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The influence of *Hypholoma fasciculare* and *Phlebiopsis gigantea* on the growth of *Heterobasidion annosum* in vitro

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The influence of two saprotrophes – isolates of *Hypholoma fasciculare* and *Phlebiopsis gigantea* on the growth of thirty three root pathogen strains – *Heterobasidion annosum* was analysed. Two methods were used. The different reaction in paired cultures among saprotrophe and pathogen isolates suggest, that one isolate of *H. annosum* is not enough to study the interaction between this pathogen and saprophytes in vitro irrespective of the method used.

Key words: *Heterobasidion annosum, Hypholoma fasciculare, Phlebiopsis gigantea.*

INTRODUCTION

Heterobasidion annosum (Fr.) Bref. causes one of the most dangerous diseases in forests all over the world (Gremmen 1970; Hubbes 1980; Mańka 1992). This fungus due to butt and root rot of coniferous and sometimes deciduous trees. Control of this pathogen is difficult since it can spread by root-to-root contact or by spores which infect stumps left in the soil after thinning in stands or clear-cutting in the next generation (Mańka 1986, 1991). *Heterobasidion annosum* can also survive as saprophyte in woody substrates for decades (Schütt et al. 1979).

The biological control is associated with of replacing the pathogen or preventing stumps by other organisms. Such as *Phlebiopsis gigantea* (Fr.: Fr.) Jülich and *Trichoderma* spp., or *Hypholoma fasciculare* (Huds: Fr.) Kummer (Twarowska 1972; Lundberg and Unestram 1981; Rhisbeth 1979; Sierota and Sternak 1993; Sierota 1995).

The objectives of this study were to determine: a – whether the growth of all using pathogen isolates is suppressed by different saprotrophe strains; b – whether the diversity of growth in paired cultures inside the pathogen population is higher than in saprotrophes.

MATERIALS AND METHODS

The pathogen. *Heterobasidion annosum* was represented by thirty three strains from P intersterility group, obtained by isolation from roots of dead Scots pines localised in two plantations (Podanin Forest District) in 1995. Each isolate belonged to different clones, which were identified on the basis of demarcation line formation (somatic incompatibility) between paired heterokaryotic cultures (S t e n l i d 1985; P i r i 1996).

The saprotrophes. Four strains of *Hypholoma fasciculare* were isolated from basidiomes, which were collected from oak stumps (Podanin, Babki and Zielonka Forest Districts) in 1996. The fifth strain was obtained from "HF" – biopreparation.

Three strains of *Phlebiopsis gigantea* were derived from Italy (Austrian pine), Finland (Scots pine) and Poland ("PG" – biopreparation).

Influence in vitro. The influence of *Hypholoma fasciculare* and *Phlebiopsis gigantea* on *Heterobasidion annosum* growth in vitro was estimated by two methods – the biotic series described by M a ń k a (1974) and the method described by M a r x (1969). All the isolates of *Hypholoma fasciculare* and *Phlebiopsis gigantea* were tested for their individual biotic effect on growth of 33 *Heterobasidion annosum* strains. Isolates were inoculated on 1.5% malt agar and the test was estimated after 10 days of incubation at room temperature according to the scale described by M a ń k a (1974).

In the second method the radial growth of mycelium was recorded (in millimetres) after 10 days of incubation at room temperature on 1.5% malt agar.

The data were subjected to analysis of variance.

RESULTS

The results of the biotic tests are presented in Table 1. The individual biotic effect for isolates of *Hypholoma fasciculare* varied from −5 (*H. fasciculare* 96016 + *H. annosum* 95109) to + 6 (*H. fasciculare* 96018 + *H. annosum* 95125). The biggest range of IBE on different strains of *H. annosum* was observed for *H. fasciculare* 96018 (from −4 to +6) and the smallest range of IBE for *H. fasciculare* 96020 (from −2 to +2). The growth of each isolate

