Variation in Pathogenicity and Virulence of Isolates of *Armillaria ostoyae* on Eight Tree Species

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ABSTRACT

Omdal, D. W., Shaw, C. G., III, Jacobi, W. R., and Wager, T. C. 1995. Variation in pathogenicity and virulence of isolates of *Armillaria ostoyae* on eight tree species. Plant Dis. 79:939-944.

Thirteen isolates representing 10 genotypes of *Armillaria ostoyae*, obtained in northern New Mexico from *Pinus ponderosa, Abies concolor, Pseudotsuga menziesii, Pinus strobiformis, Picea pungens*, and *Populus tremuloides* were used to inoculate seedlings of these hosts and *Larix occidentalis* and *Pinus contorta* var. *latifolia*. At 18 months there were no significant differences ($P > 0.05$) in mortality among the eight hosts, or in virulence across all isolates except for one isolated from *P. pungens*, which failed to infect any trees. After three growing seasons (30 months), significantly more ($P < 0.05$) *P. contorta* var. *latifolia* were infected than either *A. concolor* or *P. menziesii*. *Pinus ponderosa*, the dominant species in this region, did not differ significantly from either of the exotic species (*L. occidentalis* and *P. contorta* var. *latifolia*) in susceptibility to infection or mortality. *Populus tremuloides* was significantly ($P < 0.05$) more tolerant than conifers, being frequently infected but rarely killed. A fungal isolate's ability to incite disease was highly correlated with its production of rhizomorphs ($r = 0.94$, $P < 0.01$). Across all fungal isolates and hosts save *P. tremuloides*, the order of the isolate's ability to incite disease matched its order in killing hosts.

Species of *Armillaria* are some of the most prominent killers and decayers of deciduous and coniferous trees and shrubs (40). To date, nine biological species of *Armillaria* are known on the North American continent (9). These fungi are often referred to by the designation North American Biological Species (NABS), followed by a Roman numeral (1). Frequently, however, the root disease problem is not identified as to species, making interpretation of results from pathogenicity studies and disease conditions in the field difficult.

In this pathosystem involving so many different fungal species and genotypes, as well as innumerable hosts, one must distinguish between pathogenicity and virulence. Here, pathogenicity is defined as the ability to cause disease. Virulence is defined as the relative ability of a pathogen to cause damage on a specific host under certain environmental conditions. Percent mortality and percent mortality given infection will both be considered measures of virulence. An isolate that is unable to cause disease, and is thus nonpathogenic, cannot be virulent. Virulence in previous inoculation trials with *Armillaria* spp. has commonly been based on one or more of the following: amount of root infection, amount of mortality or time to death, or rapidity of spread. Such relatively straightforward measurements are, however, often complicated by the need to consider the role of rhizomorphs as extensions of the experimental inoculum (8).

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Accepted for publication 30 May 1995.

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Hartig (11) first demonstrated the pathogenicity of an *Armillaria* species through an inoculation experiment with trees. In North America, numerous seedling inoculation studies have resulted in valuable information on the infection biology and pathogenicity of *Armillaria* spp. (21,23, 31,33,37).

Sufficient inoculation experiments have been made with a wide enough array of isolates to convincingly demonstrate several pathogens within the genus (8). What is still unclear, however, is which species and genotypes (referred to by many as clones) (10,29,35) of *Armillaria* are pathogenic on which hosts under what conditions, and the virulence of these species and genotypes. Management implications are such that the proper identity of a species or genotype of *Armillaria* spp. on a given site might dictate the planting of less susceptible species.

Armillaria ostoyae (Romagn.) Herink (NABS I) is a widespread and aggressive pathogen on conifers in interior forests of the western United States (40). Inoculation trials with conifer seedlings have, however, given variable results. Gregory (7), Morrison (17), Rishbeth (27), and Shaw (31) have demonstrated generally moderate or high virulence of *A. ostoyae* isolates on conifer seedlings. In contrast, Mallett and Hiratsuka (14) found low virulence toward lodgepole pine seedlings using a number of isolates of *A. ostoyae* from Alberta, Canada.

