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Effects of Prescribed Burning on the Viability of *Armillaria ostoyae* in Mixed-Conifer Forest Soils in the Blue Mountains of Oregon

Abstract

This study evaluated the influence of prescribed burning, soil depth, antagonistic fungi (*Trichoderma harzianum* Rifai), and time since burning on the viability of the root pathogen *Armillaria ostoyae* (Romagnesi) Herink in wood pieces buried in the soil of a mixed-conifer forest in northeastern Oregon. Red alder (*Alnus rubra* Bong) stem segments colonized with *A. ostoyae* were buried at two soil depths in plots that were burned and not burned. Half of the *Armillaria* segments were buried with segments of *T. harzianum*. Prescribed burning in the fall significantly reduced the recovery of *A. ostoyae* immediately after the burn at a soil depth of 8 cm but not at a soil depth of 30 cm. Adding *T. harzianum* inoculum to the soil did not appear to reduce *A. ostoyae* recovery immediately after the fire, but effects appeared after several months. Differences may also be due to the timing (fall or spring) of the prescribed burns. The effects of fire either natural or prescribed on pathogenic and saprophytic fungi may greatly influence infections of woody roots, subsequent disease occurrence, and patterns of tree mortality.

Introduction

Fire has been a component of the natural environment for 350 million years (Agee 1993). In the upper elevation, mixed-conifer forests of northeastern Oregon, fire return intervals have historically ranged between 40-150 years (Mutch et al. 1993). Because of these fire patterns, stands had a dominant overstory of ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) with scattered large-diameter western larch (*Larix occidentalis* Nutt.), Douglas-fir (*Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco), and grand fir (*Abies grandis* (Dougl. ex D.Don) Lindl.). Large diameter trees were common, and insect and pathogen populations remained at endemic levels due to low stand densities and the mixed-species stands.

Fire exclusion and selective harvesting of pine and larch that began at the turn of this century have resulted in an unprecedented abundance of grand fir and Douglas-fir in many areas in the interior West (Filip 1994). Mortality from root disease and other pests is much greater in stands with more true firs (Filip and Goheen 1984, Filip et al. 1996). As a result, these stands are overstocked with diseased, suppressed, or dead grand fir, Douglas-fir, and ponderosa pine.

Prescribed burning is increasingly used throughout northeastern Oregon in an attempt to return fire to its natural role in the ecosystem. Only a few studies, however, have directly linked fire in the Pacific Northwest to the changes in the incidence or severity of root pathogens (Thies 1990). Understanding the effect of prescribed burning on root disease will help to elucidate the relationship between fire and root disease.

Several species of the fungus *Armillaria* cause a serious root disease in host plants (Shaw and Kile 1991). Until the late 1970s it was assumed that Armillaria root disease was caused by a single polymorphic species, *A. mellea* (Vahl. ex Fr.) Kummer. Several species within the genus are now recognized (Kile et al. 1993). In North America, nine species have been defined; *A. ostoyae* is the most pathogenic species for conifers (Gregory et al. 1991). In the mixed-conifer forests of northeastern Oregon, *A. ostoyae* is common and widely distributed (Schmitt et al. 1991). Schmitt found that grand fir had the highest incidence of damage from several species of root pathogens, that ponderosa pine was damaged more by *A. ostoyae* than other root pathogens, and that Douglas-fir was damaged by root pathogens in most sample strata and plant associations.

Armillaria ostoyae is a saprophytic and weak parasitic fungus that can become an aggressive tree-killing pathogen (Wargo and Shaw 1985, Shaw and Kile 1991). *Armillaria ostoyae* can survive in stumps and dead roots for at least 50 years as mycelia or rhizomorphs; they can infect adjacent living trees via mycelium across root grafts and contacts or through rhizomorphs. The black, shoestring-like rhizomorph is an important infective structure that allows the fungus to colonize large areas. The fungus attacks, colonizes, and kills apparently healthy trees of all ages, often creating canopy gaps or patches. It has proven difficult to eradicate the fungus from an infected site. At many sites, stump and root colonization has led to a substantial increase in the inoculum in the soil which contributes to the increased severity of root disease (Filip and Goheen 1984, Wargo and Shaw 1985, Shaw and Kile 1991).

