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CULTURE CONDITIONS OF *Phoma negriana* Thüm AND THE FUNGUS PATHOGENICITY TOWARDS GRAPE-VINE CANES

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Abstract. *Phoma negriana* is a little-known pathogen of grapevine canes but increasingly often it is listed in the countries of southern Europe and Asia. The work concerns a study of the opportunities for growth and development of the fungus at different temperatures, pathogenicity tests and the influence of biotechnical preparations on the growth and development of the fungus *in vitro.* Studies have shown that mycelial growth of *P. negriana* is possible at the temperature range from 5 to 32ºC with the thermal optimum from 20 to 24ºC. The optimal temperatures for the abundant sporulation was 20, 24 and 28ºC. The positive results of pathogenicity tests as well as re-isolation of the fungus from inoculated shoots confirmed the possibility of grapevine canes infection by *P. negriana*. A significant factor in facilitating infection were injuries of shoots, what indicates that the fungus is a facultative pathogen of grapevine. Biosept Active limited the growth and development of *P. negriana* significantly more strongly than Beta-Chikol. When it was applied at the concentration of 0.3%, it caused destruction and loss of fungal hyphae vitality.

Key words: grapevine, *Phoma negriana*, temperature, pathogenicity, biopreparations

INTRODUCTION

Fungi of the genus *Phoma* are widely distributed in different geographical areas of the world on plants from different botanical groups [Sutton 1980, Marcinkowska 1995, Boerema et al. 2004]. They can be specialized plant pathogens as well as poliphagous or saprotrophic fungi [Marcinkowska 2003, Boerema et al. 2004]. One of the species is *P. negriana* belonging to *Phoma* section with a typical species of this section and genus *P. herbarum* [Boerema et al. 2004]. *P. negriana* is considered an occasional pathogen of aboveground grapevine (*Vitis vinifera*) organs in Southern Europe [Gruyter et al. 1998, Boerema et al. 2004]. The prevalence of the fungus was found on the leaves, fruits and

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grapevine canes in Iran [Davari and Hajieghrari 2008]. The fungus caused shoot cankers and black rot of grapes. Typically, it infected the leaves and stems simultaneously with commonly occurring *Guignardia bidwellii, Plasmopara viticola* and *Sclerotinia sclerotiorum* [Hoffman et al. 2002, Davari and Hajieghrari 2008]. The stems, leaves and berries were particularly susceptible to infection by *P. negriana* after plant flowering. In Poland, fungal isolates were obtained during the research on the healthiness of the propagation material of grapevine in different parts of the country, mainly from shoots with symptoms of necrosis [Machowicz-Stefaniak and Król 2007]. *P. negriana,* despite its slow growth, may limit the development of various fungi species including *Alternaria alternata*, *Epicoccum purpurascens* and *Phomopsis viticola*, commonly occurring on various organs of the grapevine [Król and Machowicz-Stefaniak 2008]. This probably results from the production of secondary metabolites [Machowicz-Stefaniak and Król 2007, Król and Machowicz-Stefaniak 2008], which are known from other species in the genus [Monte et al. 1990, Giebel and Dopierała 2004, Zimowska and Machowicz-Stefaniak 2005]. The ability of secondary metabolites secretion by *Phoma* is one of the taxonomic criteria of these fungi [Monte et al. 1990, Boerema et al. 2004]. Thanks to these properties, the fungus can survive on grapevine canes in the presence of different species of phyllosphere fungi [Król and Machowicz-Stefaniak 2008].

