Coarse-scale population structure of pathogenic *Armillaria* species in a mixed-conifer forest in the Blue Mountains of northeast Oregon

B.A. Ferguson, T.A. Dreisbach, C.G. Parks, G.M. Filip, and C.L. Schmitt

Abstract: The coarse-scale population structure of pathogenic *Armillaria* (Fr.) Staude species was determined on approximately 16 100 ha of relatively dry, mixed-conifer forest in the Blue Mountains of northeast Oregon. Sampling of recently dead or live, symptomatic conifers produced 112 isolates of *Armillaria* from six tree species. *Armillaria* species identifications done by using a polymerase chain reaction based diagnostic and diploid–diploid pairings produced identical results: 108 of the isolates were *Armillaria ostoyae* (Romagn.) Herink and four were North American Biological Species X (NABS X). Five genets of *A. ostoyae* and one of NABS X were identified through the use of somatic incompatibility pairings among the putatively diploid isolates. *Armillaria ostoyae* genet sizes were approximately 20, 95, 195, 260, and 965 ha; cumulative colonization of the study area was at least 9.5%. The maximum distance between isolates from the 965-ha *A. ostoyae* genet was approximately 3810 m, and use of three estimates of *A. ostoyae* spread rate in conifer forests resulted in age estimates for the genet ranging from 1900 to 8650 years. Results are discussed in relation to possible mechanisms that influenced the establishment, expansion, and expression of these genets; the genetic structure and stability of *Armillaria*; and the implications for disease management in this and similar forests.

Résumé : La structure grossière des populations des espèces pathogènes d'*Armillaria* (Fr.) Staude a été établie sur une superficie d'environ 16 100 ha de forêt mixte de conifères relativement sèche située dans les Blue Mountains du Nord-Est de l'Oregon. L'échantillonnage de conifères vivants ou morts récemment montrant des symptômes d'infection a permis de récolter 112 isolats d'*Armillaria* sur six espèces d'arbres. L'identification des espèces d'*Armillaria* soit par la méthode diagnostique d'amplification par réaction en chaîne de la polymérase, soit par des confrontations avec des testeurs diploïdes, a produit des résultats identiques : 108 isolats correspondaient à *Armillaria ostoyea* (Romagn.) Herink et quatre appartenaient à l'espèce biologique nord-américaine X. Cinq souches distinctes de *A. ostoyea* et une souche de l'espèce biologique X ont été identifiées grâce à l'utilisation de tests d'incompatibilité somatique parmi les isolats considérés comme diploïdes. La superficie occupée par chacune des souches de *A. ostoyea* correspondait à environ 20, 95, 195, 260 et 965 ha, ce qui représente au total au moins 9,5 % de l'aire d'étude. La distance maximum entre les isolats de la souche qui occupe une superficie de 965 ha est d'environ 3810 m et l'utilisation de trois estimations du taux de progression de *A. ostoyea* dans les forêts de conifères indique que l'âge de cette souche se situe entre 1900 et 8650 ans. La discussion porte sur les mécanismes qui pourraient avoir influencé l'établissement, la progression et l'expression de ces souches, sur la structure et la stabilité génétiques d'*Armillaria* et sur les conséquences pour la gestion de la maladie dans la forêt qui a fait l'objet de cette étude ou dans des forêts similaires.

[Traduit par la Rédaction]

Introduction

Armillaria ostoyae (Romagn.) Herink, the cause of one form of Armillaria root disease, is a widespread, fungal pathogen of conifers in western North American forests (Filip and Goheen 1984; James et al. 1984; Morrison et al. 1985). The genus *Armillaria* (Fr.) Staude possesses a bifactorial, heterothallic mating system (Anderson and Ullrich 1982) in which two haploid basidiospores with compatible mating-type alleles mate to form a secondary myce-

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B.A. Ferguson. Montana Department of Natural Resources and Conservation and Idaho Department of Lands, 2705 Spurgin Road, Missoula, MT 59804-3199, U.S.A.
T.A. Dreisbach. USDA Forest Service, Pacific Northwest Research Station, Forestry Sciences Laboratory, 3200 Jefferson Way, Corvallis, OR 97331, U.S.A.
C.G. Parks.[1] USDA Forest Service, Pacific Northwest Research Station, Forestry and Range Sciences Laboratory, 1401 Gekeler Lane, LaGrande, OR 97850, U.S.A.
G.M. Filip. Forest Science Department, 216 Richardson Hall, Oregon State University, Corvallis, OR 97331-5752, U.S.A.
C.L. Schmitt. USDA Forest Service, Blue Mountains Pest Management Service Center, 1401 Gekeler Lane, LaGrande, OR 97850, U.S.A.

[1]Corresponding author (e-mail: cparks01@fs.fed.us).

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lium or genet (Guillaumin et al. 1991). Little is known regarding the rate of establishment of genets or the types of organic substrate most suitable for basidiospore infection and mating (Redfern and Filip 1991; Rishbeth 1988). Precipitation and soil moisture are hypothesized to be the main influences on the size and density of genets, however, because of their positive effects on fruiting frequency and basidiospore abundance, survival of basidiospores, and successful establishment of diploid mycelium (Anderson et al. 1979; Kile 1986; Worrall 1994). Relatively small genets of *A. ostoyae* seem to dominate in moist forest types (Rizzo et al. 1995; Smith et al. 1994; Worrall 1994), whereas larger genets appear to be more typical in drier forest types (Adams 1974; Shaw and Roth 1976) (Table 1). Studies of *A. ostoyae* population structure have shown that apparently distinct Armillaria root disease *mortality centers can be occupied by a single genet of fungus (Dettman and van der Kamp 2001a*) or multiple genets of fungus that have come in contact and formed a continuous mortality center (Dettman and van der Kamp 2001*b*; Legrand et al. 1996; Smith et al. 1994). A single, extensive genet of *A. ostoyae* can also cause multiple, discrete mortality centers with nonsymptomatic trees between them (Adams 1974; Dettman and van der Kamp 2001*b*; Shaw and Roth 1976).

