

SURVEY FOR *ARMILLARIA* BY PLANT ASSOCIATIONS IN NORTHERN ARIZONA

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ABSTRACT

Fungi in the genus *Armillaria* are associated with an important disease of deciduous and coniferous trees and shrubs in western North America. This study examined the distribution of *Armillaria* by forest habitat types on the Kaibab National Forest and northern Coconino National Forest, Arizona. Over 400 trees were examined for *Armillaria* in 76 Interior West Forest Inventory and Analysis permanent plots representing 17 different habitat types. Samples of the fungus associated with *Armillaria* root disease were collected from 23 trees and identified using DNA sequencing. All samples were determined to consist of a single species, *Armillaria solidipes* Peck [= *A. ostoyae* (Romagnesi) Herink]. Only 10 of the 76 plots and 5 of the 17 habitat types sampled had *Armillaria solidipes* present on one or more trees. *A. solidipes* was more commonly found in mixed-conifer and subalpine forests and was rare in ponderosa pine (*Pinus ponderosa* Doug. Ex. Larson & C. Larson) forests, which was consistent with previous root disease studies conducted in the Southwest. However, our estimates of *Armillaria* presence may be conservative since we did not examine entire root systems.

INTRODUCTION

Armillaria root disease has been commonly associated with root decay and mortality of deciduous and coniferous trees and shrubs and has been reported to affect more than 600 species of woody plants (Garraway et al. 1991, Morrison et al. 1991). This damaging root disease has been found in most forests of the world, but it has been reported to be more common and abundant in temperate and boreal forests than in tropical regions (Kile et al. 1991). It has been reported to occur in nearly every state of the continental United States and also reported widely in Canada and Mexico (Williams et al. 1986). This economically important tree disease has been consistently associated with fungi in the genus *Armillaria* (Fr.:Fr) Staude (Tricholomataceae), hence the common name of the disease.

The classification of the genus *Armillaria* has undergone a great deal of modification, but has now been reported to contain approximately 40 species, 10 of which have been documented in North America (Watling et al. 1991, Brazee et al. 2012, Ross-Davis 2012, Volk 2013) (Table 1). Some species of *Armillaria* reportedly survive as saprophytes in woody substrates within soil or standing dead material, while others have been demonstrated to be aggressive pathogens of living trees. The pathogenic species can kill living roots, eventually killing the infected tree, and then continue to live in dead material as saprophytes for several years (Kile et al.

1991). Species of *Armillaria* can be spread by sexually produced basidiospores, or spread vegetatively by rhizomorphs and by transfer from infected roots to healthy roots via contacts and/or grafts (Kile et al. 1991, Redfern and Filip 1991). Due to differences in pathogenicity and host preferences among the different species of *Armillaria* found in North America, forest managers require precise information on which species are present in different regions and when possible, information on the probability that *Armillaria* root disease is established in different forest types and stands (McDonald et al. 1987, Kile et al. 1991). Often trees infected with *Armillaria* root disease have been stressed to the extent that other agents, such as bark beetles, attack them and can cause death of the trees (Miller and Partridge 1974, Tkacz and Schmitz 1986, Goheen and Hansen 1993). Therefore, it is common to observe mortality caused by a complex of factors in forests infested with this disease in Arizona (Wood 1983).

In the northern Rocky Mountains, the use of habitat types (Daubenmire 1952) has been demonstrated to be a useful forest vegetation classification system for predicting the environmental conditions under which *Armillaria* root disease may be present or absent in a stand (Williams and Marsden 1982, McDonald et al. 1987, Byler et al. 1990, McDonald 1990, McDonald et al. 2005). The overall climatic conditions influencing forest habitat types are correlated with predicting the occurrence of *Armillaria*.

Table 1. Species of *Armillaria* found in North America (Baumgartner and Rizzo 2001, Kim et al. 2010, Elias-Roman et al. 2013, Brazee et al. 2013, Nelson et al. 2013, Volk 2013).