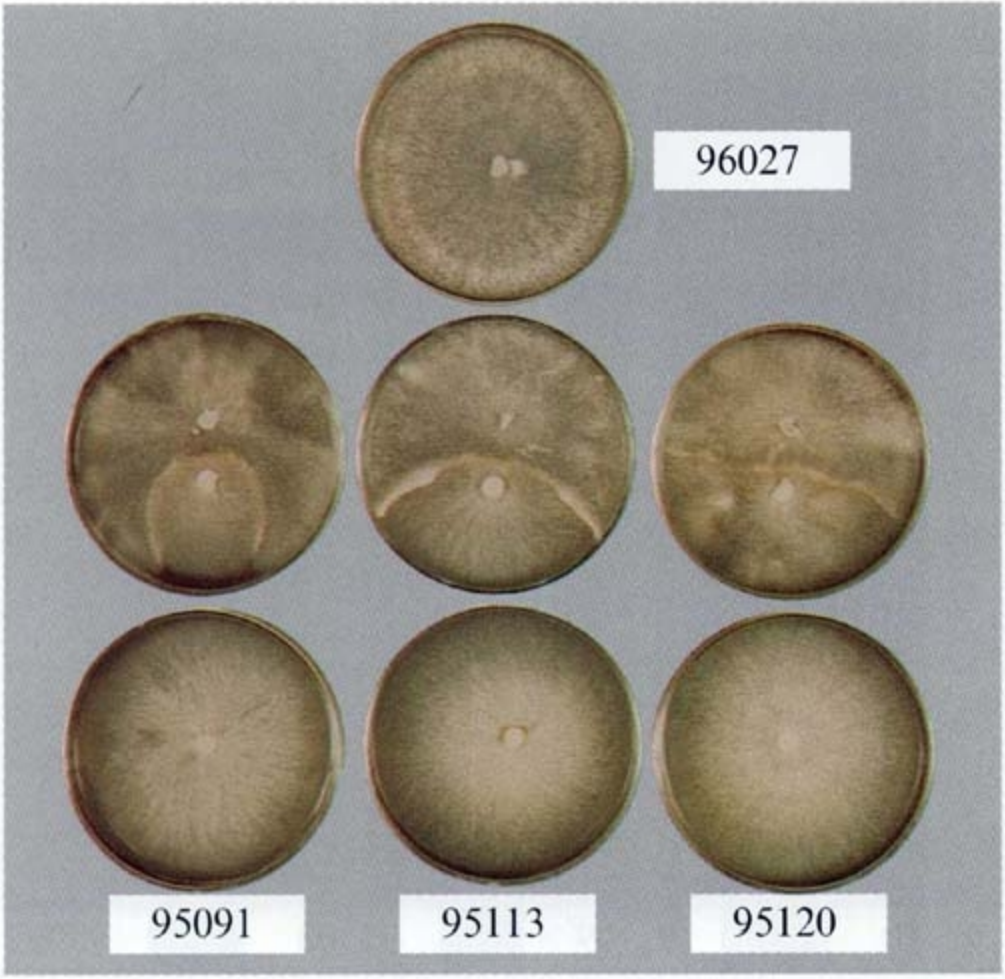

Fig. 1. *Phlebiopsis gigantea* 96027 (top) and *Heterobasidion annosum* (bottom): from left 95091, 95113, 95120 isolates in paired cultures (middle) in biotic series method