Root infections of *A. ostoyae* can be either progressive or callused (Morrison et al. 1991*b*). Progressive infections proceed distally and proximally from the point of infection with no formation of callus (Morrison et al. 1991*b*) and in most cases result in host mortality once the infection reaches the taproot or root collar (Shaw 1980; Klein-Gebbinck et al. 1991*b*). Callused, resinous lesions often form on roots of juvenile to mature trees (Robinson and Morrison 2001; Shaw 1980), remaining quiescent until the tree is killed or harvested. The fungus expands from these quiescent lesions to colonize the root system, thereby developing a high inoculum potential that can result in the death of surrounding trees over a 15- to 30-year period (Morrison and Pellow 1994; Shaw 1980; Woods 1994). Viable *A. ostoyae* inoculum can persist in belowground portions of stumps and large roots for several decades (Roth et al. 1980). Root infections in relatively dry forest types are less frequent than in relatively moist forest types, but are composed of a higher percentage of progressive lesions more likely to result in host mortality (Morrison et al. 2000).

Conifers exhibit variation both in response to infection by *A. ostoyae* (Robinson and Morrison 2001) and susceptibility to damage and mortality (Hadfield et al. 1986; Morrison et al. 1991*a*; Schmitt 2001). Hadfield et al. (1986) provided three susceptibility categories for conifers in forests east of the Cascade Range in Oregon and Washington: inland variety Douglas-fir (*Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco) and grand fir (*Abies grandis* (Dougl. ex D. Don) Lindl.) are rated as "severely damaged", ponderosa pine (*Pinus ponderosa* Dougl. ex P. & C. Laws.), lodgepole pine (*Pinus contorta* Dougl. ex Loud.), and subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.) as "moderately damaged", and western larch (*Larix occidentalis* Nutt.) as "seldom damaged". Morrison et al. (1991*a*) provided four susceptibility categories for conifers in the southern interior of British Columbia: Douglas-fir, *Abies* spp., and *Picea* spp. are rated as "susceptible", lodgepole pine as "moderately susceptible", ponderosa pine as "tolerant", and western larch as "resistant". In the Blue Mountains of northeast Oregon lodgepole pine is considered "tolerant" to *Armillaria* infection, whereas ponderosa pine has been observed to be severely damaged on some sites and moderately damaged on others (Schmitt 2001).

As with other *Armillaria* species (Kile 1983; Smith et al. 1992), vegetative growth is the primary mode of spread by *A. ostoyae* (Adams 1974; Shaw and Roth 1976; Smith et al. 1994). Once formed, diploid mycelium of *A. ostoyae* spreads via root contacts or short-distance growth of rhizomorphs through soil (Cruickshank et al. 1997; Morrison et al. 2001; Redfern and Filip 1991). Pathogenic *Armillaria* species such as *A. ostoyae* are thought to rely much more on root-to-root contact than on rhizomorphs for infection (Reaves et al. 1993; Redfern and Filip 1991), although in some cases the role of rhizomorphs appears important (Klein-Gebbinck et al. 1991*b*; Rizzo and Harrington 1993). Estimates of spread rates in the forest range from 0.22 m/year (van der Kamp 1993) in 110-year-old Douglas-fir in the central interior of British Columbia, to 0.87 m/year in naturally regenerated, young-growth ponderosa pine in south-central Washington (Shaw and Roth 1976), to 0.7 to 1.3 m/year in 20- to 27-year-old Douglas-fir plantations in the southern interior of British Columbia (Peet et al. 1996). Given time and continuity of susceptible hosts, genets of *A. ostoyae* can expand to remarkable sizes (Adams 1974; Legrand et al. 1996; Shaw and Roth 1976).

Armillaria root disease had previously been identified as the primary cause of conifer mortality in a relatively dry, mixed-conifer forest on several, adjacent watersheds in the Blue Mountains of northeast Oregon (C. Schmitt, USDA Forest Service, unpublished reports). Areas of symptomatic and dead trees ranged in size from a few trees to 100+ ha, and examination of aerial photographs revealed numerous, ring-shaped openings in the forest canopy that are characteristic of Armillaria root disease (Kile et al. 1991). Areas of nonsymptomatic forest existed among the numerous mortality centers, but we suspected this landscape contained large genets of *Armillaria*. Determining the population structure of pathogenic *Armillaria* under the influence of various habitat types, forest cover types, and wildfire regimes will help us understand the role these factors play in the establishment of genets by basidiospores, the extent to which a forested landscape can be occupied by genets of *Armillaria*, and the relation of disease expression to genet boundaries. The goal of our study was to map the coarse-scale population structure of pathogenic *Armillaria* species in this mixed-conifer forest.

Materials and methods

Study area

The study area was chosen because it has extensive symptoms of Armillaria root disease (C. Schmitt, USDA Forest Service, unpublished reports). It is approximately 12.0 × 13.5 km in size and located in the Blue Mountains of northeast Oregon on the Prairie City Ranger District of the Malheur National Forest (44°30′N, 118°25′W). Elevation ranges from 1525 to 2000 m over moderately steep topography. Climate is characterized by low to moderate annual pre-

Table 1. Examples of *Armillaria* population studies using somatic incompatibility as a method of genet identification.

