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Armillaria altimontana Is Associated with Healthy Western White Pine (*Pinus monticola*): Potential *in Situ* Biological Control of the Armillaria Root Disease Pathogen, *A. solidipes*

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Abstract: Research Highlights: Two genets of *Armillaria altimontana* Brazee, B. Ortiz, Banik, and D.L. Lindner and five genets of *Armillaria solidipes* Peck (as *A. ostoyae* [Romagnesi] Herink) were identified and spatially mapped within a 16-year-old western white pine (*Pinus monticola* Doug.) plantation, which demonstrated distinct spatial distribution and interspecific associations. Background and Objectives: *A. solidipes* and *A. altimontana* frequently co-occur within inland western regions of the contiguous USA. While *A. solidipes* is well-known as a virulent primary pathogen that causes root disease on diverse conifers, little has been documented on the impact of *A. altimontana* or its interaction with *A. solidipes* on growth, survival, and the Armillaria root disease of conifers. Materials and Methods: In 1971, a provenance planting of *P. monticola* spanning 0.8 ha was established at the Priest River Experimental Forest in northern Idaho, USA. In 1987, 2076 living or recently dead trees were measured and surveyed for *Armillaria* spp. to describe the demography and to assess the potential influences of *Armillaria* spp. on growth, survival, and the Armillaria root disease among the study trees. Results: Among the study trees, 54.9% were associated with *Armillaria* spp. The genets of *A. altimontana* and *A. solidipes* comprised 82.7% and 17.3% of the sampled isolates (n = 1221) from the study plot, respectively. The mapped distributions showed a wide, often noncontiguous, spatial span of individual *Armillaria* genets. Furthermore, *A. solidipes* was found to be uncommon in areas dominated by *A. altimontana.* The trees colonized by *A. solidipes* were associated with a lower tree growth/survival and a substantially higher incidence of root disease than trees colonized only by *A. altimontana* or trees with no colonization by *Armillaria* spp. Conclusions: The results demonstrate that *A. altimontana* was not harmful to *P. monticola* within the northern Idaho planting. In addition, the on-site, species-distribution patterns suggest that *A. altimontana* acts as a long-term, *in situ* biological control of *A. solidipes*. The interactions between these two *Armillaria* species appear critical to understanding the Armillaria root disease in this region.

Keywords: *Armillaria* biological control; *Armillaria* demography; *Armillaria ostoyae*; NABS X; *Pinus monticola*

1. Introduction

Armillaria root diseases are among the most damaging and broadly distributed root diseases of diverse woody hosts around the world (e.g., [1–3]). *Armillaria solidipes* Peck (as *A. ostoyae* [Romagnesi] Herink) is well-known as a virulent primary pathogen that causes root disease on diverse conifers within the inland western regions of the contiguous USA [4], where *A. altimontana* Brazee, B. Ortiz, Banik, and D.L. Lindner (formerly North American Biological Species X, NABS X) frequently co-occur [5]. Although *A. altimontana* is frequently considered a weak or secondary pathogen [6], little has been documented about its impact on tree health and growth. Furthermore, *A. altimontana* and *A. solidipes* are frequently found in association with western white pine (*Pinus monticola* Doug.) [5].

Western white pine is a widely distributed seral species in western North America that occupies diverse environmental conditions and habitats from ca. 52° N to 36° N latitude and 113° W to 126° W longitude [7,8]. In areas such as northern Idaho, USA, western white pine's distribution declined by 90% in fewer than 70 years in the mid-20th century [9]. This decline was due to several factors, including white pine blister rust caused by an introduced pathogen (*Cronartium ribicola* J.C. Fisch), mountain pine beetle (*Dendroctonus ponderosae* Hopkins), fire suppression, and logging [10].

Because western white pine is economically valuable and plays a critical ecological role in many forest ecosystems, genetic studies have been devoted to restoring western white pine in ecosystems where it once predominated (e.g., [8,10–12]). Successful restoration efforts must consider western white pine sources that are resistant to white pine blister rust and that are able to thrive in areas prone to Armillaria root disease, which is prevalent in northern Idaho [4]. Although previous studies have examined the adaptation to the climate using geographic features as surrogates (e.g., [13–17]) and the resistance to white pine blister rust (e.g., [18–20]), little is known about the impact of Armillaria root disease on planted western white pine.

The objectives of this study were to identify *Armillaria* isolates associated with planted western white pine at the species and genet (vegetative clone) level, to describe species/genet demography including spatial distribution, and to determine the potential influence of each *Armillaria* species and their interactions on the growth and survival of western white pine to provide baseline information for the management of Armillaria root disease in western white pine ecosystems.

2. Materials and Methods

2.1. Planting Site, Plant Materials, Planting Design, Height and Survival Measurements, and Criteria for Determining Tree Health

In 1979, Steinhoff [14] first described the Ida Creek provenance test, which was established to assess the variation in growth and survival among seed sources of western white pine from different elevations in the interior northwestern USA. The provenance test used seeds from wind-pollinated cones collected from 48 natural western white pine provenances (n = 2286 trees) from four regions (i.e., Clearwater, Kaniksu, Trestle Creek, and St. Joe) in northern Idaho, USA. The northern Idaho seed sources occurred between ca. 46° N and 49° N latitude and ranged in elevation from ca. 455 m to 1585 m [14]. The provenance test also included seeds collected from eight additional provenances (n = 86 trees) from western Washington and one provenance from the Blue Mountains, northeastern Oregon.

