Postulated Mechanism of Biological Control of Decay Fungi in Red Maple Wounds Treated with *Trichoderma harzianum*

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Scientific Contribution 1039 from the New Hampshire Agriculture Experiment Station. This research was supported in part by USDA Forest Service Cooperative Agreement 23-843 and is a portion of an M.S. thesis submitted to the Graduate School of the University of New Hampshire by the senior author.

We thank A. Shigo, Northeastern Forest Experiment Station, Durham, NH, for support and encouragement.

Accepted for publication 30 September 1980.

ABSTRACT

Smith, K. T., Blanchard, R. O., and Shortle, W. C. 1981. Postulated mechanism of biological control of decay fungi in red maple wounds treated with *Trichoderma harzianum*. Phytopathology 71:496-498.

Trichoderma harzianum has been used as a biological control agent against wood decay fungi including *Fomes connatus,* a causal organism of decay in living red maple trees. The growth of *T. harzianum* and *F. connatus* in addition to that of *Phialophora melinii*, an organism that becomes established early in the discoloration and decay process, was compared by using a basal medium containing various concentrations of gallic acid. *Trichoderma harzianum* and *P. melinii* were both tolerant of gallic acid, a major phenolic constituent of maple sapwood, but *T. harzianum* had less capacity to alleviate gallic acid inhibition of *F. connatus* than did *P. melinii*. When wounded red maples were treated with *T. harzianum* in vivo, soluble matter from the green-colored boundaries separating live, healthy sapwood from dead, infected, discolored wood had a greater phenol content than did untreated controls from which *P. melinii* was commonly isolated. It is known that pioneer fungi such as *P. melinii* help render wood susceptible to decay by reducing levels of phenols that inhibit decay fungi. Therefore, it is postulated that a mechanism of biological control of decay in red maple trees by *T. harzianum* is the replacement of pioneer fungi by *T. harzianum*.

Trichoderma harzianum Rafai was effective as a biological control agent in delaying colonization of wounds in red maple (*Acer rubrum* L.) by decay fungi (5), but the mechanism of control is not known. This study (5) and an earlier one (4) indicated that the normal pioneer fungi, particularly *Phialophora* spp., were not isolated from discolored wood when *Trichoderma* was established in wounded tissue. *P. melinii* (Nannf.) Conant, a species commonly found in maple, altered phenolic substrates inhibitory to *Fomes connatus* (Weinm.) Gill, a common decayer of maple (9). Thus, a postulated mechanism of biological control could include the following: displacement of pioneer fungi, such as *P. melinii,* by

0031-949X/81/05049603/$03.00/0

Trichoderma, the control agent; retention of a high level of phenols normally altered by the pioneer; and delayed colonization by decay fungi, such as *F. connatus,* because of the greater retention of phenols in the tissues altered after wounding.

The purpose of our study was to test the postulated mechanism by comparing the growth of *P. melinii* (pioneer), *T. harzianum* (control agent), and *F. connatus* (decayer) on media containing various levels of gallic acid, a phenolic acid found in maples (11) by determining the relative capacity of *P. melinii* and *T. harzianum* to reduce inhibition of *F. connatus* by gallic acid and by determining the levels of soluble phenols and carbohydrates in wounds treated with *T. harzianum* and from control wounds.

MATERIALS AND METHODS

Effect of gallic acid on growth. Cultures of *F. connatus, P. melinii,* and *T. harzianum* were obtained from culture collections at the Forestry Sciences Laboratory, Northeastern Forest Experiment Station, Durham, NH. These fungi were grown in a basal medium containing 4 g of carbon per liter (9) added as either glucose (10 g/L, J. T. Baker, Phillipsburg, NJ 08865); gallic acid (8.95 g/L, ICN Pharmaceuticals, Cleveland, OH 44128); or mixtures of glucose and gallic acid yielding 5, 10, and 50% gallic acid by weight of carbon. The pH of the media was adjusted to 4.5 or 6.0 with HCl or NaOH.

Fungi were grown in 250-ml Erlenmeyer flasks (four replicates per treatment) containing 25 ml of medium at 22 C. Mycelia of *T. harzianum* and *P. melinii* were harvested at 10 days, and that of *F. connatus* at 20 days on tared filter paper and oven-dried at 104 C to constant weight. Growth was recorded as milligrams of mycelial dry weight. A one-way analysis of variance was performed on the data.