Study	Forest type(s)	Location	*Armillaria* spp.	Width (m)[a]	Area (ha)[b]
Adams 1974	Ponderosa pine	Oregon, U.S.A.	*A. mellea* (s. l.)[c]	800, 1410	—
Shaw and Roth 1976	Ponderosa pine	Washington, U.S.A.	*A. mellea* (s. l.)[c]	800	—
Ullrich and Anderson 1978	Maple	Vermont, U.S.A.	*A. mellea* (s. l.)	≤50	—
Korhonen 1978	Mixed conifer – hardwood	Finland	*A. mellea* (s. str.), *A. bulbosa* (resembled), *A. ostoyae*	Normally 10–50, largest 120–150	—
Anderson et al. 1979	Ponderosa pine	Washington, U.S.A.	*A. mellea* (s. l.)[c]	400, 450	—
Kile 1983	Dry sclerophyll eucalypt	Victoria, Australia	*A. luteobubalina*	50–580	0.002–3.5
Kile 1986	Wet sclerophyll eucalypt	Tasmania, Australia	*A. hinnulea*	28[d], 76[e]	—
Klein-Gebbinck et al. 1991*a*	Lodgepole pine	Alberta, Canada	*A. ostoyae*	35–90	—
Smith et al. 1992	Hardwood	Michigan, U.S.A.	*A. gallica*	635	15
Rizzo and Harrington 1993	Mixed conifer – hardwood	New Hampshire, U.S.A.	*A. ostoyae*	Small, 30+	—
Worrall 1994	Mixed conifer – hardwood	New York, U.S.A.	*A. calvescens*	11.7[f]	0.011[f]
			A. gemina	17.1[f]	0.023[f]
			A. gallica	18.5[f]	0.027[f]
			A. ostoyae	10.6[f]	0.009[f]
Rizzo et al. 1995	Red pine – jack pine	Minnesota, U.S.A.	*A. ostoyae*	≤140	—
Legrand et al. 1996	Beech, beech – pine, pine	France	*A. ostoyae*	210	3
			A. cepistipes	130	1
			A. gallica	290	2
Dettman and van der Kamp 2001*a*	Mixed conifer	Central British Columbia, Canada	*A. ostoyae*	—	0.70–15.83
			A. sinapina	30–100	0.07–0.79
Dettman and van der Kamp 2001*b*	Mixed conifer	Southern British Columbia, Canada	*A. ostoyae*	66.1–135.1	1.12[f]
This study	Mixed conifer	Oregon, U.S.A.	*A. ostoyae*	1720–3810	95–965
			NABS X	—	2[g]

[a]Maximum width, or range of widths, between somatically compatible isolates, if provided in citation.
[b]Maximum area, or range of areas, of genets, if provided in citation.
[c]Species was most likely *A. ostoyae*.
[d]Maximum width of an apparently contiguous genet.
[e]Maximum width of an apparently discontinuous genet.
[f]Mean values.
[g]Based on field observations.

cipitation, 50 to 90 cm, that accumulates mainly as snow between October and May. Average temperatures range from –2.8 to 10.0°C with high seasonal fluctuations. The forest overstory comprises mainly inland variety Douglas-fir, grand fir, lodgepole pine, ponderosa pine, western larch, and subalpine fir. In most areas the overstory is mixed, but some stands are dominated by a single species, usually lodgepole pine. Plant associations on the cooler, moist, north- and east-facing slopes are predominantly grand fir/grouse huckleberry (*Vaccinium scoparium* Leiberg), grand fir/big huckleberry (*Vaccinium membranaceum* Dougl. ex Torr.), grand fir/twinflower (*Linnaea borealis* L.), and lodgepole pine/grouse huckleberry, while those on the warmer, drier, south- and west-facing slopes are predominantly grand fir/pinegrass (*Calamagrostis rubescens* Buckl.), grand fir/elk sedge (*Carex geyeri* Boott), Douglas-fir/pinegrass, Douglas-fir/ elk sedge, ponderosa pine/pinegrass, and ponderosa pine/elk sedge (Johnson and Clausnitzer 1992).

The structure and composition of forests in the study area have been influenced by harvesting, by fire or lack of it, and by a variety of insects and pathogens. Harvest of ponderosa pine took place on western aspects of some portions of the study area during the early to middle 1920s, while the clear-cut patches evident in Fig. 1 resulted from stand entries in the 1980s. The prominent insects and pathogens include western pine beetle (*Dendroctonus brevicomis* LeConte) on ponderosa pine; mountain pine beetle (*Dendroctonus ponderosae* Hopkins) on lodgepole and ponderosa pines; western spruce budworm (*Choristoneura occidentalis* Freeman) on grand fir and Douglas-fir; dwarf mistletoe (*Arceuthobium* spp.) on western larch, Douglas-fir, and ponderosa pine; Indian paint fungus caused by *Echinodontium tinctorium* (Ellis & Everh.) Ellis & Everh. in grand fir; annosus root disease caused by *Heterobasidion annosum* (Fr.:Fr.) Bref. in grand fir and ponderosa pine; and Armillaria root disease in a variety of species.

Field sampling

A nested sampling design was used in 1998 to examine the diversity of pathogenic *Armillaria* species and genets within individual infection centers, among infection centers within a stand, among different stands, and among watersheds that composed the study area. Sampling took place across specific areas of the landscape known to be impacted by Armillaria root disease (Fig. 1). Sample trees were either symptomatic for root disease or estimated to have been dead less than 5 years. Owing in large part to time constraints, sample trees were selected nonrandomly based on their visibility, ease of access, and to provide relatively well-spaced coverage across obviously diseased areas. Sample tree selection did not take into account tree species, crown class, or size. Samples came from lateral roots or the root collar of one to three trees in each of one to three apparently separate mortality centers within each sampled stand. Samples were refrigerated at 5°C, and isolations were performed within 2 weeks. The objectives of field sampling in 1999 were to improve sampling intensity and to determine the geographic extent of genets identified from the 1998 samples.

Isolations

Root samples were split, and three small wood chips were axenically plated on either 3% malt extract agar (45 g Difco malt extract agar, 1 L distilled H_2O) or an enhanced *Armillaria* growth media (30 g Difco malt extract, 20 g Difco dextrose, 5 g Difco peptone, 19 g Difco agar, 1 L distilled H_2O). Most wood chips were taken within 2 cm of the samples' outer surface below vigorous mycelial fans of *Armillaria* and within zones of *Armillaria* decay. Petri plates were sealed with Parafilm and stored in the dark at room temperature until pure cultures of *Armillaria* could be transferred. Pure cultures were maintained in the dark at room temperature on the enhanced *Armillaria* growth media.

Species identification

Identification of *Armillaria* species used two methods. The first (Korhonen 1978; Mallett et al. 1989; McDonald and Martin 1988) used diploid–diploid pairings, and the second used restriction fragment length polyporphisms (RFLPs) of the intergenic spacer I (IGS-I) ribosomal DNA region (White et al. 1998). *Armillaria ostoyae* and North American Biological Species X (NABS X) were considered the pathogenic *Armillaria* species most likely to occur in our study area (G. McDonald, USDA Forest Service, personal communication) and were used as identification standards. One *A. ostoyae* tester (SP9801) came from western redcedar (*Thuja plicata* Donn ex D. Don) on the southern end of Vancouver Island, British Columbia (D. Morrison, Canadian Forest Service, personal communication), whereas the second *A. ostoyae* tester (P1404) and the single NABS X tester (D82) were collected from basidiome cap tissues on Idaho's Priest River and Deception Creek Experimental Forests, respectively (J. Donley, USDA Forest Service, personal communication).

