DENDROBIOLOGY
2002, vol. 47: 51–58

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Interaction between microfungi from arable and fallow land soils and *Heterobasidion annosum in vitro*

Abstract: Werner A., Zadworny M. Interaction between microfungi from arable and fallow land soils and *Heterobasidion annosum in vitro*.
Interactions between members of microfungal communities of three arable and two fallow land soils and three P strains of *Heterobasidion annosum* were investigated *in vitro*. The effect of soil fungi on the pathogen was evaluated with two methods. In the first, the mycelial growth of *H. annosum* strains was measured and the experimental data were analysed using statistics. In the second, the biotic series method was used. The differences between the effects of soil fungi dominating in arable and fallow land soils were statistically significant. The microfungal communities of fallow land soils inhibited the growth of the pathogen more than those inhabiting arable soils. Evaluation of suppressive effect of the soil fungi on the pathogen with biotic series method showed also far more negative influence of microfungal communities of fallow land soils on the growth of *H. annosum* strains. Considering the positive effect of chemical and biological properties of the fallow land soils on the growth of Scots pine and highly suppressive effect of microfungal communities of these soils on the growth of *H. annosum* observed in the presented study, it may be concluded that arable soils left barren for many years are more beneficial for establishing of Scots pine plantations than cultivated soils.

Additional key words: biotic series method, co-culture, growth measurement, soil fungi

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Introduction

Heterobasidion annosum (Fr.) Bref. is one of the most dangerous root inhabiting pathogens, causing great damages in plantations of conifers in temperate and boreal regions of the world (Hodges 1969). It consists of three intersterility (IS) groups showing different preferences to the main host trees, namely: pines, spruces and firs (Korhonen 1978; Capretti et al. 1990). In Poland, Scots pine is the typical host of the P-group isolates of *H. annosum* (Łakomy et al. 2000).

Since mycelial growth of the fungus through soil is restricted (Rishbeth 1951), the incidence of *Heterobasidion*-root rot in managed forests is mainly related to the frequency and intensity of thinnings (Wallis 1960). The fungus infects fresh cut stumps or wounds by airborne spores, then colonizes roots and spreads on healthy trees *via* root contacts (Rishbeth 1950, 1951). Spores of *H. annosum* are known to be distributed and persistent in bulk soil. In conditions favourable for the fungus (i.e., alkaline soils, deficiency in competitive and antagonistic microorganisms, sites with previous history of the pathogen), seedlings of conifers can become infected very soon after outplanting. There is no doubt, that not only the wounds but also outplanting shock and environmental stresses can cause the thin roots vulnerable to pathogenic infection (Shoeneweiss 1981).

Although edaphic variables are considered to be one of the main regulators of the species number and population sizes (Christensen 1989), interactions between soil fungi are very complex and may strongly influence microfungal community structure (Bissett and Parkinson 1979; Nordgren et al. 1985; Widden 1986). In the opinions of Shearer and Zare-Maivana (1988) and Coleman (1985), interspecific competition and predation may even override all other effects in regulating species abundance in the substrate.

In contrast to forest soils, arable soils display higher pH, high content of nitrogen but deficiency in potassium and phosphorus. Small amount of organic matter causes a low C/N ratio. Moreover, the presence of so called "plough layer" makes capillary ascent of water difficult and destroys an aggregate structure of the surface layer (Tuszyński 1990). Low proportion of lignified tissues, which are the specific energy source of forest microbiota, creates an environment unfavourable for inhabiting by microorganisms which decide on the biological activity of forest soils or forest site-forming processes (Trojanowski and Heider 1975). In arable soils, fungi showing high competitive and antagonistic potential are scarce (Garret 1970; Krupa and Dommergues 1979). In contrast, litter and residues of woody tissues in forest soils consist a food base for many fungal species, specifically of *Trichoderma* (Sierota and Kwaśna 1998a, b). Most fungi of forest soils are known to have antagonistic properties to a number of root pathogens, including root-rot fungus *Heterobasidion annosum* – the main pathogen of conifers on former-agricultural lands (Mańka 1995; Sierota 1995).

