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Incidence, host relations and population structure of *Armillaria ostoyae* in Colorado campgrounds☆

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Abstract

Armillaria root disease is common and widely distributed in campgrounds of southwestern Colorado. *Armillaria ostoyae* spreads clonally underground and kills and decays tree roots, causing mortality or predisposing the trees to windthrow. We intensively surveyed and mapped genets (clones) of the pathogen in two campgrounds on the San Juan National Forest and one on Grand Mesa National Forest (GMNF). Three additional campgrounds on the GMNF were also surveyed. Infection (based on mycelium under the bark on or near the root collar) of all sampled live trees was 10.5% (range 7.5–15.0) inside campgrounds and 12.7% (3.3–25.9) immediately outside campgrounds, suggesting that campground construction and management practices have not exacerbated the disease. Dominant trees had significantly greater incidence of infection than trees in other crown classes. Isolates of *A. ostoyae* were obtained from 379 trees, and genets were identified by somatic incompatibility tests and variation in DNA microsatellite markers. The pathogen occurred as one large genet in the spruce-fir campground and several large genets in each of the mixed conifer campgrounds. Based on the size and distribution of the genets, the campgrounds appeared to be almost completely colonized by mosaics of centuries old genets, and little expansion of genet territories (or disease centers) appeared possible without loss of territory by another genet. The diseased trees in the campgrounds were randomly distributed rather than being organized into discrete disease centers. Live subalpine fir had significantly lower incidence of infection (7.0%) than Engelmann spruce, blue spruce and Douglas-fir (12.0, 12.3 and 15.7%, respectively), but evidence suggests a higher rate of mortality in subalpine fir. Crown thinning and dieback were useful symptoms for detection, but basal resinosis was the most efficient symptom indicating infection. Use of a combination of aboveground symptoms to select trees for more intensive examination is the most efficient approach to detection of infected trees. The disease poses difficult obstacles to long-term management of safe vegetation in developed sites.

Keywords: Forest disease; Root; Recreation; Spruce-fir; Mixed conifer; Hazard trees

1. Introduction

Armillaria root disease is the most common and widely distributed root disease in Colorado (Johnson, 1984) and poses major challenges to management of conifer forests. These challenges are particularly acute

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in developed recreation and administrative sites, where managers must deal with both hazard issues and long-term impacts on vegetation. Of mechanical failures (uprooting or snapping of roots, stems or branches) of conifers in recreation sites of the Rocky Mountain Region, 76% failed at the roots (Johnson, 1981). Root diseases are underestimated more than any other type of defect in inspections for hazardous trees (Sharon and Hubbard, 1985). Management of root diseases is further complicated because stumps as well as root systems of infected trees can provide inoculum for infection of neighboring trees. *Armillaria* species can spread somatically (clonally) underground via rhizomorphs and diseased root systems, and mortality is often more common at the edges of expanding infection centers. Also, any stress placed on living trees may predispose them to infection if the fungus is present on or near the roots (Wargo and Harrington, 1991).

In a general survey of the Rocky Mountain Region, *Armillaria ostoyae* was the only species of *Armillaria* found (Wu et al., 1996). However, further sampling and more rigorous identification tests are needed to confirm that *A. ostoyae* is the primary species responsible for mortality in conifer forests of southwestern Colorado, where the disease appears to be most common in spruce-fir and mixed-conifer forest types.

To better manage the disease, we need to compare disease incidence, symptomatology, rate of disease progress and mode of mortality among the hosts. We also need to know whether or not the disease is more severe in campgrounds than in surrounding, relatively undisturbed forests. Finally, information on spatial dynamics of the disease and related population structure of the pathogen is needed to determine whether there are discrete, expanding disease centers or continuous colonization of the stand and scattered mortality by single or multiple genets (clones).

The objectives of this study were to better elucidate the epidemiology of Armillaria root disease in recreation sites to provide a basis for development of management strategies. Here we present information on identification of the species of *Armillaria*, their host relations, disease detection, levels of disease in selected recreation sites and adjacent forests, and population structure of the pathogen.

2. Materials and methods

2.1. Field sampling

2.1.1. Sites

Two sites were sampled on the San Juan National Forest: Wolf Creek and Williams Creek Campgrounds, northeast and northwest of Pagosa Springs, CO (Table 1). Elevation is about 2425 m and precipitation averages between 65 and 90 cm annually. The forest type is mixed conifer, which occurs at relatively low elevations in southern Colorado.

Table 1
Incidence of Armillaria root disease in live trees of five host species in six campgrounds in southwest Colorado and their respective off-campground plots[a]

Campground	Forest type and elevation (m)	Engelmann spruce		Subalpine fir		White fir		Blue spruce		Douglas-fir	
		n	Infected (%)	*n*	Infected (%)	*n*	Infected (%)	*n*	Infected (%)	*n*	Infected (%)
Williams Creek	MC, 2423	693	9.4	1	0	1	0	28	25.0	2	0
Wolf Creek	MC, 2438	43	7.0	6	0	768	11.5	460	11.5	164	15.9
Big Creek	SF, 3085	560	16.8	222	6.3	0	–	0	–	0	–
Cobbett Lake	SF, 3121	710	8.5	110	8.2	0	–	0	–	0	–
Jumbo	SF, 2975	358	11.7	340	7.7	0	–	0	–	0	–
Spruce Grove	SF, 3054	832	14.4	399	6.5	0	–	0	–	0	–
Total[b]		3196	12.0 B	1078	7.0 A	769	11.4 B	488	12.3 B	166	15.7 B

MC: mixed conifer; SF: spruce-fir.

[a] Infection determined by mycelial fans under bark at or near the root collar.

[b] Total percentages of infection followed by the same letter are not significantly different ($\alpha = 0.01$) according to paired chi-square tests.

There is substantial influence from summer monsoons from the Gulf of Mexico, such that the wettest months are generally July and August. Mixed-conifer forests are composed of mixtures of white fir (*Abies concolor*), blue spruce (*Picea pungens*), Douglas-fir (*Pseudotsuga menziesii*), ponderosa pine (*Pinus ponderosa*), and sometimes other species. Wolf Creek Campground is in a mature stand dominated by the four conifer species. Williams Creek Campground is in a mosaic of mature trees, with some areas dominated by Engelmann spruce and some by blue spruce or ponderosa pine.

