

Control of growth of wood decay Basidiomycetes by *Trichoderma* spp. and other potentially antagonistic fungi

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Abstract

Isolates of *Trichoderma*, *Penicillium*, and *Aspergillus* were tested against *Trametes versicolor* and *Neolentinus lepideus* by agar interaction tests and by measuring the toxicity of culture filtrates. Some isolates were able to overgrow and kill both of the decay fungi in agar culture but others were totally ineffective. Although the filtrate from most isolates produced some growth inhibition of the two target fungi, the extent of the control varied widely. Three fungi that produced the greatest inhibitory effects (two *Trichoderma* and one *Aspergillus* isolate) were subsequently tested against a range of brown- and white-rot Basidiomycetes. The filtrates of all three isolates produced varying degrees of inhibition against each of the Basidiomycetes. However, the *Aspergillus* filtrate generally produced greater inhibition and was more effective against the white-rot Basidiomycetes. The implications of the results on the testing and use of nondecay antagonistic fungi for the biological control of wood decay are discussed.

At present, broad-spectrum poisons are the only practical method of ensuring that most wooden materials will remain free from fungal decay. While certain physical methods will protect wood from decay (e.g., excluding moisture), these are often not practical for many wooden commodities which, by the nature of their use, are subjected to fluctuating environmental conditions. Increased concern over the environmental effects of chemical biocides and associated legislative constraints on the use of some chemical treatments has meant that radical alternatives are now being sought to replace these preservatives. One possible alternative approach for protecting wood is the use of biological control systems, where microorganisms could be used to inhibit colonization and decay by wood-rotting fungi. The basic concept is to employ the natural ecological antagonisms of selected organisms (most often micro fungi) against target wood decay fungi. The idea of using biological control as a means of protecting wood from decay is not new. Early studies on the use of biological

control in wood have examined decay control in tree stumps (33), felled timbers (21,22), and electrical distribution poles (32).

In recent years, the need to find alternative wood preservation strategies has resulted in increased interest in biological control for a variety of uses in the wood preservation industry. This is mirrored by the large number of publications on this topic. Much of the research work has concentrated on the use of *Trichoderma* spp. as possible control agents for wood decay fungi (5,6,8,10,11,20,24,25, 27,28,34). *Trichoderma* has received so much attention because of the encouraging results with this genus in earlier studies (21,22,32) and the fact that *Trichoderma* spp. have been widely studied as potential control agents for a wide range of plant pathogens in agricultural systems (29,37). Other microorganisms have also been studied for application as biological control agents to protect wood from decay including bacteria (2-4,30) and other microfungi (19,23,26,34).

Biological control systems in agriculture have traditionally been used against single pests or disease agents and their success is due in part, to this specificity of interaction. In wood, however, a control agent would require a broader specificity because most wood structures can be decayed by a range of fungi. The target specificity of control agents is also likely to be peculiar to individual isolates because most micro fungi, and *Trichoderma* spp. in particular, exhibit huge interspecies and interstrain

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Forest Products Research Society 1991.
Forest Prod. J. 41(2):63-67.

variability in metabolize production (36). This paper describes work undertaken to examine the interactive effects of potential biological control strains against a range of wood decay Basidiomycetes with particular attention to the production of soluble metabolizes by the antagonists.

Materials and methods

Fifteen *Trichoderma*, 2 *Penicillium*, and 1 *Aspergillus* isolates were initially tested to find their reaction during interaction tests on agar plates against *Neolentinus* (= *Lentinus*) *lepideus* (Fr.:Fr.) Redhead and Ginns FPRL 7f) and *Trametes* (= *Coriolus*) *versicolor* L. ex Fr.) Pilate (MAD 697) as representative brown- and white-rot fungi, respectively. The *Trichoderma* isolates used in this study included five strains of *T. pseudokoningii* Rifai, two *T. polysporum* (Link. ex Pers.) Rifai (IMI 206040), and three unidentified *Trichoderma* spp. isolated from various cellulosic materials. The two *Penicillium* and one *Aspergillus* strain were isolated from the interior of a creosote-treated distribution pole.

