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Effects of Ash Leachates on Growth and Development of *Armillaria mellea* in Culture

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Abstract

Ash leachates from recently burned litter in a ponderosa pine forest in central Oregon were tested for their effects on growth and development of Armillaria mellea in culture. Two isolates were used: one from an infected western hemlock and the other from an infected ponderosa pine tree. Colonies developing from agar discs containing mycelia of the hemlock isolate and grown on a solid agar-base medium supplemented with leachates extracted from 1, 5, 10, or 20 grams of ash per liter of media had significantly lower dry weights than colonies grown on nonsupplemented medium. There were no significant differences among leachate concentrations in dry weight of colonies developing from aerial rhizomorph tips of the hemlock isolate, although these colonies had significantly greater dry weights at each concentration than did those developing from mycelia. Some colonies started with aerial rhizomoph tips of the hemlock isolate developed mycelial fans similar to those found in trees infected with A. mellea. Colonies started with mycelia of the pine isolate and grown on

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media supplemented with 5 grams of ash leachate had significantly less growth than controls or cultures grown on media with 10 grams of ash leachate. Colonies started with aerial rhizomorph tips of the pine isolate showed no significant differences in growth among the leachate treatments. At 1 and 5 grams of ash leachate, colonies started with mycelia of the pine isolate had significantly less growth than colonies started with aerial rhizomorph tips.

Keywords: Root rot, Armillaria mellea, rhizomorphs, mycelial fans, fire (forest), cations.

Introduction

Various species of Armillaria attack woody and herbaceous plants throughout the world (Raabe 1962, Shaw and Roth 1978). The fungus usually spreads disease from plant to plant by rhizomorphs or by contacts between healthy and infected roots (Garrett 1960, Shaw 1974). In forests of the Pacific Northwest, the fungus is widely distributed and causes substantial root rot of ponderosa pine (Pinus ponderosa Laws.) (Adams 1974, Johnson 1976, Shaw and others 1976). In many of these forests, fire has been a major determinant of stand structure and composition (Martin 1976, Weaver 1968). Even though fire control policies over the last 50 years may have altered stand composition to favor tree species less susceptible to Armillaria root rot than ponderosa pine (Shaw and others 1976), the interaction between fire in these forests and the activity of Armillaria has received little attention.

Heat from fire does not penetrate deeply and dissipates relatively quickly (Heyward 1937, Debano and others 1976); thus, a strong direct effect of fire on the fungus in soil is unlikely. Fire, however, can change certain site and soil characteristics that subsequently affect the fungus. For example, burning generally increases soil pH (Ahlgren and Ahlgren 1965, Tarrant 1956) and quantities of water soluble cations (Burns 1952). These changes are usually attributed to the leaching of ash minerals into the soil (Wallace 1976). Grier and Cole (1971) reported that the A and B horizons absorbed 70 to 90 percent of the ions entering soil from an ash layer. Reports by Grier and Cole also show that ion concentration of leachates in the soil solution of burned plots remain higher than the ion concentration of forest floor leachates from unburned, control plots.

Rhizomorphs of Armillaria grow well and branch profusely within litter and humus layers of soil (Redfern 1973)--those constituting the A horizons. Because most leachates from ash are absorbed into these soil layers, these leachates could affect development of A. mellea rhizomorphs, and thus the spread of root rot.

In this study, we evaluated leachates obtained from recently burned litter collected in a ponderosa pine forest for effects on growth and development in culture of two isolates of A. mellea (Vahl. ex Fr.) Kummer.

Materials and Methods

One isolate of A. mellea was obtained from a western hemlock (Tsuga heterophylla (Raf.) Sarg.) tree in western Oregon and another from a ponderosa pine in central Oregon. Both isolates were maintained on a solid medium containing 30 g malt extract, 20 g dextrose, 5 g bacto-peptone, 19 g agar, and 1 ppm benomyl per liter of distilled water. Benomyl was used to reduce colonization by fast-growing contaminate fungi such as Trichoderma spp., Penicillin spp., and Rhizopus spp. (Maloy 1974). Medium pH was adjusted to 5.8-6.0 with 1.0 N HCl prior to sterilization in an autoclave.

