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Antagonism of higher fungi to *Heterobasidion annosum* (Fr.) Bref. in laboratory conditions

Abstract: Interactions between 42 higher fungi and six strains of *Heterobasidion annosum* (Fr.) Bref., representing P and S intersterility groups were studied *in vitro*. Part of the higher fungi was represented by strains of ectomycorrhizal fungi indigenous to old disease centres caused by *H. annosum* in pine stands. Variation in antagonism to the pathogen was observed both within and between the species. The antagonism was dependent on growth rates of fungi in control. Fungi growing faster displayed a greater ability to arrest the development of the pathogen mycelia. The same tendency was observed in the strains of *H. annosum*, which generally grew faster and their growth was restricted less than that of higher fungi. Two strains of an ectedomycorrhizal fungus Mrg X, accompanied by a helper bacteria reduced mycelial extension of *H. annosum* by 72% and 76%. Among ectomycorrhizal fungi, the most effective antagonists were strains of *Xerocomus subtomentosus, Amanita muscaria, A. citrina* and *Laccaria laccata*. Three various types of interaction between the studied fungi were observed. Inhibition zone between interacting mycelia appeared often, however the fungi displayed this antagonistic property not to all strains of the pathogen. Although mycelia of *H. annosum* invaded colonies of the other fungi frequently, in none of the treatments growth of the higher fungus over the opposing colony of the pathogen was observed.

Key words: antagonism, mycorrhizal fungi, *Heterobasidion annosum*, paired cultures

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Introduction

Several mechanisms have been suggested by which mycorrhizal fungi reduce the probability of infection by root rotting pathogens (Zak 1964; Marx 1972; Perrin 1985). Besides antibiosis and physical barrier of the fungal mantle (Marx and Davey 1969) and accumulation of plant-produced antimicrobial substances, such as phenols (Duchesne et al., 1987) or volatiles (Krupa and Fries 1971; Krupa and Nylund 1972; Krupa et al., 1973), mycorrhizal fungi can reintroduce and support antagonistic, beneficial rhizosphere microorganisms (Linderman 1987). Moreover, by utilising the surplus of root carbohydrates the fungi can render the feeder roots less attractive to pathogenic root-infecting fungi (Zak 1964).

Despite the relatively high resistance of feeder roots of conifers to infection by *H. annosum*, various unfavourable changes in site conditions, which negatively influence host vitality, can make thin roots vulnerable to infection by *H. annosum* (Schoeneweiss 1975). In nurseries and young plantations coniferous trees may be infected through thin roots (Korhonen 1978; Łakomy 1998; Sierota 1998). Results of recent studies on infection of young nonsuberized, suberized and woody roots of Scots pine (Werner 1990, 1991, 1993) and Norway spruce (Asiegbu et al., 1993; Heneen et al., 1994a, 1994b) have shown

that constitutive and induced resistance factors can be overcome by various infection procedures.

The incidence of *Heterobasidion* root rot is correlated with the influence of the soil microbial community (Gibbs 1967; Mańka 1970), including mycorrhizal fungi (Moreau and Schaeffer 1959; Orłoś and Dominik 1960; Kowalski 1974).

Although a large number of microorganisms such as bacteria, actinomycetes and saprotrophic fungi antagonistic to *H. annosum*, have been studied thoroughly in laboratory conditions (Holdenrieder and Greig 1998), very little attention has been paid to mycorrhizal fungi as potential agents of biological control of *Heterobasidion* root rot.

With the persistent heavy damage caused by *H. annosum* to forestry and the necessity of reforestation of uncultivated areas or wastelands, the interest in integrated control of the pathogen has been increasing. Adoption of new trends in silviculture requires selection of appropriate mycorrhizal fungi, which would be capable of both protecting host plants against root diseases and persisting in sites exhibiting environmental stresses (Kropp and Langlois 1990). To address this question, the antagonism of higher fungi, including several mycorrhizal fungi indigenous to centres of disease caused by *H. annosum* to six strains of the pathogen was evaluated in *in vitro* co-culture petri plate studies.

Materials and methods

Higher fungi

The mycorrhizal fungi used for the screening test were represented by several strains originating from centres of the disease caused by *H. annosum* attack on first rotation pine stands in forest districts Mokrz (latitude 52°44'N longitude 16°16'E), Murzynówko (latitude 52°09'N longitude 17°24'E), Kalina (latitude 53°06'N longitude 16°47'E) Klotyldzin (latitude 53°58'N longitude 17°04'E) and Borówiec (latitude 52°16'N longitude 17°02'E) (fresh coniferous forest sites) and Lipusz (latitude 54°09'N longitude 17°46'E) (mixed fresh coniferous forest sites) formed by pine (*Pinus sylvestris* L.) with admixture of birch (*Betula verrucosa* Ehrh.), oak (*Quercus robur* L.) and maple (*Acer platanoides* L.). The age of the pine stands varied from 50 to 60 years. Simultaneously with the mycological observations (Werner and Napierała 1995, 1999; Napierała 1996), fruit bodies of higher fungi have been collected for the isolation of pure cultures. Strains of ectendomycorrhizal fungus (Mrg X) (*Ascomycotina*) were obtained from Prof. R. Pachlewski. The rest of the screened fungi (from our departmental collection) originated from several pine stands showing absence of visible symptoms of root rot caused by *H. annosum*

Isolation and maintenance of the mycorrhizal fungi in culture were performed according to the methods described by Pachlewski (1983) and Schenck (1984). Collected sporophores after cleaning and washing were surface sterilized in 95% ethanol. Then the caps of fruit bodies were split across in aseptic conditions. From the exposed interior surface small pieces of tissue were removed and placed onto nutrient agar in plates. Pp basic medium (Pachlewski 1983) was used. One liter of the medium contained 20 g glucose, 5 g maltose, 0.5 g $(NH_4)_2C_4H_4O_6$, 1 g KH_2PO_4, 0.5 g $MgSO_4 \times 7H_2O$, 0.5 ml 1% solution of $C_3H_4(OH)(COOH)_3Fe \times H_2O$, 0.5 ml 0.2% solution of $ZnSO_4$, 50 g thiamine-HCl and 15 g agar. Tissue explants were incubated in darkness at room temperature. After establishing, the mycelia emerging from the explants were aseptically transferred onto fresh nutrient agar to set up a stock culture. To avoid contamination with bacteria, Streptomycin at concentration of 100 ppm was used.

Pathogen

Heterobasidion annosum (Fr.) Bref. was represented by six strains obtained by isolation from roots of trees growing in centres of activity of the pathogen in pine and spruce stands. The strains represented two intersterility groups: S (S/2, 2c-2481, 6. 51.2) and P (W/2, K/2, P/1). The field isolates were assigned to intersterility groups basing on their ability to heterokaryotize homokaryotic tester strains of the P or S groups (Korhonen 1978).

