EFFECT OF BIOPESTICIDES ON THE GROWTH AND DEVELOPMENT OF ISOLATES OF *BOTRYTIS CINEREA* PERS., *IN VITRO* OBTAINED FROM RASPBERRY PLANTS

Anna Sapieha-Waszkiewicz[1], Barbara Marjańska-Cichoń[2], Ryszard Miętkiewski[1]*

University of Natural Sciences and Humanities, B. Prusa 14, Siedlce 08-110, Poland
[1]Department of Plant Protection
[2]Department of Vegetable Crops

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Abstract: Two *Botrytis cinerea* isolates studied in the experiment responded differently to the fungicides applied. An isolate obtained from raspberry fruit infected by the fungus, was more susceptible to the biopesticides as well as the fungicide Signum 33 WG. Efficacy of the biopesticides differed. Their efficacy, which depended on both the active ingredient and the duration of biopesticide influence on the mycelium. *B. cinerea,* was rather resistant to the biological pesticides. The growth of both the isolates was completely inhibited at a concentration that was fivefold higher than the recommended amount and the recommended concentration, but only at the initial stage of culturing. Of the biopesticides, Biosept 33 SL was most effective at controlling the growth of *B. cinerea.* No sclerotia were formed on media containing the biopesticide. Propolis also inhibited the production of spores; however, the biopesticide effectively controlled the development of mycelium only when applied at the highest rate. The synthetic fungicide Signum 33 WG is conventionally applied to control grey mould. Signum 33 WG was highly effective at controlling both the *B. cinerea* isolates.

Key words: biopesticides, grey mould, raspberry, growth, mycelium morphology

INTRODUCTION

Grey mould is one of the most widely occurring diseases of raspberry and strawberry as well as other plants. Grey mould occurs on both the cultivated and wild forms of these plants. When weather conditions favour the development of the pathogen, it is necessary to repeatedly control the disease during the growing season. Frequent applications of synthetic fungicides are associated with an increased risk of the reduced biological quality of fruit and harm to the natural environment (Huszcza 1997). It is also possible that fungi may develop resistance to fungicides. Resistance is always associated with substantial economic losses. In the case of *Botrytis cinerea* – the cause of grey mould – the development of resistance to products from the group of benzimidazoles has been observed. Repeated and one-sided applications of dicarboximidine fyngicides resulted in the occurrence of a high share of resistant forms in the *B. cinerea* (Bielenin 2007) population. Resistance and dynamically developing integrated fruit production make it necessary to search for alternative compounds to synthetic fungicides. Such compounds would be equally effective in the control of grey mould. Biopesticides, in particular those containing plant extracts and animal-derived substances offer an alternative to synthetic fungicides (Lipa and Jarosz 1990; Miętkiewski and Sapieha 1992; Niezborała and Marjańska-Cichoń 2000, 2001). Grapefruit extracts have been widely applied in plant protection. Biosept 33 SL, based on grapefruit extracts, has proven to effectively protect seeds against fungal diseases when used to treat soybeans, beans and peas (Pięta *et al.* 2005; Patkowska 2006). Grapefruit extracts have also been effectively used to protect ornamental plants (Orlikowski 2001; Saniewska 2002; Wojdyła 2004). Chitosan, the active ingredient of Biochikol 020 PC, effectively controls the development of various species of fungi, namely: *Sphaerotheca pannosa* var. *rosae, Fusarium* sp., *Rhizoctonia* sp., *B. cinerea* (Wojdyła and Orlikowski 1997; Pięta *et al*. 2004).

The objective of this work was to determine the effect of four biopesticides, compared with a synthetic fungicide, on the growth and development of *Botrytis cinerea* isolates obtained from raspberry.

