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COLONIZATION AND CONTROL OF DECAY BY *TRICHODERMA* IN DOUGLAS-FIR AND SOUTHERN PINE EXPOSED ABOVE GROUND

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INTRODUCTION

Using biological controls to protect wood against fungal attack is an attractive alternative to chemical biocides because of their environmental effects. Much research on biological control of wood-attacking fungi has involved Trichoderma spp. and, to a lesser extent, Scytalidium spp. (Freitag et al., 1991) Laboratory studies of antagonism between Trichoderma, Scytalidium. and wood decay fungi have been promising, but field studies have met with mixed results.

Glaser et al. (1969) reported that the presence of living T. viride prevented decay of pine logs by Stereum sanguinolentum. Lindgren and Harvey (1952) noticed reduced decay in Southern Pine pulpwood infected with Trichoderma. The application of T. viride to the ends of freshly cut birch bolts protected the bolts from decay (Shields and Atwell, 1963; Hume and Shields, 1975).

Most subsequent field tests have employed Binab-T powders or pellets, which are formulated in Sweden by Bio-innovation AB (Binab). The Binab-T products have been approved by the U.S. Environmental Protection Agency for non-agricultural uses. The powders contain propagules of Trichoderma spp. (ATCC 20475 and 20476, American Type Culture Collection). By combining the species, the temperature at which Trichoderma will suppress casual organisms is broadened to a range of 4C to 27C. Most 2 species stop growing below 10C to 15C. The powder is mixed with water and sprayed or brushed on the wood.

Pellets are inserted into the wood. Bruce and King (1986a) destructively sampled 166 poled inoculated with Binab-T and found Trichoderma established in 94 percent of the poles. Trichoderma could be isolated for up to 4 years following inoculation. Decay was reduced by Trichoderma but establishment of biocontrol agent was somewhat inhibited by other fungi (Bruce and King 1986b). Wood removed from the interior of a Trichoderma- treated pole 6 years after inoculation was found to be resistant to decay by Neolentinus lepideus and Antrodia carbonica. However, resistance was lost when the wood was sterilized (Bruce et al., 1991).

Morris et al. (1984) found Binab-T ineffective as a remedial treatment for N. lepideus in poles. Trichoderma effectively colonized the poles, but N. lepideus was detected in as many poles as the controls. These authors conclude that the Binab system might have some application as a protective treatment but is ineffective as a remedial treatment.

The objective of our study was to determine the ability of Binab-T pellets and wettable powder to colonize and prevent decay in Douglas-fir and Southern Pine above ground..

MATERIALS AND METHODS

Test units were inoculated with Binab-T wettable powder or pellets (Bio-Innovation AB (Binab), Algaras, Sweden). The pellets contained propagules of Trichoderma polysporum ATCC 20475 and T. harzianum ATCC 29476.

Squared Timbers

Untreated Douglas-fir and Southern Pine timbers, 15.2cm by 4.26m, were inoculated with Binab-T pellets placed into holes drilled on the upper surface. The holes were plugged with a wooden plug. The timbers were exposed on racks elevated above ground at the Valley View Exposure Site near Madison, Wisconsin, or at the Harrison National Exposure Site near Gulf port, Mississippi. Only Southern Pine timbers were exposed in Mississippi.

Treated (ammoniacal-copper-arsenic and creosote) Douglas-fir curbs on a pier near Seattle, Washington, were inoculated with Binab-T pellets in the same manner as the timbers exposed at the other sites.

Piling

Creosoted Douglas-fir piles were cut into 0.6m sections with a chainsaw and the sections randomized into different groups. The pile sections were placed upright on the ground. Binab-T wettable powder was applied by brush to the upper surface of the pile. The wettable powder was also applied combined with 5 percent ammonium bifluoride, 10 percent glucose, or both. The piles were exposed at the Gulf port site.

L-Joints

L-joint test units were constructed of Southern Pine according to Carey et al. (1981). the design of the L-joint favors penetration of rainwater into the wood end grain. Ammonium bifluoride (5 percent) was brushed on the surface of all test units. In some units Trichoderma was applied to the joint areas as follows: pellets alone, pellets plus wettable powder, and wettable powder alone. Units were exposed at the Gulf port site for 4 years.