Agar plate antagonism studies

Forty two strains of higher fungi (most of them representing ectomycorrhizal species) (Table 1) were used in comparative studies on antagonistic effects against six strains of *H. annosum*. Antagonism was studied in petri plates, according to the method described by Marx (1969). Petri plates (Ø 10 cm) containing 10 ml of Pp nutrient agar (Pachlewski 1983) were inoculated with discs (5 mm in diameter) of one-month-old mycelial mat of the mycorrhizal fungi. After establishing the mycelium on the medium, at the opposite site of the plate, in a distance of 3 cm, an inoculum of the pathogen was placed. The standard sources of the pathogen were two-week-old vegetative mycelia growing at 24°C on malt-agar medium. Inoculum discs were removed from the periphery of the cultures with a cork borer. All treatments consisted of 3 replicates. Several very slow growing mycorrhizal fungi had an advantage of one week. The cultures were incubated at 24°C in the dark. The final calculations were made after 2 weeks, when an equilibrium was reached. The fungal growth was determined in cm^2 of mycelium surface and the zone of inhibition was measured.

Table 1. Species of higher fungi used in screening tests

Species of higher fungi	Collection No.	Derivation of fungi
Amanita citrina (Schaeff.) S.F. Gray.	17–1	Collection DF. ID PAN
A. muscaria (L.) Hooker	16–1	Collection DF. ID PAN
A. muscaria	16–2	Collection DF. ID PAN
A. muscaria	16–3	Kalina
A. muscaria	16–8	Murzynówko
A. muscaria	16–11	Kalina
Cenococcum sp.	27–1	Collection DF. ID PAN
Hebeloma crustuliniforme (Bull.) Quèl.	22–1	Pachlewski´s collection
Hygrophoropsis aurantiaca (Fr.) Maire	55–1	Mokrz
H. aurantiaca	55–4	Mokrz
H. aurantiaca	55–5	Kalina
Laccaria laccata (Scop. Fr.) Berk. Et Br.	9–1	Collection DF. ID PAN
Mrg X	18–1	Pachlewski´s collection
Mrg X	18–1z	Pachlewski´s collection
Mrg X	19–1	Pachlewski´s collection
Mrg X	19–1z	Pachlewski´s collection
Paxillus involutus (Batsch) Fr.	5–1	Collection DF. ID PAN
P. involutus	5–3	Collection DF. ID PAN
P. involutus	5–8	Lipusz
P. involutus	5–13	Borówiec
P. involutus	5–14	Lipusz
P. involutus	5–15	Kalina
P. involutus	5–16	Kalina
P. involutus	5–17	Klotyldzin
Phallus impudicus Pers.	26–1	Collection DF. ID PAN
Suillus bovinus (Fr.) Knutze	15–3	Mokrz
S. bovinus	15–4	Mokrz
S. bovinus	15–5	Lipusz
S. granulatus (Fr.) Knutze	44–1	Borówiec
S. granulatus	44–2	Borówiec
S. luteus (Fr.) S.F. Gray	14–1	Collection DF. ID PAN
S. luteus	14–3	Collection DF. ID PAN
S. luteus	14–4	Collection DF. ID PAN
S. luteus	14–7	Collection DF. ID PAN
S. luteus	14–8	Collection DF. ID PAN
S. luteus	14–9	Collection DF. ID PAN
S. variegatus (Fr.) Knutze	68–1	Lipusz
Xerocomus badius (Fr.) Kühn.	2–3	Collection DF. ID PAN
X. badius	2–6	Kalina
X. badius	2–15	Kalina
X. subtomentosus (Fr.) Quèl.	1–1	Collection DF. ID PAN
X. subtomentosus	1–2	Collection DF. ID PAN

Analysis of data

The inhibiting effect of individual strain of the higher fungus on mycelial extension of the pathogen was calculated for mean area of colony from all treatments for six strains of *H. annosum*. In the same way the cumulative inhibiting effect, both of six test strains of the pathogen on individual strain of the higher fungus and of 42 higher fungi on each test strain of *H. annosum* was calculated. The individual effect of higher fungi on the growth of each test strain of *H. annosum* was estimated as a percentage of colony area reduced in paired culture in comparison with the control on a scale: 0 = to 4%, 1 = from 5% to 24%, 2 = from 25% to 49%, 3 = from 50% to 74%, 4 = from 75% to 100%. In order to evaluate variation in the mutual antagonistic influence of screened fungi, the data from each treatment were analysed using Statistica software procedures (Statistica PL 1997). Two-way analysis of variance (Anova/Manova), based on individual data, and multiple comparisons of means using Tukey's HSD test at significance level =0.05 were performed.

Results

Results of comparative studies on the interaction of fungi in paired cultures are summarized in Table 2 and 3. The screening test showed that in comparison with the ability of the studied higher fungi to arrest the development of *H. annosum* (Table 2), in the majority of cases their growth was also restricted cosiderably by the pathogen (Table 3). From among of all the screened fungi the growth of *H. annosum* was most restricted by two strains of ectendomycorrhizal fungus Mrg X (19–1z and 18–1z) and one strain of a nonmycorrhizal fungus *Hygrophoropsis aurantiaca* (55–1), which reduced the growth of the pathogen respectively by 72%, 76% and 45% in comparison with the control. Apart from a strain of *Phallus impudicus* (26–1), which does not form symbiotic association with Scots pine in pure culture, and which restricted the growth of the pathogen by 49%, the rest of the ectomycorrhizal fungi displayed weak antagonism (Table 2). In this group of fungi, the most effective in arresting the development of *H. annosum* were strains of *Xerocomus subtomentosus* (1–1 and 1–2), *Amanita muscaria* (16–3 and 16–2), *A. citrina* (17–1), *Laccaria laccata* (9–1), *Suillus bovinus* (15–4 and 15–3) and *Xerocomus badius* (2–3). In general, there was no strict correlation between the degree of inhibition of *H. annosum* by strains of mycorrhizal fungi and their ability to resist the negative influence of the opposing mycelia of the pathogen (Table 4). As examples *A. muscaria* (16–2) restricted the development of the pathogen by 25%, whereas its growth was inhibited by 39%. In contrary, *A. muscaria* (16–3), which restricted of the opposing pathogen mycelia by 24%, was inhibited by 72% in comparison with the control.

A comparison between the species indicates that strains of *A. muscaria, S. bovinus, S. luteus* and *X. subtomentosus* inhibited the growth of the pathogen more than strains of *P. involutus*. Variation between species of mycorrhizal fungi was similar to that among strains of that single species. The diversity in the ability to restrict the growth of the pathogen between several selected species of mycorrhizal fungi and among strains of the species are shown in diagrams (Figs. 1 and 2).

Analysis of variance (Table 5 and 6) revealed a highly significant variation ($p < 0.001$) between the screened higher fungi in regard to the negative influence on pathogen growth. Also variation between strains of *H. annosum* in the ability to arrest the growth of mycorrhizal fungi proved to be statistically highly significant ($p < 0.001$).

Generally, mycelial extension of the fungi in paired cultures was correlated with their growth rate in the control. Fungi growing fast arrested development of the opposing fungi more effectively. It was also valid for strains of *H. annosum*. Growth of the test strains of *H. annosum* was influenced variously by the mycorrhizal fungi (Table. 7), however their behaviour in paired cultures was apparently dependent on the growth rate in the control (Table 8).

