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BIOTIC ACTIVITY OF Phoma strasseri AND THE EFFECT OF THERMAL CONDITIONS ON THE GROWTH AND FORMATION OF THE PATHOGEN'S INFECTIOUS MATERIAL

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Abstract. *Phoma strasseri* was isolated first time from peppermint (*Mentha piperita* L.) in 2004. These species had not been found in Poland earlier. Biotic interactions between *P. strasseri* and 16 species of fungi colonizing the phyllosphere of stems and rhizomes of peppermint were determined using the biotic series method and maltose agar MA. The effect of particular fungi species on *P. strasseri* was expressed as and individual, general and summary biotic effect. Fungi from genera *Trichoderma* were found out to be the most effective and positive antagonists whereas those of *Alternaria alternata, Botrytis cinerea* and *Rhizoctonia solani* – despite the high values of IBE – were considered negative antagonists. Studies on the effect of thermal conditions pointed out that the thermal optimum for the growth of the fungus colonies ranged from 16° C to 28° C, while that for the formation of the infectious material from 24° C to 28° C. Basing on the ability of *P. strasseri* to develop in a wide range of temperatures, it was included within the group of eurythermic organisms.

Key words: black stem and rhizome rot, eurythermic organism, phyllosphere fungi, peppermint

INTRODUCTION

Fungi of the genus *Phoma* includes over 3,000 taxons. On the basis of permanent macro- and microscopic properties that appear *in vitro* under standard conditions and due to their structure and teleomorphs were divided into 9 sections [Boerema et al. 2004, Aveskamp et al. 2008]. *Phoma strasseri*, which causes one of the most serious diseases of peppermint (*Mentha piperita* L.) and which is cultivated in India, Japan, the United States and in Europe, belongs to the section of *Phyllostictoides*. Black stems and rhizomes rot, also called phomosis of mint, causes yield losses reaching even 90%.

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They are caused by the plants dying out as a result of fast degradation of stem and rhizome tissues resulting from the enzymatic decomposition of pectin compounds by polygalacturonase and macerating enzymes produced by *P. strasseri* [Melouk and Horner 1972].

The author's own studies conducted since 2004 on the healthiness of peppermint grown in south-east Poland pointed to common colonization of stems and rhizomes with symptoms of necrosis and tissue disintegration by *P. strasseri* [Zimowska and Machowicz-Stefaniak 2005, Zimowska 2007]. The pathogenic character of the fungus was confirmed by means of pathogenicity tests according to Koch's postulates [Zimowska, unpublished].

Despite common isolation of *P. strasseri* cultures from the infected stems and rhizomes, the occurrence of other fungi species was observed on those parts of plants. Those fungi included antagonistic species from genus *Trichoderma*, *Gliocladium*, *Epicoccum* as well as fungi from fast growing genus *Alternaria*, *Botrytis*, *Fusarium* and *Rhizoctonia*, which are known for their competitive abilities [Zimowska 2007].

Because the literature lacks information on the biotic interactions of *P. strasseri* and other fungi colonizing the phyllosphere of stems and rhizomes, studies were undertaken to explain this problem. Besides, the effect of thermal conditions on the growth and formation of the infectious material of the pathogen was studied in *in vitro* conditions.

MATERIAL AND METHODS

Isolate M 435 of *P. strasseri* and 16 single isolates of the accompanying species were considered in the studies on the biotic effect of *P. strasseri* (tab. 1). The fungi were selected from the author's own collection gathered as a result of extensive studies on the healthiness of peppermint conducted in the years 2004–2006 on production plantations situated in the lubelskie and świętokrzyskie voivodeships [Zimowska 2007]. The fungi were isolated from superficially disinfected organs of mint using the method of artificial cultures with a mineral medium [Zimowska and Machowicz-Stefaniak 2005]. Isolates of *P. strasseri* were obtained from the stems and rhizomes with the symptoms of necrosis and next rot of the enumerated organs (photo 1). The participation of the pathogen obtained from the stems constituted from 10.48% to 29.17% of all fungi obtained from that organ, while from the rhizomes from 17.86% to 35.63% (tab. 2). Single spore isolates of *P. strasseri* were also obtained from peppermint roots (tab. 2).

