

Jimmy L. Reaves,¹ Research Plant Pathologist, USDA Forest Service, Pacific Northwest Research Station, 3200 Jefferson Way, Corvallis, Oregon 97331

Charles G. Shaw III, Supervisory Research Plant Pathologist, USDA Forest Service, Rocky Mountain Forest and Range Experiment Station, 240 West Prospect Street, Fort Collins, Colorado 80526

and

Lewis F. Roth, Professor Emeritus, Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon 97331

Infection of Ponderosa Pine Trees by *Armillaria ostoyae*: Residual Inoculum versus Contagion

Abstract

Infection of ponderosa pine roots by *Armillaria ostoyae* was evaluated in 17 excavations of disease centers conducted 14 and 15 years after stand regeneration on ground previously treated to remove *Armillaria* inoculum. Residual inoculum was the recognizable source of infection for the first 28 trees to die in 15 of the 17 new root disease centers; the other 76 affected trees in these centers became infected by contagion from the initially attacked trees. Dead and dying trees were girdled at the root collar by host resin and *Armillaria* mycelium and had most of their roots infected. *Armillaria*-caused lesions occurred on roots of dead and dying trees and also on trees lacking above-ground disease symptoms. On symptomatic trees, the fungus was not occluded within root lesions and spread both distally and proximally from them. In contrast, the fungus was occluded within distinct lesions on roots of trees lacking above-ground symptoms. These results verify that initial infections by *A. ostoyae* on regenerated ponderosa pines result from residual, primary inoculum that significantly functions for about 12 years. As with disease development in natural stands, subsequent infections and fungal spread that generates recognizable disease centers result from contagion. This information will assist forest managers contemplating initial control measures and their determining if any subsequent treatments are needed.

Introduction

Armillaria root disease, caused by *Armillaria ostoyae* (Romang.) Herink, is sometimes so severe in the Pacific Northwest that affected ponderosa pine (*Pinus ponderosa* Laws.) stands fail to produce commercial timber for decades (Hadfield *et al.* 1986, Hagle and Shaw 1991, Kile *et al.* 1991, Shaw *et al.* 1976, Wargo and Shaw 1985, Williams *et al.* 1989). Productivity of severely impacted sites might be restored through removing infected roots of the previous stand (Arnold 1981, Morrison *et al.* 1988, 1992; Redern and Filip, 1991, Roth and Shaw, unpublished) that serve as residual inoculum and sources of primary spread to a succeeding stand (Shaw 1980). This inoculum ranges in size from large, intact root systems and stumps to small roots and fungal rhizomorphs.

Irrespective of treatment technique, machine removal of inoculum (Arnold 1981, Bloomberg and Reynolds 1988) is not complete as generally some infected material remains. Through time, *A. ostoyae* and other organisms decompose this remaining inoculum and eventually render it ineffec-

tive, but not before some disease is transmitted by contact with roots of regenerated trees (Shaw 1980). Thus, follow-up treatment might be needed to secure the initial investment in sanitation and to sustain the health of the regeneration to a harvestable age.

The need and timing of any such follow-up treatments could be more accurately determined with better knowledge of the longevity of *A. ostoyae* in residual inoculum. Furthermore, information on the time at which significant roots of the regenerated stand (contagion) is needed—the information sought through this excavation study. Knowledge gained from this study will enhance our knowledge on the vegetative spread of the fungus and thus facilitate disease control decisions.

Methods

Study Area Description

Our observations were made in a 3.6 hectare stand of young ponderosa pine located at the north base of Meadow Butte (NE1/4, Sec 10, T6N, R11E) in south central Washington state, about 15 Km northwest of Glenwood (Figure 1). The previous stand of ponderosa pine on this site had been so severely damaged by *Armillaria* root disease that

¹Present Address: USDA Forest Service, Alabama A&M University, P. O. Box 1387, Normal, AL 35762