All ash was collected from the forest floor after burning a ponderosa pine stand near Bend, Oregon. Ash leachates (AL) were obtained by mixing either 1, 5, 10 or 20 g of ash with 100 ml of distilled water and heating the solution in an autoclave for 3 minutes (hot water extracts). Each suspension was cooled for 2 hours and filtered twice under vacuum through Whatman's No. 1 filter paper. Filtrates were added to the basal medium prepared in 900 ml of distilled water and sterilized in an autoclave for 15 minutes.

Test cultures were established from stock cultures by two different methods: (1) removing a 2-mm agar disc that contained mycelia and submerged rhizomorph sections from 15-day-old pure cultures; or (2) clipping aerial rhizomorph tips measuring approximately 1 mm in length from 21-day-old pure cultures. These mycelial discs and aerial rhizomorph tips were placed on nonsupplemented medium (control) and medium supplemented with leachates obtained from extracting 1, 5, 10, or 20 g of ash. Cultures were grown in 100 x 15 mm plastic petri dishes in the dark for 21 days at 25 °C; there were 10 dishes for each concentration of ash leachate.

After incubation, all cultures were heated in an autoclave for 3 minutes to liquify agar. Colonies were then removed from the melted agar, rinsed in hot tap water, blotted on filter paper, dried in an oven for 48 hours at 90 °C, and weighed. Similar procedures have been used before to measure dry weights of A. mellea colonies grown on solid medium (Adams 1972, Cheo 1982, Shaw 1974).

For each isolate, a two-way analysis of variance using Friedman's test for unblocked data was used to evaluate the effects of ash leachate concentration and inoculum type on colony dry weight. Differences among leachate concentrations were examined within each inoculum type and differences between inoculum types were examined at each leachate concentration. Differences were judged to be significant at $P \leq 0.05$.

Results

Isolate from Hemlock

Colonies started with mycelial discs of the hemlock isolate and incubated on media supplemented with 1, 5, 10, or 20 g of AL had significantly less growth than controls; colonies incubated on 5 g of AL had significantly more growth than those on 1, 10, or 20 g of AL (table 1). There were no significant differences among leachate treatments for colonies started with aerial rhizomorph tips (table 1). At each leachate concentration, except controls, colonies started with aerial rhizomorph tips had significantly higher dry weights than those originating from mycelial discs (table 1).

In the presence of AL, regardless of concentration, formation of rhizomorphs was suppressed in all colonies originating from mycelial discs, and rhizomorph tips were located primarily within the confines of the surface mycelia (fig. 1). In contrast, colonies originating from aerial rhizomorph tips had profuse rhizomorph growth at all AL concentrations (fig. 2).

Table 1--Growth of the western hemlock isolate of Armillaria mellea on media supplemented with ash leachates

Leachate concentration	Colonies started with mycelial discs	Colonies started with aerial rhizomorph tips
Grams/liter	Dry weights (milligrams)[1/]	
Control (0)	330 a A	286 a A
1	70 b A	221 a B
5	132 c A	251 a B
10	56 b A	214 a B
20	73 b A	240 a B

[1/]Means of 10 replicates. Within each column, means followed by different lower case letters differ significantly; within each row, means followed by different upper case letters differ significantly.

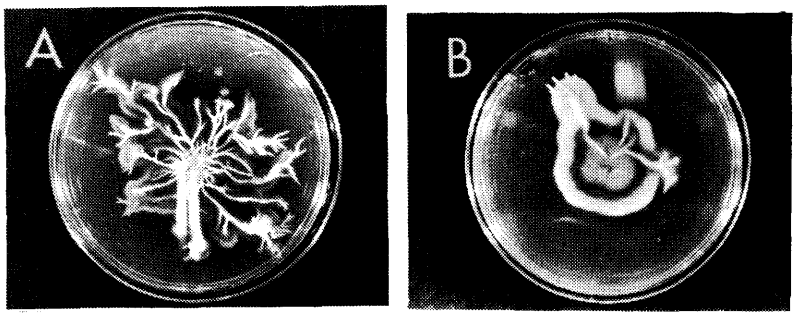

Figure 1.--Cultures of the hemlock isolate of Armillaria mellea started from mycelial discs and incubated for 21 days (view from bottom). Compared to controls (A), colonies developing on media containing 1, 5, 10, or 20 g of ash leachate exhibited a marked suppression of rhizomorph formation and growth (B).