Species	Distribution
<i>Armillaria solidipes</i> Peck/ <i>ostoyae</i> (Romagnesi) Herink	Northern conifer zone, occasionally on hardwoods
<i>A. gemina</i> Bérubé & Dessurealt	Northeastern United States, eastern Canada
<i>A. calvescens</i> Bérubé & Dessurealt	Eastern Canada to Michigan and Wisconsin
<i>A. sinapina</i> Bérubé & Dessurealt	Northern conifer zone, but typically hardwoods in the East and conifers in the West
<i>A. mellea</i> (Vahl:Fries) Kummer	Hardwood zone, mostly southeastern United States north to Iowa and Wisconsin and south to Oklahoma, Texas, and Mexico. Eastern distribution from Florida to the Appalachians to Québec. Known from Arizona, California, and Oregon but not other areas of the West
<i>A. gallica</i> Marxmüller & Romagnesi	Hardwoods in South, Northeast, Midwest, and Mexico; Arizona, California, and the Pacific Northwest
<i>A. nabsnona</i> Volk & Burdsall	Idaho, Washington, Oregon, Alaska, and British Columbia
<i>A. cepistipes</i> Velenovsky	Washington and British Columbia
<i>A. tabescens</i> (Scopoli) Emel	Southeastern United States into the Northwest, west to Ohio, also farther west and north on shores of Great Lakes and south to Veracruz, Mexico
<i>A. altimontana</i> Brazee, B. Ortiz, Banik & D. L. Lindner	Idaho, Washington, Oregon, California, and British Columbia

In the northern Rocky Mountains, habitat types characterized as cold-dry or hot-dry environments were found to be outside the ecological range of *Armillaria* and excessively cold or moist sites may also limit the growth and development of *Armillaria* and hence have little of the fungus present (McDonald et al. 1987). In the Southwest, where many of the forests are characterized by hot-dry or warm-dry climatic conditions, *Armillaria* root disease is considered important only on more mesic sites (Wood 1983). However, in northern Arizona few studies have been conducted to examine the distribution of *Armillaria* or identify the species of *Armillaria* present using the currently recognized classification of the genus. In the Southwest, habitat types are often called plant associations (Stuever 1997) and we have adopted this terminology since the two terms are synonymous.

Historically, *Armillaria* root disease was thought to be caused by a single species, *Armillaria mellea* (Vahl:Fr.) Kummer. Although Wood (1983) applied the concept of using *Armillariella* in place of *Armillaria* in a study in the southwestern United States, the use of the previous genus was invalidated by Watling et al. (1982) and is no longer used as an

obligate synonym for *Armillaria* (Volk 2013). Under the current classification system for *Armillaria*, the distribution of *A. mellea* is confined to the eastern and midwestern United States and California (Volk 2013). However, *A. mellea* was reported from southeastern Arizona on oaks (*Quercus* spp.) by Gilbertson and Bigelow (1998) based on morphology, and recently confirmed (Nelson et al. 2013). The primary species of *Armillaria* associated with a root disease of conifers throughout the western United States has now been designated as *A. solidipes* Peck [= *A. ostoyae* (Romagnesi) Herink] (Burdsall and Volk 2008), pending a vote for nomenclatural conservation (Hunt et al. 2011), and was the species morphologically identified in a study in New Mexico (Omdal et al. 1995). Gilbertson and Bigelow (1998) also reported *A. solidipes* (as *A. ostoyae*) in the Sky Islands of southeastern Arizona on several conifers and quaking aspen (*Populus tremuloides* Michx.). However, the identification of *Armillaria* species that occur in Arizona using DNA-based techniques is needed, as well as additional information on the distribution and abundance of *Armillaria* in the northern part of the state.

Today it is possible to identify species of *Armillaria* using DNA sequencing techniques (Kim et al. 2006, Ross-Davis et al. 2012). Kim et al. (2006) examined rDNA sequences for three regions and used amplified fragment length polymorphisms (AFLPs) to assess the phylogenetic relationships of several species of *Armillaria*. They concluded that ribosomal DNA intergenic spacer sequences (IGS-1) and AFLP genetic markers could be used to potentially distinguish the North American species of *Armillaria*. In addition, the phylogenetic relationships among 15 globally diverse *Armillaria* species and eight Japanese species were examined and separated using partial sequences of the translation elongation factor-1 alpha (*tef-1 α*) gene (Maphosa et al. 2006, Hasegawa et al. 2010). DNA based identification of *Armillaria* has also been used in North America and can now confidently differentiate species (Ross-Davis et al. 2012). Five clades have been identified using the *tef-1 α* gene which clearly separated the North American species of *Armillaria*. Therefore, current molecular methods have been shown to be effective at correctly identifying the currently recognized North American species of *Armillaria* (Ross-Davis et al. 2012).