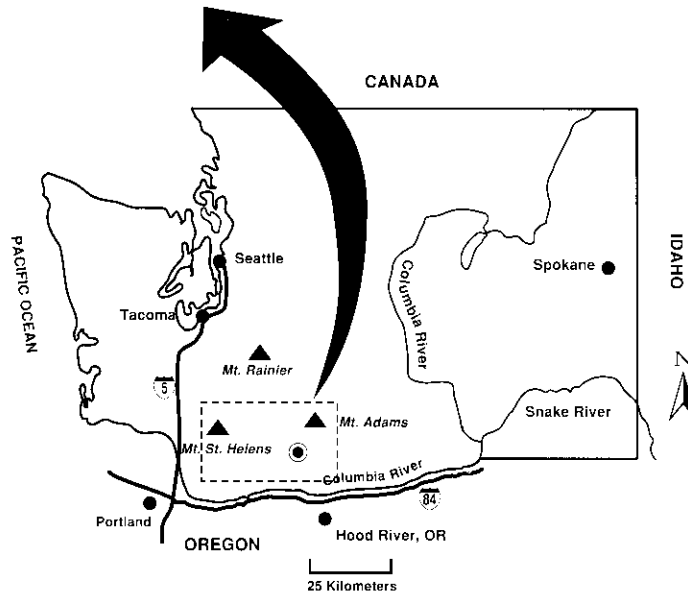
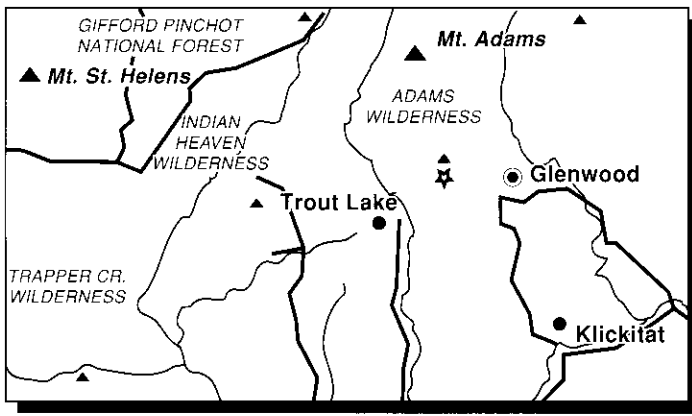


Figure 1. Location (star) of study area near Glenwood, Washington.

there was little likelihood of its persisting as a commercial, timber-producing forest (Shaw 1980, Shaw and Roth 1976, Shaw *et al.* 1976) (Figure 2). This location is part of the area that received recent attention from the nation's press during the "humongous fungus debate" (Ecenbarger 1992).

The stand was clear-cut in 1971, inoculum removed by bulldozer and raked, and the site allowed to regenerate naturally. In 1972, regeneration on the middle 1/3 of the area was destroyed and replaced with stock from Oregon and the local

source. Northern and southern thirds of the tract regenerated at natural stand densities. In 1977, trees in the middle and north thirds were thinned by pulling surplus trees to leave approximately 3113 trees/hectare.

During six of the ten years preceding death of the trees examined in this study, densities in the young stand had been somewhat reduced by annual removal of dead trees to measure effectiveness of the sanitation treatments (Roth and Shaw, unpublished). The work described here began



Figure 2. (A) A typical diseased stand located in the study area before *Armillaria ostoyae*-infected trees, snags, and stumps were removed by bulldozer; and (B) stand structure after approximately 13 years after disease treatments were administered.

after four years of not removing these dead trees, at which time mortality was present as either single trees or as small groups.

Excavation Procedure

Seventeen of these mortality groups, each with 3 or more dead trees and occupying an area ranging from 2–4 m², were evident in 1987. Mortality groups were scattered throughout the stand and thus tree spacing and size varied somewhat, but averaged roughly 10 cm DBH, 3.5 m tall, and 0.75 to 1 m apart.

Trees with no chlorotic needles, evidence of reduced leader growth, basal resinous, or mycelial fans and located several meters from each diseased group, served as controls. Control (51 total) and study trees (104 total) in the 17 disease centers were excavated with small hand tools (Shaw, 1980) to expose roots and buried materials. Resulting excavations were approximately 0.6 m wide, 0.9 m deep, and up to 6 m long. The light, uniformly textured soil derived from dacite volcanic pumice enabled excavation of residual degraded

wood, small roots, and rhizomorphs with minimal disturbance to root contacts, infection points (lesions) on roots, residual inoculum, and rhizomorphs.