Decay Resistance

Areas from Southern Pine timbers exposed at the Madison test site that were colonized by Trichoderma were removed for subsequent exposure to decay fungi. Blocks removed from the timbers were evaluated for decay resistance using the standard American Society for Testing and Materials (ASTM) soil-block test (1971). Both unsterilized and steam-sterilized blocks were tested. Postia placental M. Lars. et Lomb (MAD-698), Neolentinus (= Lentinus) lepideus (Fr.:Fr.) Redhead and Ginns (MAD-534), and Trametes (= Coriolus), versicolor (L:Fr.) Pilate (MAD-697) were the test fungi. After incubation at 27C and 70 percent relative humidity for 12 weeks, the blocks were conditioned and weighed, and weight losses were calculated to measure the extent of decay. Five replications were used for each treatment evaluated.

Treatment of Log Ends

Green Southern Pine log sections, 2.54cm thick, were flooded with a blend of Trichoderma (P74H) in 1 percent malt extract plus 0.2 percent yeast extract. The sections were exposed at the Madison site for 5 months and evaluated for sapstain discolorations in the interior wood.

RESULTS

Squared Timbers

The Southern Pine timbers treated with Binab-T pellets were well-colonized by Trichoderma (Table 1) at both the Madison and Gulf port sites. Recovery of Trichoderma reached a maximum at 20 months, but fell below 50 percent at 3 years.

Table 1. Percentage of Moisture Content and Trichoderma Recovery in Southern Pine and Douglas-fir Squared Timbers Exposed Above Ground.

Exposure Site	Time (months)	Southern Pine Recovery	Southern Pine Moisture content	Douglas-fir Recovery	Douglas-fir Moisture content
Madison	0	-	19	-	17
	8	64	20	8	17
	12	72	-	65	-
	20	96	30 +	39	19
	36	46	30 +	37	17
Gulfport	0	-	30 +	-	-
	6	32	30 +	-	-
	20	76	30 +	-	-
	36	26	30 +	-	-
Seattle	0	-	-	-	21-30 +
	12	-	-	37	21-30 +
	24	-	-	0	21-30 +

Trichoderma colonization was not as prolific in the Douglas-fir timbers as in the Southern Pine (Table 1). Maximum recovery was at 12 months in timbers exposed in Madison, but isolation of Trichoderma dropped considerably in subsequent samplings. Colonization of the Douglas-fir timbers in Seattle was much poorer than that of timbers exposed in Madison, despite very high moisture content in the Seattle timbers. the Seattle timbers were much older than the Madison timbers, and competition from other wood-inhabiting fungi might have been a factor in the poor colonization.

The spread of Trichoderma from the point of pellet insertion was determined in Douglas-fir and Southern Pine timbers exposed above ground in Madison (Table 2). As in the other Southern Pine timbers, maximum recovery occurred at 20 months. The Southern Pine was heavily colonized with Trichoderma at every distance from pellet installation. Recovery of Trichoderma dropped at every distance at 36 months. In Douglas-fir, maximum recovery of Trichoderma occurred at 8 months and dropped substantially by 36 months (Table 2). Trichoderma colonized Douglas-fir 2.54cm from pellet installation, but recovery at greater distances was very poor.

Although timbers were not tested for the presence of the Trichoderma beyond 36 months, they were left in place for observation of decay. After 5 years, decay was not present in any of the Trichoderma -treated or untreated timbers of Douglas-fir. However, visual decay developed in both Trichoderma-treated and untreated controls of Southern Pine by 5 years. Gloeophyllum trabeum fruiting bodies were observed on the pine timbers exposed in Madison; this was the only fungus isolated from the timbers.

Table 2. Recovery of Trichoderma From Exposed Southern Pine and Douglas-fir at Various Distances From Pellet Installation.

	Recovery of Trichoderma (percent) at various distances from pellet installation					
Southern Pine Time (months	2.54cm	0.3m	0.6m	0.9m	1.8m	Moisture content (percent)
0	-	-	-	-	-	19
8	16	42	50	8	8	25-40
20	92	100	83	75	75	28-30 +
36	67	75	66	50	42	28-30 +
Douglas-fir*						
0	-	-	-	-	-	17-20
8	75	17	0	8	0	18-22
20	69	8	25	8	8	23-26
36	42	0	17	17	8	24-30 +

*Douglas-fir timbers exposed in ground contact.

Piling

The heartwood interior of the Douglas-fir piling was poorly colonized by Trichoderma (Table 3). Glucose and ammonium bifluoride supplements were of no benefit in establishing. Trichoderma was not present in any of the Douglas-fir piling at 5 years. Decay was present in many piles by this time.