Because little information was available on the species of *Armillaria* that occur in northern Arizona, the hosts affected, or the distribution of the pathogen by forest type or plant association, this study was initiated in 2011 in cooperation with USDA Forest Service: Rocky Mountain Research Station (RMRS); Interior West Forest Inventory and Analysis program (IW-FIA); and Forest Health Protection, Southwest Region. Permanent plots established by IW-FIA on the Kaibab and Coconino National Forests, Arizona, were used as sampling sites. These plots were used because they allowed the accomplishment of several ancillary goals: 1) this study served as a pilot test of methodology that could potentially be added to FIA protocols, 2) because the FIA program uses a systematic sampling design, the results of this study can be used to estimate the proportion of area with *Armillaria* present, and 3) because the plots are part of a continuous forest inventory and monitoring system, the results can serve as a baseline for future comparisons.

Plots representing 17 plant associations on three ranger districts were surveyed, in which dead and living trees with or without root disease symptoms were examined for the presence of *Armillaria*. Samples of *Armillaria* were collected when observed and sent to the RMRS Forestry Sciences Laboratory in Moscow, Idaho, for positive identification based on DNA sequences of partial Large Subunit and intergenic spacer 1 (LSU-IGS1) regions of ribosomal DNA and *tef-1 α* . Here we report our

findings, which support previous reports that *Armillaria* root disease is not common in northern Arizona and that the disease is commonly associated with only one species of *Armillaria*.

METHODS

This study used permanent plots established by the IW-FIA program on the Kaibab and Coconino National Forests in northern Arizona. Plots on the North Kaibab, Williams, and Flagstaff Ranger Districts were selected based on ownership, accessibility, and plant association. A total of 76 plots were sampled that represented 17 forest plant associations (Table 2 and Fig. 1). Rather than splitting plant associations into phases, plots representing a phase were grouped under each major plant association sampled. Plots were visited during the summers of 2011 and 2012.

Plots established by IW-FIA consist of four circular subplots (radius 7.3 m, 0.02 ha) (USDA Forest Service 2011) (Fig. 2). Each unit has a central subplot with three additional subplots established 36.6 m from its center at azimuths of 360°, 240°, and 120°. Each sampling unit was classified by elevation, aspect, forest type, soil type, and plant association. Standard forest inventory data collected from the four subplots included trees species, diameter breast height (dbh, 1.4 m above the ground), tree heights, and information on downed-woody material. To avoid disturbance associated with surveys for *Armillaria* within the IW-FIA subplots, circular supplemental plots (0.04 ha) were established 36.6 m from the center of the central IW-FIA subplot at an azimuth of 300° (Fig. 2). Only one supplemental plot was established for each set of IW-FIA subplots sampled.

In each supplemental subplot, one live or dead tree of each species present in the plot was examined for the presence of *Armillaria* by each of the following dbh classes: <20, 20-40, and >40 cm whenever possible. At least three trees were examined in each plot. Dead trees or live trees with symptoms of root disease were given priority for examination over live trees with no root disease symptoms. Only the species and diameter (dbh, nearest cm) of trees sampled for the presence of *Armillaria* were recorded for each supplemental plot. Examination of trees for the presence of *Armillaria* was conducted by excavating at least one main root to a radial distance of 0.8 m from the bole. When mycelia fans were present on roots, samples were collected by carefully removing a section of the infected root so the integrity of the bark remained intact. The size of sampled root sections or mycelia fans varied depending on the size of the infected root or bole. Up to three samples were collected from each infected tree. Root samples that

Table 2. Number of plots surveyed and number of plots with Armillaria by plant association on the Flagstaff Ranger District, Coconino National Forest and the North Kaibab and Williams Ranger Districts, Kaibab National Forest, Arizona. Plant association acronyms (e.g., PSME/FEAR) and plant species classifications follow Stuever (1997). See Appendix 1 for a list of common and scientific names of plants used in the name of plant associations.