The four other campgrounds are at about 3000 m elevation on the Grand Mesa in the Grand Mesa National Forest (GMNF), north of the other campgrounds and north of Delta, CO. Annual precipitation is 90–115 cm, but most precipitation falls during winter as snow; December is the month with greatest precipitation. The forest type is spruce-fir, which occurs on relatively cool, moist, high-elevation sites and is dominated by Engelmann spruce (*Picea engelmannii*) and subalpine fir (*Abies bifolia*). The forest at Jumbo Campground is uneven-aged, with the overstory ranging from 100 to 240 years old. Aspen (*Populus tremuloides*) is interspersed in a mosaic, with cohorts 30, 65, 80 and 110 years old. The younger age classes of aspen may have arisen following clearing associated with the first use of the site for camping and then expansion and facilities development. The three other campgrounds sampled on the Grand Mesa are in forests considered even-aged, with stand ages ranging from 150 to 180 years old and with little or no aspen. In all the campgrounds, stands are dominated by spruce. Aside from campground establishment, and the occasional removal of dead trees, there has been little or no vegetation management or tree felling in these campgrounds.

2.1.2. Intensive sampling

Isolations of *Armillaria* species for population analysis were attempted at Wolf Creek, Williams Creek and Jumbo Campgrounds. In each campground, areas were delineated for intensive survey, based on prior hazard tree inspections, to include the main areas with Armillaria root disease. This included all of Wolf Creek Campground and most of the other two.

For every tree $\geq$17.8 cm (7 in.) DBH (diameter at breast height, 1.37 m), the following data were recorded: species; location (mapped by measuring azimuth and distance to reference points); designation as live, dead or stump; crown class; crown thinning ($0 =$ no significant thinning, $1 =$ low, $2 =$ moderate, $3 =$ severe and almost dead); branch dieback (scored like thinning); resinosis (above- and/or belowground); and colonization by *Armillaria* based on observations of mycelial fans under the bark. Branch dieback occurred from branch tips toward the bole, and generally in the upper two-thirds of the live crown. Examination for Armillaria infection was confined to the stem base, root collar, and major roots within 0.5 m of the collar. On living trees, the inner bark and cambium were examined for mycelial fans only when a canker (necrosis) or resinosis was visible on the outer bark. All dead-standing trees were recorded, but stumps were only recorded when they were colonized by *Armillaria*. Downed trees were recorded as stumps, but their status (uprooted or snapped) was noted.

Whenever *Armillaria* was found, samples of rhizomorphs, fans, and/or decayed wood were collected and kept at 4 °C until isolation. Isolations were attempted on benomyl/dichloran/streptomycin medium (Worrall, 1991) and repeated if the isolation failed. Pure cultures were stored on malt extract agar.

2.1.3. Additional campgrounds sampled

In three additional campgrounds (Big Creek, Cobbett Lake, and Spruce Grove, GMNF), all trees were similarly inspected, but only species, DBH, tree status (live/dead and standing/down) and colonization by *Armillaria* were recorded, and samples were not collected for isolations.

2.1.4. Comparison with adjacent forests

For all six campgrounds, we conducted similar sampling in plots adjacent to the campground to compare incidence of the disease. The sites selected had composition and topography similar to the campground, were within 50 m of the campground margin, and were as widely distributed as possible. Incidence of Armillaria root disease was not a criterion for plot placement. Four plots, 10 m $\times$ 50 m, were established adjacent to each campground. Trees in the off-campground plots were sampled in the same manner as in the respective campground plots (i.e. more data collected in plots adjacent to the three intensively sampled campgrounds).

2.2. *Armillaria* species identification

Armillaria isolates were identified to species primarily by the PCR–RFLP technique described by Harrington and Wingfield (1995). In addition, somatic compatibility between isolates indicated that the isolates were conspecific, as did common alleles of hypervariable, microsatellite loci of simple sequence repeats.

Template DNA for PCR was extracted (DeScenzo and Harrington, 1994) from representative isolates from each campground. Thermocycling conditions and reagent concentrations were as described by Harrington and Wingfield (1995). The PCR product was a portion of the intergenic spacer region (IGS-1) between the 28S and the 5S rDNA genes. The PCR product was digested with the restriction enzyme *Alu*I, electrophoresed in agarose, and stained with ethidium bromide.

2.3. Population structure

2.3.1. Somatic incompatibility

Genet identification among isolates from a given site was initially determined by somatic incompatibility tests with diploid isolates. These diploid isolates were selected as testers from across the campground. Four tester isolates were plated in the cardinal directions of an individual Petri plate of malt yeast extract agar (2% Difco malt extract and 0.2% yeast extract), and an unknown isolate was placed in the center. After 6–8 weeks, areas of confrontation between the testers and the unknown were examined, and a compatible reaction (indicating that the tester and unknown were of the same genet) was scored if the mycelia grew together without a barrage line and formed a common mycelium of uniform morphology (Harrington et al., 1992). A line of demarcation indicated somatic (vegetative) incompatibility and differing genotype (Worrall, 1997).

2.3.2. Microsatellite markers

Genetic analyses of somatic incompatibility testers was conducted using microsatellite markers to confirm that the genets identified by somatic compatibility were genetically distinct from each other, and that isolates from the same genet had the same alleles. We developed markers for two tri-nucleotide microsatellite loci from *A. ostoyae* using a procedure modified from Edwards et al. (1996), and we selected two (AOSSR27 and AOSSR84) di-nucleotide microsatellite loci from those reported by Langrell et al. (2001) for *A. osotyae* in France. Loci with the simple tri-nucleotide repeats (our markers) were identified and sequenced, and flanking PCR primers were designed to amplify these regions. We used the primers of Langrell et al. (2001) for the di-nucleotide repeat loci. For each primer pair, the forward primer was fluorescently labeled on the 5′-end with FAM, TET or HEX (IDT Inc., Coralville, IA). The primers for the tri-nucleotide locus CAG25 are ACAG25F (5′-FAM-CAT-GAC-GCC-ACG-GAT-ACC-A) and ACAG25R (5′-TCG-CTG-ACA-TGT-GCC-GAG-G), and for locus CAG77 the primers are ACAG77-F (5′-TET-AGG-CTG-GCC-GAA-TAG-TGA-AT) and ACAG77-R (5′-CTG-ATC-TGT-GAC-CTC-AAG-CA). One of the primers for AOSSR27 was labeled with HEX, and one of the primers for AOSSR84 was labeled with FAM. After PCR, the products from the four reactions (four primer pairs) of an isolate were combined and electrophoresis performed at the Iowa State DNA Sequencing and Synthesis Facility using an ABI Prism 377 gel system. GeneScan analysis software (ABI GeneScan v3.1.2) was used to estimate the PCR fragment lengths. These lengths varied by increments of three or two nucleotides in most cases, respectively, as would be expected for such loci.

