Control of wood decay by *Trichoderma (Gliocladium) virens*

I. Antagonistic properties

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1. Introduction

The control of wood-attacking fungi with fungal or bacterial biocontrol agents has recently elicited considerable research interest. Increased concern about the environmental impact of chemical wood preservatives in soil and groundwater has stimulated this trend. One of the most widely studied genera of fungal biocontrol agents is *Trichoderma (Gliocladium),* which has controlled several soil-borne pathogens under experimental conditions (R. J. COOK and K. F. BAKER, 1983). T. L. HIGHLEY and J. RICARD (1988) found that on a malt-agar medium, an isolate of *Trichoderma* virens completely inhibited growth of several white- and brown-rot fungi and killed them. This isolate also prevented decay in pine blocks exposed to brown-rot but not white-rot fungi.

Gliogard (W. R. Grace & Co., CT), a formulation of *T. virens* strain G-20 (synonym GL-2l), is registered with the U.S. Environmental Protection Agency to control damping off diseases caused primarily by *Phythium ultimum* and *Rhizoctonia solani. Trichoderma virens* produces the antibiotic, gliotoxin, an epidithiodiketopiperazine. Several studies (P. W. BRIAN and H. G. HEMMING, 1945; A. TAYLOR, 1986) have de-

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monstrated an association between gliotoxin production and *in vivo* inhibition or disease suppressive ability (S. E. WILHITE *et al.*, 1994). The purpose of the work reported here was to evaluate the effectiveness of the biofungicide, Gliogard, against wood decay and discoloring fungi in laboratory tests.

2. Methods

2.1 Fungi

Trichoderma (Gliocladium) virens (Miller *et al,)* [GL-21] was donated by W. R. Grace and Co., CT,[3] in the form of dispersible granules.

The following Basidiomycete decay fungi were used: Brown-rot- *Postia (=Poria)* placenta (Fr) M, Lars. et. Lomb. [MAD-698], *Neolentinus (=Lentinus lepideus* (Fr.:Fr:) Redhead and Ginns [MAD-534], and *Gloeophyllum trabeum* (Pers.:Fk) Murr. [MAD-617]; white-rot- *Phlebia brevisporia* Nakas, in Nakasone et Eslyn [HBB-7030-sp], *Irpex lacteus* (Fr.:Fn) Fr, [HBB-7328-sp.], and *Trametes (= Coriolus) versicolor* L. ex Fr: Pilate [MAD-697].

The mold *Trichoderma harzianum* Rifai (ATCC:20476) and stain fungus *Ceratocystis minor* (Hedgcock) Hunt (C-188) were included in the experiment to determine the effects of GL-21 metabolizes on inhibition of their growth.

2.2 Tests for antagonistic effectiveness of GL-21 in agar medium

Dual culture of GL-21 and decay fungi on malt-agar medium. A mycelial plug of each Basidiomycete fungus was placed on one edge of a Petri plate containing 2% malt extract in agar. After 2 days, the opposite edge of the plate was inoculated with a mycelial plug of GL-21. Plates without GL-21 were used as controls. The plate cultures were incubated at 27 °C and 70% relative humidity. Three replicates were used, Linear growth of the decay fungus was measured until the growth of the Basidiomycete fungus in the control plates reached the opposite side of the plate. The ability of GL-21 to kill the Basidiomycete fungi was evaluated by aseptically transferring plugs from test plates to a modified J. B. TAYLOR'S (1971) medium, as described in T. L. HIGHLEY and W. E. ESLYN (1982), to determine viability of Basidiomycetes.

Production of fungistatic metabolizes. The ability of GL-21 to produce compounds inhibitory to growth of the Basidiomycete fungi and the two discoloring fungi was determined by culture filtrate inhibition and a cellophane method (C. DENNIS and J. WEBSTER, 1971),

Filtrate Inhibition. GL-21 was grown in 500-ml flasks containing 100 ml of 2% malt extract and 0.2% yeast extract (high nitrogen) or basal salts (T. L. HIGHLEY,

[3] 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.

1973) with 0.02% NH_4NO_3 and 0.5% glucose (low nitrogen). Cultures were incubated for 7 days at 27 °C and 70% relative humidity. Mycelial mats were removed over filter paper in a Buchner funnel, and the filtrate was sterilized through a 0.20 μm sterile Millipore filter. Ten milliliters of the filtrate were placed in Petri dishes (90 mm), and 10 ml of 3% malt agar (40 °C) were added and mixed with the filtrate. Plates were inoculated with agar plugs of the decay fungi. Malt agar plates without the filtrate were inoculated with decay fungi to serve as controls. Cultures were incubated at 27 °C, and colony diameter was periodically measured until the control fungi completely covered the plate.

