ARMILLARIA OSTOYAE: GENETIC CHARACTERIZATION AND DISTRIBUTION IN THE WESTERN UNITED STATES

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ABSTRACT

Armillaria ostoyae is an important root-rot pathogen of conifers in the western United States. This thesis reviews what is currently known about *A. ostoyae* biology, distribution, and management, and provides a map of *A. ostoyae* worldwide distribution based on available literature. A range of hosts and a detailed description of host symptoms are outlined. Morphological, biological, and phylogenetic species concepts used in the identification of *Armillaria* species are described. A comprehensive assessment of strategies and concepts to manage sites impacted by this pathogen are included, with an emphasis on the role of tree stress as a predisposing factor to the pathogen.

In addition, a direct-PCR method and phylogenetic analyses were used to assess genetic diversity among genets of *A. ostoyae* in the western United States. This method defined several phylogeographic groups and showed that much of the sequence information showed heterogeneity among ribosomal DNA repeats, an indication of intraspecific or interspecific hybridization. Information on genetic diversity is important to understanding varying levels of pathogenicity within *A. ostoyae* and its diverse environmental conditions.

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DEDICATION

To my family and friends

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CHAPTER 1: Biology, distribution, and management of *Armillaria ostoyae*

Summary

This review, which emphasizes the western United States, covers *Armillaria ostoyae* biology, distribution, and management. The life cycle of *A. ostoyae* is described and many remarkable biological properties are discussed. Since *A. ostoyae* is an important root-rot pathogen of conifers, it is important for forest managers to know the current techniques for *A. ostoyae* identification. A range of hosts and a detailed description of host symptoms are reviewed, and a map of *A. ostoyae* worldwide distribution is presented based on available literature. Traditional, biological, and phylogenetic species concepts used in the identification of *Armillaria* species are summarized. The variable nature of *A. ostoyae* pathogenicity is a common theme throughout this review. A comprehensive assessment of strategies and concepts to manage sites impacted by this variable pathogen are included with emphasis on the role of tree stress as a predisposing factor to the pathogen. Silvicultural practices, biological controls, tree nutrition, fire, and other factors are all considered in respect to *Armillaria* root-rot. Hypotheses based on current research of *A. ostoyae* are presented, such as potential influences paleogeographic events on the distribution of *A. ostoyae* in western North America. This review concludes with a list of potential directions for future research on *A. ostoyae*.

1 Introduction

Armillaria ostoyae (Romagn.) Herink, one of several *Armillaria* species that can cause Armillaria root disease, is a serious pathogen of conifers of the northern hemisphere (KILE et al. 1991). While many prominent tree pathogens are introduced exotics, *A. ostoyae* is endemic across its range, and typically exists as an integral component of the ecosystems in which it resides. *Armillaria ostoyae* can often be found ubiquitously throughout a forest, not only as a pathogen, but also as a benign saprophyte on the roots of a wide array of hosts (BÉRUBÉ and DESSUREAULT 1988; MCDONALD 1991b; KILE et al. 1994). Some of the most productive forest stands occur on sites in which *A. ostoyae* frequently behaves as a saprophyte (MCDONALD 1991a; 1991b). When healthy, many native woody plants are tolerant or resistant to disease caused by pathogenic *A. ostoyae*. Armillaria root rot is often observed only when a forest stand becomes profuse with susceptible tree species that may become weakened by an array of stress factors. Susceptible trees can also be considered as ecophysiologically maladapted to a site (MCDONALD et al. 1987b, 2003; MCDONALD 1991a, 1991b, 2000). Initial establishment was favorable for these susceptible trees; however, site conditions are not optimal to support these trees into maturity. Thus, as with many predator-prey relationships, *A. ostoyae* helps eliminate and decompose weak or unfit individual trees, while leaving only the individual trees that are best suited for their environment. In this manner, *A. ostoyae* likely represents a relatively constant influence on the structure of forest stands. It has likely impacted the evolution and successional processes of trees and other associated organisms for millions of years. The long co-evolution of trees and *Armillaria* spp. may help explain why *A. ostoyae* behaves as a saprophyte more often on highly productive

sites than on marginal sites. Highly productive sites typically provide sufficient moisture and nutrients to support healthy trees through maturity.

Concern for Armillaria root disease in the forests of the western United States has been rising over recent decades. Increases in root disease of forest trees are frequently attributed to human activity (McDonald et al. 1987b). Human activity is especially prominent for root disease caused by *A. ostoyae*, where human activity has often increased tree species that are highly susceptible and contributed to tree stress. In the western United States, climax species (e.g., *Abies* spp.) have replaced many of the historical park-like stands of seral species (e.g., *Pinus* spp., *Larix* spp.). Such changes in stand compositions have occurred because of perceived economic value of susceptible trees, introduced diseases (e.g., white pine blister rust), and/or fire suppression (Gruell et al. 1982; Harvey 1994; Oliver et al. 1994; Langston 1995; Stuart 1998; Fins et al. 2002). Climax species are generally considered to be the most susceptible to Armillaria root disease as occurrence of disease is more common on these species, especially in areas that were historically dominated by seral species (McDonald 1987b).

As forest resources become increasing scarce and demand for forest products rise, it is important to manage for maximum productivity and sustainability by limiting damage from *A. ostoyae*. Because *A. ostoyae* is a primary regulator of tree growth rates and stand structure, it is a key species that must be recognized in any forest ecosystem before appropriate forest management practices or cultural activities can be determined. Occurrence and pathogenicity of *A. ostoyae* can be highly variable within and between regions, forests, and stands (Morrison 1981; Wargo and Shaw 1985; Cruickshank et al. 1997; McDonald 1998). More information is needed to better understand this

variability and the ecological roles of *A. ostoyae*. Key questions that must be addressed include: 1) Is variation in pathogenicity and virulence of *A. ostoyae* influenced by genetic variation? 2) Is genetic variation within *A. ostoyae* related to environmental factors or geographic distribution? and 3) Can information on genetic variation of *A. ostoyae* help forest managers assess risk of *Armillaria* damage?

2 Life cycle and biological properties

2.1 Basic life cycle

Members of the genus *Armillaria* are Basidiomycetes, which produce basidiospores (haploid sexual spores) on basidiocarps (fruiting bodies commonly known as mushrooms). The life cycle of *Armillaria* species in general remains somewhat hypothetical, but most *Armillaria* species are thought to be heterothallic with a bifactoroial mating system (having two mating type alleles at two incompatibility loci), while only a few species are thought to be homothallic (GUILLAUMIN et al 1991; HUGHES 2000). In this life cycle, basidospores usually germinate to produce haploid mycelium. A successful mating can occur when contact is made between two separate haploid mycelia that are sexually compatible. Immediately after mating, the resulting mycelium usually consists of dikaryotic (binucleate) cells. Clamp connections, which may form for a brief period at this stage (as is the case with *A. ostoyae*), can be used to confirm sexual compatibility or incompatibility (LARSEN et al. 1992). Subsequently, the fungus enters into its vegetative growth state, and undergoes karyogamy (nuclear fusion). Diploid mycelia and rhizomorphs that grow through the soil and tree roots account for the bulk of *Armillaria* spp. biomass. The nuclei may again separate during basidiocarp formation,

typically during the cool conditions of late autumn, before undergoing karyogamy and subsequent meiosis in the basidia to produce basidiospores, thereby completing the life cycle (KORHONEN 1980).

2.2 Rhizomorphs

One of the most distinguishing characteristics of the genus *Armillaria* is the formation of rhizomorphs. Rhizomorphs are cord-like structures composed of an aggregate of mycelium covered by a protective sheath. The sheaths act as a sort of armor for protection, which allows individuals of *Armillaria* to form expansive networks covering large areas. An individual of *A. ostoyae* found in Oregon, U.S.A. is known as one of the largest and oldest organisms on earth, occupying 965 ha with an estimated age of 1,900 to 8,650 years (FERGUSON et al. 2003). The overall structure of rhizomorphs, as reviewed by Garraway et al. (1991) and Fox (2000) is highly complex. This complex structure allows for the translocation of oxygen, water, and other nutrients that aid in growth. While the sheath around the mycelium usually forms an impenetrable barrier, rhizomorphs at the soil surface can form pores that facilitate gas exchange; mechanisms also exist for water / nutrient absorption and exudate secretion (FOX 2000). Translocation of nutrients and metabolites can occur over substantial distances (PAREEK et al. 2001). Recently, it has been shown that rhizomorph growth forms can vary by species and that pathogenicity can be predicted by these growth forms (MORRISON 2004). Rhizomorph production has also been linked to the ability of *A. ostoyae* to cause disease; genets that produce an abundance of rhizomorphs are thought to be more pathogenic (OMDAL et al. 1995; PIERCEY-NORMORE and BÉRUBÉ 2000).