The method used to study interactions between the micro fungi and decay fungi was similar to that used by Rayner and Todd (31). Mycelial plugs removed from the growing margins of cultures of either *N. lepideus* or *T. versicolor* were placed at one side of a petri dish containing malt extract agar (2% malt extract, 1.5% agar) and incubated at 27°C and 70 percent relative humidity for 4 days. After this time, cores removed from the margins of actively growing cultures of the microfungi were placed at the opposite sides of the dishes and the plates were incubated under the same conditions for up to 6 weeks. Because of the failure of many of the *Trichoderma* and the two *Penicillium* species to grow at 27°C, combinations containing these organisms were reincubated at 23°C while the remainder were reincubated at 27°C.

Plates were examined daily to determine the outcome of interactions between the organisms and were assessed on the bases of whether 1) either organism was overgrown by its competitor and the rate at which overgrowth occurred; 2) contact and overgrowth was accompanied by browning and lysis of the Basidiomycete mycelium; and 3) the Basidiomycete was completely killed by the microfungus. Lysis of the Basidiomycete mycelium was confirmed by microscopic examination of samples from the zone of contact between the two colonies where the Basidiomycete mycelium had released a brown pigment into the agar medium. In plates where the Basidiomycete had been overgrown by the antagonist, cores were plated onto malt extract agar containing 4 ppm benomyl to test viability of the decay fungus. Lack of growth from these cores after 3 weeks' incubation at 27°C indicated that the Basidiomycete had been killed by the antagonist.

In addition to interaction studies, the same 15 microfungi were tested to determine whether they produced any soluble products that were inhibitory to *N. lepideus* or *T. versicolor*. This was accomplished using a method described by Bruce et al. (9).

Each of the microfungi was grown in 100 ml of 3 percent malt extract broth for 7 days at either 23° or 27°C, and any mycelium was removed by filtration and the culture filtrate was sterilized by passing through a 0.2 µm millipore membrane. Ten ml of sterile filtrate was then

added to an equal volume of strengthened agar (2% malt extract, 3% agar) held at 50°C and poured into petri dishes to produce a solid medium for inoculation with the Basidiomycetes. Cores (7 mm diameter) removed from the margins of actively growing cultures of *N. lepideus* or *T. versicolor* were inoculated in the centers of the plates that were then incubated in the dark at 27°C. Controls were prepared by adding uninoculated malt extract broth (3%) to the strengthened agar. Three replicate plates were set up for each test. Inhibition of growth of the Basidiomycetes was recorded as the difference in mean radial growth of the Basidiomycetes in the presence or absence of the fungal filtrates after 6 days for the *T. versicolor* or 8 days for the *N. lepideus*. These values were then used to calculate the inhibition of hyphal extension as a percentage of hyphal extension in the absence of the filtrate.

After the initial screening of the culture filtrates, the three organisms that produced the highest levels of inhibition against *N. lepideus* and *T. versicolor* were selected for further testing against a wide range of wood decay Basidiomycetes.

The *T. harzianum* (T25), *T. hamatum* (T150), and *Aspergillus* isolates were incubated in 1000 ml of malt extract broth (3%) for 7 days and filter-sterilized as previously described. A 500-ml portion of the filtrate from each micro fungus was heat treated in an oven at 90°C for 2 hours to determine the effect this might have on its inhibitory activity. Solid agar plates were then made as before by adding strengthened agar to either heat-treated or nonheat-treated filtrate. Replicate plates and controls were then inoculated as previously described with cores of the following fungi: brown-rot fungi — *Coniophora puteana* (Schum.:Fr.) (MAD 515); *Fibroporia vaillantii* (DC:Fr.) Parm (FP 90877R); *Fomopsis meliae* (Underw.) Gilbn. (FPI0002R); *Gloeophyllum trabeum* (Pers.:Fr.) Murr. (MAD 617R); *Neolentinus lepideus* (Fr.:Fr.) Redhead and Ginns (MAD 534); *Antrodia* (= *Poria*) *carbonica* (Over.) Ryvet Gilbn. (MAD 141); and *Postia* (= *Poria*) *placenta* (Fr.) M. Larset Lomb. (MAD 697); white-rot fungi — *Bjerkandera adusta* (Willd.:Fr.) Karst. (L 1539sp.); *Trametes versicolor* (L. ex Fr.) Pilate (MAD 697); *Ganoderma applanatum* (Pers.) Pat. (MAD 7823s); *Heterobasidion annosum* (Fr.) Bref. (= *Fomes annosus* (Fr.) Karst.) (MAD89683); *Irpex lacteus* (Fr.:Fr.) Fr. (HHB 7328sp.); *Phanerochaete chrysosporium* Burds. (ME 446); *Phlebia brevispora* Nakas. in Nakasone et Eslyn (HHB 7030sp.); *Pycnoporus sanguineus* (L.Fr.) Murr. (FP 103380); and *Schizophyllum commune* Fr. (FP 14612R).