Although in comparison with the pathogen strains the mycorrhizal fungi grew much slower in all treatments, attempts to describe the zone of inhibition have been made. A more or less broad inhibition zone was formed between 33 strains of mycorrhizal fungi and the pathogen strains (Table 9). In some treatments a wide "demarcation zone", up to 15 mm, was formed between the interacting mycelia. In such cases, the growth of both fungi was clearly stopped. In other treatments the attempts to describe the zone of inhibition failed since the growth of both organisms was stopped at the contact line without any visible inhibition zone, or mycelia of the pathogen were not arrested by mycorrhizal fungi but grew over them (Table 9). Whenever the pathogen invaded the colonies of mycorrhizal fungi, no further growth of the latter was observed. There was a variation in the ability to form inhibition zone between the test strains of *H. annosum* and an individual strain of mycorrhizal fungus. In most cases, mycorrhizal fungi displayed such antagonistic properties to a limited number of the pathogen strains. In all screening experiments only mycelia of five strains of higher fungi, such as *A. citrina* (17–1), *H. aurantiaca* (55–1), *X. badius* (2–6) and two strains of ectendomycorrhizal fungus Mrg X (18–1z and 19–1z), exert the effective inhibition zone in co-culture with all test strains of the pathogen and were not invaded by their mycelia (Table 9). In none of the treatments growth of the mycorrhizal fungus over the opposing colony of the pathogen was observed.

Table. 2. Effect of higher fungi on growth of six strains of *Heterobasidion annosum* in paired cultures

Higher fungi	Collection No.	Mean area of colony of *H. annosum* (in cm^2)				
Hygrophoropsis aurantiaca	55–5	60,04	a*			
Suillus luteus	14–8	59.83	a			
Paxillus involutus	5–14	59.44	a			
P. involutus	5–13	58,35	a			
Hebeloma crustuliniforme	22–1	58.19	a			
Control		58.16	a			
S. bovinus	15–5	57.94	a	b		
S. granulatus	44–2	57.57	a	b		
P. involutus	5–3	57.44	a	b		
P. involutus	5–16	57.21	a	b		
S. variegatus	68–1	56.84	a	b		
P. involutus	5–17	56.05	a	b	c	
P. involutus	5–8	53.69	a	b	c	
S. luteus	14–9	53.68	a	b	c	
S. luteus	14–3	53.28	a	b	c	
Amanita muscaria	16–1	53.22	a	b	c	
P. involutus	5–1	53.22	a	b	c	
H. aurantiaca	55–4	53.04	a	b	c	
P. involutus	5–15	52.95	a	b	c	
Mrg X	19–1	52.70	a	b	c	
S. granulatus	44–1	52.61	a	b	c	
A. muscaria	16–8	52.32	a	b	c	
S. luteus	14–1	52.15	a	b	c	
A. muscaria	16–11	51.71	a	b	c	
Xerocomus badius	2–6	51.69	a	b	c	
Mrg X	18–1	51.68	a	b	c	
Cenococcum sp.	27–1	51.30	a	b	c	
X. badius	2–15	51.18	a	b	c	
S. luteus	14–4	50.78	a	b	c	
S. luteus	14–7	49.93	a	b	c	
X. subtomentosus	1–2	48.53	a	b	c	
A. muscaria	16–2	48.38	a	b	c	
S. bovinus	15–3	46.64	a	b	c	
X. badius	2–3	46.17	a	b	c	
S. bovinus	15–4	46.10	a	b	c	
Laccaria laccata	9–1	42.70	a	b	c	
A. citrina	17–1	42.58	a	b	c	
A. muscaria	16–3	40.62		b	c	
X. subtomentosus	1–1	38.19		b	c	d
Phallus impudicus	26–1	37.74		b	c	d
H. aurantiaca	55–1	32.31			c	d
Mrg X	18–1z	17.00				d
Mrg X	19–1z	14.63				d

* The same letters indicate homogenous groups according Tukeys HSD test at level =0.05

Table. 3. Inhibiting effect of *Heterobasidion annosum* test strains on growth of higher fungi in paired cultures

Higher fungi	Collection No.	Mean area of colony of higher fungi in control (in cm^2)	Mean area of colony of higher fungi in paired cultures (in cm^2)								
Amanita muscaria	16–3	36.9	10.24	a*							
Hygrophoropsis aurantiaca	55–1	16.7	7.41	a	b						
Xerocomus subtomentosus	1–1	27.2	6.34		b	c					
Control		6.28	6.28		b	c					
Laccaria laccata	9–1	14.4	5.75		b	c	d				
A. muscaria	16–2	8.7	5.36		b	c	d				
Mrg X	18–1z	15.5	4.20		b	c	d				
A. muscaria	16–11	0.9	4.02			c	d	e	f		
Mrg X	19–1z	5.5	3.39			c	d	e	f	g	
Suillus bovinus	15–3	20.0	3.09				d	e	f	g	h
Mrg X	19–1	15.0	2.78				d	e	f	g	h
Mrg X	18–1	10.2	2.71				d	e	f	g	h
X. badius	2–3	2.0	1.64					e	f	g	h
S. luteus	14–4	6.1	1.44					e	f	g	h
S. luteus	14–7	1.0	1.11					e	f	g	h
S. bovinus	15–4	6.6	1.07						f	g	h
H. aurantiaca	55–4	5.7	1.05						f	g	h
X. badius	2–6	1.8	0.98						f	g	h
S. luteus	14–9	6.3	0.96						f	g	h
Hebeloma crustuliniforme	22–1	2.7	0.86						f	g	h
S. luteus	14–1	7.3	0.83						f	g	h
Phallus impudicus	26–1	6.4	0.78						f	g	h
Cenococcum sp.	27–1	1.2	0.73							g	h
S. granulatus	44–1	1.8	0.57							g	h
A. citrina	17–1	0.5	0.51							g	h
X. subtomentosus	1–2	1.5	0.49							g	h
S. bovinus	15–5	2.8	0.46							g	h
S. luteus	14–8	6.5	0.43							g	h
S. variegatus	68–1	3.7	0.42							g	h
P. involutus	5–17	1.2	0.39							g	h
P. involutus	5–15	0.8	0.36							g	h
P. involutus	5–1	0.8	0.34							g	h
H. aurantiaca	55–5	7.2	0.33							g	h
P. involutus	5–8	1.0	0.31								h
S. luteus	14–3	1.0	0.28								h
S. granulatus	44–2	0.7	0.27								h
P. involutus	5–13	1.3	0.26								h
A. muscaria	16–11	0.9	0.23								h
A. muscaria	16–8	0.6	0.22								h
P. involutus	5–16	1.1	0.22								h
X. badius	2–15	1.2	0.21								h
P. involutus	5–14	1.1	0.18								h
P. involutus	5–3	1.3	0.14								h

* The same letters indicate homogenous groups according Tukeys HSD test at level =0.05

