

Differential Response of *Poria weirii* to Phenolic Acids from Douglas-fir and Red Alder Roots

JAMES M. TRAPPE

C. Y. LI

K. C. LU

W. B. BOLLEN

Abstract. In previous studies the phenolic acids, p-coumaric, ferulic, syringic, and vanillic, have been quantitatively determined from hydrolyzed extracts of red alder roots, and p-coumaric and vanillic, from Douglas-fir roots. Alder roots resist infection by *Poria weirii*, whereas Douglas-fir roots are highly susceptible. In the present study, the compounds alone and in all combinations were tested for effects on growth of two genotypes of *P. weirii* in vitro. The combination of all compounds, as found in alder root hydrolysates, inhibited growth of both *Poria* isolates. The p-coumaric-vanillic combination associated with Douglas-fir root hydrolysates inhibited one isolate and stimulated the other. The two isolates differed markedly in response to several other combinations of the compounds and in their effects on pH of the medium. Phenolic substances probably participate in the *Poria*-resistance system of alder. Physiological strains of *P. weirii* that effectively attack any susceptible host may exist, but not all strains are likely to be equally pathogenic on all hosts or all root parts of a host (bark vs. sapwood vs. heartwood, for example). Biological control of *P. weirii*, seeming anomalies in behavior of *P. weirii* in nature, and breeding for host resistance are discussed. *Forest Sci.* 19: 191-196.

Additional key words. *Alnus rubra*, *Pseudotsuga menziesii*, roots, root-rot fungi, tree diseases, fungi.

GROWTH OF *Poria weirii* (Murr.) Murr., a widely spread and lethal root pathogen of many conifers in western North America, is significantly affected by several phenolic compounds in vitro (Li *et al.* 1969). Quantitative analyses of hydrolyzed extracts from roots of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), a *Poria*-susceptible species, revealed three of the previously tested substances: vanillic, p-coumaric, and p-hydroxybenzoic acids. Extracts from roots of red alder (*Alnus rubra* Bong.), a *Poria*-resistant species, contained vanillic, p-coumaric, ferulic, and syringic acids (Li *et al.* 1972). In both species concentrations of the substances in hydrolysates were markedly lower per unit dry weight of roots than the concentrations previously tested on *P. weirii* in vitro. Of

these five substances, all but ferulic acid have also been found in hydrolyzed extracts of soil under red alder (Li *et al.* 1970b).

The unhydrolyzed forms of these compounds in roots and soil are presently unknown. Regardless of their original forms, they doubtlessly account for only part of the probably complex biochemical systems involved in relative resistance to *P. weirii*. However, further study of the reaction of

The authors are, respectively, Principal Mycologist, Microbiologist, Principal Plant Pathologist, and Principal Microbiologist, Forestry Sciences Lab., Pacific Northwest Forest and Range Exp. Sta., USDA Forest Service, Corvallis, Ore. Dr. Bollen is also Professor, Dep. of Microbiology, Oregon State Univ. This study was supported in part by National Science Foundation Grant GB-18650. Manuscript received Sept. 21, 1972.

P. weirii to these compounds can increase understanding of the nature of host resistance, the factors affecting growth and survival of the pathogen, and genotypic interactions of pathogen and host. This study was designed to determine the effects of four of the phenolic substances at concentrations found in hydrolyzed root extracts on growth and pH relations of two isolates of *P. weirii*. The fifth substance, p-hydroxybenzoic acid, was not available to us in purified form at the time the study was conducted.

Materials and Methods

Two isolates of *P. weirii* were selected on the basis of growth rate in vitro: T-55, relatively slow-growing, isolated from an infected Douglas-fir near Randall, south of Mount Rainier, Washington; and T-124, relatively fast-growing, isolated from an infected Douglas-fir near Quilcene, east side of the Olympic Peninsula, Washington. Both isolates have been maintained in culture on identical media for more than 5 years.

Trione's (1964) liquid synthetic medium, modified by substituting 10 g glucose for 20 g sucrose and kept at the post-autoclaving pH 5.5, served as the basal medium for all treatments. The two isolates were grown in the basal medium for 21 days at room temperature, then removed from the medium, consolidated by centrifugation, washed three times with double-distilled water, and homogenized aseptically in a Waring blender in a volume of double-distilled water adjusted to provide equal concentrations of inoculum for all treatments.

