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FROM STUDIES ON POSSIBILITY OF PROTECTING BLUE SPRUCE (*Picea pungens* Engelm.) AGAINST FUNGI. PART I. LABORATORY ASSESSMENT OF ANTIFUNGAL ACTIVITY OF SELECTED FUNGICIDES

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Abstract: The antifungal activity of two fungicides, i.e. Rovral Flo 255 SC and Sportak Alpha 380 EC was determined by *in vitro* tests to 12 fungal species isolated from blue spruce (*Picea pungens*) and its cultivar Glauca. It was found that Sportak Alpha 380 EC has the highest antifungal activity and the widest range of application to all fungi under examination. Rovral Flo 255 SC has shown significantly poorer activity.

Key words: *Picea pungens*, fungi, control, fungicides

INTRODUCTION

In Poland the health status of blue spruce (*Picea pungens* Engelm.) in urban green areas become more and more important problem [Bartyńska i Mirski 2005, Werner i in. 2001]. The authors present the results of studies on identification of new fungal species that have not been included yet into the list of spruce pathogens. In the protection program for coniferous plants against diseases one can find only recommendations pertaining spruce protection against *Rhizosphaera kalkhoffii* and the fungi belonging to the genus *Fusarium*, causing fusarium root rot in plant nurseries [Łabanowski i in. 2001]. However, there is no information on protecting this plant against other fungi isolated from the spruce and identified in recent years [Bartyńska i Mirski 2005, Werner i in. 2001]. The aim of this paper is to recognize a possibility of protecting blue spruce against fungi that infest this tree by assessing activity of two fungicides, namely Rovral Flo 255 SC and Sportak Alpha 380 EC.

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MATERIAL AND METHODS

An assessment of antifungal activity of fungicides was one of the stages of studies carried out in Kraków in the years 2002–2004 on health status and possibility of protection of blue spruce against fungi.

The antifungal activity and range of application for Rovral Flo 255 SC (of contact action) and Sportak Alpha 380 EC (of systemic and deep action) were evaluated by *in vitro* tests for fungi isolated from tissues (needles and shoots) of diseased trees. The most frequently isolated fungi were selected for testing, namely *Acremonium tubakii, Anthostomella conorum, Arthrinium* state of *Apiospora montagnei, Aureobasidium pullulans, Botryodiplodia rubi, Fusarium camptoceras, F. moniliforme* var. *lactis, Penicillium canescens, Phoma pomorum, Rhizosphaera kalkhoffii, Ulocladium consortiale* and *Zythiostroma pinastri* [Bartyńska i Mirski 2005].

The laboratory tests were carried out by media with amendments [Kowalik i Krechniak 1961]. The fungicide Rovral Flo 255 SC was tested at concentrations of 0.075%; 0.15% and 0.225%, while Sportak Alpha 380 EC at 0.025%; 0.05% and 0.075%. The concentrations of fungicides under examination corresponded respectively to the recommended dose in the plant protection program (second value), the recommended dose reduced by half (first value) and increased by half (third value). When selecting fungicide concentrations the guidelines for coniferous plants protection against diseases were taken into account [(Łabanowski i in. 2001].

During the test fungicides were introduced directly into liquid and slightly cooled PDA agar. After thorough mixing with the agar the obtained suspension was poured into Petrie dishes of 70 mm in diameter. Afterwards, an agar disk with mycelium of fungi under examination was placed centrally into each dish for each combination. A medium without amendments with a disk of appropriate fungus was used as the control for each combination. The test was carried out in three repetitions for each combination. The antifungal efficiency of fungicides under consideration was then calculated form Abbot's formula [Kowalik i Krechniak 1961]:

$$I = \frac{C - T}{C} \cdot 100$$

where: I – fungus linear growth inhibition index (percentage), C – fungus colony diameter in the control combination, T – fungus colony diameter in combination containing a specified fungicide concentration in the agar.

Finally, an effect of fungicides under examination of fungal biology (mycelium morphology, presence of spores, sporification intensity, presence of endosporous forms) was determined.

RESULTS AND DISCUSSION

The tested fungicides showed different effect on the mycelium growth of fungal species depending on oil type and its concentration in the medium (table 1).