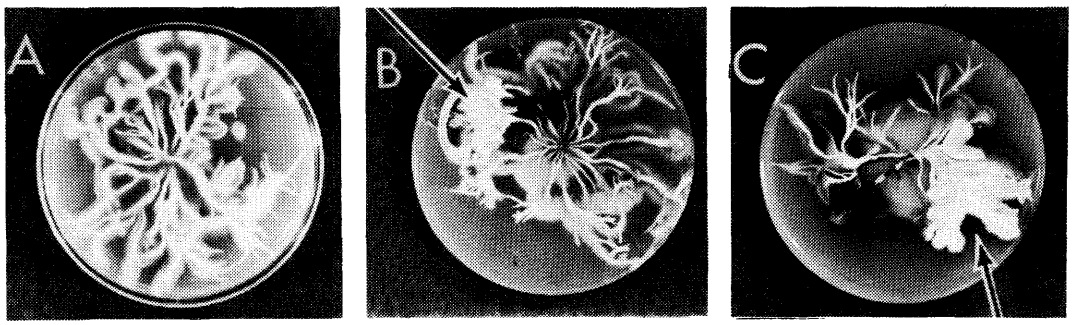

Figure 2.--Cultures of the hemlock isolate of Armillaria mellea started from aerial rhizomorph tips and incubated for 21 days (view from bottom). Compared to controls (A) and culture started from mycelial discs (fig. 1), colonies developing on media supplemented with any concentration of ash leachate did not express a marked suppression of rhizomorph formation or growth. Several colonies developing on media amended with 1, 10, or 20, g of ash leachate developed mycelial fans similar to those found in infected trees (arrows, B, C).

Mycelial fans similar to those found beneath the bark of infected pine trees developed at the interface of the agar medium and the petri dish bottom in 80, 50, and 50 percent of the colonies started with aerial rhizomorph tips and grown on media with 1, 10, or 20 g of AL, respectively (fig. 2B, 2C). No such fans developed on control medium or that containing 5 g of AL (fig. 2A). Patterns of rhizomorph branching in colonies originating from aerial rhizomorph tips were similar at all concentrations of AL, but were different from branching patterns of colonies orginating from mycelial discs (figs. 1 and 2).

Isolate from Pine

Colonies started with mycelial discs of the pine isolate and incubated on media supplemented with 5 g of AL had significantly less growth than controls or colonies incubated on media supplemented with 10 g of AL. For colonies started with aerial rhizomorph tips, there were no significant differences in dry weight among leachate treatments (table 2). Colonies started with mycelial discs had significantly less growth than colonies started with aerial rhizomorph tips at 1 and 5 g of AL (table 2).

Colonies orginating from mycelial discs or aerial rhizomorph tips grew vigorously over the media at all concentrations of AL (fig. 3). No mycelial fans developed, nor were variations in rhizomorph morphology observed between the two types of inoculum (fig. 3).

Table 2--Growth of the ponderosa pine isolate of Armillaria mellea on media supplemented with ash leachates

Leachate concentration	Colonies started with mycelial discs	Colonies started with aerial rhizomorph tips
Grams/liter	Dry weights (milligrams)[1/]	
Control (0)	208 a A	305 a A
1	132 ab A	342 a B
5	105 b A	214 a B
10	267 a A	285 a A
20	181 ab A	258 a A

[1/]Means of 10 replicates. Within each column, means followed by different lower case letters differ significantly; within each row, means followed by different upper case letters differ significantly.

