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Antagonistic activity of xylotrophic mushrooms against pathogenic fungi of cereals in dual culture

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Summary. The antagonistic activity of 17 species of xylotrophic Basidiomycotina (*Coriolus versicolor, Flammulina velutipes, Ganoderma lucidum, Hypholoma fasciculare, H. sublateritium, Kühneromyces mutabilis, Lentinula edodes, Lentinus tigrinus, Pholiota alnicola, Ph. aurivella, Ph. destruens, Pleurotus ostreatus, P. cornucopiae, Polyporus squamosus, P. subarcularius, P. varius* and *Schizophyllum commune*) against 4 fungi (*Bipolaris sorokiniana, Fusarium culmorum, Gaeumannomyces graminis* var. *tritici* and *Rhizoctonia cerealis*), responsible for foot and root diseases of winter cereals, were tested in dual-culture experiments on potato-dextrose agar. Almost all tested mushroom species markedly inhibited mycelial growth of the four phytopathogenic fungi, antagonistic activity of *P. ostreatus, H. fasciculare, G. lucidum, L. tigrinus* and *S. commune* being particularly strong. Inhibiting activity mainly comprised two reactions: deadlock, consisting in mutual inhibition at a distance or at mycelial contact, and replacement, consisting in initial deadlock followed by partial or complete overgrowth.

Key words: wood-decaying macromycetes, microfungi, wheat, biological control.

Introduction

Xylotrophic mushrooms are widely distributed in nature. They occur in climates ranging from tundra to the tropics and colonize a wide range of soils. They have different mechanisms for survival and proliferation, including physical attack of other fungi and the production of biologically active metabolites, such as antibiotic compounds (Steglich, 1981; Anke *et al.,* 1983; Hautzel *et al.,* 1990; Toyota and Hostettmann, 1990; Becker *et al.,* 1994; Steinmetz *et al.*, 1995; Badalyan,1998; Badalyan *et al.*, 1998). These fungi therefore have the potential for a variety of biocontrol applications (Badalyan, 2001).

Many fungi are human, animal and plant pathogens. Against most of these fungi, particularly plant pathogens, there are no effective biological control methods at present. The interactions between xylotrophic mushrooms and such fungi could be of interest for the development of biocontrol methods.

Antagonistic activity of mushrooms against fungi has been reported by many authors. In particular Chaumont *et al.* (1982) showed that aqueous extracts of 67 species of Basidiomycotina in the genera *Boletus, Collybia, Cortinarius, Hebeloma* and *Lactarius* were active against *Cytospora* sp., *Fusarium oxysporum, Graphium ulmi*, *Rhizoctonia solani* and *Stereum purpureum* in *in vitro* tests.

To our knowledge no data are available on the activity of mushrooms against pathogenic fungi

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causing foot and root diseases in cereals. These mushrooms may provide biocontrol of the fungi and the diseases they cause.

The aim of this study was to investigate the antagonistic activity of 17 xylotrophic mushrooms against 4 phytopathogenic fungi in dual-culture experiments. The phytopathogenic fungi tested are all soil-borne and they cause the most important foot and root diseases of cereals in temperate areas of the world.

Materials and methods

Test organisms

The following isolates of xylotrophic mushrooms were used: *Coriolus versicolor* (Fr.) Quèl., LEM 1Cv; *Flammulina velutipes* (Curt.: Fr.) Sing., LEM III2Fv; *Ganoderma lucidum* (Fr.) Karst., LEM 1Gl; *Hypholoma fasciculare* (Fr.) Kumm., LEM 1Hf; *H. sublateritium* (Fr.) Kumm., LEM 2Hs; *Kühneromyces mutabilis* (Schaeff.: Fr.) Sing. & Sm., LEM 319Km; *Lentinula edodes* (Berk.) Sing., LEM 351Le; *Lentinus tigrinus* (Bull.: Fr.) Sing., LEM 2Lt; *Pholiota alnicola* (Fr.) Sing., LEM 55Phal; *Ph. aurivella* (Fr.) Kumm., LEM 72Phau; *Ph. destruens* (Brond.) Gill., LEM 1Phd; *Pleurotus cornucopiae* Roll., LEM 1Pc; *P. ostreatus* (Jacq.: Fr.) Kumm., LEM 19Po; *Polyporus subarcularius* (Donk) Bond., LEM 2Ps; *P. squamosus* (Huds.: Fr.) Fr., LEM Psq; *P. varius* (Pers.: Fr.) Fr., LEM 20Pv; and *Schizophyllum commune* Fr., LEM 1Schc. All isolates were from the collection of the Laboratory of Experimental Mycology (LEM) of Yerevan State University (Armenia). Mycelium of these mushrooms was obtained by plating small portions of fruiting bodies onto 2% malt-extract-agar (MEA; Difco laboratories, Sparks, MD, USA) with 100 mg l^{-1} chloramphenicol. The cultures thus obtained were maintained as mycelium in tubes on 2% MEA at 5°C in the dark. All fungi were previously screened for biological activity in inter-specific pairings (Badalyan, 1998).