Plates were then incubated at 27°C. The inhibition of growth of the Basidiomycetes was recorded as before after 5 days' incubation for *T. versicolor*, *B. adusta*, *P. brevispora*, and *I. lacteus*; 6 days for *F. meliae* 7 days for *P. placenta*, 8 days for *N. lepideus*, *S. commune*, *P. chrysosporium*, *G. trabeum*, *H. annosum*, *F. vaillantii*, and *G. applanatum*; 11 days for *C. puteana* and *P. sanguineus*; and 15 days for *A. carbonica*.

Results and discussion

Interaction studies

A wide variety of reactions were produced by the microfungi against the two Basidiomycetes (Table 1). All five of the *T. pseudokoningii* species and one of the uniden-

TABLE 1. — Interactions between potential antagonists and *N. lepeudeus* and *T. versicolor* in an agar plate system.^a

Antagonist	<i>Neolentinus lepeudeus</i>	<i>Trametes versicolor</i>
<i>Aspergillus</i> isolate	-	-
<i>Penicillium</i> spp. pole isolates		
X	-	-
S	-	+
Unknown <i>Trichoderma</i> isolates		
K	++	++
L	+	+
M	-	+
Binab <i>Trichoderma</i> isolate	-	+
<i>T. longibrachiatum</i> (IMI 53408)	--	--
Root isolates		
<i>T. pseudokoningii</i>		
T22	++	++
T33	++	++
T51	++	++
T55	++	++
T64	++	++
<i>T. polysporum</i>		
T38	-	--
T190	+	-
<i>T. viride</i>		
T40	+	+
<i>T. harzianum</i>		
T25	-	-
<i>T. hamatum</i>		
T150	+	-

^a ++ = rapid overgrowth and killing of Basidiomycete without browning of mycelium; + = slower overgrowth and killing of Basidiomycete accompanied by browning and lysis of mycelium; - = browning and lysis of Basidiomycete mycelium at point of contact but no subsequent overgrowth and killing; and -- = eventual overgrowth of the antagonist by the Basidiomycete.

tified *Trichoderma* strains (K) quickly overgrew and killed both Basidiomycete fungi. Unlike an earlier study (6), however, no obvious signs of lysis accompanied the overgrowth and killing of the Basidiomycetes by these *Trichoderma* isolates.

The *Trichoderma viride* isolate and one of the unidentified *Trichoderma* strains (L) also completely overgrew and killed the two target fungi, but in this instance the slower overgrowth was accompanied by browning and lysis of the mycelium of the decay fungi. Of the remaining *Trichoderma* isolates, *T. hamatum* and one of the *T. polysporum* species overgrew and killed *L. lepeudeus* but only produced lysis and browning along the zone of contact with *T. versicolor*, failing to overgrow the Basidiomyceta despite extended incubation periods. Conversely, the mixed Binab *Trichoderma* culture and the third unidentified *Trichoderma* strain (M) overgrew and killed the *T. versicolor*, but only produced lysis at the point of initial contact with the *N. lepeudeus*. The remaining three *Trichoderma* isolates did not completely kill either Basidiomycete and *T. longibrachiatum* was eventually overgrown by the Basidiomycete cultures. The two *Penicillium* isolates and the *Aspergillus* isolate produced intense browning and lysis of the Basidiomycete along the line of contact with the antagonist, but very little overgrowth of the decay fungi occurred, except that *T. versicolor* was eventually overgrown and killed by one of the *Penicillium* isolates (S).

