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INFLUENCE OF SOME COMPOUNDS ON DEVELOPMENT OF *SPHAEROTHECA PANNOSA* VAR. *ROSAE*

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Abstract. Influence of triforine (standard), garlic juice, Antifung 20 SL (20% vermicompost), Atpolan 80 EC (76% mineral oil), Biosept (grapefruit juice) and Atonik AL on development of *Sphaerotheca pannosa* var. *rosae* was evaluated. Garlic juice, Atpolan 80 EC and Biosept applied as rose spray gave similar effect in the control *S. pannosa* var. *rosae* as triforine. Observations under scanning electron microscop indicated that 24 hrs after rose spraying all tested compounds cause almost complete collapse of conidia and hyphe.

Key words: biopreparation, oil, *Sphaerotheca pannosa* var. *rosae*, rose, control, scanning electron microscopy

I. INTRODUCTION

Powdery mildew caused by *Sphaerotheca pannosa* var. *rosae* is one of the most frequent and important diseases of roses. To control the pathogen many fungicides are recommended (Wojdyła 1999). In recent years some oil compounds as well as bioproducts (Antifung 20 SL, Bioczos BR) were registered in Poland. Additionally, effectiveness of Atonik AL, Biosept and Polyversum against plant diseases and their stimulatory effect on plant growth and development are under evaluation. High efficacy of Antifung 20 SL (Wilk et al. 1996), Atonik (Wojdyła and Orlikowski 1999), Atpolan 80 EC (Wojdyła 1998) Biosept (Wojdyła unpublished) and garlic juice (Wojdyła 1996) against *S. pannosa* var. *rosae* on roses was shown in previous experiments. Unfortunately no information on direct action of these compounds on fungus growth is available.

It was shown that vermicompost, incorporated into substrates before planting, killed some nematodes (Szczech and Brzeski 1994; Ribeiro et al. 1998) as well as *Plasmodiophora brassicae*, *Fusarium oxysporum* and *Phytophthora cryptogea* (Szczech and Brzeski 1994, Wilk and Orlikowski 1998). Moreover the preparation was highly effective in protection of roses against *Sphaerotheca pannosa* var. *rosae* (Wilk et al. 1996).

In earlier research Atonik AL was used to increase yield of pepper (Panajotov et al. 1997; Djuma'ijak 1986), sour cherry (Chitu et al. 1998) or apple (Kopecky 1995; Koupil 1997). Moreover, Saniewska (1999) reported good effectiveness of this product against *Puccinia antirrhini* on snapdragon while Wojdyła (unpublished) showed its inhibitory effect on *Puccinia horiana*, *Diplocarpon rosae*, and *Melampsora epitea*.

Oil compounds widely used to improve parameters of spraying mixtures i.e. to decrease surface tension, slow down evaporation and increase penetration into plant tissues (Zdonek et al. 1986; Shama et al. 1998; Wojdyła 1998) were found also effective in protec-

tion against some pathogens. Northover and Schneider (1993) using mineral oils against *Venturia inaequalis* and *Podosphaera leucotricha* noted efficacy of 61-81% and 99%, respectively. Azam et al. (1998) proved that 0.5% rape oil can control *Uncinula necator* at a similar level as sulphur fungicides and fenarimol. Also soya oil was highly effective against *Plasmopara viticola* (Redl and Bauer 1990). Plant and mineral oils used every 10 days in protection of ornamental trees and shrubs against powdery mildew were similarly effective as fungicides (Chauwel et al. 1998).

Results of Lipa and Jarosz (1991), Saniewska and Orlikowski (1994), Orlikowski et al. (1995), Saniewska (1995) and Wojdyła (1996) indicate the possibility of using dried and powdered garlic or garlic juice in control many phytopathogens. Ajoen, a compound found in garlic, proved to be the most active ingredient of these preparations (Lipa and Jarosz 1988; Lipa and Jarosz 1991; Reimers et al. 1993; Saniewska 1995). Qvarnstrom and Ramert (1992) showed high effectiveness of 5% garlic juice against *Diplocarpon rosae* on roses. Moreover 1-5% garlic juice applied every 7 days protected 48% of cucumber plants against *Erysiphe cichoracearum* (Qvarnstrom 1992).

The aim of the research presented was to evaluate the effectiveness of bioproducts (Antifung 20 SL, Biosept, garlic juice), oil (Atpolan 80 EC) and plant growth stimulator (Atonik AL) against *Sphaerotheca pannosa* var. *rosae* and their direct influence on fungus development.

II. MATERIAL AND METHODS

1. Evaluation of effectiveness of tested preparations against *Sphaerotheca pannosa* var. *rosae*

Plant material: Trials were conducted on three rose varieties: 'Madelon' and 'Sonia' grown in glasshouse in 10 cm pots (plants obtained from in vitro culture), 'Madelon' cultivated on beds under poly film tunnel and 'Lampion' grown in the field.

Tested compounds: The compounds used in the trials are shown in Table 1 (Saprol 190 EC was used as a standard fungicide).