Table 4. Mutual inhibition of mycelial extension expressed as percentage of colony area reduced in comparison with control

Higher fungi	Collection No.	Inhibition of mycelial extension in comparison with control (in %)	
		Pathogen	Higher fungi
Amanita citrina	17–1	1	0
A. muscaria	16–1	18	57
A. muscaria	16–2	25	39
A. muscaria	16–3	24	72
A. muscaria	16–8	7	63
A. muscaria	16–11	11	75
Cenococcum sp.	27–1	0	39
Hebeloma crustuliniforme	22–1	10	68
Hygrophoropsis aurantiaca	55–1	45	56
H. aurantiaca	55–4	10	82
H. aurantiaca	55–5	0	94
Laccaria laccata	9–1	21	60
Mrg X	18–1	13	73
Mrg X	18–1z	72	24
Mrg X	19–1	22	83
Mrg X	19–1z	76	38
P. involutus	5–1	14	39
P. involutus	5–3	4	89
P. involutus	5–8	4	69
P. involutus	5–13	5	77
P. involutus	5–14	6	84
P. involutus	5–15	3	55
P. involutus	5–16	10	80
P. involutus	5–17	8	69
Phallus impudicus	26–1	49	88
Suillus bovinus	15–3	11	87
S. bovinus	15–4	25	84
S. bovinus	15–5	7	84
S. granulatus	44–1	9	71
S. granulatus	44–2	2	61
S. luteus	14–1	14	87
S. luteus	14–3	14	72
S. luteus	14–4	2	76
S. luteus	14–7	23	0
S. luteus	14–8	7	93
S. luteus	14–9	17	85
S. variegatus	68–1	12	84
Xerocomus badius	2–3	0	18
X. badius	2–6	0	45
X. badius	2–15	12	83
X. subtomentosus	1–1	36	77
X. subtomentosus	1–2	22	68

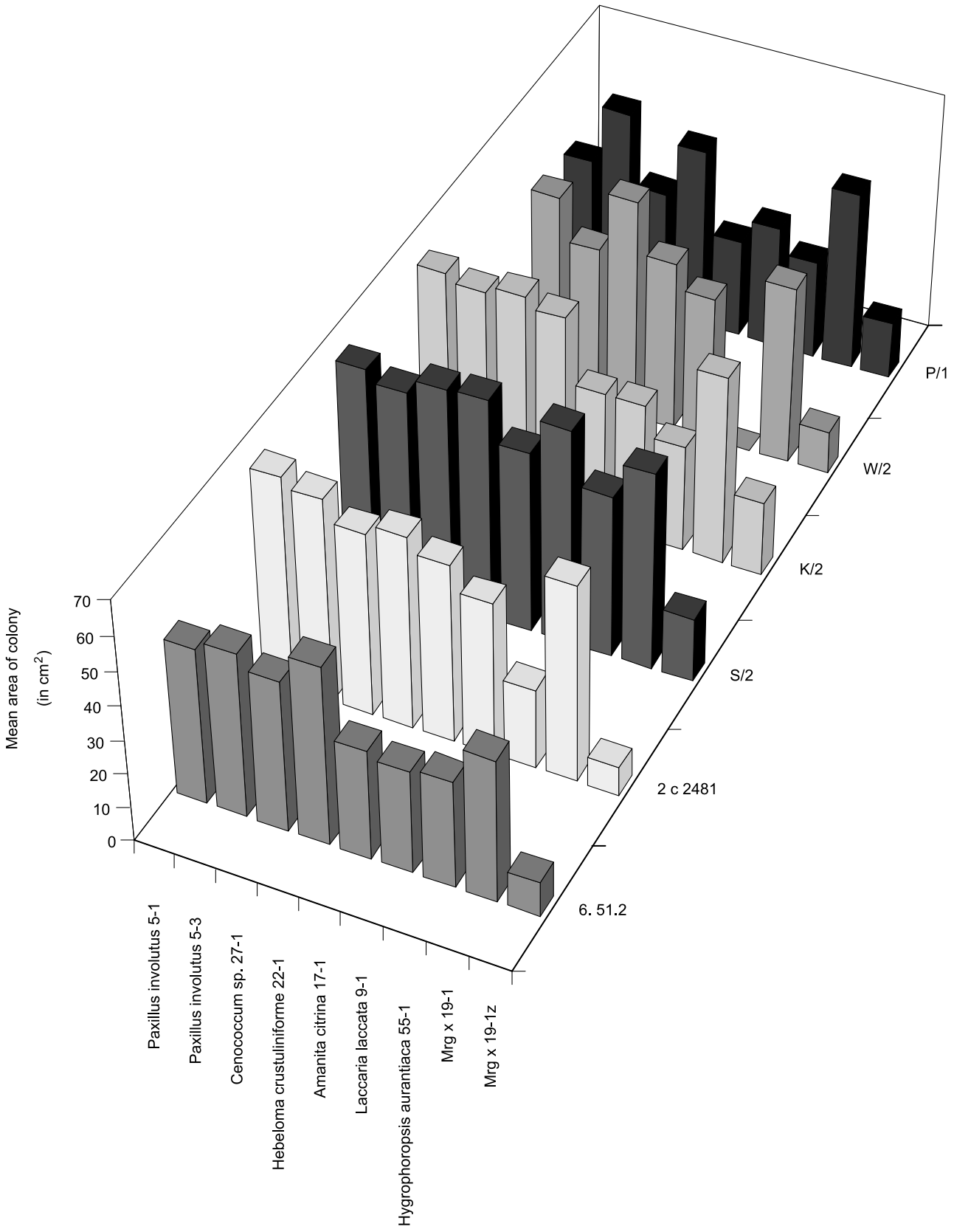

Fig. 1. Mean areas of colonies of six strains of *Heterobasidion annosum* in paired cultures with several strains of higher fungi