Microfungal community structure of arable soils is related to agriculture crop (Mikołajska and Majchrzak 1996) and changes along with crop rotation. According to Stahl and Christensen (1992), the members of fungal communities under oligotrophic conditions are less territorial, while mycelia on resource-rich microhabitats are more combative and territorial. As was shown by Werner et al. (2001), microfungi forming communities of the resource-rich arable soils were generally more numerous and quantitatively more variable. The behavior of the fungi *in vitro* with co-occurring mycorrhizal fungi was much more territorial than that of fungi dominating in resource-poor soils of fallow lands (Werner and Zadworny 2001a). Since resource-poor soils determine inhabiting them by microfungi of similar requirements for limited and more unified resources, the competitive rather than antagonistic interactions may be the principial regulator of composition of microfungal communities of such microhabitats. Nevertheless, the dominance in the soils of fallow lands species of *Trichoderma* may cause the final combined effect of such communities on the growth of other soil fungi, including root-pathogenic fungi, far more inhibiting.

Mańka (1970) has found a correlation between functional structure of microfungal communities operating in the sphere of thin roots of Scots pine and incidence of *Heterobasidion*-root rot. It is well known that the damages caused by *H. annosum* are the heaviest on fertile, risky sites, characterized by the absence of fungi showing competitive and antagonistic potential against the pathogen. In contrast to arable soils, the fallow land soils are less fertile, but show higher C/N ratio and lower pH (Aerts et al. 1995), which favour inhabiting the upper layer of soils abundant in plant residues by species of *Trichoderma* (Strzelczyk 1988; Tanaka et al. 1998).

The objective of the study was to examine the *in vitro* interactions between soil fungi dominating in soils of three agricultural and two fallow lands and three P strains of *Heterobasidion annosum*. To evaluate the effect of the studied microfungal communities on the growth of the pathogen strains the biotic series method (Mańka 1974) and measurements of the fungal growth in co-culture according to the method proposed by Eckstein and Liese (1970) were used.

Materials and methods

Soils and isolation of fungi

Samples of three arable soils were collected at Bukowiec, Złotniki and Poznań and two soils of fallow lands were collected at Mieczewo and Błażejewko in Poland. After preparing the average samples of each soil, saprobic fungi were isolated with Warcup's (1950) soil plate method modified by Johnson and Mańka (1961) (Mańka 1964). The chemical properties, species of fungi inhabiting the soils and their frequencies were described by Werner et al. (2001). The fungi dominating in the soils and used in the study are listed in Table 1.

Heterobasidion annosum strains

Heterobasidion annosum was represented by three strains of the P-intersterility group: 95107, 97067, 96051, originated from the collection of Dr. P. Łakomy (Department of Forest Pathology, University of Agriculture, Poznań, Poland).

Petri dish cultures

Each soil fungus was grown opposite each of the pathogen strains in Petri dishes on Malt extract agar (Difco) (30g malt extract, 15g agar in 1 l distilled water, pH 5.5). The standard sources of soil fungi and pathogen strains were two-week-old mycelia growing at 24°C on malt agar medium. The fungi were inoculated as discs (5 mm in diameter) of mycelial mats at 3–4 cm away from each other and incubated at 24°C in the dark. The fungi grown individually served as control. All treatments were replicated three times. The fungal growth was checked once a day and aver-