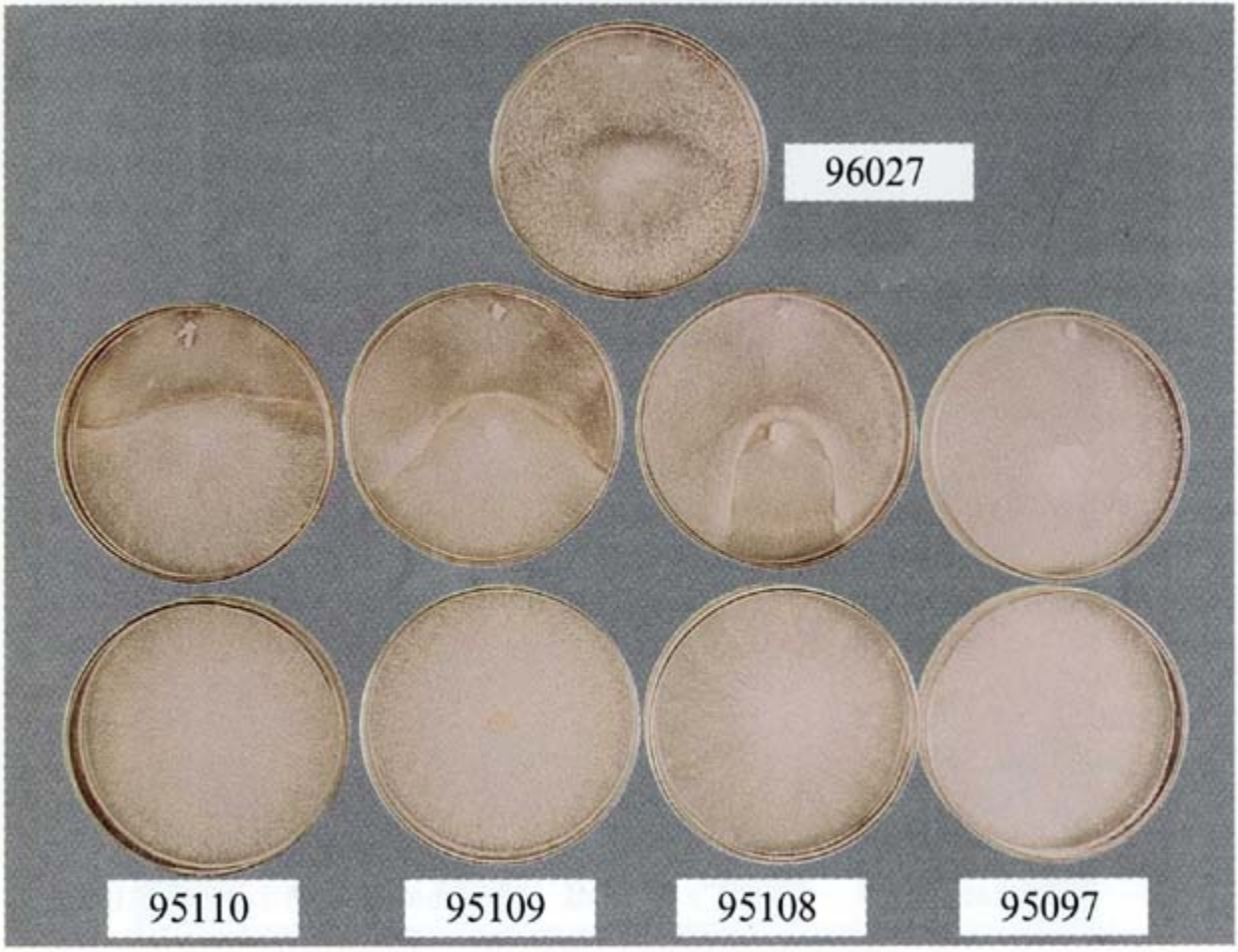

Fig. 2. *Phlebiopsis gigantea* 96027 (top) and *Heterobasidion annosum* (bottom): 95110, 95109, 95108, 95097 isolates in paired cultures (middle) in Marx's method

Table 1

Some examples of individual biotic effect of *Hypholoma fasciculare* and *Phlebiopsis gigantea* isolates influence on the growth of *Heterobasidion annosum*

Isolate No	*Heterobasidion annosum*									
	95090	95093	95095	95099	95104	95107	95117	95120	95122	95125	95133
H. fasciculare 96016	$+2^{**1}$	$+3^{ns}$	-1^{**}	$+1^{ns}$	-4^{**}	-2^{*}	$+2^{**}$	-2^{**}	0^{ns}	$+2^{**}$	-4^{ns}
96018	−2	+4	−2	−1	−1	0	+1	+3	+1	+6	+1
96019	+2	+2	−1	−1	−1	−2	+1	0	0	+5	−1
96020	+1	+2	0	−2	−2	−2	0	0	−1	+2	−1
96024	−1	+3	0	+1	−2	0	+1	+3	0	+2	−1
Ph. gigantea 96027	$+6^{ns}$	$+6^{ns}$	$+2^{ns}$	$+3^{**}$	$+3^{*}$	$+3^{ns}$	$+2^{ns}$	$+2^{*}$	$+8^{ns}$	$+5^{ns}$	$+5^{**}$
96028	+8	+7	+3	+7	+4	+5	+5	+3	+8	+7	+4
96029	+7	+6	+5	+5	+4	+5	+4	+3	+6	+5	+3