Because the Jemez District of the Santa Fe National Forest in New Mexico includes forest stands that are infested with, and have a long history of, Armillaria root disease (15), it was selected for this study. Our specific objective was to compare the resistance, to New Mexican isolates of *A. ostoyae,* of ponderosa pine (*Pinus ponderosa* Douglas ex P. Laws. & C. Laws.), white fir (*Abies concolor* (Gordon & Glend.) Lindl. ex Hildebr.), Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), southwestern white pine (*Pinus strobiformis* Engelm.), blue spruce (*Picea pungens* Engelm.), and aspen (*Populus tremuloides* Michx.), all tree species native to the Jemez Mountains. Western larch (*Larix occidentalis* Nutt.) and lodgepole pine (*Pinus contorta* Douglas & Loud. var. *latifolia* Engelm. ex S. Watts) were also tested, larch because of its inherent resistance to Armillaria root disease (18) and lodgepole pine because it is common in similar habitats elsewhere. Trials were performed in a shade house and in the field to see if host age or the artificial conditions imposed by the shade house had any effect on isolate pathogenicity and virulence.

Conifer seedlings are an obvious choice for pathogenicity tests because of their susceptibility to infection by *Armillaria* spp. However, circumstantial evidence from several parts of the world indicates that young trees are more prone to infection from *Armillaria* spp. than older trees of the same species (22,25,27). Field inoculation of established trees is an integral step toward understanding pathogenicity of isolates. Due to failed field inoculations, our analysis will be limited to the shade house trial.

MATERIALS AND METHODS

Test facility. A shade house, located on the campus of Colorado State University and constructed of slatted snow fence, was used for these trials. Photosynthetically active radiation incident on the canopy (measured in the shade house on a cloudless 6 October 1993 at 1500 h) was 720 μmol m^{-2} s^{-1}—a level higher than that used by Entry et al. (3) for fully illuminated seedlings. An automatic irrigation system with overhead sprinklers was installed for even watering. Weed barrier cloth covered 5 to 15 cm of gravel on which the potted trees were placed. Pots were placed edge-to-edge, and unsterilized top soil was packed in the gaps to reduce temperature and moisture fluctuations in the pots.

Plant sources. Seedling type, source, and age for the eight host species are presented in Table 1. Seedlings were potted in April 1989 in 4-liter black plastic pots containing a commercial potting mix composed of 55% Canadian sphagnum peat, 25% perlite, and 20% vermiculite by volume.

Fungal sources and identification. Thirteen isolates of *Armillaria* were collected from the Jemez District of the Santa Fe National Forest, NM (35°50′N, 106°35′W) in September, 1988 (Table 2). Isolates were collected from roots of diseased ponderosa pine, southwestern white pine, blue spruce, Douglas-fir, white fir, and aspen (isolates PP1, PP3, PP4, WP1, WP2, BS1, BS2, DF1b, DF2, WF1, WF2, A2, and A4), all located within approximately 10 km^2. The diploid *Armillaria* isolates were first paired in culture with each other to identify relationships among isolates, and then paired in culture with haploid tester strains of known NABS of *Armillaria* spp. to identify species relationships (9). Recent research indicates that somatic incompatibility appears to be a reliable method for delineating genotypes of *Armillaria* (10,29,35).

Inoculum production and seedling inoculation. Inocula of 13 *Armillaria* isolates growing in 15-cm-long branch segments of red alder (*Alnus rubra* Bong.) were prepared by Shaw's method (31). Over the 3-month incubation period, sterile, distilled water was added to replace water lost due to fungal use or evaporation, to maintain 2.5 cm of water in the jars at all times.

Seedlings were inoculated according to the method of Shaw et al. (34). A section of plastic pipe, 2.0 cm in diameter and 15.2 cm long, had been placed directly adjacent to each seedling during planting. The pipe was removed and an inoculum segment was placed so that it was in contact with the taproot. Autoclaved, noninoculated branch segments were used for control seedlings. Pots were placed in the shade house in May 1989. The experimental design was a randomized complete block, with each of 15 blocks containing eight host species inoculated with 14 isolate treatments (13 isolates and one control).

Pathogenicity of *Armillaria ostoyae*. Inoculated seedlings were assessed periodically for symptoms of root disease from May 1989 to October 1991. Seedlings that died during this period were removed and symptoms and signs of root disease were recorded. Containers with uninoculated seedlings were placed in the sites vacated by dead seedlings to maintain the integrity of the planting environment. We terminated the study after 30 months.