MATERIALS AND METHODS

Two isolates of the fungus *B. cinerea* isolated from raspberry canes (X1) and fruit (X2) were used for analysis. An experiment was conducted to determine the effect of the biopesticides Bioczos S (active ingredient – garlic extract 10 ml from 10 g garlic pulp), Biosept 33 SL (active ingredient – grapefruit extract 33%), Biochikol 020 PC (active ingredient – chitosan 2%) and Propolis (active in-

*Corresponding address:
sapieha@ap.siedlce.pl

gredient – propolis 2.5%) as well as the synthetic fungicide Signum 33 WG (active ingredient – pyraclostrobin 6.7% + boscalid 26.7%) on the growth and development of *B. cinerea* colonies.

Pesticides were applied at the following basic concentrations:

- Bioczos S – 5%,
- Biosept 33 SL – 0.5%,
- Biochikol 020 PC – 0.5%,
- Propolis – 1.5%,
- Signum 33 WG – 3% .

The biopesticides were applied in these combinations:

- fivefold higher than the recommended rate (A),
- the rate which is recommended for application in fruit farming (B),
- the rate fivefold lower than the recommended amount (C).

The fungicides tested were added at rate A to sterile Potato dextrose agar (PDA) cooled to a temperature of around 55°C. Successive concentrations of the biopesticides were obtained by the dilution method. The medium was poured into sterile Petri dishes (8 cm in diameter) and, 24 hours later, they were inoculated with the fungus.

Each combination was performed in 4 replicates. Experiments were done in two series. The control consisted of colonies growing on pure PDA medium. The temperature of culturing was 22°C.

The growth of the colonies was monitored after 24 hours and 96 hours; at the time when the control colonies completely covered the plates. Additionally, the colony development and morphology of both *B. cinerea* isolates were observed on the 20th day of culturing.

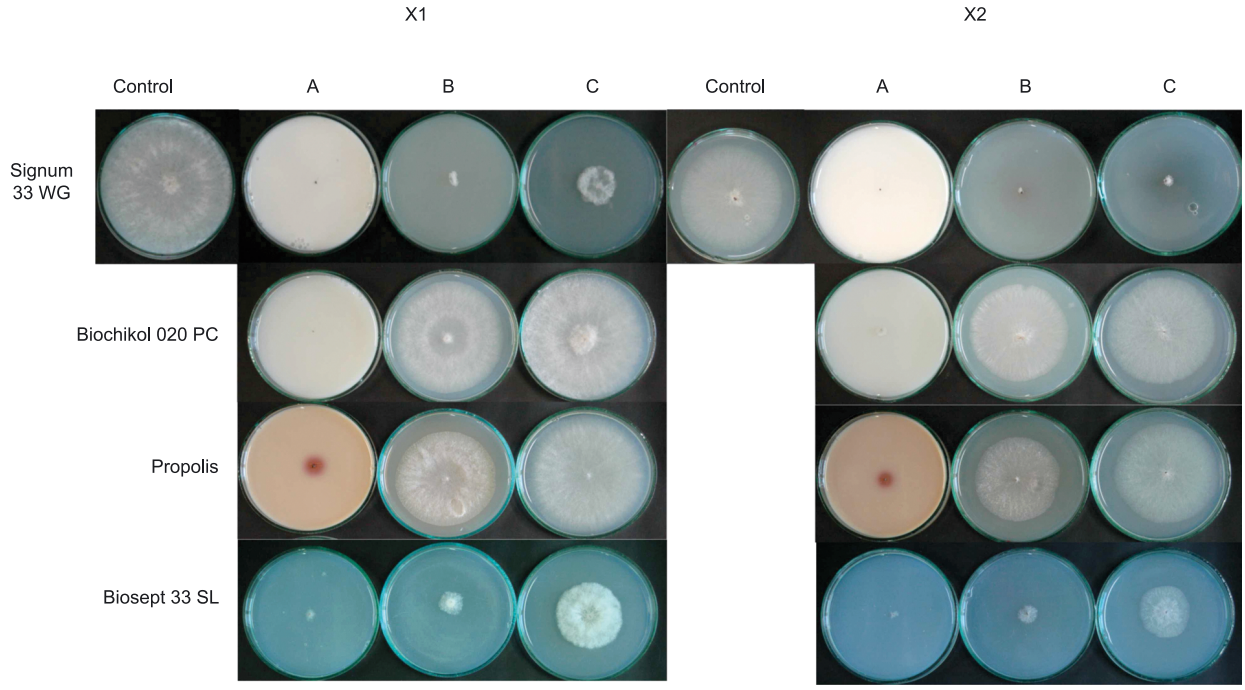
The results are presented as colony size expressed as a percentage of the control.