Because literature lacks information on the biotic effect of *P. strasseri*, the studies considered different fungi species, regardless of the frequency of their isolation [Zi-mowska 2007]. The studies were conducted using the method of biotic series method [Mańka 1974, Mańka and Mańka 1992, Mańka 1995], on a maltose agar MA [De Gruyter et al. 2002]. This method was adopted for fungi colonizing the plants' phyllosphere [Zimowska 2004, Machowicz-Stefaniak et al. 2008]. Two discs of 3 mm in diameter from 14-day-old cultures, one of *P. strasseri* and one of the fungus representing the studied community, were taken. They were placed mycelium down, 2 cm apart in the center of the Petri dish, on the solidified medium. The dishes with single fungi species constituted the control. For each experimental combination, 4 dishes were con-

260

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Smooton of furneme	IBE*	2004		2005		2006	
Gatunek grzyba		Frequency Częstotliwość	GBE**	Frequency Częstotliwość	GBE**	Frequency Częstotliwość	GBE**
Alternaria alternata (Fr.) Keissler	9+	128	+768	198	+1188	100	+600
Botrytis cinerea Pers.	9+	35	+210	15	06+	21	+126
Epicoccum purpurascens Ehrenberg	+	S	+5	3	+3		
Fusarium avenaceum (Fr.) Sacc.	+2	108	+216	127	+254	73	+146
Fusarium culmorum (W.G.Sm.) Sacc.	+4	142	+568	119	+476	100	+400
Fusarium equiseti (Corda) Sacc.	+	115	+115	128	+128	106	+106
Fusarium axysporum Schlecht.	-1	98	-98	73	-73	50	-50
Phoma exigua var. exigua Desm.	+3	12	+36	6	+27	25	+75
Phoma heteroderea Chen. Dickson & Kimbrough	+3			8	+24	10	+30
Rhizoctonia solani Küchn	L+	6	+63	12	+84	21	+147
Trichoderma harzianum Rifai	+8	5	+40	10	+80	5	$^{+40}$
Trichoderma koningii Oud.	+8	23	+184	19	+152	33	+264
Penicillium verrucosum Dierckx var. cyclopium (West.) Samson Stolk et Hadlok	-1			9	-9	5	-5
Gliocladium catenulatum Gilman et Abbott	ų	8	-24	4	-12	10	-30
Gliocladium fimbriatum Gilman et Abbott	ς-	11	-33	7	-21	11	-33
Gliocladium roseum Bainier	ς-	ю	6-	9	-18	9	-9
Number of isolates		702		744		576	
SBE***			+2041		+2376		+1810

IBE* – Individual Biotic Effect – Indywidualny Efect Biotyczny, GBE** – General Biotic Effect – Ogólny Efekt Biotyczny, SBE*** – Summary Biotic Effect – Sumaryczny Efekt Biotyczny.

Table 2. Participation of *Phoma strasseri* isolates in fungal communities obtained from pep-
permint (*Mentha piperita* L.) with the symptoms of black stem and rhizomes rotTabela 2. Udział izolatów *Phoma strasseri* wśród grzybów uzyskanych w latach 2004–2006

z mięty pieprzowej (*Mentha piperita* L.) z objawami czarnej zgnilizny łodyg i rozłogów