Biological control of *Armillaria* spp., although promising *in vitro*, has been unsuccessful for the most part *in situ*. Species of *Trichoderma* are reported to be the principal invaders of wood colonized by *Phellinus weirii* (Murr.) Gilb., the cause of laminated root rot (Nelson 1982, Goldfarb et al. 1989). Yang and Hood (1992) reported that a species of *Trichoderma* was markedly antagonistic toward *A. novae-zelandiae* (Stevenson) Herink. and *A. limonea* (Stevenson) Boesewinkel on freshly cut radiata pine (*Pinus radiata* D. Don) segments as compared to its interaction with eight other common saprophytic fungi. Munnecke et al. (1981) showed that *Trichoderma* spp. were responsible for the death of *Armillaria* mycelia on citrus trees after *Armillaria* had been "stressed" by fumigation with carbon disulfide in California. Filip and Roth (1977) found a significant increase in *Trichoderma* spp. in ponderosa pine stumps treated with methyl bromide or vorlex as compared to untreated stumps in central Washington.

Biological control of *Armillaria* is difficult because effective populations of antagonistic fungi are not sustainable under normal field conditions. Because prescribed fire treatments can influence antagonistic soil microorganisms (Margaris 1977, Parmeter 1977), judicious use of fire may indirectly help minimize Armillaria root disease. In central Oregon, Reaves et al. (1984) found that ash leachates from prescribed burns in ponderosa pine forests reduced the growth of *A. ostoyae* in cultures. Isolates of *Trichoderma* spp. obtained from burned soils were more antagonistic to *A. ostoyae* in culture than isolates from unburned soils (Reaves et al. 1990).

The purpose of our study was to determine whether prescribed burning reduces the viability of *A. ostoyae* in wood segments buried in a mixed-conifer forest soil. We were specifically interested in four issues: (1) whether there is a significant difference in the viability of *A. ostoyae* inoculum segments between burned and unburned treatments; (2) whether the treatment difference is associated with the depth of buried segments (8 cm vs. 30 cm); (3) whether the treatment difference is associated with the presence of *Trichoderma harzianum*; and (4) whether the treatment difference is associated with time since the prescribed burning treatment (over a year).

Methods

Research Site

The study was conducted in the Genesis Demonstration Area (T12S, R34E, Sec. 23; T13S, R34E, Sec. 1) in the Prairie City Ranger District, Malheur National Forest of Oregon. Four plant associations occur in the research area: *Abies grandis/Calamagrostis rubescens*, *A. grandis/Carex geyeri*, *A. grandis/Vaccinium membranaceum*, and *A. grandis/V. scoparium* (Johnson and Hall 1990). Stands in the study area contained dying and dead grand fir and Douglas-fir due to an outbreak of western spruce budworm (*Choristoneura occidentalis* Freeman) from 1980 to 1992.

Stands in the research area were part of a timber sale during the late 1970s and early 1980s. The most recent sales included regeneration harvests in mixed-conifer stands, overstory removals, and precommercial thinnings in ponderosa pine and fir stands. Harvesting, which began in 1978, fell victim to the timber buy-back program of the early 1980s and was not completed until 1991 after timber resale in 1987.

Three units in the study area were selected because of their prescribed fire treatment priority. Units A and B were selectively harvested in order to reduce excess stand density and favor ponderosa pine and western larch. Unit H was clearcut. Prescribed fire was planned in all three units in order to reduce fuel loads and create planting spots.

Fungal Inoculation

Because of the uneven distribution of Armillaria root disease in the study area, an isolate of *Armillaria ostoyae* from northeastern Oregon was used to artificially inoculate the treatment blocks. *Trichoderma* spp. were isolated from the soil at the research sites. A preliminary laboratory test was performed in order to screen for the *Trichoderma* isolate that was the most antagonistic to *A. ostoyae*; an isolate of *T. harzianum* was used as the inoculum.