The seedlings were established and maintained in a replicated nursery trial for 3 years. In 1971, the seedlings from the nursery trial were outplanted (as 3–0 seedlings stock) to a field plot known as "Ida Creek" within the Priest River Experimental Forest in northern Idaho, USA. The Ida Creek field plot was ca. 0.8 ha in size and was located on a north-facing slope at an elevation of 970 m.a.s.l. (northeast plot corner located at ca. 48°21′48.75″ N and 116°49′25.36″ W). In the year prior to planting, Ida Creek was cleared of naturally grown, mature western white pine, western larch (*Larix occidentalis* Nutt.), western hemlock (*Tsuga heterophylla* [Raf.] Sarg.), grand fir (*Abies grandis* [Douglas ex D. Don] Lindley), and western redcedar (*Thuja plicata* Donn ex D. Don) with only a minimal disturbance.

The seedlings where planted with a spacing of 1.2 m × 1.2 m. For each provenance, the seedlings were planted in 25-tree-row plots aligned from east to west that were randomized within two blocks. Each 25-tree-row plot included seedlings from five families arranged as five tree-row plots per family (n = 2372 trees); however, all provenances were not fully represented after planting.

Measurements on the survival, height growth, and diameter at breast height (DBH) growth were collected over multiple years with the last measures taken at the end of the 1987 growing season. Surveys of the *Armillaria*-associated mycelial fans, wood rot, rhizomorphs, and basal resinosis were conducted in 1987, and the blister rust bole cankers were recorded in 1982 and 1987. Based on missing tree data, a ca. 12% mortality is assumed to have occurred from the first year of establishment (1971) through 1982. Because the cause of mortality was largely unknown, this early mortality (n = 296 trees), which may have also included tree positions that were never planted, was excluded from the analysis.

2.2. Armillaria Survey and Isolate Collection

During 1987, 16 years after outplanting, the root collar and major lateral roots of each tree within the planting (2076 living or recently dead trees = 2372 total trees—296 from early mortality) were inspected for *Armillaria* by excavating 0.3-m deep for a distance of ca. 0.5- to 1-m away from the stem in three cardinal/ordinal directions. The trees were considered to have signs and/or symptoms of Armillaria root disease only if they were (1) alive with mycelial fans, wood rot, or both or (2) recently dead and displayed basal resinosis in addition to mycelial fans, wood rot, or both, which indicates that the tree was alive when it was infected with *Armillaria* spp. Live trees with no mycelial fans or wood rot where considered healthy even if epiphytic rhizomorphs were found on the roots. Epiphytic rhizomorphs, mycelial fans, and rotten wood were used as sources to establish 1221 isolates in culture following the methods of McDonald et al. [21] (Table 1).

2.3. Armillaria Genet and Species Identification

Following the isolations of *Armillaria* in 1987, somatic incompatibility pairing tests among all isolates were used to identify individual genets that comprised the isolates, and isolates that fused in culture to form homogeneous colonies were considered as belonging to the same genet (e.g., [22–24]) (Table 1). The somatic pairing of each genet was used to separate the genets at the species level [22,23,25–34]. The initial species identification was performed by the somatic pairing (e.g., [28,29,32,35]) of each genet 2–16 times against well-characterized reference isolates of *A. solidipes* (15–32 reference isolates), *A. sinapina* Bérubé and Dessur (two reference isolates), and *A. altimontana* (8–20 reference isolates considered as NABS X when the pairing tests were performed), which were available at the time. The somatic pairing test was replicated once (Table S1). Each somatic pairing reaction was interpreted twice by separate individuals, and the pairing tests were coded to allow "blind" readings (i.e., the genet and reference isolate were unknown to the reader). Isolate pairings that resulted in intraspecific compatibility (colorless antagonism) were considered as belonging to the same species. Pairings that resulted in a black line (pseudosclerotial plate) or other incompatible reactions were considered as belonging to different species [35]. The species identification of each *Armillaria* genet was further verified by the DNA sequencing of a partial translation elongation factor-1α (*tef1*) for at least one isolate following the methods of Ross-Davis et al. [36]. Multiple *Armillaria* isolates (derived from rhizomorphs, mycelial fans, and rotten wood) of the same genet on the same tree were considered as a single isolate for the analyses of pathogenicity and impacts on tree growth and survival.

Table 1. The *Armillaria* isolates, genets, and species collected in 1987 from 2076 western white pine (*Pinus monticola*) planted in a 0.8-ha site at the Priest River Experimental Forest in northern Idaho, USA.

Species	Genet	# of Isolates	GenBank Accession No. [a]
A. altimontana	G1	63	MH879011
	G2	947	MH879012
	Total	**1010**	
A. solidipes			
	G3	11	MH879013
	G4	26	MH879014
	G5	19	MH879015
	G6	27	MH879016
	G7	128	MH879017
	Total	**211**	
A. altimontana & A. solidipes			
	Total	**1221**	

[a] The partial translation elongation factor 1α (*tef1*) sequence.