The phytopathogenic fungi used were *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoem., LM BY; *Fusarium culmorum* (W.G. Smith) Sacc., LM 20.91; *Gaeumannomyces graminis* (Sacc.) Von Arx & Olivier var. *tritici* Walker, LM 14.95 and *Rhizoctonia cerealis* Van der Hoeven, LM 15.98. The codes of isolates (LM) are the accession numbers of cultures from the collection of the Dipartimento di Protezione Valorizzazione Agroalimentare, University of Bologna (Italy). They were isolated from wheat plants from cereal fields in Italy. The cultures were stored in tubes on Difco potato-dextrose-agar (PDA) under mineral oil at 5°C in the dark.

Each fungal species was transferred from stored cultures onto PDA plates and cultured at 23°C in the dark to obtain colonies 3–4 cm in diameter. Mycelial plugs 2 mm in diameter were cut from the edge of these cultures and used as inoculum for the experiments.

Dual-culture experiments

Competitive interactions between the xylotrophic mushrooms and the cereal pathogenic fungi were evaluated in dual-culture experiments on Petri dishes (90 mm diameter) containing 20 ml PDA (Difco). In each dish, two 2-mm diameter mycelial disks, one from mushroom colonies and one from fungal colonies, were placed on the agar surface 30 mm apart. Immediately after inoculation, the plates were sealed with plastic film and incubated in darkness at 24°C for 30 days. Colony growth and the type of interaction were examined daily under a stereomicroscope. The mushroom and fungal isolates were paired in all possible combinations. Three replicates were prepared for each pairing. A total of 204 pairings was examined. The antagonistic ability of each fungal organism was determined using a rating scale for the 3 main types of reactions (A, B, C) and 4 sub-types (C_{A1}, C_{B1}, C_{A2} and C_{B2}). Type A and B was *deadlock* (mutual inhibition, in which neither organism was able to overgrow the other) at mycelial contact (A), or at distance (B); type C *replacement*, overgrowth without initial deadlock. The intermediate subtypes scored were: C_{A1} partial, and C_{A2} complete, replacement after initial deadlock with mycelial contact; C_{B1} partial, and C_{B2} complete, replacement after initial deadlock at a distance. The following score was assigned to each type or sub-type of reaction: A=1; B=2; C=3; C_{A1}=3.5; C_{B1}=4; C_{A2}=4.5; C_{B2}=5. The antagonism index (AI) was then calculated for each fungal species using the following formula:

$$AI=A(n\times1)+B(n\times2)+C(n\times3)+C_{A1}(n\times3.5)+C_{B1}(n\times4)+C_{A2}(n\times4.5)+C_{B2}(n\times5)$$

where n= frequency of each type or sub-type of reaction.

The presence of dense zones of mycelium, ag-

gregated structures such as mycelial cords, pigmented hyphae, exudate droplets, dark pseudosclerotial lines, and fruiting body primordia in the interaction zones was also noted.

Results and discussion

The interactions between xylotrophic mushrooms and cereal pathogenic fungi in dual cultures on PDA are shown in Table 1. Two types of competitive interaction were observed: deadlock, consisting in mutual inhibition after mycelial contact (Fig. 1A) or at a distance (Fig. 1B), and replacement, consisting in the inhibition of one organism, followed by partial (Fig. 1D) or complete overgrowth by the replacing fungus.