Seedlings were removed from pots and their roots were examined for rhizomorphs. Rhizomorphs were considered "extensive" if they occurred on the exterior of the root ball, "moderate" if they were restricted to the inoculum stick, or "absent." Roots were carefully extricated, by hand, from soil and dipped in water to remove any remaining potting mix. Roots were then examined for rhizomorphs that were in contact with the roots and had possibly initiated infections. Also noted were lesions or resin soaking, either at the root collar or elsewhere on the root systems. If a lesion or resin-soaked region was discovered, then the presence or absence of rhizomorphs was noted and bark was removed to look for a mycelial fan in the cambial region. A final status was determined for each seedling: dead, infected (i.e., fans in the cambial region), or healthy. Chlorotic foliage and mycelial fans in the cambial region were used to identify infected seedlings; cause of death or decline was also assessed.

Isolations from 101 seedlings and inoculum sticks, representing each isolate of *Armillaria* sp. and tree species, were made onto ortho-phenylphenol medium (30) without antibiotics. A partially balanced, incomplete block design (2) was used to select seedlings and inoculum sticks for these isolations. If selected seedlings were infected, then isolations were attempted from seedling roots and the inoculum stick. If a selected seedling was not infected, then the next diseased seedling of the same species was examined. There was considerable bacterial contamination in these isolations. We transferred the most seriously contaminated cultures to media containing either streptomycin (25 μg/ml), ampicillin (100 μg/ml), or kanamycin (50 μg/ml).

Statistical analysis. Differences in ability to cause infection and mortality among *Armillaria* isolates, and consistency of effect among host species, were analyzed using logistic regression (36). Because interaction between fungal isolates and tree species was nonsignificant ($P > 0.05$), differences in infection and mortality were analyzed by the comparison of many proportions procedure (5). The association between disease and rhizomorph production was examined using Pearson's correlation (36). Differential survival among species was assessed by comparing life tables between all pairs of species (12). This set of test results was interpreted using Holm-adjusted *P* values to obtain an overall Type I error of $\alpha = 0.05$ (42).

RESULTS

Isolate identification. The 13 isolates were found to represent 10 different genotypes of *A. ostoyae* (Table 2). Somatic incompatibility studies indicated that isolates WP1 and PP1, DF2 and PP4, and WF1 and PP3 were somatically compatible pairs and were probably of the same genotype, respectively. These species and genotype identifications were independently confirmed (G. McDonald, *personal*

Table 1. Sources of seedlings used in the pathogenicity trial

Species	Nursery	Age	Source	Elevation (m)
Western larch	Cour d'Alene, Idaho	2-0 BR[x]	Flathead, Mont.	1,515
Blue spruce	Ft. Collins, Colo.	2-0 BR	NA[y]	NA
Aspen	Ft. Collins, Colo.	2-0 C[z]	Cameron Pass, Colo.	2,575
Lodgepole pine	Bessey, Nebr.	2-0 BR	Pike-San Isabel, Colo.	3,182
White fir	Wind River, Oreg.	2-0 BR	Dechutes, Oreg.	1,818
Douglas-fir	Albuquerque, N.M.	3-0 BR	Lincoln, N.M.	2,727
Ponderosa pine	Albuquerque, N.M.	2-0 BR	Gila, N.M.	NA
SW white pine	Albuquerque, N.M.	2-0 BR	Lincoln, N.M.	NA

[x] Bare root.
[y] Not available.
[z] Containerized.

Table 2. Source and genotype identity of *Armillaria ostoyae* isolates from the Jemez Mountains, New Mexico

Isolate[z]	Source	Genotype
WP1	Infected roots of white pine	1
PP1	Infected roots of ponderosa pine	1
DF2	Infected roots of Douglas-fir	2
PP4	Infected roots of ponderosa pine	2
DF1b	Roots of dead Douglas-fir	3
WF1	Infected roots of white fir	4
PP3	Infected roots of ponderosa pine	4
BS2	Infected roots of blue spruce	5
BS1	Roots of dead blue spruce	6
WF2	Roots of dead white fir	7
A2	Roots of dead aspen	8
A4	Roots of dead aspen	9
WP2	Infected roots of white pine	11

[z] Letters abbreviate host from which the isolate was obtained.

communication). A logistic regression was performed on the infection proportions of these isolates. No statistical difference in the ability to infect their hosts was found between isolates WF1 and PP3 (P = 0.9168), WP1 and PP1 (P = 0.4811), and DF2 and PP4 (P = 0.214).