2.4. Statistical Analyses

Whether the *Armillaria* species were spatially autocorrelated was assessed using Moran's I using the spdep [37] package in R [38]. Moran's I is a statistical measure of spatial autocorrelation. A Moran's I value of 0 indicates a perfect random dispersion. Increasingly more positive values indicate an increasing clustering with the value of 1 indicating a perfect clustering.

The relationship between the *Armillaria* status (i.e., trees colonized by *A. altimontana*, *A. solidipes*, both *Armillaria* species, or neither *Armillaria* species) and the height growth, DBH growth, and survival of the study trees was assessed using generalized linear mixed-effects models (GLMM) using the lme4 package [39] in R. GLMM was used because its treatment of random effects (e.g., genetic and spatial variation) can provide a better fit of the data than the traditional ANOVA [40,41]. The model selection was based on minimizing the Akaike Information Criterion (AIC). The GLMM equation used for analysis of height was

$$Y_{ijklmno} = U + A_i + R_j + F[P(R)]_{jkl} + R(B)_{mo} + C(B)_{no} + e_{ijklmno} \quad (1)$$

where $Y_{ijklmno}$ is the observed ith tree of the jth maternal family in the kth provenance in the lth region of seed origin in the mth row in the nth column in the oth block; U is the overall mean; A_i is the *Armillaria* status effect; R_j is the effect of the region of seed origin; $F[P(R)]_{jkl}$ is the effect of the maternal tree family within the provenance and region of origin; $R(B)_{mo}$ is the row effect (north to south) within the block; $C(B)_{no}$ is the column effect (east to west) within the block; and $e_{ijklmno}$ is the random residual error. The *Armillaria* status and region of seed origin were treated as categorical fixed effects. The effect of the family was treated as a categorical random; the row and column effects were treated as a continuous random. The main effects of the seed source elevation and provenance were treated as random but were excluded from the final models because neither improved the model fit in comparison to the models that excluded the variables. The models for DBH and survival followed the above description for the height model but excluded the region of seed origin effect. In addition, the DBH model included a site density effect (the number of neighboring trees (0 to 8) for each tree) which was treated as a continuous random. The model residuals were checked for normality and homoscedasticity by a visual inspection of the residual plots (Figure S1). The statistical significance of the fixed effects was tested by comparing the likelihoods of the nested models using a maximum likelihood. The likelihood ratio testing for GLMMs with random effects having few levels can be unreliable [40,42]. Thus, the region (n = 4) was treated as a fixed effect. In addition, the likelihood ratio tests were confirmed with bootstrap tests using the pbmn function [43] in R. The differences between the *Armillaria* status groups were

separated by Tukey-adjusted comparisons using the emmeans function [44] in R, which is appropriate for unbalanced group data (i.e., *Armillaria* status) [45]. The GLM function in R was used for a logistic regression to assess the significance of colonization by *Armillaria* species with disease symptoms.

Provenances from Washington and Oregon were excluded from these regression analyses because of a limited representation. Therefore, the regression analyses were exclusively focused on seed sources originating from northern Idaho that were collected from the same general region as the study site. In addition, white pine blister rust infections were identified in 2% (n = 49 trees) and 12% (n = 237 trees) of trees during the 1982 and 1987 assessments, respectively (Note that 41 trees infected with blister rust in 1982 survived to be included in the tally for 1987). White pine blister rust was not significantly ($p > 0.05$) correlated to the occurrence of *Armillaria* spp. (data not shown). Because white pine blister rust can cause decreased growth and increased mortality (e.g., [46]), trees with blister rust (n = 245) were removed from the statistical analyses of the *Armillaria* impact to avoid confounding blister rust influences on the growth and survival of western white pine. After excluding early dead, "control" trees (presumably planted to maintain spacing within the study), nonlocal, and white pine blister rust-afflicted trees, a total of 1694–1758 trees, depending on missing data, were included in the regression analyses (Table S2). The data used for the analyses are available in the Supplementary Materials (Table S3).

3. Results

3.1. Armillaria Species, Genets, and Isolates

Armillaria was found in association with 54.9% (n = 1139 trees) of the 2076 western white pine trees (including nonlocal, control, and white pine blister rust-afflicted trees) at the Ida Creek plantation. A total of 1221 *Armillaria* isolates were recovered and established in culture. The somatic pairing tests demonstrated that all isolates collected across the 0.8-ha planting were comprised within seven genets, which were numbered G1–G7 (Table 1). The results of the original somatic pairing tests are shown in the Supplementary Materials (Table S1). In general, Genets 1 and 2 showed ≥89.5% intraspecific compatibility with the *A. altimontana* reference isolates, ≤3.7% compatibility with the *A. solidipes* reference isolates, and ≤25% compatibility with the *A. sinapina* reference isolates, which indicated that Genets 1 and 2 belong to *A. altimontana*. In contrast, Genets 3–7 showed 46.1%–78.1% compatibility with the *A. solidipes* reference isolates, ≤12.5% compatibility with the *A. sinapina* reference isolates, and ≤6.3% compatibility with the *A. altimontana* reference isolates, which indicated that Genets 3–7 belonged to *A. solidipes*. Based on the *tef1* sequences, Genets 1 and 2 showed a 99% similarity to *A. altimontana*; whereas, Genets 3, 4, 5, 6, and 7 showed a 99% similarity to *A. solidipes*. For each of the seven *Armillaria* genets (two genets of *A. altimontana* and five genets of *A. solidipes*), the *tef1* sequences are deposited in the GenBank (MH879011–MH879017). Thus, based on the somatic compatibility tests and *tef1* sequencing, Genets 1 and 2 were confirmed as *A. altimontana* and Genets 3, 4, 5, 6, and 7 were confirmed as *A. solidipes*. The genets of *A. altimontana* (Genets 1 and 2) and *A. solidipes* (Genets 3–7) contained *tef1* sequences that were ≤94% similar. Similarities of the *tef1* sequences from *A. altimontana* (Genets 1 and 2) and *A. solidipes* (Genets 3–7) with additional *Armillaria* species are compared in Table S4.