^{**1} – significant differences in IBE of influence of all saprotrophes isolates on the growth of one *H. annosum* isolate

^{**2} – significant differences in IBE of influence of one saprotrophe isolate on the growth of all *H. annosum* isolates

** – a = 0.01

of *H. fasciculare* was suppressed by some isolates of *H. annosum*. Moreover the growth of other pathogen isolates was restricted by the same saprotrophe isolate. Only three isolates of *H. annosum* were suppressed by all *H. fasciculare* isolates and two isolates of *H. fasciculare* were suppressed by all the isolates of the pathogen. The isolate from the biopreparation was suppressed by the highest number of *H. annosum* isolates (20). The analysis of variance displayed significant differences in IBE estimation for each *H. fasciculare* isolate influence on the growth of all the *H. annosum* isolates. In 12 cases the influence of all *H. fasciculare* isolates on the growth of one *H. annosum* isolate did not differ significantly.

Isolates of *Phlebiopsis gigantea* suppressed the growth of the all *Heterobasidion annosum* isolates. The IBE for *Ph. gigantea* varied from +2 (*Ph. gigantea* 96027 + *H. annosum* 95095; Fig. 1) to +8 (e.g. *Ph. gigantea* 96028 + *H. annosum* 95090). The biggest range of IBE on different isolates of *H. annosum* was estimated for *Ph. gigantea* 96027 (from +2 to +8) and the smallest one for *Ph. gigantea* 96029 (from +3 to +8). The analysis of variance showed significant differences in IBE estimation for each *Ph. gigantea* isolate influence on the growth of all the *H. annosum* isolates. In 22 cases the differences in the influence of all *Ph. gigantea* isolates on the growth of one *H. annosum* isolate were not statistically significant.

Table 2

Some examples of mycelium growth percentage of saprotrophe and pathogen in comparison to the control

	Heterobasidion annosum								
	95095	95100	95101	95107	95108	95109	95122	95126	95127
H. fasciculare 96016	$96^{ns}/83^{**}$	$83^{**}/101^{ns}$	$96^{ns}/146^{*}$	$200^{**}/94^{ns}$	$100^{ns}/99^{ns}$	$86^{*}/125^{**}$	$86^{*}/89^{*}$	$130^{**}/112^{*}$	$109^{ns}/85^{*}$
H. fasciculare 96018	$40^{**}/144^{**}$	$43^{**}/109^{ns}$	$48^{**}/154^{**}$	$43^{**}/91^{ns}$	$43^{**}/109^{ns}$	$40^{**}/135^{**}$	$48^{**}/88^{*}$	$46^{**}/118^{*}$	$43^{**}/100^{ns}$
H. fasciculare 96019	$62^{**}/139^{**}$	$54^{**}/90^{*}$	$67^{**}/154^{**}$	$70^{**}/88^{*}$	$65^{**}/105^{ns}$	$60^{**}/125^{**}$	$57^{**}/88^{*}$	$70^{**}/108^{ns}$	$67^{**}/87^{**}$
H. fasciculare 96020	$60^{**}/143^{**}$	$47^{**}/109^{ns}$	$57^{**}/143^{**}$	$57^{**}/107^{ns}$	$50^{**}/107^{ns}$	$55^{**}/130^{**}$	$50^{**}/89^{*}$	$55^{**}/118^{*}$	$57^{**}/90^{ns}$
H. fasciculare 96024	$71^{**}/139^{**}$	$68^{**}/107^{ns}$	$74^{**}/160^{**}$	$63^{**}/88^{*}$	$63^{**}/103^{ns}$	$63^{**}/132^{**}$	$60^{**}/88^{*}$	$63^{**}/115^{*}$	$71^{**}/96^{ns}$
Ph. gigantea 96027	$99^{ns}/26^{**}$	$92^{ns}/85^{*}$	$100^{ns}/31^{**}$	$100^{ns}/47^{**}$	$101^{ns}/38^{**}$	$102^{ns}/63^{**}$	$88^{*}/65^{**}$	$95^{ns}/49^{**}$	$99^{ns}/33^{**}$
Ph. gigantea 96028	$100^{ns}/19^{**}$	$89^{*}/73^{**}$	$94^{ns}/25^{**}$	$98^{ns}/48^{**}$	$100^{ns}/37^{**}$	$100^{ns}/41^{**}$	$88^{*}/60^{**}$	$93^{ns}/66^{**}$	$66^{**}/75^{**}$
Ph. gigantea 96029	$133^{**}/26^{**}$	$72^{**}/95^{ns}$	$116^{*}/56^{**}$	$93^{ns}/74^{**}$	$114^{*}/74^{**}$	$103^{ns}/107^{ns}$	$105^{ns}/78^{**}$	$105^{ns}/58^{**}$	$84^{**}/51^{**}$

