppe 1999], *P. foveata* comb. nov. *Boeremia foveata* [Giebel and Dopierała 2004] and *P. strasseri* comb. nov. *Boeremia strasseri* [Zimowska 2012]. The present studies show that the harmfulness of the tested isolates of *P. complanata* was not limited only to causing symptoms of necrosis on the leaves and stems of angelica but also the inhibition of schizocarps germination and

induction of germs necrosis. This fact indicates the possibility of infection of angelica in the earliest phase of plants developing, what may be result from the presence of the infectious material of the pathogen in the soil [Machowicz-Stefaniak et al. 2008, Zalewska 2010]. Although it is believed that the life cycle of the *Phoma sensu lato* is influenced by the occupied niche, it is relatively similar for all pathogenic species [Aveskamp et al. 2008]. In the absence of a suitable host, most species survive as saprobes on decaying material of infected host plants in the form of chlamydospores or pycnidia. *P. complanata* does not form chlamydospores, but it forms pycnidia with scleroplectenchymatic, thick walls, which were observed in the present studies and they are likely to survive the unfavorable period for the fungus and the new conidia formed inside could be the primary source of infection for the germ of angelica.

Studies on the ultrastructure of the inoculated stems with conidial suspension pointed to the penetration of the pathogen into the tissue through stomata without the formation of the adhesive structure in the form of appressorium. Species belonging to genus *Phoma sensu lato* can infect plants by forming or not forming an appressorium [Roustaee et al. 2000]. Penetration without appressorium formation was also reported for *Phoma lingam* [Hammond et al. 1985], *P. narcissi* [Saniewska and Dyki 1997], and *P. macdonaldii* [Roustaee et al. 2000]. Formation of an appressorium by fungi depends on the plant organ which the pathogen infects. Moreover, the formation of this adhesive structure is affected by such factors as the presence of epicuticular waxes , rigidity, and surface hardness of infected organs [Höhl et al. 1990]. There are several types of natural openings in plants which may be penetrated by fungal hyphae. Infection of the stems and leaves of angelica by *P. complanata* occurred through the stomata, and injuries to the covering tissue raise the efficiency of inoculation, which indicates on the facultative parasitism of the fungus. The stomata are considered to be the most common route for the pathogen to enter the plants [Roustaee et al. 2000]. This route of penetration has been reported for other pathogenic fungi such as *P. linguam* [Sock and Hoppe 1999], *Phomopsis diachenii* [Machowicz-Stefaniak et al. 2012], *Septoria tritici* [Cohen and Eyal 1993], *S. apicola* [Donovan et al. 1990] and *S. carvi* [Zalewska 2013]. Observation of conidia germination in the furrows of angelica stems and the subsequent presence of the pathogen mycelium is justified. It is known that spores of most fungi are preparing for infection by taking water from the environment and swelling to achieve an adequate state of hydration. Water is collected in the furrows of angelica stems, creating favorable conditions for the germination of spores. Accumulation of infectious material of *P. complanata* also takes place in these places.

The present studies allowed to explain the preliminary stage of infection of the stems of angelica by *P. complanata* and the interaction between the pathogen and the host plant.

CONCLUSIONS

1. *P. complanata* should be considered as the facultative pathogen of angelica.
2. Penetration of the pathogen takes place through stomata and injury of covering tissue makes more efficient inoculation.

3. In the initial stage of infection the fungus do not create adhesive structure in the form of appressorium.

4. *P. complanata* caused necrosis of germs, stems and leaves of angelica as well as the fungus limited schizocarp germinated.

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PATOGENICZNOŚĆ *Phoma complanata* (Tode) Desm. DLA ARCYDZIĘGLA LITWORA (*Archangelica officinalis* Hoffm.)

Streszczenie. Arcydzięgiel litwor (*Archangelica officinalis* Hoffm.) może być zasiedlany przez różne gatunki grzybów, w tym również przez grzyby *Phoma sensu lato*. Przeprowadzone badania dotyczą gatunku *Phoma complanata* wyizolowanego z nadziemnych i podziemnych części arcydzięgla w latach 2009–2011. Testy patogeniczności przeprowadzono zgodnie z postulatami Kocha, z uwzględnieniem różnych metod inokulacji. Potwierdziły one patogeniczny charakter polskich izolatów *P. complanata* oraz izolatu referencyjnego dla wybranych organów arcydzięgla. Obserwacje przebiegu procesu infekcji wykonano przy użyciu skaningowego mikroskopu elektronowego (SEM). Na podstawie przeprowadzonych badań *P. complanata* uznano za fakultatywny patogen arcydzięgla. Grzyb wnika do tkanek przez aparaty szparkowe bez tworzenia na końcu strzępki kiełkowej struktury adhezyjnej w postaci appresorium.

Słowa kluczowe: testy patogeniczności, metody inokulacji, skaningowy mikroskop elektronowy – SEM

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