Because of the difficulty and expense of establishing field studies, agar-based systems similar to the one described are used in most initial screening of biological control systems. Often, however, control agents fail when sub-

TABLE 2. — Inhibition of hyphal extension of *N. lepeudeus* and *T. versicolor* when grown on media containing culture filtrate from potential antagonists.

Antagonist	Inhibition	
	<i>Neolentinus lepeudeus</i>	<i>Trametes versicolor</i>
	----- (%) -----	
<i>Aspergillus</i> isolate	59	83
<i>Penicillium</i> spp. pole isolates		
X	14	32
S	58	70
Unknown <i>Trichoderma</i> isolates		
K	22	16
L	13	13
M	23	20
Binab <i>Trichoderma</i> isolate	22	24
<i>T. longibrachiatum</i> (IMI 53408)	16	26
Root isolates		
<i>T. pseudokoningii</i>		
T22	30	24
T33	0	4
T51	2	18
T55	14	4
T64	37	25
<i>T. polysporum</i>		
T38	10	5
T190	16	8
<i>T. viride</i>		
T40	8	28
<i>T. harzianum</i>		
T25	95	30
<i>T. hamatum</i>		
T150	75	73

sequently tested in field conditions. Baker and Cook (1) outlined some of the major reasons for this lack of transferability, not least the fact that some of the active metabolites produced in nutrient-rich media may not be produced in the nutrient-limited field environment. It would therefore seem important that the influence of the nutrient conditions on the antagonistic traits of *Trichoderma* isolates be determined before suitable media-based screening systems are developed for testing *Trichoderma* spp. against wood decay fungi. An alternative strategy, however, would be to carry out screening on wood using a system such as that proposed by Freitag and Morrell (18).

Trichoderma spp. are known to possess a variety of mechanisms to combat other fungal species. These include: fungistatic and fungicidal volatile and nonvolatile metabolites (7,14,36); a high inoculum potential by rapid colonization and removal of available nutrients (21,22); and mycoparasitism by hyphal coiling associated with the production of a range of lytic enzymes including chitinase and B(1-3) glucanase (13,15-17,35).

The variation in efficacy and specificity of the results of the interaction studies involving *Trichoderma* isolates may therefore reflect the combined effects of some, if not all of these mechanisms of antagonism. The relative importance of each mechanism will also be greatly influenced by the environmental conditions in which the *Trichoderma* and its target are exposed. It is possible that the screening conditions might favor one antagonistic mode while under different environmental conditions other antagonistic mechanisms may be the determining factor affecting the outcome of any interaction.

The effect of the culture filtrates from the range of antagonists on Basidiomycete growth was most variable (Table 2). Their effect on growth showed no correlation to the ability of the individual antagonists to kill the Ba-

sidiomycetes in interaction studies. In fact, the culture filtrates from *T. pseudokoningii* isolates, which were the most rapid killers of the Basidiomycetes, produced little or no inhibition of growth of either of the two Basidiomycetes. Similarly, isolates that produced metabolites capable of causing significant inhibition in Basidiomycete growth (e.g., *T. harzianum* T25), would not be selected using traditional cross plating screening methods.

The effects of the culture filtrates from the *Aspergillus*, *T. hamatum* (T150), and *T. harzianum* (T25) against the range of brown- and white-rot fungi are presented in Table 3. These results indicate that the specificity of action of the filtrates was variable for both of the *Trichoderma* spp. and *T. harzianum* generally was more effective against the brown-rot fungi. The target specificity of the *Trichoderma* species is evident even at the strain level. While the filtrate from *T. harzianum* inhibited the hyphal extension of *N. lepideus* (FPRL 7F) by 95 percent, growth of *N. lepideus* (MAD 534) was inhibited by only 28 percent. The inhibitory effect of the *T. hamatum* (T150) filtrate was reduced by heating. Since this isolate is known to produce large quantities of chitinase and B(1-3) glucanase enzymes (data not presented), denaturation of these by the heating process may account for the reduced levels of inhibition. The remaining activity in the filtrates may be due to the toxicity of other, more stable metabolites. The filtrate of the *Aspergillus* isolate was much more effective at inhibiting the growth of the Basidiomycete organisms than the two *Trichoderma* isolates, and in some instances totally restricted the growth of the decay fungi. It was particularly effective against the white-rot fungi, except for *P. brevispora*, where it had no effect.