Ranger district	Plant association	Number of plots	Plots with <i>Amillaria</i>
Flagstaff	Subalpine fir/Nevada pea (ABLA/LALAL3)	1	0
	White fir/Creeping barberry (ABCO/MARE11)	1	0
	Douglas-fir/Arizona fescue (PSME/FEAR)	2	0
	Douglas-fir/Creeping barberry (PSME/MARE11)	1	0
	Douglas-fir/Gambel oak (PSME/QUGA)	1	1
	Ponderosa pine/Arizona fescue (PIPO/FEAR)	7	1
	Ponderosa pine/Blue grama (PIPO/BOGR2)	7	0
	Ponderosa pine/Mountain muhly (PIPO/MUMO)	5	0
Williams	Douglas-fir/Mountain muhly (PSME/MUMO)	1	0
	Ponderosa pine/Arizona fescue	7	0
	Ponderosa pine/Blue grama	3	0
	Ponderosa pine/Mountain muhly	2	0
North Kaibab	Subalpine fir/Common juniper (ABLA/JUCO6)	5	3
	Engelmann spruce/Fleabane (PIEN/EREX4)	3	0
	Engelmann spruce/Maple (PIEN/ACGL)	1	0
	Blue spruce/Dryspike sedge (PIPU/CAFO3)	4	2
	Blue spruce/Fleabane (PIPU/EREX4)	1	0
	White fir/Arizona fescue (ABCO/FEAR)	2	0
	White fir/Creeping barberry	4	2
	White fir/Dryspike sedge (ABCO/CAFO3)	3	0
	Douglas-fir/Arizona fescue	1	0
	Douglas-fir/Gambel oak	1	0
	Ponderosa pine/Arizona fescue	4	1
	Ponderosa pine/Blue grama	2	0
	Ponderosa pine/Mountain muhly	3	0
	Ponderosa pine/Gambel oak (PIPO/QUGA)	4	0
Total		76	10