2.4. Spatial analyses

In the three intensively sampled campgrounds, we conducted *K*-order nearest neighbor analyses of all trees (including dead and downed trees and stumps) versus the subset of those with *Armillaria* using the software tools provided by Crimestat II (Levine, 2002). The distribution of infected trees was compared with that of all trees. Rectangular correction for edge effects was used, and the bounding rectangle of the dataset with the larger distribution (all trees) was used for both datasets.

3. Results

3.1. Incidence on host species

Considering all sampled trees together, 11.1% of live trees in and around the sampled campgrounds had

detectable Armillaria infection. Aspen, narrowleaf cottonwood (*P. angustifolia*) and ponderosa pine were uncommon in our sample and were rarely found with Armillaria root disease, and incidence of Armillaria infection of these species was excluded from the analyses (Table 1). With all campgrounds and off-campground plots combined, chi-square analysis showed that disease incidence in live trees varied significantly ($P < 0.0001$) among the five most common tree species. Pairwise comparisons among species showed that subalpine fir had significantly ($P < 0.001$) lower incidence of infection than each of the other species (Table 1). No other differences were significant.

Although this study was not designed to assess mortality, counts of standing-dead trees, downed trees and stumps colonized by *Armillaria* could be used to explore differences among species in mortality caused by Armillaria root disease. These assessments assume that all stumps or dead trees with *Armillaria* fans were pathogenically colonized. Because complete counts of stumps were not made (only stumps with *Armillaria* fans were recorded), numbers of live trees were used to determine relative amounts of Armillaria-caused mortality. Most of the available data were for Engelmann spruce and subalpine fir at two campgrounds (Jumbo and Williams) and their off-campground plots. For Engelmann spruce, there were 77 colonized, dead trees or stumps and 1094 live trees. For subalpine fir, there were 70 dead trees or stumps colonized by *A. ostoyae* and 347 live trees. Chi-square analysis of these counts showed a highly significant difference ($P < 0.0001$), indicating significantly higher mortality due to the disease in subalpine fir than in Engelmann spruce.

3.2. Comparison with off-campground plots

Incidence of the disease varied among the six campgrounds and between the six campgrounds and their respective off-campground plots (Table 2). Overall, incidence of infection inside campgrounds (10.5%) was somewhat less than outside campgrounds (12.7%). Three campgrounds had significantly lower incidence inside than outside the campground, and two campgrounds had the reverse condition. When a conservative Bonferroni correction was applied (multiplying P by the number of campgrounds to reduce the chances of a type 1 error; Bland and Altman, 1995), only one difference was significant at $\alpha = 0.05$. In this case Wolf Creek Campground had significantly lower incidence of Armillaria root disease inside the campground (10.8%) than outside the campground (25.9%).

3.3. Symptoms

3.3.1. Crown thinning

The incidence of Armillaria root disease varied significantly ($P < 0.0001$) among trees of the different crown thinning classes according to a chi-square test (Table 3). Disease incidence was higher in trees with moderate crown thinning (27%) than in trees with light or no crown thinning (20 and 8%, respectively).

Table 2
Incidence of Armillaria root disease in live trees in six campgrounds and their respective off-campground plots in southwest Colorado[a]

Campground	On-campground		Off-campground		Corrected P[b]
	Infection (%)	No. of trees	Infection (%)	No. of trees	
Cobbett Lake	7.5	720	15.0	100	0.068
Williams	8.1	764	12.5	88	0.984
Jumbo	10.1	643	3.3	91	0.215
Wolf Creek	10.8	1470	25.9	54	0.004
Spruce Grove	11.2	1139	19.4	93	0.119
Big Creek	15.0	680	5.9	102	0.077
Total	10.5	5416	12.7	528	

[a] All tree species (including non-hosts) are included. Infection determined by mycelial fans under bark at or near the root collar.
[b] Incidence of disease within each campground was compared for significant differences ($\alpha = 0.05$) with the off-campground plots using chi-square test with the Bonferroni correction. Corrected P: the calculated P times the number of campgrounds tested (6).

Table 3
Incidence of Armillaria infection in trees in various symptom classes in three intensively sampled campgrounds and their respective off-campground plots[a]

Symptom	Class	Percentage of all trees[b]	Incidence of infection (%)	Percentage of all infected trees
Crown thinning	None	84.5	8.3	68.9
	Light	14.1	20.1	27.9
	Moderate	1.2	27.0	3.2
	Severe	0.2	0.0	0.0
Branch dieback	None	76.2	7.1	53.2
	Light	20.8	17.7	36.2
	Moderate	2.6	40.5	10.3
	Severe	0.4	7.7	0.3
Resinosis	None	92.5	4.8	45.9
	Aboveground	4.4	52.6	24.1
	Belowground only	3.2	90.7	29.9

[a] Infection determined by mycelial fans under bark at or near the root collar.
[b] Based on 3067 trees with data on crown thinning, 3067 trees with data on branch dieback, and 3075 trees with data on presence and position of resinosis.

However, less than one-third of the diseased trees expressed any crown thinning. No Armillaria root disease was detected in the six trees with severe crown thinning.

3.3.2. Branch dieback

The incidence of Armillaria root disease varied among the trees of different dieback classes based on chi-square analysis ($P < 0.0001$). Trees with moderate dieback had an infection incidence of 41%, which was greater than that of trees with light dieback, which was in turn greater than that of trees without dieback (Table 3). Only 1 of 13 trees with severe dieback was infected. Almost half (47%) of infected trees expressed some level of branch dieback.

3.3.3. Resinosis

Trees with resinosis on the lower stem or belowground were much more likely to be infected than were trees without resinosis (Table 3). Incidence of resinosis in trees infected with Armillaria was higher than the incidence of crown thinning or dieback, with a majority of infected trees expressing resinosis (57%; this figure includes 35 trees for which data on

Table 4
Relationships between infection in live trees and resinosis above and/or below the soil line by species in three intensively sampled campgrounds and their off-campground plots[a]

Species	Number of trees[b]	Incidence of infection (%) among trees with resinosis	Occurrence of resinosis (%) among infected trees
Engelmann spruce	1094	61.4 B[c]	80.9 A
Douglas-fir	166	95.0 A	73.1 AB
Blue spruce	488	81.1 A	71.7 AB
Subalpine fir	347	42.4 C	53.9 B
White fir	769	87.5 A	15.9 C
Total trees	2864	67.0	57.7

[a] Infection determined by mycelial fans under bark at or near the root collar.
[b] All trees, infected and uninfected, with and without resinosis.
[c] Within a column, values followed by the same letter are not significantly different according to a series of paired chi-square tests, $\alpha = 0.05$.