Trichoderma spp. have been shown to reduce the incidence of decay by *N. lepideus* in the heartwood of creosoted distribution poles (8,10, 11). Furthermore, wood removed from the interior of a *Trichoderma*-treated pole 6 years after inoculation was found to be resistant to decay by *N. lepideus* and *P. carbonica* but not to *T. versicolor* (12). Similarly, Highley and Ricard (20), using ASTM soil

block tests, found that *Trichoderma* spp. generally were able to protect wood from brown-rot fungi but were not as effective against white-rot fungi. The results described in this paper showed a similar pattern for the filtrate from *T. harzianum*. The apparent inability of *Trichoderma* to control decay by white-rot organisms would obviously preclude its use in many wood structures. However, since internal decay of pole interiors in the United Kingdom is caused predominantly by brown-rot fungi, particularly *N. lepideus* (6), this selectivity of action is not necessarily a problem for this particular application. Alternatively, greater strain selection may provide *Trichoderma* isolates that are effective against a wider range of target fungi.

Further study of the *Aspergillus* isolate is warranted in view of the inhibition produced by the culture filtrate of this organism. While this fungus did not overgrow and kill the Basidiomycetes when grown in competition on agar plates, it caused lysis of the decay fungi at the point of contact. Since this organism was isolated from a pole interior it may be well suited for application to wood as a living control agent.

From the results presented here, and in many other recent research articles in the area of wood preservation, it is clear that biological control provides a useful addition to the use of chemical preservatives for limiting fungal decay. Modes of antagonism of organisms (including *Trichoderma* spp.) are complex, and unlike the mode of action of most chemicals, may be subject to change under different environmental conditions. This point is important and must be carefully considered during the evaluation of control systems, particularly during screening tests for potential control agents. For example, if control of a particular decay agent in wood occurs via mycoparasitism, then screening control agents on nutrient-rich media that favor production of soluble antibiotics will be wholly inappropriate. Instead an assay for lytic enzymes may be better suited for identifying the most efficient antagonist. This may, in turn, avoid expensive field failures

TABLE 3. — Percent inhibition of typical hyphal extension of brown- and white-rot fungi when grown on media containing filtrates from selected antagonists.

Basidiomycetes	Inhibition ^a					
	<i>T. harzianum</i> (T25)		<i>T. hamatum</i> (T150)		<i>Aspergillus</i>	
	unheated	heated	unheated	heated	unheated	heated
White-rot	----- (%) -----					
<i>B. adusta</i>	36	36	54	13	90	92
<i>T. versicolor</i>	25	25	74	11	82	81
<i>G. applanatum</i>	16	2	11	0	100	100
<i>H. annosum</i>	54	43	46	12	75	83
<i>I. lacteus</i>	10	8	0	1	61	57
<i>P. chrysosporium</i>	4	7	0	2	100	100
<i>P. brevispora</i>	5	8	54	10	-3	1
<i>P. sanguineus</i>	5	10	3	0	100	100
<i>S. commune</i>	-5	-10	7	2	86	93
Brown-rot						
<i>C. puteana</i>	83	80	33	22	67	83
<i>F. vaillantii</i>	67	16	12	7	38	60
<i>F. meliae</i>	41	28	23	11	35	38
<i>G. trabeum</i>	24	19	6	6	74	74
<i>N. lepideus</i>	28	22	39	20	66	65
<i>P. carbonica</i>	27	6	6	6	23	23
<i>P. placenta</i>	34	10	18	2	25	30

^a Negative values indicate that growth stimulation has occurred.

that diminish the confidence of industry in the concept of biological control for wood preservation.

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