Pathogenicity and virulence. In the shade house, isolates of *A. ostoyae* infected 574 of 1,532 (38%) inoculated seedlings, though more than 99% of all inoculum sticks appeared to be viable. Due to lost labels, 28 inoculated seedlings were omitted from the analysis. Infected seedlings were identified first by foliar symptoms (chlorosis) and then by mycelial fans under the bark. The first seedling killed by *A. ostoyae* appeared 5 months after inoculation (Fig. 1). Thirty-nine percent (226/574) of the infected seedlings expressed no aboveground symptoms during 30 months of exposure to *A. ostoyae*. Disease progress appeared to be slow since it was not until 12 months into the study that more than one seedling died during a month.

However, not all isolates were pathogenic (Table 3). Isolate BS2, obtained from an infected blue spruce, was unable to infect any seedlings, including blue spruce. All other isolates were pathogenic, but not on all hosts. We find it interesting that, even though there was no significant interaction ($P > 0.05$) between fungal isolates and tree species, isolate DF1b did not infect white pine, and both isolates BS1 and WP2 failed to infect white fir.

Both frequency of disease among hosts and frequency with which isolates incited disease varied significantly ($P < 0.05$) (Table 3). White fir became infected much less frequently than most other host tree species, regardless of fungal isolate. Although there was a great deal of variability among isolates, WF2 was the most virulent, inciting disease more frequently than most of the other isolates (Table 3).

Seedling mortality differed significantly among fungal isolates ($P < 0.05$) (Table 3). Isolates that incited disease most frequently also killed their hosts most frequently. With aspen as the exception, there was a similar species ranking for both infection and mortality. Although aspen frequently became infected, the fungus rarely killed this host.

With one exception (isolate BS2), percent mortality of hosts given infection by isolates of *A. ostoyae* was indistinguishable (Table 3). The hosts, on the other hand, showed significant differences ($P < 0.05$) in percent mortality once they became infected (Table 3). Many infected lodgepole pine and western larch seedlings died, whereas aspen was more tolerant and rarely succumbed to the pathogen. This "mortality given infection" category reduces the variation among isolates, since 12 of 13 are not significantly different in spite of differences of 15 to 72% in infection and 4 to 37% in mortality. As such, this measure of isolate virulence may have limited utility.

Survival curves varied greatly among the different hosts (Fig. 1). Not only did fewer aspen perish throughout the study, but it took nearly 28 months for the first one to die. At the other extreme, lodgepole pine died at a significantly faster rate ($P < 0.05$) than most other species. In this study we observed a seasonal relationship with mortality (Fig. 1). The percentages of trees killed during the four seasons were: December to February, 2%; March to May, 20%; June to August, 31%; September to November, 47%.

In spite of the close contact between inoculum sticks and host root systems, generally the taproot, production of rhizomorphs was nearly a requisite for infection —as indicated by the strong correlation between the ability of an isolate to incite disease and its production of rhizomorphs ($r = 0.94$, $P < 0.01$). Those isolates that most frequently produced rhizomorphs (i.e., WP1, WP2, WF1, A2, PP3, WF2,