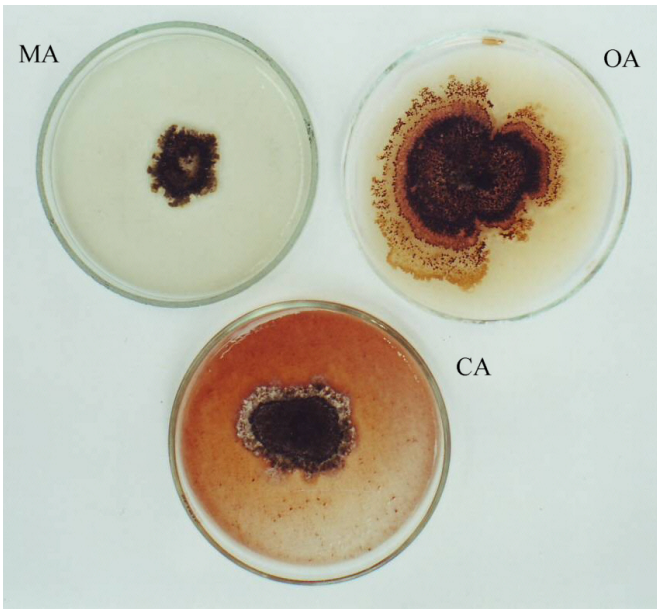
Due to the repeated occurrence of *P. negriana* on grapevine, and considering the lack of information on the biology of the pathogen, the conditions of the occurrence and harmfulness to vines, the studies were carried out on thermal requirements, pathogenicity and sensitivity of *P. negriana* towards some biotechnical preparations.

MATERIAL AND METHODS

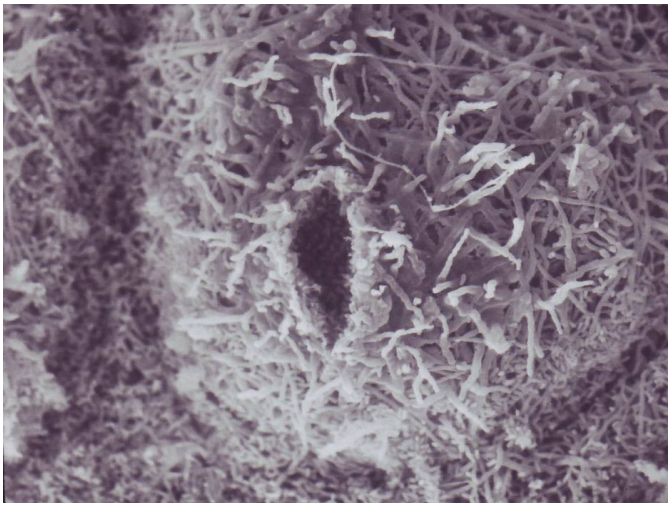
Four native isolates of *P. negriana*: W 1205, W 1308, W 1075 and W 1432 were used for the study of thermal requirements of the fungus, while for the study of the pathogenicity isolates: W 1075, W 1175 and W 1410 were used, and for the study on the limitation of the growth of the fungus only one isolate W 1075 was used (phot. 1, 2, 3). Identification of one spore cultures of the fungus, according to the current rules of taxonomy *Phoma* spp. [Boerema i in. 2004], and the micro-morphological characterization of the fungus were presented in an earlier paper by Machowicz-Stefaniak and Król [2007]. All isolates of the fungus were obtained from grape-vine canes with the symptoms of spots and from dying stems of small plants taken from the farms near Lublin and Warszawa provinces (phot. 4). Moreover, the canes of grapevine cv. Schuyler and biotechnical preparations Biosept Active (33% grapefruit extract) produced by Cintamani Poland and Beta-Chikol (2% chitosan) produced by Gumitex Polifarm in Łowicz were studied.

Studies of thermal requirements. The growth of each isolate colony was estimated at the temperatures -6ºC, 5ºC, 10ºC, 16ºC, 20ºC, 20ºC, 24ºC, 28ºC and 32ºC on oat agar medium. The culture was kept on a solidified medium in Petri dishes. The medium was inoculated with a 7-day-old inoculum of the fungus – a disc with the diameter of 3 mm. For each isolate four replications were prepared. The culture was kept for 14 days. The evaluation criteria adopted in the study consisted of the linear growth rate, the diameter