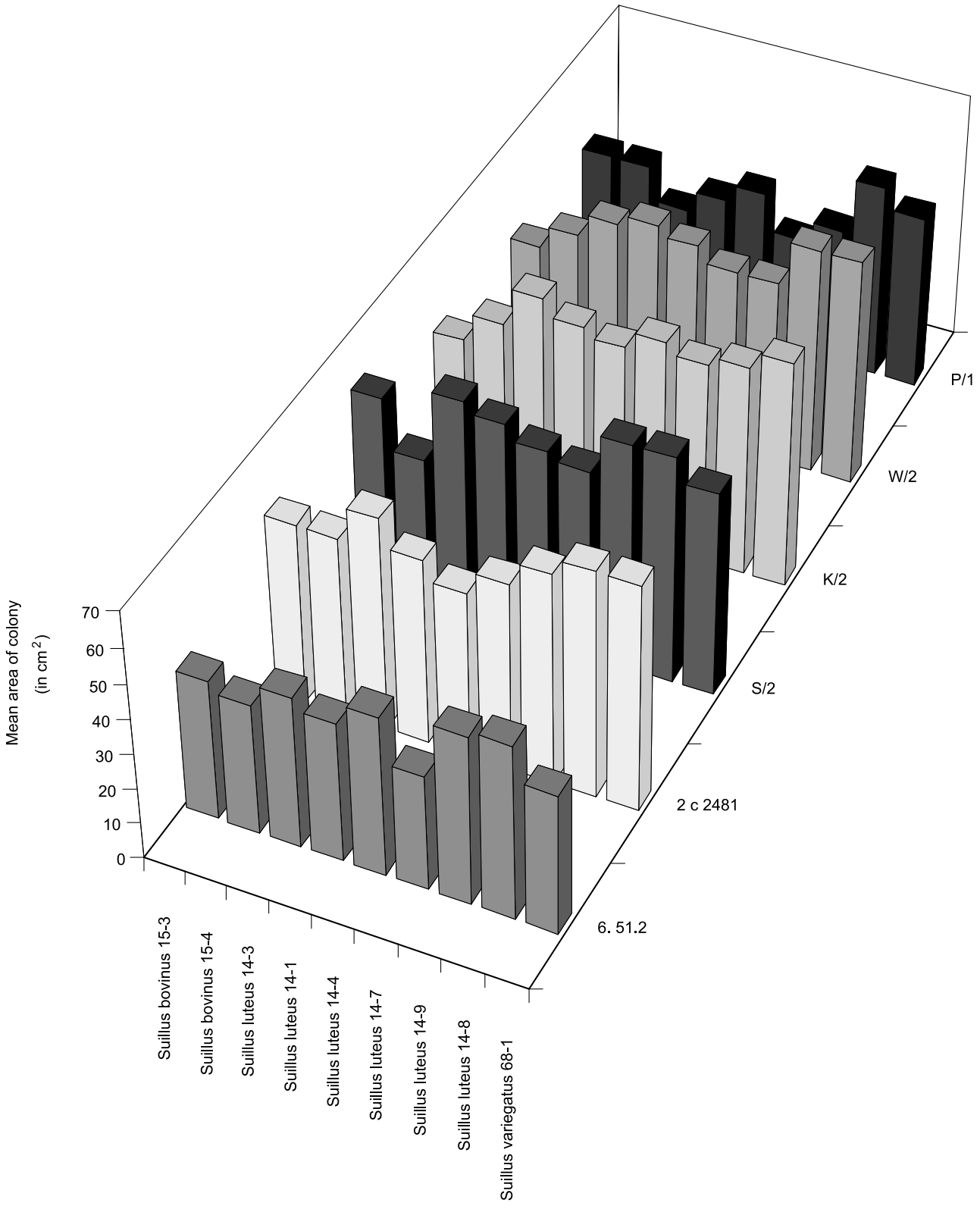

Fig. 2. Mean areas of colonies of six strains of *Heterobasidion annosum* in paired cultures with several strains of *Suillus bovinus*, *S. luteus* and *S. variegatus*

Table 5. Analysis of variance for mycelial extension (in cm^2) of 42 strains of higher fungi in paired cultures with six strains of *Heterobasidion annosum*

Source of variation	df	MS	F	*p*
Higher fungi (F)	42	98.1409	43.6166	0.0000
Replications (R)	2	2.2858	1.0159	0.3627
FxR	84	1.4327	0.6367	0.9946
Error	623	2.2501		
Total	751			

Table 6. Analysis of variance for mycelial extension (in cm^2) of six strains of *Heterobasidion annosum* in paired cultures with 42 strains of higher fungi

Source of variation	df	MS	F	*p*
Heterobasidion annosum (P)	6	7484.69	22.1164	0.0000
Replications (R)	2	34.650	0.1024	0.9027
PxR	12	26.793	0.0792	0.9999
Error	731	338.422		
Total	751			

Plate tests showed that there are various types of interactions between mycorrhizal fungi and the pathogen strains. In general, they can be divided into three main types.

1. The two fungi equally restricted each other. The zone of inhibition was clearly recognizable (Fig. 3).

2. The growth of interacting fungi was restricted to a greater or lesser degree. The zone of inhibition was not observed. Mycelial extension was ceased on contact line or the pathogen marginally invaded the colony of the mycorrhizal fungus (Fig. 4).

3. The growth of the mycorrhizal fungus was arrested and its mycelium was completely invaded by the pathogen (Figs. 5).

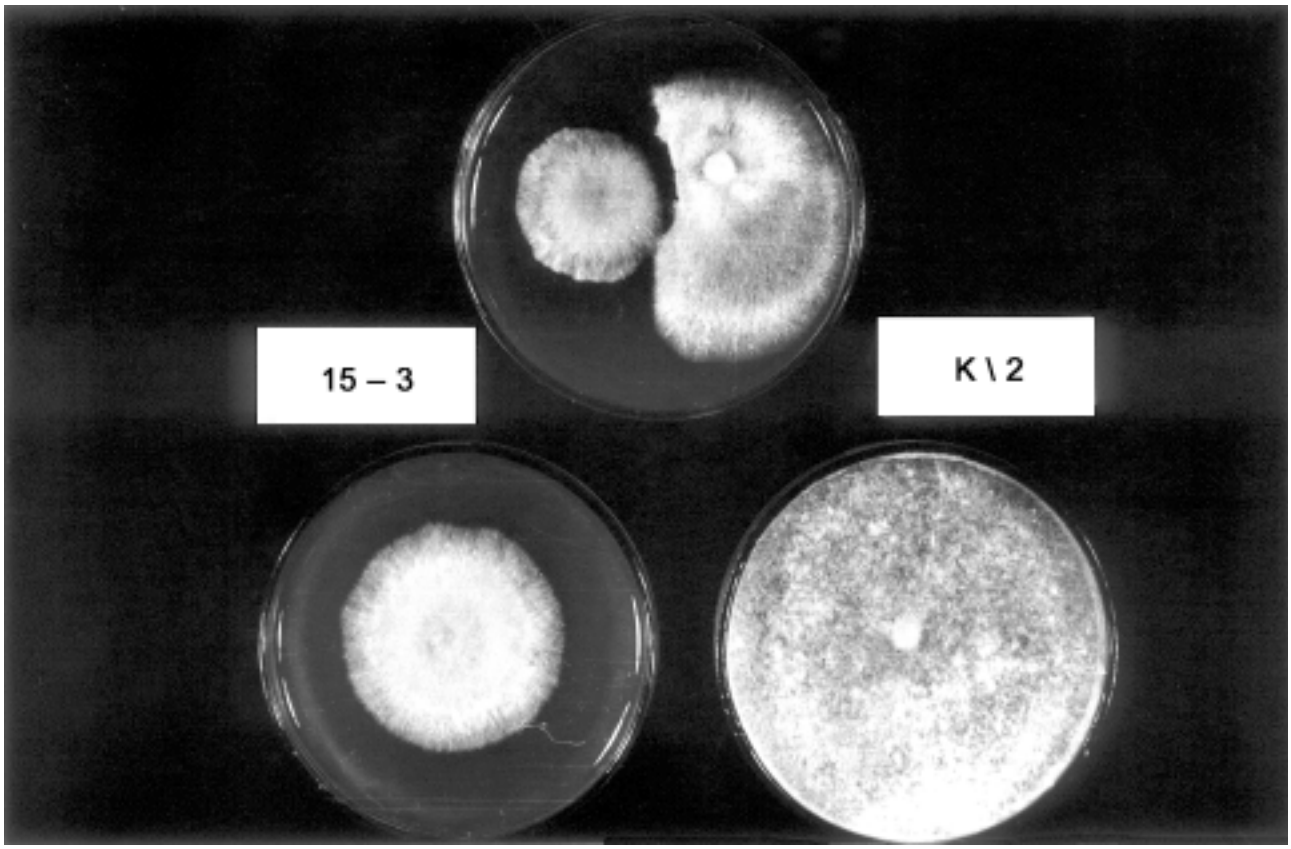

Fig. 3. Interaction between *Suillus bovinus* (15–3) and *Heterobasidion annosum* (K/2)