Relative capacity to alter effect on gallic acid. *P. melinii* and *T. harzianum* were grown on basal media containing either glucose or gallic acid as a sole source of carbon. Initial pH was adjusted to 4.5 for *P. melinii* and 6.0 for *T. harzianum*, since these respective pH levels yielded the most dry weight in the growth studies and are within the range found in healthy red maple wood. At 10 days, each medium was filtered. The filtrates were used to prepare the following media: unamended glucose filtrate, unamended gallic acid filtrate, and gallic acid filtrate amended with glucose to yield 5, 10, and 50% gallic acid by initial weight of carbon. The media then were filter sterilized, transferred aseptically to sterile 250-ml Erlenmeyer flasks (25 ml per flask), inoculated with *F. connatus*, and incubated for 20 days. After incubation four replicate flasks were harvested as above.

Field study area. Six red maple trees (13–28 cm dbh) at the Kingman Experimental Research Farm, Madbury, NH, were selected for wounding. Each tree received four wounds in each of two whorls. The whorls were at 1.0 and 1.75 m aboveground, with the wounds evenly spaced around the trunk diameter. The wounds were made with a 1.4 cm drill bit to a depth of 5 cm.

Inoculum was prepared by growing *T. harzianum* in the basal medium containing 10 g of glucose per liter. Seven-day-old cultures were chopped 5 sec in a Waring Blendor with an equal volume of glycerol (J. T. Baker, Phillipsburg, NJ 08865). The wounds were made in July and were treated immediately after they were inflicted. Approximately 2 ml of chopped *T. harzianum* in glycerol was applied by swabbing the wood exposed by wounding it with a small paint sponge saturated with the suspension. Glycerol alone was used as a control. Each tree received both the glycerol control and the *T. harzianum* inoculum. The application of treatments was randomized with respect to whorl height.

The six trees were harvested 1 yr later in July 1978. Wood sections 1 × 3 × 5 cm were split from 5-cm-thick serial disks cut through each whorl and immediately above and below each whorl. The wood sections were freeze-dried. Shavings were planed from three different areas of the wood sections: sapwood, discolored wood, and the green-colored boundary between the sapwood and discolored wood. The shavings were ground in a Wiley mill to pass an 850-μm sieve and the resulting tissue samples were used for chemical analysis of extractable material.

Chemical analyses. Half-gram samples of ground wood were extracted by constant stirring with 50 ml of distilled water under reflux in a boiling water bath for 1 hr. Total soluble phenols and total soluble carbohydrates were determined as described by Shortle (6).

RESULTS

T. harzianum produced the most dry weight in 100% glucose medium, pH 6.0 (Table 1). Growth of *T. harzianum* was significantly less at 50/50% gallic acid/glucose, and only a small amount of growth was supported at 100% gallic acid. *P. melinii* produced the most dry weight in 100% glucose, pH 4.5. Growth of *P. melinii* was significantly less at 50/50% gallic acid/glucose, but the fungus also produced substantial dry weight with gallic acid as a sole source of carbon. *F. connatus* produced the most dry weight in 100% glucose medium, pH 6.0. Growth of *F. connatus* was significantly less at 5/95% and 10/90% gallic acid/glucose, and was totally inhibited when the amount of gallic acid was 50% or more.

Filtrates of *P. melinii* were more effective than were filtrates of *T. harzianum* in promoting the growth of *F. connatus* in media containing greater amounts of gallic acid (Table 2). Culture filtrates of *T. harzianum* originally containing 10% or more gallic acid supported no growth of *F. connatus*, whereas culture filtrates of *P. melinii* originally containing 10 and 50% gallic acid supported growth totalling 14 and 6 mg dry weight, respectively.

Significantly more soluble dry matter was found in the green-colored boundary between sapwood and discolored wood than in either the sapwood or discolored wood (Table 3). Discolored wood had significantly less soluble dry matter than either of the other two tissues. Treatment of the wounds with *T. harzianum* did not

TABLE 1. Growth of *Trichoderma harzianum, Phialophora melinii,* and *Fomes connatus* on basal medium containing glucose and gallic acid as carbon sources

Carbon source[a]		Mean dry wt of mycelium (mg)[b]					
		T. harzianum		*P. melinii*		*F. connatus*	
GA (%)	Glu (%)	4.5[c]	6.0	4.5	6.0	4.5	6.0
	100	47	77	102	89	26	41
5	95	56	68	98	78	17	20
10	90	61	70	96	82	4	6
50	50	26	34	68	47	0	0
100		4	10	41	8	0	0
LSD ($P < 0.05$)		5.7		13.2		4.5	

[a] Carbon added to basal medium as glucose (Glu) and/or gallic acid (GA) to yield 4 g of carbon per liter.
[b] Means of four observations at 10 days for *T. harzianum* and *P. melinii* and 20 days for *F. connatus*.
[c] Initial pH of medium.