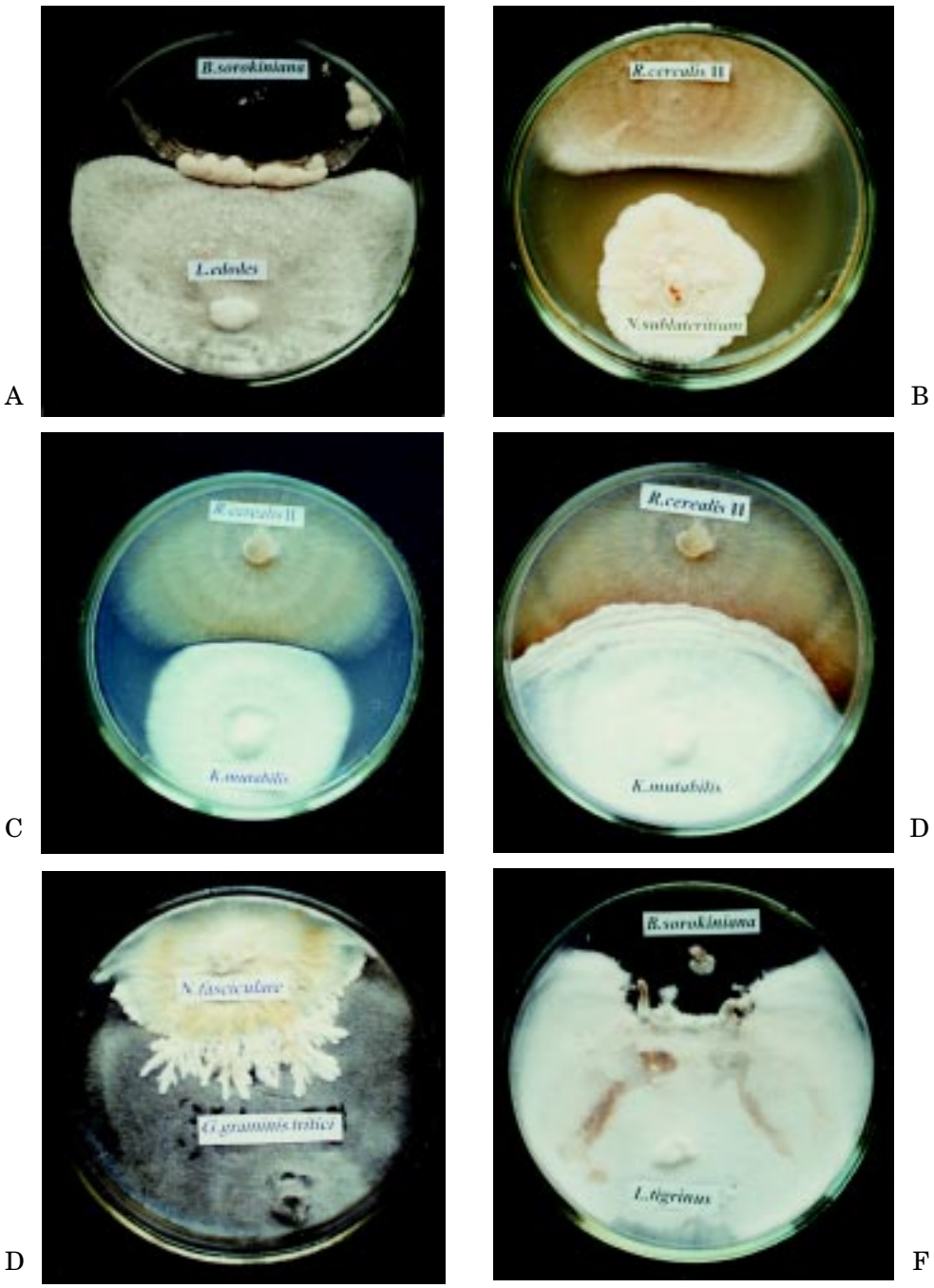
On the basis of the AI values, mushrooms were divided into three groups: I - active (AI>15): *P. ostreatus, H. fasciculare, G. lucidum, L. tigrinus, S. commune*; II - moderately active (AI=15-10): *Ph. alnicola, F. velutipes, C. versicolor, Ph. aurivella, K. mutabilis, L. edodes, P. squamosus*; and III - weakly active (AI <10): *Ph. destruens, H. sublateritium, P. cornucopiae, P. varius, P. subarcularius*. In a previous study (Badalyan, 1998), mushrooms belonging to group I were found to have strong antagonistic/biological activity in dual culture even against xylotrophic species, indicating that the AI is relatively constant for each species and can be used for bio-ecological characterisation. Establishing the AI is the first step in screening mushrooms for physiological and biological activity.