2.3 Rate of spread

Spatial clustering analysis supports that *A. ostoyae* spreads by means of subterranean growth rather than clonal spores (DETTMAN and VAN DER KAMP 2001a). Variable rates of spread have been recorded. A survey of *A. ostoyae* in a stand of 110-year-old Douglas-fir [*Pseudotsuga menziezii* (Mirb.) Franco] in British Columbia (Canada) showed that the disease progressed at a rate of 22 cm per year between 1989 and 1992 (VAN DER KAMP 1993), while another study within a plantation showed the fungus expanded radially at a rate of up to 2 m per year (PEET et al. 1996)

2.4 Other biological properties

Armillaria ostoyae has many unique and intriguing biological properties, some of which may harbor great benefits to mankind. Bioluminescence, bioremediation, medicinal properties, and mycorrhizal associations comprise some of the more interesting attributes of *A. ostoyae*. Bioluminescence of *A. ostoyae* has apparently drawn the attention of man since ancient times, when man reportedly used chunks of bark laden with mycelial fans to mark paths during the night (SCHAECHTER 1997). It is not understood why different individuals and species of *Armillaria* exhibit variable levels of light emission (FOX 2000). *Armillaria* spp. are also members of the so called "white rot fungi", these fungi are known to decompose various environmental contaminants. Like other "white rot fungi", *Armillaria* spp. can produce powerful enzymes that have the potential to decompose various toxic compounds; other "white rot fungi" have been shown to degrade harmful substances such as chlorophenols, polycyclic aromatic hydrocarbons (PAHs), and 2,4,6-trinitrotokuene (TNT) (KIM and SONG 2000; RAMA et. al. 2000; SCHLOSSER et al. 2000;

RHO et al. 2001). In addition, an *Armillaria* sp. is a mycorrhizal symbiont of orchids in Asia. Tubers from *Gastrodia eleta* Bl. species of orchids have been used by the Chinese for centuries as a treatment for an array of medical problems, including stiffness, dizziness, and headaches. Modern research has confirmed that *G. eleta* is effective in treating neurasthenia, epilepsy, neuralgia, vertigo, hyperlipemia, and hypertension. These tubers have become endangered due to over collection within their limited distribution. Recently, supplements of *Armillaria* sp. have been found as an effective alternative to *G. eleta* because medicinal components of *G. eleta* consist primarily of metabolites that are produced by *Armillaria* sp. (DHARMANANDA 2002).

3 Disease detection and *Armillaria ostoyae* identification

3.1 Host symptoms

Stunted growth, crown fading/thinning, tree death, resinosis, fungal fruiting bodies, rhizomorphs, mycelial bark fans, spongy decay, and zone lines are all signs or symptoms of Armillaria root disease (HADFIELD et al. 1986; MALLETT 1992). Crown symptoms such as needle color, density, terminal branch growth, and dead branches can all be used with some success to detect root disease in general (OMDAL et al. 2004). However, disease caused by *A. ostoyae* often goes undetected until it reaches a very severe stage, because roots are often heavily colonized before above-ground symptoms are readily evident (WHITE and MORRISON 1999). Severely infected trees often show signs of resinous (resin soaked bark at the base of the tree). Because mycelial bark-fans are often formed after or during tree death by saprophytic individuals of *Armillaria* species, close inspection of the bark and *Armillaria* mycelium is needed to detect whether *A. ostoyae*

may be the primary pathogen responsible for mortality of a tree. It should also be noted that infection by pathogenic *Armillaria* spp. frequently acts as a predisposing (stress-causing) agent to attacks by other pests, such as bark beetles. Mycelium of pathogenic *A. ostoyae* penetrate several different layers of the bark, where as mycelial bark fans of saprophytic individuals are normally only produced in a single layer in the cambial area under the bark (per. comm. G. I. MCDONALD). Other symptoms, such as resinosis and wound periderm formation, are only produced by a living tree, thereby providing evidence of infection by pathogenic *Armillaria* spp. Mycelial penetration through several layers of bark likely signifies *A. ostoyae* across its geographic range. However, the geographic range of *A. ostoyae* does overlap with other pathogenic species of *Armillaria*, and a small percentage of individuals from primarily saprophytic species can apparently act as pathogens in some situations. Thus, positive identification of *A. ostoyae* must be performed through morphological, biological, and/or molecular genetic techniques.

3.2 General *Armillaria ostoyae* identification

RAY (1704), MICHELI (1721), and BATTARRA (1755) are recognized as the first individuals to have recorded species from the genus of *Armillaria* (WATLING et al. 1982, 1991). Since then, the nomenclature, taxonomy, and identification of *A. ostoyae* and other *Armillaria* species has been frequently revised and continues to increase in complexity. Presently, it is estimated that around 40 species of *Armillaria* exist in the world (WATLING et al. 1991; VOLK and BURDSALL 1995). Ten *Armillaria* species are known to exist in the U.S.A., including both primarily pathogenic and mostly saprophytic species that have variable levels of pathogenicity (Table 1-1). Many identification problems are

Table 1-1. **North American *Armillaria* species and relative pathogenicity**

Pathogenicity	Species	NABS[1,2]	Primary host	Selected references (North America)
High	*A. mellea*	VI	Hardwoods	Bruhn et al. 2000, Baumgartner and Rizzo 2001b
High	*A. ostoyae*	I	Conifers	Morrison et al. 1985a, Wargo and Shaw 1985
High	*A. tabescens*	Unspecified	Hardwoods	Bruhn et al. 2000
Moderate	*A. gemina*	II	Hardwoods	Bérubé and Dessureault 1989, Blodgett and Worrall 1992a
Moderate	*A. nabsnona*	IX	Hardwoods	Morrison et al. 1985a, Volk et al. 1996
Low	*A. calvescens*	III	Mixed	Bérubé and Dessureault 1989, Blodgett and Worrall 1992a
Low	*A. cepistipes*	XI	Mixed	Morrison et al. 1985a, Banik et al. 1996
Low	*A. gallica*	VII	Mixed	Blodgett and Worrall 1992a, Baumgartner and Rizzo 2001b
Low	*A. sinapina*	V	Mixed	Morrison et al. 1985a, Bérubé and Dessureault 1988
Rarely pathogenic	Unnamed	X	Mixed	Morrison et al. 1985a, Banik et al. 1996, McDonald et al. 2003

[1]NABS= North American Biological Species

[2]Anderson and Ullrich 1979

derived from the lack of suitable voucher collections (AMMIRATI 1979; WATLING et al. 1982) and the lacking consensus amongst three prevalent methods of species differentiation. In recent decades, traditional methods of identification using morphological techniques have been strongly augmented by biological and phylogenetic species concepts.