The distributions of the *A. altimontana* and *A. solidipes* genets within the 16-year-old planting of western white pine are depicted in Figure 1. The *Armillaria* genets likely spanned outside of the experimental planting; however, Genet 2 (*A. altimontana*) spanned at least 109 m, and Genet 7 (*A. solidipes*) spanned at least 120 m (Figure 1). Within the western white pine plantation, *A. altimontana* was found most frequently, with occurrence on 48.7% of trees (n = 1010 trees), whereas *A. solidipes* was found on 10.2% of the trees (n = 211 trees). Both *A. altimontana* and *A. solidipes* co-occurred in association with 4% of the trees (n = 82 trees). It is especially noteworthy that in only one case (a tree colonized by *A. solidipes*) were different genets of the same *Armillaria* species found in association with the same tree. In addition, one isolate of *A. gallica* (identified by the *tef1* sequences) was found ca. 2.5 m

away from the planting, which indicates that *A. gallica* can potentially exist in a similar habitat to the planting site.

Figure 1. The *Armillaria* species and genet distribution within the Ida Creek plantation (0.8 ha) at the Priest River Experimental Forest, Idaho, USA: Each square pixel represents 1.2 m × 1.2 m (a single tree location), and the colored pixels indicate trees associated with *Armillaria altimontana*, *A. solidipes*, or both. Cells split diagonally show the two unique genets that were identified on that single tree. The grey pixels indicate trees where neither *A. altimontana* nor *A. solidipes* were found or trees that were either missing or died early from unknown causes. The sequences (*tef1*) from G1–G7 (MH879011–MH879017) are deposited in GenBank.

The mapped distribution of *Armillaria* species showed patterns of clustering within species that were supported by Moran's I for *A. altimontana* (I = 0.61, $p < 0.0001$) and, to a much lesser extent, *A. solidipes* (I = 0.178, $p < 0.0001$). Thus, trees colonized by *A. altimontana* were more likely to have neighboring trees colonized by the same species.

3.2. *Armillaria* Association with Disease

Of the 1758 trees that remained in the analyses after the dataset was refined to exclude nonlocal provenances, control, and white pine blister rust-afflicted trees, 7.5% of trees (including 1.5% of recently dead trees) showed signs/symptoms of Armillaria root disease (i.e., mycelial fans or rotten wood on living trees or mycelial fans or rotten wood on recently dead trees with basal resinosis), while 46.1% of living/recently dead trees contained only epiphytic rhizomorphs (Table 2). Disease signs/symptoms were observed on 13.9% of the living/recently dead trees ($n = 131$) colonized by *Armillaria* ($n = 942$ trees) (Table 2).

Table 2. The proportion of *Armillaria* isolates associated with diseased or apparently healthy (epiphytic rhizomorphs only) trees from among 1758 western white pine (*Pinus monticola*). [a] Within the Ida Creek plantation (0.8 ha) at the Priest River Experimental Forest, Idaho, USA.

	Living Trees	Dead Trees	# of Trees
Clean (no *Armillaria*) [b]	797 (45.3%)	19 (1.1%)	816
Epiphytic rhizomorphs (no disease) [c]	807 (45.9%)	4 (0.2%)	811
Diseased [d]	105 (6%)	26 (1.5%)	131

[a] The dataset was refined to exclude the nonlocal provenances, control, and white pine blister rust-afflicted trees. [b] Clean = no rhizomorphs, mycelial fans, rotten wood, or dead trees with this condition and no basal resinosis. This category includes two trees with rotten wood or mycelial fans but no resinosis. [c] Epiphytic rhizomorphs = no mycelial fans or rotten wood but colonized by *Armillaria* (not associated with disease). [d] Diseased = mycelial fans or rotten wood on living trees or mycelial fans or rotten wood on recently dead trees with basal resinosis.

The occurrence of disease symptoms on colonized trees differed significantly (X^2 [1, N = 942] = 807.79, $p < 0.001$) by the *Armillaria* species. In particular, 74.7% of the *A. solidipes* isolates and only 1.8% of the *A. altimontana* isolates were associated with disease symptoms on living or recently dead trees (Table 3). Conversely, 98.2% of the *A. altimontana* isolates and 25.3% of the *A. solidipes* isolates were not associated with causing disease (Table 3). On diseased trees where both *Armillaria* species were found (n = 62 trees in refined dataset), the *A. solidipes* isolates were derived from mycelial fans or rotten wood, which indicates that *A. solidipes* was causing disease, whereas almost all *A. altimontana* isolates were derived from epiphytic rhizomorphs and were not associated with causing disease. In one case, however, an *A. solidipes* isolate was derived from a mycelial fan and an *A. altimontana* isolate was derived from rotten wood on the same tree.

