

Relative Capacities of Filamentous and Non-filamentous Bacteria from Two Forest Soils To Inhibit *Phellinus weirii* in Culture

Abstract

Alder and conifer soil were examined for *Streptomyces* spp. and non-filamentous bacteria antagonistic to *Phellinus weirii*. Conifer soil supported higher populations *Streptomyces* spp. and higher percentage of *Streptomyces* species antagonistic to *P. weirii* than did alder soil. The populations of non-filamentous bacteria were low in both soils. Alder soil, however, had a higher percentage of non-filamentous bacteria antagonistic to *P. weirii* than did conifer soil. Identified *Streptomyces* show that different species are present in each soil type.

Introduction

Phellinus weirii (Murrill) Gilbertson [= *Poria weirii* (Murrill) Murrill] causes serious root rot of many species of native conifers in western North America. The fungus can survive for many years in stumps and roots of dead and living trees, and it may infect roots of adjacent host trees when ectotrophic mycelia pass from one to another at points of root contact.

Some evidence suggests that damage to susceptible conifers is suppressed by the presence of red alder, *Alnus rubra* Bong. (Trappe, 1972; Nelson *et al.* 1978). Alder soils contain phenolic compounds shown to inhibit growth of *P. weirii* in vitro (Li *et al.*, 1972) and high levels of nitrogen including NO₃ (Franklin *et al.*, 1968), a form of N not utilized by *P. weirii* (Li *et al.*, 1967). Researchers have also shown significant differences in microbial populations between alder and conifer soil (Lu *et al.*, 1968; Wicklow *et al.*, 1974). The relative numbers or percentage of microbes antagonistic to *P. weirii*, however, have not been reported. Soil microbes, in addition to nitrogen and phenolic compounds, might influence disease development. In this study, we examine differences in populations of *Streptomyces* and non-filamentous bacteria antagonistic to *P. weirii* in adjacent stands of red alder and of mixed conifers, primarily Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco].

This study was conducted at Cascade Head Experimental Forest of coastal Oregon, an area chosen for its history of intensive research and available scientific information.

Methods and Materials

Three soil samples were collected from one alder stand and from one conifer stand at two-month intervals for one year. Each sample consisted of three sub-samples taken approximately 60 cm from the base of a tree to a depth of 64 cm after the first 5 cm of top litter had been removed. The soil was immediately taken to the laboratory and screened through a 6-mm sieve.

Soil dilution plates (1:50,000 dil.) were made in triplicate from 20 gm each of the soil samples using sodium albuminate agar (pH 6.8) as the selective medium for *Streptomyces* and non-filamentous bacteria. The plates were incubated at 28° C for eight days and colonies counted. A double-layer plate technique (Li *et al.*, 1969) was used to test antagonism towards *P. weirii*. The fungus was first grown in malt broth, then the mycelium was washed in sterile, distilled water, and homogenized. One ml of the homogenate was pipetted into each petri plate and 10 ml of 1 percent water-agar was poured on top and allowed to solidify. Ten ml of malt yeast peptone (MYP) agar medium was layered over the water-agar and the surface inoculated with bacterial colonies, randomly selected from the dilution plates. Double-layer plates were incubated at 26° C for two weeks, and antagonism was recorded if a zone of inhibition was produced around a bacterial colony (Fig. 1).

Totals of 2144 *Streptomyces* colonies from conifer soil and 1571 colonies from alder soil were tested for their antagonism to *P. weirii*. Of the non-filamentous bacteria,