Table 5
Incidence of Armillaria infection and efficiency of detection when using multiple symptoms in trees at three intensively sampled campgrounds and their respective off-campground plots[a]

Symptoms	Percentage trees with one or more symptoms[b]	Incidence of infection (%)	Percentage of all infected trees detected
Crown thinning or branch dieback	30.1	17.3	54.4
Crown thinning, branch dieback or aboveground resinosis	32.2	18.9	63.0
Crown thinning, branch dieback, or resinosis above- or belowground	34.4	23.1	78.2

[a] Infection was determined by mycelial fans under bark at or near the root collar.
[b] Based on 3032 trees with data on crown symptoms and presence and position of resinosis, and 3067 trees with data on crown symptoms and resinosis, regardless of position of the resinosis.

location of resinosis was missing). Resinosis below the soil line was diagnostic for the disease, as 92% of the trees with belowground resinosis (at least in part) were infected by *Armillaria*. Resinosis that occurred above the soil line was often due to other causes, such as wounds, so its diagnostic value for Armillaria root disease was not as great as belowground resinosis.

However, these overall figures on resinosis hide major differences among species in expression of above- or belowground resinosis (Table 4). Infected Engelmann spruce trees most consistently had resinosis, followed by Douglas-fir and blue spruce. Only about half of infected subalpine fir trees had resinosis, and only 16% of the infected white fir had resinosis.

3.3.4. Multiple symptoms

We computed the efficiency of combinations of symptoms in detecting infected trees (Table 5). The incidence of infection in the combined-symptom categories was not much higher than that for individual symptom classes (Table 3), but the percentage of infected trees in the combined groups was substantially higher. Trees with crown symptoms (thinning or branch dieback) included 54% of all infected trees (Table 5). When aboveground resinosis was added to the symptom group, 63% of infected trees were included. Adding belowground resinosis resulted in inclusion of nearly 80% of infected trees.

3.4. Tree size and crown class

The mean DBH of all infected trees (35 cm; $n = 633$) was significantly greater ($P \leq 0.0001$ according to a *t*-test) than that of uninfected trees (33 cm; $n = 5062$). The difference in diameter was greatest in blue spruce and Douglas-fir, but the tests of those species individually did not show significant

Table 6
Incidence of Armillaria infection by species and crown class in three intensively sampled campgrounds in southwest Colorado and their respective off-campground plots[a]

Species	Dominant		Codominant		Intermediate		Suppressed	
	No. of trees	Incidence of infection (%)	No. of trees	Incidence of infection (%)	No. of trees	Incidence of infection (%)	No. of trees	Incidence of infection (%)
Engelmann spruce	62	16.1	491	9.0	444	10.1	96	11.5
Blue spruce	88	15.9	205	12.2	156	10.3	37	10.8
Douglas-fir	32	18.8	64	20.3	61	11.5	9	0
Subalpine fir	2	0	175	5.1	149	9.4	18	5.6
White fir	60	20.0	174	9.8	405	12.6	129	6.2
Total trees[b]	244	17.2 A	1109	9.7 B	1215	10.9 B	289	8.3 B

[a] Infection determined by mycelial fans under bark at or near the root collar.
[b] Total percentages of infection followed by the same letter are not significantly different according to paired chi-square tests ($\alpha = 0.01$).

difference because of the relatively small sample sizes (488 blue spruce and 166 Douglas-fir).

Dominant trees had significantly higher incidence of infection than trees in all other crown classes (Table 6). That difference was strongest in the spruces and white fir. Suppressed trees generally had low incidence of infection.

3.5. Species identification and population structure of Armillaria

3.5.1. Species identification

Representative tester isolates from each of the three campgrounds were identified to species using the PCR test. Digestion of the IGS PCR product with *Alu*I gave the unique profile for *A. ostoyae* (Harrington and Wingfield, 1995). Other isolates were identified based on somatic compatibility with identified isolates or by microsatellite alleles in common with identified isolates. In total, 93 isolates were tested by one or more of these criteria, and each proved to be *A. ostoyae*.

3.5.2. Somatic incompatibility

At each campground, isolates scattered across the campground were selected for somatic incompatibility testing with all other isolates from that campground. For Williams Creek Campground we selected 16 testers, 8 testers were selected from Wolf Creek, and 4 were selected from Jumbo Campground. Five somatic incompatibility groups were found at Williams Creek, though there were some ambiguous reactions, and not every isolate proved compatible with a tester. Seven somatic compatibility groups were found among the Wolf Creek isolates, again with some ambiguous reactions. Only one somatic incompatibility group was detected from Jumbo Campground.

3.5.3. Microsatellite markers

Alleles of approximately 356 and 359 bp were found for locus CAG25, and alleles of 328, 331, 334, 337, 340, and 349 bp were found for locus CAG77 (Table 7). For the di-nucleotide repeat AOSSR27, alleles of approximately 143, 145, 147, 149, and 151 bp were found, and alleles of 126, 128, 132, 134, and 136 bp were found for locus AOSSR84.

Isolates were homozygous or heterozygous for the four loci. The genet identified at Jumbo Campground was homozygous for three of the loci, but bands of three sizes were identified for the SSR84 locus (Table 7). Similar alleles were found at the three campgrounds, thus supporting the contention that only one species of *Armillaria* was present at these three campgrounds. However, each compatibility group had a unique set of alleles for the four loci, and no genotype was found at more than one campground (Table 7).

Table 7
Alleles (size of PCR product in bp) of four microsatellite loci found among 14 genets of *Armillaria ostoyae* at three campgrounds in Colorado

Campground	Genet	Number of isolates tested	Microsatellite alleles[a]			
			CAG25	CAG77	AOSSR27	AOSSR84
Williams Creek	WM-G1	2	356/359	328	143/147	134/137
	WM-G2	12	359	328	143/151	126
	WM-G3	17	359	328/331	145/147	126
	WM-G4	10	359	328/334	143/147	126
	WM-G7	18	359	331	143/147	134
	WM-G9	2	356/359	328	143/147	134
Wolf Creek	WF-G3	2	356	340/349	149	136
	WF-G5	2	359	328	145	134
	WF-G8	2	359	328/331	143/147	126
	WF-G10	6	359	328/331	143/147	128/136
	WF-G11	4	359	328/331	143	134
	WF-G13	5	359	328/337	143	134
	WF-G14	2	356	337/340	n.d.	134
Jumbo	J-G1	10	359	328	143	128/132/136

[a] The two alleles at heterozygous loci are separated by a slash.