Table 1. An effect of fungicides on inhibiting mycelium linear growth (%)
Tabela 1. Wpływ badanych fungicydów na zahamowanie rozrostu liniowego (%) grzybni testowanych gatunków grzybów

Fungus Grzyb	Fungicide and its concentration/ I = inhibition index (%) Fungicyd i jego stężenie / I = współczynnik zahamowania (%)						Control Kontrola
	Rovral Flo 255 SC			Spotrak Alpha 380 EC			
	0.075%	0.15%	0.225%	0.025%	0.05%	0.075%	
Acremonium tubakii W. Gams	83.81 c*	83.57 c	86.91 b	100 a	100 a	100 a	0 d
Anthostomella conorum (Fuckel) Sacc.	88.57 d	89.77 c	97.14 b	100 a	100 a	100 a	0 e
Arthrinium state of *Apiospora montagnei* Sacc.	86.43 d	89.14 c	90.00 b	100 a	100 a	100 a	0 e
Aureobasidium pullulans (de Bary) Arnaud	22.82 b	10.91 d	13.01 c	100 a	100 a	100 a	0 e
Botryodiplodia rubi Syd.	82.63 c	82.86 b	82.86 b	100 a	100 a	100 a	0 d
Fusarium camptoceras Wollenw. et Reinking	50.24 c	48.81 d	55.24 b	100 a	100 a	100 a	0 e
Fusarium moniliforme Sheld var. *lactis* (Pir. et Rib.) Bilai	57.63 d	59.53 b	58.81 c	100 a	100 a	100 a	0 e
Penicillium canescens Sopp	7.68 d	55.49 b	27.46 c	100 a	100 a	100 a	0 e
Phoma pomorum Thüm	83.81 c	84.06 c	87.39 b	100 a	100 a	100 a	0 d
Rhizosphaera kalkhoffii Bubák	82.94 b	80.70 d	82.02 c	100 a	100 a	100 a	0 e
Ulocladium consortiale (Thüm.) Simmons	76.67 d	100 a	96.67 b	100 a	100 a	95.24 c	0 e
Zythiostroma pinastri (Karst.) Höhn.	41.91 d	59.29 c	83.10 b	100 a	100 a	100 a	0 e

* Values marked with the same letter in columns have no significant differences at P = 0.05 (Duncan test)
* Wartości oznaczone takimi samymi literami w wierszach nie różna się istotnie przy p = 0,05 testu Duncana.

The very high antifungal activity and the widest range of application were found for Sportak Alpha 380 EC, regardless of concentrations under investigation; already at the recommended concentration reduced by half this fungicide effectively inhibited the mycelium growth for all fungi under consideration (photo 1–4).

Rovral Flo 255 SC showed slightly poorer antifungal activity. This fungicide had the most inhibitory effect to mycelium of *U. consortiale* and *A. conorum*; and slightly poorer effect on *F. camptoceras* and *F. moniliforme* var. *lactis* (50–60%). One could expect more effective activity of this fungicide to fungi belonging to the genus *Fusarium*, as this preparation is recommended for protecting against fusarium root rot. Per-

haps better antifungal activity of this fungicide to *Fusarium* spp. could be achieved at higher concentrations (tab. 1). The poorest inhibitory effect on mycelium growth was recorded for genera *Penicillium* and *Aureobasidium*. In other fungal species, i.e. *Arthrinium* state of *A. montagnei, A. tubakii, B. rubi, Ph. pomorum, Rh. kalkhoffii* and *Z. pinastri* the mycelium growth was inhibited by 40–90% (tab. 1).