Table 1. Frequency of soil fungi from three arable soils and two fallow lands

Stand	Soil fungi	Frequency in %
Bukowiec arable soil	*Penicillium waksmanii* Zaleski	17.85
	Trichoderma aureoviride Rifai	13.26
	Colletotrichum coccodes (Wallr.) Hughes	7.14
	Epicoccum nigrum Link	5.61
	Epicoccum purpurascens Ehrenb. ex Schlecht.	5.61
	Mucor hiemalis Wehmer	4.08
		53.53
Poznań arable soil	*Penicillium commune* Thom	37.68
	Trichoderma viride Pers.	17.87
	Penicillium waksmanii Zaleski	8.21
	Arthrinium phaeospermum (Corda) M. B. Ellis	2.41
		66.17
Złotniki arable soil	*Trichoderma virens* (Mill. Gidd et Foster) von Arx	16.21
	Penicillium commune Thom*	9.45
	Penicillium janczewski Zaleski*	6.75
	Trichoderma viride Pers	5.40
	Mortierella sp.*	4.05
	Mortierella thaxteri Biorling*	4.05
	Penicillium aurantiogriseum Dierckx	4.05
	Trichoderma koningii Oudem.	4.05
		54.01
Błażejewko fallow land	*Trichoderma koningii* Oudem.	72.38
	Trichoderma hamatum (Bon.) Bain	14.24
	Penicillium waksmanii Zaleski	5.52
	Cladosporium herbarum (Pers.) Link	1.16
		97.47
Mieczewo fallow land	*Trichoderma koningii* Oudem.	58.66
	Trichoderma harzianum Rifai	22.96
	Penicillium vinaceum Gilman et Abbott	13.77
	Trichoderma hamatum (Bon.) Bain	1.04
	Zygorhynchus moelleri Vuill.	1.04
		93.30

* Fungi additionally used in evaluation of the effect of microfungal communities on the growth of *H. annosum* with biotic series method

age growth rate of each fungus was calculated as was described by Eckstein and Liese (1970). Two way analysis of variance (Anova/Manova), based on individual data and comparisons of mean values using Tukey's HSD test at significance level $p<0.05$, and Student's test were performed using the statistical analysis software Statistica PL 1997 (StatSoft Polska Inc., USA).

After ten days of incubation at 24°C in the dark, individual (IEB) effects of screened fungi were evaluated according to scale used in biotic series method (Mańka 1974). General (GEB) and summary (SEB) biotic effects of the studied soil fungi communities on *H. annosum* were calculated.

Results

Measurements of soil fungi and *H. annosum* growth in co-culture

Effects of all the studied soil fungi, microfungal communities representing five soils and two types of soils (i.e., arable and fallow land soils) on the growth of *H. annosum* strains are presented in Table 2.

All the soil fungi showed great variation in their influence on the growth of three strains of the pathogen. The effect was negative and statistically significant (Fig. 1). Comparing the soil effect, there were insignificant differences in the inhibition of the pathogen growth by the fungal communities representing soils from five localities ($p<0.112$) and highly signifi-

Table 2. Analysis of variance, separately for the growth of 23 soil fungi representing three arable and two fallow land soils, microfungal communities of five stands, and two types of soils in co-culture with three P strains of *Heterobasidion annosum*

Source of variation	df	MS	*P*	Source of variation	df	MS	*P*	Source of variation	df	MS	*P*
Saprotrophs (S)	22	5.448	0.000	Stand (S)	4	3.230	0.112	Soils (S)	1	9.275	0.022
H. annosum (H)	2	41.644	0.000	*H. annosum* (H)	2	37.961	0.000	*H. annosum* (H)	2	36.139	0.000
S × H	44	4.161	0.000	S × H	8	4.949	0.004	S × H	2	7.236	0.018
Error	138	0.548		Error	192	1.700		Error	201	1.765	
Total	210			Total	210			Total	206		

cant differences between two types of soils ($p<0.022$) (Tab. 2). The fungal communities of fallow land soils inhibited significantly the pathogen growth more than the fungal communities of arable soils (Fig. 2). The fungi of both types of soils similarly inhibited the growth of each of the strains of *H. annosum* (Figs. 3A and 3B).