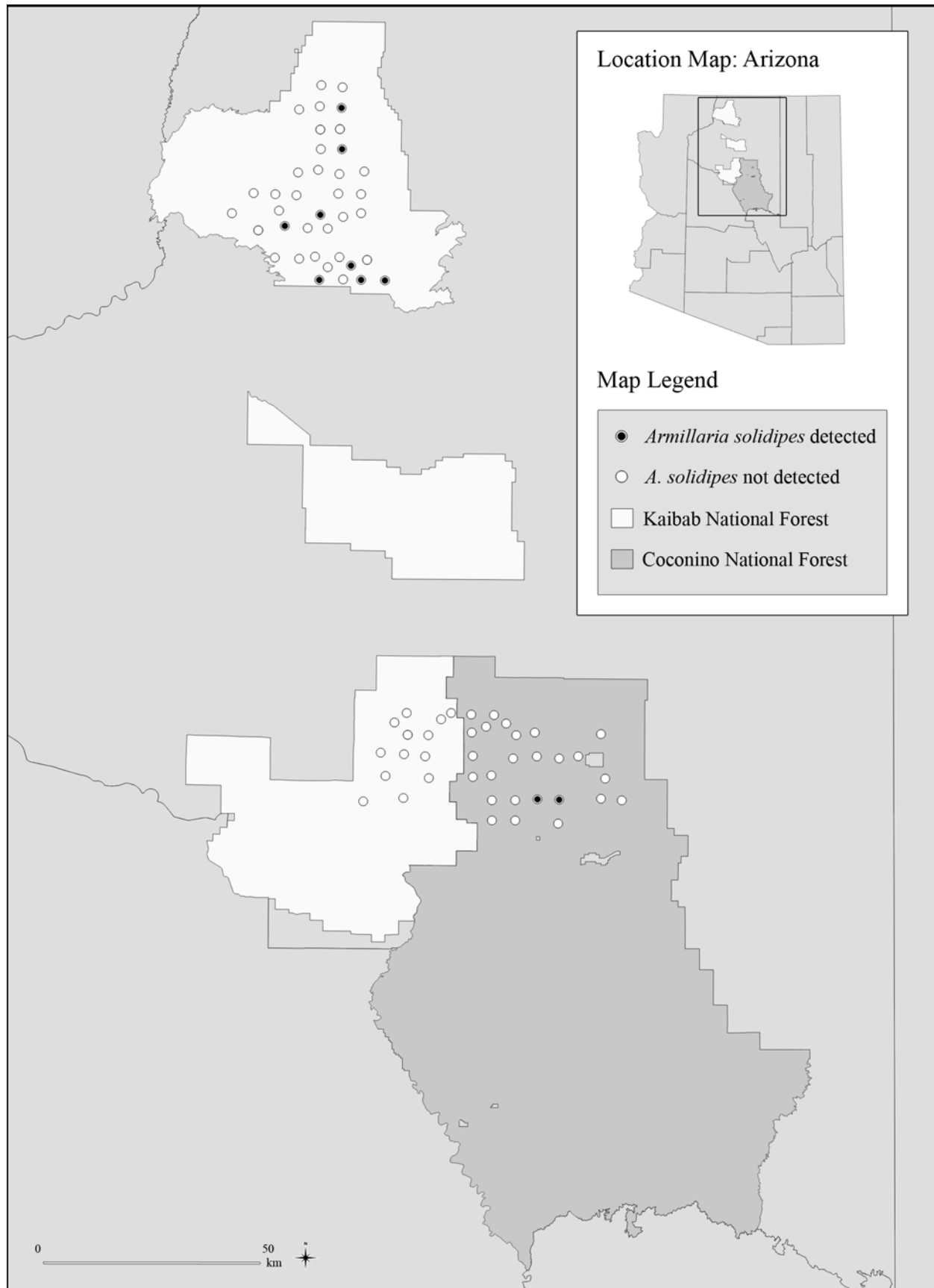


Figure 1. Approximate locations of 76 plots surveyed for *Armillaria* on the Kaibab and Coconino National Forests, Arizona.

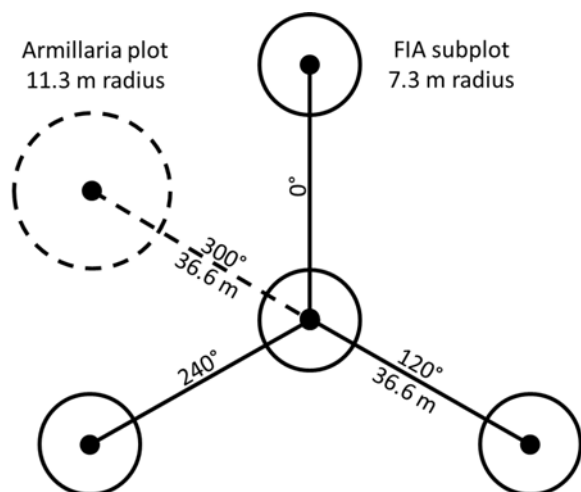


Figure 2. Plot design for Intermountain West Forest Inventory and Analysis sample plots with the supplemental *Armillaria* plot placed at a 300° azimuth from the center of the plot cluster.

appeared to be colonized by *Armillaria* were collected from 42 trees. Each *Armillaria* sample was placed in a paper bag and all the bags collected for a tree were placed in a Ziploc bag and kept in a cooler while in the field and then refrigerated.

Within 2 weeks, samples were mailed overnight on ice to the RMRS Forestry Sciences Laboratory, Moscow, Idaho. *Armillaria* was isolated from mycelial fans, rhizomorphs, or wood; then established in culture on 3% malt-agar medium (3% malt extract, 1.5% peptone, 3% glucose, and 1.5% agar) and incubated at 22°C in the dark. Template DNA was collected directly from scrapings of actively growing mycelia or by using DNeasy Plant Mini DNA extraction kits (Qiagen Inc., Valencia, CA, USA) to extract DNA from ca. 100 mg of mycelia that was cultured on 0.2-μm-pore nylon filters (Millipore Corp., Billerica, Massachusetts, USA) overlaying the culture medium. Extracted DNA samples were used for PCR and sequencing of the partial Large Subunit and intergenic spacer 1 (LSU-IGS1) regions of the ribosomal DNA was used to identify which species of *Armillaria* was associated with each sample (Kim et al. 2006). Species identify was confirmed using the *tef-1α* gene (Ross-Davis et al. 2012). *Armillaria* sequences were identified based on similarity (>95%) and phylogenetic placement using SplitsTrees4 with default parameters (Huson and Bryant 2006) when compared against reference sequences from Kim et al. (2006), Hanna et al. (2007), Ross-Davis et al. (2012) and/or Elias-Roman et al. (2013).

RESULTS

A total of 76 supplemental 0.04-ha plots were inspected for *Armillaria* representing 17 plant

associations (Table 2). A total of 10 plots had one or more trees with *Armillaria*: 8 of these plots were on the North Kaibab Ranger District (Kaibab Plateau) and two were on the Flagstaff Ranger District (Table 2 and Fig. 1). This represented 21% and 8% of the plots surveyed on those districts, respectively. *Armillaria* was not detected on the Williams Ranger District. A single FIA plot in Arizona has an expansion factor of approximately 2,500 ha, depending on the actual number of plots surveyed at a given point in the inventory cycle. Therefore, the simplified estimate is that the survey area of this study was approximately 190,000 ha, at least 25,000 ha (13%) of which would be expected to have *Armillaria* present. However, our estimates of *Armillaria* presence may be conservative since we did not examine entire root systems.