Cellophane method. The ability of GL-21 to produce fungistatic metabolizes was also determined by the cellophane method of C. DENNIS and J. WEBSTER (1971). Sterile cellophane was placed over a malt-agar medium and inoculated in the center with GL-21. After 3 days of growth at 27 °C, the cellophane was removed and the plate was inoculated with the target fungus. Cultures were incubated as previously noted, and colony diameter was measured.

2.3 Tests for antagonistic effectiveness of GL-21 in wood

Southern Pine or maple test blocks (25 mm × 25 mm × 9 mm, with the 9-mm dimension in the grain direction) were treated with GL-21 by several methods and tested for decay resistance in soil-block tests. To determine if live GL-21 was necessary to render blocks resistant to decay, some blocks were sterilized after treatment with propylene oxide or sterile filtrates of GL-21 were used to treat blocks.

Blocks vacuum impregnated with culture filtrate or homogenate. GL-21 was grown in 500-ml flasks containing 100 ml of 2% malt extract and 0.2% yeast extract. Cultures were incubated for 7 days at 27 °C and 70% relative humidity. Then, the cultures were homogenized for about 10 seconds in a Waring blender or mycelial mats were removed from the liquid cultures over a Buchner funnel, and the filtrate was sterilized by passage through a 0.20-μm sterile Millipore filter. Blocks were vacuum impregnated with the homogenate or sterilized filtrate and dried down in absorbent-lined pans for 3 hours. One set of treated blocks was placed immediately into soil-block bottles. A portion of the homogenate-treated blocks was incubated for 6 weeks on glass rods over agar in soil-block bottles containing 1.5% water agar or 2% malt extract. Blocks were then sterilized with propylene oxide and placed into soil-block tests.

Blocks treated with GL-21 granule, Maple or Southern Pine blocks were placed into soil-block bottles, and one side of the block was inoculated with a granule of GL-21 and the opposite side with a Basidiomycete fungus.

Blocks treated with GL-21 over soil medium. Blocks were mass treated with GL-21 using a modified soil-block technique. Soil (1,200 g ovendry) was placed in an aluminum pan measuring 23 cm × 32 cm × 6 cm. Deionized water was added to bring the moisture content of the soil up to 40%, and 24 sweetgum feeders (25 mm × 25 mm × 0.2 mm) were placed on the surface of the soil. The pan was covered with foil and autoclave for 30 min. at 121 °C, Test blocks were treated with GL-21 culture homogenate or GL-21 granules and placed into pans. The blocks were incubated for 21 days at 27 °C and 70% relative humidity. Following incubation, blocks were propylene oxide sterilized and placed into soil-block decay tests.

2.4 Eradication of decay fungi by GL-21

Southern Pine blocks were exposed to *P. placenta* and *G, trabeum* and maple blocks to *T. versicolor* in soil-block tests for 2 weeks. The blocks were then treated with a GL-21 culture blend and replaced in soil-block bottles inoculated with the noted decay fungi. Incubation was 10 weeks.

2.5 Soil-block test of pretreated wood

The blocks pretreated with GL-21 were evaluated for decay resistance using the standard American Society for Testing and Materials (ASTM, 1971) soil-block test. Bottles were incubated at 27 °C and 70% relative humidity in the dark. Percentage weight loss, the measure of decay, was calculated from the weight of decayed blocks after equilibration at 70% relative humidity and 27 °C, Original weight was recorded after similar equilibrations. Five replications were used for each treatment.

3. Results

In dual cultures of GL-21 and wood decay fungi, in a malt-agar medium, GL-21 quickly overgrew the decay fungi, completely inhibiting their growth and killing all of them.

Pretreatment of Southern Pine and maple blocks by vacuum impregnation with a culture blend of GL-21 or a GL-21 granule prevented

Table 1: Decay resistance of GL-21 treated and untreated (control) Southern Pine and maple blocks.

		Weight loss[a] produced by wood-rotting Basidiomycete (%)			
Wood	Fungus	Culture blend unsterilized	Filtrate sterilized	Grandule	Control
Maple					
	White rot				
	Trametes versicolor	0 ± 0	22.3 ± 2.8	0 ± 0	30.4 ± 12.2
	Phlebia brevispora	0 ± 0	31.2 ± 12.6	0 ± 0	39.2 ± 2.6
	Irpex lacteus	0 ± 0	49.0 ± 10.7	0 ± 0	56.6 ± 9.4
Southern Pine					
	Brown rot				
	Posta placenta	0 ± 0	27.4 ± 2.3	0 ± 0	31.8 ± 3.6
	Neolentinus lepideus	0 ± 0	18.0 ± 1.1	0 ± 0	23.9 ± 2.3
	Gloeophyllum trabeum	0 ± 0	33.7 ± 1.4	0 ± 0	40.8 ± 2.0

[a] Mean weight loss with standard deviation (10 weeks exposure).