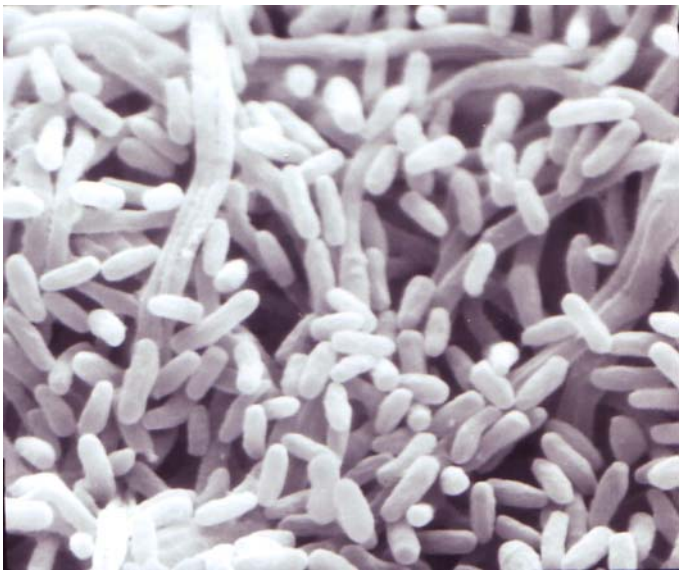
of 14-day-old colonies subjected to the statistical analysis. The appearance of colonies and the rate of pycnidia formation and secretion of conidia were observed to 42 day of cultivation [Machowicz-Stefaniak et al. 2012a, b].



Phot. 1. 14-day-old colonies of *Phoma negriana*, W 1410 on malt agar – MA, oat agar – OA and cherry agar – CA (photo E. Zajęcka)



Phot. 2. Pycnidium of *Phoma negriana* (SEM), 610 × magnification (photo M. Wróbel)



Phot. 3. Conidia of *Phoma negriana* (SEM) 4200 × magnification (photo M. Wróbel)

Phot. 4. Grapevine stems from which *Phoma negriana* was isolated (photo E. Król)

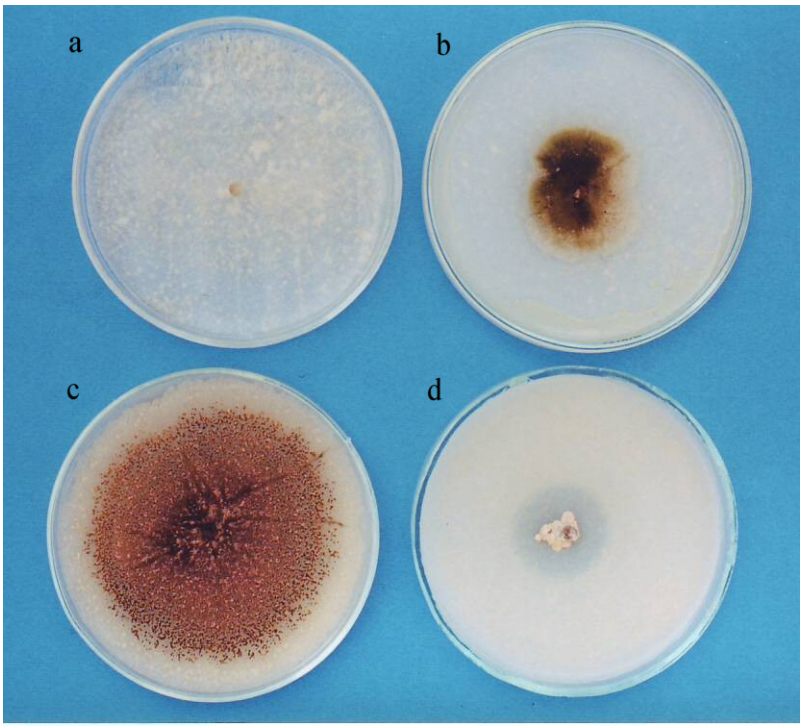
Studies of *P. negriana* pathogenicity. Grape-vine canes were cut into pieces 8–10 cm in length, which were disinfected in 50% ethanol for 1 min and then washed 3 times for 3 min. in sterile distilled water, after which they were placed in sterile moist chambers. In each moist chamber 10 those canes were placed. Three mm inoculum of the fungus overgrown with *P. negriana* mycelium was placed on the cane sections injured with a scalpel (combination I) and on those that were not injured (combination II).