3.3 Morphological identification

Armillaria ostoyae can be identified somewhat reliably with detailed morphological characterization of basidiocarps and knowledge of *Armillaria* spp. distribution. However, *A. ostoyae* is closely related with *A. borealis* Marxmüller & Korhonen and *A. gemina* Bérubé & Dessur. and hybrids may exist with intermediate morphological characteristics (BÉRUBÉ and DESSUREAULT 1988; HARRINGTON and WINGFIELD 1995; PIERCEY-NORMORE et al. 1998; HANNA et al. 2003a, 2004). Other species, such as *A. sinapina* Bérubé & Dessur., *A. calvescens* Bérubé & Dessur., and *A. gallica* Marxmüller & Romagn., are difficult or impossible to differentiate using only morphology. Another drawback of morphological identification is that basidiocarp formation is seasonal and sporadic (PÈREZ-SIERRA and HENRICOT 2002). ROMAGNESI (1970, 1973), KORHONEN (1978), MARXMÜLLER (1982), MARXMÜLLER and PRINTZ (1982), GREIG and STROUTS (1983), and ROLL-HANSEN (1985) have all described European *A. ostoyae* based on basidiocarp morphology (ROLL-HANSEN 1985). Detailed morphological characterization of North American *A. ostoyae* basidiocarps has been provided by BÉRUBÉ and DESSUREAULT (1988), who were able to distinguish *A. ostoyae* from *A. sinapina*, *A. mellea sensu stricto* (Vahl:Fr) P.Kumm., and *A. lutea* Gillet. They also saw justification

in synonymy between North American and European *A. ostoyae*. Basidiocarps of *A. ostoyae* can generally be distinguished from other species by blackish-brown, olive-colored, or yellow scales on the cap and often well-developed scales on the stem (ROLL-HANSEN 1985). These basidiocarps are also often found in clusters at the base of their host, in contrast with primarily saprophytic *Armillaria* spp. that are more commonly found as single fruiting bodies (per. comm. G. I. MCDONALD). Rhizomorph characteristics can also be used to help distinguish *Armillaria* spp. (ROLL-HANSEN 1985; MORRISON 2004).

3.4 Biological species identification

The genus of *Armillaria* was once recognized as comprising only one species (*Armillaria mellea*) until HINTIKKA (1973) demonstrated the presence of a bifactoroial sexual compatibility system within *Armillaria* that could be used to distinguish species through mating tests (WATLING et al. 1991). *Armillaria mellea sensu lato* is the name now used for all *Armillaria* individuals described or identified before 1973, while individuals that continue to hold this species name after 1973 may be referred to as *A. mellea sensu stricto*. HINTIKKA's findings led to the establishment of a "biological species concept" for identifying *Armillaria* species that was used by KORHONEN (1978, 1980) to distinguish five European Biological Species (EBS), ANDERSON and ULLRICH (1979) to distinguish ten North American Biological Species (NABS), and Nagasawa (1991) to identify six biological species, known as Nagasawa's (Nag.) groups, in Honshu, Japan (OTA et al. 1998, FUKUDA et al. 2003). Subsequently, two (NABS IV and VIII) of the original ten biological species recognized in North America were later found by ANDERSON (1986) to

be superfluous, and two new species have since been included [*A. cepistipes* Velen. and *A. tabescens* (Scop.) Emel] to keep the North American species total at ten (VOLK and BURDSALL 1995, Table 1-1). The "biological species concept" recognizes species based on mating behavior; individuals that are *interfertile* are of the same species, while those that are *intersterile* are not (ANDERSON et al. 1980). After examining interrelatedness among continental groups, it was concluded that *A. ostoyae* is present in all three continents as the previously known biological species EBS C, NABS I, and Nag. C (ANDERSON et al. 1980; OTA et al. 1998; FUKUDA et al. 2003). However, the "biological species concept" led to the delimitation of more *Armillaria* species than could be readily recognized by morphology alone (WATLING et al. 1982).

A variety of tests based on the "biological species concept" can be used in *Armillaria* identification. Somatic incompatibility tests use paired diploid-diploid cultures in the lab to produce mycelial reactions (ADAMS 1974; SHAW and ROTH 1976; KORHONEN 1978; KILE 1983; MALLET and HIRATSUKA 1986; WORRALL 1994; WU et al. 1996). A reaction in which a black line develops between cultures signifies incompatible separate species, a clear line signifies the same species, and fusion indicates cultures of the same individual genet. Other tests based on the "biological species concept" include haploid-diploid or haploid-haploid interfertility tests (SIEPMANN 1987; GUILLAUMIN et al. 1991; RIZZO and HARRINGTON 1992; WU et al. 1996). Tests using diploid material have the advantage of allowing for the use of more commonly available rhizomorphs and mycelial fans. Although such tests can be used reliably to identify isolates, some inconsistencies can occur. A small percentage of individual genets may exhibit compatibility or partial compatibility with more than one species. Such inconsistencies

are perhaps attributable to interspecific hybridization. In addition, somatic pairing tests require considerable time and expertise to perform, and researchers using the same tester isolates have sometimes obtained differing or ambiguous results (HARRINGTON and WINGFIELD 1995).

3.5 Molecular genetic identification

A third set of methods for *Armillaria* species identification is based on molecular genetic data. In theory, molecular genetic tests can be conducted on samples derived from any fungal material. The study and use of molecular genetic information for *Armillaria* identification has continued to grow over recent years, as new molecular based technologies have become available. Molecular genetic techniques offer several advantages over morphological and biological methods of identification, among them are high-resolution markers (e.g., DNA sequences) that allow the detection of differences and similarities between and within individuals. These DNA differences also allow detailed phylogenetic and phylogeographic analyses, which provide insights into intraspecific and interspecific hybridization, species and sub-species evolution, and modes of dispersal (PEREZ-SIERRA et al. 2000). In theory, genetic markers could also be linked to important ecological factors, such as virulence, pathogenicity, and environmental adaptation. As reviewed by PÈREZ-SIERRA et al. (2000), DNA-based methods have been growing in popularity as a means to identify *Armillaria* species. Earlier studies by ANDERSON and STASOVSKI (1992) and HARRINGTON and WINGFIELD (1995) provided the basis for a proliferation in DNA-based identification of *Armillaria* spp., especially methods using Polymerase Chain Reaction (PCR). Various PCR methods have been used to discern *A.*

ostoyae from other *Armillaria* species (ANDERSON and STASOVSKI 1992; HARRINGTON and WINGFIELD 1995; VOLK et al. 1996; BANIK et al. 1996; WHITE et al. 1998; KIM et al. 2000; and others as reviewed by PEREZ-SIERRA et al. 2000). These methods have also shown a close relationship of *A. ostoyae* to both *A. borealis* and *A. gemina* (ANDERSON and STASOVSKI 1992; HARRINGTON and WINGFIELD 1995; CHILLALI et al. 1998a, 1998b). HARRINGTON and WINGFIELD (1995) also speculated at evolutionary intermediates between *A. ostoyae* and *A. borealis*, and BÉRUBÉ and DESSUREAULT (1988) reported that these species exhibit similar morphology. Phylogenetic studies also indicate that *A. ostoyae* is similar to *A. gemina* (ANDERSON and STASOVSKI 1992; PIERCEY-NORMORE et al. 1998), and some individuals appear to contain hybrid DNA sequences that may represent a combination of the two species (HANNA et al. 2003a, 2004).

4 Distribution, hosts, and pathogenicity

Armillaria ostoyae has a circumboreal distribution and a wide host range (Fig 1-1, Table 1-2). Variability in the damage and effects of *A. ostoyae* on a forest stand is a common theme across its distribution. In western North America, BENTON and EHRLICH (1940) were perhaps the first to recognize the need to characterize the great variability within *Armillaria mellea sensu lato*, which they acknowledged as a common cause of root disease in western white pine (*Pinus monticola* Douglas) within northern Idaho forests. *Armillaria ostoyae* has since been recognized to vary in both virulence and pathogenicity (OMDAL et al. 1995; MCDONALD 1998; MORRISON and PELLOW 2002). Even individuals that are derived from close geographical areas can exhibit considerable variability. Comparisons of two *A. ostoyae* genets from the Priest River Experimental Forest in

northern Idaho revealed that one genet formed bark fans (signifying a highly pathogenic individual) on 88% of the trees on which it was found; whereas, the other genet appeared only as epiphytic rhizomorphs on 77% of the trees where it was collected (MCDONALD 1998).