	Ν	umber (and percent) of	isolates	– Liczba (ora	az procent) izolatów	
Plant organs]	Lubelskie province woj. lubelskie			iętokrzyskie province woj. świętokrzyskie	
Organy rośliny	P. strasseri	other species of fungi pozostałe gatunki grzybów	total razem	P. strasseri	other species of fungi pozostałe gatunki grzybów	total razem
Stems – Łodygi	75 (10.46)	717	792	194 (29.17)	471	665
Rhizomes – Rozłogi	120 (17.86)	672	792	210 (35.63)	279	589
Leaves – Liście		364	364		499	499
Roots – Korzenie	3 (0.43)	699	702	20 (4.20)	456	476
Total – Ogółem	198 (7.47)	2452	2650	424 (19.02)	1805	2229



- Photo 1. Black stems rot (a), and rhizomes rot (b) of peppermint caused by *P. strasseri*. Photo B. Zimowska
- Fot. 1. Czarna zgnilizna łodyg (a) i rozłogów (b) mięty pieprzowej powodowana przez *P. strasseri*. Fot. B. Zimowska

sidered which were treated as replications. They were kept in a thermostat at the temperature of 24° C. The biotic effect was estimated on the basis of an 8-degree scale after 10 days of common growth, and in the case of *Gliocladium* spp. the observation was extended to 34 days [Łacicowa 1989]. While evaluating the biotic effect, the overgrowth of the fungus colony by the accompanying fungus, the occurrence of the inhibition zone between two colonies and growth inhibition of the colony of one of the fungi were taken into consideration [Mańka 1995]. In the case of the overgrowth of *P. strasseri* by other fungi species, the studies examined changes in the appearance of morphological structures, i.e. the hyphae and conidia. The biotic effect of the accompanying fungi representing the phyllopshere of the stems and the rhizomes of peppermint and *P. strasseri* was expressed as an individual biotic effect (IBE) [Mańka 1974]. Next, the general biotic effect (GBE) was estimated which was the product of the individual biotic effect and the multiplicity of the occurrence of particular fungi species. The algebraic sum of general biotic effects made it possible to determine the summary biotic effect (SBE), which reflected the effect of all studied species of phyllosphere fungi on *P. strasseri* in the years 2004–2006. A positive value of IBE indicates the growth inhibition of the pathogen, whereas a negative value of IBE points to the lack of growth inhibition of the pathogen's colony. "O" value means a neutral effect of both fungi on each other [Mańka 1974, Mańka 1995].

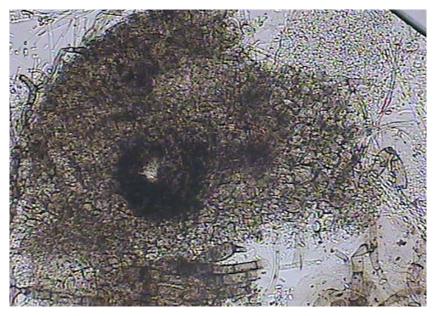
Five isolates were chosen at random for the studies on the effect of thermal conditions on the growth and formation of the infectious material of P. strasseri: isolates M 365 and M 324, obtained from the infected rhizomes of peppermint cultivated in the świętokrzyskie voivodeship, isolates M 435 and M 398 obtained from the dying out stems of plants from the plantations situated in the lubelskie voivodeship and the model isolate of CBS 126.93 obtained from the central bank of fungi Centraalbureau voor Schimmelcultures in the Netherlands. Each isolate grew at the temperatures of -6°C, 5°C, 10°C, 16°C, 20°C, 24°C, 28°C and 32°C. The culture was kept on a maltose agar MA, which was inoculated with the isolates of the studied fungi. The inoculation material were discs with the diameter of 5 mm cut out from 14-days' mother colonies that grew on MA medium at the temperature of 24°C. Four repetitions were used for each isolate. Observations of the linear growth of the colonies of the studied isolates and the formation of morphological structures were kept for 14 days [Zimowska 2002, 2010]. The colony diameter was measured every other day. Simultaneously, macroscopic features of the colonies and the development of pycnidia and conidia were observed. Results obtained from the experiment were statistically analyzed using a two factor analysis of variance (Anova) according to SAS program.