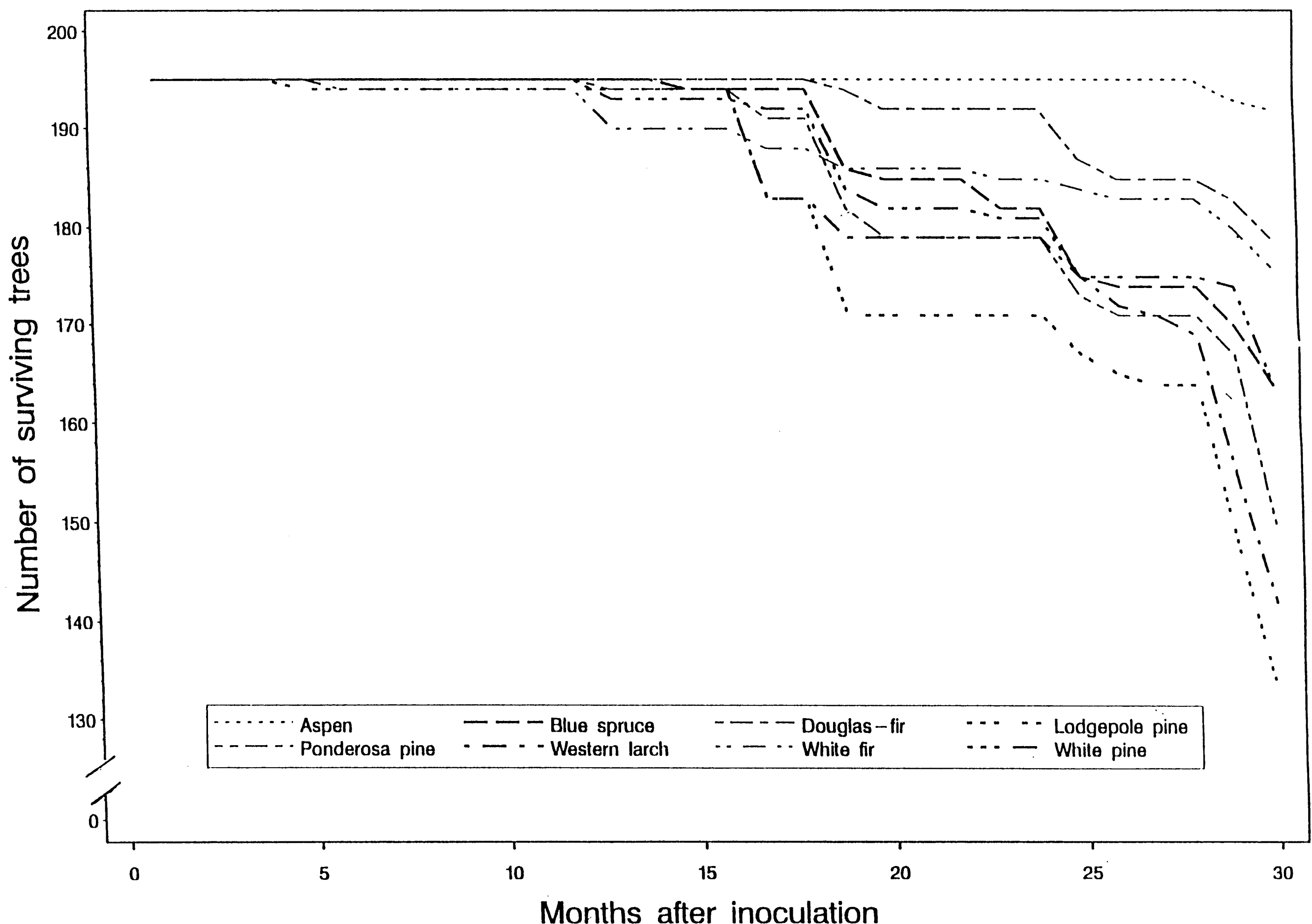

Fig. 1. Mortality of eight tree species during the 30 months after inoculation with 13 isolates from 10 genotypes of *Armillaria ostoyae*.

PP1) incited the highest frequency of disease (Table 4). Isolate BS2, which was unable to infect any host, produced significantly fewer ($P < 0.05$) rhizomorphs than any other isolate (Table 4). In only 1% of 574 cases was disease present in the apparent absence of rhizomorphs. Similarly, all of the lethal lesions associated with the 258 seedlings killed by *A. ostoyae* in this experiment appeared to have been initiated by rhizomorphs.

Reisolation from inoculum segments and diseased seedlings. After three growing seasons, each isolate was successfully reisolated from an inoculum stick and successful reisolations were made on 54 of 101 (54%) attempts. On no occasion was a fungus other than the one inoculated onto the stick recovered. One of the 120 uninoculated control sticks was found with external rhizomorphs, but no fungal decay. Failure to obtain a clean culture from these rhizomorphs precluded its identification.

We also attempted 34 reisolations from diseased seedlings and on 26 (76%) occasions we were successful. These 26 were then paired in culture with the isolate with which each respective seedling was originally inoculated; in every instance, isolates were somatically compatible with the fungal isolates with which the seedlings were originally inoculated. In all of the unsuccessful reisolations, contamination, rather than lack of *A. ostoyae* viability, appeared to be the cause of nonrecovery.