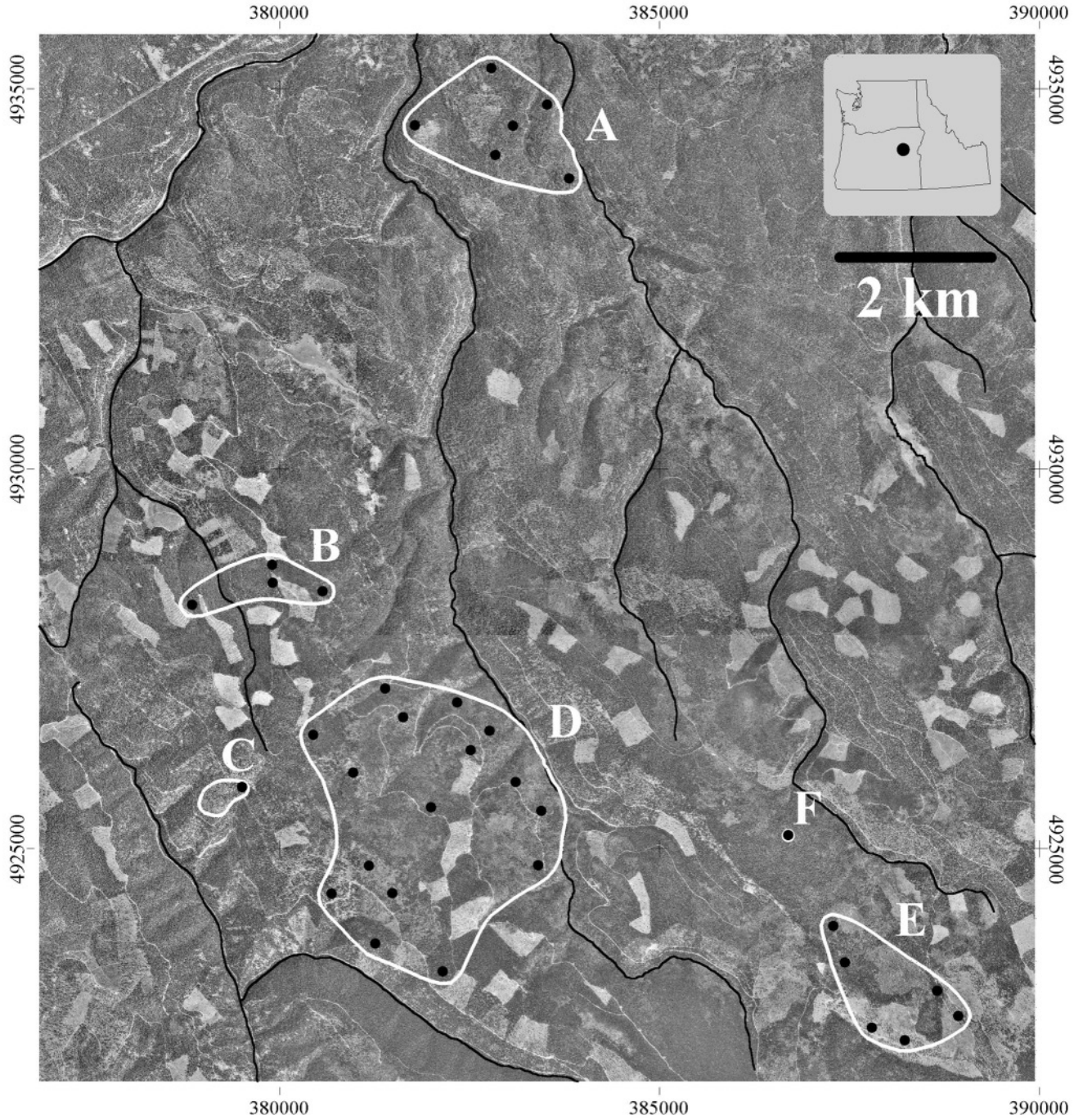
Diploid–diploid pairings for species identification followed the specific methods of Wu et al. (1996). The plate pairings were placed in clear plastic bags and stored in the dark at room temperature for 24 to 28 days, at which time reactions between mycelia were interpreted. Reactions were categorized as follows: (*i*) the formation of a nearly hyphae-free gap between opposing mycelia, interpreted as a pairing between two genets of the same species, and (*ii*) the formation of a nearly hyphae-free gap between opposing mycelia with a black line in the growth medium, interpreted as a pairing between different *Armillaria* species (see Korhonen 1978; Mallett et al. 1989).

Species determination by using White et al.'s (1998) diagnostic for *Armillaria* consisted of DNA extraction from actively growing mycelia by using the methods of Gardes and Bruns (1993), amplification of the IGS-I region via polymerase chain reaction (PCR), digestion of the amplification product with the restriction enzyme ALU-I, and separation of IGS-I fragments on an agarose gel. This diagnostic was performed for all 87 isolates collected in 1998 by using the two testers of *A. ostoyae* (SP9801 and P1404) and the one of NABS X (D82) as standards. The diagnostic was repeated by David Rizzo at the University of California, Davis, on a subset of isolates representing four of the *A. ostoyae* genets and the single NABS X genet.

Genet identification

Isolates were grouped into genets by using somatic incompatibility (SI), the self–nonself recognition system in basidiomycetes (Todd and Rayner 1980; Worrall 1997). The

Fig. 1. Genets of *Armillaria ostoyae* (A–E) and NABS X (F). White lines represent putative genet boundaries, black circles within genet boundaries represent locations where one to six isolates were collected, and black lines represent watershed drainages.



genetic basis of SI is not well understood (Worrall 1997), although Guillaumin (1998) presented preliminary evidence that *A. ostoyae* is heterozygotic for two, closely linked SI loci. Reactions for genet identification were categorized the same as for species identification with the addition of a third reaction, fusion of opposing mycelia, interpreted as a pairing between the same species and genet. Two investigators interpreted all plate pairings independently, conferring when initial interpretations differed.