The time of death for each study tree was determined for three preceding years by examining the color and retention of needles; trees killed earlier were recorded as dead more than three years. The number and location of lesions on each root system and, where possible, the inoculum source for each infection was recorded. Each disease center was sketched to show healthy roots, infected roots, root lesions, root grafts, root contacts, and location of residual inoculum (Figures 3 and 4). Roots with mycelial fans beneath the bark were recorded as infected. Living roots without such fans or lesions were recorded as healthy. Isolations were made from randomly selected infected roots, but no cultures were obtainable from residual inoculum. All isolates recovered from infected roots were identified as *A. ostoyae* by the *in vitro* cultural methods of Morrison *et al.* (1985)—the fungus that caused the extensive damage in the former stand on this site (Shaw 1984).

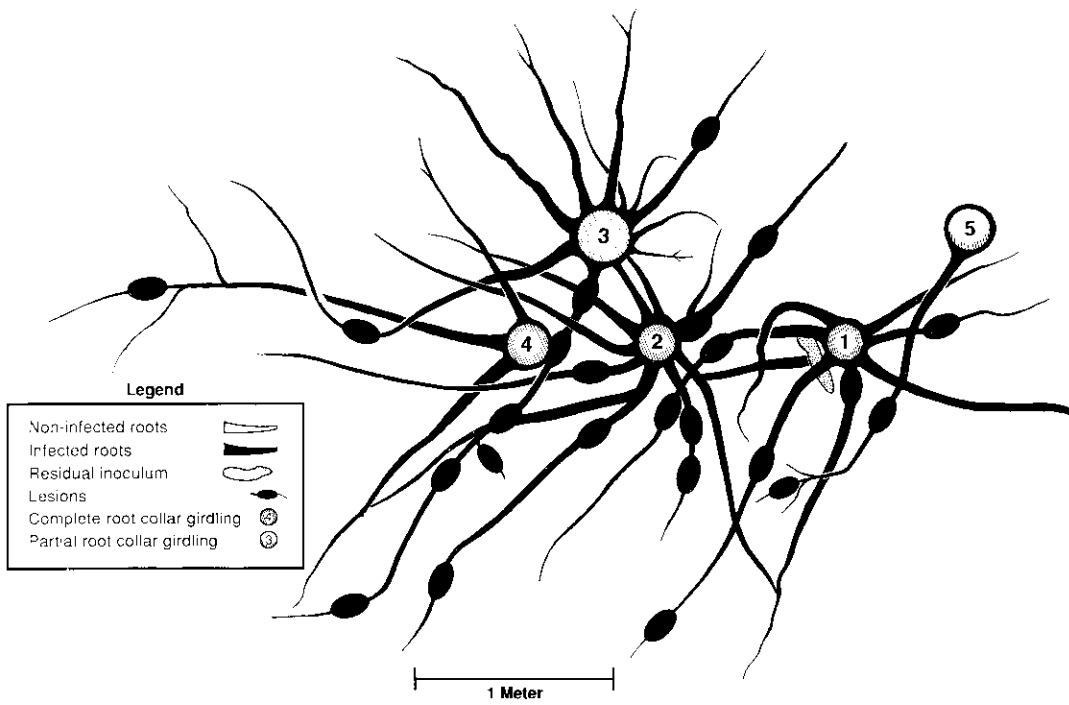


Figure 3. Schematic representation of a disease center (No. 15) showing rhizomorphs and numerous lesions on root systems. Increasing numbers represent the chronology of death.