RESULTS AND DISCUSSION

The effect of biopesticides on *B. cinerea* mycelium differed and depended on both the active ingredient and biopesticide concentration (Figs. 1–6). The growth of *B. cinerea* fruit isolate (X2) was completely inhibited 24 hours after inoculation, irrespective of the biopesticide and its concentration (Figs. 2–5). The cane isolate (X1) responded differently; low rates (C) of Bioczos S, Biosept 33 SL and Biochikol 020 PC only markedly retarded its growth (Figs. 2–4). The first 24 hours after inoculation, Propolis was most toxic for *B. cinerea*, irrespective of the rate, and prevented the growth of both the isolates (Fig. 5).

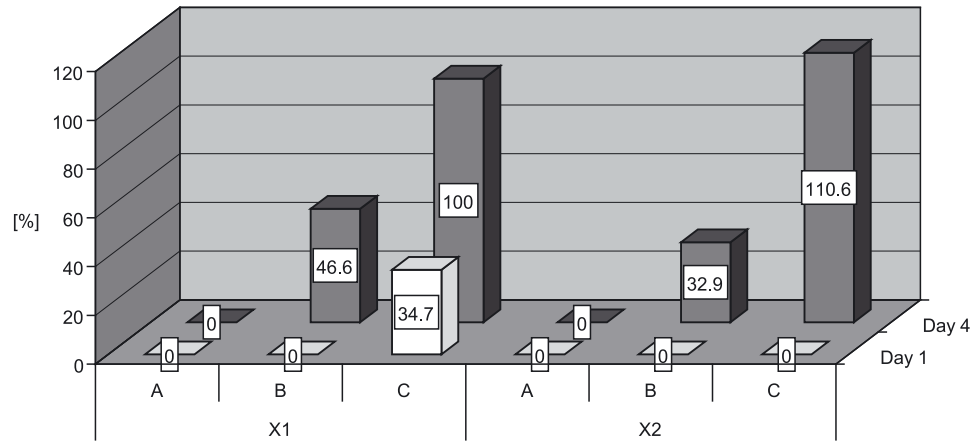
After 76 hours, the toxicity of the biopesticides was much lower and varied. Machowicz-Stefaniak *et al.* (1995) reported that efficacy of the garlic biopesticide dropped as the experiment progressed. The authors demonstrated that, following an application of the extract, inhibition of *B. cinerea* mycelium growth lasted for 8 days of incubation. In the present experiment only Bioczos S and Propolis permanently inhibited the growth of both the isolates when applied at the highest rate (A) (Fig. 2, 5).

After an application of the recommended rate (B) of the biopesticides, only Bioczos S and Biosept 33 SL markedly inhibited the growth of *B. cinerea.* Toxicity of the above products to *B. cinerea*, Biosept 33 SL in particular,



