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POSSIBILITIES OF GROWTH AND DEVELOPMENT SUPPRESSION OF *Topospora myrtilli* (Feltg.) Boerema ON ARTIFICIAL MEDIA AND STEMS OF HIGHBUSH BLUEBERRY (*Vaccinium corymbosum* L.)

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Abstract. In presented studies was undertaken the attempt to determine the effectiveness of such preparations as Biochikol 020 PC and Biosept 33 SL in limiting the growth of *T myrtilli in vitro*. Moreover, the protective effect of these biopreparations and Dithane M45 80WP for highbush blueberry stems was determined. The studies carried out i*n vitro* indicated that Biochikol 020 as well as Biosept 33 SL significantly limited the growth and development of pathogen. Very strong inhibition of hyphae growth and its deep degradation were caused by Biosept 33 SL in concentration 0.3%.
Similarly, in experiment on protecting action of selected preparations for stems of highbush blueberry against *T. myrtilli*, Biochikol 020 and Biosept 33 SL inhibited the growth of pathogen. But its protective effects were weaker in comparison to Dithane M45 80WP because after the application of this preparation the development of disease symptoms caused by *T. myrtilli* was significantly lower.

Key words: Topospora myrtilli, Vaccinium corymbosum, biopreparations, fungicides

INTRODUCTION

The fungus *Topospora myrtilli* (Feltg.) Boerema belongs to dangerous pathogen of ericaceous plants, including highbush blueberry (*Vaccinium corymbosum* L.). It causes canker of stems and fruit rot in all regions of this plant crop over the world [Oudemans et al. 1998, Stromeng and Stensvand 2001, Szmagara and Machowicz-Stefaniak 2005]. Symptoms of disease in the form of ellipsoidal canker spots of chestnut-brown colour with a brighter centre and a red-purple edge are located mainly in lower parts of stems. Infection occurs in period from bud swelling to falling down of leaves, through leafstalk marks or intact tissue of one or two-years-old stems [Weingartner and Klos 1975,

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Borecki and Pliszka 1978, Stromeng and Stensvand 2001, Szmagara and Machowicz-Stefaniak 2004].

Destructive action of *T. myrtilli* lead to dying of stems above infection place or even all shrubs. Fungus can also cause significant losses on fructified and well-kept plantations and during fruit storage [Oudemans et al. 1998, Stromeng and Stensvand 2001, Szmagara and Machowicz-Stefaniak 2005].

Suppression of pathogenic fungi growth by preparations of organic origin as, i.e. Biochikol 020PC and Biosept 33SL is thought to be perspective. Active compounds included in these preparations have possibilities to inhibit the growth of bacteria and fungi affecting crop plants [Wojdyła and Orlikowski 1997, Woedtke 1999, Pięta et al. 2004, Król 2005].

By reason of significant harmfulness of *T. myrtilli* were carried out the studies on possibilities of suppression of its growth *in vitro* and *in vivo* by selected preparations to protection the stems of plantings of highbush blueberry against the infection of this pathogen.

MATERIALS AND METHODS

Three isolates of *Topospora myrtilli* obtained from highbush blueberry stems (Bw 1535, Bw 1577, Bw 2462) and biopreparations Biochikol 020PC and Biosept 33SL were selected to investigations. To experiment with Biochikol 020PC were used four concentrations: 0.01%; 0.025%; 0.05%; 0.1% of preparation and poor malt extract agar (Difco) with decreased to a half amount of maltose per litre [Wojdyła and Orlikowski 1997, Pięta et al. 1998, Pastucha 2001]. Biosept 33SL was used in 0.05%; 0.1%; 0.2%; 0.3% concentrations with the standard PDA medium [Orlikowski et al. 2001].

Suitable amounts of each preparations were added to sterilised and cooled medium. Subsequently, on solid medium the inocula of fungus, i.e. discs of particular isolates taken from 10-day-old sporulating colonies, were put. The incubations were carried out at 22°C. Four replications were used for each isolate of fungus and each tested concentration of preparation. Colonies of the fungus cultivated on malt extract agar without the additions of biopreparations were used as a control material. After 4 and 8 days the diameter of colonies were measured and results were submitted to statistical analysis. Materials to microscopic observations were taken from different sites of each Petri dish.