Armillaria was found in 5 of the 17 plant associations sampled. It was most often found in plant associations that represented warm-dry climatic conditions of the subalpine or mixed-conifer forest types (Stuever 1997); the subalpine fir/common juniper (ABLA/JUCO6), white fir/creeping barberry (ABCO/MARE11), and Douglas-fir/Gambel oak (PSME/QUGA) plant associations (Tables 2 and 3). *Armillaria* was also detected in one plant association that represented cold-wet climatic conditions of the mixed conifer forest type; the blue spruce/dryspike sedge (PIEN/CAFO3) plant association. Although 11 plots were sampled that represented three additional subalpine (PIEN/ACGL, PIEN/EREX4, and ABLA/LALAL3) and four mixed conifer forest plant associations (PIPU/EREX4, ABCO/FEAR, ABCO/CAFO3, and PSME/MUMO), *Armillaria* was not detected in any of them (Table 2). *Armillaria* was only found in one ponderosa pine plant association, ponderosa pine/Arizona fescue (PIPO/FEAR), and although 18 plots were sampled for this plant association, the pathogen was only found in two plots; one each on the Kaibab and Coconino National Forests (Table 2). Although 25 plots representing three other ponderosa pine plant associations (PIPO/BOGR2, PIPO/MUMO, and PIPO/QUGA) were sampled, *Armillaria* was not detected.

A total of 343 conifers and 82 deciduous species were surveyed in the 76 plots that were examined for *Armillaria* (Table 4). Of these, only 19 conifers and four quaking aspen had evidence of *Armillaria* (mycelial fans) on their roots or main boles that were detected by the survey method used. Rhizomorphs were rare with only two short segments collected from the bark of mycelial fan samples. The distribution of the 425 trees examined for *Armillaria* by diameter classes was: <20 cm – 239 trees; 20-40 cm – 117 trees; >40 cm – 69 trees. Of

Table 3. Number of trees surveyed (first number) and number of trees with *Armillaria* (second number) by tree species for the five plant associations where *Armillaria* was observed. Plant association acronyms follow Stuever (1997) and see Table 2.

Plant association	Ponderosa pine	Quaking aspen	Douglas-fir	White fir	Engelmann spruce	Blue spruce
PIPO/FEAR	44/3	0/0	0/0	0/0	0/0	0/0
PSME/QUGA	4/1	3/3	2/0	0/0	0/0	0/0
ABCO/MARE11	11/2	9/0	9/1	6/2	0/0	2/0
PIPU/CAFO3	7/3	6/0	0/0	3/0	3/2	3/0
ABLA/JUCO6	11/1	14/1	10/2	6/0	12/2	0/0
Total	76/10	32/4	21/3	15/2	15/4	5/0

the 23 trees in which *Armillaria* was detected, 11 were in the <20 cm class, 10 were in the 20–40 cm class, and two were in the >40 cm class. *Armillaria* was detected primarily on dead trees; 19 of the 23 trees (83 %).

The two most commonly surveyed tree species were ponderosa pine (*Pinus ponderosa* Dougl. ex Larson & C. Larson) and quaking aspen. Both of these species were infrequently associated with *Armillaria*; it was found on only 10 ponderosa pines (5%) and four aspen (6%) (Table 4). Four other conifers that were sampled in the subalpine and mixed-conifer plant associations were Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), white fir (*Abies concolor* (Gord. & Glend.) Hilldebr.), Engelmann spruce (*Picea engelmannii* Parry ex Engelm.), and blue spruce (*Picea pungens* Engelm.). *Armillaria* was also detected on three of these species and the incidence of infection was again very low: Douglas-fir (7 %), white fir (5 %), and Engelmann spruce (15 %) (Table 4). Only a few trees of the following species were examined for *Armillaria* within the sample plots and the pathogen was not detected: Gambel oak (*Quercus gambelii* Nutt.), New Mexican locust (*Robinia neomexicana* Gray), Utah juniper (*Juniperus osteosperma* (Torr.) Little), Colorado pinyon pine (*Pinus edulis* Engelm.), limber pine (*Pinus flexilis* James), and subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.).