3.5.4. Genet delimitation

Most isolates were found to be members of a multi-tree genet based on the compatibility tests and/or based on a unique set of alleles at the four microsatellite loci. Not all of the isolates showed somatic compatibility with testers, and some isolates could not be tested because they died or were contaminated. Because of the difficulty and cost associated with microsatellite analyses, only representative isolates could be "fingerprinted" with the microsatellite markers. With few

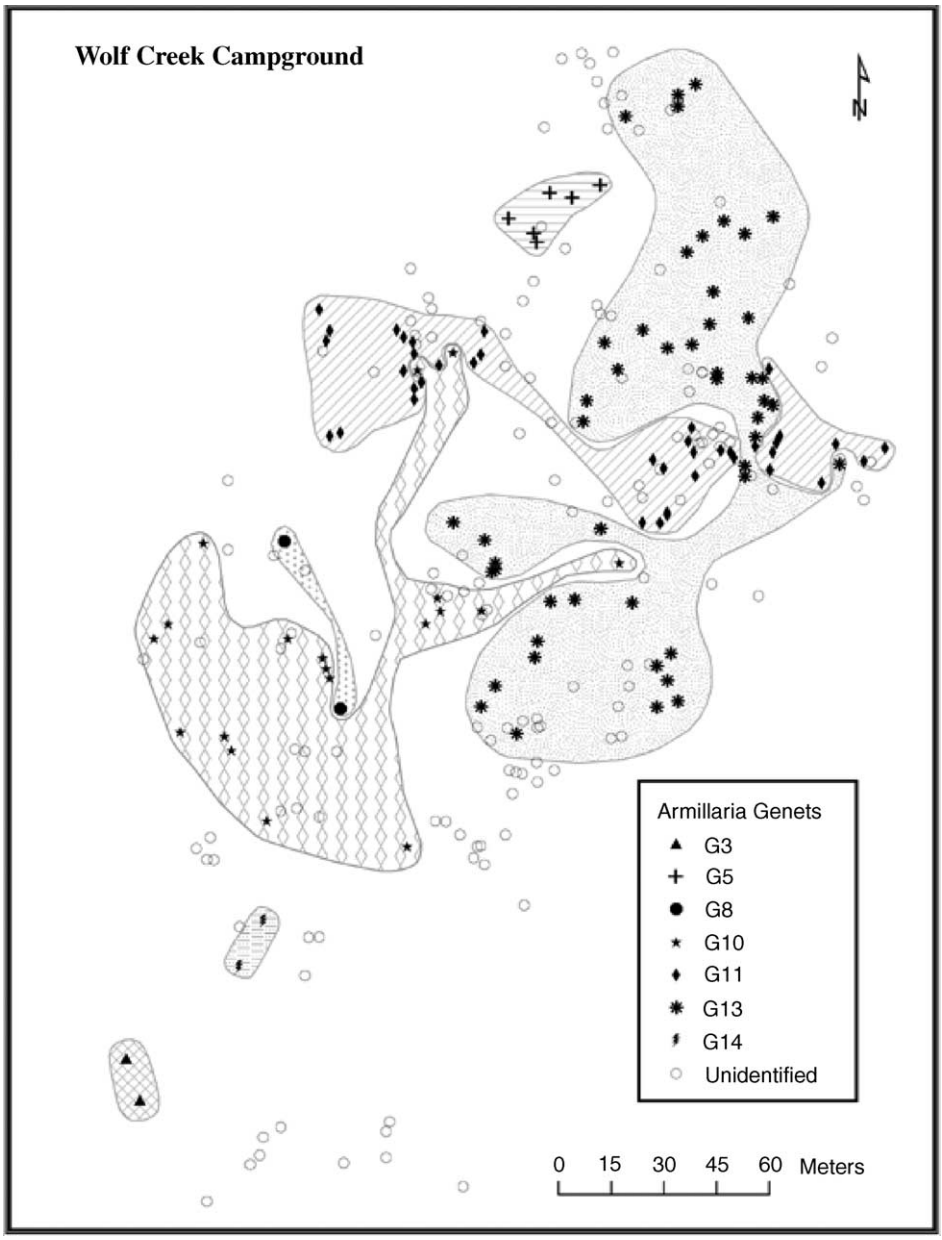

Fig. 1. Distribution of trees and stumps colonized by *A. ostoyae* and the distribution of genets in Wolf Creek Campground. Open circles represent occurrences of the pathogen that were not identified to genet.

exceptions, there was excellent agreement between genet determination based on somatic incompatibility and microsatellite markers. Ambiguous somatic incompatibility reactions were seen between the isolates of genet WF-G8 and the two isolates of genet WF-G10, though isolates of these two genets differed at locus AOSSR84 (Table 7). Some individual isolates showed unique combinations of microsatellite alleles but were not compatible with other isolates and may have been single-tree genets (data not shown in Table 7).