RESULTS

Out of 16 tested fungi, 11 inhibited the growth of *P. strasseri*, which is testified to by the positive values of IBE (tab. 1). The maximally positive values of +8 occurred in the case of *Trichoderma koningii* and *T. harzianum* (tab. 1). *Trichoderma* spp. colonies overgrew the inoculum of *P. strasseri*, making the growth and sporulation of the pathogen impossible. Besides, *Trichoderma* spp. caused degradation and dying out of *P. strasseri* hyphae (photo 2) as well as destruction and decomposition of the pycnidia (photo 3). The fungi species that considerably inhibited the growth of 10-days' pathogen colonies were *Rhizoctonia solani*, *Botrytis cinerea* and *Alternaria alternata* because the value of the individual biotic effect was, respectively, +7, +6 and +6 (tab. 1). Besides the dynamic growth of *R. solani* and *B. cinerea* mycelium on the surface of *P. strasseri* colonies, the studies observed the presence of sclerotia of both fungi species. Scarce, single pycnidia were observed on the surface of the pathogen colonies with the common growth of each of the enumerated two species and *P. strasseri*. The species



- Photo 2. Degradation of *P. strasseri* hyphae caused by *Trichoderma* spp. \times 500. Photo E. Zalewska
- Fot. 2. Degradacja strzępek P. strasseri spowodowana przez Trichoderma spp. \times 500. Fot. E. Zalewska



- Photo 3. Destruction of *P. strasseri* pycnidium caused by *Trichoderma* spp. \times 500. Photo E. Zalewska
- Fot. 3. Destrukcja piknidium *P. strasseri* spowodowana przez *Trichoderma* spp. \times 500. Fot. E. Zalewska

Biotic activity of Phoma strasseri and the effect of thermal conditions on the growth...

265

that inhibited the growth of 10-days' colonies of P. strasseri only to a small extend were Fusarium avenaceum, F. culmorum and F. equiseti since the values of IBE were, respectively, +2, +4 and +1 (tab. 1). Besides, no changes were observed in the structure of the pathogen hyphae and pycnidia filled with conidia were abundantly formed on the whole surface of P. strasseri colonies. In the case of F. oxysporum, IBE value was negative, which testifies to the lack of the inhibiting effect of that fungus on P. strasseri (tab. 1). A similar relationship was observed in the case of the common growth of the pathogen with Penicillium verrucosum var. cyclopium (tab. 1). The colonies of Gliocladium spp. met the colony of P. strasseri after 17 days of common growth, while after 20 days they overgrew 1/4 of the surface of the pathogen colony. After 34 days of common growth, the whole colony of P. strasseri was covered with the mycelium of the tested species from genus *Gliocladium*. The hyphae of the hyperparasite wound around the pathogen hyphae, growing inside them and inside the conidia, causing their degeneration. Phoma exiqua var. exiqua and P. heteroderae proved to be the species that limited the growth of P. strasseri colonies only to a limited extent since the value of IBE for those fungi was +3 (tab. 1). Besides, with the common growth of *P. strasseri* with the fungi enumerated above, a 3 mm inhibition zone was observed.

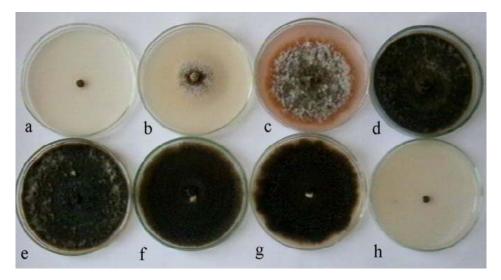


Photo 4. 14-day-old colonies of *P. strasseri* on malt agar at temperature: -6°C (a); 5°C (b); 10°C (c); 16°C (d); 20°C (e); 24°C (f); 28°C (g); 32°C (h). Photo E. Zalewska