Garlic juice was prepared according to the procedure described by Lipa and Jarosz (1991). Concentrations of different compounds used in trials are given in Tabs. 2-6. Spraying was started from 6 to 12 days after when first disease symptoms were noted and plants were sprayed at weekly intervals, and Antifung 20 SL was also applied at two-week intervals. As a wetting agent Citowett AL was used at the concentration of 0.02% in all treatments.

Table 1

Compounds used for the control of *Sphaerotheca pannosa* var. *rosae*

Commercial name	Active ingredient
Antifung 20 SL	0.20 dcm^3 vermicompost per 1 dcm^3
Atonik AL	0.03 dcm^3 sodium 2-nitrophenolate per 1 dcm^3
	0.02 dcm^3 sodium 4-nitrophenolate per 1 dcm^3
	0.01 dcm^3 sodium 5-nitroguaiacolate per 1 dcm^3
Atpolan 80 EC	76% mineral oil
Bioscpt	60% grapefruit juice
Garlic	0.5-1% garlic juice
Saprol 190 EC	190 g triforine per 1 dcm^3

Evaluation of effectiveness of treatments was conducted according to the six-grade scale described by Wojdyła and Orlikowski (1992): 0 – no symptoms, 1 – up to 1% of shoot area covered with fungus mycelium, 2 – from 1.1 to 5%, 3 – from 5.1 to 10%, 4 – from 10.1 to 20%, 5 – over 20% of shoot area covered with fungus. The effectiveness of treatments was assessed 6 and 11 weeks after the first spraying on the field grown plants, and after 2 or 4 weeks on plants under cover.

The experiments were set as the randomised blocks design with 4 replicates and 1 (under cover) or 5 (in the field) plants in each replicate.

2. Microscopic observations

Plants of rosa 'Madelon' grown in poly film tunnel were sprayed with tested compounds three days after the first disease symptoms occured. After 24 h leaves were collected and effects of tested compounds on fungus mycelium and spores were studied in scanning electron microscope.

III. RESULTS

1. Evaluation of effectiveness of tested preparations against *Sphaerotheca pannosa* var. *rosae*

Antifung 20 SL: At the moment of first spraying over 20% of leaf area was covered with mycelium of *S. pannosa* var. *rosae* (Tab. 2). Two weeks after spraying only slight decrease of the infection level was noted. After next 2 weeks the strongest infection was found on unprotected, control plants. Depending on frequency of treatments infection level was reduced by 30.4 or 40%. Antifung 20 SL used every week was more effective than used at two-week intervals. The product left a thick deposit on the plant leaves drastically decreasing quality of cut flowers and presumably reducing photosynthesis.

Atonik AL: In the first experiment, after two treatments level of infection was reduced by 50% comparing to control plants and after next two treatments the difference was over 38% (Tab. 3). In the second experiment reduction of infection level was about 43% after first two treatments and almost 28% after four treatments (Tab. 3). However, in both experiments Atonik AL was less effective than Saprol 190 EC.

Table 2

Effectiveness of Antifung 20 SL in the control of *Sphaerotheca pannosa* var. *rosae* on field growing roses cv. Mercedes: degree of rose shrubs infection

Initial infection level and beginning of experiment: 1999.06.28 = 5.0

Treatment	Concn. in %	Frequency of spray. in day	Weeks after first spraying	
			2	4
Control	–	–	5.00 b	5.00 b
Saprol 190 EC	0,15	7	4.00 a	3.48 a
Antifung 20 SL	25	7	4.23 a	3.00 a
Antifung 20 SL	2	14	4.23 a	3.48 a

Note: Means followed by the same letter for each column do not differ at a 5% level of significance (Duncan's multiple range t-test).

Table 3

Effectiveness of Atonik AL in the control of *Sphaerotheca pannosa* var. *rosae* on greenhouse roses cv. Sonia: degree of rose shrubs infection

Initial infection level and beginning of experiment in trials: I – 1997.07.09 = 1.0*
II – 1997.07.23 = 1.7**

Treatment	Conc. in %	Weeks after first spraying			
		I*		II**	
		2	4	2	4
Control	–	2.30 c	4.85 c	3.75 c	4.97 c
Saprol 190 EC	0.15	0.49 a	0.38 a	0.23 a	0.58 a
Atonik AL	0.1	1.22 b	3.00 b	2.15 b	3.60 b

Note: see Table 2.

Atpolan 80 EC: Spraying was started when infection level was 4.1 (Tab. 4). After two treatments with Atpolan 80 EC almost 50% reduction of infection level was observed. Next two treatments resulted in infection reduction by 67% comparing to unprotected control plants. After four treatments Saprol 190 EC was significantly more effective than Atpolan 80 EC against *S. pannosa* var. *rosae* (Tab. 4).