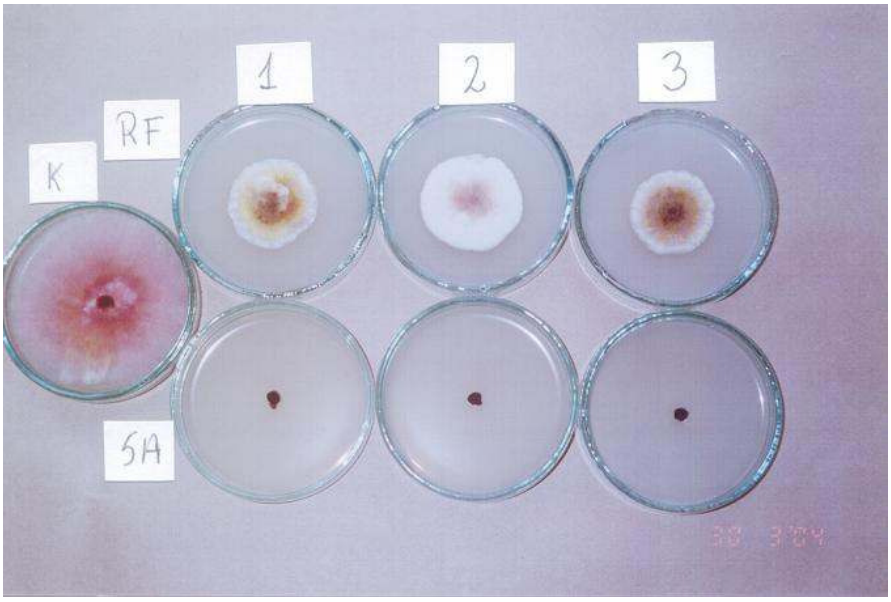

Photo 1. An effect of fungicides on linear growth *Fusarium camptoceras* (K – control; RF, SA – Rovral Flo 255 SC and Sportak Alpha 380 EC, respectively; 1 – concentration 0.075% for Rovral Flo i 0.025% for Sportak Alpha; 2 – concentration 0.15% for Rovral Flo i 0.05% for Sportak Alpha; 3 – concentration 0.225% for Rovral Flo and 0.075% for Sportak Alpha)

Fot. 1. Wpływ fungicydów na rozrost liniowy *Fusarium camptoceras* (K – kontrola; RF, SA – odpowiednio Rovral Flo 255 SC i Sportak Alpha 380 EC; 1 – stężenie 0,075% dla Rovralu Flo i 0,025% dla Sportaku Alpha; 2 – stężenie 0,15% dla Rovralu Flo i 0,05% dla Sportaku Alpha; 3 – stężenie 0,225% dla Rovralu Flo i 0,075% dla Sportaku Alpha)

It should be noted that an increasing concentration of this fungicide in the agar led to more effective inhibition of mycelium linear growth. This indicates that it would be justified to perform further studies by using this preparation at higher concentrations.

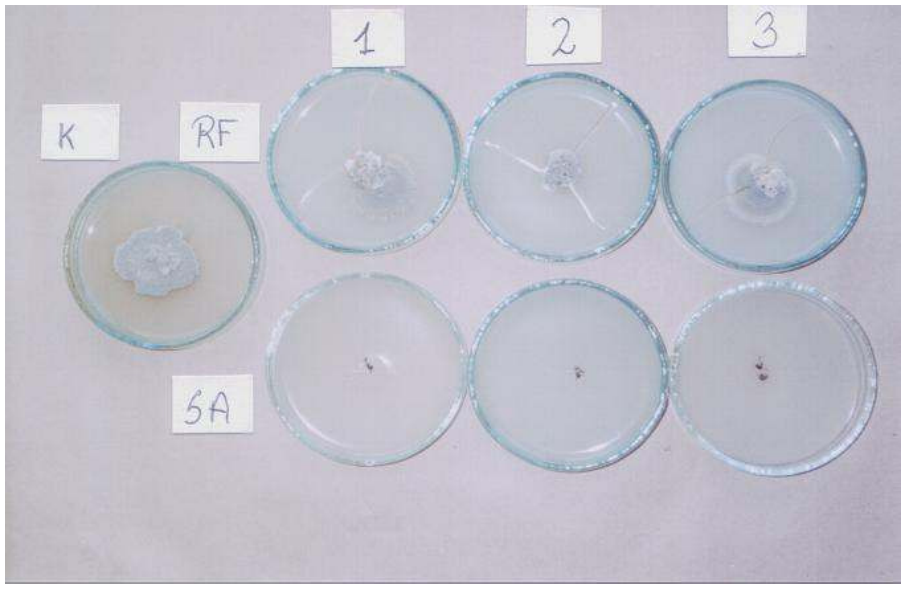

Photo 2. An effect of fungicides on linear growth *Penicillium canescens* (K – control; RF, SA – Rovral Flo 255 SC and Sportak Alpha 380 EC, respectively; 1 – concentration 0.075% for Rovral Flo i 0.025% for Sportak Alpha; 2 – concentration 0.15% for Rovral Flo i 0.05% for Sportak Alpha; 3 – concentration 0.225% for Rovral Flo and 0.075% for Sportak Alpha)

Fot. 2. Wpływ fungicydów na rozrost liniowy *Penicillium canescens* (K – kontrola; RF, SA – odpowiednio Rovral Flo 255 SC i Sportak Alpha 380 EC; 1 – stężenie 0,075% dla Rovralu Flo i 0,025% dla Sportaku Alpha; 2 – stężenie 0,15% dla Rovralu Flo i 0,05% dla Sportaku Alpha; 3 – stężenie 0,225% dla Rovralu Flo i 0,075% dla Sportaku Alpha)