Table 3. The *Armillaria* species and genets associated with disease on western white pine (*Pinus monticola*) planted within the 0.8-ha Ida Creek site, Priest River Experimental Forest, northern Idaho, USA [a].

Species	Genet	# of Isolates not Associated with Disease [b]	# of Isolates Associated with Disease [b]	Total # of Isolates/Genet
A. altimontana	G1	43	0	43
	G2	723	14	737
	Total	766 (98.2%) [c]	14 (1.8%) [d]	780
A. solidipes	G3	2	7	9
	G4	18	4	22
	G5	6	8	14
	G6	3	22	25
	G7	12	80	92
	Total	41 (25.3%) [c]	121 (74.7%) [d]	162

[a] Trees (n = 942) colonized by *Armillaria* from among 1758 western white pine: Isolates of the same genet derived from epiphytic rhizomorphs, a mycelial fan, and/or rotten wood from a single tree were counted as a single isolate. In one case, Genet 2 and Genet 5 were associated with disease symptoms on the same tree. [b] Disease signs and symptoms = mycelial fans, rotten wood, or both on living trees or mycelial fans, rotten wood, or both on recently dead trees with basal resinosis. [c] The percent of isolates not associated with disease (e.g., epiphytic rhizomorphs). [d] The percent of isolates associated with disease.

3.3. Growth and Survival of Western White Pine in Relation to Armillaria spp.

The height, DBH, and survival of western white pine differed significantly by *Armillaria* colonization status (i.e., trees colonized by *A. altimontana*, *A. solidipes*, both species, or neither species; Table 4). The height, DBH, and survival were greatest among trees colonized by *A. altimontana* (6.7 m, 7.5 cm, and 99%, respectively), while a lower height, DBH, and survival occurred among trees with no *Armillaria* (6.4 m, 7.2 cm, and 98%, respectively), trees colonized by *A. solidipes* (5.8 m, 6.7 cm, and 84%, respectively), and tree colonized by both *A. altimontana* and *A. solidipes* (5.8 m, 6.8 cm, and 79%, respectively). Post hoc Tukey tests showed that the height and survival of trees colonized by *A. altimontana* differed significantly ($p < 0.05$) from all other *Armillaria* colonization statuses (Figure 2). In contrast and in agreement with Rehfeldt et al. [16] and Steinhoff [14], the provenance and elevation of the seed source was not identified as an important source of variation for predicting the height, DBH, or survival of western white pine.

Table 4. A summary of the model comparison tests for the effects of *Armillaria* status and region of seed origin on western white pine (*Pinus monticola*) height, diameter (DBH), and survival at the Ida Creek site at the Priest River Experimental Forest in northern Idaho, USA.

Trait	Model	Test df (Chi Df)	Test Deviance (Chisq)	*p*-Value
Height	*Armillaria* status (F)	3	31.92	<0.0001
	Region (F)	3	9.86	0.0198
	Armillaria status × region (F)	9	4.75	0.8552
	Family within provenance and region (R)	1	37.505	<0.0001
	Row within block (R)	1	10.447	0.0012
	Column within block (R)	1	104.35	<0.0001
DBH	*Armillaria* status (F)	3	21.453	<0.0001
	Family within provenance and region (R)	1	24.221	<0.0001
	Site density (R)	1	9.095	0.0026
	Row within block (R)	1	3.429	0.0641
	Column within block (R)	1	23.297	<0.0001
Survival	*Armillaria* status (F)	3	102.36	<0.0001
	Family within provenance and region (R)	1	31.087	<0.0001
	Row within block (R)	1	24.8	<0.0001
	Column within block (R)	1	24.607	<0.0001

Notes: The model comparison using a maximum likelihood testing was confirmed with similar results using parametric bootstrapping: For each response variable, a sub-model that excluded the listed term was compared to a full model that included all the terms listed for the respective response variable except the interaction. The testing for the interaction of *Armillaria* status × region of seed origin compared the full model versus a full model with the interaction. Variables treated as fixed or random are indicated by (F) or (R), respectively.