A hierarchy of SI pairings was used to maintain manageable numbers of plates. Isolates collected from the same mortality centers in 1998 were first paired against each other; if all were from the same genet then one isolate was chosen for further use. The resulting subset of isolates, each representing a mortality center within the same stand, was then paired together in all combinations; if all were from the same genet then one isolate was chosen for further use. The resulting subset of isolates, each representing mortality centers from different stands, was then paired against each other in all combinations as the final step in genet identification. Genet identity for isolates collected in 1999 was determined by first pairing all isolates from each collection site against

one another to confirm congenet origin. Pairings were then constructed between one isolate from each of the collection sites and a subsample of the 1998 isolates that consisted of two isolates from genet A, two from genet B, one from genet C, and the tester isolates SP9801, P1404, and D82.

Once each isolate was identified to species and genet, a map of the isolate collection points and approximate genet boundaries was constructed using geographic information system (GIS) software (ArcView 3.2, ESRI, Redlands, Calif.) with digital orthophotoquads as background imagery. Placement of genet boundaries relied primarily on locations of collection sites, but also incorporated observations on the extent of tree mortality and presence of physical barriers such as relatively wide, perennial streams, and evidence of root disease on aerial photographs. Once putative genet boundaries were established, the area of each was calculated to the nearest 5 ha within ArcView. Genet age was estimated to the nearest 50 years by measuring the distance between the furthest collection points within each genet, dividing that distance in half to obtain a maximum genet "radius", and then dividing that by three independent growth rate estimates for *A. ostoyae* in Douglas-fir and ponderosa pine (van der Kamp 1993; Shaw and Roth 1976; Peet et al. 1996). This method of estimating genet age should be considered conservative because if genet growth has been asymmetrical from the original infection point, the longest genet "radius", and thus the calculated age, would be greater. In addition, changes in host continuity may have constricted the genets at some point in their history, and this cannot be accounted for by considering current genet widths only.

Results

Field sampling

We obtained 87 isolates of *Armillaria* in 1998 from five conifer species (Table 2), representing 54 mortality centers spread among 21 forest stands. The 25 *Armillaria* isolates obtained in 1999 came from five conifer species at 14 locations. The total of 112 *Armillaria* isolates collected over both years came from six conifer species (Table 2). Eighty-nine percent of isolates came from grand fir and Douglas-fir (Table 2): 49 from dead trees and 51 from live trees. Examination of plant associations at 86 of 87 collection sites in 1998 showed that 49% (42) represented relatively cooler, moist sites, largely grand fir/grouse huckleberry (31 of 42), whereas 51% (44) represented relatively warmer, drier sites, largely grand fir/pinegrass (28 of 44) (Johnson and Clausnitzer 1992). Forty-six percent of the 1998 collection sites had at least some evidence of prior harvest activities.

Species identification

The diploid–diploid species identification pairings revealed that 4 of the 87 isolates collected in 1998 were NABS X, and the remaining 83 were *A. ostoyae*. All 25 isolates collected in 1999 were identified as *A. ostoyae* with diploid–diploid pairings. Results from our PCR-based species identification (White et al. 1998) matched those from the diploid–diploid pairings in all cases. There were two distinct RFLP patterns: one, with the two largest bands equaling 310 and 300 kilobases, matched *A. ostoyae*, whereas the other, with the two largest bands equaling 400 and 180 kilobases, matched NABS X. A subsequent PCR-based identification performed on a subset of these isolates confirmed our findings (D. Rizzo, University of California, Davis, personal communication).

Table 2. *Armillaria* isolations by host, collection year, and host condition.

Host	1998 (D, L)[a]	1999 (D, L)	Total (D, L)	Host totals
Abies grandis	28, 26	14, 4	42, 30	72 (64%)
Pseudotsuga menziesii	5, 20	2, 1	7, 21	28 (25%)
Pinus ponderosa	2, 2	2, 0	4, 2	6 (5%)
Pinus contorta	1, 2	1, 0	2, 2	4 (4%)
Abies lasiocarpa	0, 1	0, 0	0, 1	1 (1%)
Larix occidentalis	0, 0	1, 0	1, 0	1 (1%)
All hosts	36, 51	20, 5	56, 56	100

[a]Trees that were dead (D) or living (L) at time of sample collection.

The four isolates of the putative *Armillaria* species NABS X (Banik and Burdsall 1998) came from three dead grand fir and one symptomatic Douglas-fir, all approximately 50 years old and within an approximately 2-ha area of scattered, dead or symptomatic trees. Thin mycelial fans were observed on two of the grand fir and the Douglas-fir, but no resinosis was observed at the root collars of these trees.

Genet identification

We identified five genets of *A. ostoyae* and one genet of NABS X (Fig. 1, Table 3) by using SI. The 83 isolates of *A. ostoyae* collected in 1998 came from genets A, D, and E (Fig. 1). Two more genets, B and C (Fig. 1), were discovered in 1999 as part of our effort to better define the boundaries of genet D to the west and northwest. Four of the *A. ostoyae* genets, A, B, D, and E, extended across ridges that defined watershed boundaries. Two genets, A and D, may have had further expansion blocked along portions of their eastern edges because of host disruption caused by perennial streams (Fig. 1), as no disease symptoms were observed on hillsides opposite areas of obvious infection. None of the *A. ostoyae* genets abutted, overlapped, or were contained within the bounds of a larger genet.

Discussion

Summary

This study has confirmed the existence of extensive, possibly millennia-old *A. ostoyae* genets in the relatively dry, mixed-conifer forests east of the Cascade Range; extended the known maximum size of an *A. ostoyae* genet to approximately 965 ha; and provided the first coarse-scale map of the population structure of pathogenic *Armillaria* species in this forest type (Fig. 1). Genet density of *A. ostoyae* was extremely low by our sampling methods, but cumulative colonization of the study area by *A. ostoyae* was at least 9.5%. *Armillaria ostoyae* was identified as the predominant cause of symptomatic and dead conifers on this landscape, adding to the evidence that *A. ostoyae* is a primary, virulent pathogen on North America conifers (Kile et al. 1991; Morrison et al. 1985; White et al. 1998). One genet of NABS X was found acting as a pathogen, but this site was not investigated

Table 3. Characteristics of *Armillaria* genets.