Based on DNA sequences of the LSU-IGS1 and the *tef-1α* gene, isolates from the 23 trees were all identified as *Armillaria solidipes* (= *A. ostoyae*).

Table 4. Number of trees surveyed and number of trees and percentage with *Armillaria* by tree species in 76 plots on the Kaibab and Coconino National Forests, Arizona.

Tree species	Number examined	Number with <i>Armillaria</i>	Percent with <i>Armillaria</i>
Ponderosa pine	189	10	5
Quaking aspen	65	4	6
Douglas-fir	46	3	7
White fir	43	2	5
Engelmann spruce	27	4	15
Blue spruce	20	0	0
Gambel oak	11	0	0
Utah juniper	7	0	0
Colorado pinyon pine	6	0	0
Limber pine	4	0	0
New Mexico locust	6	0	0
Subalpine fir	1	0	0
Total	425	23	5

DISCUSSION

Recently, it was determined that *A. solidipes* was the appropriate name for the species associated with *Armillaria* root disease of conifers in the West (Burdalls and Volk 2008); however, Hunt et al.

(2011) argued that *A. solidipes* is an ambiguous species which is probably not conspecific with *A. ostoyae* and as a result have proposed that *A. ostoyae* be conserved over *A. solidipes*. Thus, the appropriate taxonomy for the *Armillaria* sp. that was recovered in this study remains controversial. Other species of *Armillaria* that have been reported in Arizona include *A. mellea* and *A. gallica* Marxmüller & Romagnesi. The reports of *A. mellea* have been from the Chiricahua, Pinaleno, Santa Catalina, and Santa Rita Mountains in southeastern Arizona on three species of oak (Gilbertson and Bigelow 1998) and the report of *A. gallica* is from the Mogollon Rim on Douglas-fir in north-central Arizona (Nelson et al. 2013). Other species of *Armillaria* that are of interest in Arizona include *A. sinapina* (Table 1) and a recently discovered undescribed species from the State of Mexico, Mexico (Elias-Roman et al. 2013). However, it is improbable that these taxa are the common species of *Armillaria* associated with root disease in northern Arizona based on our results, but further sampling may discover that these other species are more common than what our findings suggest.

Armillaria solidipes can be highly pathogenic on conifers throughout the western United States, but we also found it infecting aspen in subalpine forests on the north Kaibab Plateau and in mixed conifer forests on the San Francisco Peaks. *Armillaria* was most often found on dead trees and was probably associated with mortality of the infected trees. However, insects that attack weakened trees (particularly bark beetles of conifers) may have also contributed to their death. Mortality of ponderosa pine in northern Arizona has been shown to sometimes involve a complex of *Armillaria* root disease and bark beetles (*Dendroctonus* spp. and *Ips* spp., Scolytidae) or dwarf mistletoe (*Arceuthobium* spp., Viscaceae), or both (Wood 1983). However, we did not examine trees for the presence of insects or other diseases. *Armillaria solidipes* generally exists as both a pathogen and a saprophyte, but can exist in a nonpathogenic state in some forests (Redfern and Filip 1991). However, since we primarily found *Armillaria* associated with dead trees that displayed symptoms of disease, the populations we sampled in northern Arizona are considered pathogenic and likely contributed to the death of the infected trees.

In comparison to the northern Rocky Mountains, northern Arizona evidently has a relatively low incidence of *Armillaria* root disease. McDonald et al. (1987) reported they found *Armillaria* associated with over 30% of each tree species they sampled in Washington, Idaho, and Montana. Our results found that the incidence of *Armillaria* on the

trees we examined was only 15% or less. Wood (1983) also found a relatively low incidence of *Armillaria* root disease in Arizona. The highest incidence of the disease he reported for Arizona was for mixed conifer/spruce-fir forests on the Apache-Sitgreaves National Forest where 24% of the dead trees he examined were infected with *Armillaria*.