TABLE 2. Growth of *Fomes connatus* in media derived from culture filtrates of *Trichoderma harzianum* and *Phialophora melinii*

Carbon source[a]		Mean dry wt of *F. connatus* mycelium (mg)[b]			
		T. harzianum filtrate[c]		*P. melinii* filtrate	
GA (%)	Glu (%)	Uninoculated	Inoculated	Uninoculated	Inoculated
	100	40	21	30	22
5	95	17	32	12	32
10	90	1	0	0	14
50	50	0	0	0	6
100		0	0	0	0
LSD ($P < 0.05$) = 4.5					

[a] Carbon added to basal medium as glucose (Glu) or gallic acid (GA) and incubated 10 days with or without inoculum before filtration; filtrates were then inoculated with *F. connatus* without amendment (100% Glu, 100% GA) or after amendment of the gallic acid filtrate with glucose to yield 5%, 10%, and 50% gallic acid by initial weight of carbon (4 g/L).
[b] Mean of four observations at 20 days.
[c] Initial pH of *T. harzianum* media, 6.0; *P. melinii*, 4.5; pH at time of inoculation with *F. connatus* was 7.6 and 7.8, respectively.

TABLE 3. Water-soluble substances from sapwood, discolored wood, and the green-colored boundary separating these tissues in wounded red maple treated with *Trichoderma harzianum*

Substance	Mean dry wt of soluble substance (mg/g wood)[a]						LSD ($P = 0.05$)
	Sapwood		Boundary		Discolored wood		
	Control[b]	Treated	Control	Treated	Control	Treated	
Soluble dry matter	41	42	73	77	20	21	12.4
Carbohydrate, total	21	25	24	23	10	10	9.4
Phenol, total	14	12	34	49	5	5	11.7

[a]Mean of three observations expressed as milligrams substance per gram of moisture-free wood.
[b]Control = wounds treated with glycerol; treated = wounds treated with *T. harzianum* spores in glycerol.

significantly affect the amount of soluble dry matter measured in any of the tissue that was examined.

Total soluble carbohydrates were equivalent in sapwood and the boundary zone, but decreased in discolored wood. Treatment with *T. harzianum* had no measurable effect on the amount of total carbohydrates measured in any of the tissues examined.

Total soluble phenols occurred in the greatest concentration in the boundary zone, in lesser concentration in sapwood, and in the least concentration in discolored wood (Table 3). Treatment with *T. harzianum* was associated with a greater concentration of phenols in the boundary zone between sapwood and discolored wood.

DISCUSSION

Suggested mechanisms for the biological control activity of members of the genus *Trichoderma* are hyperparasitism, antibiosis, lysis, and competition for nutrients (1,2,13). Our study suggests another mechanism for the biological control of decay in living trees.

When *T. harzianum* was applied to wounds in maple and persisted, colonization by decay fungi was delayed (5). Isolation data indicated that *T. harzianum* had replaced the pioneer organisms (4,10) such as *P. melinii*. Pioneer fungi have been shown to be more tolerant of phenols than are decay fungi, and the role of these organisms in removing inhibitory substances from wood has been proposed for living trees (8,12) and wood products (3). When trees are wounded and the exposed tissue is infected by pioneer organisms followed by decay fungi, the tree responds by walling-off the infected area with phenol-enriched tissues (7). By forcing *T. harzianum* to occupy the niche normally occupied by pioneers, we hypothesize that the response of the tree becomes more effective and decay fungi are inhibited for extended periods of time.

Results of our in vitro culture experiments indicated *T. harzianum* to be as tolerant of phenols as is *P. melinii*, but the former does not alter the concentration of gallic acid (a major phenolic constituent of maple sapwood [11]) to the same extent as the latter. When *T. harzianum* was used to treat wounds of maple sapwood in vivo, a greater concentration of soluble phenols was observed at the sapwood-discolored wood boundary than in untreated controls. This indicates that *Trichoderma* either promotes a strong tree response or helps maintain the tree response by replacing phenol-degrading pioneers as postulated. The replacement mechanism does not appear to be operative in the discolored wood of columns after 1 yr, but the replacement effect during early column formation may have long residual effects without continuous maintenance of different levels of phenols. Culture studies in our laboratory by H. W. Pottle (*unpublished*) showed no evidence of mycoparasitism of *T. harzianum* on either *P. melinii* or *F. connatus*. Certainly no single mechanism can explain how a process involving a complex of tree response, multiple-sequential infection, and colonization taking place over many years is regulated, but replacement of phenol-degrading pioneers by *Trichoderma* may play an important part.

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