Table 4

Effectiveness of Atpolan 80 EC in the control of *Sphaerotheca pannosa* var. *rosae* on roses cv. Madelon (plastic tunnel): degree of rose shrubs infection

Initial infection level and beginning of experiment: 1997.07.21 = 4.1

Treatment	Conc. in %	Weeks after first spraying	
		2	4
Control	–	4.50 b	4.57 c
Saprol 190 EC	0.15	2.15 a	0.76 a
Atpolan 80 EC	0.2	2.27 a	1.50 b

Note: see Table 2.

Table 5

Effectiveness of Biosept in the control of *Sphaerotheca pannosa* var. *rosae* on roses cv. Madelon (plastic tunnel): degree of rose shrubs infection

Initial infection level and beginning of experiment in trials: I – 1998.05.21 = 3.1*
II – 1998.07.27 = 4.0**

Treatment	Conc. in %	Weeks after first spraying			
		I*		II**	
		2	4	2	4
Control	–	3.62 c	3.63 d	4.67 e	4.55 e
Saprol 190 EC	0.15	0.25 a	0.50 a	1.09 a	1.00 a
Biosept	0.15	1.21 b	1.89 c	2.44 d	2.50 d
Biosept	0.1	1.00 ab	1.18 b	2.10 c	2.15 c
Biosept	0.2	0.56 a	1.00 b	1.50 b	1.85 b

Note: see Table 2.

Biosept: In the first experiment, depending on the concentration used, from 67% to 85% reduction of infection level was noted after two treatments (Tab. 5). After next two treatments infection reduction was between 52.1% and 72.5% comparing to control plants. In the second experiment spraying was started when infection level was 4.0 (Tab. 5). After two treatments with Biosept infection level of treated plants was from 48% to 68% lower than of untreated plants and these relations were the same after next two treatments. Biosept was highly effective in control of *S. pannosa* var. *rosae* and efficacy of treatments increased with increasing concentration of the fungicide. However, Biosept was less effective than Saprol 190 EC.

Garlic juice: At the time of the first treatment no disease symptoms were noted on the plants. After 6 weeks of experiment level of infection of protected plants was very low (Tab. 6). In the first experiment, after the following 5 treatments, level of infection on plants sprayed with garlic juice was dependend on concentration and was 40% or 45.2% lower than on unprotected plants. At the same time in the second experiment, reduction of infection was 59% and 65%, respectively. Lower concentration of garlic juice was significantly less effective than higher concentration.

Table 6

Effectiveness of garlic juice in the control of *Sphaerotheca pannosa* var. *rosae* on field growing roses cv. Lampion: degree of rose shrubs infection

Initial infection level and beginning of experiment in trials: I – 1994.06.20 = 0.0*
II – 1994.06.27 = 0.0**

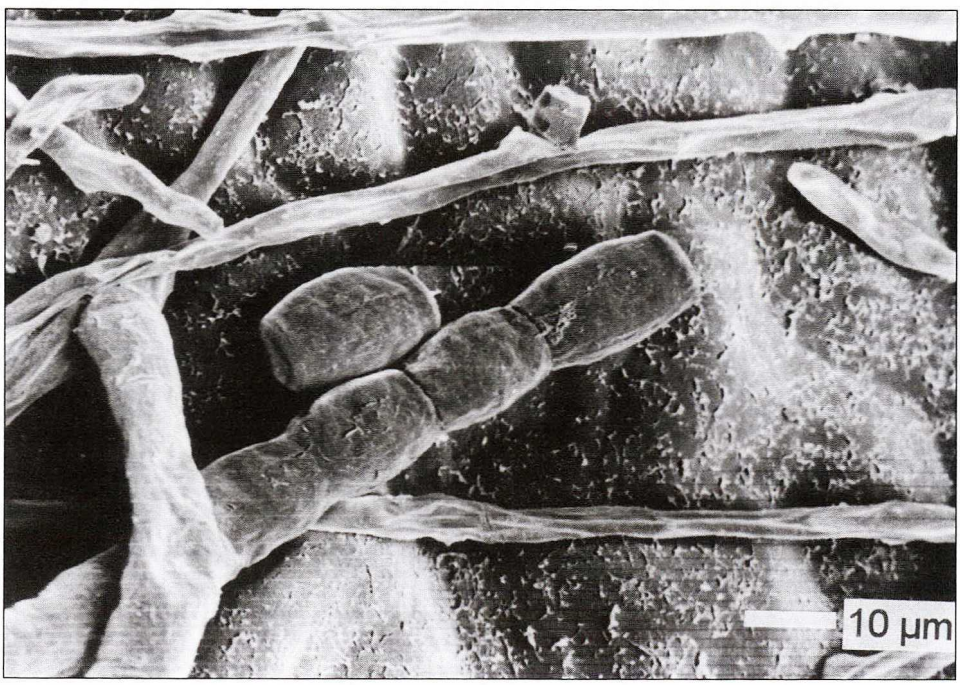
Treatment	Conc. in %	Weeks after first spraying			
		I*		II**	
		6	11	6	11
Control	–	3.64 b	5.00 c	2.45 c	5.00 d
Saprol 190 EC	0.15	0.05 a	2.90 b	0.00 a	1.15 a
Garlic	0.5	0.24 a	3.00 b	0.05 b	2.05 c
Garlic	1.0	0.11 a	2.74 a	0.00 a	1.75 b

Note: see Table 2.