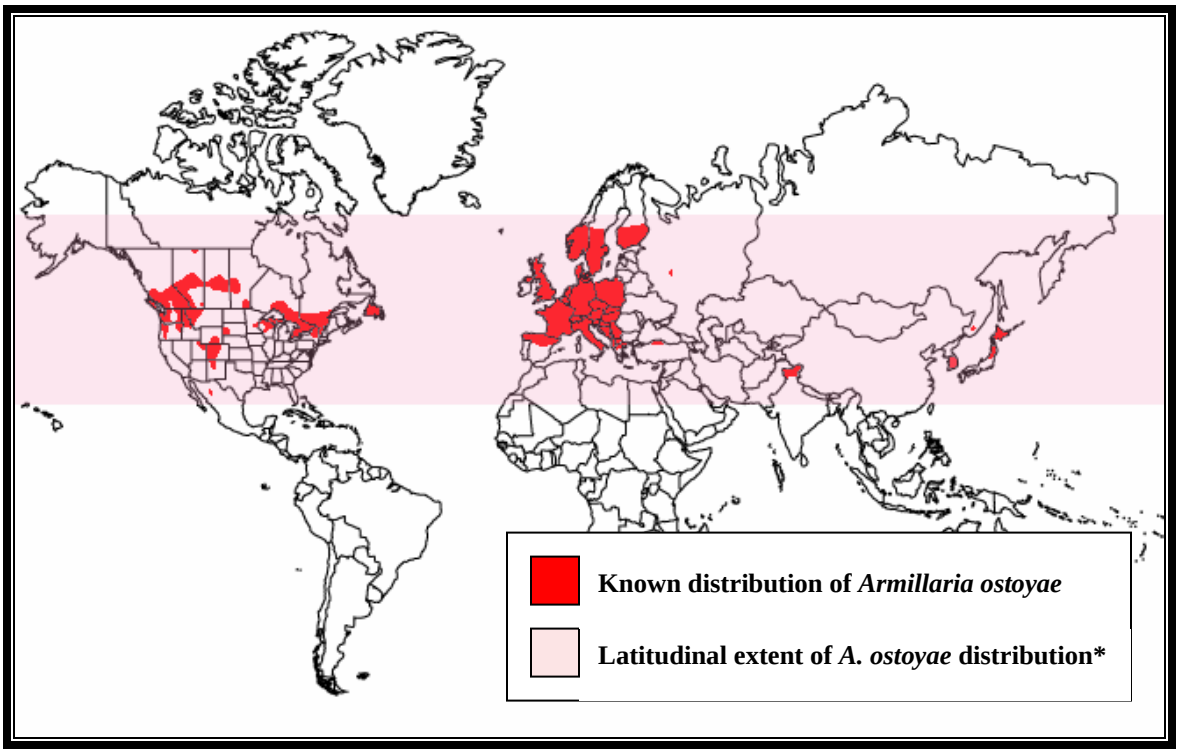
4.1 Asia

Armillaria ostoyae is thought to be prominent on both conifers and hardwoods in Asia although most reports are of its saprophytic nature (KILE et al. 1994). The genus *Armillaria* exists as an important pathogen of larch in China; however, little work has been done to identify it to species. Although *A. tabescens* and *A. gallica* have been associated with disease in China, most Armillaria root disease in China has been attributed to *A. mellea sensu lato* (KILE et al. 1994; MOHAMMED et al. 1994). On the Japanese island of Hokkaido, *A. ostoyae* specifically has been noted to be mycorrhizal with orchids (TERASHIMA et al. 1998). However, the distribution of *A. ostoyae* needs confirmation in other portions of Asia, including the Kashmir region on India where *A. ostoyae* has been tentatively identified (WATLING and GREGORY 1980; KILE and WATLING 1988).

4.2 Europe

Throughout Europe, *A. ostoyae* is nearly ubiquitous on suitable hosts that occur between the latitudinal ranges of 40°N to 63°N (Fig 1-1, Table 1-2). In this region, *A. ostoyae* is most common as a saprophyte, a pathogen of stressed trees, or a pathogen in young conifer plantations (KILE et al. 1994; KWASNA et al. 2001). However, *A. ostoyae* can behave as a primary parasite in rare cases, such as on mountain pine (*Pi. uncinata* Mill. ex Mirb.) in the French Pyrenees (DURRIEU et al. 1981; KILE et al. 1994). Although

Fig. 1-1. **Circumboreal distribution of *Armillaria ostoyae*.**



* Though not yet reported, it is reasonable that *Armillaria ostoyae* may occur throughout much of central Asia, the Middle East, and other areas within its latitudinal extent.

Table 1-2. ***Armillaria ostoyae*** **distribution and host genera**

Location	Associated Host Genera	Selected References
Asia		
India (Kashmir)		Watling and Gregory 1980
Japan	*Abies, Acer, Betula, Gastria, Quercus, Larix, Picea, Pinus*	Mohammed et al. 1994, Ota et al. 1998, Terashima et al. 1998, Tosa et al. 2004
Korea	*Pinus, Quercus*	Sung et al. 1991
Russia	unspecified	Hanna 2004
Turkey	unspecified	Pekşen and Karaca 2003
Europe		
Albania	*Abies, Cupressus, Juniperus, Picea, Pinus, Betula, Fagus*	Lushaj et el. 2003
Austria	unspecified	Halmschlager and Kowalski 2004
Czech Republic	*Abies, Pinus, Betula, Quercus, Pseudotsuga*	Lochman et al. 2004
Denmark	unspecified	Marxmüller and Printz 1982, Roll-Hansen 1985
England	*Corylopsis, Pinus, Picea, Pseudotsuga*	Risbeth 1982, 1983, Gregory 1989, Roll-Hansen 1985, Perez Sierra et al. 1999
Finland	*Alnus, Pinus, Fragaria*	Korhonen 1978, 1980, Anderson et al. 1980, Mohammed et al. 1994, Hanna et al. 2004
France	*Abies, Picea, Pinus, Quercus, Sarothamnus, Ulex*	Romagnesi 1970, 1973, Jacques-Félix 1977, Korhonen 1978, Guillaumin and Berthelay 1981, Lung-Escarmant et al. 1985, Roll-Hansen 1985, Mohammed et al. 1994, Chillali et al. 1998a, Chillali et al. 1998b, Langrell et al. 2001, Lung-Escarmant and Guyon 2004
Germany	*Betula, Fagus, Picea, Pinus, Quercus, Sorbus*	Marxmüller 1982, Roll-Hansen 1985, Schulze et al. 1997, Sicoli et al. 2003
Greece	*Abies, Juniperus, Picea, Pinus, Fagus*	Tsopelas 1999
Ireland (northern)	*Prunus*	Anonymous February 10, 2005
Italy	*Abies, Picea*	Anselmi and Minerbi 1989, Intini 1989, Kile et al. 1994, Sicoli et al. 2002, 2003

Table 1-2. **(continued)**

Location	Associated Host Genera	Selected References
Europe (continued)		
Norway	*Picea*	Roll-Hansen 1985, Żółciak 2004
Poland	*Picea, Pinus, Quercus*	Korhonen 1978, Guillaumin and Berthelay 1981, Roll-Hansen 1985, Łakomy 1996, 1998, Perez Sierra et al. 1999, Kwaśna 2002, Kwaśna et al. 2004
Russia	*Picea*	Selochnik et al. 2004
Serbia	unspecified	Keca et al. 2004
Slovenia	unspecified	Munda 1997, Tsopelas 1999
Spain	*Pinus*	Aguín Casal et al. 2003
Sweden	*Picea*	Sicoli et al. 2003
Switzerland	*Picea, Pinus, Pseudotsuga, Larix*	Prospero et al.2003a, 2003b, 2004; Rigling et al. 2003
North America		
Alberta, Canada	*Abies, Pinus, Populus, basidiocarps*	Mallett and Hiratsuka 1987; Mallett 1990, 1992; Morrison and Mallett 1996; Mallet and Maynard 1998; Mallett and Volney 1999; Morrison and Pellow 2002; Pankuch et al. 2003
British Columbia (southern), Canada	*Alnus, Larix, Pinus, Pseudotsuga*	Anderson and Ullrich 1979; Morrison et al. 1985, 1992, 2000, 2001; Bloomberg and Morrison 1989; Reaves and McWilliams 1991; van der Kamp 1993, 1995; Morrison and Mallett 1996; Cruickshank et al. 1997; White et al. 1998; White and Morrison 1999; Chapman and Xiao 2000; Cruickshank 2000, 2002; Dettman and van der Kamp 2001a, 2001b; Robinson and Morrison 2001; Morrison and Pellow 2002; DeLong et al. 2002; van der Star 2003; Chapman et al. 2004; Robinson et al. 2004