Red alder (*Alnus rubra* Bong.) stem segments were obtained in the Oregon Coast Mountains. Segments were approximately 12 cm x 2.5 cm (with bark) and were soaked overnight in tap water; 4-6 were placed vertically into glass jars. Jars were 1/4 filled with water and autoclaved at 120°C for one hour. The segments were cooled overnight and inoculated with *A. ostoyae* grown on 3% malt agar. Jars were incubated in the dark at (18-24°C) for 3 months until the segments were well colonized by *A. ostoyae*. *Trichoderma-harzianum*-inoculum segments were similarly prepared from autoclaved red alder stem segments, but were incubated for one month.

Experimental Design and Plot Establishment

Two blocks each were randomly established in units A, B, and H in the study area: blocks 1 and 2 in unit A, blocks 3 and 4 in unit B, and blocks 5 and 6 in unit H. Each block was designed with a split-plot configuration. The use of prescribed burning was the whole-plot factor, and soil depth and presence of *Trichoderma* were the sub-plots factors. Each 12 m x 24 m sub-block (burned or unburned) contained four plots (Figure 1). Treatments were randomly assigned to the plots. Fifteen red alder stem segments colonized with *Armillaria* were buried in each plot before burning; five segments were buried for each of three sampling times (1 day, 1 month, and 2 months after burning). A *Trichoderma*-inoculum segment was buried adjacent to and touching an *Armillaria*-inoculum segment where indicated (AT). An iron stake was placed at each corner and at the center of each plot. The position of each inoculum segment was measured from the closest iron stake and recorded on a map for future sampling.

The configuration was a 2^3 factorial design; the treatments were burned or unburned, 8 or 30 cm soil depth, and presence or absence of *Trichoderma* inoculum. All segments were randomly assigned to each treatment and buried between August and September 1993. Before the prescribed fire treatment, woody debris was evenly scattered over each plot so that burning was uniform.

Prescribed Burning

Unfortunately, local weather and the availability of personnel at the Prairie City Ranger District affected our ability to field burn. Instead of burning all six blocks in the fall of 1993 as planned, the fire treatment occurred at three different times over two years.

Blocks 5 and 6 in H unit were burned for an hour on 27 October 1993 (Figure 2). The woody debris and litter layer on both blocks was barely consumed; soils were moist to the touch. On 31 October 1993, the burned plot of block 6 was reburned for four hours; the first sampling of segments occurred the next day, the second sampling occurred in June 1994, and the third in August 1994.

Block 3 in unit B and block 5 in unit H were burned on 8 June 1994, nine months after inoculation. The fire which lasted one hour and was extinguished with water, completely consumed the woody debris and litter layer (Figure 3). We began sampling the next day, sampled a second time in July, and a third time in August.

Blocks 1, 2, and 4 in units A and B were burned on 27 October 1994, one year after inoculation. The fire lasted one hour and completely consumed the woody debris and litter layer. Water was used on all plots to extinguish the fire. Sampling for Blocks 1, 2, and 4 was done only on the day after the burn. These 3 blocks received the same treatment, so data could be statistically analyzed.

Inoculum Recovery

Following each prescribed fire treatment, five segments from each treatment were randomly selected in order to test the viability of *A. ostoyae* at each sampling time. Segments were excavated individually, bagged in separate paper bags for each treatment, and stored at 1-2°C until they were processed in the laboratory. We attempted to isolate the fungi by splitting each segment aseptically and plating five wood chips (1 x 1 x 1 mm) onto a selective medium for *Armillaria* (2% malt agar with 1.5 ppm each of prochloraz, benomyl, and thiabendazole; 30 ppm rose bengal; and 100 ppm streptomycin sulfate; Goldfarb et al. 1989). The recovery rate of *A. ostoyae* was scored from 0-5 based on the number of isolates recovered from that segment. The number of *A. ostoyae* isolates recovered was converted to 0-100% scale. Treatment means were the average of the 15 segments in that treatment.