Table 7. Inhibition of mycelial extension of six strains of *Heterobasidion annosum* in paired cultures with 42 strains of higher fungi expressed in degrees

Higher fungi	Collection No.	Strains of *H. annosum*					
		W/2	2c 2481	S/2	P/1	6.51.2.	K/2
Amanita citrina	17–1	0	0	0	+1	0	+2
A. muscaria	16–1	+1	0	0	+2	+2	+1
A. muscaria	16–2	+1	+1	0	+2	+2	+2
A. muscaria	16–3	+2	+1	0	+2	+2	+1
A. muscaria	16–8	+1	0	0	0	+2	0
A. muscaria	16–11	+1	+1	+2	0	+1	0
Cenococcum sp.	27–1	+2	+1	0	0	0	0
Hebeloma crustuliniforme	22–1	0	+1	0	+1	+1	+1
Hygrophoropsis aurantiaca	55–1	–*	+3	+1	+2	+2	+2
H. aurantiaca	55–4	+1	0	0	0	+2	+1
H. aurantiaca	55–5	0	0	+1	0	0	0
Laccaria laccata	9–1	0	+2	0	+2	+2	+2
Mrg X	18–1	0	+1	0	+1	+2	0
Mrg X	18–1z	+4	+3	+3	+3	+4	+4
Mrg X	19–1	+1	0	0	+1	+2	+1
Mrg X	19–1z	+4	+4	+3	+3	+4	+3
Paxillus involutus	5–1	+2	0	0	+1	+1	0
P. involutus	5–3	0	0	0	0	+1	0
P. involutus	5–8	0	0	+1	0	+1	0
P. involutus	5–13	+1	0	+1	0	0	0
P. involutus	5–14	+2	0	+1	0	0	0
P. involutus	5–15	0	0	+1	0	+1	0
P. involutus	5–16	+1	0	+2	0	0	0
P. involutus	5–17	+1	0	+2	0	0	0
Phallus impudicus	26–1	–	+2	+2	+3	+1	+2
Suillus bovinus	15–3	0	+1	+1	+1	+2	+1
S. bovinus	15–4	+1	+1	+2	+2	+2	+1
S. bovinus	15–5	+1	0	+1	0	0	0
S. granulatus	44–1	0	0	+1	0	+1	+1
S. granulatus	44–2	0	0	+1	0	0	0
S. luteus	14–1	0	+1	0	+2	+2	0
S. luteus	14–3	0	0	0	+2	+1	0
S. luteus	14–4	0	+1	+1	+1	+1	0
S. luteus	14–7	+1	–	+1	+2	+2	+1
S. luteus	14–8	0	0	0	+1	+1	+1
S. luteus	14–9	+1	+1	0	+2	+2	+1
S. variegatus	68–1	+1	0	+1	+1	+2	0
Xerocomus badius	2–3	0	0	0	0	0	0
X. badius	2–6	+1	+1	0	0	–	0
X. badius	2–15	+1	0	+1	0	+2	0
X. subtomentosus	1–1	–	+2	0	+3	+2	+2
X. subtomentosus	1–2	+2	0	0	+3	+2	+1

* – lack of observation

Scale: 0: 0–4% +1: 5–24% +2: 25–49% +3: 50–74% +4: 75–100%

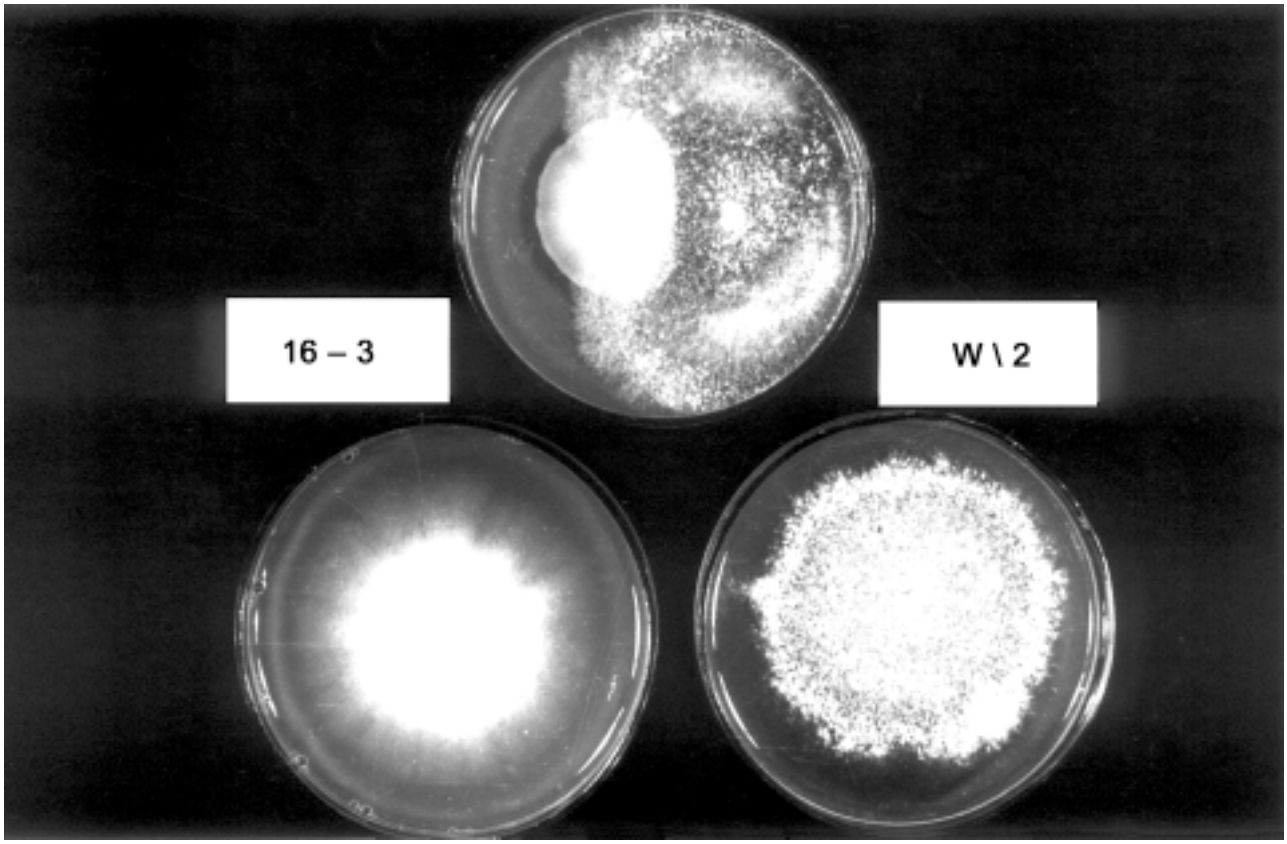

Fig. 4. Interaction between *Amanita muscaria* (16–3) and *Heterobasidion annosum* (W/2)