Table 1-2. **(continued)**

Location	Associated Host Genera	Selected References
North America (continued)		
Chihuahua, Mexico	*Abies, Pinus, Pseudotsuga*	Shaw 1989, Hanna 2003b, 2004
Colorado, U.S.A.	*Abies, Picea, Pinus, Pseudotsuga*	Wu et al. 1996, Omdal et al. 2004, Worrall et al. 2004
Idaho, U.S.A.	*Abies, Pinus, Pseudotsuga*	Entry et al. 1991, Kim 1999; Kim et al. 2000, Hanna et al. 2003b, 2004
Manitoba, Canada	unspecified	Morrison and Mallett 1996
Michigan, U.S.A.	*Abies, Acer, Alnus, Betula, Malus, Populus, Prunus, Pinus, Picea, Thuja*	Proffer et al. 1987; Smith et al. 1990; Larsen et al. 1992, Smith et al. 1994, Banik et al. 1995
Minnesota, U.S.A.	*Pinus, Abies, Alnus, Amelanchier, Betula, Corylus, Populus, Vaccinium*	Banik et al. 1995, Rizzo et al. 1995, Kromroy 1999
Montana, U.S.A.	unspecified	Hanna et al. 2003b, 2004
New Hampshire, U.S.A.	*Abies* , *Picea* , *Pinus* , *Acer* , *Betula* , *Tsuga* , *Fagus* , *Prunus*	Ullrich and Anderson 1978, Anderson and Ullrich 1979, Harrington 1987; Harrington et al. 1989; Wargo 1989, Rizzo and Harrington 1988, 1993; Kim 1999; Kim et al. 2000, Wargo and Carey 2001; Hanna et al. 2003b, 2004
New Mexico, U.S.A.	*Abies, Picea, Pinus, Populus, Pseudotsuga, Larix*	Omdal et al. 1995, Omdal et al. 2004, Hanna et al. 2003b, 2004
New York State, U.S.A.	*Acer, Fagus, Picea, Pinus, Betula, Tsuga, Pseudotsuga*	Ullrich and Anderson 1978, Anderson and Ullrich 1979, Blodgett 1990, Blodgett and Worrall 1992a, 1992b; Frontz et al. 1998
Newfoundland, Canada	*Abies, Betula, Picea*	Bérubé 2000, Piercey-Normore and Bérubé 2000

Table 1-2. **(continued)**

Location	Associated Host Genera	Selected References
North America (continued)		
Ontario, Canada	*Acer, Betula, Carya, Fagus, Fraxinus, Ostrya, Prunus, Quercus, Tilia, Abies, Picea, Pinus, Tsuga*	Ullrich and Anderson 1978, Anderson and Ullrich 1979, Dumas 1988; Whitney 1988,1997; Wiensczyk et al. 1997, McLaughlin 2001; Morrison and Pellow 2002
Oregon, U.S.A.	*Abies, Pseudotsuga, Pinus, Larix*	Reaves and McWilliams 1991, Morrison and Pellow 2002, Filip et al. 2002, Ferguson et al. 2003, Hanna et al. 2003b, 2004
Pennsylvania, U.S.A.	unspecified	Frontz et al. 1998
Quebec, Canada	*Acer, Betula, Pinus*	Piercey-Normore et al. 1998
Saskatchewan, Canada	unspecified	Mallett 1992
South Dakota, U.S.A.	*Picea, Pinus, Populus*	Wu et al. 1996, Kallas 1997, Kallas et al. 2003
Utah, U.S.A.	unspecified	McDonald 1998, Hanna et al. 2003b, 2004
Vermont, U.S.A.	*Acer, Abies, Betula, Picea*	Ullrich and Anderson 1978, Anderson and Ullrich 1979, Anderson et al. 1980, Mohammed et al. 1994
Wisconsin, U.S.A.	*Abies, Picea, Pinus, Acer, Populus, Quercus, Betula*	Banik et al. 1995, 1996, Kromroy 1999, Kromroy 2004
Washington State, U.S.A.	*Abies, Picea, Pinus, Pseudotsuga, Rubus, Salix, Tsuga*	Ullrich and Anderson 1978, Anderson and Ullrich 1979, Entry et al. 1991, Reaves and McWilliams 1991, Banik et al. 1996, McDonald 1998, Kim 1999. Kim et al. 2000, Roth et al. 2000, Hanna et al. 2003b, 2004
Wyoming, U.S.A.	*Abies, Pinus*	Wu et al. 1996

it is mainly associated with conifers, *A. ostoyae* can grow and produce rhizomorphs on hardwood species in Europe, and it has been implicated as a potential contributor to oak decline (REDFERN 1975; RISHBETH 1982; DAVIDSON and RISHBETH 1988; GREGORY 1989; HOOD et al. 1991; HALMSCHLAGER and KOWALSKI 2004).

4.3 Eastern North America

In eastern North America, *A. ostoyae* is found in Newfoundland and throughout most of the states and provinces surrounding the Great Lakes (Fig 1-1, Table 1-2). Though the species was not identified, STANOSZ and PATTON (1987) reported that an *Armillaria* sp. may affect aspen (*Populus* sp.) sucker stand development in eastern North America. Subsequently, BANIK et al. (1995) showed that *A. ostoyae* was more pathogenic to aspen than *A. sinapina* and *A. gallica*. In central and southern Ontario, *A. ostoyae* was commonly associated with conifers; whereas diverse *Armillaria* species were associated with hardwoods (MCLAUGHLIN 2001). *Armillaria ostoyae* causes similar mortality on Ontario sites dominated by maple (*Acer* spp.) and oak (*Quercus* spp.) (MCLAUGHLIN 2001). In contrast, a low incidence of *A. ostoyae* infection was found on sugar maple (*Acer saccharum* Marsh.) in New York state (BLODGETT and WORRALL 1992b).

4.4 Western North America

For the most part, *A. ostoyae* is common in western North America, wherever suitable hosts and habitats occur. General distribution is known in the Rocky Mountains from Chihuahua, Mexico to a northern limit of 53°N along the British Columbia/Alberta border, along the Pacific coast and Cascades from southern Oregon to the same limit of

53°N in British Columbia, through central Alberta over through central Manitoba, and in the Black Hills of South Dakota (Figure 1-1, Table 1-2). MALLETT (1992) also recognized one atypical occurrence in Alberta near the border of the Northwest Territories.

Similar to other areas of the world, *A. ostoyae* primarily associates with coniferous hosts in western North America (WARGO and SHAW 1985; CRUICKSHANK et al. 1997). However, it has a very wide host range, and can even infect hardwood shrubs. Thus, *A. ostoyae* can persist in areas devoid of conifers (TARRY and SHAW 1966; ADAMS 1974; SHAW 1975; MORRISON 1981; WILLIAMS and MARSDEN 1982; MCDONALD et al. 1987a, b). In general, the most susceptible hosts are Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] and true firs (*Abies* spp.), while cedar (*Thuja* spp.) and pines (*Pinus* spp.) are the least susceptible (MORRISON 1981; HADFIELD et al. 1986; HAGLE and GOHEEN 1988).

Armillaria root rot negatively affects commercial timber production in western North America by causing tree mortality, reduction in tree growth, and predisposition to windthrow (CRUICKSHANK 2000; DETTMAN and VAN DER KAMP 2001a). Damage from *A. ostoyae* varies widely by location, host, and individual genet, with some genets appearing extremely pathogenic while others appear only weakly pathogenic (SHAW and LOOPSTRA 1988; MUGALA et al.1989; HOOD et al. 1991; OMDAL et al. 1995; DETTMAN and VAN DER KAMP 2001b). In some situations, even hosts generally considered to be resistant, such as ponderosa pine (*Pi. ponderosa* Douglas ex Lawson & C. Lawson) can become greatly damaged by *A. ostoyae* infection (SHAW et al. 1976). In interior forests of western North America, Douglas-fir is one of the most susceptible tree species (MORRISON 1981;

MORRISON et al. 1992; ROBINSON and MORRISON 2001), while the coastal variety of Douglas-fir seldom succumbs to infection (JOHNSON et al. 1972; ROBINSON and MORRISON 2001). Mortality caused by *A. ostoyae* is common on trees of all size classes within interior British Columbia, but such mortality is rare in trees older than 25 years old on the coast (MORRISON 1981; WARGO and SHAW 1985; CRUICKSHANK et al. 1997).