Table 3. Recovery of Trichoderma and Presence of Decay in Piling Tops Treated With Binab-T Wettable Powder.

Treatment	Total Number of Piles	Number of Piles with Trichoderma* (at 16 months)	Number of Piles with decay (at 5 years)
Ammonium bifluoride + Trichoderma	11	2	8
Ammonium bifluoride	5	0	0
Ammonium bifluoride + glucose + Trichoderma	5	1	3
Trichoderma	9	4	1
Trichoderma + glucose	6	0	3
Control	5	1	3

*Trichoderma was not isolated from the piles at 5 years of exposure.

L-Joints

Ammonium biofluoride (5 percent) was applied to the joint area of the L-joints because fluoride is reported to stimulate Trichoderma (Lindgren and Harvey, 1952). The effect of ammonium biofluoride on stimulation of Trichoderma could not be determined because ammonium biofluoride alone protected the L-joints during the 4 year exposure.

Trichoderma was isolated from all units throughout the 4 year exposure at the Gulfport site (Table 4). However, decay was present by 2 years in some units that were not treated with ammonium biofluoride.

Table 4. Decay of Southern Pine L-Joints Treated with Binab-T Pellets. Binab-T Wettable Powder, and Ammonium Biofluoride.

Treatment		Decay Rating[a]			
Ammonium biofluoride[b]	Binab-T	1 year	2 years	3 years	4 years
Yes	No	0	0	0	0
No	No	0	0	0	0-20
No	Pellets	0	0-40	0-40	0-80
Yes	Pellets	0	0	0	0
No	Powder	0	0	0	0-40
Yes	Powder	0	0	0	0
No	Pellets and Powder	0	0	0	40
Yes	Pellets and Powder	0	0	0	0
Control[c]	-	0	20	60	60

[a]O-no evidence of decay, 20-decay suspected, 40-decay definite, but limited, 60-general decay, 80-decay warrants replacement of unit, 100-failure.
[b]All test units received surface treatment with 5 percent ammonium biofluoride. Some units were treated in the joint area as well.
[c]No surface or joint treatment with ammonium biofluoride or Binab-T.

Decay Resistance

Both unsterilized and sterilized wood removed from Trichoderma -colonized Southern Pine timbers after 3 years was not resistant to decay by Postia placenta or Trametes versicolor in soil-block tests (Table 5). Weight loss in unsterlized Trichoderma -treated pine caused by Neolentinus lepideus was considerably reduced compared to that of untreated controls. However, decay resistance was lost when the wood was sterilized.

Table 5. Decay Resistance of Trichoderma-Treated Southern Pine Timbers.

Decay fungus	Weight loss (percent)		
	Unsterilized treated timbers	Steam-sterilized treated timbers	Untreated controls
Neolentinus lepideus	11	33	39
Postia placenta	54	57	54
Trametes versicolor	6	13	7

DISCUSSION

In this study, we examined the ability of a commercial preparation of Trichoderma to colonize and survive in Douglas-fir and Southern Pine timbers exposed above ground in various climatic areas. Under field conditions Trichoderma readily colonized Southern Pine (mostly sapwood) squared timbers and L-joints, but the percentage of recovery in the squared timbers dropped considerably in the third year of exposure. Trichoderma was isolated up to 1.8m from the point of pellet insertion in the Southern Pine timbers. Colonization of the Douglas-fir heartwood by Trichoderma is squared timbers and piling exposed above ground was much poorer than that in Southern Pine. Douglas-fir timbers exposed in ground contact in Madison were better colonized, but recovery was very poor beyond 2.54cm from pellet insertion.

The Binab-treated Southern Pine timbers in this study were better colonized than the interior of creosoted Scotch pine distribution poles treated with Binab-T pellets (Bruce and King, 1986a,b; Bruce et al., 1990). Recovery of Trichoderma from the heartwood center of the distribution poles was only 25.6 percent after 18 months and 23.6 percent after 6 years. Trichoderma did not appear to develop and colonize throughout the poles. Bruce et al. (1990) attributed the lack of distribution to resident fungi, but another factor may have been the high natural durability of heartwood and lack of nutrients for favorable growth.

Although Trichoderma was able to develop and spread through the Southern Pine timbers, it did not stop decay. The timbers were eventually decayed by Gloeophyllum trabeum. In previous laboratory tests (Highley and Ricard, 1988), we found that Trichoderma did not control G. trabeum decat.