Statistical Analysis

Percent recovery of *A. ostoyae* was tabulated by burning treatment, soil depth, and presence of *Trichoderma*. Data from blocks 1, 2, and 4 were examined by using analysis of variance for a 2^3 factorial with an F test (P<0.05; SAS Institute, Inc. 1994). We explored the main and interactive effects of prescribed burning, depth of inoculum, and the presence of *Trichoderma* on the percentage recovery of *A. ostoyae*. Comparisons were made between all combinations of treatments.

Results

There were no significant differences among blocks 1, 2, and 4 (P = 0.94) in the percentage recovery of *Armillaria* immediately after the prescribed burn in October 1994. The effect of prescribed burning on *Armillaria* viability was significant (P = 0.0138; Table 1). The interaction between the prescribed burn and soil depth on *Armillaria* viability was also significant (P = 0.0138). The 3-way interaction of prescribed burning, soil depth, and the presence of *Trichoderma* (P = 0.3003), the interaction between prescribed burning and the presence of *Trichoderma* (P = 0.4313), and

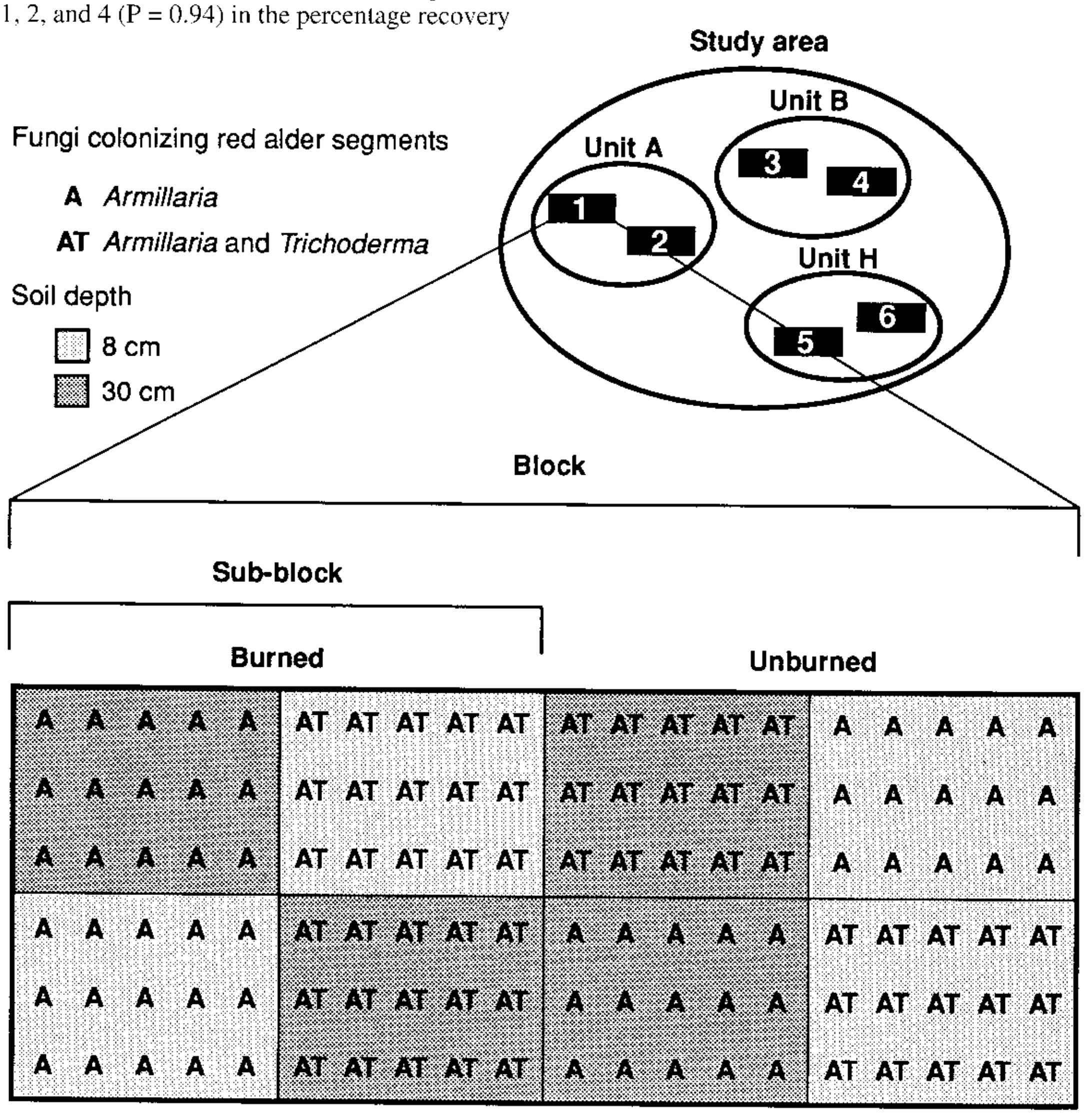

Figure 1. Diagram of the plot configurations and randomized treatment applications within each block. Red alder segments, colonized by *Armillaria* (A) were buried 8 or 30 cm under the soil surface (soil depth). In some cases, a segment colonized by *Armillaria* and a segment colonized by *Trichoderma* were buried together (AT). The plots in which these segments were buried were subsequently subjected to prescribed burning treatments.

Figure 2. Field crews igniting debris near plots used to determine effects on *Armillaria* viability.

Figure 3. Typical plot after burning and prior to sampling for *Armillaria* viability.

the interaction between soil depth and the presence of *Trichoderma* (P = 0.9193) did not significantly affect the viability of *Armillaria*.

Prescribed Burning and Soil Depth