2. Microscopic observations

Control (untreated plants): Fungus hyphae densely covered epidermis of upper side of leaves. Numerous appresoria were formed in the places where hyphae were in contact with plant cells (Fig. 1). Typical barrel-shaped cells of conidiophores were visible. On the epidermis, often along the conidiophores, single spores were visible and some of them germinating. Sections of mycelium had the appearance of tissues with low turgidity suggesting its dehydratation (Fig. 2).

Saprol 190 EC: Mycelium on the surface of infected tissues was destroyed. However, some hyphae which were in contact with leaf surface, as well as some conidiophores were only partially destroyed (Fig. 3). Spores separated from conidiophores visible between hyphae



Figs. 1, 2. Hyphae, conidiophores and spores on surface leaf untreated plants

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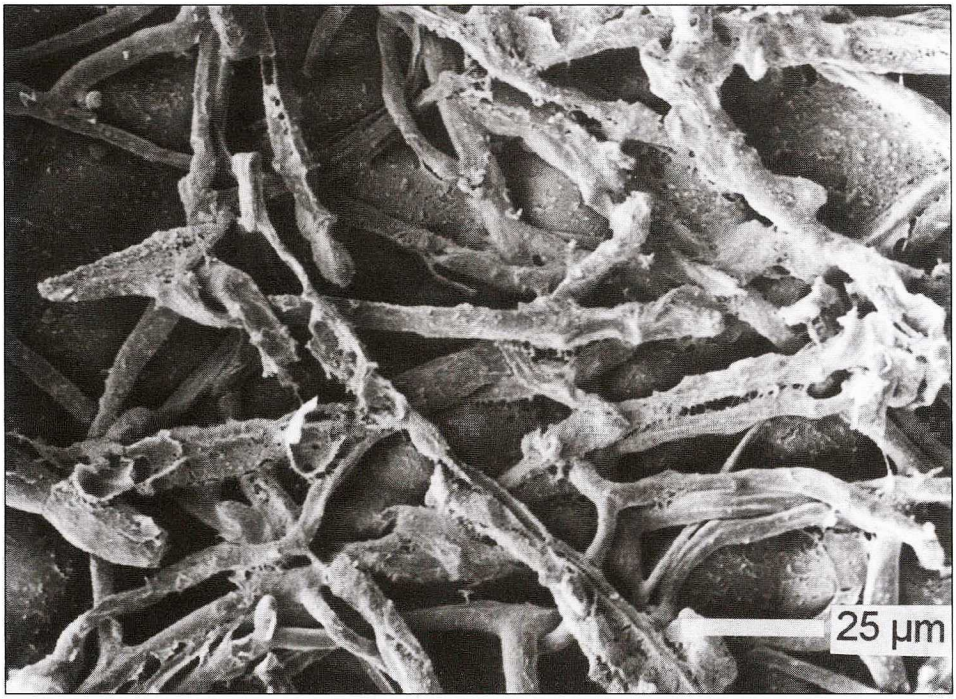
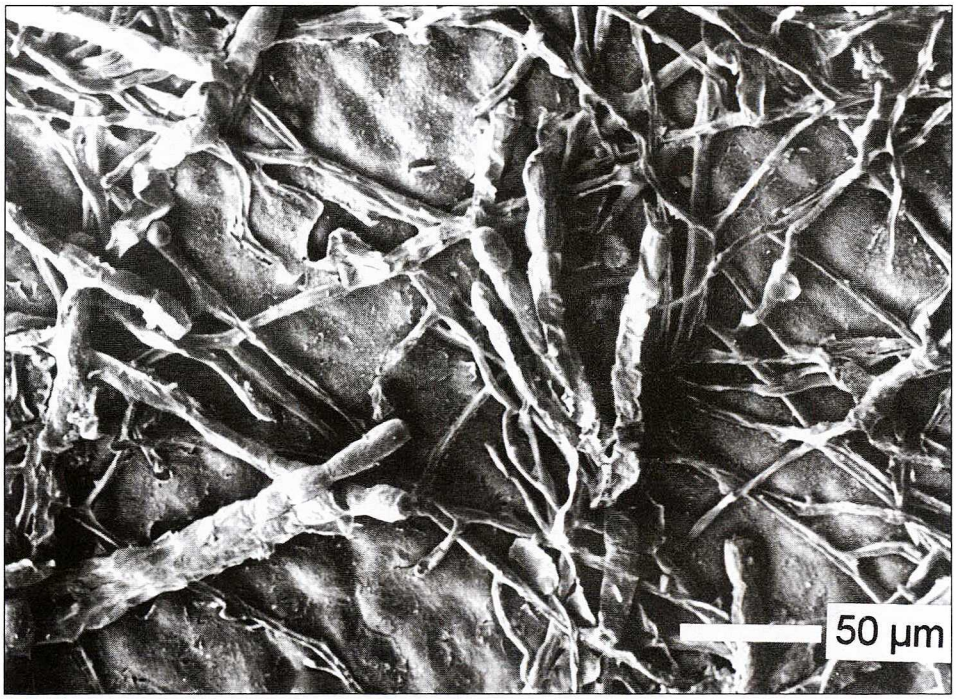
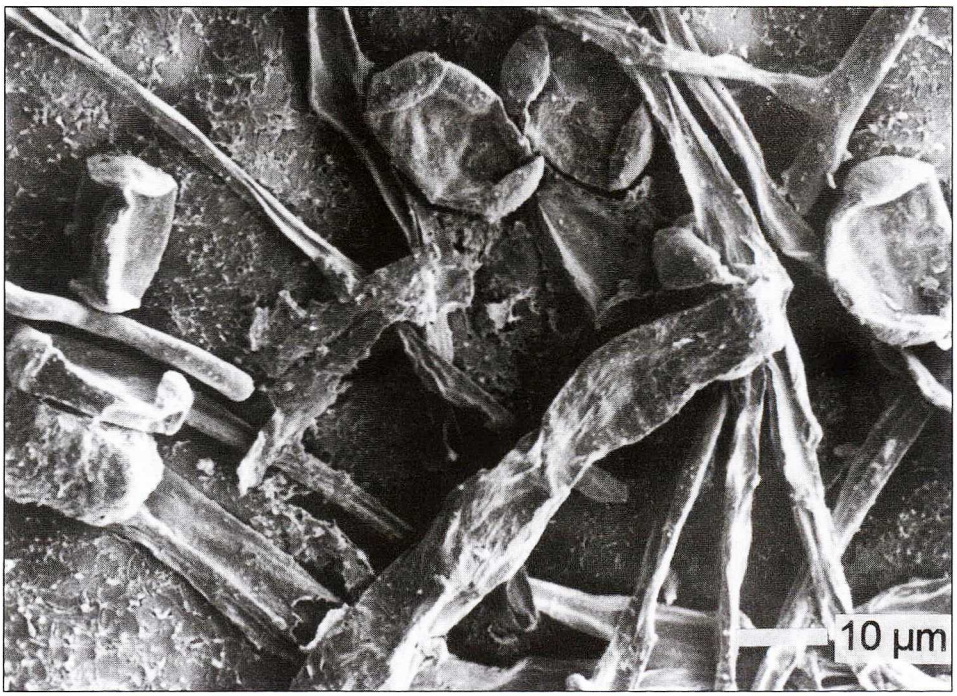