Table 2: Decay resistance of GL-21 treated blocks removed from soil-block tests and retested for decay resistance after sterilization.

Wood	Fungus	Weight loss[a] produced by wood-rotting Basidiomycete (%)	
		Pretreated with GL-21	Control
Maple			
	White rot		
	Trametes versicolor	45.6 ± 1.6	39.0 ± 3.7
	Phlebia brevispora	6.8 ± 1.3	3.8 ± 2.3
	Irpex lacteus	43.6 ± 5.2	40.0 ± 3.3
Southern Pine			
	Brown rot		
	Posta placenta	30.2 ± 9.7	47.0 ± 4.2
	Neolentinus lepideus	24.4 ± 0.8	32.2 ± 3.0
	Gloeophyllum trabeum	47.3 ± 2.1	47.9 ± 5.7

[a] Mean weight loss with standard deviation (8 weeks exposure).

Table 3: Decay resistance of Southern Pine and maple blocks sterilized after treatment with GL-21 over soil medium.[a]

Wood	Fungus	Weight loss[b] produced by wood-rotting Basidiomycete (%)		
		Culture blend unsterilized	Grandule	Control
Maple				
	White rot			
	Trametes versicolor	53.7 ± 3.1	56.4 ± 2.3	51.3 ± 1.3
	Phlebia brevispora	20.9 ± 2.3	25.8 ± 3.9	18.9 ± 2.5
	Irpex lacteus	61.5 ± 8.1	63.9 ± 6.6	56.0 ± 8.9
Southern Pine				
	Brown rot			
	Posta placenta	12.5 ± 18.6	57.1 ± 6.5	48.1 ± 2.2
	Neolentinus lepideus	17.4 ± 2.1	20.3 ± 2.6	24.5 ± 2.3
	Gloeophyllum trabeum	44.7 ± 6.9	58.7 ± 2.8	43.1 ± 3.3

[a] Blocks were treated with a culture blend of GL-21 or GL-21 granule, incubated for 3 weeks over soil, and proplylene oxide sterilized prior to exposure to decay fungi.

[b] Mean weight loss with standard deviation (10 weeks exposure).

attack by all the decay fungi in soil-block tests (Table 1). Blocks that had been treated with a sterilized culture blend were only slightly less decay resistant than the untreated controls. Table 2 shows the decay resistance of the GL-21 treated blocks removed from the soil-block test and retested for decay resistance after steam sterilization. There was no residual toxicity to the decay fungi in the absence of living antagonist. In the same manner, blocks sterilized with propylene oxide after treatment with culture blend or granule and incubated for 3 weeks over a soil medium before exposure to decay fungi were susceptible to attack by all decay fungi (Table 3).

The decay resistance of blocks sterilized with propylene oxide after treatment with culture blend of GL-21 over a low nutrient (water agar) or high nutrient (malt agar) medium for 6 weeks is shown in Table 4. Blocks treated by incubation over the malt-agar medium suffered slightly less weight loss than those incubated over water agar, but weight loss was still high.

Table 4: Decay resistance of Southern Pine and maple blocks sterilized after treatment with GL-21 over a low and high nutrient agar medium.[a]

		Weight loss[b] produced by wood-rotting Basidiomycete (%)		
Wood	Fungus	Water agar	Malt agar	Control
Maple				
	White rot			
	Trametes versicolor	39.0 ± 1.7	37.4 ± 3.4	46.4 ± 1.8
	Phlebia brevispora	20.0 ± 6.2	19.4 ± 2.4	19.4 ± 1.5
Southern Pine				
	Brown rot			
	Posta placenta	50.6 ± 3.7	35.1 ± 5.3	49.6 ± 1.9
	Neolentinus lepideus	29.0 ± 3.4	17.0 ± 6.9	26.8 ± 1.7
	Gloeophyllum trabeum	43.6 ± 5.3	25.3 ± 4.0	39.4 ± 3.1

[a] Blocks were treated with a culture blend of GL-21, incubated for 6 weeks over water agar or 2% malt agar, and propylene oxide sterilized prior to exposure to decay fungi.

[b] Mean weight loss with standard deviation (10 weeks exposure).