The 16 chemical treatments included controls plus four phenolic acids, alone and in all possible combinations. The compounds were added as reagent grade authentic¹ at the concentrations Li *et al.*

¹Sources of the compounds were: p-coumaric acid, Mann Research Lab., New York; ferulic and vanillic acids, Sigma Chemical Co., St. Louis; syringic acid, Eastman Organic Chemicals, Rochester. Mention of products or companies does not constitute endorsement by the U.S. Department of Agriculture.

(1972) determined on a dry root weight basis (Table 1). Prepared stock solutions of syringic acid in double-distilled water and the other compounds in 25 percent ethanol were adjusted to pH 5.0 with 1N NaOH and sterilized by filtration through ultra-fine porosity fritted glass discs. Appropriate volumes of each stock solution were pipetted into 250-ml Erlenmeyer flasks containing 47 ml of basal medium. Sterile water and ethanol were then added in suitable ratio so that all flasks contained 50 ml of medium with the same quantities of ethanol and varying only in content of phenolic acids. The pH of all media was 5.5 after these additions.

Four flasks of each chemical treatment were aseptically inoculated with 0.2 ml mycelial suspension containing 0.2 mg of isolate T-55; and four, with T-124. The flasks were then randomly arranged in a dark incubator at 20°C. After 23 days incubation, mycelia were removed from each flask, wash-filtered through tared Whatman No. 42 filter paper, dried in a circulating oven at 80°C for 2 days, and weighed to the nearest 0.1 mg. Final pH of the medium in each flask was determined to the nearest tenth unit.

Data were submitted to analysis of variance with orthogonal comparisons of main effects and interactions. Significant second and higher order interactions proved difficult to interpret in a biologically meaningful way because of apparent synergisms and antagonisms between certain compounds in combination. The Duncan multiple range test was therefore applied to compare individual treatment means.

Differences Between Isolates

Mean Colony Weight. With all chemical treatments combined in analysis of variance, colony weight of isolate T-124 (293 mg) was significantly greater than that of T-55 (269 mg). The range of mean weights over all treatments was somewhat broader for T-55 than for T-124.

Final pH. With all chemical treatments combined, the mean of final pH values of the medium was significantly greater for isolate T-55 (pH 5.9) than for T-124 (pH

TABLE 1. Mean mycelial weights and final pH of media for two isolates of *Poria weirii* grown in control media and media containing phenolic compounds alone and in all combinations.¹

Phenolic ² compound	Weight (mg)		pH	
	Isolate T-55	Isolate T-124	Isolate T-55	Isolate T-124
<i>O</i>	312b	324b	4.4a	3.7d
<i>C</i>	302b	331ab	5.4bc	3.1a
<i>F</i>	264cd	259de	6.0de	3.5abc
<i>S</i>	274c	281d	5.7cd	3.3a
<i>V</i>	227ef	275d	7.4h	3.4ab
<i>CF</i>	245de	328ab	6.6g	3.4ab
<i>CS</i>	336a	347a	4.6a	3.0a
<i>CV</i>	226ef	345a	7.2h	2.9a
<i>FS</i>	263cd	248e	5.9cd	3.7bcd
<i>FV</i>	263cd	274d	6.0de	3.7d
<i>SV</i>	247d	300c	6.2ef	3.2a
<i>CFS</i>	314b	259de	5.2b	3.6abcd
<i>CFV</i>	268c	270d	5.6cd	3.5abc
<i>CSV</i>	221f	301c	7.6h	3.2a
<i>FSV</i>	266cd	273d	6.3fg	3.6bcd
<i>CFSV</i>	268c	272d	4.6a	3.7cd
Ave.	269	293	5.9	3.4

¹ Rounded-off mean of four replicates for each treatment. Means within columns not sharing a common postscript letter differ significantly at the 95 percent confidence level with the Duncan multiple range test. Uninoculated media had a pH of 5.5.

² *O* control media, no phenolic substance

C p-coumaric acid, 80 mg/l

F ferulic acid, 150 mg/l

S syringic acid, 60 mg/l

V vanillic acid, 30 mg/l

3.4). Indeed, the lowest value of the range of pH for T-55 over all treatments was significantly greater than the highest value of the range for T-124 when individual treatments were compared (Table 1). In both cases, the mean of final pH values of controls was lower than the starting pH. For T-124 this was also true for all other treatments, whereas for T-55 most treatments other than controls ended the experiment with a higher pH. In general, the larger the mean colony weight of a treatment, the less was the mean of final pH values of the median. Exceptions to this trend occurred with certain treatments as detailed below, especially for isolate T-124.

Effects of Phenolic Compounds

In the following discussion, phenolic compounds are referred to by abbreviations given in Table 1.

p-Coumaric Acid (*C*). With the isolates combined in analysis of variance, the mean mycelial weight of all treatments including *C* (290 mg) was significantly greater than that of all treatments lacking *C* (272 mg).