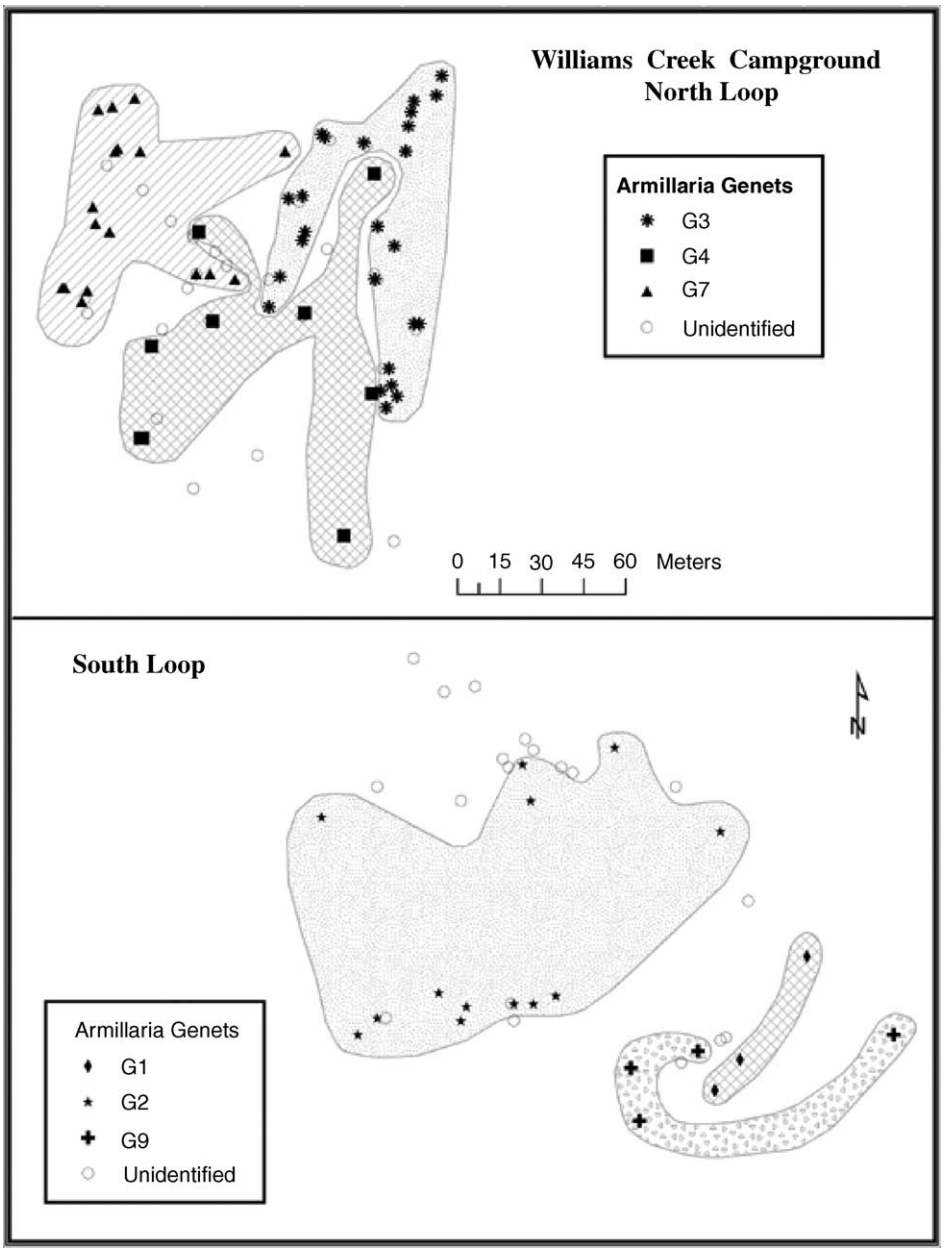

Fig. 2. Distribution of trees and stumps colonized by *A. ostoyae* and the distribution of genets in Williams Creek Campground. Open circles represent occurrences of the pathogen that were not identified to genet.

Seven, six and one multi-isolate genets were identified at Wolf Creek, Williams Creek and Jumbo Campgrounds, respectively (Figs. 1–3). The lengths of the largest identified genets at Williams Creek, Wolf Creek and Jumbo Campgrounds were at least 320, 209, and 297 m, respectively. Most or all of the genets likely were larger, but we were not able to assign isolates to them because of technical difficulties, or the genets extended beyond the plot boundaries. Eleven isolates in off-campground plots adjoining Wolf Creek and Williams Creek Campgrounds were also identified to genet. In most cases these isolates belonged to genets also identified in the respective campground, but two isolates near Williams Creek belonged to a genet that was not found inside the campground.

3.6. Distribution of infected and uninfected trees

In the three mapped campgrounds (four distributions because Williams Creek was sampled in two discontinuous areas), nearest-neighbor indices for all trees indicated clustering at the scales observable in our sampling (Fig. 4). Infected trees tended to be less clustered than all trees and approximated a random distribution at larger scales. In Jumbo Campground, for example, clustering of all trees was equivalent to that of infected trees at very small scales, but the

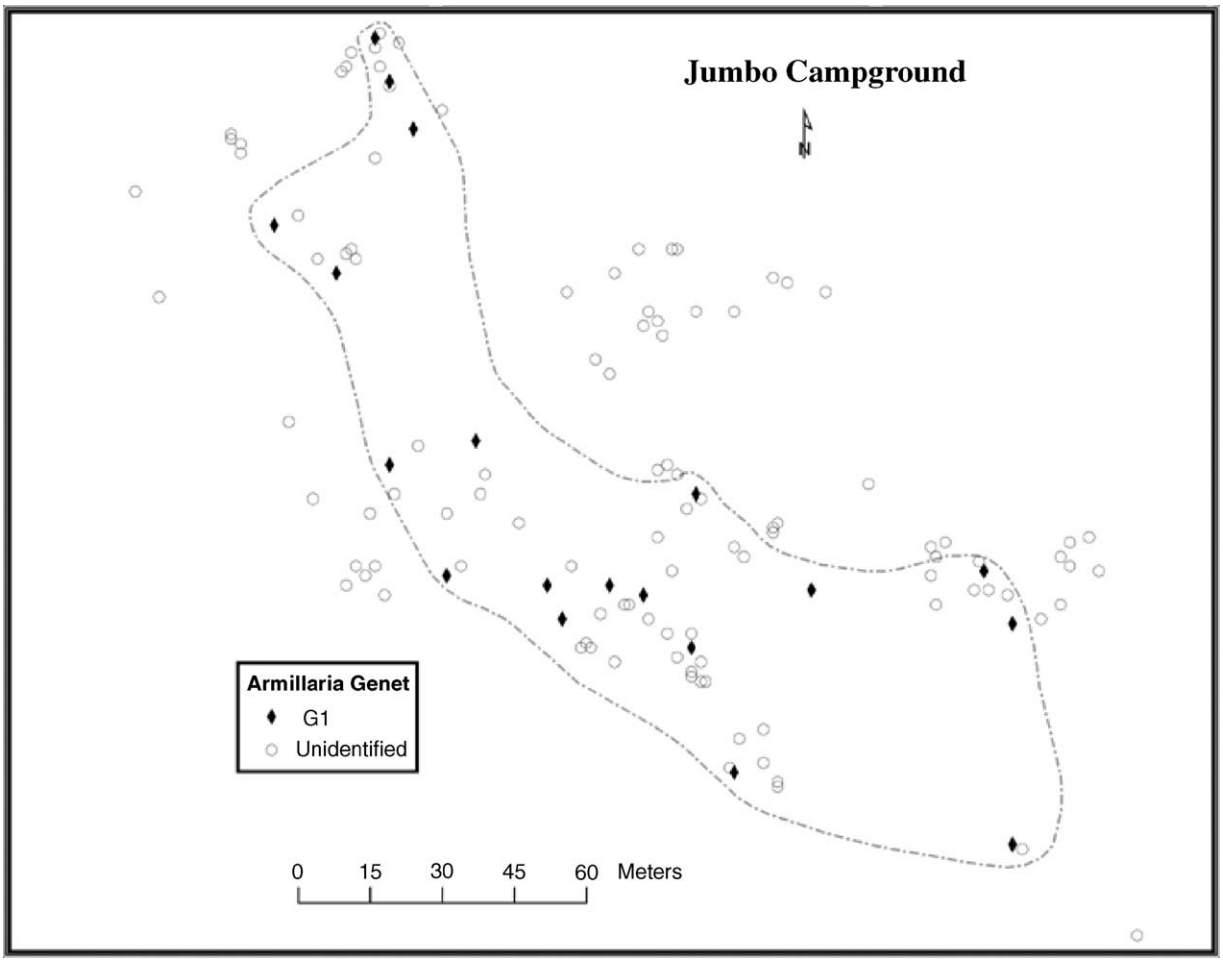

Fig. 3. Distribution of trees and stumps colonized by *Armillaria ostoyae* and the distribution of the identified genet in Jumbo Campground. Open circles represent occurrences of the pathogen that were not identified to genet.

