Phytopathol. Mediterr. (2004) 43, 44–48

Interactions between xylotrophic mushrooms and mycoparasitic fungi in dual-culture experiments

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Summary. Seventeen wood-decaying mushroom species (*Coriolus versicolor*, *Flammulina velutipes*, *Ganoderma* sp., *Hypholoma fasciculare*, *H. sublateritium*, *Kühneromyces mutabilis*, *Lentinula edodes*, *Lentinus tigrinus*, *Pholiota alnicola*, *Ph. aurivella*, *Ph. destruens*, *Pleurotus cornucopiae*, *Pl. ostreatus*, *Polyporus subarcularius*, *Po. squamosus*, *Po. varius* and *Schizophyllum commune*) were paired with three *Trichoderma* species (*T. harzianum*, *T. pseudokoningii*, and *T. viride*) and *Clonostachys rosea* in dual-culture experiments on an agar-based medium. Xylotrophic mushrooms and mycoparasitic fungi in general showed similar competitive ability; deadlock, or mutual inhibition after mycelial contact, was observed in 45.6% of pairings, while stable inhibition at a distance occurred in 4.4% of pairings. Replacement, or overgrowth of xylotrophic mushroom by a mycoparasitic fungus was observed in 29.4% of pairings; the opposite, overgrowth of the xylotrophic mushroom on the mycoparasitic fungus in 20.6%. of pairings. Of the xylotrophic mushrooms, *Pl. ostreatus*, *Ganoderma* sp., *F. velutipes* and *H. fasciculare*, showed the highest competitive ability against mycoparasitic fungi. Of the mycoparasitic fungi, *T. harzianum* showed the strongest competitive activity against xylotrophic mushrooms.

Key words: xylotrophic mushrooms, mycoparasitic fungi, interactions.

Introduction

Competition is a fact in any natural fungal community and plays an important role in determining the distribution and abundance of fungal species (Widden, 1997). Inter-specific interactions between fungi help explain not only changes in the structure of fungal communities, but also the context within which fungi can be exploited to control pathogens under natural or semi-natural conditions. However, only limited information is available on competition between fungi (Widden, 1997). Fungal species in the genera *Trichoderma* and *Gliocladium* are cosmopolitan in the soil, on decaying wood and on vegetable matter and some species are frequently dominant in widely varying habitats. This may be attributable to the varied metabolic capability of *Trichoderma* species and their competitive nature (Gams and Bissett, 1998). *Trichoderma* can colonise wood by using non-structural carbohydrates, such as sugar and starch, and then they limit colonisation by secondary invaders such as wood-decaying mushrooms (Smith *et al*., 1981; Klein and Eveleigh, 1998). Moreover, *Trichoderma* have also been shown to inhibit wood fungi by producing volatile and non-volatile me-

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tabolites (Bruce *et al.,* 1984; Bettucci *et al.*,1988). Conversely, the mycelium of some wood-rotting mushrooms is boosted by some *Trichoderma* strains (Dick and Hutchinson, 1966; Hutchinson and Cowan, 1972; Bettucci *et al*., 1988).

In this study seventeen species of wood-decaying mushrooms were paired with four species of mycoparasitic fungi to understand their interactions. Most of these mushrooms in previous dual-culture experiments had already shown their antagonistic effectiveness against the soil-borne phytopathogenic fungi *Bipolaris sorokiniana*, *Fusarium culmorum, Gaeumannomyces graminis* var. *tritici,* and *Rhizoctonia cerealis,* the antagonistic activity of *Pleurotus ostreatus*, *Hypholoma fasciculare, Ganoderma lucidum*, *Lentinus tigrinus* and *Schizophyllum commune* being particularly strong (Badalyan *et al*., 2002).