DISCUSSION

Pathogenicity and virulence. The overall levels of infection and mortality caused by *A. ostoyae* in this experiment were generally higher than those observed by others (3,14). We found considerable variation in the virulence of isolates, measured as percent mortality, within a local population of *A. ostoyae.* Although all but one of 13 isolates were able to cause infection on eight tree species, not all of these isolates caused mortality on all tree species. Other studies also indicated that isolates that caused the most infection also killed the most trees (19,33).

Our results suggest that the variation in pathogenicity and virulence may be the result of each isolate's relative infection efficiency. All pathogenic isolates were able to kill their hosts; the differences among isolates were the rates at which they killed their hosts. Similar within-species variability has been found for the ability of *Armillaria* species, including *A. ostoyae*, to utilize alcohols and phenolic compounds (32). Thus, there is a need to evaluate several isolates before conclusions are made about the pathogenicity or virulence of species or genotypes of *Armillaria.*

That isolate BS2 of *A. ostoyae* was nonpathogenic on all host species tested is perplexing, as it was isolated from an infected tree. Although this isolate appeared to incite disease in the field, at least on the blue spruce from which it was isolated, it may have been acting saprobically. Isolate BS2 remained viable on four of the six (67%) inoculum sticks examined, and produced rhizomorphs on 26% of the 120 sticks inoculated. The production of rhizomorphs by this isolate (BS2) was significantly less ($P < 0.05$) than that of all other isolates. In inoculation trials, North American and European isolates of *A. ostoyae* have generally been moderately or highly virulent toward conifer seedlings (8). We know of no other pathogenicity trials of this duration in which an isolate of *A. ostoyae* was nonpathogenic.

Seasonal patterns of mortality, similar to the one we observed, were observed by Wilbur et al. (41) on peach trees inoculated with *A. mellea* sensu lato. However, they noted a sharp reduction in mortality in the fall months whereas, in our study, mortality remained high throughout October. Minimal mortality occurred during winter months, which may coincide with the dormant period of both host and pathogen. Disease development and mortality were highest in late summer and fall. Perhaps the seedlings were entering dormancy and were unable to defend against the pathogen, which was still actively growing.

Morrison et al. (18) suggest that there is little difference in susceptibility to *A. ostoyae* among field-grown conifers less than 15 years old. We found this may not al-

Table 3. Mortality/infection of eight host species caused by 13 isolates of *Armillaria ostoyae* 30 months after inoculation of the 2-0 and 3-0 seedlings in a shadehouse in Colorado

	Host inoculated[u]											
Isolate	White fir	White pine	Ponderosa pine	Douglas-fir	Aspen	Western larch	Blue spruce	Lodgepole pine	Total	Infection (%)[v]	Mortality (%)	Mortality given infection (%)[w]
BS2	0/0[x,y]	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0 a	0 a	0 a
DF2[z]	0/0	3/4	0/2	0/2	0/5	3/5	0/4	1/4	7/26	22 bc	6 abc	27 ab
PP4[z]	1/2	0/2	1/1	1/2	0/4	0/2	1/3	1/3	5/19	16 b	4 ab	26 ab
BS1	0/0	0/1	0/3	0/1	0/3	3/4	1/2	1/4	5/18	15 b	4 ab	28 ab
A4	0/1	0/1	3/5	0/1	0/6	3/5	0/2	1/3	7/24	20 bc	6 abc	29 ab
WP1[z]	0/3	3/7	6/10	2/2	0/11	10/10	3/7	8/13	32/63	53 de	27 cde	51 b
PP1[z]	2/6	3/9	4/10	1/7	1/6	7/11	2/9	8/9	28/67	57 de	24 bcde	42 b
WP2	0/0	3/7	4/5	3/7	0/8	5/8	4/6	4/8	23/49	42 cd	20 bcde	47 b
WF1[z]	4/5	4/5	6/13	0/5	0/9	7/9	3/9	4/10	28/65	57 de	25 cde	43 b
PP3[z]	1/4	4/8	9/10	0/4	1/10	3/9	6/12	12/13	36/70	58 de	30 de	51 b
A2	5/7	6/10	3/6	1/9	0/8	4/6	4/10	8/10	31/66	57 de	27 cde	47 b
WF2	3/6	6/9	7/12	5/8	1/11	7/11	5/13	9/13	43/83	72 e	37 e	52 b
DF1b	0/1	0/0	2/3	3/7	0/1	2/4	2/4	4/4	13/24	21 bc	11 abcd	54 b
Total	16/35	32/63	45/80	16/55	3/82	54/84	31/81	61/94	258/574			
Infection (%)	18 r	33 rst	42 st	28 rs	42 st	46 st	42 st	50 t				
Mortality (%)	8 rs	17 st	23 t	8 rs	2 r	29 t	16 bt	32 t				
Mortality given infection (%)	46 st	51 st	55 st	29 s	4 r	64 r	38 bt	65 t				