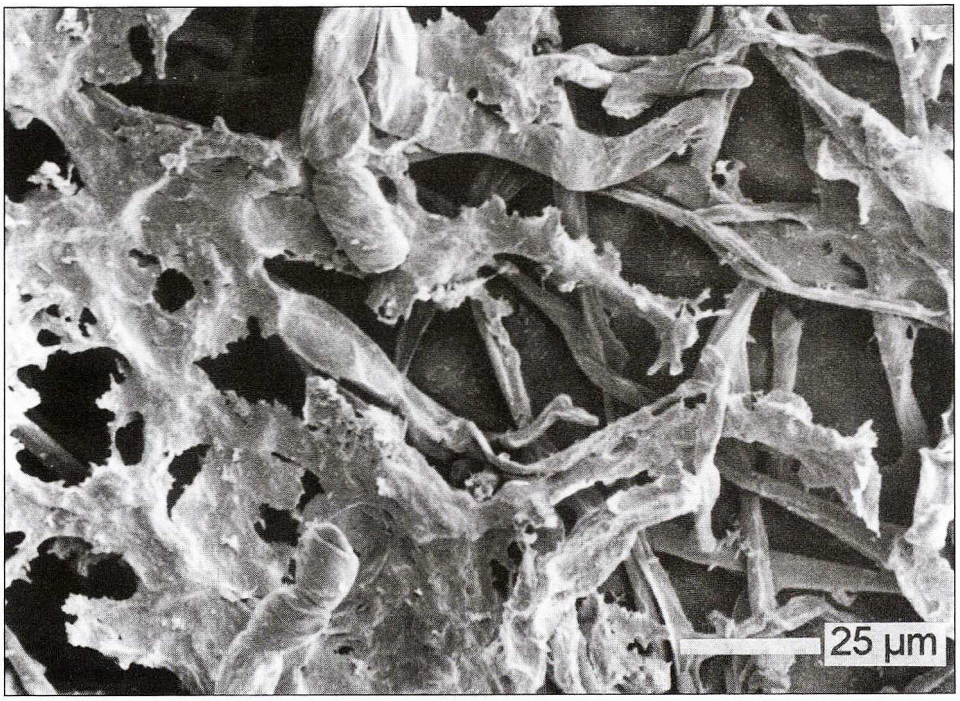
Figs. 3, 4. Almost completely destroyed conidia and hyphae after Saprol 190 EC application



