Effects of Some Phenolic Compounds on the Growth of Phialophora melinii and Fomes connatus

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ABSTRACT

The ability of *Phialophora melinii* and *Fomes connatus* to tolerate and to utilize certain phenolic compounds was studied in vitro to help explain successional patterns of fungi in living trees. *Phialophora melinii,* which is often isolated in advance of *F. connatus* in columns of discolored and decayed tissue in sugar maple, tolerated and utilized phenolic compounds which were toxic to *F. connatus*. The capacity of *P. melinii* to alter phenolic compounds, and thereby permit growth of *F. connatus,* was indicated. The effects of pH, manganese concentration, nitrogen source, amount of glucose present, and means of sterilization on the growth of these fungi in media containing phenolic compounds were also investigated. Phytopathology 61:552-555.

Phialophora melinii (Nannf.) Conant is commonly isolated from discolored and decayed tissues of sugar maple, *Acer saccharum* Marsh., and other species of deciduous hardwoods (12, 13, 14). Studies of the physiology of *P. melinii* and *Polyporus glomeratus,* a decay fungus often associated with *P. melinii,* indicated that *P. melinii* grew well in culture on levels of certain phenolic compounds that inhibited the growth of *P. glomeratus* (W. C. Shortle, *unpublished data.*). The inhibitory effect of phenolic compounds on some decay fungi has been demonstrated in culture and in living trees (8, 11). The role of phenolic compounds in host-pathogen interactions is well known (7).

The apparent differences between nonhymenomycetous fungi, such as *P. melinii,* and decay fungi with which they are associated, in their ability to utilize phenolic compounds could help account for successional relationships in living trees. It has been suggested that *Trichocladium canadense,* another nonhymenomycetous fungus which is often found in advance of decay fungi, utilizes the oxidized phenolic compounds in discolored wood in advance of *Fomes igniarius* (6).

Our purpose was to determine the ability of *P. melinii* to utilize and to detoxify phenolic compounds that inhibit or prevent the growth of *Fomes connatus* (Weinm.) Gill., an important decay fungus on sugar maple.

MATERIALS AND METHODS.—Two isolates of *Phialophora* sp., identified as *P. melinii,* were used. The isolates came from discolored tissue associated with the decay of *Fomes connatus* in sugar maple. The isolate of *F. connatus* (RLG-5660-S-SP) used came from the U.S. Forest Disease Laboratory, Laurel, Md.

The basic medium used to test the growth of *P. melinii* and *F. connatus* contained 10 g/liter D-glucose, 2 g/liter asparagine, and a basal medium of salts, buffer, trace elements, and vitamins as described by Lilly & Barnett (9). Phenolic compounds were substituted as carbon sources for all or part of the D-glucose at a rate equivalent to 10 g/liter D-glucose (4 g/liter carbon). The fraction of the carbon source comprised by a phenolic compound was reported as the ratio of that compound to glucose based on the wt of carbon.

NH_4NO_3 or KNO_3 was substituted for asparagine in some media at a rate equivalent to 2 g/liter asparagine (0.4 g/liter nitrogen). Yeast extract was used in some media at a rate of 2 g/liter.

In treatments where manganese was added, a stock solution of $MnSO_4 \cdot H_2O$ was used. The stock solution was added as part of the distilled water used to make the medium so that the final concentration of Mn^{++} of the treatment solution was 10, 100, or 500 ppm.

The pH of each medium was adjusted to 4.5 or 6.0 using 5 N NaOH or 5 N HCl. The fungi were grown in 25 ml of medium in 250-ml Erlenmeyer flasks. Sterilization was done by autoclaving at 15 psi and 121 C for 15 min or by filter sterilization using a Seitz filter. Seitz filtration gave results comparable to those using Millipore filtration. The sterilized media were inoculated with mycelium chopped 5 sec from 12-day cultures of *P. melinii* or 21-day cultures of *F. connatus*.

After incubation at 25 C for various lengths of time, depending on the rate of growth, the mycelium was harvested and oven-dried at 105 C for 24 hr in tared 10-ml beakers. Growth was reported as oven-dried wt of mycelium.

Regrowth medium.—To determine the ability of *P. melinii* to alter the inhibitory properties of phenolic compounds, *P. melinii* was grown 14, 18, and 22 days on the basic medium in which gallic acid had been substituted for D-glucose. The mycelium was removed by filtration, and the culture medium was resterilized by filter sterilization. This resterilized solution was mixed with a glucose solution to yield a gallic acid-to-glucose ratio equivalent to those used to test the growth of *P. melinii* and *F. connatus*. The ratio was based on the original gallic acid content of the treatment solution which contained 4 g/liter carbon.

RESULTS.—*Phialophora melinii* utilized gallic acid as a carbon source (Table 1). It grew on all media at

pH 4.5 containing gallic acid as a carbon source. At this low pH level, the gallic acid was not oxidized to the dark-colored oxidation products that formed in media at pH 6.0. However, as *P. melinii* grew on the gallic acid medium, the color did change from light to dark, which indicated the oxidation of gallic acid to products of unknown nature. This change was accompanied by an increase in pH.