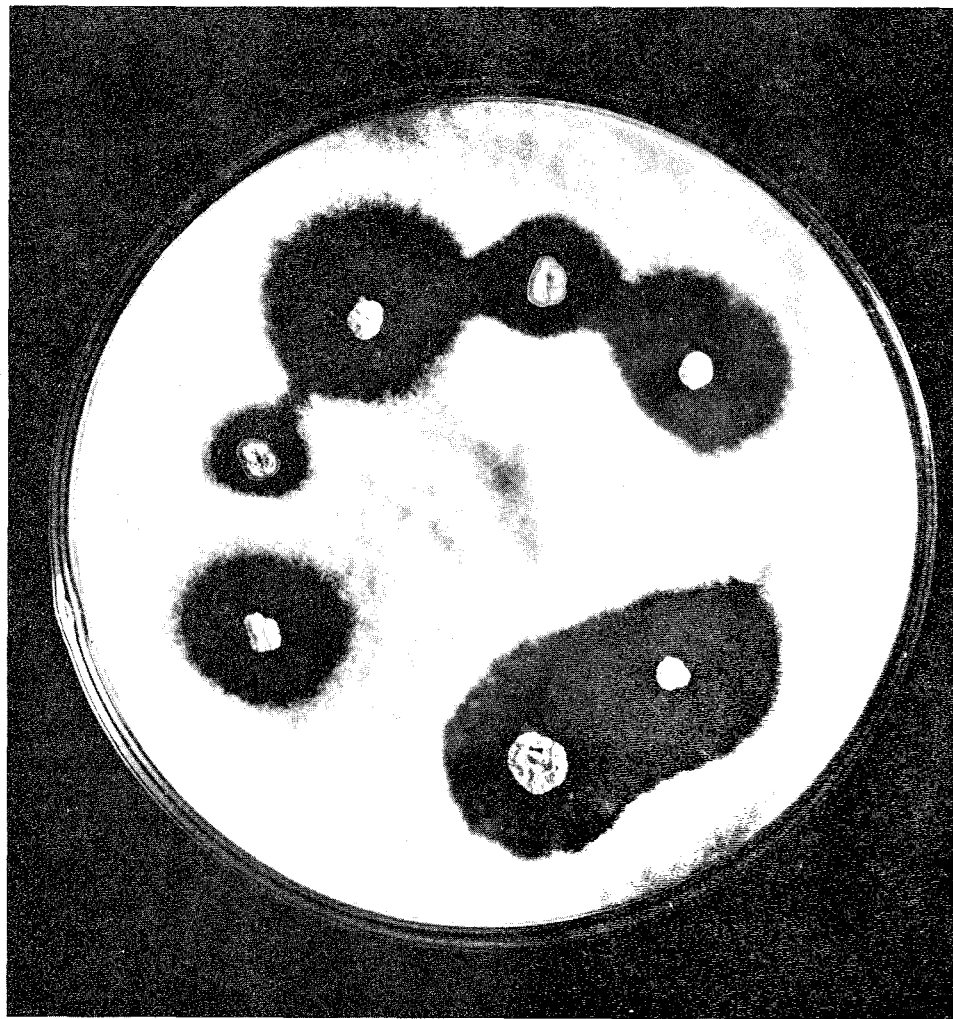


Figure 1. Inhibition of *P. weirii* by *Streptomyces* isolated from conifer soil.

462 from conifer soil and 797 from alder soil were tested for their antagonism to *P. weirii*.

Differences in the percentages of antagonistic *Streptomyces* and non-filamentous bacteria between the two soil-types were tested for significance by two factor analysis of variance, "ANOVA" (Neter and Wasserman, 1974).

Twenty colonies of *Streptomyces* antagonistic to *P. weirii* were randomly selected from cultures grown from the October samples taken from each of the two stands. These organisms were identified by methods, and on media, described by Shirling and Gottlieb (1966, 1968 a, 1968 b, and 1969) as modified by Kuster (1972). Color of aerial mycelium and reverse side of mycelium, color of soluble pigment, melanin reaction, and morphology of sporophores and spores noted. Carbohydrate utilization and growth rates were determined with sucrose, D-mannitol, L-arabinose, D-cylose, D-fructose, rhamnose, I-inositol, and raffinose. Glucose agar was used as a positive control and media without carbohydrate as a negative control. Electron micrographs were used to determine sporophore and spore morphology.

Results

The percentage of *Streptomyces* isolates antagonistic to *P. weirii* was significantly higher ($p \leq .05$) in those isolated from conifer soil than from alder soil at all sampling times (Fig. 2). Twenty-seven percent of the *Streptomyces* from conifer soil and 14 percent of the *Streptomyces* from alder soil were antagonistic to *P. weirii*. The proportion of antagonists differed among the months of isolation ($p \leq .05$), but the greatest mean responses for both conifer (48 percent) and alder (28 percent) sites occurred in October.

Conifer soil, in addition to having the higher percentage of antagonistic *Streptomyces*, had a higher total number of *Streptomyces* isolates for the two sampling times when counts per gram of soil were calculated: $2.0 \times 10^5/g$ in January and $1.8 \times 10^6/g$ in April. The population in alder soil was $8.0 \times 10^4/g$ in January and $1.5 \times 10^6/g$ in April.

None of the 40 antagonistic *Streptomyces* isolates identified from October samples were common to both soils. Of the 20 conifer soil isolates, 15 were identified as *S. parvulus* Waksman and Gregory; 4 as *S. aureomonopodiales* Krasilnikov and Yuan; and 1 as *S. hawaiiensis* Cron, Whitehead, Hooper, Heinemann and Lein. From the 20 alder isolates, 11 were identified as *S. plicatus* (anon.); 4 as *S. gelaticus* Waksman and Henrici; 4 as *S. atroolivaceus* Preobrazhenskaya, Blinov and Ryabova; and 1 as *S. rochei* Berger, Jampolsky and Goldberg.

In contrast to *Streptomyces* results, a significantly greater proportion ($p = .05$) of non-filamentous bacteria from alder soil were antagonistic to *P. weirii* (21 percent) than from conifer soil (4 percent) for six sampling times combined (Fig. 2). However, non-filamentous bacteria were far less numerous (organisms/g soil) than *Streptomyces* in either soil. The population of non-filamentous bacteria in alder soil was $5.6 \times 10^4/g$ in January and $2.3 \times 10^5/g$ in April. In conifer soil it was $3.1 \times 10^4/g$ in January and $1.3 \times 10^5/g$ in April.