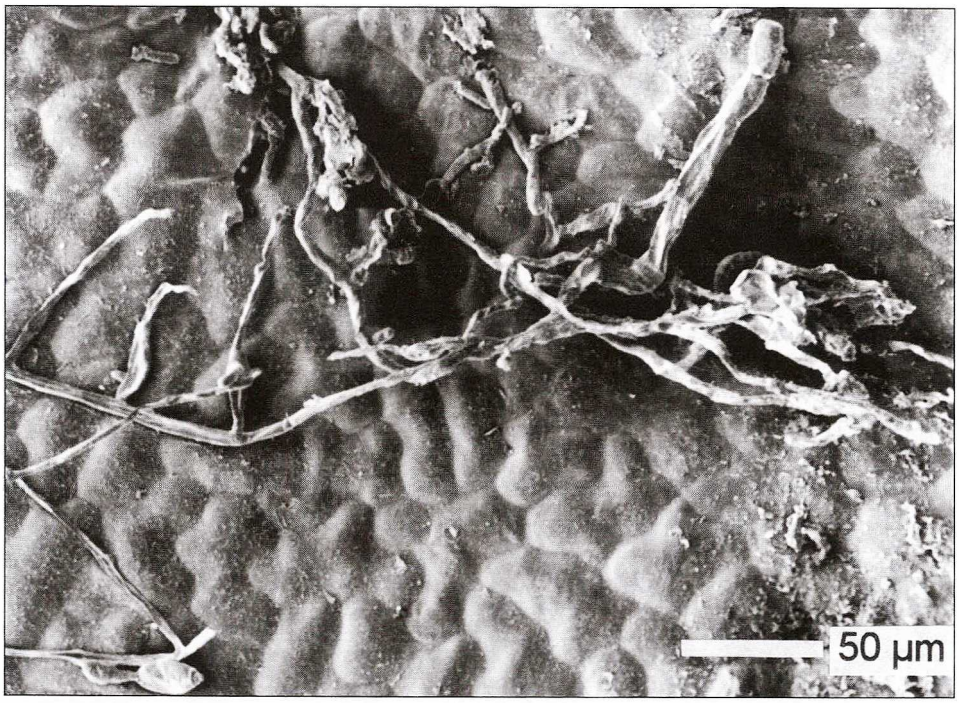


Figs. 5, 6. Most of hyphae, conidiophores and spores completely collapsed after Antifung 20 SL application

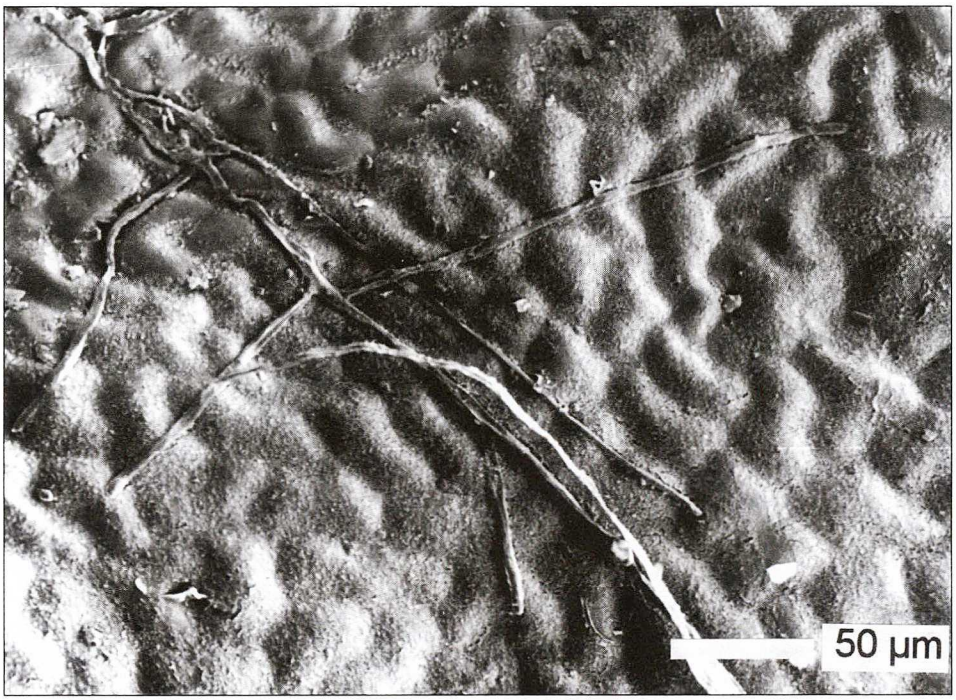
Figs. 7, 8. Most of spores, conidiophores and hyphae completely collapsed after Atonik AL application

