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Studies on Insect Killing by *Scopulariopsis brevicaulis* (*Sacc.*) Bainier (*Moniliales, ser. Annellorsporae*)

Badanie właściwości owadobójczych *Scopulariopsis brevicaulis* (*Sacc.*) Bainier (*Moniliales, ser. Annellorsporae*)

Scopulariopsis brevicaulis (*Sacc.*) Bainier commonly occurs in various natural environments of Europe, Asia, Australia, North and South America. It has been isolated from different types of soil and from products of plant and animal origin (Morton and Smith 1963, Domsch and Gams 1970).

Scopulariopsis brevicaulis belongs to fungi that have been frequently isolated from insects, including those associated with agricultural, horticultural and forest trees cultivation (Kalviš11 1970ň Żłowacka-Pilot 1974, Bałazy 1976, Lipa 1976, Machowicz-Stefaniak 1976). However, attempts at artificial infection of various insects by *S. brevicaulis* have often been of poor efficiency (Kalviš11 1970ň Żłowacka-Pilot 1974, Bałazy 1976, Machowicz-Stefaniak 1976). It seems that isolation of *S. brevicaulis* from dead insects in their natural habitats constitutes insufficient proof that it is the cause of death of these insects. Therefore, the purpose of these studies was to find the vital requirements of *S. brevicaulis* and to explain its possible pathogenic connections with insectcs.

MATERIAL AND METHODS

The studies have been based on one-spore cultures of *Scopulariopsis brevicaulis* strain M.n. – 602 that had been obtained from the larvae L3 *Malacosoma neustria L.* The fungus culture was grown on the media: potato dextrose agar (PDA), pepton agar (PA) and Czapek Dox (CzD) at 5°C, 10°C, 15°C, 20°C, 25°C, 30°C, 35°C according to Machowicz-Stefaniak (1988). The effect of the culture medium and temperature on the fungus growth was determined on the basis of the culture medium and temperature on the fungus growth was determined on the basis of the linear growth of a colony and sporulation intensity and the data obtained were statistically analyzed using the Duncan's test (Oktaba 1965).

Moreover in a laboratory experiment, the pathogenic effect of *S. brevicaulis* on mature larvae of *Anthonomus pomorum L.*, *Laspeyresia pomonella L.*, *Malacosoma neustria L.* and *Achroea grisella F.* was examined. As the infective material was the conidia obtained from one – spore cultures cultivated on the PA medium for 21 days at 25°C were used. The infectious suspension contained

3×10^7 spores in 1 ml distilled water. Two different ways aiming to contact insects with the fungus spores, described in detail in an earlier paper (Machowicz – Stefaniak and Miczulski 1985) were applied.

Test I. Inoculation of larvae through application of drops of water suspension of *S. brevicaulis* spores onto the dorsal side of the larva's abdomen.

Test II. Infecting larvae and simultaneous pierçing of their cuticle with a needle. In both experimental cases control combinations were set up. Each single experiment included 40 larvae of each insects species. The insects were reared at 24 – 25°C in the way described by Machowicz – Stefaniak (1987). Reisolation of the fungus species from dead larvae was performed according to Koch's rules.

RESULTS AND DISCUSSION

Differentiation in the growth rate of the *S. brevicaulis* colonies depending on the temperature and medium is presented in table 1. At 25°C the diameter of the 16 day old fungus colony grown on each medium was significantly higher than at the remaining temperatures. The fungus growth was fairly intensive at 30°C since the diameter of the 16 day old colonies was significantly higher than the diameter of the colonies growing on the media examined at temperatures above 30°C and below 20°C. Moreover, at 25°C and 30°C the highest colony diameter was reached by the fungus grown on PA and it was significantly higher than the colony diameter on CzD and PDA (tab. 1) Similarly, at 20°C and 15°C the size of the *S. brevicaulis* colonies on CzD and PDA was significantly lower than that on PA. At 35°C, 10°C and 5°C the colony growth was very slow on all applied media.

Sporulation intensity of *S. brevicaulis* on the media examined was fairly high at a temperature range of 15 to 30°C (tab. 2). Only at 30°C and 25°C the number of conidia formed on 1 cm^2 PA medium was significantly higher than on PDA and CzD media. At 5°C the fungus did not form spores (tab. 2).