In western North America, another common theme of Armillaria root disease is it appears to become more common as management intensifies (BLENIS et al. 1987; MCDONALD et al. 1987b; HOOD et al 1991). Most timber practices that involve harvesting tend to increase Armillaria root disease (REDFERN and FILIP 1991; CRUICKSHANK et al.1997; MORRISON et al. 2000, 2001; DETTMAN and VAN DER KAMP 2001b). *Armillaria ostoyae* is also a primary concern in young plantations, especially plantations of highly susceptible Douglas-fir where disease can cause mortality (BUCKLAND 1953; PIELOU and FOSTER 1962; JOHNSON et al. 1972; HOOD and MORRISON 1984; MORRISON et al. 1985a,b; BOOMBERG 1990; HOOD et al. 1991).

Increased likelihood of windthrow also raises concerns of potential injury and liability at campgrounds where Armillaria root rot is present (WORRALL et al. 2004; HAGEL et al. 2004). Surveys for *A. ostoyae* in southwestern Colorado found infection levels of 10.5% on live trees within the campgrounds and 12.7% just outside the campgrounds (WORRALL et al. 2004). In some instances, precautionary removal of trees within the campgrounds has been deemed necessary (HAGEL et al. 2004).

5 Potential influences of paleogeographic events on the distribution of *A. ostoyae* in western North America

Distribution of *A. ostoyae* in the western North America raises some intriguing questions because it does not always coincide with seemingly suitable host and habitat distribution. *Armillaria ostoyae* is yet to be reported from Alaska and California; however, another species (NABS X), which typically co-exists with *A. ostoyae* in much of the interior northwestern United States, has been identified in California (McDonald 1998; Baumgartner and Rizzo 2001a). Another interesting paradox occurs in an interior region (i.e., central and southern Idaho, part of southwestern Montana, and northwestern Wyoming) where *A. ostoyae* has not been found, even though apparently suitable habitat occurs and other primarily saprophytic *Armillaria* species (e.g., NABS X) have been found (per. comm. G. I. McDonald). The absence of *A. ostoyae* in these areas is perhaps attributable to a series of volcanic events. Beginning ca. 17 million years ago, a series of "supervolcanos" began erupting in northern Nevada and southeast Oregon. The volcanic eruptions proceeded northeastward, eventually reaching the Yellowstone area ca. 2 million years ago (Smith and Siegel 2000). The Yellowstone "supervolcanos" have erupted on a cycle of roughly every 600,000 years and are thought to have completely removed all life forms in their paths of destruction (Smith and Siegel 2000). These volcanic events may have eliminated *A. ostoyae* from some regions, but questions remain about why other *Armillaria* spp. occur in these regions. Perhaps *A. ostoyae* is less adept at dispersal and recolonization (e.g., via spores) than other *Armillaria* species. A similar scenario is found in British Columbia where the range of *A. ostoyae* is more limited than its saprophytic counterparts. Specifically, *A. sinapina* is found much further north than *A.*

ostoyae (MORRISON et al. 1985). The glacial maximum ca. 18,000 years ago covered much of Canada and "pushed" most life forms to non-glaciated areas to the south. The northern most occurrence of *A. ostoyae* in British Columbia is near what may have been an ice-free corridor that extended to the east of the Rocky Mountains (PIELOU 1991; WEIN 1999). Phylogeographic studies of *A. ostoyae* throughout Canada may reveal important insights into the theory that several nunataks (areas of cryptic refugia) existed well out into the ice sheets on high elevation mountains and plateaus (PIELOU 1991; WEIN 1999). It has also been suggested that deep caves may have acted as nunataks because subfreezing surface temperatures have little effect on temperatures in the below-ground caves where several life forms exist (HOLSINGER 1981). Although natural caves have not been surveyed for *Armillaria* spp., *Armillaria* rhizomorphs and mycelia were found 91 m below ground within an underground acid-mine drainage system in Pennsylvania (HINCE and ROBBINS 2003). In that site, iron bacteria-laden pools known as "yellow boy" sediment were densely colonized by *Armillaria* rhizomorphs as "thick as pencils" (HINCE and ROBBINS 2003).

6 Management strategies and concepts

Growing evidence suggests that predisposition to stress is a major factor in the rise of Armillaria root disease caused by *A. ostoyae* (SHIGO 2000; SZYNKIEWICZ and KWAŚNA 2004). In areas of high Armillaria risk, it is generally recommended that steps be taken to reduce tree stress before disease reaches a critical state. Prevention is the least expensive and most effective measure to reduce loss caused by *A. ostoyae* (SCHMITT 2001). In areas heavily colonized by *A. ostoyae*, a critical evaluation of disease impact and forest

management objectives is required to ensure that the level of loss justifies any control measures (WARGO and SHAW 1985; WHITNEY 1988).

6.1 Ecophysiological maladptation and local provenance

It has been suggested that many presently existing trees have a greater susceptibly to Armillaria root disease because they are ecophysiologically maladapted to their current habitat (MCDONALD et al. 2003). Many maladapted tree species and/or genotypes exist at a site because of inappropriate management practices that have altered stand composition or allowed planting of trees or seed derived from non-local seed sources. Altered stand composition is common within interior-northwestern North America, where fire suppression has favored climax species [e.g., true firs (*Abies* spp.) and Douglas-fir] over seral species [e.g., pines (*Pinus* spp.) and larch (*Larix* spp.)]. However, seral tree species are generally more resistant and/or tolerant to Armillaria root disease. Thus, recommended management for Armillaria root disease generally favors regeneration of seral tree species or the planting of seral tree species derived from local seed sources (SINGH and RICHARDSON 1973; WHITNEY 1988). While non-local seed sources may have an adequate level of fitness for a chosen site, it is normally safer to choose seed of local provenance from a viewpoint of sustaining ecological stability, maintaining genetic diversity, and ensuring that trees are adapted to the site (WILKINSON 2001; SACKVILLE HAMILTON 2001). Trees grown in this manner are less likely to experience plant stress that can exacerbate Armillaria root rot.

6.2 Tree planting

Injuries caused by planting may render trees more susceptible to Armillaria root rot. Some suggest using container-grown rather than bare-root trees (SINGH and RICHARDSON 1973; WHITNEY 1988), though containers may also cause root deformations that predispose trees to infection by *Armillaria* spp. (LIVINGSTON 1990; PIERCEY-NORMORE and BÉRUBÉ 2000). For this reason, seeding or natural regeneration has been recommended for areas with high risk for Armillaria root rot (SINGH and RICHARDSON 1973; WHITNEY 1988).

6.3 Mechanical removal

Stump removal and root raking can help reduce *Armillaria* spp. inoculum (MORRISON 1981; WHITNEY 1988; ROTH et al. 2000; STURROCK 2000); however, it is likely impossible that all the inoculum can be removed from a site using mechanical means. In addition, stump removal is expensive and also not a suitable option on sites with steep slopes where erosion is a concern (MORRISON and MALLET 1996).