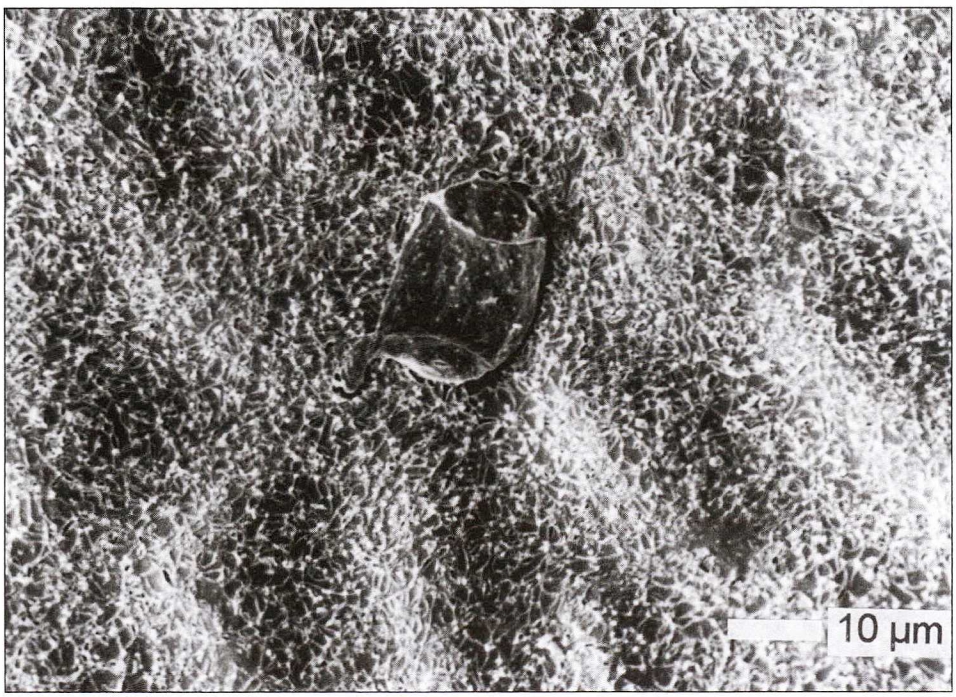


Figs. 9, 10. Almost completely destroyed hyphae and spores after Atpolan 80 EC application



Figs. 11, 12. Almost completely destroyed hyphae and spores after Biosept application





Figs. 13, 14. Complete collapsed of hyphae and spores after application of garlic juice

were misshaped. Large-particle residue of fungicide were present on fungus mycelium, and between parallel hyphae membranous, perforated structures could be seen. Almost all hyphae had sunken walls with numeroeus cracks, perforations and narrowings (Fig. 4).

Antifung 20 SL: Surface of a leaf and hyphae had rough and granular structure. Cuticule exhibited many cracks and loose flakes of wax. Fragments of mycelium were destroyed by over 90%. Hyphae walls were sunken and corrugated with perforations and numerous narrowings. However, fragments of mycelium without visible changes in shape and structure were also present (Fig. 5). Many contiguous fragments of mycelium were agglutinated. Numerous spores were completely destroyed (Fig. 6).

Atonik AL: Fragments of leaf surface were densely covered with fungus hyphae. Only on small fragments of leaf mycelium was completely destroyed (Fig. 7). Strongly deformated parts of conidiophores or their bases were visible next to unaffected ones. Some spores were of concave-convex shape while some were similar to these on untreated leaves (Fig. 8).

Atpolan 80 EC: After this treatment characteristic lobed and shredded structures covered parts of mycelium (Fig. 9). Besides these of a regular and unchanged shape, many hyphae and spores were completely degenerated or with visible deformations suggesting partial loss of turgidity. Barrel-shaped spores were present next to partially deformated or completely destroyed ones (Fig. 10). Conidiophores with sunken walls as well as typical for untreated leaves were observed.

Biosept: Only sparse fragments of mycelium were found on leaf surface. Most of them were separated from leaf surface, loosely suspended above it (like spiderweb). Almost all hyphae were degenerated, some of them in fragments of different shapes and sizes with irregular edges (Fig. 11). Deformated spores were often elongated (Fig. 12). Terminal parts of conidiophores (potential spores) were least deformated.

Garlic juice: Only sparse fragments of mycelium were observed on the examined leaves. In this treatment leaf surface was different from other treatments. Cuticule had characteristic fibrilar-granular structure consisting presumably of cuticular waxes. Fungus hyphae were strongly flattened, cracked and seemed to be pressed into the leaf cuticule. Places on leaf surface from where fungus fragments fell apart were smooth and darker than surrounding surface. Spores were sparse, flattened and curved. They were pressed onto the leaf cuticule (Figs. 13, 14).

IV. DISCUSSION

Tested compounds were highly effective against *S. pannosa* var. *rosae*. Some of them showed the same (garlic juice) or almost the same effectiveness (Anfifung 20 SL, Atpolan EC, Biosept) as standard fungicide Saprol 190 EC. Only Atonik AL was much less effective than Saprol 190 EC.