Mean weights of colonies of both isolates grown on media with *C* only did not differ from controls (Table 1). However, when *C* was combined with the inhibitory *S*, a significant synergistic stimulation of growth was shown with both isolates.

Mycelial weights with *CF* and *CV* significantly exceeded those of *F* and *V* for isolate T-124, whereas for T-55 these combinations were respectively as strongly inhibitory as *F* and *V* alone. For T-55, addition of *C* to *FS* completely counteracted the inhibition exerted by *FS* alone, but *CSV* was significantly more inhibitory than *SV* alone. *C* appeared not to influence the effects of the remaining combinations of phenolics.

The mean of final pH values of media of all treatments with *C* was significantly lower (4.6) than that of all treatments without *C* (4.8), conforming on the average with the overall inverse relationship between mycelial growth and final pH of the medium. Occasionally the trend was otherwise but lacked any perceptible pattern (Table 1).

Ferulic Acid (F). With the isolates combined in analysis of variance, the mean mycelial weight of all treatments including *F* (271 mg) was significantly lower than that of all treatments lacking *F* (290 mg).

Mean weights of colonies of both isolates grown on media with *F* only were significantly lower than the controls (Table 1). For isolate T-124, addition of *F* to *C* and *V* did not significantly affect mycelial growth. Inhibition by *F* was increased when it was added to *S* and all combinations of *C*, *S*, and *V*. The stimulatory effects of *CS* and *CV* on T-124 were reversed to strongly inhibitory by addition of *F*. Isolate T-55, in contrast, responded quite the opposite in several treatments incorporating *F*: *FV*, *CFV*, and *CFSV* resulted in significantly greater mean weights than did *V*, *CV*, and *CSV*, respectively.

The mean of final pH values of the medium of all treatments with *F* (4.7) did not differ significantly from that of all treatments without *F* (4.6). The significant *F* x isolate interaction, however, revealed that for T-55 the mean pH value of all *F* treatments was significantly lower than that of all non-*F* treatments, whereas for T-124 the opposite prevailed. Since presence of *F* in combination with other compounds tended to increase growth of T-55 and decrease that of T-124 (Table 1), the *F* treat-

ments conformed on the average to the general inverse relationship between colony weight and final pH.

Syringic Acid (S). With the isolates combined in analysis of variance, the mean mycelial weight of all treatments including *S* (280 mg) did not differ significantly from that of all treatments lacking *S* (282 mg). However, the significant *S* x isolate interaction revealed that for isolate T-55 the mean mycelial weight of all treatments including *S* was higher than that of all treatments lacking *S*, whereas the opposite was true for T-124.

Mean weights of colonies of both isolates grown on media with *S* alone were significantly lower than the controls (Table 1). *CS* significantly stimulated growth over controls for both isolates. Combination of *S* with other compounds resulted in variable responses by the isolates. Some additions of *S* were stimulatory, some inhibitory, and some had no effect.

The final mean of pH values of media of all treatments with *S* (4.6) was significantly lower than that of media lacking *S* (4.8). A significant *S* x isolate interaction revealed that this difference resulted solely from T-55 (pH 6.1 without *S*, 5.7 with *S*). No significant difference occurred with T-124. In general, however, the *S*-containing treatments followed the trend of inverse relationship between final pH and mycelial growth (Table 1).

Vanillic Acid (V). With the isolates combined in analysis of variance, the mean mycelial weight of all treatments including *V* (268 mg) was significantly lower than that of all treatments lacking *V* (293 mg).

Mean weights of both isolates grown on media with *V* only were significantly lower than those grown on control media (Table 1). For isolate T-55, addition of *V* to other compounds frequently resulted in increased inhibition. Most strikingly, the strongly stimulatory effect of *CS* on T-55 was reversed to strongly inhibitory by addition of *V*. On the other hand, *V* added to *CF* significantly increased growth over *CF* alone. Isolate T-124 responded quite differently in several treatments. Addition of

V to *S* and *FS* produced a significant increase of growth; to *CS* and *CF*, significant decrease; and to the other combinations, no change in effect.

The mean of final pH values of the media of all treatments with *V* (4.9) was significantly greater than that of treatments lacking *V* (4.5). With only few exceptions, treatments with *V* conformed to the general trend of decreasing final pH with increasing mycelial weight.