Wood (1983) only reported that 1, 7, and 12% of the dead ponderosa pines examined on the Kaibab, Apache-Sitgreaves, and Coconino National Forests, respectively, had *Armillaria* root disease. In New Mexico, he only found *Armillaria* root disease on 8% of the dead ponderosa pines surveyed on the Carson and Santa Fe National Forests. Our results also demonstrate a relatively low incidence of *Armillaria* on ponderosa pine in northern Arizona. This was particularly evident in the ponderosa pine habitat types sampled where *A. solidipes* was only found on 3 of the 110 ponderosa pines we surveyed; all 3 were in the PIPO/FEAR plant association (Table 3). Most of the infected ponderosa pines we observed (6 of 10 trees) were in mixed conifer forests. McDonald et al. (1987) hypothesized that *Armillaria* was probably completely absent from the ponderosa pine and limber pine plant associations in the northern Rocky Mountains. Our results indicated that *Armillaria* may also be absent from, or at least rare in, most ponderosa pine plant associations in northern Arizona, but additional surveys are needed in these plant associations because our sample sizes were relatively small compared to those of McDonald et al. (1987).

Wood (1983) reported that 38% of the dead trees examined in the subalpine forests and 15% of the dead trees in the mixed-conifer forests of New Mexico had *Armillaria* root disease. Although we did not survey a large number of plant associations (4) or plots (10) in the subalpine forests of northern Arizona, the incidence of *Armillaria* was the highest (11% of the trees examined) in one of the plant associations (ABLA/JUCO6) compared to the other plant associations surveyed (PIEN/ACGL, PIEN/EREX4, and ABLA/LALAL3). We also found *Armillaria* in three mixed-conifer forest plant associations (ABCO/MARE11, PSME/QUGA, and PIEN/CAFO3). This suggested that additional sampling should be completed in the subalpine and mixed-conifer forests of northern Arizona, particularly on the Kaibab Plateau where *Armillaria* has been observed to be more common in those forest types than in the ponderosa pine type (Wood 1983). Of the 38 plots we surveyed on the Kaibab Plateau in subalpine and mixed conifer forests, 21% were infested with *Armillaria*. In addition, we found that 15% of the Engelmann spruce examined were infected with *Armillaria*, which was the highest

incidence of infection for a tree species surveyed. Therefore, it would be worthwhile to sample additional habitat types dominated by Engelmann spruce to better determine the incidence of *Armillaria* in subalpine and mixed-conifer forests elsewhere in Arizona. Our results and those reported by Wood (1983) and McDonald et al. (1987) have demonstrated that *Armillaria* is evidently more prevalent in mixed-conifer and subalpine forests than it is in ponderosa pine forests and this relationship should be investigated in other regions of the West.

The methodology used in this study has potential for use in the FIA inventory and monitoring program. The use of an additional subplot, along with the survey/sampling methods used, allows for application of this sampling protocol with little concern about adverse impact to the regular FIA plot footprint. Expanded employment of this methodology would permit statistically valid estimation of the minimum area with *Armillaria* present, as well as the ability to estimate change in the area infested by *Armillaria* over time. Future surveys using IW-FIA plots could use two, or possibly three, supplemental 0.04 ha plots which would increase the accuracy for estimates of the presence of *Armillaria*, yet continue to minimize the disturbance to the sampling site.

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Appendix 1. Common and scientific names of plants used for plant associations (Stuever 1997).

Common name	Scientific name
corkbark fir (subalpine fir)	<i>Abies bifolia</i> A.Murray (<i>Abies lasiocarpa</i> (Hooker) Nuttall)
white fir	<i>Abies concolor</i> (Gordon & Glendinning) Hildebrand
Engelmann spruce	<i>Picea engelmannii</i> Parry ex Engelmann
blue spruce	<i>Picea pungens</i> Engelmann
ponderosa pine	<i>Pinus ponderosa</i> Douglas ex Lawson & C. Lawson
Douglas-fir	<i>Pseudotsuga menziesii</i> (Mirbel) Franco
Gambel oak	<i>Quercus gambelii</i> Nuttall
Rocky Mountain maple	<i>Acer glabrum</i> Pursh
common juniper	<i>Juniperus communis</i> L.
creeping barberry	<i>Mahonia repens</i> (Lindley) G. Don
Nevada pea	<i>Lathyrus lanszwertii</i> Kellog
blue grama	<i>Bouteloua gracilis</i> (Kunth) Griffiths
Arizona fescue	<i>Festuca arizonica</i> Vasey
mountain muhly	<i>Muhlenbergia montana</i> (Nuttall) Hitchcock
fleabane	<i>Erigeron eximius</i> Greene
dryspike sedge	<i>Carex foenea</i> Willdenow