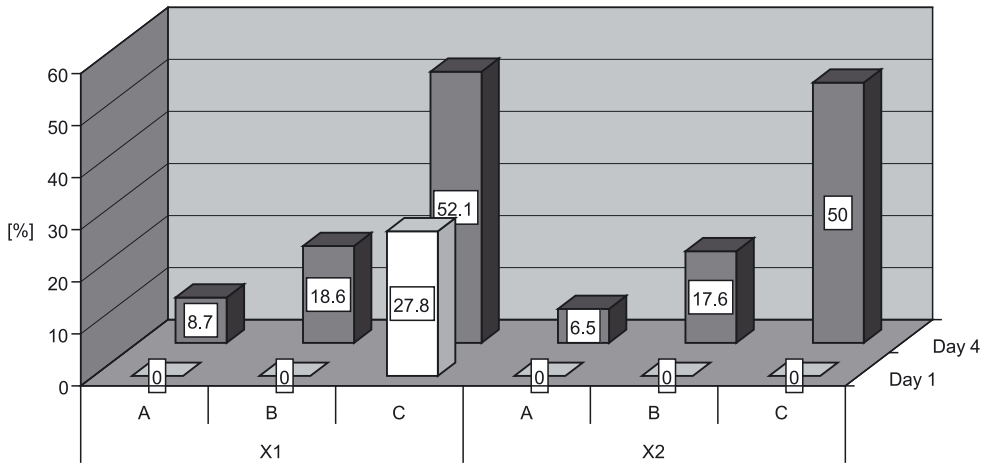
A – concentration 5-times higher than recommended
B – recommended concentration
C – concentration 5-times lower than recommended
X1 – can
X2 – fruit

Fig. 1. Twenty-days old *B. cinerea* colonies on medium with biopreparations, and synthetic fungicide



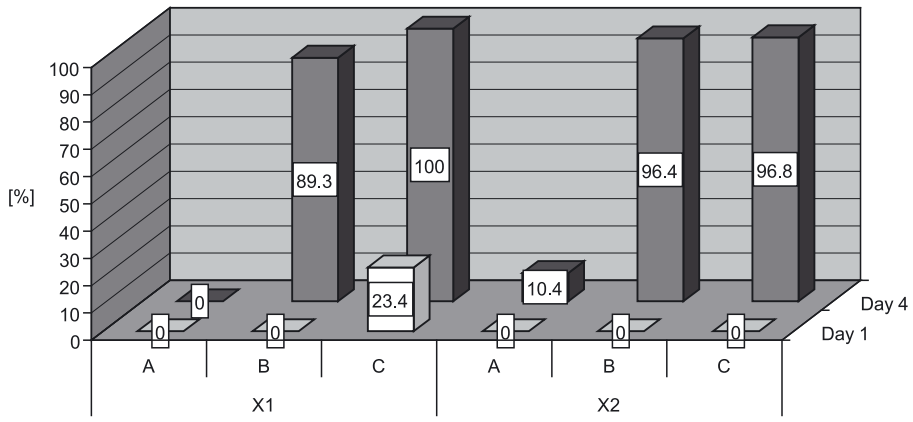
A – concentration 5-times higher than recommended
B – recommended concentration
C – concentration 5-times lower than recommended
X1 – can
X2 – fruit

Fig. 2. Diameter of colonies of the fungi *B. cinerea* on medium with biopreparation Bioczos SL (in % compared to the control)



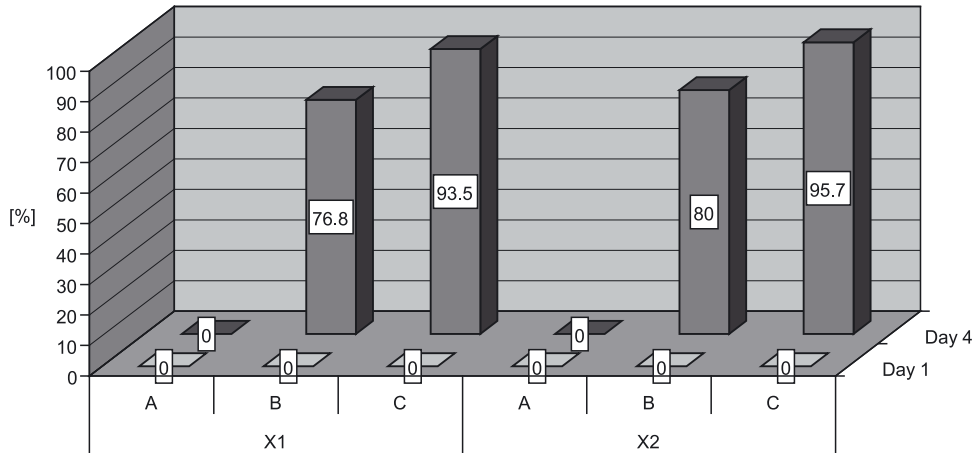
Explanations are as in fig. 2

Fig. 3. Diameter of colonies of the fungi *B. cinerea* on medium with biopreparation Biosept 33 SL (in % compared to the control)