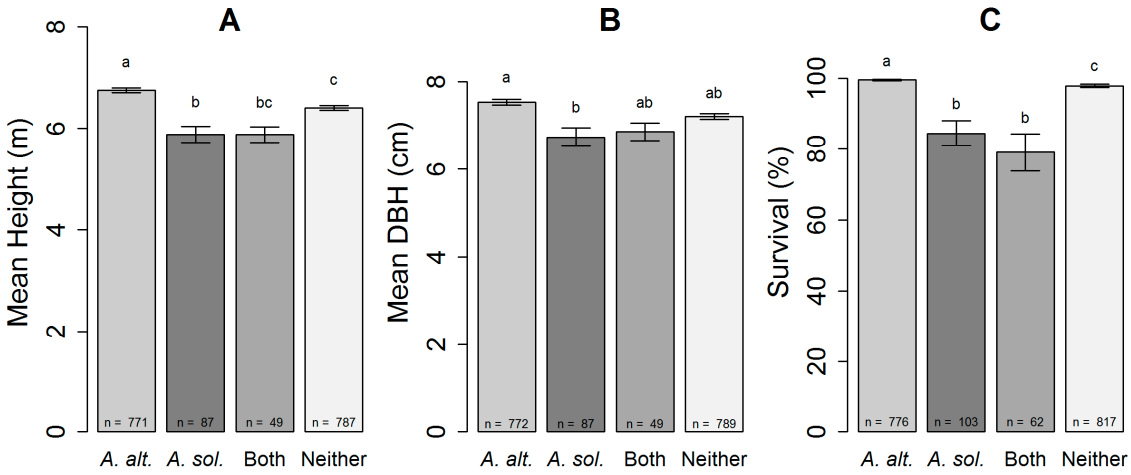

Figure 2. (**A**) The mean height, (**B**) mean diameter at breast height (DBH), and (**C**) percent survival among western white pine (*Pinus monticola*) growing in association with *Armillaria altimontana* (*A. alt*.), *A. solidipes* (*A. sol*.), both *A. altimontana* and *A. solidipes* (Both), or neither *Armillaria* species (Neither) at 16 years post-planting at the Priest River Experimental Forest, northern Idaho, USA. The height and DBH measures were not available for every live tree in the dataset. The bars show the standard error. Means sharing a lower case letter within each bar graph (i.e., A, B, and C) are not significantly different ($p < 0.05$) by Tukey-adjusted means separation.

4. Discussion

It is well-known that the *Armillaria* species play diverse ecological roles in forest ecosystems, including roles as virulent pathogens of diverse woody plants, beneficial saprophytes, and mycorrhizal symbionts [2,3]. In a 16-year-old western white pine plantation spanning 0.8 ha, we found and characterized two genets of *A. altimontana* and five genets of *A. solidipes* that exhibited distinct spatial distribution and interspecific associations (Table 1; Figure 1). The presence of the Armillaria root disease clearly corresponded with the *Armillaria* species present. In particular, the *A. solidipes* isolates were frequently (74.7%) associated with Armillaria root disease (Table 3). This finding is consistent with

the well-documented pathogenic role of *A. solidipes* (as *A. ostoyae*) on conifers in North America [4,47]. Although western white pine is generally considered tolerant to Armillaria root disease [21], planted or young western white pine seedlings have been found to be more susceptible in areas where *Armillaria* pathogens are common [48]. In contrast, a large majority (98.2%) of the *A. altimontana* isolates were not associated with Armillaria root disease. In addition, the tree height, DBH, and survival differed significantly ($p < 0.001$) by *Armillaria* colonization status (Table 4) with trees colonized only by *A. altimontana* exhibiting a greater growth (i.e., height and DBH) and survival in comparison with trees colonized only by *A. solidipes*, by both *Armillaria* species, and not associated with *Armillaria* (Figure 2). These findings support the hypothesis that *A. altimontana* is typically nonpathogenic or a weak pathogen and further suggest that *A. altimontana* could perhaps behave as a beneficial symbiont, epiphyte, or both in western white pine under certain conditions.

The association of *A. altimontana* with the slightly enhanced growth and survival of western white pine was unexpected and warrants further investigation. Part of this increased growth and survival is likely due to the absence of pathogenic *A. solidipes*, but trees associated with *A. altimontana* also exhibited better growth and survival than trees growing in association with no *Armillaria*. Possible explanations for the increased growth and survival of *A. altimontana*-associated western white pine over trees with no *Armillaria* associations are potentially complex. *Armillaria altimontana* may play an ecological role as a beneficial root or rhizosphere symbiont. Alternatively, *A. altimontana* could contribute to or reflect a soil environment and microbial community that are conducive to the growth and survival of western white pine. With similarities in their geographic distributions (see [49]), it can also be speculated that *A. altimontana* and western white pine may have a long history of coevolution.

Armillaria genets can only spread via the vegetative growth of rhizomorphs and/or mycelia (e.g., root-to-root contact [3], which allows additional inferences based on the spatial distribution of the *Armillaria* species and genets within the Ida Creek planting. For example, Genet 2 of *A. altimontana* occupied a span of $\geq$109 m and Genet 7 of *A. solidipes* occupied a span of $\geq$120 m within the study plot boundaries (Figure 1). Based on an estimation by van der Kamp [50] that *A. solidipes* (as *A. ostoyae*) spreads at a rate of 0.22 m yr^{-1} in interior British Columbia, Canada, we very conservatively estimate that the *A. altimontana* genet has been present on the site for at least 248 years and that the *A. solidipes* genet has been present for at least 273 years, assuming a 0.22 m yr^{-1} growth in each direction from the midpoint of each genet's distribution within the plot. The age of these genets is almost certainly much older considering these genets most likely spread beyond the plot boundaries.

Another intriguing observation is that the *A. altimontana* and *A. solidipes* genets were not contiguously distributed. For example, Genet 1 (*A. altimontana*) spanned a 28-m gap where the genet was not present (Figure 1) and Genet 4 (*A. solidipes*) spanned an 81-m gap (Figure 1). Such patterns of spatial distribution suggest that genets of *A. altimontana* and *A. solidipes* have apparently been expanding/contracting and interacting over centuries. However, biotic/abiotic environmental factors that contribute to the expansion or contraction of *A. altimontana* and *A. solidipes* remain unknown. Because this study plot is in an experimental forest on a site that was not previously harvested or managed before site preparation (see [51–53]), these influencing factors likely represent natural processes, such as interspecies competition, microclimate change, natural succession, tree development, fire, insect attack, and other factors that influence stand structure or composition. Further investigations are warranted to determine the environmental factors that enhance or suppress site occupancy by *A. altimontana* or *A. solidipes*. Information from such studies could provide critical insight into management approaches to suppress *A. solidipes* and/or enhance *A. altimontana*.