Materials and methods

Test-organisms

The xylotrophic mushrooms tested were: *Coriolus versicolor* (Fr.) Quel., LEM1Cv; *Flammulina velutipes* (Curt.: Fr.) Sing., LEMIII2Fv; *Ganoderma* sp. (Fr.) Karst., LEM1G; *Hypholoma fasciculare* (Fr.) Kumm., LEM1Hf; *H. sublateritium* (Fr.) Kumm., LEM2Hs; *Kühneromyces mutabilis* (Schaeff.: Fr.) Sing. & Sm., LEM319Km; *Lentinula edodes* (Berk.) Sing., LEM351Le; *Lentinus tigrinus* (Bull.: Fr.) Sing., LEM2Lt; *Pholiota alnicola* (Fr.) Sing., LEM55Phal; *Ph. aurivella* (Fr.) Kumm., LEM872Phau; *Ph. destruens* (Brond.) Gill., LEM1Phd; *Pleurotus cornucopiae* Roll., LEM1Pc; *Pl. ostreatus* (Jacq.: Fr.) Kumm., LEM19Po; *Polyporus subarcularius* (Donk) Bond., LEM2Ps; *Po. squamosus* (Huds.: Fr.) Fr., LEM3Psq; *Po. varius* (Pers.: Fr.) Fr., LEM20Pv; and *Schizophyllum commune* Fr., LEM1Schc. All the isolates are deposed in the collection of the Experimental Mycology Laboratory of Yerevan State University, Armenia. Their origin, isolation and maintenance techniques are reported in Badalyan *et al*. (2002).

The mycoparasitic fungi tested were *Clonostachys rosea* (Link: Fr.) Schroers, Samuels and Gams, LM4Cr, *Trichoderma harzianum* Rifai, LM11Th, *T. pseudokoningii* Rifai, LM8Tp, and *T. viride* Pers.: Fr., LM9Tv. The isolates were from the collection of the Dipartimento di Protezione e Valorizzazione Agroalimentare, University of Bologna, Italy. Test cultures were stored in tubes on special low-nutrient Niremberg agar (Niremberg, 1981) at 5°C in the dark.

Xylotrophic mushrooms and mycoparasitic fungi were transferred from stored cultures to 2% malt-extract-agar (Difco laboratories, Sparks, MD, USA) and potato-dextrose-agar (PDA, Difco) Petri dishes respectively and cultured at 24°C to obtain colonies 3–4 cm in diameter. Mycelial plugs 2-mm in diam. were cut from the edge of these colonies and used as inoculum in pairing experiments.

Dual-culture experiments

Competitive interactions between wood-decaying mushrooms and mycoparasitic fungi were studied in dual-culture experiments on PDA in Petri dishes (Badalyan *et al*., 2002). In each dish, two 2-mm diameter mycelial disks, one from a mushroom colony and one from a mycoparasitic fungus, were placed on the agar surface 30 mm apart. The mushrooms and mycoparasitic fungi were paired in all possible combinations. Three replicates were prepared for each pairing. Plates were examined daily under a stereomicroscope to study the interaction process. A rating scale with 3 types (A, B and C) and 4 sub-types (C_{A1}, $C_{B1,}$ C_{A2} and C_{B2}) of reactions was used for each fungus, where: A, deadlock, mutual inhibition, in which neither organism was able to overgrow the other after mycelial contact; B, deadlock at a distance i.e. without mycelial contact; C, replacement, overgrowth without initial deadlock; C_{A1}, partial replacement after initial deadlock; C_{A2}, complete replacement after initial deadlock; C_{B1}, partial replacement after initial deadlock at a distance; 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 calculated for each fungal species using the formula:
$AI = \sum n \times i$ where n= number (frequency) of each type or sub-type of reaction; i= corresponding score.

Results and discussion

The competitive effect of xylotrophic mushrooms and mycoparasitic fungi was broadly similar; deadlock after mycelial contact occurred in 45.6% of pairings and deadlock at a distance in 4.4 % of pairings. Mycoparasitic fungi overgrew the xylotroph-

ic mushrooms in 29.4% of pairings; while the mycoparasitic fungi were overgrown by xylotrophic mushrooms in 20.6% of pairings.

Of the xylotrophic mushrooms, *Pl. ostreatus, Ganoderma* sp. and *F. velutipes* had the strongest competitive effect (Tab.1). These mushrooms showed against mycoparasitic fungi the same strong antagonistic activity they showed towards some cereal pathogenic fungi in previous *in vitro* dual-culture experiments (Badalyan *et al*., 2002). *Pl. ostreatus* had the highest antagonism index value. This fungus completely replace *C. rosea* and *T. pseudokoningii*, and partially replaced *T. harziamun*. Barrages with brown pigmented zones were observed in interactions areas. These could be associated with laccase activity and formation of melanin compounds that protected mushroom hyphae from micro-organism attack; some studies suggest that mushrooms release laccases as part of a defensive response against mycelial invasion and that these enzymes help the fungi to adapt to environmental stress or to antagonists (Rayner *et al.,* 1994; Savoie *et al*., 2001).