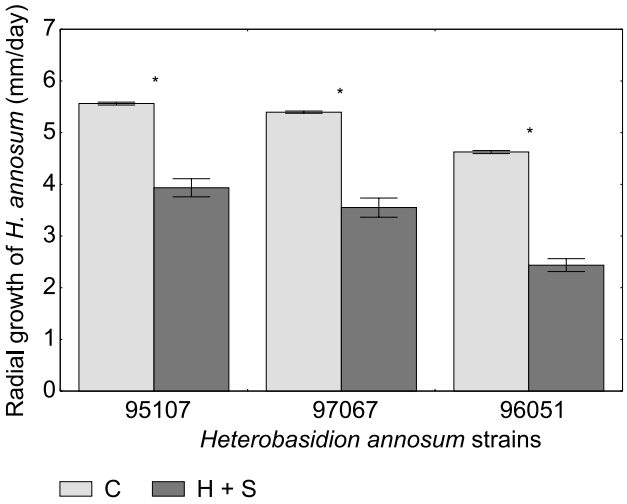

Fig. 1. Growth of three strain of *Heterobasidion annosum* in co-culture with microfungi of arable and fallow land soil (H+S) in comparsion with control (C). *=$P<0.05$ according to Student's test

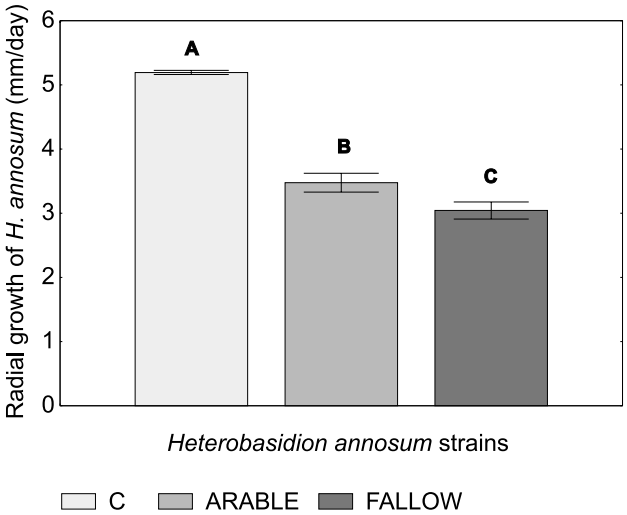

Fig. 2. Growth of *Heterobasidion annosum* in co-culture with microfungi of arable (ARABLE) and fallow land soils (FALLOW) in comparsion with control (C). Means designated by the same letter differ significantly at the 5% level using Tukey's HSD test

Evaluation of suppressive effect of microfungal communities on the growth of *H. annosum* with biotic series method

Individual biotic effects (IBE) of each component of the microfungal communities, general biotic effects (GBE) and summary biotic effects are presented in Table 3.

The microfungal communities of arable soils showed far less negative effect on the pathogen growth when compared with highly suppressive effects of microfungal communities of fallow land soils. In the case of soils from Błażejewko and Mieczewo the summary biotic effects were very high (+1858 and +1724, respectively), whereas from among arable soils, the most suppressive effect of microfungal community of soil from Złotniki was only + 75.

Discussion

The results of the study suggest far more unfavourable influence of microfungal communities of fallow land soils on the growth of *H. annosum* than those of arable soils. It was confirmed by measurements of the pathogen growth in co-culture with soil fungi and analysis of variance of the experimental data and by evaluating the effect of the communities on the pathogen with the method of biotic series.

Traditionally, studying the effect of soil fungi community on a pathogen with the method of biotic series, 10–15 most frequently occurring fungal species that build up over 80% of the community are used (Mańka 1995). In the presented study we decided to take into consideration less number of fungal species. Nevertheless, the selected fungi were dominant in both frequency in soils and influence on the pathogen growth. In the case of arable soils (Bukowiec, Poznań and Złotniki), they built up 53.53, 66.17 and 54.05% of the communities, whereas the rest of their components were less frequent and in a consequence of this, their efect on the pathogen growth was insignificant. By contrast, the selected soil fungi of fallow lands