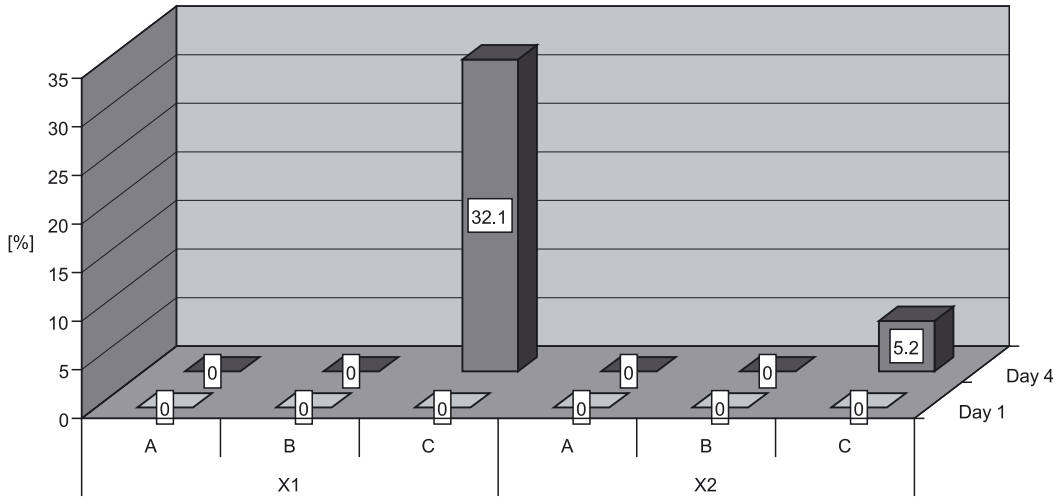
Explanations are as in fig. 2

Fig. 4. Diameter of colonies of the fungi *B. cinerea* on medium with biopreparation Biochikol 020 PC (in % compared to the control)



Explanations are as in fig. 2

Fig. 5. Diameter of colonies of the fungi *B. cinerea* on medium with biopreparation Propolis (in % compared to the control)



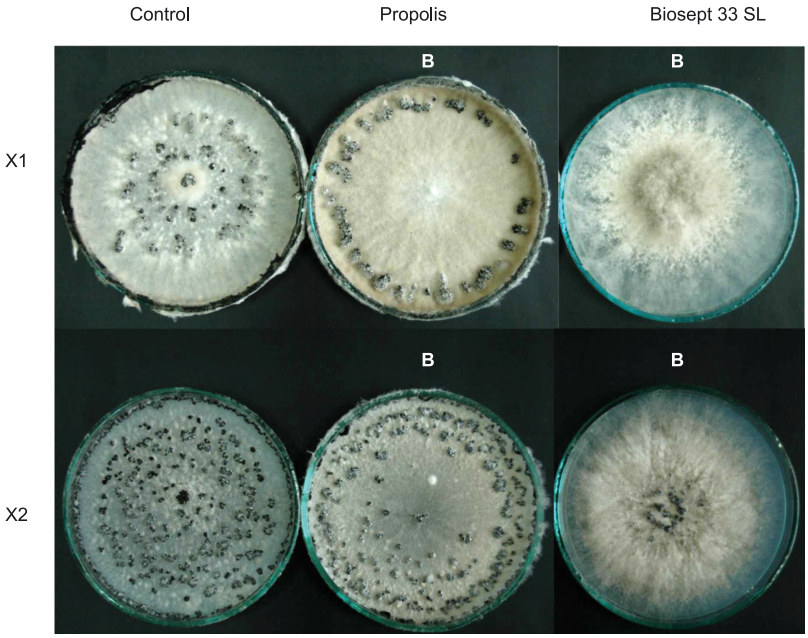
Explanations are as in fig. 2

Fig. 6. Diameter of colonies of the fungi *B. cinerea* on medium with preparation Signum 33 WG (in % compared to the control)