For each isolate and manner of infection three replicates were made. Control canes were both injured (control I) and not injured tissues (control II), which were placed on 3 mm discs of oat agar medium. The moist chambers with the study material were placed in a thermostat at the temperature 20ºC for three weeks. Observations of the forming necroses, their length, formation of pycnidia and conidia were carried out for 20 days. After this time, reisolation of *P. negriana* according to Koch's postulates was performed. For this purpose a small fragment of the tissue containing the place of inoculation was taken and after disinfection was placed on malt agar medium [Machowicz--Stefaniak et al. 2012a].

Effect of biopreparations on *P. negriana.* The studies were conducted *in vitro*. The method was applied in which the test preparation was added in the utility form to the sterile medium cooled to 5°C, and the inoculum of the tested fungus was put on the medium [Borecki 1984, Machowicz-Stefaniak and Zalewska 2011]. Biosept Active was tested at the concentrations of 0.05%, 0.1%, 0.2% and 0.3% on PDA medium [Machowicz-Stefaniak and Zalewska 2011], whereas Beta-Chikol at the concentrations of 0.01%, 0.025%, 0.05% and 0.1% on poor agar medium with the amount of maltose reduced by half in 1 dm^3 [Pięta et al. 1998, Machowicz-Stefaniak and Zalewska 2011]. The experiment was conducted in two series. The measure of the toxic activity of the tested preparations was estimated as a percentage of inhibition of 4- and 8- day-old colonies on the medium with preparations in relation to the control colonies [Kowalik and Krechniak 1961, Machowicz-Stefaniak and Zalewska 2011], and possibly the changes in the appearance of morphological structures. In the case of no fungus growth on the medium containing the preparation the type of toxic activity was determined [Borecki 1984].

RESULTS

Growth of the fungus at different temperatures. The observed isolates of *P. negriana* at the temperature of -6ºC did not grow (phot. 5a). However, after moving the plates to the temperature of 20°C, scarce substrate hyphae appeared after 6 days. After another 3 days the poor mycelium of the fungus got covered with numerous pycnidia, which sporulated very intensively, owing to which the colony took the creamy pink appearance. The mycelial growth of *P. negriana* isolates growing at the temperatures of 5°C and 10°C were observed after 4–8 days of culture. However, further growth of the colony was slow. Similarly, at the temperature of 32°C the mycelium grew very slowly, although the first hyphae were observed just after 2–4 days of culture. At 32°C the mycelium had an unusual appearance, it was white-gray, very dense, thick and felty (phot. 5 d), and after moving to room with moderate temperature a delicate creeping hyphae formed on the margin of the colony. The studied isolates of *P. negriana* at the range of temperature from 16°C to 28°C formed mycelium just after two days of incubation. Initially, the mycelium was white with a delicate, floccose structure. During the successive days, when pycnidia were formed, it took on darker colouring (phot. 5b, c), and the colour of the reverse changed from cream to dark green.



Phot. 5. 14-day-old colonies of *Phoma negriana,* W 1205 growing at the temperature: a – -6ºC, b – 16ºC, c – 24ºC i d- 32ºC (photo E. Zajęcka)

The sporulation was also depended on temperature (tab. 1). At the temperature of -6°C and 32°C the fungus did not form any pycnidia or conidia. At the temperatures of 5°C and 10°C pycnidia began to form, respectively after 16 and 12 days, but their number was small. At the temperature ranging from 16°C to 28°C pycnidia formed abundantly on the whole surface of the colony, but the earliest, i.e. after 4–6 days of culture

Table 1. Effect of temperature on *Phoma negriana* sporulation (data for 4 strains)