Filtrates of GL-21 incorporated into high or low nitrogen media inhibited the growth of the decay fungi and the discoloring fungi (Table 5). However, only *T. versicolor* and *P. placenta* were 100% inhibited by the GL-21 filtrates. Inhibition of the white-rot fungi was greater in the low nitrogen medium, and inhibition of the brown-rot and discoloring fungi

was greatest in the high nitrogen medium. In the cellophane test for antibiotic production, growth of all decay fungi and the discoloring fungi on malt-agar was completely inhibited.

GL-21 was able to prevent additional decay by *P. placenta* and *T. versicolor* but not *G. trabeum* (Table 6).

Table 5: Fungistatic ability of GL-21 in agar medium.

		Inhibition of growth (%)		
		Filtrate[a]		Cellophane test
		Low N	High N	
White rot				
	Trametes versicolor	100.0	100.0	100
	Phlebia brevispora	73.3	51.8	100
	Irpex lacteus	76.9	45.1	100
Brown rot				
	Postia placenta	100.0	100.0	100
	Neolentinus lepideus	72.0	87.9	100
	Gloeophyllum trabeum	70.0	75.4	100
Mold and stain				
	Trichoderma harzianum	25.9	60.0	100
	Ceratocystis minor	55.6	72.2	100

[a] Filtrate from GL-21 grown on a basal salts medium containing 0.02% nitrogen plus 0.5% glucose (Low N) or 2% malt extract and 0.2% yeast extract (High N).

Table 6: Ability of GL-21 to arrest decay.

	Weight loss[a] produced by decay fungus (%)		
		After 10 weeks	
	Prior to GL-21	Treated with GL-21	Untreated controls
Postia placenta	9.7 ± 0.6	1.8 ± 0.4	60.0 ± 1.0
Gloeophyllum trabeum	24.6 ± 0.8	10.7 ± 3.6	52.8 ± 1.7
Trametes versicolor	6.7 ± 0.4	0.9 ± 0.4	47.8 ± 2.5

[a] Mean weight loss produced by decay fungi in wood prior to exposure to GL-21 was subtracted from final weight loss obtained. Southern Pine was decayed by *P. placenta* and *G. trabeum* and maple by *T. versicolor*.

4. Discussion

In these laboratory studies, a commercially formulated *Trichoderma virens* (GL-21) was an effective pretreatment for the protection of wood against decay by all white- and brown-rot fungi that were examined. Dual-culture experiments on malt-agar also showed that GL-21 was fungicidal to the decay fungi. In a previous study (T. L. HIGHLEY and J. RICARD, 1988), a *T. virens* isolate was unable to control decay by white-rot fungi. Variation in antagonistic properties of *Trichoderma* isolates is very common. The broad spectrum of activity exhibited by GL-21 against the decay fungi in the present study lends credence to the use of *T. virens* as an effective biocontrol agent of wood decay.

Trichoderma (Gliocladium) virens strains (C. R. HOWELL and R. D. STIPANOVIC, 1983) produce a number of well-established antifungal agents, such as gliovirin and gliotoxin. GL-21 produced metabolizes in agar medium that completely inhibited growth of the decay fungi in the cellophane test. However, wood treated with metabolizes of GL-21 was not decay resistant nor was wood resistant to decay fungi after death of GL-21. Most fungitoxic metabolizes are destroyed by high temperatures, thus it is not surprising that decay resistance was lost when treated blocks were steam sterilized. However, treated blocks also lost their biocontrol activity when gas sterilized. Why the metabolizes were inhibitory in agar but not in wood is unknown. Possibly metabolizes are unstable in wood, and the living mycelium of GL-21 must be present to provide a fungitoxic concentration of metabolizes. Also, the relative contribution of antibiosis to the overall biocontrol activity of GL-21 in wood is not known. If competition and parasitism are major contributors to the mechanism of antagonism, living mycelium must be present to be effective.

In a previous study (T. L. HIGHLEY and J. RICARD, 1988), a *T. virens* isolate was unable to stop already established decay by *P. placenta* and *T. versicolor* in wood. However, in this study, GL-21 was effective in preventing additional decay in wood by these fungi. Although GL-21 was able to quickly overgrow and kill *G. trabeum* in agar interaction, it was unable to stop additional decay in wood by this fungus. Contributing to this apparent anomaly, between agar and wood, are the differences in nutrients in wood and agar. Also worthy of consideration is the fact that weight loss in *G. trabeum* decayed wood prior to exposure to GL-21 was substantially greater than that produced by the other two decay fungi. Thus, if the *G. trabeum* decay had been at an early stage of development, GL-21 might have acted therapeutically in wood.