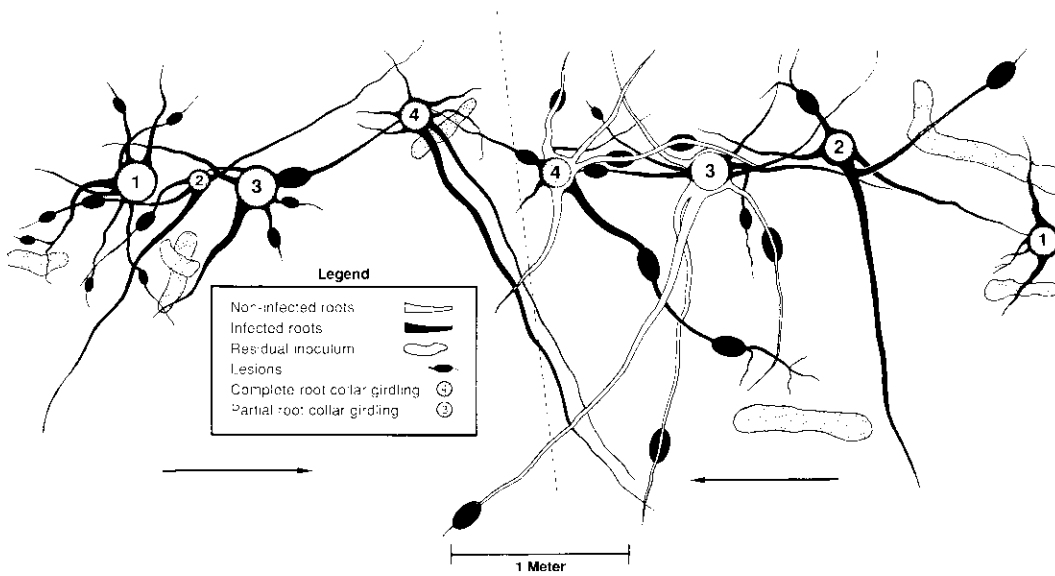


Figure 4. A schematic representation of two disease centers that may have coalesced to form one large center (No. 6). Probable direction of disease spread is denoted by arrows; the outer boundary of each "center" ends at the dotted line. Increasing numbers represent the presumed chronology of death in each center at one year intervals.

Results

The first trees to die in 15 of the 17 disease centers were infected via residual inoculum near or contacting the root collar (Figures 3 and 4). Other dead trees in these centers (Table 1), were infected and subsequently killed by contagion. In one instance, an infected root only 0.5 cm in diameter from a previously killed tree was grafted to the taproot of an adjacent tree, resulting in its infection. The chronosequence of tree death in disease centers No. 4 and No. 11 (Table 1) could not be determined; residual inoculum was not found and needles were absent from all dead trees—indicating that they died at least 3 years prior to our excavations.

TABLE 1. Distribution of infection by *Armillaria* on ponderosa pine roots by source—residual or secondary inoculum

Root Disease Center No. ^{a,b}	No. of Trees and (roots) ^c Examined	No. of Trees Infected From:	
		Residual	Secondary
1	4 (13)	1	3
2	10 (31)	3	7
3	6 (35)	2	4
4	3 (16)	0	3
5	8 (17)	2	6
6	8 (41)	2	5
7	3 (10)	1	2
8	8 (41)	1	7
9	3 (22)	1	2
10	7 (27)	2	5
11	6 (27)	2	6
12	12 (25)	4	8
13	7 (21)	3	4
14	3 (15)	1	3
15	6 (27)	2	4
16	5 (17)	1	4
17	5 (31)	2	3
Total	104 (400)	28	76

^aRoot disease centers were excavated in the summers of 1987 and 1988, 14 to 15 years after stand establishment.

^bThe original source of infection in root disease centers No.'s 4 and 11 could not be traced to residual inoculum (see text).

^cTotal number of primary roots examined on all trees in each center is in parentheses.

Residual material found near root collars of dying or recently killed trees ranged from small, deteriorated root fragments to intact pieces of wood that supported rhizomorphs to rare residual inoculum still containing the stringy, water-soaked wood

typical of advanced decay caused by *Armillaria*. No firm wood from the previous stand was unearthed nor did we strike any large, residual tap roots.

Nearly all residual inoculum found away from the root collars was badly deteriorated with no apparent *Armillaria* mycelial fans or rhizomorphs—it was usually distinctly gray in color, dry, and easily crumbled. This deteriorated inoculum was not associated with root collars or even roots of the present stand, suggesting that only a small portion of the residual inoculum functioned in perpetuating disease. These observations do not, however account for the probability that some fragments of this nature may have been previously functional and in contact with root collars of dead trees pulled between 1973 and 1983. The limited residual inoculum found in excavations around control trees was usually not in contact with roots or was only associated with their distal portions and not root collars.

In two instances, residual inoculum was responsible for infection of trees on opposite edges of reasonably large centers and roots of these trees intermingled with those from other trees in the center (Figure 4). Previously distinct, small centers appear to have coalesced by the time of our examination to form one larger center of now indistinguishable origin (Figure 4).