Genet	Species	Isolates	Width (m)[a]	Area (ha)[b]	Age (years)[c]
A	*A. ostoyae*	26	2150	260	1100, 1250, 4900
B	*A. ostoyae*	6	1720	95	900, 1000, 4000
C	*A. ostoyae*	2	—[d]	20[d]	—[d]
D	*A. ostoyae*	61	3810	965	1900, 2200, 8650
E	*A. ostoyae*	13	2030	195	1000, 1150, 4600
F	NABS X	4	—[e]	2[e]	—[e]

[a]Maximum width of genet (nearest 10 m) as measured between the most distant collection points.

[b]Area (nearest 5 ha) measured within putative genet boundaries as shown in Fig. 1.

[c]Age (nearest 50 years). The left estimate is based on a spread rate of 1.0 m/year (Peet et al. 1996), the center estimate, on 0.87 m/year (Shaw and Roth 1976), and the right estimate, on 0.22 m/year (van der Kamp 1993).

[d]Estimations of width and age were not made since genet was represented by only two, proximal isolates. Genet boundary (Fig. 1) and area estimate were based on field observations.

[e]Locations of NABS X isolates composing genet F were not recorded accurately enough to estimate width and age; area estimate is based on field observations of affected area.

further to examine whether other root pathogens may have been predisposing these trees to NABS X infection.

Genet establishment

The population structure of *A. ostoyae* on our study area is most like those found in similar, relatively dry forest types (Adams 1974; Anderson et al. 1979; Shaw and Roth 1976), and to a lesser extent similar to that of *Armillaria luteobubalina* Watling & Kile in dry, eucalypt forests of Australia (Kile 1983). It contrasts dramatically, in terms of genet size and density, with the population structure of *Armillaria* spp., including *A. ostoyae*, found in relatively moist forest types (Table 1). Probable explanations for these differences in genet size involve the effects of moisture on fruiting frequency and basidiospore abundance, basidiospore survival, and establishment of diploid mycelia (Termorshuizen 2000; Wargo and Shaw 1985); limitations on conspecific genet expansion imposed by SI (Todd and Rayner 1980); and competition for resources (Rizzo et al. 1995; Worrall 1994). However, little is known of suitable conditions and substrate for basidiospore establishment (Rishbeth 1988), and surveys comparing fruiting abundance and frequency of *Armillaria* species among dry and moist forest types are lacking.

The number of genets identified on our study area (Fig. 1) suggests that the rate of establishment of *A. ostoyae* is very low. However, if the only genets that are able to persist need to possess rare fitness traits, or combinations of traits, such as competitive ability, genetic stability, or wide host range, the rate of establishment per se might be higher than results suggest, whereas genet survival would be low. The simplest explanation, however, would seem to be that successful establishment of genets is a function of moisture regime. In a more moist, mixed-conifer forest type in northwest Montana (B. Ferguson, unpublished data), the coarse-scale population structure of *A. ostoyae* appeared to be intermediate between that found in studies from eastern North America (Table 1) and results from the current study. In the Montana investigation, 52 *A. ostoyae* genets were identified across roughly 3000 ha. One genet of 100+ ha was delineated, but the majority ranged in size from the area occupied by a few dead or symptomatic trees to tens of hectares.

Genet expansion and disease expression

One assumption regarding Armillaria root disease is that fire is a limiting factor owing to (*i*) promotion of an open-grown forest structure that reduces root contacts and keeps infected root systems better isolated (Shaw and Roth 1976), and (*ii*) promotion of seral species such as western larch, ponderosa pine, and lodgepole pine that are more tolerant to root infections (Mallett and Volney 1999; Robinson and Morrison 2001). An intriguing question, therefore, is the mechanisms by which these genets of *A. ostoyae* expanded to their current size in these fire-influenced forests (Maruoka 1994). We propose two possible explanations. First, *A. ostoyae* may be as capable of efficient spread in a forest composed of larger, less dense, less root-disease-susceptible species as it is in a forest composed of smaller, more dense, more disease-susceptible species. Second, the forest structure and composition during the early to middle postglacial period may have been more amenable to spread of *A. ostoyae* than it has under the influence of natural fire regimes of the last several hundred years (Agee 1994; Maruoka 1994).

A scenario can be constructed for the efficient spread of *A. ostoyae* in a low-density forest composed of a high proportion of seral species such as lodgepole pine, western larch, ponderosa pine, and to a lesser extent Douglas-fir. On mature trees of moderately to highly resistant species, such as western larch and on some sites ponderosa pine, *A. ostoyae* exists primarily within resinous root lesions (Robinson and Morrison 2001; Reaves et al. 1993; Roth et al. 1980; Shaw 1980), although the potential for these lesions to produce rhizomorphs capable of causing significant intertree infection is questioned (D. Morrison, personal communication). Such lesions often girdle a root, however, resulting in colonization of the distal portion while the portion proximal to the lesion continues to resist fungal advance (Robinson and Morrison 2001; Reaves et al. 1993; Shaw 1980). In these instances the inoculum potential of the colonized portion of the root likely allows for successful fungal transfer at root contacts (D. Morrison, personal communication). This type of spread would tend to be cryptic and relatively slow. However, the death of scattered, large trees prior to the era of selective harvest and fire suppression would have provided occasional "pulses" of high inoculum potential that over the next several decades would either kill adjacent trees or form quiescent lesions on their root systems (Roth et al. 1980; Shaw and Roth 1976). These trees would then become the future sources of inoculum pulses within the mature stand as they succumbed.

Fire and (or) bark beetles may have been two of the forces that helped create these pulses of inoculum and subsequent waveform mortality. Since *Armillaria* can grow from quiescent root infections to colonize the biomass of a root system after a tree is harvested (Shaw et al. 1976; Wargo and Shaw 1985; Woods 1994), it is likely that root systems of trees

killed by fire are also colonized in this fashion. If so, root systems of scattered, large trees, or patches of such trees, killed by medium- to high-severity fires within genet boundaries would increase the inoculum load and potential of *A. ostoyae* (Shaw et al. 1976; Wargo and Shaw 1985), resulting in either increased mortality along the interface between burned and nonburned overstory trees or the infection of subsequent regeneration. Infection by *A. ostoyae* may result in increased levels of ethanol within the host, as shown with other root-infecting fungi (Kelsey and Joseph 1998; Kelsey et al. 1998). Ethanol in combination with host resins, commonly present on *A. ostoyae* infected roots as a response to fungal infection (Morrison et al. 1991*b*), can act synergistically as a kairomone for bark- and root-feeding beetles (Schroeder and Lindelow 1989), thereby increasing the likelihood that *A. ostoyae* infected trees are attacked by bark beetles (Bartos and Schmitz 1998; Goheen and Hansen 1993).