The recovery of *Armillaria* decreased significantly from 36 to 5% at soil depths of 8 cm in burned plots but remained unchanged at soil depths of 30 cm (Table 2). The recovery of *Armillaria* was lowest in the burned and highest in the unburned treatments at a soil depth of 8 cm. The mean recovery of *Armillaria* at the 8 cm depth with prescribed burning was 5% which is not statistically different than 0% recovery (P = 0.43); thus *Armillaria* at a soil depth of 8 cm had virtually no viability after the prescribed burning). The mean recovery of *Armillaria* in unburned plots was 36% at 8 cm and 19% at 30 cm; however, depth itself did not have a significant effect on the percentage recovery of *Armillaria* in unburned plots (P = 0.8059; Table 1).

Season of Burning

Block 6 in Unit H was burned in the fall 1993 and sampled three times: fall 1993, spring 1994, and fall 1994. Because this was the only block effectively treated in fall 1993, there was no statistical analysis. In plots treated with prescribed burning, the recovery of *Armillaria* appeared to remain the same at the 30 cm soil depth regardless of the time of sampling; however, recovery decreased at the 8 cm depth at the second sampling. In unburned plots, the recovery of *Armillaria* at a depth of 8 cm was higher than at 30 cm at the second and third samplings.

TABLE 1. Results of an analysis of variance of the effects of prescribed burning (Burn), soil depth, and presence of *Trichoderma* (*Trich*) on the viability of *Armillaria* inoculum.

Source	NDF[1]	DDF[1]	Type III F[1]	Pr > F[1,2]
Burn	1	14	7.92	**0.0138**
Soil depth	1	14	0.06	0.8059
Trich	1	14	0.14	0.7181
Burn x Depth	1	14	7.92	**0.0138**
Burn x *Trich*	1	14	0.66	0.4313
Depth x *Trich*	1	14	0.01	0.9193
Burn x Depth x *Trich*	1	14	1.16	0.3003

[1]NDF = numerator degrees of freedom; DDF = denominator degrees of freedom; Type III F = Type III F-value; and Pr> F = probability greater than F-value.

[2]Bolded values are significant.

TABLE 2. The percentage recovery of *Armillaria ostoyae*, in northeastern Oregon, at two soil depths and at burned and unburned sites.

Treatment	Soil depth (cm)	Mean[1]	SE
Burned	8	5 (0.23)a[2]	0.29
Burned	30	19 (0.95)ab	0.29
Unburned	8	36 (1.83)b	0.29
Unburned	30	19 (0.95)ab	0.29

[1] Means are the percentage and actual number (in parentheses) of viable isolations of *A. ostoyae* out of a total of five attempts from each of 15 segments per treatment.