^{ns} – the mycelium size in pairing cultures doesn't differ signifficantly in comparision to the control

* – the mycelium size in pairing cultures differ significantly on a = 0.05 in comparison to the control

** – the mycelium size in pairing cultures differ significantly on a = 0.01 in comparison to the control

The results of the method described by M a r x are presented in Table 2. In most cases *H. fasciculare* isolates restricted the growth of *H. annosum* cultures. The pathogen growth was inhibited by 1 – 53% (Fig. 2). The isolate of *H. fasciculare* used in the biopreparation restricted the growth of the greatest number of *H. annosum* isolates. The growth stimulation was also estimated for *H. annosum* (1 – 60%) cultures. In paired cultures a reduced growth reduction of *H. fasciculare* isolates was observed. Only in three cases the cultures of *H. fasciculare* isolate from the biopreparation was bigger in comparison to the control (9%, 30% and 100%). The analysis of variance showed significant differences between the growth of *H. fasciculare* and *H. annosum* isolates in paired cultures. Only *Hypholoma fasciculare* cultures from the biopreparation did not differ significantly from the control in about 50% of the cases.

Each isolate of *Phlebiopsis gigantea* inhibited the growth of all the *H. annosum* isolates by 3% – 81%. In one case only the pathogen culture was bigger than the control (7%). The pathogen isolates also restricted the growth of *Ph. gigantea* by 1% – 36%. In most cases (20) the cultures of *Ph. gigantea* (96027) from the biopreparation were bigger than the control. The analysis of variance showed significant differences between.

DISCUSSION

In both experiments the saprotrophs influence on the growth of *Heterobasidion annosum* isolates was different. The results of IBE and the diameter of cultures were significantly different for each pathogen isolate. Moreover the same type of relation was observed for saprotrophe isolates. All the authors used only one isolate of *Heterobasidion annosum* in biotic series (P r z e z b ó r s k i 1974; M a ń k a et al. 1991; S i e r o t a 1995). It was demonstrated that the isolates derived from different stocks reacted differently in paired cultures in both methods. In addition some of these *H. annosum* cultures were isolated from roots of killed trees localised in one mortality gap. M a ń k a et al. (1993) showed that only one isolate of species from fungal community may be use as a representative. However the isolates of the same species from different fungal communities would exert a different individual biotic effect on the same pathogen growth (M a ń k a et al. 1993; K o w a l s k i 1989; K u r z a w i ń s k a 1992, 1993). W e r n e r et al. (1995) indicated that various *H. annosum* stocks could be inhibited in a different way by soil saprotrophes. It seems that one isolate of *H. annosum* is not enough to estimate the influence of saprotrophe on pathogen in paired cultures. The IBE for influence of *H. fasciculare* on the growth of different *H. annosum* stock varied from −4 to +6 and of *Phlebiopsis gigantea* from +2 to +8. S i e r o t a (1996) tested two isolates of *H. fasciculare* with

H. annosum. He found that the IBE for the isolate derived from stump roots of the 1st generation pine of post-agricultural land was +3 and for the isolate derived from the roots of the 2nd generation: +4. P r z e z b ó r s k i (1974) also used in the biotic series two isolates of *H. fasciculare* that were isolated from Scots pine stumps localised in two different stands. The IBE in this case was the same −3. The results of Marx tests were also significantly different. The growth of *H. fasciculare* isolate from biopreparation was inhibited by 20 pathogen isolates in biotic series. At the other hand this saprotrophe isolate restricted the growth of 12 isolates of *H. annosum* but in the same time its growth was suppressed by 14 pathogen isolates in Marx test. This test makes it possible to determine the influence of saprotrophe on the pathogen growth and also the opposite situation in paired cultures. Not only the restriction but also the stimulation of mycelium growth could be estimated by means of the Marx test. M a t k o w s k i (1996) used seven isolates of the *Pyrenochaeta terrestris* (Hansen) Gorenz, Walker et Larson and one *Fusarium oxysporum* isolate in biotic series. The IBE varied from +3 to −3.