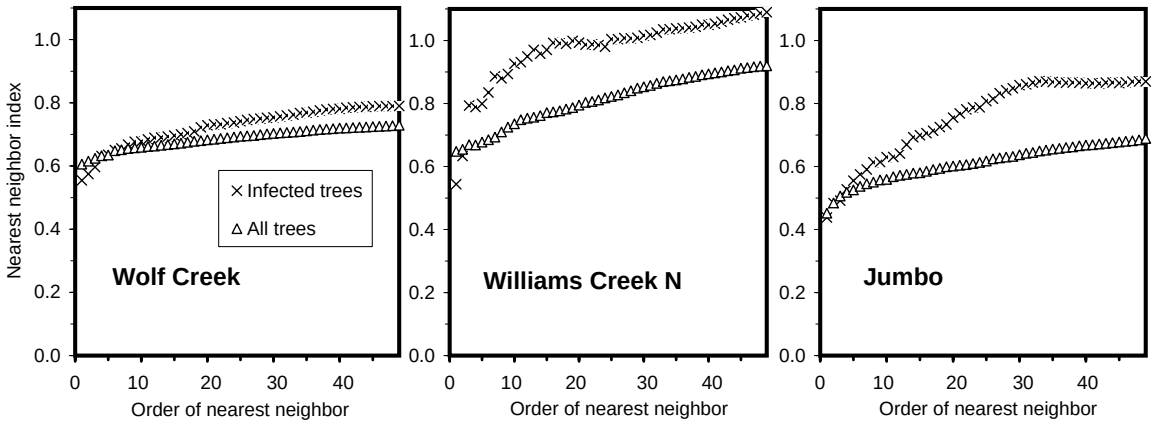

Fig. 4. Results of a *K*-order nearest neighbor analysis of infected trees (live, dead and stumps) only and all trees (including uninfected) at three campgrounds. An index of 1.0 indicates spatial randomness and an index approaching 0 indicates extreme clustering.

distribution of infected trees was more random than that of all trees at larger scales (Fig. 4). The only exception was that, in the northern section of Williams Creek Campground and in Wolf Creek Campground, the first order of nearest neighbor of infected trees (i.e. pairs) was slightly more clustered than that of all trees.

4. Discussion

The forest floors of the campgrounds we sampled in southwest Colorado were well colonized by large genets of *A. ostoyae*. The incidence of Armillaria-infected trees in each of the campgrounds was high, and clearly, the incidence of Armillaria root disease at these sites is greater than we detected. Only the root crown and immediately adjacent roots were examined, and we were limited by the need to avoid significant tree damage in our sampling. The proportion of infections that were detected may vary with host species, tree size, and other factors, but the five conifer species most commonly encountered each showed substantial levels of disease.

4.1. Incidence on host species

All members of the Pinaceae in the Rocky Mountain Region are hosts of Armillaria root disease, but subalpine fir generally has been considered one of the most susceptible hosts. In a road-side survey of root disease mortality centers in southern Colorado, 57 centers were found in subalpine fir, and only two were in Engelmann spruce (James and Goheen, 1981). Lodgepole pine and ponderosa pine are also common hosts in the region (Johnson, 1984), but the disease in lodgepole pine is generally confined to trees less than 20 years of age (Johnson, 1999; Sharon, 1988), and ponderosa pine is infected only in certain localities. Engelmann spruce and other hosts are usually considered to be less commonly infected or more tolerant to Armillaria root disease. The low incidence of disease that we found in living subalpine fir, relative to other species, is not consistent with these opinions and the limited available data.

However, incidence of disease in living hosts is only one aspect of comparing host susceptibility. Disease dynamics must also be considered. Engelmann spruce may survive with the disease for an extended period, meaning more live infected trees, but a lower mortality rate. Extended survival (‘‘tolerance’’) may actually be more hazardous in a recreation setting because extensive root decay and mechanical failure can occur before development of crown symptoms and death. Subalpine fir, on the other hand, may die quickly after the disease is established, so that there are relatively few live infected trees but a higher mortality rate. Indeed, our limited data on mortality indicated greater mortality associated with the disease in subalpine fir than in Engelmann spruce. This may be due to a greater rate of disease progress after infection, but

it may also be due to a difference in the bark beetles on the respective hosts. The western balsam bark beetle (*Dryocoetes confusus*), which attacks subalpine fir, may more selectively and effectively kill root-diseased trees than does the spruce beetle (*Dendroctonus rufipennis*) and other bark beetle species on *Picea* spp. Information is needed on disease dynamics, bark beetle biology and mode of death to more fully understand differences in disease incidence among the hosts.

4.2. Comparison with off-campground plots

Although the incidence of Armillaria root disease in living trees was lower in most of the campgrounds than in their off-campground plots, the data do not show a consistent and significant trend. Lower incidence in campgrounds could be due in part to removals for hazard tree management, in which trees with branch dieback or crown thinning might be removed. However, such tree removals were rare in these campgrounds and would not have greatly affected disease incidence in living trees. Other aspects of campground use may also decrease incidence of the disease.

Our data do not support the hypothesis that the disease is more severe in campgrounds than in neighboring forests. Our data do suggest that the disease is much more common in the forest at large than has been considered heretofore.

4.3. Symptoms

There are two aspects to the usefulness of a symptom in detecting disease. One is the diagnostic value of the symptom, as measured by the incidence of disease in trees with the symptom. The other is the consistent expression of the symptom, as measured by the percentage of infected trees that express the symptom. To the extent that a symptom is not diagnostic, one would make a type 1 error, rejecting the null hypothesis when in fact a symptomatic tree is uninfected. To the extent the symptom is not expressed, one makes a type 2 error (accepts the null hypothesis) and misses infected trees. The perfect symptom would be expressed only by, and by all, infected trees.