Fot. 4. 14 dniowe kolonie *P. strasseri* na pożywce maltozowej w temperaturze: -6°C (a); 5°C (b); 10°C (c); 16°C (d); 20°C (e); 24°C (f); 28°C (g); 32°C (h). Fot. E. Zalewska

The studies on the effect of thermal conditions pointed to considerable differentiation in the growth of *P. strasseri* colonies cultured at different temperatures. None of the studied fungi isolates formed the air mycelium at the temperatures of -6° C and 32° C (photo 4). When the cultures had been removed from the temperature of -6° C to 24° C, the fungus isolates formed an olive-brown air mycelium 4 days afterwards. Single pycnidia, with conidia inside, appeared after 6 days of the culture. After P. strasseri cultures were removed from the temperature of 32°C to the temperature of 24°C, none of the fungus isolates renewed its growth. At the temperatures ranging from 16°C to 28°C, a dynamic growth of the colonies was observed already 4 days later of the culture. All isolates formed an olive-brown air mycelium with an olive-brown reverse. The edge of the colonies was regular (photo 4). At the temperature of 10°C, white fluffy air mycelium hyphae appeared around the inoculum after 6 days of the culture. In 10 days, the mycelium overgrew the surface of the medium and the reverse took on an olive-green colour. At the temperature of 5°C, loose mycelium hyphae were not observed until the 8th day of the colony. Earlier, only a poor substrate mycelium was visible. The formation of pycnidia (photo 5) at the temperatures ranging from 20°C to 28°C was observed after 6 days of the culture, and at the temperatures from 10°C to 16°C after 8 days of the culture. From 35 to 50 pycnidia per 1 cm² were formed at the temperatures from 24-28°C, whereas at the temperatures ranging from 16-20°C there were 15 to 25 of them. Cream-salmon colour drops of the conidial exudate coming out of the pycnidia (photo 6) were visible at the temperatures 24-28°C already after 4 days, while at the temperature of 16°C after 12 days of the culture. The formation of single pycnidia with scarce conidia in them were observed at the temperature of 5°C after 10 days of the culture; however, no drop of the exudate was observed at the top of the pycnidia till the last day of the culture.



Photo 5. Pycnidia with ostioles of *P. strasseri*; SEM × 790. Photo M. Wróbel Fot. 5. Piknidia *P. strasseri* z ujściami; SEM × 790. Fot M. Wróbel

266

Acta Sci. Pol.

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isolates	60	32°C		7	28°C		(1	24°C			20°C		1	16°C		1	10°C			5°C			-6°C	
	x	p1	\mathbf{p}_2	x	p1	\mathbf{p}_2	×	h	\mathbf{p}_2	x	рı	p_2	x	ц	\mathbf{p}_2	×	ld	p_2	×	¹ d	\mathbf{p}_2	x	\mathbf{p}_1	\mathbf{p}_2
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365 5	5.0	e	A	83.8	q	В	90.06	а	V	90.0	а	V	90.06	а	A	73.8	c	D	31.8	р	C	5.0	e	Α
4	5.0	e	¥	90.06	а	A	90.0	а	V	90.06	а	V	85.5	q	В	78.3	c	C	36.0	р	В	5.0	e	A
435 5	5.0	p	A	88.8	а	A	90.06	а	V	90.06	а	V	90.06	а	A	80.3	q	В	28.5	c	D	5.0	р	A
398 5	5.0	р	A	90.06	а	A	90.0	а	V	90.0	а	V	90.06	а	A	78.8	q	C	38.5	ပ	В	5.0	р	V

x - diameter of colonies in mm - średnica kolonii w mm,
p₁ - differences depending on temperature for isolate - róźnice w zależności od temperatury dla danego izolatu,
p₂ - differences between isolates at given temperature - róźnice między izolatami w danej temperaturze,
The means differ in a significant way if they are not marked with the same letter - Wartości oznaczone ta samą literą nie róźnią się istotnie.