6.4 Biological control

Although tests have been limited to small research plots, biological control may offer one of the most promising strategies to reduce negative impacts of *Armillaria* root disease on a large scale. Similar to guidelines for seed sources, biological control agents of local origin may represent a prudent choice from an ecological viewpoint of maintaining biological diversity and stability. Fortunately, many of the organisms being considered as biological controls are common to forests in which *A. ostoyae* occurs. Bacterial species of

Pseudomonas have been shown to be antagonistic towards *A. ostoyae in vitro*; however, little is known about bacterial relationships with *A. ostoyae* in the forest soil (DeLong et al. 2002). Fungal genera, such as *Pleurotus*, *Hypholoma*, and *Trichoderma*, have also been shown to be antagonistic towards *A. ostoyae in vitro* (REAVES et al. 1990; CHAPMAN and XIAO 2000). Early field trails by CHAPMAN et al. (2004) demonstrated biological control of *Armillaria* sp. using *Hypholoma fasciculare* (Fries) Kummer; their study showed considerably less mortality in the plots treated with *H. fasciculare* compared to the control plots. *Trichoderma* spp. from burned plots appeared to be more antagonistic towards *Armillaria* in culture. REAVES et al. (1990) found that *Trichoderma* isolates that were recovered after a fire event were more antagonistic towards *A. ostoyae in vitro* than those from unburned areas. It is likely that other fungi and bacteria also exhibit potential for biological control of *A. ostoyae*, but more studies are needed to identify these organisms. One source of biological control agents for *A. ostoyae* may lie within the genus *Armillaria*. Other *Armillaria* species are known to be predominately saprophytic and may be the best-suited organism to occupy and compete in the same niche as *A. ostoyae*. Known relationships of *A. ostoyae* with other *Armillaria* species are variable. On some sites, pathogenic and non-pathogenic individuals seem to exclude each other indicating competition; however, on other sites the different species occur within the same area (MCDONALD 1991b; MCDONALD 2003; PROSPERO et al. 2003). In related studies, an antagonistic relationship has been observed with pathogenic *A. mellea sensu stricto* and saprophytic *A. gallica* (BRUHN et al. 2000).

While *A. ostoyae* genets apparently compete directly with each other across their range and show very little geographic overlap (WORRALL et al. 2004, per. comm. G. I.

MCDONALD), their response and recognition of other species of *Armillaria* may be based on multiple interacting factors. In areas of limited resources, these fungi perhaps have genetically encoded mechanisms that signal each other's presence. The unnamed species, NABS X, is one example of a saprophytic species that seemingly interacts with *A. ostoyae* under certain conditions (MCDONALD et al. 2003; Figs. A1-1, A1-2, A1-3). It is interesting to note that *A. nabsnona* appears to have evolved from the Rockies group of *A. ostoyae* (HANNA et al. 2004). A recent study by HANNA et al. (2003a) has found rDNA (internal transcribed spacer) sequences from NABS X that appears to be a hybrid sequence derived from *A. nabsnona* Volk & Burdsall and *A. sinapina.* Is it possible that NABS X has inherited recognition genes from its ancestor *A. ostoyae* that are only triggered under certain environmental conditions?

6.5 Fire

Armillaria ostoyae is a common component of forests that have frequent (i.e., less than 100-year) fire intervals, and it has been shown that *A. ostoyae* can persist through fire events (SCHMITT 2001; FERGUSON et al. 2003). While fire cannot be used to eliminate *A. ostoyae* from a site, it should be considered in any plan in which preventative strategies are needed for managing Armillaria root rot. Fire may be important in managing *A. ostoyae* on several levels: 1) REAVES et al. (1990) showed that fire may maintain populations of microorganisms that are antagonistic towards *A. ostoyae*; 2) Fire promotes the growth and regeneration of resistant seral tree species, while limiting highly susceptible climax tree species; and 3) While a single fire can kill trees, which can subsequently become colonized by *Armillaria* (SCHMITT 2001), frequent fire events may

also prevent the buildup of downed woody debris that serves as an inoculum reservoir. For these reasons, fire prescriptions must be site specific and consider the long-term goals of the site.

6.6 Silvicultural practices

After selective cutting, stumps can become colonized by *A. ostoyae*, thereby increasing inoculum on the site (MORRISON et al. 2001). The overall effect of selective cutting on Armillaria root rot is directly related to the long-term prescription of the site after cutting. Selective cutting to promote seral species may be a beneficial measure to protect against further proliferation of the disease. However, selective cutting may create wounds on remaining trees that can facilitate infection by *Armillaria* sp. (PANKUCH et al. 2003). In addition, selective cutting may promote attack from bark-beetles by providing a well-defined target or providing suitable substrates for beetle reproduction. Anecdotal evidence suggests that pre-commercial thinning increases damage from *A. ostoyae* within the interior British Columbia but not in coastal forests (CRUICKSHANK et al. 1997). Koenigs (1969) found an increase of Armillaria root disease in thinned western redcedar (*Thuja plicata* D. Don). In contrast, other studies have concluded that pre-commercial thinning does not increase Armillaria root disease (JOHNSON and THOMPSON 1975; FILIP et al 1989; CRUICKSHANK et al. 1997). Following thinning on British Columbia sites, between 12-52% of stumps became colonized by *Armillaria* spp., allowing for a great increase in inoculum on these sites (CRUICKSHANK et al. 1997). That study also found that better growth rates of juvenile coastal trees correspond to the trees ability for callus formation around root lesions, thus protecting the trees from *Armillaria* infection. Interior

cedar and Douglas-fir grew slower, did not form wound callus as quickly, and were more susceptible to Armillaria root disease. Inoculum build-up can perhaps be minimized by limiting cuts of large-diameter trees, because large-diameter trees are apparently more likely to become colonized by *Armillaria* spp. (VAN DER KAMP 1995; MORRISON et al. 2000).

6.7 Nutrition

Severity of Armillaria root disease has been linked to soil-nutrient levels, because severe Armillaria root disease has been observed on sites with nutrient deficiencies (SHIELD and HOBBS 1978; SINGH 1983; FILIP et al. 2002). Common perceptions and research results on the effects of nutrition supplementation on Armillaria root disease are mixed. SHIGO (2000) states, "fertilizers will benefit the pathogens more than the tree," while others suggest that proper nutrition could increase disease resistance of trees by increasing their phenol/sugar ratios (MYSZEWSKI et al. 2002). MOORE et al. (2000) and ENTRY et al. (1991) have both shown that higher phenol/sugar ratios in tree roots correspond with increased resistance to Armillaria root disease, and lower phenol/sugar ratios correspond with a higher incidence of the root disease. Furthermore, MOORE et al. (2000) also showed that the decreases in phenol/sugar ratio corresponded with decreases in the productivity of the habitats, and MCDONALD et al. (1987b) also suggested that damage from Armillaria root disease increases as site productivity decreases. *Armillaria ostoyae* is a genetically diverse and pathogenically variable species that occupies widely ranging habitats with varying soil types and parental substrates, therefore site-specific prescriptions of nutritional supplementation may be needed to achieve optimal benefits.

While nutritional supplementation shows promise, an improper prescription may be detrimental to a site. In northwestern Washington, U.S.A., one particular site subjected to variable nutritional supplementation has been dubbed “the square plots of death” due to damage from Armillaria root disease (pers. comm. J.A. MOORE and P. CANNON, as illustrated by Fig. A1-1).

6.8 Host genetics

The phenol/sugar ratio within Douglas-fir roots can also vary by genotype (MYSZEWSKI et al. 2002). Like nutritional supplementation to increase phenol/sugar ratios, the use of host genotypes that have a high phenol/sugar ratio in their root system may provide protection against Armillaria root disease while maintaining or increasing site productivity (MYSZEWSKI et al. 2002). Such approaches may have merit; however, the importance of locally adapted trees must also be considered. Interactions between tree genotype and environment may produce unintended consequences that result is increased plant stress. Because plant stress can be a predisposing factor of Armillaria root disease, environmental adaptation must be considered along with other genetically encoded disease-resistance mechanisms. Thus, any introduction of non-local stock should be considered carefully for other long-term implications within the forest ecosystem.