Genetically different isolates of *Heterobasidion annosum* show higher variability of growth than saprophytes in paired cultures. Irregular damages caused by this pathogen and different disease spreading in Scots pine stands suggest that the variability concerns also the virulence of isolates belonging to different clones.

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REFERENCES

G r e m m e n J. 1970. The actual situation of research and control of the root rot fungus (*Fomes annosus*) in the Netherlands. Proc. 3rd Int. Conf. on *Fomes annosus*, IUFRO, Denmark, 1968: 33–36.

H u b b e s M. 1980. *Fomes annosus* in Canada. In: L. Dimitri (ed.). Proc. of 5th conference on root and butt rots. Kassel: Intern. Union Forestry Res. Organisa: 40–42.

K o w a l s k i S. 1989. Study on the effect of mycorrhizosphere fungi on growth of *Heterobasidion annosum* in plantation in a zone of strong pollution with industrial emissions. proc. of the 7th Int. Conf. on Root and Butt Rots, 9–16 August 1988, British Columbia, Canada. Ed. D.J. Morrison. for. Can. Pacif. For. Cent. Can. V8Z 1M5: 477–485.

K u r z a w i ń s k a H. 1992. The effect of fungal communities in soil environment of potato crop on the growth of pathogens *Fusarium sulphureum* Schl. and *Fusarium coeruleum* (Sacc.) Booth. Phytopathol. Polon. 16: 5–16.

K u r z a w i ń s k a H. 1993. The effect of fungi isolated from soil environment on agents of dry rot of potato – *Fusarium sulphureum* Schl. and *Fusarium coeruleum* (Sacc.) Booth. Phytopathol. Polon. 18: 13–18.

Lundberg A., Unestram T. 1981. Antagonism against *Fomes annosus*. Comparison between different test methods *in vitro*. Mycologia. 70: 107–115.

Mańka K. 1974. Zbiorowiska grzybów jako kryterium oceny wpływu środowiska na choroby roślin [Fungal communites as a cryterion for estimating the effect of the environment on plant diseases]. Zesz. Probl. Post. Nauk Rol. 160: 9–23.

Mańka K. 1986. Trzebieże jako zabiegi przeciwdziałające występowaniu huby korzeni (*Heterobasidion annosum* (Fr.) Bref.) i zamieraniu pędów sosny (*Gremmeniella abietina* (Lagerb.) Morelet) [Thinnings as a treatment couneracting the occurrence of *Heterobasidion annosum* (Fr.) Bref. and *Gremmeniella abietina* (Lagerb.) Morelet]. Sylwan 7: 1–8.

Mańka K. 1991. Huba korzeni. Folder nr 6 z serii Choroby Drzew Leśnych. PWRiL, Poznań.

Mańka K. 1992. Fitopatologia leśna. PWRiL, Warszawa.

Mańka K., Kwaśna H., Babkiewicz M., Kazimierczak T. 1991. Biotic resistance of soil to plant pathogens. Proceedings of the first conference of the European Foundation for Plant Pathology "Biotic interactions and soil-borne diseases". Netherlands Soc. Plant Pathol. Elsevier, Amsterdam–Oxford–New York–Tokyo: 311–315.

Mańka M., Dymek A., Piechowska K. 1993. Biotic series method for evaluation of soil fungi effect on plant pathogenic fungi. I. Raplicability of individual biotic effect measurement in the biotic series method. Phytopathol. Polon. 18: 87–98.

Marx D. H. 1969. The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. Phytopathol. 59: 153–163.

Matkowski K. 1996. Wstępne badania nad wzajemną reakcją pomiędzy *Pyrenochaeta terrestris* (Hansen) Gorenz, Walker et Larson i *Fusarim oxysporum* Schlecht. Choroby Roślin a Środowisko. Materiały z sympozjum naukowego, Poznań, 27–28.06.1996: 329–336.