Of the mycoparasitic fungi, *Trichoderma* isolates showed higher competitive activity than the *C. rosea* isolate; *T. harzianum* antagonism was particularly strong. In most pairings the fungus partially or completely overgrew the mushroom mycelium, or stopped after contact the paired colony. *Pl. ostreatus*, as mentioned above, was the only fungus that was not sensitive to *T. harzianum*. All tested *Trichoderma* isolates operated via contact antagonism, as shown by the absence of inhibition areas between paired mycelia. This suggests that under the conditions of the experiments, the isolates did not produce volatile/non-volatile metabolite(s) active against the mushrooms, or that if they did the concentration of such metabolite(s) was not sufficient to act at a distance.

The dominance of *Pl. ostreatus* over *Trichoderma* is interesting in view of the fact that different species of *Trichoderma* are responsible for a wide-

Table 1. Interactions between mycelia of xylotrophic mushrooms and mycoparasitc fungi *Clonostachys rosea, Trichoderma harzianum, T. pseudokoningi* and *T. viride* in pairings on agar-based medium.

Xylotrophic mushroom	Type of interaction [a]				
	Clonostachys rosea	*Trichoderma harzianum*	*Trichoderma pseudokoningii*	*Trichoderma viride*	Total antagonism index
Coriolus versicolor	A	A	C_{A1}*	A	3.0
Flammulina velutipes	C_{B1}	C_{A2}*	C_{B1}	C_{B1}	12.0
Ganoderma sp.	C_{A1}	A	C_{A2}	C_{A1}	12.5
Kühneromyces mutabilis	A	C_{A2}*	A	A	3.0
Lentinula edodes	A	C_{A1}*	A	A	3.0
Lentinus tigrinus	A	A	A	A	4.0
Hypholoma fasciculare	C_{A1}	A	A	C_{A1}	9.0
Hypholoma sublateritium	B	C_{A1}*	A	A	4.0
Pholiota alnicola	A	C_{A2}*	A	C_{A1}*	2.0
Pholiota aurivella	C_{A1}	C_{A2}*	C_{A1}*	C_{A1}*	3.5
Pholiota destruens	B	C*	C*	C*	2.0
Pleurotus cornucopiae	A	C_{A1}*	C_{A1}*	C_{A1}*	1.0
Pleurotus ostreatus	C_{A2}	C_{A1}	C_{A2}	A	13.5
Polyporus squamosus	B	C_{A1}*	C_{A1}*	C_{A1}*	2.0
Polyporus subarcularius	A	A	A	A	4.0
Polyporus varius	C_{B1}	C_{A1}*	A	A	6.0
Schizophyllum commune	C_{A1}	A	A	A	6.5
Total antagonism index	13.0	44.5	26.0	26.0	

[a] A, deadlock after mycelial contact; B, deadlock at a distance; C, overgrowth without initial deadlock; C_{A1}, partial replacement after initial deadlock at contact; C_{A2}, complete replacement after initial deadlock at contact; C_{B1}, partial replacement after initial deadlock at a distance; C_{B2}, complete replacement after initial deadlock at a distance.

*, The mycoparasitic fungus overgrew the xylotrophic mushroom. In the other replacement reactions the mushroom overgrew the mycoparasitic fungus.