Control of G. trabeum is important because this fungus is a prevalent cause of decay of pine on the exterior of buildings and decay of other pine products off the ground and subject to rain wetting.

On the other hand, Neolentinus lepideus is easily controlled by Trichoderma in laboratory tests, but control in field tests is rather disappointing (Bruce et al., 1990). Bruce and King, (1986b) reported that inoculation of poles with Binab pellets either before or after infection with N. lepideus reduced the incidence of decay approximately 50 percent. However, in another study, Bruce et al. (1990) found that prior inoculation reduced decay only 17 percent. If N. lepideus was established prior to pole infection, no reduction in decay occurred, The authors concluded that for successful control of N. lepideus the fungus must be eradicated in the early stages of colonization. Failure of Trichoderma to prevent decay to a greater extent was probably due to poor colonization of the heartwood interior.

A biocontrol treatment would have had a better chance for success as a groundline treatment for poles than as a treatment for aboveground wood because of the continual moisture. It is difficult to envisage active control of decay by antagonists in aboveground structures, such as window frames and waterfront curbing, when the moisture content fluctuates substantially. Any biological control agent would have to maintain itself during the times when the timber remained dry, only becoming active when wetting occurred. On the other hand, if the biocontrol agent left behind a fungistatic residue, resistance to decay might be improved. Trichoderma has been reported to produce fungistatic substances (Bruce and King, 1983; Bruce and Highley, 1991). However, this evidently did not occur in the field exposure in our tests because wood removed from Trichoderma -infected areas of pine timbers and sterilized was not decay-resistant. (Bruce et al., 1991) found similar results with sterilized wood removed from Trichoderma -treated poles after 7 years. However, the authors found that unsterilized wood was resistant to N. lepideus but not to Trametes versicolor. Likewise, in the present study, unsterilized Trichoderma -treated wood removed from pine timbers reduced decay by N. lepideus, but not T. versicolor or Postia placenta,

Although Trichoderma did not successfully control decay in Douglas-fir and Southern Pine exposed above ground in our study, there are many examples of successful control of wood-inhibiting fungi by Trichoderma. In our study, control of stain in green pine log sections was successful. Hulme and Shields (1975) found that Trichoderma protected freshly-cut birch bolts from decay. Lindgren and Harvey (1952) prevented decay by Peniphora by spraying green bolts with ammonium biofluoride and Trichoderma. Pottle et al. (1977) showed that be pruning wounds of a red maple could be protected for more than 2 years by inoculating the wounds with a suspension of Trichoderma. In a comprehensive study that compared various chemical commercial compounds with Trichoderma preparations, Mercer and Kirk (1984) found that Trichoderma out-performed chemical compounds, both in acceleration of callus formation by sapwood and in prevention of basidiomycete infection of heartwood. The biggest success story for Trichoderma as a bioprotectant is its use on fruit trees to protect cud silver leaf by basidiomycete Chondrostereum purpureum (Dobos and Richard, 1974). Chondrostereum purpureum spores infect the tree via contact with unprotected wood, most often pruning wounds. The preventative effect of Trichoderma in this disease was clearly demonstrated (Groslande, 1970).

The success stories cited here deal with relatively short-term protection of wood against attacking fungi. Thus, initial research on the use of biocontrol agents to control wood-attacking fungi should emphasize short-term protection, such as protection of lumber and logs during drying. Control of decay in wood products has to be very long-term, many years, if not decades, For a biocontrol organism to accomplish this would be very difficult unless the organism spreads throughout the wood and deposits as residual fungistatic material that remains after the death of the organism or alters the substrate to make it nutritionally undesirable to the decay fungus.

SUMMARY

In this study, we determined the ability of a biological control product of Trichoderma to colonize and prevent decay in Douglas-fir and Southern Pine timbers exposed above ground. Trichoderma colonized Southern Pine timbers but did not prevent decay from Gloeophyllum trabeum. Douglas-fir timbers were poorly colonized by Trichoderma. Recovery of Trichoderma from timbers decreased after 3 years. Material removed from timbers colonized by Trichoderma resisted decay by Neolentinus lepideus, but not Postia placenta or Trametes versicolor. Any decay prevention was lost, however, when the wood

was sterilized prior to exposure to the decay fungi. Treatment of green Southern Pine log ends with Trichoderma protected the wood from sapstain fungi.

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Note: The use of trade or firm names in this publication is for reader information and does not imply endorsement by the U.S. Department of Agriculture of any product or service.

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