Antifung 20 SL used weekly reduced development of *S. pannosa* var. *rosae* by 40%. This confirms the results obtained earlier by Wilk et al. (1996). The microorganisms present in Antifung 20 SL and introduced on the leaf surface treated with the preparation may in some cases become inactivated and die in short time due to lack of water as well as high temperature and solar radiation. This fungicide probably acts in two steps. Presumably their chemical ingredients play active role just after the treatment. In contrast, the effect of activity of microorganisms become visible only after few days. It is probably why this fungicide is highly effective against soil-borne pathogenes (Wilk and Orlikowski 1998). This is because microorganisms contained in it find in soil favourable growth conditions. The disadvantage of this preparation is an intensive white deposit left on treated plants. In microscopic observations strong deformation of fungus fragments was noted. However, some spores, especially those situated on the tips of conidiophores were not deformated. They may become a source of inoculum. The fungicide is mostly active on the surface in direct contact with mycelium and spores.

Atonik AL used to control *S. pannosa* var. *rosae* decreased to some extent development of the pathogen. The results agree with those obtained by Saniewska (1999) where, Atonik AL was effective against *Puccinia antirrhini* on snapdragon. Significantly lower efficacy of this fungicide observed in field comparing to Saprol 190 EC, was confirmed by microscopic analysis. The latter proved that many fragments of the fungus were not destroyed by Atonik AL. Spores that had not been destroyed could infect neighbouring plants.

Atpolan 80 EC and Biosept strongly reduced fungus development. Microscopic observations showed almost complete degeneration of mycelium, conidiophores and conidia, but field efficacy of these fungicides was lower than of Saprol 190 EC. Probably fragments of mycelium and some spores were not destroyed by these fungicides, which is in agreement with earlier observations (Wojdyła 1998). Not complete destruction of mycelium and spores may be a result of induction of air bubbles on leaf surface which reduce penetration of spraying mixture onto the leaf surface. Not destroyed spores may become a source of infection.

Garlic juice was highly effective against *S. pannosa* var. *rosae* probably due to the action of ajoen (Reimers et al. 1993). The preparation used for spraying roses strongly reduced development of the fungus. Effectiveness of garlic juice increased with the increase of its concentration from 0.5 to 5% (Wojdyła 1996). Effectiveness was the highest when the preparation was used soon after first symptoms of disease had been noted. Microscopic observations made 24 h after treatment showed that fungus hyphae were flattened and disintegrated which suggests direct action. This was confirmed in the field where as efficacy of garlic juice approached up to 100%.

V. CONCLUSIONS

1. Among tested compounds Antifung 20 SL, Atpolan 80 EC, Biosept and garlic juice were the most effective against *S. pannosa* var. *rosae*.
2. Antifung 20 SL, Atpolan 80 EC, Biosept, garlic juice and Saprol 190 EC caused deformation and disintegration of fungus hyphae, conidiophores and conidia.
3. Antifung 20 SL used weekly was more effective than when it was used every two weeks.
4. Increasing concentration of Biosept and garlic juice resulted in increased efficacy.
5. Garlic juice at the concentration 1% was as effective as Saprol 190 EC.

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WPŁYW NIEKTÓRYCH ZWIĄZKÓW NA ROZWÓJ *SPHAEROTHECA PANNOSA* VAR. *ROSAE*

STRESZCZENIE

W przeprowadzonych badaniach polowych oraz w uprawie róż pod osłonami oceniano aktywność biologiczną biopreparatów (Antifung 20 SL), Biosept, sok z czosnku, oleju (Atpolan 80 EC) i stymulatora wzrostu roślin (Atonik AL) do zwalczania *S. pannosa* var. *rosae*. Krzewy róż opryskiwano co 7 lub 14 dni. W drugiej części badań przy użyciu mikroskopu skaningowego oceniano wpływ badanych związków na grzyba. W tym celu krzewy róż uprawiane pod osłonami opryskano jeden raz, a po 24 godzinach pobrano liście do obserwacji.

Badane preparaty wykazywały wysoką skuteczność w zwalczaniu *S. pannosa* var. *rosae*. Niektóre z nich dorównywały (sok z czosnku) bądź nieznacznie ustępowały (Antifung 20 SL, Atpolan 80 EC, Biosept) skutecznością fungicydowi standardowemu Saprol 190 EC. Zdecydowanie niższą skuteczność wykazywał Atonik AL. Z kolei wzrost stężenia preparatów Biosept oraz soku z czosnku wiązał się ze wzrostem ich skuteczności. Natomiast Antifung 20 SL stosowany co 14 dni wykazywał niższą skuteczność aniżeli przy aplikowaniu co 7 dni.

Już po 24 godzinach od wykonania opryskiwania w obserwacji przeprowadzonej przy użyciu mikroskopu skaningowego stwierdzono, że badane związki powodowały prawie całkowite spłaszczenie i deformację grzybni, trzonków oraz zarodników konidialnych. Jednak w przypadku preparatu Atonik AL tylko fragmenty grzybni były całkowicie zniszczone. Z kolei po zastosowaniu oleju Atpolan 80 EC stwierdzono rozpad fragmentów grzyba, a następnie ich łączenie się w płatowate, strzępiaste – dosyć duże struktury.