[u] Number of original seedlings inoculated for each genotype x seedling species = 15; Total number of seedlings/tree species = 195; Total number of seedlings for each fungal isolate = 120.

[v] Numbers followed by different letters differ significantly ($P < 0.05$); "r" through "t" relate to rows (overall host differences) and a thru e relate to columns (overall isolate differences).

[w] Represents the percentage of infected trees that were killed by *A. ostoyae.*

[x] Numerator equals the number of infected seedlings that died.

[y] Denominator equals the number of infected seedlings.

[z] Isolates DF2 and PP4, WP1 and PP1, and WF1 and PP3 were shown through somatic incompatibility studies to represent the same genotypes, respectively.

ways be the case. For example, lodgepole pine was significantly ($P < 0.05$) more susceptible than either white fir or Douglas-fir to *A. ostoyae* (Table 3). To attribute this result to lodgepole pine being an exotic host may be spurious, as the seed sources for the other hosts included in this study were not from the site and therefore had no more previous exposure to the pathogen's genotype than did lodgepole pine. Had the seedlings in this trial been intentionally stressed (e.g., drought or insect feeding), differences among species might not have been so apparent, as stress has been shown to increase seedling susceptibility to attack by *Armillaria* spp. (3,20). In fact, the high number of asymptomatic, infected seedlings suggests that these seedlings experienced little stress during the experiment.

The time to symptom expression varies considerably in inoculation studies with *Armillaria* spp. Several studies indicate that certain isolates generally take longer than others to cause visible, aboveground symptoms of infection (7,24). Two-year-old, field-grown, bare-root lodgepole pine seedlings showed symptoms of disease within 1 month of being inoculated with *A. mellea* sensu stricto (14), and most of the seedlings that died did so within 25 days of inoculation. MacKenzie and Shaw (13) observed mortality within 6 months of planting, and within 27 months *Armillaria* had killed 16% of the Monterey pine (*Pinus radiata* D. Don) seedlings planted on a site freshly cleared of indigenous mixed hardwood forest. In contrast, Patton and Riker (21) found that in field inoculations with red pine (*Pinus resinosa* Aiton) and eastern white pine (*Pinus strobus* L.), the first infection occurred 27 months after inoculation, whereas the last recorded infection was 96 months after inoculation.

The length of time that a pathogenicity trial is monitored can affect the pathogenicity and virulence rankings. We began to observe considerable mortality caused by *A. ostoyae* only after 15 to 18 months (Fig. 1). Had we terminated the experiment at 18 months, the susceptibility ranking of the hosts would have differed markedly from that obtained after 30 months. Furthermore, we would not have had as clear an understanding of the virulence of the fungal isolates, because by 18 months relatively few seedlings expressed disease symptoms, and even fewer had been killed. At 18 months, there were no significant differences ($P > 0.05$) in mortality among the eight species and no significant differences ($P > 0.05$) in virulence across all 13 fungal isolates. Similarly, three isolates (BS1, BS2, and A4) had not induced symptoms on any hosts, and Douglas-fir and aspen had yet to express any symptoms.

Table 4. Rhizomorph production by 13 isolates of *Armillaria ostoyae*

Isolate	Inoculum sticks producing rhizomorphs (%)[y]	Infections (%)[z]
BS2	26 a	0 a
BS1	55 b	15 b
DF1b	56 b	21 bc
A4	56 b	20 bc
DF2	67 b	22 bc
PP4	68 b	16 b
WP1	82 c	53 de
WP2	86 c	42 cd
WF1	86 c	57 de
A2	88 c	57 de
PP3	92 c	58 de
WF2	93 c	72 e
PP1	94 c	57 de

[y] Samples, within the same column, followed by different letters differ significantly ($P < 0.05$).