Several lines of evidence suggest that *A. altimontana* is acting as a biological control agent against *A. solidipes* via competitive exclusion in the environment associated with this study site (ca. 1987). Firstly, the genet distributions indicate that both species likely extended across the majority of the plot at one point in time (Figure 1), yet *A. altimontana* dominates the site at the time of the study (1987). The dominance of *A. altimontana* on the site is supported by the Moran's I values of

spatial autocorrelation that shows the distribution of *A. altimontana* was highly clustered (I = 0.61) in comparison to the distribution of *A. solidipes*, which exhibited much lower clustering (I = 0.178). Furthermore, subsequent surveys from 2014–2018 found little evidence of Armillaria root disease caused by *A. solidipes* in the plot. Secondly, the 0.8-ha plot was relatively homogenous and was planted with trees of the same species and age, which suggests that environmental variation should have little influence on the distribution patterns between *Armillaria* species. Moreover, the ecological amplitude of *A. solidipes* appears to encompass and extend beyond that of *A. altimontana* [5], which suggests that the observed species distribution patterns were likely influenced by factors apart from environmental variation. Finally, the biological control of *A. solidipes* by *A. altimontana* is consistent with species distribution patterns across the region. For example, on sites where *A. altimontana* was absent, large continuous *A. solidipes* genets have been reported, such as a 965-ha mortality center in northeast Oregon [54]. The single genet of *A. solidipes* from that study was found to span 3.8 km with an estimated age of 1900–8650 years. In contrast, on multiple sites in northern Idaho and western Washington, USA where *A. altimontana* and *A. solidipes* were found to co-occur, other studies [55,56] have reported distribution patterns for *A. solidipes* similar to the present study that are considerably more modest. Although the aforementioned evidence provides support that *A. altimontana* is competitively excluding *A. solidipes* in the rhizosphere or surrounding soil within the study site, more studies are needed to confirm this occurrence.

Based on the competitive exclusion principle [57], sympatric organisms that compete for the same ecological niche cannot exist in stable equilibrium. Accordingly, this principle provides one basis for the function of biological control agents. One means of biological control can occur when a biological control agent has a competitive advantage over a pathogen within a shared niche. Biological control could occur when the biological control agent is already dominating the niche or has a competitive advantage, such as a faster growth or reproduction. Raziq [58] suggested that a biological control agent or antagonist of an *Armillaria* pathogen should have the capacity to inhabit wood and the rhizosphere. For example, *Hypholoma fasciculare* (Huds.:Fr.) P.Kumm., a wood-rotting basidiomycete, has demonstrated the potential to competitively displace *A. solidipes* (as *A. ostoyae*) after stump inoculations [59]. Similarly, five species of saprobic lignicolous fungi (*H. fasciculare*, *Ganoderma lucidum* [Curtis] P.Karst, *Phanerochaete velutina* [DC.] P.Karst., *Schizophyllum commune* Fries, and *Xylaria hypoxylon* [(L.) Grev.]) exhibited the potential for the competitive exclusion of *Desarmillaria tabescens* (Scop.) R. A. Koch and Aime (as *A. tabescens* [Scop.] Emel) and *A. mellea* (Vahl) P.Kumm. under controlled inoculation studies [60]. Based on stump surveys in southeastern Alaska, Shaw [61] hypothesized that colonization by a weakly pathogenic *Armillaria* spp. could deter the spread of the root pathogen *Heterobasidion annosum s.l.* (Fr.) Bref. When considering potential biological control agents for Armillaria root disease, the nonpathogenic or weakly pathogenic *Armillaria* spp. should not be overlooked because of their potential saprophytic ability to compete with pathogenic *Armillaria* spp. for the same or a similar ecological niche in the rhizosphere and soil surrounding the host tree.

The in vitro antagonism between different *Armillaria* spp. is well-established, and the "black line" zone of antagonism provides the basis for species identification based on the pairing tests of somatic incompatibility (e.g., [29,31,35]). Furthermore, in nature, the distributions among interspecific *Armillaria* genets typically do not appear to be randomly interspersed. Even so, the potential in situ antagonism as a basis for competitive exclusion between or among *Armillaria* spp. is difficult to interpret conclusively. For example, several previous studies have also examined patterns of interspecific interactions: (1) *A. gemina* Bérubé and Dessur. and *A. solidipes* (as *A. ostoyae*) in a mixed-species forest stand in New Hampshire, USA [23]; (2) *A. calvescens* Bérubé and Dessur., *A. gemina*, *A. gallica* Marxm. and Romagn., and/or *A. solidipes* (as *A. ostoyae*) in different forest types of New York, USA [33]; (3) *A. ostoyae*, *A. gallica*, and/or *A. cepistipes* Velen. in hardwood and mixed forests of France [34]; and (4) *A. gallica* and *A. mellea* in mixed hardwood forests of California, USA [62]. The overlapping distributions observed in many of these studies have been interpreted as reflecting the differences in ecological niches and colonization strategies (e.g., [23,26,34]). In a similar study

of the *Armillaria* distribution, Bruhn et al. [63] reported that the occurrence of *A. gallica* may reduce the activity of other more virulent *Armillaria* species in mixed oak forests of the Ozark Mountains of southeastern Missouri, USA.