The protective effect of selected preparations to highbush blueberry stems was determined in the same trial of studies. The study material consisted of potted, well-developed plantings of highbush blueberry of Jersey cultivar, an isolate of *T. myrtilli* (Bw 2462) and preparations: Biochikol 020PC, Biosept 33SL and Dithane M45 80WP. The investigations were carried out under climatic chamber conditions. The stems of plants, directly after inoculation with fungus, were sprayed with one of biopreparations or fungicide. The infection material was consisted of 2 mm discs of *T. myrtilli* taken from 14-day-old sporulating colonies cultivated on PDA medium. The inocula of fungus were placed on pricked stems. Solutions 0.5% Biochikol 020PC, 0.1% %, Biosept 33SL and 0.3% Dithane M45 80WP were applied. Concentrations of preparations were

used in accordance with recommendations of producer and according to Wojdyła and Orlikowski [1997] and Orlikowski et al. [2002].

Positive control material were the stems of plantings with damaged epidermis, on which discs of fungus inoculum was placed and sprayed with sterile distilled water. Negative control material were the stems with damaged epidermis and sprayed with sterile distilled water. Each combination of the experiment included sixty places with the damaged epidermis. Forty plantings of Jersey cultivars were used in studies.

Prepared in this way plants were placed in climatic chamber and sprayed with sterile distilled water once a day. During the experiment, from 6 a.m. to 8 p.m., the light intensity was at the level 3200 luxes and temperature 22°C, and from 8 p.m. to 6 a.m. plants stayed at dark conditions and 18°C. The humidity of the air in the climatic chamber was 90–95%.

Observations of health status and estimation of the effectiveness of preparations were performed in 6-days intervals. During each observation the occurrence or lack of disease symptoms were established, what lead to determine the number of effective infections. After the appearance of first disease symptoms were performed the measurements of created necroses and number of *T. myrtilli* pycnidia per 1 cm^2.

In the last step of experiment, the fungus was reisolated from each inoculated site on stems according to Koch's rules.

RESULTS

The performed studies indicated that the diameters of four-day-old and eight-day-old colonies of *T. myrtilli* cultivated on medium enriched with Biochikol 020 PC, as well as Biosept 33 SL, were significantly smaller than diameters of control colonies. In the case of Biochikol 020 PC, the smallest diameters of four-day-old colonies, 6.12 mm and 6.88 mm, were observed for the isolate Bw 2462 cultivated on medium containing respectively 0.1% and 0.05% of this biopreparation. The growth of colony of Bw 1535 isolate was the most inhibited at 0.1% of preparation and diameter equalled 7.62 mm. Size of Bw 1577 isolate colony on medium enriched with Biochikol 020 PC in four tested concentration did not differ significantly. Whereas, mean size of *T. myrtilli* colony was significantly lower at 0.05% and 0.1% Biochikol 020 PC than at 0.01% and 0.025% concentration of this preparation (tab. 1).

On the other hand, the smallest diameter of eight-day-old colonies, equalled 6.62 mm was noted in the case of Bw 2462 isolate, cultivated on medium enriched with 0.1% of preparation and it was significantly lower in comparison with diameter of control colony (27 mm) (tab. 1). The sizes of eight-day-old colonies of Bw 1535 and Bw 1577 isolates on medium containing particular concentrations of Biochikol 020 PC did not differ significantly. Only in the case of Bw 2462 isolate, the diameters of eight-day-old *T. myrtilli* colonies on medium with 0.05% and 0.1% Biochikol 020 PC were significantly lower than on medium containing 0.01% and 0.025% of this preparation (tab. 1). It was also observed that after 8 days of growth, irrespective of Biochikol 020 PC concentration in medium, occurred changes in the macroscopic appearance of colony in each studied *T. myrtilli* isolates. The colonies of fungi cultivated on me-

Table 1. Influence of Biochikol 020 PC on size of *Topospora myrtilli* colony
Tabela 1. Oddziaływanie Biochikolu 020 PC na wielkość kolonii *Topospora myrtilli*

Isolate Izolat	Concentration – Stężenie									
	0.01%	0.025%	0.05%	0.1%	control kontrola	0.01%	0.025%	0.05%	0.1%	control kontrola
	diameter of 4-day-old colonies, mm średnica 4-dniowych kolonii, mm					diameter of 8-day-old colonies, mm średnica 8-dniowych kolonii, mm				
Bw 1535	10.50 de	11.75 e	9.00 cd	7.62 abc	14.12 f	12.12 c	12.00 c	10.5 bc	9.38 abc	24.00 d
Bw 1577	9.25 cd	8.25 bc	8.88 cd	8.38 bc	15.75 f	10.00 bc	9.25 abc	9.75 bc	9.50 abc	33.38 f
Bw 2462	9.25 cd	8.62 bcd	6.88 ab	'6.12 a	15.25 f	11.38 bc	10.88 bc	9.00 ab	6.62 a	27.00 e
	$NIR_{0.05}$ = 1.88					$NIR_{0.05}$ = 2.91				
Average Średnia	9.67 c	9.54 c	8.25 b	7.38 a	15.04 d	11.17 c	10.71 bc	9.75 ab	8.50 a	28.12 d
	$NIR_{0.05}$ = 0.86					$NIR_{0.05}$ = 1.33				