The metabolizes of GL-21 were also inhibitory to the growth of the wood discoloring fungi, *Trichoderma harzianum* and *Ceratocystis minor*,

in agar medium. Additional work is planned on the metabolizes from GL-21 for application to green timber during drying. If successful, these naturally produced fungitoxic compounds, or their analogues, may have commercial application for short-term protection of green wood against discoloring fungi.

5. Summary

Antagonistic characteristics of a commercial biofungicide, *Trichoderma (Gliocladiurn) virens* (GL-21, W. R. Grace and Co., CT), were evaluated against three white-rot fungi, *Trametes versicolor, Phlebia brevispora, Irpex lacteus,* and three brown-rot fungi, *Postia placenta, Neolentinus lepideus, and Gloeophyllum trabeum*. In dual cultures of *T. virens* and wood decay fungi, *T, virens* rapidly overgrew and killed the decay fungi. Pretreatment of Southern Pine and maple blocks with *T. virens* prevented weight loss by all decay fungi in the soil-block tests. *T. virens* colonized blocks, treated with propylene oxide to kill the antagonist, were not decay resistant. Filter-sterilized filtrates from *T. virens* showed fungistatic effect against the decay fungi in agar medium. However, weight loss in wood blocks treated with filter-sterilized filtrates of *T. virens* was only slightly reduced when exposed to decay fungi in soil-block tests. Thus, *T. virens* must be alive to prevent wood decay.

Zusammenfassung

Bekämpfung der Holzfäule durch *Trichoderma (Gliocladium) virens* I. Antagonistische Eigenschaften

Es wurden die antagonistischen Eigenschaften eines handelsüblichen Biofungizids, *Trichoderma* (*Gliocladium*) *virens* (GL-21, W. R. Grace and Co., CT) gegenüber den 3 Weißfäulepilzen *Trametes versicolor, Phlebia brevispora* und *Irpex lacteus* und den 3 Braunfäulepilzen *Postia placenta, Neolentinus lepideus* und *Gloeophyllum trabeum* untersucht. In Doppelkulturen von *T. virens* und den jeweiligen holzzerstörenden Pilzen überwuchs T. *virens* die holzzerstörenden Pilze sehr schnell und tötete sie ab. Eine Vorbehandlung von 'southern pine'- und Ahorn-Klötzchen mit *T. virens* verhinderte in Erde-Klötzchen-Versuchen das Wachstum aller holzzerstörenden Pilze. Holzklötzchen, die von *T. virens* besiedelt waren und anschließend mit Propylenoxid behandelt wurden, erwiesen sich als nicht widerstandsfähig gegen Pilzbefall. Sterilisierte Filtrate von *T. virens* zeigten in einem Agar-Medium fungistatische Wirkung gegenüber den holzzerstörenden Pilzen. Der Gewichtsverlust von Holzklötzchen, die mit Filtraten von *T. virens* behandelt worden waren, sank in den Erde-Klötzchen-Versuchen nur geringfügig. Daraus wurde geschlossen, daß nur Lebendkulturen von *T. virens* Holzfäule verhindern können.

Résumé

Contrôle des pourritures du bois par *Trichoderma (Gliocladium) virens* I. Propriétés antagonistes

Nous avons établi les propriétés antagonistes d'un fongicide commercial, *Trichoderma* (*Gliocladium*) *virens* (GL-21, W. R. Grace and Co., CT), contre 3 pourritures blanches, *Trametes versicolor, Phlebia brevispora, Irpex lacteus* et 3 pourritures brunes, *Postia placenta, Neolentinus lepideus* et *Gloeophyllum trabeum*. Lorsque *T. virens* est inoculé avec les pourritures, *T. virens* pousse rapidement et élimine les pourritures. Le prétraitement des échantillons de pins et d'érable avec *T. virens* contrôle la perte de poids due aux pourritures dans tous les échantillons soumis au test dans le sol. *T. virens* colonise les échantillons de bois traités avec de l'oxyde de propylène, qui est utilisé pour tuer les champignons antagonistes, cependant ces échantillons de bois n'ont pas résisté aux pourritures. Les filtrats de *T. virens* prestérilisés montrent une activité fongicide contre les champignons des pourritures sur milieux gélosés. Cependant, les pertes de poids des échantillons de bois traités avec les filtrats de *T. virens* prestérilisés, sont seulement légèrement réduites, lorsqu'ils sont soumis aux pourritures dans un test dans le sol. Ce qui suggère que ce sont seulement les cultures actives de *T. virens* qui peuvent contrôler la pourriture du bois.

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