Of the cereal pathogenic fungi only *F. culmorum* showed combative ability against some xylotrophic mushrooms of groups II and III: *K. mutabilis, L. edodes, H. sublateritium, Ph. aurivella* and *Ph. destruens*. The more highly combative group I mushrooms on the other hand were not overgrown by *F. culmorum*. *Gaeumannomyces graminis* var. *tritici, B. sorokiniana* and *R. cerealis* showed low combative ability against xylotrophic fungi, being strongly inhibited by all test-

Table 1. Antagonism index values (in square brackets) and competitive reactions between mycelium of xylotrophic mushrooms and cereal pathogenic fungi in pairings on potato dextrose agar medium.

Xylotrophic mushroom	Origin	Cereal pathogenic fungus			
		Gaeumannomyces graminis var. *tritici* [8]	*Bipolaris sorokiniana* [9]	*Fusarium culmorum* [25]	*Rhizoctonia cerealis* [9]
Coriolus versicolor [11.5]	Armenia	C_{A1}[a]	C_{A1}	A	C_{A1}
Flammulina velutipes [12]	Armenia	C_{B1}	C_{A1}	C_{A1}	A
Ganoderma lucidum [17]	Armenia	C_{A2}	C_{A2}	C_{A2}	C_{A1}
Kühneromyces mutabilis [10.5]	Germany	A	C_{A2}	C_{A2}*	C_{B2}
Lentinus edodes [10]	France	C_{B1}	A	C_{A1}*	C_{B2}
Lentinus tigrinus [17]	Armenia	C_{A2}	C_{A1}	C_{A2}	C_{A2}
Hypholoma fasciculare [17.5]	France	C_{A2}	C_{B2}	C_{A2}	C_{A1}
Hypholoma sublateritium [8]	Armenia	B	C_{B1}	C_{B1}*	B
Polyporus squamosus [10]	Armenia	C_{B1}	C_{B1}	A	A
Polyporus varius [7]	Armenia	B	B	A	B
Polyporus subarcularius [7]	Armenia	B	B	A	B
Pleurotus ostreatus [18]	Armenia	C_{A2}	C_{A2}	C_{A2}	C_{A2}
Pleurotus cornucopiae [8]	Italy	C_{B1}	B	A	A
Pholiota alnicola [13]	France	C_{B1}	C_{A1}	A	C_{A2}
Pholiota aurivella [10.5]	France	C_{B1}	B	C_{A1}*	C_{A2}
Pholiota destruens [9]	Armenia	A	C_{B1}	C_{A1}*	C_{B1}
Schizophyllum commune [15.5]	Armenia	C_{B2}	C	C_{A2}	C

[a] A, deadlock with mycelial contact; B, deadlock at a distance; C, overgrowth without initial deadlock; C_{A1}, partial replacement after initial deadlock with contact; C_{A2}, complete replacement after initial deadlock with contact; C_{B1}, partial replacement after initial deadlock at a distance; C_{B2}, complete replacement after initial deadlock at a distance; * overgrowth of the mushroom by the phytopathogenic fungus. In all other replacement reactions the mushroom overgrew the phytopatogenic fungus.