The fungicides under examination shown a differentiated impact on saprobionts having a beneficial effect on plants, e.g. *Au. pullulants* [Kowalski i Sadłowski 1993, Patkowska 2003]. In compliance with Warren [1974] this fungus shown a high susceptibility to benomyl contained in Sportak Alpha 380 EC, while this species demonstrated a low susceptibility to dicarboxsimide contained in Rovral Flo 255 SC. The latter finding is consistent with the results reported by Lima et al. [2003].

Similar reaction to benomyl was recorded also for other saprobionts, for instance belonging to genus *Penicillium* as well to species *Pe. canescens* described in this paper. The reaction to dicarboximide was higher compared to that of *Au. pullulans.* One can conclude that benomyl may cause improper growth of saprobionts, thus enhancing occurrence of pathogens [Warren 1974].

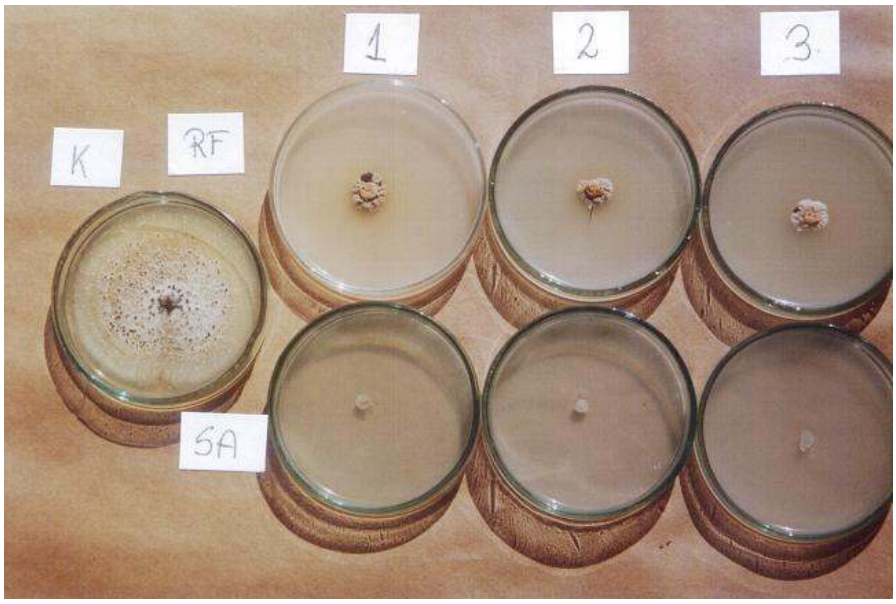

Photo 3. An effect of fungicides on linear growth *Rhizosphaera kalkhoffii* (K – control; RF, SA –Rovral Flo 255 SC and Sportak Alpha 380 EC, respectively; 1 – concentration 0.075% for Rovral Flo i 0.025% for Sportak Alpha; 2 – concentration 0.15% for Rovral Flo i 0.05% for Sportak Alpha; 3 – concentration 0.225% for Rovral Flo and 0.075% for Sportak Alpha)

Fot. 3. Wpływ fungicydów na rozrost liniowy *Rhizosphaera kalkhoffii* (K – kontrola; RF, SA – odpowiednio Rovral Flo 255 SC i Sportak Alpha 380 EC; 1 – stężenie 0,075% dla Rovralu Flo i 0,025% dla Sportaku Alpha; 2 – stężenie 0,15% dla Rovralu Flo i 0,05% dla Sportaku Alpha; 3 – stężenie 0,225% dla Rovralu Flo i 0,075% dla Sportaku Alpha)

Also the effect of fungicides under examination on some properties of tested fungi was differentiated (tab. 2). Sportak Alpha 380 EC at concentration of 0.075% did not inhibit the mycelium development in *Ulocladium consortiale* but no sporification of this fungus was observed. Its hyphae were thinner than those of the control, colorless and without cross partitions. The signs of hypha dieback can be observed in this medium. In other combinations with this fungicide no mycelium growth was recorded for tested fungi.