Tab.1. Effect of temperature and of culture medium on the size of 16 – day old colonies of *Scopulariopsis brevicaulis*

Culture medium temperature	Diameter of 16 – day – old Scopupularopis brevicaulis colonies (mm)								
	PDA			CzD			PA		
	M	P1	P2	M	P1	P2	M	P1	P2
35°C	30.71	a	A	32.25	a	A	30.00	a	A
30°C	50.00	a	B	59.25	b	B	77.50	c	B
25°C	59.62	a	C	69.50	b	C	81.25	c	C
20°C	42.75	a	D	46.12	a	D	71.75	b	B
15°C	29.25	a	A	25.25	a	E	46.75	b	D
10°C	1.87	a	E	0.87	a	F	3.25	a	E
5°C	0.75	a	E	0.75	a	F	0.75	a	E

PDA – potato – dextrose – agar
CzD – Czapek – Dox
PA – pepton – agar
M mean
P1 – differences depending on culture medium at given temperature – small letters
P2 – differences depending on temperature on given culture – capital letters
Means differ significantly ($P<0.01$) if they are not marked with the same letter

Tab. 2. Sporulation intensity of 16 day old *Scopulariopsis brevicaulis* cultures depending on temperature and culture medium

Culture medium temperature	Number of spores from 1 cm^2 culture								
	PDA			CzD			PA		
	M	P1	P2	M	P1	P2	M	P1	P2
35°C	678.344	a	AB	242.038	a	B	500.000	a	BC
30°C	1.237.515	a	A	1.098.728	a	A	1.958.599	b	A
25°C	754.777	a	AB	764.331	a	AB	1.805.732	b	A
20°C	786.624	a	AB	931.371	a	A	713.376	a	BC
15°C	717.834	a	AB	611.465	a	AB	1.047.771	a	B
10°C	407.643	a	B	280.255	a	B	219.745	a	C
5°C	0		no sporulation				0		

Tab. 3. *Scopulariopsis brevicaulis* reisolates obtained from dead larvae

Treatment	Species of insects	Larval instars	Number (%) of dead larve from which the fungus was reisolated			
			Test I (40x2)	Test II (40x2)		
			days after infection			
			5 10 15	5	10	15
Application of spore suspension	Anthonomus pomorum L.	L4	no reisolation	5(12.5)	5(12.5)	7(17.5)
	Laspeyresia pomonella L.	L5		2(5)	5(12.5)	6(15)
	Malacosoma neustria L.	L4		4(10)	7(17.5)	9(22.5)
	Achroea grisella F.	L4		6(15)	9(22.5)	16(40)
Application of water	Anthonomus pomorum L.	L4	no reisolation	no reisolation		
	Laspeyresia pomonella L.	L5				
	Malacosoma neustria L.	L4				
	Achroea grisella F.	L4				

Observation of insect's larvae artificially infected with S. brevicaulis showed that their feeding was less intensive than that of the control larvae. The first dead individuals appeared five days after infection, which fell on the period of pupation (tab. 3). The mortality was regularly higher in the test when the larvae skin was injured. The dead larvae were initially soft with black necrotic spots 1 & 4 mm in diameter on the dorsal side of abdomen, then they dried up and most often they became flat. Only few dead individuals of *Anthonomus pomorum* and *Achroea grisella* underwent the process of mummification.

Mycological analysis of the dead larvae showed that in test I they had not been infected with the fungus examined because no *S. brevicaulis* was reisolated from any of the individuals treated with the fungus suspension (tab. 3). However, in test II where possibilities of infection through the skin injury had been assumed, the examined fungus was reisolated from 7 (17,5 %) dead larvae of *A. pomorum*, 6 (15 %), *L. pomonella* 9 (22,5 %). *M. neustria* and 16 (40 %) *A. grisella* (tab. 3).

Although in literature *S. brevicaulis* has been regarded as tolerant towards temperature fluctuations (Morton and Smith 1963, Domsch and Gams 1970), the present studies indicate that it reacts favourably to rather increased tempe-

rature. It has been determined that the fungus growth is most intensive at 25°C – 30°C, whereas sporulations occurs at a wider temperature range i. e. 15°C – 30°C. The pepton agar medium appeared to be most favourable for the mecelium growth and sporulation of *S. brevicaulis*, similarly as in cases of the majority of common entomogenous hyphomycetes (Machowicz – Stefaniak 1988). The results imply that *S. brevicaulis* can be regarded as a facultative pathogen of insects, however, cuticle scratches seem to be a major factor of the infection process initiation. There is no mention of chitin decomposition in summarized data on biochemical activity of this fungus (Domsch et al. 1980). Most probably it is not able to produce chitynolytic enzymes or their activity is too weak to perforate insect cuticle.

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STRESZCZENIE

Celem pracy było poznanie wymagań życiowych *S. brevicaulis* oraz wyjaśnienie jego ewentualnych powiązań chorobotwórczych z owadami. Hodowlę grzyba prowadzono na pożywce ziemniaczano-glukozowej, Czapek-Dox i peptonowej w temperaturze 5°C, 10°C, 15°C, 20°C, 25°C, 30°C, 35°C. Uzdolnienia owadobójcze grzyba badano dla larw *A. pomorum, L. pomonella, M. neustria, A. grisella*, wzrost grzyba przebiega najintensywniej w temperaturze od 25°C do 30°C, a zarodnikowanie od 15°C do 30°C. Najkorzystniejszym podłożem dla wzrostu grzybni i zarodnikowania *S. brevicaulis* jest pożywka peptonowa. Badany grzyb można uznać tylko warunkowym patogenem owadów ze zranionym oskórkiem.