There was no significant difference in growth whether the medium was autoclaved or filter-sterilized, which indicated that autoclaving did not alter the response to gallic acid. The growth of *P. melinii* on media containing inorganic nitrogen sources in which the only carbon was in gallic acid clearly indicated the ability to utilize gallic acid. Adding high amounts of manganese increased the growth significantly.

Phialophora melinii failed to grow on all media containing gallic acid as a carbon source at pH 6.0 unless high concentrations of manganese were present. It did not grow at pH 3.3. Dark-colored oxidation products produced at the high level of pH were apparently toxic to *P. melinii*. The toxic action was nullified when high concentrations of manganese were present. The dark-colored products of tannic acid allowed growth, so they may be less toxic.

Toxic effects of the oxidation products at pH 6.0 were nullified by the presence of another carbon source, glucose, even at relatively low concentrations (Table 2). The growth at both levels of pH was greater as the glucose increased. At the low level of gallic acid, there may be a stimulatory growth effect.

Phialophora melinii tolerated and grew well in the presence of several phenolic compounds (Table 3); whereas similar rates were toxic to *F. connatus*. The growth of *F. connatus* was completely inhibited by all compounds except tyrosine which caused some inhibition. The mean oven-dried wt of mycelium at 25 days for *F. connatus* on glucose alone plus basal medium was 137 mg. When tyrosine was added at the rate of 1:10 and 1:1, growth was decreased to 55 and 66 mg, respectively.

The data indicated that ortho-dihydroxyphenolic compounds, such as catechol, are much more inhibitory to *P. melinii* than are meta- or para-dihydroxyphenols, such as resorcinol and hydroquinone, respectively. At the low rate, 1:10, only cinnamic acid and pyrogallol completely inhibited the growth of *P. melinii*. The growth on the phenolic amino acid, tyrosine, and on the trihydroxyphenolic acid, gallic acid, was not significantly different from that on glucose alone. Good growth was obtained on both resorcinol and hydroquinone. Phenol, catechol, and coumarin were inhibitory. At the high rate, 1:1, all the compounds except tyrosine, resorcinol, hydroquinone, and gallic acid were completely inhibitory to *P. melinii*. Of these compounds, resorcinol and hydroquinone, were strongly inhibitory.

TABLE 1. Growth of *Phialophora melinii* on gallic acid under varying conditions of pH, manganese concentration, and method of sterilization

Treatment[a]	pH		Color		Days of growth	Mean[b] oven-dried wt. of mycelium (mg)
	Initial	Final	Initial	Final		
Glucose						
	6.0	4.6			8	81 ± 17
	6.0	7.9			12	116 ± 6
	4.5	6.8			8	121 ± 5
Gallic acid						
	6.0		Dark	Dark	12	0
Filter sterilized[c]	6.0		Dark	Dark	12	0
	4.5	8.1	Light	Dark	14	61 ± 8
Filter sterilized	4.5	8.5	Light	Dark	14	59 ± 3
	3.3		Light	Light	12	0
Gallic acid with inorganic N source						
NH_4NO_3	6.0		Dark	Dark	12	0
	4.5	7.6	Light	Dark	14	48 ± 3
KNO_3	4.5	8.5	Light	Dark	14	43 ± 2
Gallic acid with manganese added						
10 ppm Mn++	6.0		Dark	Dark	12	0
100 ppm Mn++	6.0	6.2	Dark	Dark	12	2[d]
500 ppm Mn++	6.0	7.0	Dark	Dark	12	46 ± 5
500 ppm Mn++	4.5	7.9	Light	Dark	14	86 ± 4
Tannic acid	6.0	5.4	Dark	Dark	12	22 ± 3

[a] Treatment refers to compounds added to a basal medium of asparagine or an inorganic N source, buffer, salts, trace elements, and vitamins (9).

[b] Means based on six observations (three replications/isolate per treatment). The 95% confidence limits are reported for each treatment mean.

[c] All treatments were autoclaved at 15 psi and 121 C unless noted otherwise.

[d] Mean based on three observations because only one isolate grew at this level of manganese.

TABLE 2. Growth of *Phialophora melinii* under varying concentrations of gallic acid and glucose

Ratio of gallic[a] acid to glucose	pH		Color		Days of growth	Mean[b] oven-dried wt of mycelium (mg)
	Initial	Final	Initial	Final		
Glucose alone	6.0	4.6			8	81 ± 17
1:10	6.0	5.7	Dark	Dark	8	122 ± 10
1:1	6.0	5.6	Dark	Dark	8	92 ± 17
1:1 filter-sterilized[c]	6.0	4.9	Dark	Dark	8	39 ± 4
10:1	6.0	6.9	Dark	Dark	12	33 ± 4
20:1	6.0	7.0	Dark	Dark	12	21 ± 3
Glucose alone	4.5	6.8			8	121 ± 5
1:10	4.5	6.5	Light	Dark	8	138 ± 2
1:1	4.5	6.2	Light	Dark	8	104 ± 4

[a] Ratio of gallic acid to glucose based on the wt of carbon added to a basal medium of asparagine, buffer, salts, trace elements, and vitamins (9).
[b] Means based on six observations (three replications/isolate per treatment). The 95% confidence limits are reported for each treatment mean.
[c] All treatments were autoclaved at 15 psi and 121 C unless noted otherwise.