A B C D D F

Fig. 1. Combative interactions between xylotrophic mushrooms and phytopathogenic fungi in dual cultures on potato dextrose agar. (A) Deadlock with mycelial contact between *L. edodes* and *B. sorokiniana* 20 days after inoculation. (B) Deadlock at a distance between *H. sublateritium* and *R. cerealis* 10 days after inoculation. (C) Initial deadlock between *K. mutabilis* and *R. cerealis* 7 days after inoculation, followed by replacement (D) of the pathogenic fungus by dense mycelial waves of *K. mutabilis* 7 days later. (E) Replacement of *G. graminis* var. *tritici* by mycelial cords of *H. fasciculare* 21 days after inoculation. (F) Replacement of *B. sorokiniana* by *L. tigrinus* 30 days after inoculation. Note the presence of fruiting body primordia of *L. tigrinus* in the interaction zone.

Table 2. Frequency of type and subtype of interactions between mycelium of xylotrophic mushrooms and pathogenic fungi of cereals in dual culture experiments on potato dextrose agar medium, expressed as a percentage of the total number (204) of pairings tested.

Deadlock		Replacement of cereal pathogenic fungi by xylotrophic mushrooms		Replacement of xylotrophic mushrooms by cereal pathogenic fungi	
Sub-type	%	Sub-type	%	Sub-type	%
A	17.6	C	2.9	C	0
B	14.7	C_{A1}	13.2	C_{A1}	4.4
		C_{A2}	23.5	C_{A2}	1.6
		C_{B1}	14.7	C_{B1}	1.5
		C_{B2}	5.9	C_{B2}	0
Total	32.3	Total	60.2	Total	7.5

For legend see Table 1.

ed mushrooms and eventually overgrown by those of group I.

The frequency of each type and sub-type of reaction is shown in Table 2. Replacement was more frequent (60.2+7.5%) than deadlock (32.3%). In 60.2% of pairings the mushrooms overgrew the pathogenic fungi. Complete replacement was slightly more frequent (29.4%) than partial replacement (27.9%). Overall 92.5% of pairings led to prevention and limitation of fungal growth. This clearly indicated that the mushrooms were combative against the pathogenic fungi, and that they usually operated via contact antagonism.

Although replacement is common in Basidiomycotina interactions, its mechanism remains unclear. It may be brought about by lysis and parasitism on a large scale, or in some cases overgrowing mycelium may simply smother the overgrown mycelium (Rayner and Webber, 1984). The specificity of the interactions between individual mushrooms and pathogenic fungi even in the present study, together with the formation of dense mycelium, suggests that some form of recognition response was involved (Rayner and Webber, 1984). Dense aerial mycelium in the interaction zones was usually produced by both mushrooms and fungi when there was deadlock, but only by the overgrowing organism when there was replacement. In pairings between *K. mutabilis* and *R. cerealis*, dense waves of *K. mutabilis* mycelium advanced across to the *R. cerealis* colony (Fig.1 C, D). In pairings of *H. fasciculare* with *B. sorokiniana*, *G. graminis* var. *tritici* and *F. culmorum* (Fig. 1E), the colonies of the fungus were replaced by dense mycelial cords produced by the xylotrophic mushroom. Fruit-body primordia occurred only in pairings of *L. tigrinus* with *B. sorokiniana* (Fig. 1F).

In the replacement reaction the overgrowing species changed its rate of growth at least three times. Changes in the growth rate were more frequent in pairings between *Ph. alnicola* and *B. sorokiniana,* between *G. lucidum* and *B. sorokiniana*, and between *L. edodes* and *G. graminis tritici*. Often the overgrown species presented exudate droplets and a yellowish brown pigmentation. Strong pigmentation occurred in colonies of *F. culmorum* paired with *L. edodes* and *C. versicolor* and in *R. cerealis* paired with *K. mutabilis*. Changes in colony colour were due to diffusible metabolites produced by overgrowing species: a brown pigmentation indicated mycelial phenoloxidase or peroxidase activity (Li, 1981, 1983).

Conclusions

All tested xylotrophic mushrooms were competitive against the cereal pathogenic fungi. They produced metabolites with antifungal properties. The most active mushrooms, *P. ostreatus, H. fasciculare, G. lucidum, S. commune* and *L. edodes*, are now being paired with the pathogenic fungi in semi-natural substrates in the presence of wheat seedlings for further study.

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