[z] Across all species, see Table 3.

Production of rhizomorphs may influence the optimum duration of inoculation trials with *Armillaria* spp. (7). Gregory (7), Redfern (24), and Rishbeth (28) indicate that some isolates take longer than others to cause visible, aboveground symptoms of infection. Shaw (31), testing the pathogenicity of *A. ostoyae* isolates on seedlings of ponderosa and Monterey pines, observed a significant positive correlation between the percentages of inoculum segments producing rhizomorphs and the percentage of seedlings infected. We also found a strong positive association ($P = 0.0001$) between rhizomorph production and virulence and the presence of rhizomorphs at lethal lesions. This relationship was expected because rhizomorphs are considered the main means of infection in temperate regions (26).

Although pathogenicity is not necessarily related to rhizomorph production, our results do suggest that virulence is related to rhizomorph production. These results contrast markedly with Mallett and Hiratsuka (14) in which significant mortality was observed even though rhizomorphs were absent from branch segments infested with *A. ostoyae* and from infected seedlings. Perhaps conditions in their greenhouse and growing medium were conducive to mycelial growth and infection, a situation not likely in the field.

There does not appear to be a relationship between the hosts from which the different fungal isolates were collected and the susceptibility of that host to infection. For example, the isolates collected from white fir were the most virulent, yet white fir was significantly less susceptible to infection than most other species (Table 3). Similarly, ponderosa pine was moderately susceptible to infection, yet isolates collected from ponderosa pine were highly variable in their virulence (Table 3). One probable explanation for this poor association may be that there is little host/pathogen specificity. For example, multiple isolates of the same genotypes (1,2,4) were originally obtained from two different hosts (Table 2).

The behavior of white fir in the shade house differs markedly from that observed in the field. Strong field evidence suggests that *Abies* spp. are highly susceptible to *Armillaria* (4,16). In our experiment, however, white fir was the least susceptible to infection by *A. ostoyae* (Table 3).

White fir trees in the field are subjected to stresses such as drought and insect defoliation that were not imposed on the seedlings in this trial. These stresses may increase their susceptibility to Armillaria root disease (39). In contrast, two studies (20,38) suggest that there is a reduction in the frequency of infection by *A. ostoyae* as defoliation stress increases. However, these studies are limited by their lack of adequate replication and by artificial means of defoliation.

Our pathogenicity study was completed on young trees in non-forest conditions, so applying our findings directly to the field is not feasible. Thus, confirming field studies are needed to see how management of Armillaria root disease may be influenced by tailoring the tree species planted based on the pathogenicity and virulence of pathogen genotypes present on a site, or determining the host species present on a site that may serve as symptomless carriers (i.e., aspen on sites where conifers are harvested). Similarly, reportedly resistant species such as larch (18) need to be field tested against the pathogen genotypes present.

As part of this study, we attempted field inoculations in the Jemez Mountains with four isolates of *A. ostoyae*. Utilizing *A. ostoyae*–colonized branch segments of red alder, we inoculated three roots on each of 83 pole-sized trees. We have not reported our results since after 3 years in the field only 10% of the inoculated roots became infected. Other researchers have experienced similar field inoculation failures with *Armillaria* spp. (6). Thus, a workable inoculation technique is needed for dry ecosystems so further field pathogenicity studies can be completed.

This study clarifies the pathogenicity and virulence of *A. ostoyae* on eight forest tree species with which it is frequently associated. Pathogenic variation within the species may be considerable, which suggests that it may be spurious to report findings of *A. ostoyae*, and probably other *Armillaria* spp., at the species level. Equally variable is the virulence of genotypes found within the species. Variations in both virulence and pathogenicity are suggestive of the need to evaluate the responses of several isolates before one can draw meaningful inferences about the character or nature of species and genotypes of *Armillaria*.

ACKNOWLEDGMENTS

The assistance of Rudy King, Deb Carter, and Jill Janssen with study design and statistical analysis is greatly appreciated.

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