Oat agar medium			
temperature (ºC)	beginning of pycnidia formation	numer of pycnidia on the surface of colony	beginning of conidia secretion
-6	lack	lack	lack to 42 day
5	16–42 day	sparse	lack to 42 day
10	12–19 day	sparse	12–23 day
16	14–20 day	numerous	16–22 day
20	6 day	mass on whole surface of colony	6–8 day
24	4–6 day		6–8 day
28	6 day		6 day
32	lack	lack	lack to 42 day

at 20°C, 24°C and 28°C. At the temperatures of 20°C, 24°C and 28°C the pycnidia of *P. negriana* abundantly secreted beige or beige-pink, thick drops of conidia after 6–8 days. However, at 10°C and 16°C very scanty exudate of conidia appeared after 12–23 days of culture (tab. 1).

Table 2. Diameter (mm) of 14-day-old colonies of *Phoma negriana* on oat agar medium at various temperature

Isolates	Temperature							
	-6ºC	5ºC	10ºC	16ºC	20ºC	24ºC	28ºC	32ºC
	P1 P2	P1 P2	P1 P2	P1 P2	P1 P2	P1 P2	P1 P2	P1 P2
W 1075	0.0 a A	8.5 a b A	17.0 b A	35.8 c A	53.0 d A	64.5 e B	29.0 c A	16.0 b A
W 1205	0.0 a A	10.0 a A	24.0 b A	32.4 bc A	64.3 d B	83.5 e C	37.6 c A	9.5 a A
W 1308	0.0 a A	15.0 b A	46.3 d B	30.9 c A	90.0 e C	53.9 d A	51.3 d B	10.4 b A
W 1432	0.0 a A	11.0 b A	24.0 c A	39.3 d A	54.4 e AB	59.6 e AB	35.3 d A	10.0 ab A

Values differ significantly if they are not marked with the same letter
P1 – differences depending on the temperature for one isolates – small letters
P2 – differences between isolates for one temperature – capital letters
$LSD_{0.05}$ = 10.2

Table 3. Effect of temperature on the size of 14 days *Phoma negriana* colony on oat agar medium (mean for 4 strains)

Temperature in ºC	Diameter of colony in mm	
-6	0.0	A
5	11.13	B
10	27.94	C
16	34.72	D
20	65.38	E
24	65.41	E
28	38.29	D
32	11.47	B

$LSD_{0.05}$ = 4.05

The significantly largest diameter of colony 14-days-old, amounting 83.5 mm, was found for isolate W1205 at the temperature 24°C. On the other hand, the diameter of the colony of isolate W1308 – 90.0 mm was the highest at 20°C (tab. 2). The significantly smallest diameters of 14-day-old colonies had all tested isolates at the temperatures of 5°C and 32°C, and at 10°C with the exception of isolate W1308. At the temperature of 16°C the diameter of colonies of all isolates was significantly smaller than that of isolates growing at the temperatures 20°C and 24°C. At 28°C the diameter of the colonies of the studied isolates was significantly lower than at 20°C and 24°C, with an exception of isolate W 1308 (tab. 2). The average size of 14-day-old colonies, irrespective of the

isolate, was the highest and was not significantly different at 20°C and 24°C (tab. 3). On the other hand, the average size of the colony was not significantly different at 28°C and 16°C, as well as 32°C and 5°C. However, at 10°C the average size of the colony was significantly higher than the size of colonies growing at 5°C and 32°C (tab. 3).

Study of *P. negriana* pathogenicity. As results of the conducted inoculation of canes shown in the combination I the mycelium of *P. negriana* developed to 4th days, and in the combination II from 10 to 14 days on inoculum used for the infection of canes (tab. 4). In combination I, after 6 days from inoculation and after 14–18 days in combination II single, spidery hyphae moved onto the canes tissue, around the place of infection. In the course of time, the mycelium of *P. negriana* gradually overgrew the further parts of canes (tab. 4).