In turn, Rovral Flo 255 SC had no effect on the tested fungi species *Acremonium tubakii*, *Aureobasidium pullulans*, *Penicillium canescens* and *Ulocladium consortiale*, while changes of fungi features were observed in other combinations. The sporification stimulation was observed in the fungus *Arthrinium* state of *Apiospora montagnei* (at all concentrations), and in *Fusarium camptoceras* at concentration of 0.225%. Hyphae of *Arthrinium* state of *A. montagnei* were evidently thickened (at concentrations of 0.075%

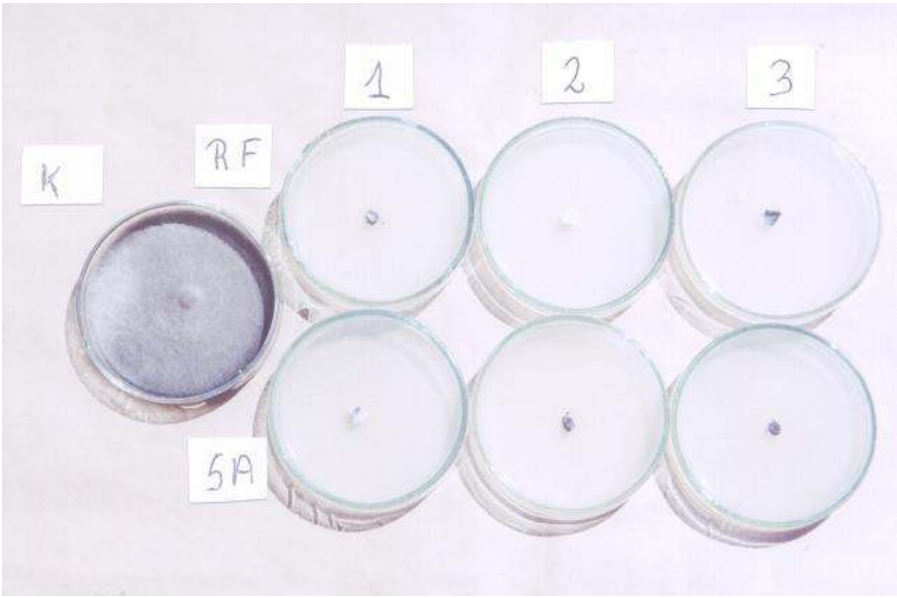

Photo 4. An effect of fungicides on linear growth *Ulocladium consortiale* (K – control; RF, SA – Rovral Flo 255 SC and Sportak Alpha 380 EC, respectively; 1 – concentration 0.075% for Rovral Flo and 0.025% for Sportak Alpha; 2 – concentration 0.15% for Rovral Flo i 0.05% for Sportak Alpha; 3 – concentration 0.225% for Rovral Flo and 0.075% for Sportak Alpha)

Fot. 4. Wpływ fungicydów na rozrost liniowy *Ulocladium consortiale* (K – kontrola; RF, SA – odpowiednio Rovral Flo 255 SC i Sportak Alpha 380 EC; 1 – stężenie 0,075% dla Rovralu Flo i 0,025% dla Sportaku Alpha; 2 – stężenie 0,15% dla Rovralu Flo i 0,05% dla Sportaku Alpha; 3 – stężenie 0,225% dla Rovralu Flo i 0,075% dla Sportaku Alpha)

and 0.225%). This preparation significantly reduced sporification in *F. moniliforme* var. *lactis* (0.15 and 0.225%), and no perithecium was observed in *Anthostomella conorum* while hyphae were considerably thickened. No pycnidium formation and hypha thickening were observed in *Botryodiplodia rubi*, *Rhizosphaera kalkhoffii* and *Zythiostroma pinastri*. Light brown hypha coloring not observed in the control culture was recorded for *Rh. kalkhoffii.* The preparation at all concentrations stimulated intensive production of chlamydospores in *Phoma pomorum*, while hyphae were very thin (tab. 2).

After analyzing an effect of this preparation on the fungal species under examination one can see its harmful effect on morphology and development, as no sporification was found in most of the fungi under consideration.