Rhizomorphs were rarely encountered “free” in the soil and never where they appeared to function as primary inoculum. They were found occasionally beneath the bark on functioning residual inoculum but more often they occurred epiphytically on infected and non-infected roots of the regeneration. When present on infected roots, rhizomorphs usually occurred at lesions and were embedded in the surrounding, resin-soaked soil.

Roots of *Armillaria*-killed trees had abundant lesions and invariably had mycelial fans extending beneath the bark, usually along the entire length of the root. Numerous lesions also were found on roots of control trees; however, all except one of these lesions were occluded and mycelial fans were absent on these roots. On the 372 roots from symptomatic trees, 163 lesions were found. Some roots had only one lesion, others had as many as 10, often in a “beadlike” arrangement along the root. Of the 395 roots examined on control trees, 46 lesions were found with a maximum of three on any one root.

Sampling was inadequate among various stand densities to determine how density might effect the epidemiological process we encountered; however, within the generally high range we studied, density appeared to have little effect on disease development.

Discussion

When treated in 1971, the study site was occupied by a disease-depleted stand of young-growth pine and a few, scattered, old-growth trees. We suspect that the various sanitation treatments left groups of extensively decayed tap roots of pole-sized trees more or less *in situ*. Stronger, medium-sized roots were largely removed from the soil by treatments which also may have scattered smaller, broken pieces of *Armillaria* infected roots somewhat uniformly across the site. It appears to be this latter material that is accountable for most subsequent disease centers and was encountered 16 years later in our excavations.

In unmanaged forest, early mortality of trees regenerating onto areas infested with *Armillaria* develops from infections on roots that are near or in contact with previously killed trees (Adams 1972, 1974; Wargo and Shaw 1985). As seedlings enlarge into trees, contacts among roots of neighboring trees arise, become more common, allow for tree-to-tree spread of the fungus, and lead to patch development of root disease (Shaw 1980). Damage may be confined to a few early losses, but it also can extend marked through subsequent tree-to-tree spread of the pathogen (Rykowski 1984, Wargo and Shaw 1985). Our data show that the dynamics of root disease development in managed stands on infested sites are similar to those in preceding natural stands, even where extensive inoculum removal operations have been conducted.

The development of underground signs and symptoms of fungal attack (i.e. lesions, rhizomorphs, mycelial fans, basal resinosis) on individual trees also appear to be analogous to that described for older natural stands in this area (Shaw 1980), including the occurrence of multiple lesions on a single root. These lesions likely developed as rhizomorphs grew epiphytically along roots and penetrated the cambium through openings in bark scales (Woeste 1956, Redfern 1978). This behavior and contacts with residual inoculum, which was largely exhausted by the time of our

study, and with diseased roots of other young trees may account for the many lesions we encountered on distal portions of root systems. In older trees, Shaw (1980) found distal spread of the fungus from such resinous lesions on live roots which resulted in the fungus residing as a perthophyte. Perhaps the lesions examined in this study were too young or the trees too small to express this condition.

This study demonstrated that the dynamics of infection on sites treated for root disease can change from primary, residual inoculum to contagion in the early stages of stand development, some 10 to 12 years after regeneration. Even small, infected roots on trees killed by contacting residual inoculum can act as secondary inoculum and spread *Armillaria* to other pines.

We assume that these expanding infection centers will continue to enlarge via contagion as the trees age—as they do in untreated, naturally regenerated stands (Shaw 1980, Shaw *et al.* 1976). If the extent of resulting damage is somewhat offset by the development of any possible resistance or tolerance to the fungus as the trees age (Morrison *et al.* 1992), remains to be seen. Relating eventual damage in treated stands to the effectiveness of inoculum reduction through sanitation (Roth and Shaw, unpublished) will be particularly important to managers contemplating initial control actions and in their determining if any follow-up treatments (Morrison *et al.* 1992) may be needed.

These data also can aid efforts to model the dynamics and impact of *Armillaria* root disease in forest stands (Shaw *et al.* 1991, Stage *et al.* 1990) by providing site specific data for equations that calculate the longevity and effectiveness of inoculum and "time to death" multipliers for infected trees (Stage *et al.* 1990). Such local adjustments to the Western Root Disease Model are necessary to increase the accuracy and utility of predictions for disease spread and impact that foresters must make during development of management plans.

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