Table 4. Development of disease symptoms on grape-vine canes inoculated with *Phoma negriana*

Combination of experiment	Isolate	Development of *P. negriana* mycelium on inoculated canes	Formation of necrosis	Formation of pycnidia
Combination I Inoculation of wounded canes	W 1075	to 4th day – hyphae on inoculum	14 day	18 day
	W 1175	from 6th day – on canes around the place of inoculation	14 day	18 day
	W 1410	from 8th day – on further parts of canes	14 day	18 day
Combination II Inoculation of not wounded canes	W 1075	10–14 day – hyphae on inoculum	16 day	18 day
	W 1175	14–18 day on canes around the place of inoculation	18 day	18 day
	W 1410	16–20 day – on further parts of canes	18 day	20 day
Control I wounded canes		lack of mycelium growth	lack	lack
Control II not wounded canes		lack of mycelium growth	lack	lack

First symptoms of disease on the inoculated canes occurred after 14 days in combination I and after 16–18 days in combination II (tab. 4). They were black, necrotic spots covering the stem tissue around the site of infection, which then got larger along it (phot. 6). Pycnidia filled with spores characteristic of and similar to *P. negriana* formed on the diseased tissues in both combinations of the experiments.

After 20 days of the experiment, it was found that the number of successful infections was significantly higher on the injured canes than on those that were not injured (tab. 5). In combination I the average number of infected canes was 16 and it was significantly higher than the average number amounting to 7.3 in combination II. In neither control were the diseases symptoms observed on the test canes. On the canes in combination I there occurred black, necrotic spots from 12.8 to 18.6 mm, an average of 15.9 mm in the length. This value was significantly higher than the average length of the necrosis, amounting to 9.5 mm on the canes in combination II (tab. 5).

Phot. 6. Necrotic spots on the stems inoculated with *Phoma negriana* – a, control – b (photo E. Zajęcka)

Table 5. Results of inoculation of grape-vine canes cv. Schuyler with *Phoma negriana* after 20 days

Combination of experiment	isolate	Number o studied canes	Number of canes with symptoms of necrosis	Mean length of necrosis on diseased canes	Number of canes from which *P. negriana* was reisolated
Combination I Inoculation of wounded canes	W 1075	30	17	12.8	18
	W 1175	30	12	16.2	17
	W 1410	30	19	18.6	25
	mean	30	16 a	15.9 a	20 a
Combination II Inoculation of not wounded canes	W 1075	30	6	8.3	4
	W 1175	30	8	8.4	3
	W 1410	30	8	11.7	9
	mean	30	7.3 b	9.5 b	5.3 b
Control I wounded canes		30	0 c	0 c	0 b
Control II Not wounded canes		30	0 c	0 c	0 b
$LSD_{0.05}$			4.9496	4.5733	7.0807

Values marked with the same letter do not differ significantly

Table 6. Effect of Beta-Chikol on the growth of *Phoma negriana*

Studied isolate	Concentration									
	0.01%	0.025%	0.05%	0.1%	control	0.01%	0.025%	0.05%	0.1%	control
W 1075	percentage of inhibition of 4-day-old colonies					percentage of inhibition of 8-day-old colonies				
Mean	9.3 a	-11.62 b	6.97 a	-13.95 b	0.0 ab	6.11 a	18.33 a	7.22 a	12.22 a	0.0 a
$LSD_{0.05}$	14.36					32.11				

Values marked with the same letter do not differ significantly

Table 7. Effect of Biosept Active on the growth of *Phoma negriana*

Studied isolate	Concentration									
	0.05%	0.1%	0.2%	0.3%	control	0.05%	0.1%	0.2%	0.3%	control
W 1075	percentage of inhibition of 4-day-old colonies					percentage of inhibition of 8-day-old colonies				
Mean	37.31 b	54.87 a	49.99 a	58.53 a	0.0 c	67.42 a	69.72 a	69.26 a	66.51 a	0.0 b
$LSD_{0.05}$	10.13					7.69				

Values marked with the same letter do not differ significantly

As a result of reisolation, *P. negriana* isolates were obtained from all canes showing disease symptoms as well as from canes without symptoms in combination I. On the other hand, in combination II the fungus was reisolated only from a small number of canes showing the symptoms of necrosis (tab. 5). All cultures of *P. negriana* obtained from inoculated grape-vine canes had macro- and microscopic features corresponding to the isolates used for inoculation.