Discussion

We did not substantiate that the alder soil we sampled had antagonistic populations sufficient to inhibit *P. weirii*. On the contrary, our tests showed that the conifer soil supported higher populations of *Streptomyces* with a higher percentage of *Streptomyces*

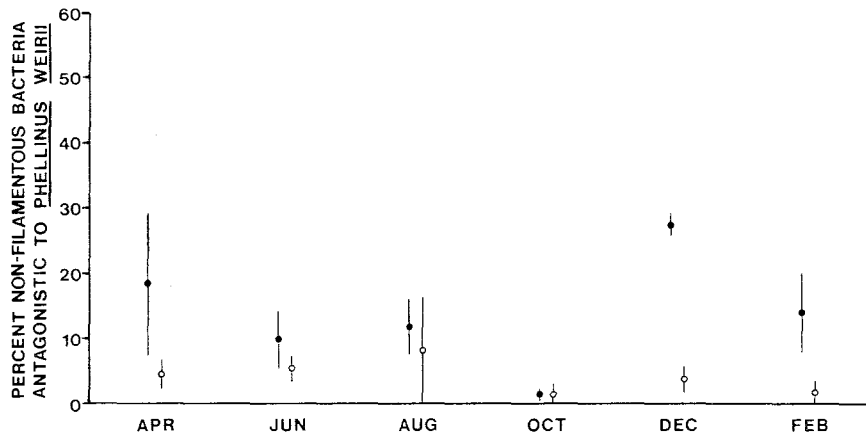
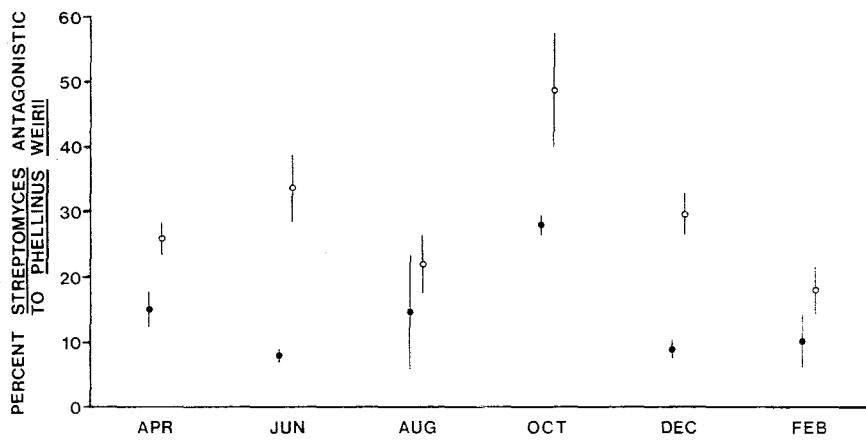


Figure 2. Comparison of the percentage of *Streptomyces* and non-filamentous bacteria antagonistic to *P. weirii* in conifer and alder soil. Open dot represents the mean value in conifer soil. Closed dot represents the mean value in alder soil. The bar lines denote standard error.

isolates antagonistic to *P. weirii* than did the alder soil. The difference in populations might be attributed to the soil pH (5.3 in conifer vs. 4.6 in alder). Normally, in agricultural soils, it is considered that a soil pH of 5.0 or less will limit the growth of most strains of actinomycetes (Alexander, 1961), and many forest soils of pH 4.6 or less have only small numbers of actinomycetes (Jensen, 1930). Alder soil, however, did have a greater proportion of non-filamentous bacteria antagonistic to *P. weirii*; but owing to the relatively small populations, it appears these bacteria had little influence on the total antagonistic microbial population.

Christensen *et al.* (1962), Christensen and Whittingham (1965), and Wicklow *et al.* (1974) have shown that fungal populations of the soil reflect the species composition of higher vegetation. Wicklow *et al.* (1974) also found relatively few dominant species of fungi in alder and conifer stands. Our results parallel these findings in that we found both a distinctly different antagonistic *Streptomyces* population in conifer vs. alder soil and an apparent dominance by relatively few species, none of which were

common to both soils. These results suggest a very specific and definable microbial community may exist in forest soils related to the dominant vegetative communities growing there.

The alder soil studied here was low in populations of what Baker and Cook (1974) called "resident antagonists," (bacteria and actinomycetes). Therefore, the opportunities for biological control based on fungistasis and antagonism found in this soil are probably reduced as compared to the conifer soil. Since sample sites were limited to a single area, conclusions cannot be broadly extrapolated. More intensive work needs to be done in soils under different tree species to determine whether the differences in microbial populations are consistent and can be related to the behavior of *P. weirii*.

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