Table 2. An effect of fungicides on some features (spore and hyphae appearance, sporification intensity, presence of endosporous forms) of the tested fungal species compared to those of the control cultures

Tabela 2. Wpływ badanych fungicydów na niektóre cechy testowanych gatunków grzybów (wygląd zarodników i strzępek grzybni, obfitość zarodnikowania, obecność utworów przetrwalnikowych) w porównaniu z kulturami kontrolnymi

Fungus Grzyb	Control Kontrola		Fungicide and its concentration / Fungus features Fungicyd i jego stężenie / Cechy grzybów					
			Rovral Flo 255 SC			Spotrak Alpha 380 EC		
			0.075%	0.15%	0.225%	0.025%	0.05%	0.075%
Acremonium tubakii	Single spores, Chlamydospores present in mycelium	1* 2*	No differences			No mycelium growth in the medium with amendments		
Anthostomella conorum	Perithecium with ascuses, no ascospores	1	No differences	No perithecium				
		2	Thickened hyphae					
Arthrinium state of *Apiospora montagnei*	Single spores	1	Considerably stimulated					
		2	Thickened hyphae	No differences	Thickened hyphae			
Aureobasidium pullulans	Abundance sporification	1 2	No differences Brak różnic					
Botryodiplodia rubi	Large quantity of piknidium with conidia	1	Pycnidum formation reduced					
		2	Thickened hyphae					
Fusarium camptoceras	Large quantity of macroconidia	1	Considerably stimulated sporification					
		2	No differences					

Fusarium moniliforme var. *lactis*	Average quantity of micro- and macroconidia, Hypae light pink	1	No differences	Reduced sporification			
		2	No differences				
Penicillium canescens	Abundance sporification, Hyphae colourless	1					
		2					
Phoma pomorum	No piknidium with conidia, Chlamydospores present in mycelium	1					
		2	Thin hyphae, numerous chlamydospores				
Rhizosphaera kalkhoffii	Large quantity of piknidium with conidia, Hyphae colourless	1	No pycnidium				
		2	Thickened hyphae, light brown colored				
Ulocladium consortiale	Large quantity of conidia, Hyphae dark brown	1	No differences	No mycelium growth in the medium with amendments	No differences	No mycelium growth in the medium with amendments	No differences
		2					Thin, colorless, without partitions, dying
Zythiostroma pinastri	Large quantity of piknidium with conidia	1	No pycnidium			No mycelium growth in the medium with amendments\	
		2	Thickened hyphae				

* 1 – sporification; 2 – hyphae appearance
* 1 – zarodnikowanie; 2 – wygląd strzępek

CONCLUSIONS

1. Based on the tests presented above it is recommended to implement in horticultural practice a chemical method for protecting blue spruce against fungi if no effective other methods (agrotechnical and biological) can be used.

2. Among fungicides under examination Sportak Alpha 380 EC shows the highest effectiveness and a wide range of application. It is recommended to test this preparation at concentration reduced by half compared to that of recommended so far, i.e. 0.025%, because of high antifungal activity even at such concentration.

3. Rovral Flo 255 SC shows considerably poorer antifungal activity compared to that of Sportak Alpha 380 EC (lower by 10.19% to 67.14%), as well as narrower range of activity to the tested fungi. Nevertheless, this fungicide seems to toxic to fungi by reducing sporification to some degree and thinning its cell walls.

4. This preparation can be recommended to protect blue spruce against the following fungi only: *Acremonium tubakii, Anthostomella conorum, Arthrinium* state of *Apiospora montagnei, Botryodiplodia rubi, Phoma pomorum, Rhizosphaera kalkhoffii, Ulocladium consortiale*; in principle at any of test concentrations. The fungicide shows considerably lower activity to other fungi.

5. Due to diversified reaction of fungi to concentration of Rovral Flo 255 SC in the agar, it would be justified to test its effectiveness at a wider concentration range.

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Z BADAŃ NAD MOŻLIWOŚCIĄ OCHRONY ŚWIERKA KŁUJĄCEGO (*Picea pungens* Engelm.) PRZED GRZYBAMI. CZEŚĆ I. LABORATORYJNA OCENA SKUTECZNOŚCI GRZYBOBÓJCZEJ WYBRANYCH FUNGICYDÓW

Streszczenie: W badaniach *in vitro* określano grzybobójczą aktywność dwóch fungicydów, tj. Rovralu Flo 255 SC i Sportaku Alpha 380 EC, w stosunku do 12 gatunków grzybów wyizolowanych z roślin świerka kłującego (*Picea pungens*) i jego odmiany Glauca. Najszersze spektrum działania oraz wysoką aktywność grzybobójczą wykazał się Sportak Alpha 380 EC, bo wobec wszystkich badanych gatunków grzybów. Natomiast Rovral Flo 255 SC był znacznie słabszy.

Słowa kluczowe: *Picea pungens*, grzyby, zwalczanie, fungicydy

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