Values marked with the same letter do not differ significantly – Wartości oznaczone tą samą literą nie różnią się istotnie

Table 2. Influence of Biosept 33 SL on size of *Topospora myrtilli* colony
Tabela 2. Oddziaływanie Bioseptu 33 SL na wielkość kolonii *Topospora myrtilli*

Isolate Izolat	Concentration – Stężenie									
	0.05%	0.1%	0.2%	0.3%	control kontrola	0.05%	0.1%	0.2%	0.3%	control kontrola
	diameter of 4-day-old colonies, mm średnica 4-dniowych kolonii, mm					diameter of 8-day-old colonies, mm średnica 8-dniowych kolonii, mm				
Bw 1535	9.12 b	8.75 b	8.12 b	3.00 a	14.88 c	10.75 d	9.37 bcd	8.37 bcd	3.00 a	34.88 e
Bw 1577	9.00 b	7.12 b	7.62 b	7.12 b	15.00 c	9.62 cd	8.38 bcd	8.12 bcd	8.38 bcd	33.00 e
Bw 2462	7.12 b	7.12 b	5.25 ab	5.37 ab	15.25 c	7.50 bcd	7.75 bcd	5.38 ab	6.00 abc	34.25 e
	$NIR_{0.05}$ = 4.05					$NIR_{0.05}$ = 4.05				
Average Średnia	8.41 b	7.67 b	7.00 ab	5.17 a	15.04 c	9.29 c	8.50 bc	7.29 ab	5.79 a	34.04 d
	$NIR_{0.05}$ = 1.85					$NIR_{0.05}$ = 1.85				

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dium with Biochikol 020 PC were brighter, i.e. white-grey, in comparison to control colony of lemon-green colour. The hyphae of aerial mycelium formed a loose structure, as distinguished from felty mycelium of control colonies.

In the case of Biosept 33 SL use, after four days of *T. myrtilli* cultivation, the minimal sizes of colonies were observed at 0.3% concentration. The size of colonies of pathogen at Bw 1535 and Bw 2462 isolates growing on medium containing tested concentrations of Biosept 33 SL did not differ significantly (tab. 2). Similarly, isolate Bw 1535 of *T. myrtilli* after eight days of growth on medium containing 0.3% of preparation formed significantly the lowest colony and has minimal increment (tab. 2).

The highest diameter of colony at 0.05% concentration of preparation after four days of cultivation was noted at isolates Bw 1535 and Bw 1577, respectively 9.12 mm and 9.0 mm, whereas after eight days diameter reached 10.75 mm at Bw 1535 isolate, but these values were significantly smaller in comparison to control (tab. 2).

The performed experiments indicated changes in macro- and microscopic appearance of *T. myrtilli* colonies cultivated on medium containing Biosept 33 SL. Both four- and eight-day-old colonies of pathogen, regardless of biopreparation concentration, were dark grey colour. The hyphae of aerial mycelium were significantly shortened, thickened and formed compact, fluffy-hairy structure, whereas the surface of control colony was bright lemon-green and felty. Moreover, hyphae of tested isolates cultivated in the presence of 0.2 and 0.3% concentrations of Biosept 33 SL demonstrated the symptoms of high degeneration.

In the studies on protection action of selected preparations to highbush blueberry stems the first disease symptoms on the stems was observed on positive control and in Biosept 33 SL combinations after ten days of experiment. Single, necrotic spots, 3–5 mm diameter were found on stems around the place of infection. These spots were characteristic around shape and chestnut-brown colour.

After next six days of observations, necrotic spots were observed on all plants of positive control, preventively treated with biopreparations and on the part of plants treated with Dithane M45 80 WP. Necrotic spots increased, achieved the length ranging from 5 mm to 15 mm.

The single pycnidia on surface of necrosis on stems protected with biopreparations were appeared after 30 days of observations. Significantly more pycnidia were on necrosis spots of positive control stems.

The most places with symptoms of necrosis, i.e. 75% were observed on stems of positive control, and its mean percentage was significantly higher than in the case of plants protected with Dithane M45 80 WP and negative control combination (tab. 3).