Effect of biopreparations on *P. negriana.* Beta-Chikol at the concentration of 0.1% caused the greatest percentage of inhibition of 4-day-old colonies amounting to 9.3, while at the concentration of 0.05% preparations the percentage of inhibition was 6.97 (tab. 6). Those values did not differ significantly from each other but were significantly higher than in control and at the concentrations of the preparations 0.025 and 0.1%. It was shown that for the latter two concentrations the effect of the preparations was stimulating. The percentage of growth inhibition of 8-day-old colonies of *P. negriana* at various concentrations ranged from 6.11 to 18.33 but those values did not differ significantly; however, they were significantly higher than in control. The colonies of this fungus growing in the presence of Beta-Chikol formed pycnidia with conidia. Microscopic observations showed that the fungus formed a mass of tangles of large hyphae in the form of long ropes. Clearly enlarged vacuoles were found inside the cells of those hyphae.

Biospet Active, regardless of the concentration of preparations in the medium, strongly inhibited the development of *P. negriana* after 4, as well as 8 days of cultivation (tab. 7). The highest percentage of growth inhibition of 4-day-old colonies amounting to 58.53, 49.99 and 54.87 occurred on the medium with preparations at the concentration 0.3, 0.2 and 0.1%, respectively. Those values were significantly higher than the percentage of inhibition of *P. negriana* colony growth at the concentration of 0.05% of Biosept Active. A strong inhibition of 8-day-old colony growth of the fungus was, depending on the concentration, from 66.51 to 69.72 and the values did not differ significantly from each other but were significantly higher than in control (tab.7). Microscopic observations showed that in the presence of Biosept Active in the medium, particularly at the concentration of 0.3%, hyphae of *P. negriana* were thickened, dark, and contained numerous enlarged vacuoles and also cytoplasm came off from the cell walls. The colonies, regardless of the concentration of the preparation, did not at all form pycnidia or conidia of the fungus.

DISCUSSION

The obtained results and their statistical analysis showed that the mycelial growth of *P. negriana* is possible in a wide temperature range, i.e, from 5 to 32°C, with the thermal optimum from 20 to 24°C. This suggests that the fungus may be harmful to grapevines grown in the ground and under cover. Demonstrating that *P. negriana* has the ability to maintain a viable mycelium at a temperature not favorable for its growth, i.e. -6.5, 10 and 32°C indicates the possibility of survival of this fungus in grapevine canes both in hot and moderate climates. It also indicates that the low temperature did not eliminate the fungus from the plant material during the storage of mother canes.

Pycnidia and conidia formation in the fungus culture, and the intensity of their creation were clearly related to temperature. The optimum for intensive sporulation was 20, 24 and 28°C. It seems that the hot and humid seasons will favor the formation of large quantities of infectious material and plant infection. Likewise, for various species of the genus *Phoma*, the optimal conditions for infections occur at high relative humidity and the temperatures from 25 to 27°C [Spotts 1977, 1980, Pezet and Jermini 1989].

The results of infection tests indicated that infection of grapevine canes by *P. negriana* was possible, which was documented by positive results of fungus re-isolation from inoculated shoots. Because of the importance of injuries in the infection process, the fungus should be considered as an occasional pathogen of grapevine. Although the disease symptoms caused by *P. negriana* are non-specific for grapevine, the presence of black spots on the shoots may suggest their colonization by this fungus only or together with other pathogenic fungi [Stojanovič 1986].

The non-specific nature of the symptoms of canes infection by *P. negriana* is proved by the lack of fungus re-isolation from the inoculated shoots, despite the presence of necrotic spots on them. On the other hand re- isolation of *P. negriana* cultures from canes that were inoculated but did not show any disease symptoms indicates on possibilities for the asymptomatic fungus development inside the host plant tissues. This phenomenon, often observed in the case of other pathogens of bark and wood [Król 2005, 2006], creates the danger of spreading cane pathogens with cuttings produced from apparently healthy shoots.