The alternative explanation for development of these genets, that historical forest structure and composition has been more amenable to establishment and expansion of *A. ostoyae* genets, is highly problematic. Climate fluctuations during the last 10 000 years in the Pacific Northwest (Thompson et al. 1993) may have resulted in periodic development of a more dense forest composed of a higher proportion of mid- to late-successional, *Armillaria*-susceptible hosts, such as grand fir. However, the regional trend toward a moister climatic pattern has apparently occurred in the late Holocene; the early and mid-Holocene were warmer and drier than at present (Long et al. 1998; Thompson et al. 1993). Therefore, it appears that conditions during the last several thousand years were actually more favorable for growth of a more root-disease-susceptible forest than earlier postglacial conditions. However, because of the high level of uncertainty in the age calculations for these genets (Table 3), their origin cannot be correlated with climatic conditions of any specific era.

The existence of these extensive *A. ostoyae* genets challenges the view that colonization by this fungus has increased within the relatively dry forests of eastern Oregon and Washington owing to recent, human-caused shifts in forest structure and composition (Gast et al. 1991; Hessburg et al. 1994). This perception may have arisen owing to increases in root disease symptoms and mortality within the boundaries of extensive, long-lived genets where fungal inoculum had historically been more balanced with a less dense, less root-disease-susceptible forest (Byler and Hagle 2000; Goheen and Otrosina 1998; Hansen and Lewis 1997; Shaw et al. 1976). Selective harvesting, fire suppression, and grazing have altered forest structure and composition in this forest type over the last 100–150 years, so that many forests once dominated primarily by pine and western larch are now dominated primarily by Douglas-fir and grand fir (Agee 1994; Heyerdahl et al. 2001). This has likely resulted in increased mortality within genet boundaries and perhaps a slightly increased rate of spread at genet boundaries. However, these changes have not been present long enough to substantially impact genet expansion. The most conservative age estimate for genet D is 1900 years (Table 3), suggesting that it has persisted for several forest generations. If lower estimates of spread rates for *A. ostoyae* are more accurate (van der Kamp 1993), or if genet expansion has been asymmetrical, slowed by periodic disturbances in host distribution or has at times constricted, the age of this genet may be 8500+ years. Therefore, these genets of *A. ostoyae* have apparently expanded to their current size under the influence of natural forest dynamics, fire regimes, and climatic variation, not because of the effects of fire suppression or selective harvesting on forest structure and composition. Disease expression in areas long occupied by *A. ostoyae* has increased, but not *A. ostoyae* per se.

Under the influence of historic fire regimes, the pattern of mortality within these genets was likely more diffuse, occurring primarily as single, scattered trees or small groups of trees. This was the situation described by Roth et al. (1980) in a root-diseased, old-growth ponderosa pine forest in south-central Washington, where many of the scattered snags that died prior to the first harvest entry displayed evidence of *Armillaria* infection. Fire suppression allowed establishment of dense second-growth stands of ponderosa pine, and subsequent, partial cutting of the old-growth left large stumps that acted as foci for virulent root disease centers (Roth et al. 1980; Filip and Goheen 1984). Such changes in forest structure and composition have, in a sense, destabilized the host–pathogen relation in favor of the fungus (Morrison and Mallett 1996; Shaw et al. 1976). *Armillaria ostoyae* does not require predisposition to kill mature, vigorous trees of susceptible species when inoculum potential is high (Rosso and Hansen 1998), but its strategy for colonization and expansion in this forest type likely makes use of species of all susceptibility and vigor levels. Susceptible species are killed outright by the fungus, while the root systems of resistant species are colonized from quiescent root lesions after the tree is killed by harvesting, bark beetles, or fire.

Fire histories of the last four centuries show that frequent, low- to moderate-severity fires in this forest type resulted in a more structurally homogenous, lower-density forest often composed of a higher proportion of seral species (Agee 1994; Maruoka 1994; Hagle and Goheen 1988). Occasional severe fires resulted in landscape mosaics of stand age and composition (Agee 1994; Maruoka 1994). This fire regime could affect the levels of mortality within genets in two ways. First, the proportion of species highly susceptible to *A. ostoyae*, such as Douglas-fir and grand fir, was lower (Agee 1994; Heyerdahl et al. 2001; Maruoka 1994), although almost certainly still of consequence to the dynamics of this pathosystem. Second, the age structure of susceptible species would have been skewed toward younger age-classes because of frequent fires; once infected by *A. ostoyae*, the smaller root systems of these trees would not have the same inoculum potential or life-span as would larger Douglas-fir and grand fir that have developed with fire suppression (Hagle et al. 2000; Morrison and Pellow 1994). It is unlikely, however, that fire substantially reduces levels of existing *Armillaria* inoculum. In a watershed adjacent to our study area, Filip and Yang-Erve (1997) found that low-intensity fires reduced the viability of *A. ostoyae* inoculum blocks buried at 12 cm but not those buried at 30 cm. Finally, it is the very existence of these extensive, possibly

millennia-old genets of *A. ostoyae* that provides strong, although indirect, evidence that fire has little long-term impact on established genets.

A final consideration regarding disease expression across these genets is continuity of the pathogen; does the fungus exist more or less everywhere within putative genet boundaries or only where disease is being expressed? Lack of suitable hosts over portions of a genet for an extended period may in theory exhaust fungal inoculum and result in the formation of ramets or spatially distinct patches of a genet (see Adams 1974; Dettman and van der Kamp 2001*b*). Host reestablishment could then act as a bridge that allows ramets to recombine. Root disease surveys in our study area have shown that mortality centers within genet boundaries tend to be spatially distinct and highly variable in size (C. Schmitt, USDA Forest Service, unpublished reports), but our sampling methods could not answer the question of whether the fungus exists across both asymptomatic and diseased areas within putative genet boundaries (Fig. 1). However, it is possible that these genets of *A. ostoyae* are contiguous, existing in asymptomatic portions of the forest as root lesions and (or) epiphytic rhizomorphs on less susceptible conifers, hardwoods, and shrubs (Adams 1974; McDonald et al. 1987; Tarry and Shaw 1966). Confirmation would require systematic, fine-scale sampling on transects across diseased and asymptomatic portions of these genets (Fig. 1).