[2] Means followed by a different letter are significantly (P<0.05) different according to ANOVA (SAS Institute, Inc. 1994).

Following the burning treatment, the recovery of *Armillaria* appeared to decrease with time in the presence of *Trichoderma* but increased in the absence of *Trichoderma*. Without the burning treatment, recovery of *Armillaria* increased at the second sampling, then decreased at the third sampling, especially in the presence of *Trichoderma*.

Block 3 in Unit B was burned in spring 1994 and sampled three times: June, July, and August 1994. Block 5 in Unit H was also burned in the spring, but the unburned plot was accidently destroyed. The spring burn seemed to have no effect on the recovery of *Armillaria* during the sampling period. At the second and third sampling, *Armillaria* recovery in burned plots at the 8 cm depth was higher than recovery at 30 cm. The presence or absence of *Trichoderma* appeared to have little affect on *Armillaria* recovery.

Discussion and Conclusions

There was a significant reduction in *Armillaria* recovery immediately following a fall burn. The recovery of *Armillaria* was lowest (5%) in burned plots and highest (36%) in unburned plots in segments at a soil depth of 8 cm. The results of this field study suggest that the heat from the fire was able to kill a high proportion of *Armillaria* at a soil depth of 8 cm but not at 30 cm. The fact that most segments were scorched by the fire in all three blocks further supported the conclusion that the reduction in *Armillaria* recovery was caused by heat. Hood and Sandberg (1989) reported that there was a highly significant reduction in the yield from isolates of rhizomorphs, although no significant change in rhizomorph frequency, after felling and burning of forest vegetation in New Zealand.

Trichoderma harzianum can kill *Armillaria* mycelium in agar media in laboratory tests. The shift in the microbial balance from ash leachates in forest soils (Reaves et al. 1990) that favors the antagonist *Trichoderma* following a prescribed fire is a rather slow process compared to lab tests. We did not find a decrease in *Armillaria* with *Trichoderma* in samples taken immediately after the fire, although 8-10 months after the prescribed burning we found a decrease in *Armillaria* recovery. Additional research is needed in order to evaluate whether the reduction in *Armillaria* recovery is short-term. Also, there are large natural populations of *Trichoderma* spp. in forest soils that we did not monitor.

There was no apparent difference in the recovery of *Armillaria* between burned and unburned plots after the spring burning treatment. This differs from the results after the fall burning; we found a significant difference between burned and unburned plots at the 8 cm soil depth. More studies are needed to establish the timing and intensity of fire that are necessary to effectively reduce *Armillaria* recovery.

Prescribed burns should have occurred in all blocks in fall 1993. Instead, prescribed burning occurred at three different times over two years. Use of prescribed burning on public land involves a systematic series of decisions. The steps in this process include examining the proposed burn unit, developing prescriptions, obtaining permits and resources, addressing public concerns, checking weather and fuel conditions, selecting ignition patterns and tools, conducting the burns, and mopping up after completion.

Waiting for suitable weather conditions is a primary constraint in prescribed burning. Both the fuel and atmospheric conditions must be dry enough to support combustion, yet not so dry that escape of the fire is likely. This creates very narrow windows for treatment given the spring snow cover, dry and hot summers, and rainy autumns in eastern Oregon.

Woody roots that are naturally infected with *Armillaria* are normally present at depths much greater than were inoculum segments in our study. Obviously, an operational burn with conditions similar to our study would only destroy a small portion of the total *Armillaria* inoculum. Unless the burn was hotter or more penetrating, a situation that could lead to soil damage and significant nutrient loss, the direct effects of prescribed fire on *Armillaria* populations appear to be negligible. The long-term effect of burning as related to changes in *Trichoderma* populations and subsequent indirect effects on *Armillaria,* however, may be more important. Except for Reaves et al. 1984, 1990, and our study there are no studies on fire and root disease in interior western forest ecosystems. Long-term studies that explore the effects of prescribed burning on naturally infected roots in soil need to be conducted.

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