has been confirmed in the experiments by Dłużniewska (2005) who found that the growth *in vitro* of the pathogen was markedly inhibited by Biosept 33 SL. Additionally, the biopesticide was toxic to the fungal spores; preventing their germination. In turn, an addition of Bioczos BR to the medium on the fourth day of incubation inhibited the growth of *B. cinerea* mycelia (Sapieha-Waszkiewicz *et al.* 2000) by almost 60%, which was also demonstrated in the present study. Again, fruit isolate X2 was slightly more susceptible to Bioczos S and Biosept 33 SL. Biochikol 020 PC and Propolis applied at rate B were barely toxic to *B. cinerea* (Figs. 4–5); the colony size was reduced by about 14%, on average, compared with the control. Poor efficacy of chitosan (Biochikol 020 PC), in the control of *B. cinerea* was reported by Wojdyła and Orlikowski (1997). They found that the biopesticide inhibited the development of the disease on rose petals only during the first three days of the experiment when applied at the concentrations of 0.1% and 0.15%. Our findings in the present work were similar.

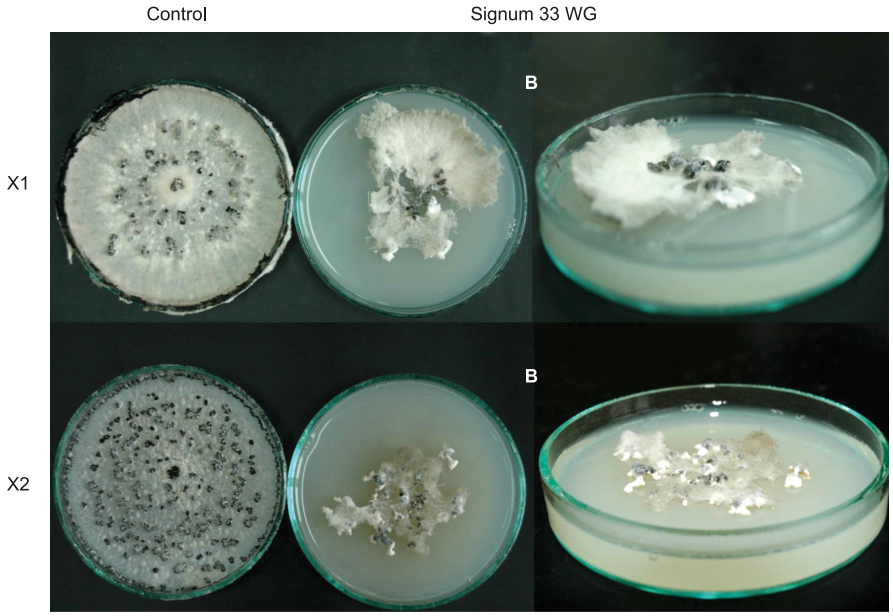
The lowest concentration (C) of the biopesticides was hardly toxic to *B. cinerea*. The size of its cultures was comparable to the control. The sole exception was Biosept 33 SL which markedly inhibited the growth of both the isolates. Towards the end of the experiment the isolates were around 50% smaller than the control colonies (Fig. 3). Also studies by Sapieha-Waszkiewicz *et al.* (2001) demonstrated that Biosept 33 SL was a highly effective biopesticide in the control of *B. cinerea.* Colony growth was markedly inhibited at all the concentrations examined. Moreover, when the biopesticide was applied at a concentration fivefold lower than the recommended rate, the size of colonies was 38% of the control colonies.