B. Zimowska

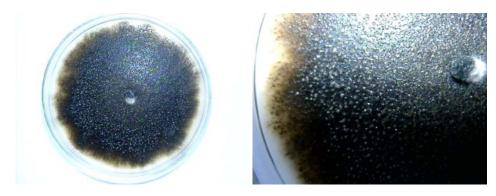


Photo 6. 14-day old colony of *P. strasseri* at 24°C and drops of conidial exudates (a); × 15 (b). Photo E. Zalewska

It was proved statistically that the diameter of 14-days' colonies of *P. strasseri* isolates growing at the same temperature did not generally differ significantly, with an exception of the isolates growing at the temperatures of 10°C and 5°C (tab. 3). Besides, at the temperature of 28°C, only the diameter of the colony of M 365 isolate was significantly larger than the colony diameter of the other isolates, while at the temperature of 16°C such a relationship was observed for isolate M 314 (tab. 3). The greatest diameter was reached by the studied isolates of *P. strasseri* at the temperatures of 16°C, 20°C, 24°C and 28°C, and it was considerably different from the colonies of isolates cultured at the temperatures of 5°C and 10°C, except isolate CBS 126.93, which grew at the temperature of 10°C (tab. 3).

DISCUSSION

The present studies showed that with the majority of the tested fungi the IBE value was positive, which suggests that *P. strasseri* growth can be inhibited by those fungi in the phyllosphere of peppermint stems and rhizomes. It is worth noticing that small positive values were obtained in the case of *Fusarium* spp., which belong to fast growing, toxin-forming fungi pathogenic towards a lot of herbal plants [Reuveni 1982, Machowicz-Stefaniak and Zalewska 2004, Zalewska and Machowicz-Stefaniak 2004]. This can point to competitive abilities of *P. strasseri* towards *Fusarium* spp., which finds its justification in the production of a large amount of the infectious material by *P. strasseri* and its ability for fast, dynamic growth, which was confirmed by the studies on the effect of thermal conditions on the pathogen's growth. Therefore, if in natural conditions *P. strasseri* causes primary infection of peppermint stems and rhizomes, facultative parasites from genus *Fusarium* may find it difficult to enter into a parasitic contact with the tissues of peppermint plants. The thesis is confirmed by the results of the mycological analysis of the infected stems and rhizomes of mint growing in the

268

Fot. 6. 14-dniowa kolonia *P. strasseri* w temperaturze 24°C i krople wydzieliny konidiów (a); \times 15 (b). Fot. E. Zalewska

świętkorzyskie voivodeship, where the frequency of isolating *P. strasseri* from the rhizomes was higher than that of fungi from genus *Fusarium*, and it was only slightly smaller in the case of the stems [Zimowska 2007].

Negative values of IBE obtained after 10 days of common growth of *Gliocladium* spp. and *P. strasseri* result from the manner of their antagonistic effect. Those fungi are mainly known for their abilities for antibiosis and overparasitism; hence, the full antagonistic effect of those fungi is visible only after 30–40 days, which was observed in the present studies and earlier, for such pathogens as *Botrytis cinerea, Septoria carvi* and *Seimatosporium hypericinum* [Machowicz-Stefaniak 1998, Zimowska 2004, Machowicz-Stefaniak et al. 2008].

A strong inhibiting effect of fungi from genus *Trichoderma* on *P. strasseri*, which is shown in complete overgrowth of the mycelium and degradation of hyphae and conidia, should be positively estimated in the practical aspect of using the strains of *T. har-zianum* and *T. koningii* in biological control of *P. strasseri*. Strong competitive abilities of *Trichoderma* spp. resulting from the production of endo- and exoenzymes, toxic metabolites and from overparasitism [Papavizas 1985, Fokkema 1995] were used, for example, in India, to control *Fusarium oxysporum* f.sp. *cumini* [Singh et al. 2007], in Canada to limit the occurrence of the wilt of purple coneflower caused by *Sclerotinia sclerotiorum* [Chang et al. 2006], as well as in Argentina to control coriander alternariosis caused by *Alternaria alternata* [Sandoval et al. 2006].