Piri T. 1996. The spreading of the S type of *Heterobasidion annosum* from Norway spruce stumps to the subsequent tree stand. Eur. J. For. Path. 26: 193–204.

Przezbórski A. 1974. Zagadnienie huby korzeni na tle mikologicznej analizy pniaków sosnowych. Zesz. Probl. Post. Nauk. Roln. 160: 47–86.

Rishbeth J. 1979. Modern aspects of biological control of *Fomes* and *Armillaria*. Eur. J. For. Path. 9: 331–340.

Sierota Z. 1995. Rola grzyba *Phlebiopsis gigantea* (Fr.: Fr.) Jülich w ograniczaniu huby korzeni w drzewostanach sosny zwyczajnej (*Pinus sylvestris* L.) na gruntach porolnych. Prace IBL. 810, seria A.

Sierota Z. 1996. Biotic activity of fungal communities against a pathogen – a proposal of evaluation with the use of "biotic effect coefficients". Phytopathol. Polon. 12: 5–13.

Sierota Z., Sternak A., 1993. Zagrożenia II generacji sosny ze strony huby korzeni na gruntach porolnych. Las Polski 19: 10–11.

Stenlid J. 1985. Population structure of *Heterobasidion annosum* as determined by somatic incompatibility, sexual incompatibility, and isoenzyme patterns. Can. J. Bot. 63: 2268–2273.

Schütt P., Schuck H. J., Lautenschlager K., Prestleu W., Stimm B. 1979. *Fomes annosus* sporocarps – their abundance on decayed logs left in the forest. Eur. J. For. Path. 9: 57–61.

Twarowska I. 1972. Badania nad zwalczaniem huby korzeniowej metodą biologiczną [Investigation on the biological control of the root rot]. Prace IBL 405: 1–55.

Werner M., Werner A., Andrzejak R. 1995. Antagonistic effect of some soil fungi on *Fusarium oxysporum* and *Heterobasidion annosum* in laboratory experiments. Environmental Biotic Factors In Integrated Plant Disease Control. European Foundation for Plant Pathology; 3rd Conference Polish Phytopatol. Soc., 5–9. 09. 1994: 611–616.

Wpływ *Hypholoma fasciculare* i *Phlebiopsis gigantea* na wzrost *Heterobasidion annosum* in vitro

Streszczenie

Testowano wpływ pięciu izolatów *Hypholoma fasciculare* i trzech *Phlebiopsis gigantea* na wzrost 33 izolatów patogena *Heterobasidion annosum* typu P in vitro. W tym celu posłużono się dwiema metodami: metodą szeregów biotycznych opisaną przez Mańkę (1974) oraz metodą opisaną przez Marxa (1969). Indywidualny efekt biotyczny określony dla kultur dwugrzybowych *H. fasciculare* + *H. annosum* był różny i wynosił od −5 do +6. Tylko trzy izolaty patogena były ograniczane przez wszystkie izolaty *H. fasciculare*, natomiast wzrost dwóch izolatów saprotrofa był ograniczany przez wszystkie izolaty *H. annosum*. Indywidualny efekt biotyczny w testach *Ph. gigantea* wynosił od +2 do +8. Jednoczynnikowa analiza wariancji wykazała statystycznie istotne różnice w ocenach wpływu każdego izolatu *H. fasciculare* i *Ph. gigantea* na wszystkie izolaty *H. annosum*. Na podstawie testu Marxa stwierdzono, że w większości przypadków izolaty *H. fasciculare* ograniczały wzrost patogena (1−53%). Obserwowano także stymulację wzrostu *H. annosum* (1−60%) oraz jednocześnie ograniczenie wzrostu izoaltów *H. fasciculare*. Z kolei każdy izolat *Ph. gigantea* ograniczał wzrost wszystkich izolatów patogena (3−81%), ale niektóre izolaty saprotrofa były równocześnie ograniczane przez *H. annosum* (1−36%). W wielu przypadkach jednoczynnikowa analiza wariancji wykazała ststystycznie istotne różnice pomiędzy wielkościami grzybni testowanych izolatów w kulturze dwugrzybowej w porównianiu z grzybniami kontrolnymi.