built up more than 90% of their microfungal communities. Predominance in the communities of fallow lands species of *Trichoderma* explains the highly suppressive effect of these soils on the pathogen growth. Most of *Trichoderma* species produce soluble and volatile antimicrobial substances (Dennis and Webster 1971a, b). Moreover, several of them are known to show mycoparasitic capabilities towards soil microfungi and soilborne pathogens due to biosynthesis of wall degrading enzymes, such as cellulases, chitinases and glucanases (Benhamou and Chet 1996; Calistru et al. 1997; Elad et al. 1982, 1983, 1984; Inbar and Chet 1994; Jacobs and Kamoen 1986). *Trichoderma* spp. restrict the growth of *H. annosum in vitro* and under natural conditions (Sierota 1976; Kwaśna 1997). Specifically, the species of *Trichoderma* gain high frequencies and easily restrict or eliminate wood decomposing fungi at low pH. This may, to a certain degree, explain a low ability to restrict the growth of mycorrhizal fungi inhabiting living roots observed *in vitro* and under greenhouse conditions by Werner and Zadworny (2001a and 2001b).

According to the studies by Sierota and Kwaśna (1998a and 1998b), application of natural substrates to abandoned farmland soils changed the structure of microfungal communities. Increased frequency of *Mucorales*, *Trichoderma*, and *Penicillium* species following the addition of pine sawdust resembled the structure of microfungal communities of deciduous forests and caused an increase in the amount of carbon, nitrogen, potassium and calcium, and C/N ratio (Sierota and Kwaśna 1998a and 1998b). The microfungal community structures of the fallow land soils used in the presented study, and described by Werner et al. (2001), resembled far more those of the post agricultural land soil after application of pine sawdust by Sierota and Kwaśna (1998a and 1998b) than before the treatment. A lower pH and a higher amount of plant residues in the upper horizon of the fallow land soils were most probably responsible for higher frequency of species involved in the mineralization processes and created an environment favouring the increase in population sizes of microorganisms effective in reducing the diseases caused by soilborne pathogens. Such conditions are known to stimulate the plant growth in sandy, nutrient-depleted and even dune soils (Bääth and Söderström 1980; Wicklow and Whittingham 1974; Wohrlab et al. 1963). In the studies by Werner and Zadworny (2001a, b), a substantial growth stimulation of Scots pine seedlings was observed on soils of the same fallow lands, even in the lack of mycorrhizal fungi. Although mycorrhizal seedlings grown on arable and fallow land soils did not vary significantly in the above-ground growth, a lower shoot : root ratio was still observed in the group of plants grown on the fallow land soils. Also mycorrhizal and nonmycorrhizal plants inoculated with *H. annosum* on the fallow land soils showed a lower shoot : root ratio than those grown on arable soils. Considering the positive effect of chemical and biological properties of the fallow land soils on the growth of Scots pine seedlings (Werner and Zadworny 2001a), a better adaptation of mycorrhizal fungi to these soils (Werner and Zadworny 2001b) and highly suppressive effect of microfungal communities of these soils on the growth of *H. annosum* observed in the presented study, it may be concluded that arable soils left barren for many years are more beneficial for establishing of Scot pine plantations than cultivated soils.