Unlike the biopesticides, the synthetic fungicide Signum 33 WG was highly toxic to *B. cinerea* (Fig. 6). Its highest and recommended rates inhibited the growth of the fungus during the whole experiment. *B. cinerea* susceptibility to rate C of Signum 33 WG was slightly lower – both the isolates grew slowly. Once again, isolate X2 was much more susceptible to the fungicide as the size of its colonies was 5.2% of the control. The growth of raspberry cane isolate X1 was more dynamic with the size of fungal colonies being 32.1% of the control (Fig. 6).



B – recommended concentration

Fig. 7. Morphology of 20-day-old colonies *B. cinerea* on medium with preparations Propolis and Biosept 33 SL



X1 – can
X2 – fruit
B – recommended concentration

Fig. 8. Morphology of 20-day-old colonies *B. cinerea* on medium with fungicide Signum 33 WG

Observations made on day 20 of the experiment demonstrated that the colonies of both the *B. cinerea* isolates started to develop on media containing all the biopesticides, irrespective of their concentration. The morphology of colonies growing on media containing Bioczos S and Biochikol 020 PC did not differ from the morphology of the control. Higher concentrations of Propolis inhibited the development of sclerotia, with complete lack of sclerotia on media containing Biosept 33 SL (Fig. 7). Signum 33 WG was the only plant protection product which completely destroyed mycelia of both the *B. cinerea* isolates (Fig. 8) when applied at the highest rate. At the remaining two rates, the development of colonies of both the isolates was very slow and their mycelia were very deformed and came away from the dish. Moreover, less sclerotia were produced compared with the control (Fig. 8).

CONCLUSIONS

1. Sensitivity of *B. cinerea* to the biopesticides applied in the experiment differed depending on the isolate. The cane isolate was much more resistant to the biopesticides.
2. The biological pesticides were barely toxic to *B. cinerea*. The development of both the isolates was inhibited, but only at the beginning of the experiment. This was the time when the biopesticides were applied at the recommended rate or the rate which was fivefold higher than the recommended amount.
3. Of the biopesticides examined in the study, Biosept 33 SL was the strongest inhibitor of *B. cinerea* whereas the effect of Biochikol 020 PC was the poorest.
4. The effect of Propolis applied at the recommended rate and the rate which was fivefold higher than the recommended amount was comparable to the effect of the remaining biopesticides. Additionally, it inhibited formation of *B. cinerea* spores.
5. The fungicide Signum 33 WG was highly toxic towards both the isolates of *B. cinerea*.

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POLISH SUMMARY

WPŁYW BIOPREPARATÓW NA WZROST I ROZWÓJ *IN VITRO* IZOLATÓW *BOTRYTIS CINEREA* PERS., POCHODZĄCYCH Z KRZEWÓW MALIN

Wykorzystane w doświadczeniu dwa izolaty *Botrytis cinerea* różniły się podatnością na zastosowane fungicydy. Izolat pochodzący z porażonych owoców maliny okazał się wrażliwszy na zastosowane biopreparaty oraz fungicyd Signum 33 WG. Skuteczność zastosowanych preparatów była zróżnicowana i zależała zarówno od substancji aktywnej, jak i czasu oddziaływania preparatu na grzybnię. Preparaty biologiczne okazały się mało toksyczne dla *B. cinerea.* W dawce 5-krotnie wyższej i zalecanej tylko w początkowej fazie hodowli całkowicie hamowały wzrost obu izolatów. W grupie biopreparatów, Biosept 33 SL najsilniej ograniczał wzrost *B. cinerea*, na podłożach zawierających ten preparat nie obserwowano tworzenia się sklerocjów grzyba. Ograniczenie wytwarzania form przetrwalnikowych obserwowano również po zastosowaniu Propolisu, jednak preparat ten skutecznie ograniczał wzrost grzybni tylko po aplikacji najwyższej dawki. Standardowo stosowany do zwalczania szarej pleśni syntetyczny fungicyd Signum 33 WG, cechował się wysoką skutecznością w stosunku do obu izolatów *B. cinerea.*