Although crown thinning indicated an increased likelihood of Armillaria infection, infection was not detected in most trees with crown thinning. Also, most infected trees did not have noticeable crown thinning. Thus, while crown thinning has some value in detecting the disease, its diagnostic value and expression are low.

If one excludes trees with severe branch dieback from consideration, dieback is a somewhat useful symptom. It was more diagnostic than crown thinning, in that a higher percentage of trees with dieback (particularly moderate) were infected. It was also more reliably expressed than crown thinning, as nearly half of infected trees showed dieback. However, reliance solely on branch dieback would still result in misdiagnosis of most trees.

Resinosis was highly diagnostic, especially when the resin was detected below the soil line. Even trees with resin above the soil line are much more likely to be infected than trees without resinosis. Resinosis, properly evaluated, is nearly perfect as a diagnostic symptom, and correct diagnoses will almost always be made when the symptom is present. However, its absence is less conclusive. With Engelmann spruce the probability of missing infected trees by relying on resinosis is relatively low, but it increases with the other species.

The percentage of infected trees showing aboveground symptoms in this study (63%; Table 5) is identical to the same datum for Armillaria root rot in mature conifers in two plots in a partially cut stand in the dry climate zone of southern interior British Columbia (Morrison et al., 2001). However, in that study, dead trees were included in the calculation, symptoms were limited to resinosis and lesions, and root systems were completely extracted from the soil for diagnosis, so virtually all infections were detected.

Because of labor costs and potential damage to living trees, not every tree in a campground can be inspected for the most diagnostic sign of Armillaria root disease, that is, mycelial fans under the bark of the major lateral roots or the base of the tree. Aboveground symptoms are needed to identify which trees are to be intensively examined for mycelial fans. We found that multiple aboveground symptoms are more powerful than any single symptom in the detection of Armillaria root disease. Intensive investigation of trees with either crown thinning or branch dieback would result in detection of more than half of the infected trees. Including aboveground resinosis with

the crown symptoms (32% of the trees in these campgrounds) would result in detection of almost two-thirds of the infected trees. If trees were examined for belowground resinosis, detection efficiency would be even higher.

4.4. Armillaria species identification

Only one species of *Armillaria*, *A. ostoyae*, was identified at the three campgrounds. This is not surprising as it is the only identified species of *Armillaria* from the central and southern Rockies (Wu et al., 1996), and the species most commonly associated with conifer mortality in North America east and north of California. The PCR–RFLP test for species determination (Harrington and Wingfield, 1995) was performed on only a few testers from each campground, but these isolates were somatically compatible with many more isolates and shared microsatellite alleles with others.

4.5. Population structure and distribution of infected trees

The forest floor of each of the campgrounds appeared to be colonized by one or more interlocking genets in a mosaic pattern. Consistent with earlier studies of *A. ostoyae* (Rizzo and Harrington, 1993; Rizzo et al., 1995; Worrall, 1994), each genet generally occupied a discrete area, though some genets appeared to be divided or fragmented, presumably from invasion by other genets.

The campground in the spruce-fir forest, Jumbo, was occupied by one large genet, but the campgrounds in mixed-conifer forests, Wolf Creek and Williams Creek, were occupied by multiple genets. These mixed-conifer forests have more late-season rainfall, which may be more conducive to mushroom production and initiation of new infection centers (Rizzo and Harrington, 1993; Harrington and Wingfield, 1998). More extensive sampling would be needed to determine if this represents real differences between the forest types, but genets found in all three campgrounds were relatively large, as has been found for *A. ostoyae* genets in other parts of the western USA. Although our genets could be much larger than our sampling showed, this apparently continuous colonization of the forest floor by *Armillaria* genets in our campgrounds, in some cases with multiple genets in a small area, contrasts with eastern Oregon, where genets of *A. ostoyae* up to 965 ha have been found separated by large forested areas apparently uncolonized by *Armillaria* (Ferguson et al., 2003).

Radial spread rate of *A. ostoyae* has been estimated at roughly 1 m per year in young conifer stands (Peet et al., 1996; Shaw and Roth, 1976) and 0.22 m per year in a 110-year-old stand of Douglas-fir (van der Kamp, 1993). Using the slower rate for mature stands, the largest of the genets found in the three campgrounds are estimated to be at least 700–900 years old. Because most genets are bordered by other, competing genets, the genets are probably not expanding significantly. Thus, the genets are likely much older than the above estimates. The genets were undoubtedly present before the campgrounds were established around 1950, and the sizes of the genets are probably not greater in the campgrounds than in the surrounding forest.

These mature genets appear to be beyond the expansion phase, at least in the mixed-conifer forest type, and may survive largely by colonizing susceptible trees within their established territories. There is no expanding margin of a genet, and hence no concentration of mortality at the periphery of a disease center, as is sometimes found in forests with little summer rainfall, where mushroom production is rare and infection centers are relatively few (Harrington and Wingfield, 1998). There was no evidence of spatial clustering of the diseased trees, and management strategies based on discrete disease centers (Morrison et al., 1991; Williams et al., 1986) appear to be inappropriate here. Current management practices do not appear to be consistently increasing or decreasing the incidence of the disease within the campground relative to the surrounding forest.

4.6. Management implications

The high level of Armillaria root disease in these campgrounds, and in similar developed forest sites, presents a management challenge on two fronts: (a) infected trees are hazardous and (b) mortality from the disease and removal of hazard trees make it difficult to ensure a sustainable forest cover. Effective and practical measures for reducing future infection in infested campgrounds are not available. Management therefore

must be oriented toward avoiding the disease or reducing current incidence of advanced infections by efficient detection and removal of diseased trees.

Our data show that these campgrounds were established in stands that were already infested by the pathogen and suggest that the incidence of infection in living trees was not strongly influenced by campground use and management. Site selection is thus a critical factor, and disease avoidance, where possible, should be an effective management approach. Potential sites should be surveyed for incidence of root disease before establishing or relocating campgrounds. An additional measure would be to avoid areas with highly susceptible tree species, most notably Douglas-fir, Engelmann spruce and blue spruce, or these species may be selectively removed to create roadways and make clearings. Infected trees, in particular, should be identified and removed before facilities are laid out and constructed.

Based only on location relative to infected trees, all trees have equal likelihood of infection, so targeting trees for removal based on proximity to known diseased trees is not supported by our data. Inspections should be designed to maximize efficiency of detection of diseased trees. Careful inspection of all root systems in a campground for symptoms and signs is not practical on a yearly basis, but our data suggest that about two-thirds of trees with mycelial fans at or near the root collar could be detected by examining the root collars of only those trees with crown thinning, branch dieback or aboveground resinosis.

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