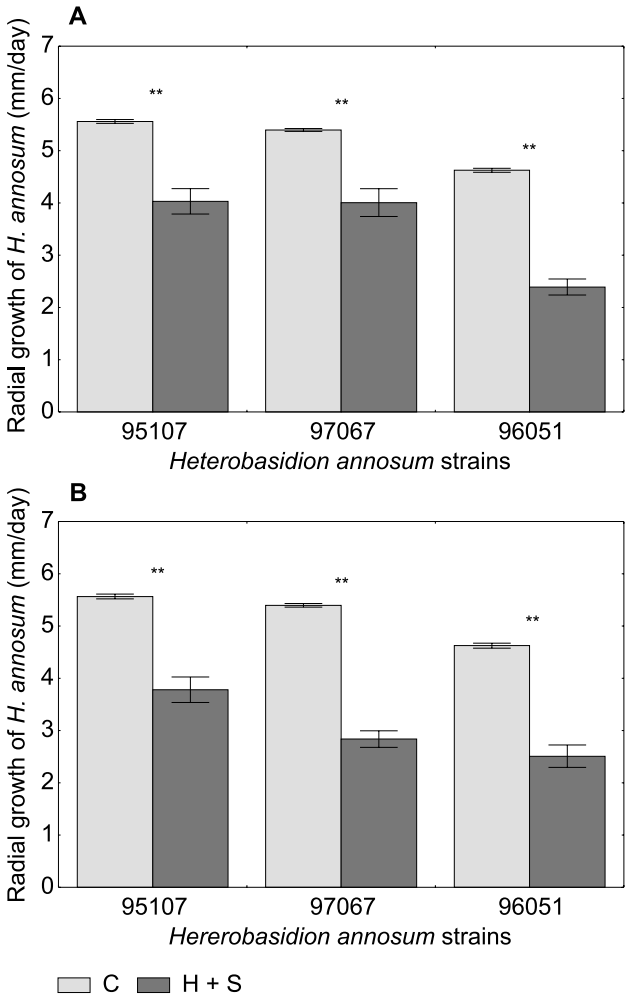

Fig. 3. Growth of three strains of *Heterobasidion annosum* (H+S) in pesence of microfungi of arable soils (A) and fallow land soils (B). **=$P<0.001$ according to Student's test

Acknowledgement

This study was sponsored by the Polish Committee for Scientific Research, grant No 6 PO4F 003 12.

Table 3. Effect of members of microfungal community of three arable and two fallow land soils on P – strains *Heterobasidion annosum* in co-culture

Stand	Soil fungi	Frequency	Strains of *Heterobasidion annosum*					
			96051		97067		95107	
			IBE	GBE	IBE	GBE	IBE	GBE
Bukowiec	*Penicillium waksmanii*	35	–3	–105	–3	–105	–2	–70
	Trichoderma aureoviride	26	+7	+182	+7	+182	+7	+182
	Colletotrichum coccodes	17	–5	–85	–1	–17	–1	–17
	Epicoccum nigrum	11	–3	–33	–2	–22	–3	–33
	Epicoccum purpurascens	11	–5	–55	–3	–33	–5	–55
	Mucor hiemalis	8	+8	+64	+6	+48	+6	+48
	Summary biotic effect (SBE)	+ 25						
Poznań	*Penicillium commune*	78	–2	–156	–1	–78	–2	–156
	Trichoderma viride	37	+8	+296	+7	+259	+7	+259
	Penicillium waksmanii	17	–5	–85	–6	–102	–5	–85
	Arthrinium phaeospermum	5	+2	+10	–4	–20	0	0
	Summary biotic effect (SBE)	+ 47						
Złotniki	*Trichoderma viride*	12	+2	+24	+3	+36	+6	+72
	Penicillium commune	7	+3	+21	+2	+14	+3	+21
	Penicillium janczewski	5	+4	+20	+2	+10	+3	+15
	Trichoderma virens	4	+7	+28	+2	+8	+1	+4
	Mortierlla sp.	3	–1	–3	–3	–9	–2	–6
	Mortierella thaxteri	3	–3	–9	–2	–6	–3	–9
	Penicillium aurantiogriseum	3	–2	–6	+2	+6	+3	+9
	Trichoderma koningii	3	–1	–3	–2	–6	–2	–6
	Summary biotic effect (SBE)	+ 75						
Błażejewko	*Trichoderma koningii*	249	+6	+1494	+7	+1743	+6	+1494
	Trichoderma hamatum	49	+6	+294	+7	+343	+6	+294
	Penicillium waksmanii	19	–1	–19	–2	–38	–1	–19
	Cladosporium herbarum	4	0	0	–2	–8	–1	–4
	Summary biotic effect (SBE)	+ 1858						
Miczewo	*Trichoderma koningii*	281	+6	+1686	+6	+1686	+5	+1405
	Trichoderma harzianum	110	+2	+220	+2	+220	+2	+220
	Penicillium vinaceum	68	–1	–68	–2	–136	–2	–136
	Trichoderma hamatum	5	+7	+35	+6	+30	+7	+35
	Zygorhynchus moelleri	5	–2	–10	–1	–5	–2	–10
	Summary biotic effect (SBE)	+ 1724						

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