Research on the direct influence of biotechnical preparations *in vitro* on the growth and development of *P. negriana* indicates the need for further testing. The results confirm the ability of Beta-Chikol, especially Biosept Active, to limit the fungus growth and cause changes in its macro and microscopic features, which had been demonstrated in the case of other pathogens [Pięta et al. 2004, Patkowska 2009a, b].

Biosept Active, containing in its composition an extract from pulp and seeds of grapefruit, was an effective biopreparation. The active compounds contained in this product inhibit the growth of microorganisms and induce plant resistance [Wojdyła 2001, Orlikowski et al. 2002]. Biosept Active significantly inhibited the growth and development of *P. negriana* in all tested concentrations. The direct effectiveness of Biosept Active *in vitro* was observed after 4 days of its influence on fungal colonies, whereas after 8 days this biopreparation caused complete inhibition of pycnidia formation and therefore the sporulation of the fungus. The demonstrated killing effect of Biospet Active resulted from destruction and loss of fungus hyphae viability [Machowicz-Stefaniak and Zalewska 2011, Zalewska et al. 2013]. This type of interaction is the most useful, in terms of the practical use of the preparation.

Beta-Chikol slightly limited the growth and development of *P. negriana* and its inhibitory effect *in vitro* was insufficient. Chitosan contained in this biopreparation is generally used on plants to induce their resistance mechanisms, so its effect on the pathogen takes place via the plant metabolism [Pospieszny 1997].

High capacity of Biosept Active in reducing *in vivo* the growth and development of *P. negriana* suggests that this formulation can be recommended for further testing.

CONCLUSIONS

1. The growth of *P. negriana* is possible in the temperature range from 5 to 32°C, but the optimum temperature for their growth is 20–24°C, and for abundant sporulation 20–28°C.

2. Positive results of infection tests and re-isolation of fungus from inoculated canes indicate the possibility of their infection by *P. negriana*. Necrotic spots on the shoots are the symptom of disease caused by the fungus.

3. Infection of the shoots mainly by the injured tissue indicates the facultative nature of *P. negriana* parasitism.

4. The demonstrated ability of *P. negriana* for asymptomatic development in the shoot tissues may contribute to the spread of the pathogen with the cuttings produced from apparently healthy canes.

5. Biosept Active limited the growth and development of *P. negriana* significantly more strongly than Beta-Chikol.

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WARUNKI WZROSTU *Phoma negriana* Thüm I PATOGENICZNOŚĆ GRZYBA DLA PĘDÓW WINOROŚLI

Streszczenie. *Phoma negriana* jest mało znanym patogenem pędów winorośli, aczkolwiek coraz częściej notowanym w krajach południowej Europy i Azji. Praca dotyczy

możliwości wzrostu i rozwoju grzyba w różnej temperaturze, testów patogeniczności i oddziaływania preparatów biotechnicznych na wzrost i rozwój grzyba *in vitro*. Badania wykazały, że wzrost grzybni *P. negriana* jest możliwy w zakresie temperatury od 5 do 32ºC, przy optimum termicznym od 20 do 24ºC. Za optymalną temperaturę dla intensywnego zarodnikowania uznano 20, 24 i 28ºC. Pozytywne wyniki testów infekcyjnych oraz reizolacja grzyba z inokulowanych pędów wskazały na możliwość porażania łozy winorośli przez *P. negriana.* Czynnikiem istotnie ułatwiającym infekcję były zranienia pędów, co wskazuje, że grzyb jest fakultatywnym patogenem winorośli. Biosept Active istotnie silniej ograniczał wzrost i rozwój *P. negriana* aniżeli Beta-Chikol. Zastosowany w stężeniu 0,3% powodował destrukcję i utratę żywotności strzępek grzyba.

Słowa kluczowe: winorośl, *Phoma negriana*, temperatura, patogeniczność, biopreparaty

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