The sympatric distribution of *A. altimontana* and *A. solidipes* found in this study and others (e.g., [55,56]) is similar to a previous study in European forests where the saprotrophic/weakly pathogenic *A. cepistipes* can occur sympatrically with the pathogenic *A. ostoyae* [26]. The sympatric distributions of *Armillaria* spp. in our study and perhaps other aforementioned studies seem likely attributable to the *in situ* antagonism between species that results in competitive exclusion.

To the extent that the observed pattern reflects competitive exclusion, *A. altimontana* may offer potential biological control against *A. solidipes* as was originally proposed by McDonald [64]. However, trees colonized by both *A. altimontana* and *A. solidipes* displayed a reduced growth and survival similar to trees colonized by only *A. solidipes* (Table 4), which suggests that once *A. solidipes* has colonized the cambial tissue of living roots, it may have a competitive advantage over *A. altimontana* as a pathogen on substrates within a living host. Understanding the biotic (e.g., [65–76]) and abiotic (e.g., [5]) environmental factors that influence the dynamic interactions of *A. altimontana* and *A. solidipes* seems key to the development of management practices for Armillaria root disease in coniferous forests of the interior western North America.

The disparate ecological roles of *A. altimontana* and *A. solidipes* in association with western white pine further confirms the need to accurately identify *Armillaria* spp. on a site before developing and applying appropriate forest management practices. The DNA-sequence data indicate that previous somatic pairing tests provided the accurate identification of *A. altimontana* and *A. solidipes*; however, *tef1* sequencing appears to be a more reliable and faster method for identifying *Armillaria* spp. in North America [36,77–81].

It remains inconclusive if and how *A. altimontana* functions to limit the distribution of *A. solidipes*. Further insights into this relationship could provide a unique basis for developing novel methods to manage Armillaria root disease in association with western white pine. Additional studies are needed to determine the extent of this relationship with other forest species, geographic regions, and climates. Complex interactions among the soil microbial communities undoubtedly play critical ecological roles, such as biological control, decomposition, nutrient cycling, symbioses, etc.; however, the role of these microbial communities is not well-understood in forest ecosystems. Soil metagenomic and metatranscriptomic techniques (e.g., [80]), coupled with environmental data, such as soil properties, moisture, temperature, plant coverage, etc., could provide greater insight into the soil microbial communities and ecological functions that favor *A. altimontana*, increase tree growth and survival, and reduce Armillaria root disease [81].

5. Conclusions

In a 16-year-old western white pine plantation spanning 0.8 ha, we found and characterized two genets of *A. altimontana* and five genets of *A. solidipes* that exhibited distinct spatial distribution and interspecific associations. The spatial distributions of individual *Armillaria* genets were wide and noncontiguous, and *A. solidipes* was found to be uncommon in areas dominated by *A. altimontana.* Trees colonized by *A. solidipes* were associated with a substantially higher incidence of root disease, a lower tree growth (i.e., height and DBH), and a lower tree survival than trees colonized only by *A. altimontana* or trees with no *Armillaria* colonization. In addition, trees colonized only by *A. altimontana* were associated with a slightly greater height growth and survival than trees not associated with *Armillaria.* These findings support the hypothesis that *A. altimontana* is typically nonpathogenic or a weak pathogen and further suggest that *A. altimontana* may act to competitively exclude *A. solidipes* in the soil and/or rhizosphere and could perhaps behave as a beneficial symbiont, epiphyte, or both in western white pine under certain conditions. Understanding the interactions of these two *Armillaria* species appears critical to understanding and developing novel management approaches for Armillaria root disease in this region.

Supplementary Materials: The following are available online at http://www.mdpi.com/1999-4907/10/4/294/s1, Table S1. The somatic pairing tests to identify the *Armillaria* genets among the isolates collected from western white pine (*Pinus monticola*) within a plantation at the Ida Creek site, Priest River Experimental Forest, northern Idaho, USA. Table S2. The number of western white pine (*Pinus monticola*) trees by region of seed origin and group status used for the analyses or results presented in Figure 1; Figure 2 and Tables 1–4 for the Ida Creek site, Priest River Experimental Forest, northern Idaho, USA. Table S3. The tree height, DBH, survival, *Armillaria* colonization status, and disease signs and symptoms data for western white pine (*Pinus monticola*) trees at the Ida Creek plantation, Priest River Experimental Forest, Idaho, USA. Table S4. The Sequence Identity Matrix for *Armillaria* genets from Ida Creek plantation at the Priest River Experimental Forest, Idaho, USA vs. themselves and the North American *Armillaria* standards. Figure S1. A graphical summary of (A) the height (Equation (1)) and (C) diameter at breast height (DBH) model fits for residuals vs. observed and the Q-Q plots for (B) height (Equation (1)) and (D) the DBH models of western white pine (*Pinus monticola*) trees growing in Ida Creek plantation within the Priest River Experimental Forest in northern Idaho, USA.

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