The other species of the tested fungi, i.e. *A. alternata, B. cinerea, R. solani* or *Phoma exigua* var. *exiqua,* cannot be regarded as positive antagonists since they belong to the species that are pathogenic towards a lot of herbal plants, including peppermint [Karla et al. 2004, Zimowska 2007, Machowicz-Stefaniak et al. 2008].

The present studies on the effect of thermal conditions on the growth and formation of the infectious material showed that the vegetative growth of *P. strasseri* colonies is possible within a wide range of temperatures, i.e. from 5°C to 28°C. The temperature of 32°C was considered negative for the growth and development of the fungus. The temperature of -6°C did not prove destructive towards the pathogen as after moving the cultures to the temperature of 24°C after 14 days of the experiment, the fungus isolates resumed their growth and even started to sporulate. This can suggest the ability of P. strasseri mycelium to survive on the infected parts of peppermint where in early spring, when the temperature reaches the values over 0°C, the infectious material is formed, which spreads the pathogen at the time of vegetation. The temperature between 16°C and 28°C can be regarded as the thermal optimum for the fungus growth, while the range of temperatures from 24°C to 28°C is optimum for the formation of the infectious material. Basing on the present studies, P. strasseri can be called an eurythermic species [Roustaee et al. 2000] since – contrary to stenothermic species which can survive only small ranges of temperatures - it can develop in a wide range of temperatures. The thermal requirements of P. strasseri are similar to those of other species from genus Phoma, e.g. P. exigua var. exigua [Zhao and Shamoun 2006], P. lingam [Vanniasingham and Gilligan 1988] or P. macdonaldii [Roustaee et al. 2000]. It follows from the present studies that the thermal conditions in Poland during the vegetation of mint are conducive to the development of the fungus and the formation of a large amount of the

infectious material, which can result in a dynamic development of disease symptoms on peppermint rhizomes and stems.

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BIOTYCZNA AKTYWNOŚĆ Phoma strasseri ORAZ WPŁYW WARUNKÓW TERMICZNYCH NA WZROST I TWORZENIE MATERIAŁU INFEKCYJNEGO PATOGENA W WARUNKACH in vitro

Streszczenie: *Phoma strasseri* wyizolowano po raz pierwszy z roślin mięty pieprzowej (*Mentha piperita* L) w 2004 r. Gatunek ten wcześniej nie był notowany w Polsce. Biotyczne interakcje pomiędzy *P. strasseri* a 16 gatunkami grzybów zasiedlających fylosferę łodyg i rozłogów mięty pieprzowej określono metodą szeregów biotycznych, stosując pożywkę maltozową MA. Oddziaływanie poszczególnych gatunków grzybów na *P. strasseri* wyrażano indywidualnym, ogólnym oraz sumarycznym efektem biotycznym. Za najbardziej efektywnych i pozytywnych antagonistów uznano grzyby z rodzaju *Trichoderma. Alternaria alternata, Botrytis cinerea, Rhizoctonia solani* pomimo wysokich wartości IBE uznano za negatywnych antagonistów. Badania nad wpływem warunków termicznych wykazały, że optimum termiczne dla wzrostu grzyba mieści się w zakresie temperatury od 16°C do 28°C, a dla tworzenia materiału infekcyjnego od 24°C do 28°C. Na podstawie wykazanej zdolności do rozwoju *P. strasseri* w szerokim zakresie temperatury zaliczono go do organizmów eurytermicznych.

Slowa kluczowe: czarna zgnilizna łodyg i rozłogów, organizm eurytermiczny, grzyby fylosferowe, mięta pieprzowa

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