Poor growth of *F. connatus* on gallic acid medium has been reported as a characteristic of the fungus (4, 5, 10). *Fomes connatus* did not grow when gallic acid was added alone or with glucose in a rate of 1:1 or 1:10 to the basal medium at pH 6.0 or 4.5, whether asparagine or yeast was used as a nitrogen source. Using yeast as a nitrogen source, instead of asparagine, greatly increased the growth of *F. connatus* when glucose was used alone; but the fungus still failed to grow in the presence of gallic acid. The mean oven-dried wt of mycelium at 25 days was 33 mg at pH 6 and 5 mg at pH 4.5 when asparagine was used, and 135 mg at pH 6 and 152 mg at pH 4.5 when yeast was used.

After gallic acid present in a medium was acted upon by *P. melinii, F. connatus* grew (Table 4). Growth was greatest at the 2 low levels of gallic acid; but limited growth occurred at the high rate (1:1), when *P. melinii* had been grown on the gallic acid medium for 14 days.

TABLE 3. Growth of *Phialophora melinii*[c] on various phenolic compounds

Compound[a]	Mean[b] oven-dried wt of mycelium (mg)	
	Ratio of compound to glucose *P. melinii*	
	1:10	1:1
Glucose alone	135	
Tyrosine	135	113
Phenol	44	0
Catechol	9	0
Resorcinol	116	20
Hydroquinone	111	8
Pyrogallol	Trace	0
Gallic acid	135	102
Cinnamic acid	0	0
Coumarin	25	0

[a] Compounds added with glucose in a ratio of 1:10 or 1:1 based on wt of carbon to a basal medium of asparagine, buffer, salts, trace elements, and vitamins (9).
[b] Means based on three replications/isolate per treatment.
[c] Growth at 9 days. Initial pH 4.5.

The action of *P. melinii* undoubtedly makes many complex changes in the medium.

DISCUSSION.—This study has indicated the potential of *Phialophora melinii* not only to tolerate and utilize certain phenolic compounds which are toxic to *F. connatus,* but to alter the compounds so they are no longer toxic. The total phenolic content of clear sugar maple tissue was shown to be 3 times that of discolored tissue from which *P. melinii* was isolated (16). It is this discolored tissue in living trees which is invaded by decay fungi. As clear tissues are discolored, the pH increases (6, 14). As the dark pigments are produced in the gallic acid medium by *P. melinii,* there is also an increase in pH.

The ability of *Phialophora* spp. to utilize a wide range of nitrogen and carbon sources and to grow over a wide range of pH has been demonstrated (1). The presence of vitamins and unknown substances soluble in alcohol, acetone, ether, and chloroform extracted from wood were stimulatory to the growth of *Phialophora* spp. (2, 3). Phenolic compounds could have been present in these fractions. All these factors could make *Phialoph-*

TABLE 4. Growth of *Fomes connatus* on gallic acid medium after the growth of *Phialophora melinii*

Ratio of gallic[a] acid to glucose	Mean[b] oven-dried wt of mycelium (mg)		
	Days of growth of *P. melinii*		
	14	18	22
Glucose alone	48 ± 5	50 ± 9	53 ± 9
1:20	19 ± 17	15 ± 3	18 ± 1
1:10	26 ± 13	25 ± 7	33 ± 4
1:1	5 ± 8	0	0
Gallic acid alone	0	0	0

[a] Ratio of weight of carbon of gallic acid before growth to glucose added after growth to basal medium of asparagine, buffer, salts, trace elements, and vitamins (9).
[b] Means based on four replications/isolate per treatment. The 95% confidence limits are reported for each treatment mean. Growth at 25 days.

ora spp. a primary invader with which decay fungi could not compete.

It should be noted that the apparent oxidation of gallic acid before inoculations produced a toxic or inhibitory effect on the growth of *P. melinii*. Early discolorations in the living tree may be caused by host response to wounding through which the phenolic compounds present are oxidized to a more toxic state. If the reaction is sufficiently strong, invasion may be prevented or inhibited. Changes in manganese concentration in living trees (15) could help overcome the toxic effects observed in culture. The carbohydrate level could also be important.

The capacity of nonhymenomycetous fungi and decay fungi to respond to different levels of various phenolic compounds probably varies greatly. Preliminary studies by the authors on other decay fungi showed that *F. igniarius* can tolerate low levels of phenolic compounds, while *Polyporus glomeratus* behaves much like *F. connatus*. The differences in the capacity of decay fungi to tolerate certain phenolic compounds may help account for differences observed in the ability of several of these fungi to degrade wood in various stages of discoloration and decay (14).

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