Similarly, plants protected with Biosept 33 SL and Biochikol 020 PC indicated symptoms of numerous necrosis, and mean percentage of places with symptoms was 9.00% and 5.25% respectively, and did not differ significantly from positive control (tab. 3). The size of necrosis on stems of plants protected with Biosept 33 SL, in positive control and treated with Biochikol 020 PC were 8.35 mm, 7.65 mm and 6.00 mm, respectively. These values were significantly higher than obtained in combination with Dithane M45 80 WP and negative control (tab. 4). The highest mean number of pycnidia, equalled 18.18 units per 1 cm^2, was observed on necrosis of stems in positive control and on necrosis of stems protected with Biosept 33 SL, where occurred 15 units per 1 cm^2.

Development of disease symptoms was significantly weaker or did not occurred on stems of plants protected with Dithane M45 80 WP. Diameters of necrosis ranged from 4 mm to 10 mm, and mean size of necrosis was 1.95 mm (tab. 4). On necrosis of stems of these plants did not observed the presence of pycnidia, like in the case of negative control stems (tab. 4).

After 56 days of experiments on stems of plants protected with fungicide disease symptoms did not intensified and looked like in 30th day.

During all time of observations did not noticed disease symptoms on stems of negative control plants, which maintained healthy appearance, in comparison to positive control.

Table 3. Protection of highbush blueberry against *Topospora myrtilli* infection by selected preparation (after 27 days)

Tabela 3. Ochrona pędów borówki wysokiej przed infekcją *Topospora myrtilli* za pomocą wybranych preparatów (po 27 dniach)

Variants of experiment Warianty doświadczenia	Number of infected places on stems Liczba infekowanych miejsc na pędach	Number of places – Liczba miejsc, %			
		with symptoms of necrosis z objawami nekrozy	average średnie	from which *T.myrtilli* was isolated z których reizolowano *T. myrtilli*	average średnie
Dithane M45 80 WP	60	13 (21.66%)	3.25 ab	28 (46.66%)	7.00 b
Biochikol 020 PC	60	21 (35.00%)	5.25 abc	28 (46.66%)	7.00 b
Biosept 33 SL	60	36 (60.00%)	9.00 bc	35 (58.33%)	8.75 b
Positive control Kontrola pozytywna	60	45 (75.00%)	11.25 c	29 (48.33%)	7.25 b
Negative control Kontrola negatywna	60	0.00	0.00 a	0.00	0.00 a
$NIR_{0.05}$			7.11		4.71

Values marked with the same letter do not differ significantly
Wartości oznaczone tą samą literą nie różnią się istotnie

Table 4. Size of necrosis and number of *Topospora myrtilli* pycnidia on highbush blueberry stems after 27 days from use of biopreparates

Tabela 4. Wielkość powstałych nekroz i liczba piknidiów *Topospora myrtilli* na pędach borówki wysokiej po 27 dniach od zastosowania biopreparatów

Variants of experiment Warianty doświadczenia	Average size of necrosis, mm Średnie wielkości nekroz, mm	Average number of pycnidia per 1 cm^2 Średnia liczba piknidiów na 1 cm^2
Dithane M45 80 WP	1.95 a	0.00 a
Biochikol 020 PC	6.00 b	4.62 a
Biosept 33 SL	8.35 b	15.00 b
Positive control Kontrola pozytywna	7.65 b	18.18 b
Negative control Kontrola negatywna	0.00 a	0.00 a
$NIR_{0.05}$	4.09	6.13

Values marked with the same letter do not differ significantly
Wartości oznaczone tą samą literą nie różnią się istotnie

Tested fungus was reisolated from 58.33% of places on stems sprayed with Biosept 33 SL, from 46.66% places treated with Biochikol 020 PC and Dithane M45 80 WP and from 48.33% places on stems of positive control. These values did not differ significantly (tab. 3).

DISCUSSION

Performed studies on effect of Biosept 33 SL and Biochikol 020 PC on *T. myrtilli* confirmed its capacity to direct suppression of fungi growth and causing macro- and microscopic changes of fungus, what correlate with results obtained by Wojdyła and Orlikowski [1997], Pięta et al. [2004], Król [2005]. The studies indicated also existence of intra-species diversification of *T. myrtilli.* It was expressed in morphological variability of colonies of isolates cultivated under different conditions and diversified reaction of some isolates on action of both tested biopreparations. Bw 1577 and Bw 2462 isolates exceptionally strongly reacted on Biochikol 020 PC, and Bw 1535 isolate on Biosept 33SL. Similar relationship was found by other authors, indicated that efficiency of chitozan under *in vitro* conditions depends mainly on susceptibility of species or isolate of fungus [Alan and Hadwiger 1979]. Unique high efficiency of tested biopreparations was noted for Bw 1535 isolate at the highest used concentrations, what was shown in relation to other pathogens by Orlikowski and Skrzypczak [2001] and Król [2005].