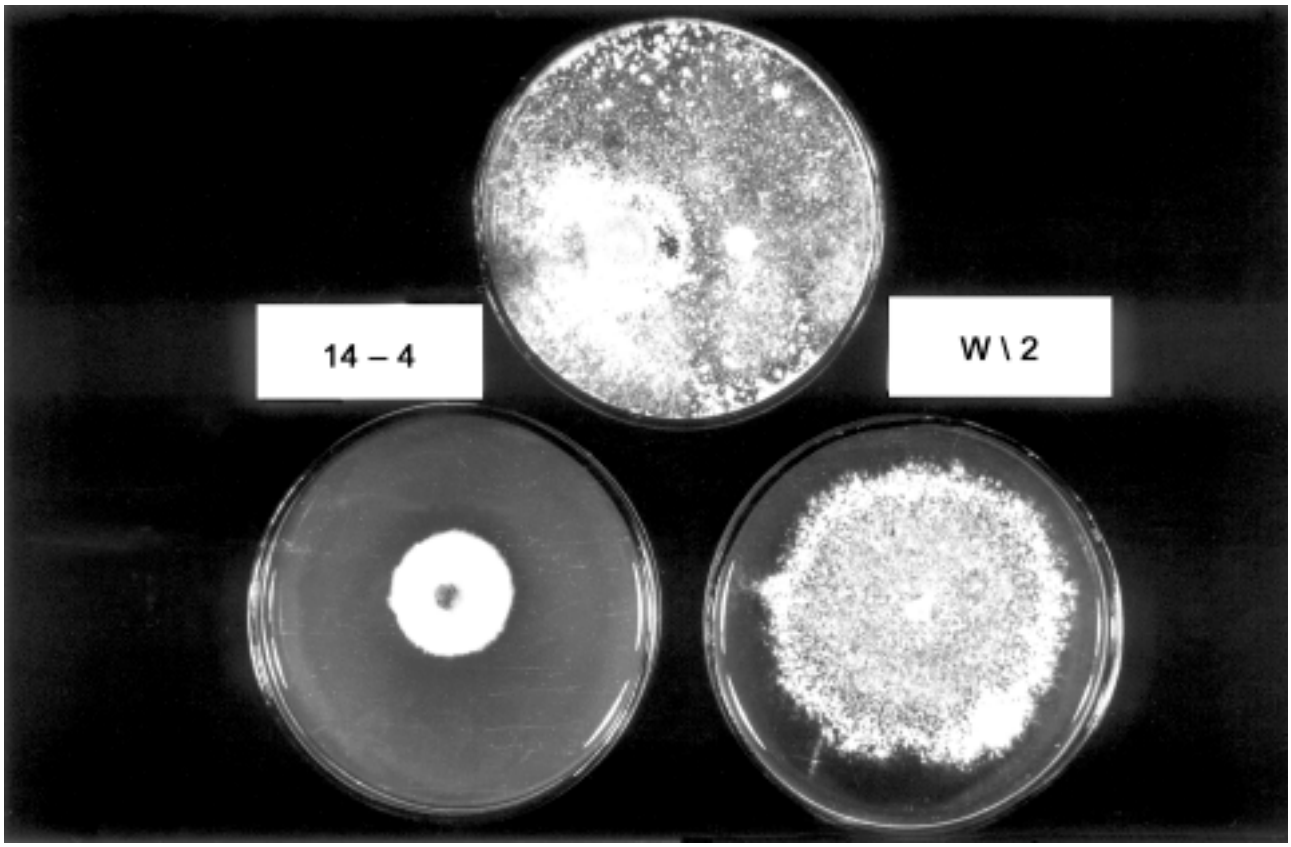

Fig. 5. Interaction between *Sullus luteus* (14–4) and *Heterobasidion annosum* (W/2)

Table 8. Cumulative inhibiting effect of 42 strains of higher fungi on growth of test strains of *Heterobasidion annosum* in paired cultures

Strains of *H. annosum*	Intersterility group	Mean area of colony in control (in cm^2)	Mean area of colony in paired cultures (in cm^2)				
K/2	P	67.0	59.458	a*			
Control		58.0	58.030	a			
2c 2481	S	65.0	57.611	a	b		
P/1	P	62.3	51.252		b	c	
6.51.2	S	60.6	47.139			c	
S/2	S	50.7	45.112			c	
W/2	P	42.2	36.281				d

* The same letters indicate homogenous groups according Tukey's HSD test at level =0.05

Discussion

It has been shown many times that mycorrhizal fungi inhibit growth of phytopathogenic fungi *in vitro*. Some of them can protect conifer seedlings from root decay caused by species of *Fusarium*, *Pythium*, *Rhizoctonia* and *Phytophthora* (Duchesne et al. 1989b).

Antagonism of ectomycorrhizal fungi to *H. annosum* was studied previously by Hyppel 1968a, 1968b; Cervinkova 1990; Hanso 1993. Hyppel (1968a) in co-culture petri plate studies tested 85 strains of mycorrhizal fungi and the pathogen, and found among them strong antagonists, strains with weak antagonistic properties and strains lacking this feature. Results of the present study confirm the great variation among the higher fungi in the antagonism to *H. annosum*. It is noteworthy that variation in this regard between strains of several species was even wider than interspecific variation.

The inhibition of fungal growth *in vitro* are linked with the production of antibiotics (Marx 1972; Sylvia and Sinclair 1983; Sampagni et al. 1985; Kope and Fortin 1988) and mycolytic activity (de Vries and Wessels 1972; Peberdy 1979). In the soil environment the effects of antibiotics are modified due to the varying concentration of nutrients, the presence of microbial population and the physicochemical properties of the soil (Strzelczyk 1988). Moreover, culture conditions and physiological age of mycelia affect the kind and amount of the produced antimicrobial compounds (Omura and Tanaka 1984; Vandamme 1984; Duchesne et al. 1989a) and lytic enzymes (Peberdy, 1976; Farquar and Peterson 1990). This permits only a very careful transfer of results obtained *in vitro* to a soil system.