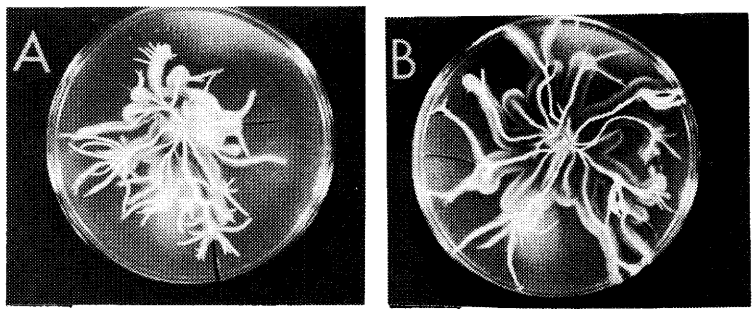

Figure 3.--Cultures of the pine isolate of Armillaria mellea incubated for 21 days (view from bottom). Cultures started with either mycelial discs (A) or aerial rhizomorph tips (B) exhibited profuse rhizomorph development at all concentrations of ash leachate.

Discussion

Isolates from both hemlock and pine showed a general reduction in growth when exposed to various concentrations of AL, although the reduction was not always statistically significant. Interestingly, extracts from dried foliage of many plant species present in the ponderosa pine forest type have a stimulating effect on growth of A. mellea in culture (Adams 1972); such foliage had contributed litter to the forest floor where we burned and collected ash.

In general, AL appeared to have a less pronounced effect on growth of A. mellea colonies originating from aerial rhizomorph tips than from mycelial discs. We attribute this difference to the presence of meristematic tissue within rhizomorph tips (Motta 1969) that allow the fungus to respond rather quickly to the different growing conditions created by AL.

For colonies of the hemlock isolate started with mycelia, ash leachates appear to inhibit growth at a concentration of less than 1 g per liter. A threshold level for the pine isolate is not clear because cultures grown at 5 g AL showed significantly less growth than controls, but those at 10 g had significantly more growth than those at 5 g. Additional concentrations between the 5 g intervals we tested must be evaluated to determine the concentration at which ash begins to inhibit growth in culture.

For both the hemlock and pine isolates, any negative effect of AL on growth of colonies started with aerial rhizomorph tips must occur at a concentration greater than the 20 g AL that we tested.

AL reduced, but did not totally inhibit, formation of rhizomorphs in colonies originating from mycelial discs of the hemlock isolate. Formation of rhizomorphs in such colonies of the pine isolate, however, appeared unaffected by leachates. This difference is probably related to differences between isolates of A. mellea, as they are known to vary in several cultural characteristics (Benton and Ehrlich 1941, Gibson 1961, Lisi 1940, MacLean 1950, Raabe 1966, 1969). These variations make it difficult to infer what effects ash resulting from forest fire may have on the fungus when it leaches through the soil and contacts rhizomorphs.

Mycelial fans similar in appearance to those found in trees infected by A. mellea formed in some cultures originating from aerial rhizomorph tips of the hemlock isolate that were growing on media amended with AL. Their presence may be attributed to morphology of the rhizomorph tip and to the influence of AL on it. Rhizomorphs produce mycelia and invade host cambium as a result of contact between a rhizomorph tip and a host (Redfern 1978). The development of mycelial fans in the aforementioned cultures suggests that their formation may be induced by a chemical factor(s) in addition to the "rhizomorph-host contact phenomenon." Ash has been reported to contain high concentrations of various cations (Grier and Cole 1971). In preliminary studies,[1/] A. mellea grown on medium amended with magnesuim carbonate or any combination of magnesuim carbonate and either potassium or calcium carbonate produced small fan-like rhizomorphs. These cations may stimulate production of mycelial fans in certain isolates of A. mellea.

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Metric Equivalents

1 gram (g) = 0.03527 ounce
1 liter (l) = 1.0567 quarts
1 milliliter (ml) = 0.001056 quart
1 millimeter (mm) = 0.03937 inch
°C = 5/9 (°F - 32)

[1/] J. Reaves, unpublished data.

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