In the case of other isolates were stated significant suppression of its colonies growth independently on the level of biopreparation concentration, what was found in relation to chitosan and soil born fungi [Wojdyła and Orlikowski 1997, Pięta et al. 1998]. To treatment of plants by chitosan accompanying such phenomena like intensified lignification and production of phytoalexins and hydrolytic enzymes, which are inductive resistance factors and can occur simultaneously. Chitosan is known in literature as a compound which mobilize plants to rapid resistance reaction on pathogen attack with so-called elicitors, it means inductors of resistance [Pośpieszny and Struszczyk 1994, Pośpieszny 1997].

Probably, owing to these mechanisms of action chitosan contained in tested biopreparation, occurred significant suppression of growth of colony, changes in morphological structures and macroscopic appearance of *Topospora myrtilli* and number of infection of highbush blueberry stems. Many authors earlier gave attention to this in relation to other microorganisms [Pośpieszny and Struszczyk 1994, Pośpieszny 1997, Pięta et al. 1998, Maćkowiak and Pośpieszny 2002, Pięta et al. 2004]. It can be assumed that this biopreparation as the inductor of resistance stimulated plants of highbush blueberry after artificial *T. myrtilli* infection to defensive reactions and slight limited the number of effective infections. Many authors observed positive effect of protective action of chitosan in control of various plants diseases and ascribed its ability to induction of multi-direction defensive reactions [Borkowski et al. 2002, Orlikowski et al. 2002, Pięta et al. 2004].

Similar, compounds presented in grapefruit extract, contained in Biosept 33SL, inhibit the germination of spores and limit the growth of germ hyphae by dehydratation of cytoplasm. Biopreparation cause also delay of size of soil born pathogens population and deformation its spores and decomposition of hyphae [Pięta et al. 2004]. Active compounds contained in this biopreparation inhibit development of microorganisms and induce resistance of plants. It can be assumed that the way of action caused significant

suppression of growth of *T. myrtilli* colonies and changes in morphological structures of fungus and limit the growth of pathogen on highbush blueberry stems.

The efficiency of tested biopreparations is insufficient, but presented results concern preliminary studies and despite of significant activity in suppression of *T. myrtilli* growth it is necessary to continue the experiments.

In subsequent studies should be taken into account the preventive application of Biosept 33 SL in concentrations of 0.2%, 0.3% or 0.1% on plants because of efficiency of mentioned concentrations. It was confirmed by results of the authors' own studies, as well as by Saniewska [2002].

CONCLUSIONS

1. Performed studies indicated an existence of intra-species diversification of *T. myrtilli*, what was expressed in morphological variability and diversified reaction of some isolates on action of biopreparates.

2. Obtained results concerning direct effect of biopreparations *in vitro* as well as on protection of highbush blueberry stems against *T. myrtilli* infection, indicate on necessity of further studies in aspect of highbush blueberry protection against to this pathogen.

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MOŻLIWOŚCI OGRANICZANIA WZROSTU I ROZWOJU *Topospora myrtilli* (Feltg.) Boerema NA SZTUCZNYCH PODŁOŻACH ORAZ NA PĘDACH BORÓWKI WYSOKIEJ (*Vaccinium corymbosum* L.)

Streszczenie. W prezentowanych badaniach podjęto próbę określenia skuteczności preparatów Biochikol 020 PC i Biosept 33 SL w ograniczaniu wzrostu *in vitro T. myrtilli*. Ponadto określano wpływ ochronnego działania tych biopreparatów oraz Dithane M45 80WP dla pędów borówki wysokiej. Przeprowadzone badania *in vitro* wykazały, że zarówno Biochikol 020, jak i Biosept 33 SL znacząco ograniczały wzrost i rozwój patogenu. Bardzo silne hamowanie wzrostu strzępek i ich głęboką degradację powodował przede wszystkim Biosept 33 SL w stężeniu 0,3%. Podobnie w doświadczeniu nad ochronnym działaniem wybranych preparatów dla pędów borówki wysokiej przed *T. myrtilli*, Biochikol 020 i Biosept 33 SL hamowały rozwój patogenu. Jednak ich ochronne działanie było słabsze w porównaniu z Dithane M45 80WP, po zastosowaniu którego rozwój objawów chorobowych powodowanych przez *T. myrtilli* był znacznie mniejszy.

Słowa kluczowe: *Topospora myrtilli, Vaccinium corymbosum*, biopreparaty, fungicydy

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