Under the culture conditions of this study the majority of the mycorrhizal fungi displayed weak negative effect on the growth of the pathogen strains. At the same time, the fungi were characterized by a relatively slow growth in control, whereas the studied strains of *H. annosum* grew rapidly. The antagonism between fungi in paired cultures was apparently dependent on their growth rates. Strains of mycorrhizal fungi characterized by a faster growth displayed a greater ability to inhibit the growth of *H. annosum*. A similar tendency was observed in the studied strains of the pathogen. Mycolytic activity is known to occur in various fungi from all taxonomic groups (Paberdy 1976, 1979; Hashiba and Yamada 1982). In studies by Farquar and Peterson (1990) *Fusarium oxysporum*, a soil-born pathogen caused protoplast release from *Paxillus involutus*. Previous investigations on the effects of *H. annosum* on mycorrhizal fungi have not reported this phenomenon. Also this study did not provide any information on the mechanisms involved in restriction of the growth of mycorrhizal fungi by the pathogen. Although *H. annosum* displays a very low competitive saprotrophic ability in a soil environment (Rishbeth 1950; Gibbs 1967), studies on cell wall modification of mycorrhizal fungi in co-culture with different strains of the pathogen are needed.

Life functions of mycorrhizal fungi depend mainly on the supply of carbohydrates and many other compounds from the host-plant. The activity of mycorrhizal fungi, including their antagonistic abilities, is also modified by the mycorrhizosphere microorganisms. Thus, it seems that the antagonism of mycorrhizal fungi in paired cultures in the absence of soil microorganisms and of the host plant which stimulates growth of mycorrhizal fungi may reflect the growth tendencies of the studied fungi on a medium, rather than their antagonistic properties *in vivo*.

A relatively high level of pathogen growth inhibition in plate tests was recorded for two strains of the ectendomycorrhizal fungus Mrg X, accompanied by a helper bacteria (unpublished information), marked with symbols 18–1z and 19–1z. Bacteria-free cultures (18–1 and 19–1) inhibited the growth the pathogen to a lesser extend. This suggests that the antagonism of those fungi was affected mostly by their interaction with the bacteria. In forest conditions the growth of pathogens and mycorrhizal fungi is conditioned by a complex of various factors, including the presence of

Table 9. Types of final situation between higher fungi and *Heterobasidion annosum* in paired cultures

Higher fungi	Collection No.	Inhibition zone (in mm)	Invasion P* M	Invasion M P
Amanita citrina	17–1	0–9	–	–
A. muscaria	16–1	0	– +	–
A. muscaria	16–2	0	– +	–
A. muscaria	16–3	0–3	– +	–
A. muscaria	16–8	0–6	– +	–
A. muscaria	16–1	0–6	– +	–
Cenococcum sp.	27–1	0–8	– +	–
Hebeloma crustuliniforme	22–1	0	– +	–
Hygrophoropsis aurantiaca	55–1	1–3	–	–
H. aurantiaca	55–4	0–1	– +	–
H. aurantiaca	55–5	0–2	– +	–
Laccaria laccata	9–1	2–4	– +	–
Mrg X	18–1	0	+	–
Mrg X	18–1z	6–10	–	–
Mrg X	19–1	0	+	–
Mrg X	19–1z	7–13	–	–
Paxillus involutus	5–1	0–3	– +	–
P. involutus	5–3	0	+	–
P. involutus	5–8	0–2	– +	–
P. involutus	5–13	0–1	– +	–
P. involutus	5–14	0–1	– +	–
P. involutus	5–15	0–2	– +	–
P. involutus	5–16	0–1	– +	–
P. involutus	5–17	0–3	– +	–
Phallus impudicus	26–1	3–10	– +	–
Suillus bovinus	15–3	2–5	– +	–
S. bovinus	15–4	0–3	– +	–
S. bovinus	15–5	0–3	– +	–
S. granulatus	44–1	0	– +	–
S. granulatus	44–2	0–1	– +	–
S. luteus	14–1	0–4	– +	–
S. luteus	14–3	0	+	–
S. luteus	14–4	0–3	– +	–
S. luteus	14–7	0–15	– +	–
S. luteus	14–8	0	– +	–
S. luteus	14–9	0–2	– +	–
S. variegatus	68–1	0–3	– +	–
Xerocomus badius	2–3	0–14	– +	–
X. badius	2–6	0–11	–	–
X. badius	2–15	0–8	– +	–
X. subtomentosus	1–1	0–10	– +	–
X. subtomentosus	1–2	0–9	– +	–

*P – pathogen; M – higher fungus
– – lack of invasion; –+ – sporadic invasion; + – constant invasion

saprotrophic microorganisms. Study on the effects of several soil fungi characteristic of forest soils and farmland soils on the growth of mycorrhizal fungi and the same strains of *H. annosum* showed that the growth of the pathogen was markedly limited by expansive fungi of the genus *Trichoderma* (Werner et al., 1995a and 1995b). Among the studied soil fungi only few inhibited the growth of mycorrhizal fungi to some extent, whereas the majority had no effect or even slightly stimulated their growth.

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Badania nad antagonizmem pomiędzy grzybami mikoryzowymi a grzybem *Heterobasidion annosum* (Fr.) Bref. w warunkach *in vitro*

Streszczenie

W warunkach *in vitro* przebadano wpływ czterdziestu dwóch szczepów grzybów wyższych na ograniczanie wzrostu sześciu szczepów *Heterobasidion annosum* należących do dwóch intersterylnych grup P i S. Badania prowadzono według metody opisanej przez Marxa (1969). Grzyby hodowano w szalkach Petriego, na pożywce Pp (Pachlewski 1983). Po dwóch tygodniach mierzono powierzchnię kolonii obu grzybów (w cm^2), strefę inhibicji (w mm) oraz opisano sytuację zaistniałą na szalce. Badane grzyby wyższe wykazywały znaczne zróżnicowanie stopnia ograniczania wzrostu grzyba *H. annosum*. Najlepszymi antagonistami w stosunku do sześciu badanych szczepów patogena okazały się szczepy grzyba ektendomikoryzowego oznaczonego symbolem Mrg X (18–1z i 19–1z), których grzybnie wykazywały obecność bakterii, szczep grzyba ektomikoryzowego z gatunku *Amanita muscaria* (16–3), szczep grzyba niemikoryzowego *Hygrophoropsis aurantiaca* (55–1) oraz grzyba *Phallus impudicus* (26–1) nie tworzącego z sosną symbiozy. Obok zmienności międzygatunkowej stwierdzono dużą zmienność wewnątrzgatunkową. Różnice pomiędzy szczepami z gatunków: *A. muscaria, Suillus luteus, Paxillus involutus* czy *H. aurantiaca* były niekiedy bardziej znaczące niż pomiędzy gatunkami.

Badane grzyby mikoryzowe cechowały się stosunkowo wolnym wzrostem na pożywce, natomiast większość badanych szczepów *H. annosum* wzrastała szybko. Stwierdzono zależność pomiędzy szybkością wzrostu a stopniem negatywnego oddziaływania na wzrost grzybów w bikulturach. Szczepy grzybów wyższych charakteryzujące się szybszym wzrostem przejawiały większą zdolność hamowania wzrostu szczepów grzyba *H. annosum* i jednocześnie wykazywały większą tolerancję na obecność patogena.