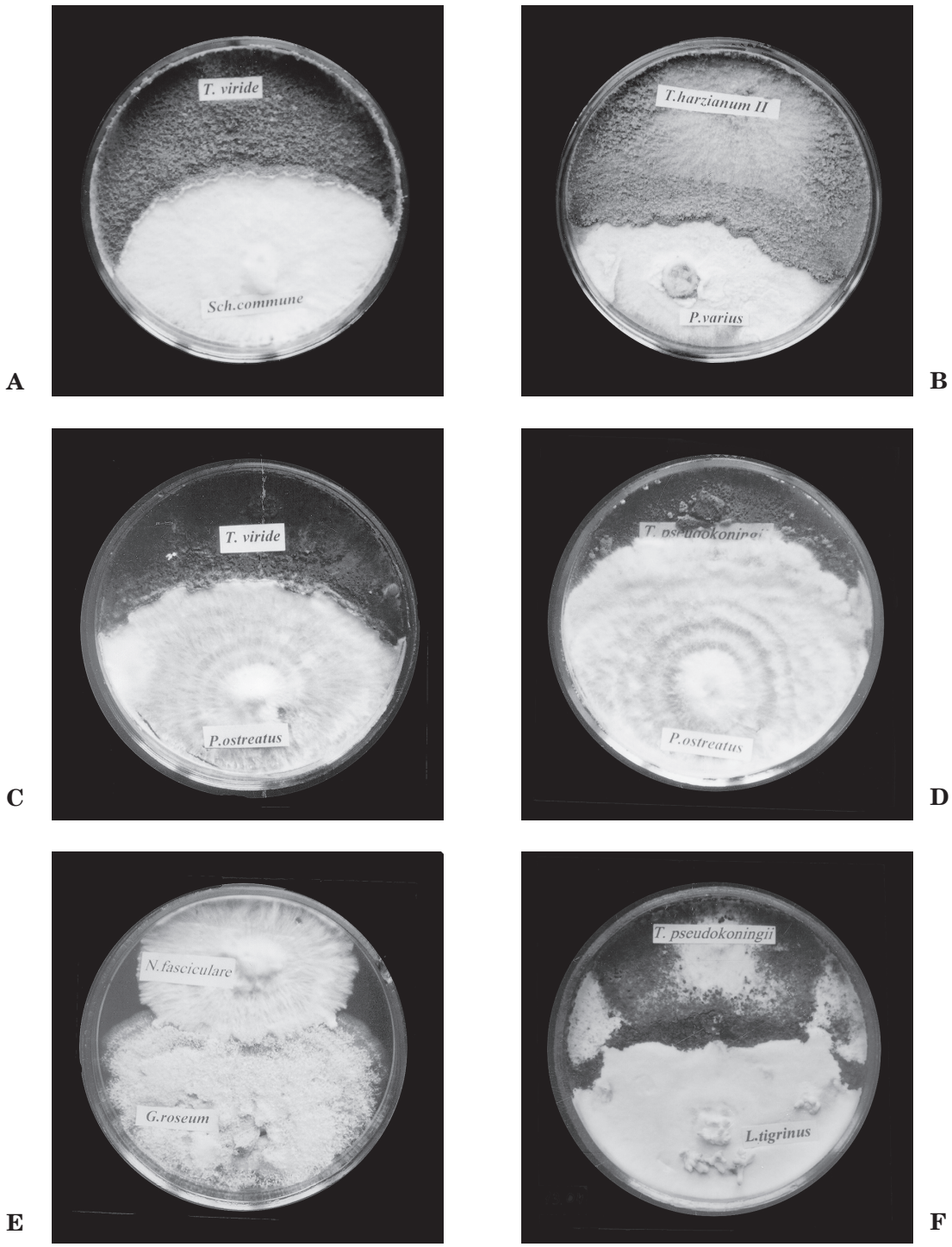

Fig. 1. Comparison between xylotrophic mushrooms and mycoparasitc fungi in dual cultures on agar-based medium. (A) Deadlock after contact between *Schizophyllum commune* and *Trichoderma viride*. (B) Partial replacement of *Polyporus varius* by *T. harzianum*. (C) Deadlock after contact between *Pleurotus ostreatus* and *T. viride*. (D) Replacement of *T. pseudokoningii* by *Pl. ostreatus*. (E) Initial replacement of *Clonostachys rosea* by *Hypholoma fasciculare*. (F) Deadlock after contact between *Lentinus tigrinus* and *T. pseudokoningii*.

spread green mould that has occurred in the last 15 years in Europe and North America, causing severe losses in *Agaricus bisporus*, *Lentinus edodes* and *Pleurotus* spp. (Ospina-Giraldo *et al*., 1999). Against this disease there are no effective control methods at present. On the *Trichoderma–Pleurotus* interaction, Reper and Penninckx (1987) in dual-culture experiments over a range of temperatures and pH values observed that where the mycelia of *Pl. ostreatus* and *T. hamatum* made contact, the growth of the former ceased, whereas that of the latter was unaffected; moreover, these authors reported that the mycoparasitic fungus affected the growth and fructifications of the oyster mushroom mainly by producing a diffusible non-volatile toxin which killed *Pl. ostreatus*. In contrast, the isolate of *Pl. ostreatus* used in our study showed strong combative ability against all tested *Trichoderma* isolates; the mechanism of this particular interaction is now under investigation to obtain information that may be useful for the control of green mould during mushroom cultivation.

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Accepted for publication: March 23, 2004