CV and *CFSV*. These combinations found in roots of Douglas-fir (*CV*) and red alder (*CFSV*) merit special mention. For both isolates, *CFSV* significantly inhibited mycelial growth as compared to controls. *CV* significantly stimulated growth over that of controls for T-124; for T-55, in contrast, *CV* significantly inhibited growth as compared both to controls and *CFSV*.

Final mean of pH values of the medium of these treatments followed the general trend of inverse relationship with mycelial weight except for *CFSV* with isolate T-55. The final mean pH of this treatment was among the lowest of the experiment.

Discussion

The wide genetic diversity among clones of *P. weirii* reported by Childs (1963), Morrison (1969), and others is strongly confirmed by our results. The isolates used in the experiments were selected strictly because one, T-55, grows slowly on synthetic media and the other, T-124, grows rapidly. However, in many cases the two isolates responded quite differently to phenolic compounds incorporated into the media alone or in combination.

Among the more interesting differences are the responses to *F*, *V*, *CV*, and *CFS*. Although *F* and *V* alone were inhibitory to both isolates, their effects in combination with other compounds were generally reversed between the two isolates. *F* was associated with a trend toward increased mycelial weight of T-55 and decreased mycelial weight of T-124, although the opposite was true for *V*. The *CV* combination determined earlier for roots of

Douglas-fir (Li *et al.* 1972) significantly inhibited growth of T-55 compared to controls but significantly stimulated that of T-124.

At the same time, these two physiologically diverse isolates responded in similar ways to several compounds and combinations of compounds. *CS* was significantly stimulatory to both, thereby meriting consideration in development of media for optimum growth of *P. weirii*. *CFSV*, the combination found in roots of the *Por*-resistant red alder, was significantly inhibitory to both isolates. *CSV* compounds qualitatively determined from soil under alder (Li *et al.* 1970b) also significantly inhibited both isolates, although concentrations in soil were probably lower than in this experiment.

The inhibition of *P. weirii* growth by the *CFSV* combination lends credence to the involvement of such compounds in resistance of alder to *P. weirii*. However, since inhibition was only 12 to 16 percent of mycelial weight production, alder's resistance clearly involves more, such as other phenolic compounds, long-chain fatty acids (Li *et al.* 1970a), and undefined components. Judging from the synergistic and antagonistic effects exhibited by the various combinations of phenolic compounds that we studied, a biochemical "mechanism" of resistance in alder or other resistant species can be defined only by the sum of its parts.

The discrepancy between the two isolates in final pH of the medium reflects metabolic differences that could be important in pathogenesis and in the host's ability to regulate the pathogenic mechanism.

The striking differences between the two isolates in response to several combinations of the tested substances and in acid metabolism indicates notable physiological strain differentiation in *P. weirii*. The results suggest some working hypotheses about the pathogen and about unexplained features of its behavior in forests.

Physiological strains of *P. weirii* which attack effectively any susceptible host could well exist. But, as Morrison (1969) concluded, not all strains are likely to be

equally virulent on all hosts. Furthermore, not all strains can be expected to attack different parts of a host (bark, cambium, sapwood, and heartwood) with equal virulence when these parts vary in their combinations and concentrations of inhibitors and stimulators.

The physiological diversity of *P. weirii* coupled with its apparently slight ability to establish new infections by spores may account for some of its unexplained behavior in nature. For example, western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) is a susceptible host but often remains apparently healthy in infection centers where Douglas-fir is being killed (Childs 1970). In some large infection centers containing Douglas-fir and western hemlock, the stands are being converted to pure hemlock by differential killing of the Douglas-fir. Such centers may contain a genotype of *P. weirii* that is inhibited by the combination of compounds in hemlock.

Different combinations of compounds in various root tissues could account for seemingly anomalous differential infections in nature. Thus, root bark of a host species might contain combinations of compounds strongly inhibitory to a genotype of *P. weirii*, but root wood might lack an inhibitory combination or even contain a stimulatory one. In this case, entry of *P. weirii* might be restricted to wounds and root grafts. The disease pattern in an infection pocket would then be erratic. Trees with roots wounded or grafted to roots of infected neighbors would have the disease, and others would escape.

Relative proportions of the compounds in combination were not varied in this study. Radwan (1972) demonstrated that concentrations of chlorogenic acid in foliage varies markedly between clones of Douglas-fir. Presumably, Douglas-fir geno-

types can also vary in relative concentrations of *Poria*-affecting compounds in roots. The possibility of inherent, widely effective, defense mechanisms in some genotypes of susceptible hosts cannot be dismissed. However, the potential genetic variability within *P. weirii* reduces the likelihood of success in development of broadly resistant host genotypes.

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