Genetic stability of *Armillaria* genets

We only examined SI loci for genet identity, but speculate that the *A. ostoyae* genets on this landscape would display the same lack of genetic variation as genets of *Armillaria gallica* Marxm. & Romagn. (Hodnett and Anderson 2000; Smith et al. 1990, 1992). Hodnett and Anderson (2000) examined *A. gallica* genets for genetic variation resulting from somatic mutation and found no variation in nucleotide sequence. They proposed that either *A. gallica* has a mutation rate that is less than expected, or that the rate of cell division in *A. gallica* rhizomorphs was substantially less than assumed. Saville et al. (1998) suggested that *Armillaria* species may avoid accumulating deleterious genetic mutations, a process known as Muller's ratchet, by mitochondrial DNA recombination, but this would not explain how mutations are dealt with during possibly eons of mitotic division of nuclear DNA in long-lived genets.

One genetic interaction that may well occur across extensive genets is the establishment of haploid mycelium followed by genetic exchange with the established diploid mycelium (Rizzo and May 1994; Carvalho et al. 1995). Rizzo and May (1994) found two outcomes in haploid–diploid matings of *A. ostoyae*; migration by the diploid nucleus into the haploid mycelium followed by replacement of the haploid nucleus, or formation of putatively stable, binucleate (2N + N) mycelia. The ecological role of such binucleate mycelia in factors such as pathogenicity, however, is unknown (Rizzo and May 1994). Carvalho et al. (1995) reported that haploid–diploid matings of *A. gallica* resulted in three outcomes: (*i*) replacement of the haploid nuclei by the diploid nuclei, (*ii*) recombination, or (*iii*) a triploid condition. Replacement of the haploid nucleus by the diploid was reported as the most common outcome in both *A. gallica* (Carvalho et al. 1995) and *A. ostoyae* (Rizzo and May 1994) and is thought to be one mechanism that maintains genetic stability of the established diploid. Kim et al. (2000) found evidence for infrequent triploidy in *A. ostoyae*, but, as with the binucleate condition (Rizzo and May 1994), the potential effect of heteroploidy on the ecology of *A. ostoyae* is unknown.

Implications for disease management

A conservative estimate, based on a study area of 16 100 ha and a combined *A. ostoyae* genet area of approximately 1545 ha, is that 9.5% of our study area is within the boundaries of *A. ostoyae* genets (Fig. 1, Table 3). Long-term management plans need to identify and account for such extensive *A. ostoyae* genets because of their potential to affect forest growth and mortality (Bloomberg and Morrison 1989; Mallett and Volney 1999), to act as refugia for endemic bark beetle populations (Bartos and Schmitz 1998), to increase accumulation of coarse woody debris (Hansen and Lewis 1997; K. Fields, USDA Forest Service, unpublished data), and perhaps most importantly to affect forest structure and succession (Byler and Hagle 2000; Hansen 1999; Hansen and Goheen 2000).

If the management goal in this forest is to limit disease expression, a conservative approach to root disease management should be taken in the asymptomatic forest within genet boundaries because latent infections of *A. ostoyae* may be present on many asymptomatic trees (Morrison et al. 2000; Robinson and Morrison 2001; Shaw 1980; Woods 1994). Management within genet boundaries needs to emphasize maintenance of seral species, such as western larch and ponderosa pine, and removal of the most susceptible mid- to late-seral species such as grand fir and Douglas-fir (Morrison and Pellow 1994; Morrison and Mallett 1996; Morrison et al. 1991*a*). Silvicultural approaches that emphasize seral species are recommended even for stands with low levels of Armillaria root disease (Filip and Goheen 1984; Morrison and Mallett 1996). Selective cutting in such stands is the least favorable option as it would likely result in an ever-increasing inoculum load among the remaining crop trees (Morrison et al. 2001; Morrison and Mallett 1996; Roth et al. 1980). However, if spread of *A. ostoyae* in this forest type is equally efficient regardless of forest structure and composition, then these management recommendations will only help to reduce disease impacts within genet boundaries, not limit expansion of the genet.

Study limitations

This study area was purposely selected because it had extensive symptoms of Armillaria root disease, so incidence and extent of disease cannot be considered typical for random landscapes in this forest type. In addition, SI was the only technique used for genet identification. Comparisons of SI, mating type, and molecular techniques for delineation of *Armillaria* genets have shown that SI provides identical (Smith et al. 1992, 1994) or nearly identical results (Dettman and van der Kamp 2001*a*; Guillaumin et al. 1996; Kile 1983; Rizzo et al. 1995; Rizzo and Harrington 1993). Guillaumin et al. (1996) compared methods of *Armillaria* genet identification, including analyses of SI, mitochondrial DNA via RFLPs, nuclear DNA via randomly amplified polymorphic DNA, mating-type alleles, and isoenzymes, and

concluded that SI is a "valid technique" for epidemiological studies of *Armillaria* species. Anderson and Kohn (1995) and Rizzo et al. (1995), however, recommend use of selectively neutral markers in addition to SI, or mating-type tests to distinguish genets that expanded mitotically following a single mating event from those that resulted from matings of mycelia originating from sibling basidiospores.

Conclusions

We hypothesize that for this relatively dry forest type: (*i*) establishment of *A. ostoyae* genets is a rare event owing more to the influence of moisture regime than forest structure and composition; (*ii*) expansion of *A. ostoyae* genets occurs with near-equal efficiency regardless of forest structure or composition; (*iii*) the level of symptom expression across genets depends on forest structure and composition; (*iv*) extensive *A. ostoyae* genets develop because of host continuity and lack of spatial restrictions imposed by conspecific genets; and (*v*) fire does not impose natural controls on well-established *A. ostoyae* genets.

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