7 Future studies

Thus far, most studies on *A. ostoyae* have focused on basic biology, ecological roles, distribution, and disease management. Continued research is needed in all these areas, as many critical questions remain. The expression of Armillaria root rot is related to the

geographical distribution and genetic variation within the pathogen and the host and their interactions with the biotic and abiotic environment (McDonald 1991b). Because Armillaria root rot results from complex interactions among host, pathogen, and environment, it can be difficult to determine influences of specific factors. Great advancements in molecular genetics and geographic information systems provide an ideal opportunity to provide insights into the ecological interactions of Armillaria root rot. Molecular genetics approaches will help assess the contribution of pathogen genetics to the ecological roles of *A. ostoyae* as a pathogen or saprophyte. Similarly, molecular genetics can help determine how host genetics contributes to resistance/susceptibility to Armillaria root rot. In addition, molecular genetics provide an avenue to study how evolutionary and geographic histories of *Armillaria* spp. influence their current ecological roles. Geographic information systems provide another powerful tool to link geographic variation in pathogen genetics to geographic variation in the environment, and examine relationships of paleogeographic events and cultural practices to *Armillaria* genetics and ecological behavior. The integration of molecular genetics and geographic information systems will help develop models to predict risk of Armillaria root disease. Risk prediction will help forest managers choose appropriate management practices to limit damage caused by *A. ostoyae* and/or maintain balanced natural ecosystems.

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References

ADAMS, D. A., 1974: Identification of clones of *Armillaria mellea* in young-growth ponderosa pine. Northwest Science **48,** 21-28.

AGUÍN CASAL, O.; SIERRA, A. P.; SABARIS ROMA, M.; MANSILLA VÁZQUEZ, J. P., 2003: Identification of *Armillaria* spp. in north-west Spain using molecular techniques. In: Root and Butt Rots of Forest Trees, 10th International Conference on Root and Butt Rots: Proc. of the IUFRO WP 7.02.01 Meeting. Québec City, Canada, September 16-22, 2001. Ed. by LAFLAMME, G.; BÉRUBÉ, J. A.; BUSSIÉRES, G. Natural Resources Canada, Canadian Forest Service, Laurential Forestry Centre, Sainte-Foy, Québec. Information Report LAU-X-126. pp. 24-26.

AMMIRATI, J., 1979: Chemical studies of mushrooms: The need for voucher collections. Mycologia **71,** 437-441.

ANDERSON, J. B., 1986: Biological species of *Armillaria* in North America: Redesignation of groups IV and VIII and enumeration of voucher strains for other groups. Mycologia **78**, 837-839.

ANDERSON, J. B.; ULLRICH, R. C., 1979: Biological Species of *Armillaria mellea* in North America. Mycologia **71,** 402-414.

ANDERSON, J. B.; KORHONEN, K.; ULLRICH, R. C., 1980: Relationships between European and North American Biological Species of *Armillaria mellea*. Experimental Mycology **4,** 87-95.

ANDERSON, J. B.; STASOVSKI, E., 1992: Molecular phylogeny of northern hemisphere species of *Armillaria*. Mycologia **84,** 505-516.

ANONYMOUS, February 10, 2005: *Armillaria ostoyae*. Northern Ireland Fungus Group. [Online] Available http://www.nifg.org.uk/species/atlas2.htm?item=NBNSYS0000037776 [Last accessed February 10, 2005]

ANSELMI, N.; MINERBI, S., 1989: Root rots involved in forest decline in Italy. Proceedings of the 7th International Conference on Root and Butt Rots, Vernon and Victoria 1988. pp. 503-512.

BANIK, M. T.; PAUL, J. A.; BURDSALL, H. H. JR., 1995: identification of *Armillaria* species from Wisconsin and adjacent areas. Mycologia **87,** 707-712.

BANIK, M.T.; VOLK, T.J.; BURDSALL, H. H. JR., 1996: *Armillaria* species of the Olympic Peninsula of Washington state, including confirmation of North American biological species XI. Mycologia **88,** 492-496.

BATTARA, A., 1755: *Fungorum agri ariminensis historia*. Faventia.

BAUMGARTNER, K.; RIZZO, D. M., 2001a: Distribution of *Armillaria* species in California. Mycologia **93,** 821-830.

BAUMGARTNER, K.; RIZZO, D. M., 2001b: Ecology of *Armillaria* spp. in mixed-hardwood forests of California. Plant Dis. **85,** 947-951.

BENTON, V. L.; EHRLICH, J., 1940: Variation in culture of several isolates of *Armillaria mellea* from western white pine. Phytopath. **31,** 803-811.

BÉRUBÉ, J. A., 2000: *Armillaria* species in Newfoundland. Can. J. For. Res. **30,** 507-512.

BÉRUBÉ, J. A.; DESSUREAULT, M., 1988: Morphological characterization of *Armillaria ostoyae* and *Armillaria sinapina* sp. nov. Can. J. Bot. **66,** 2027-2034.

BÉRUBÉ, J. A.; DESSUREAULT, M., 1989: Morphological studies of the *Armillaria mellea* complex: Two new species *A. gemina* and *A. calvescens*. Mycologia **81,** 216-225.

BLENIS, P.; HIRATSUKA, Y.; MALLETT, K., 1987: Armillaria root rot in Alberta. Agriculture and forestry Bulletin. **10,** 4-5.

BLODGETT, J. T., 1990: Ecology of *Armillaria* species in New York forests. MS Thesis, State University of New York College of Environmental Science and Forestry, Syracuse, NY, U.S.A. 69 pp.

BLODGETT, J. T.; WORRALL, J. J., 1992a: Distributions and hosts of *Armillaria* species in New York. Plant Dis. **76,** 166-170.

BLODGETT, J. T.; WORRALL, J. J., 1992b: Site relationships of *Armillaria* species in New York. Plant Dis. **76,** 166-170.

BLOOMBERG, W.J., 1990: Effect of stand conditions on advance of *Phellinus weirii* in Douglas-fir plantation. Phytopathology **80,** 553-559.

BLOOMBERG, W. J.; MORRISON, D. J., 1989: Relationship of growth reduction in Douglas-fir to infection by Armillaria root disease in southeastern British Columbia. Phytopathology **79,** 482-487.

BRUHN, J. N.; WETTEROFF, J. J. JR.; MIHAIL, J. D.; KABRICK, J. M.; PICKENS, J. B., 2000: Distribution of *Armillaria* species in upland Ozark Mountain forests with respect to site, overstory species composition and oak decline. Forest Pathology **30,** 43-60.

BUCKLAND, D. C., 1953: Observations on *Armillaria mellea* in immature Douglas-fir. Forestry Chronicle **29,** 344-347.

CHAPMAN, B.; XIAO, G., 2000: Inoculation of stumps with *Hypholoma fasciculare* as a possible means to control Armillaria root disease. Can. J. Bot. **78,** 129-134.

CHAPMAN, B.; XIAO, G.; MYERS, S., 2004: Early results from field trials using *Hypholoma fasciculare* to reduce *Armillaria ostoyae* root disease. Can. J. Bot. **82,** 962-969.

CHILLALI, M.; WIPF, D.; GUILLAUMIN, J.-J.; MOHAMMED, C.; BOTTON, B., 1998a: Delineation of the European *Armillaria* species based on the sequences of the internal transcribed spacer (ITS) of ribosomal DNA. New Phytol. **138,** 553-561.

CHILLALI, M.; IDDER-IGHILI, H.; GUILLAUMIN, J.-J.; MOHAMMED, C.; LUNG-ESCARMANT, B.; BOTTON, B., 1998b: Variation in the ITS and IGS regions of ribosomal DNA among the biological species of European *Armillaria*. Mycol. Res. **102,** 533-540.

CRUICKSHANK, M. G.; MORRISON, D. J.; PUNJA, Z. K., 1997: Incidence of *Armillaria* species in precommercial thinning stumps and spread of *Armillaria ostoyae* to adjacent Douglas-fir trees. Can. J. For. Res. **27,** 481-490.

CRUICKSHANK, M., 2000: Volume loss of Douglas-fir infected with *Armillaria ostoyae*. In: Proceedings, From Science to Management and Back: A Science Forum for Southern Interior Ecosystems of British Columbia. Ed. by HOLLSTEDT, C.; SUTHERLAND, K.; INNES, T. Kamloops, B.C.: Southern Interior Forest Extension and Research Partnership, pp. 127-129.

CRUICKSHANK, M. G., 2002: Accuracy and precision of measuring cross-sectional area in stem disks of Douglas-fir infected by Armillaria root disease. Can. J. For. Res. **32,** 1542-1547.

DAVIDSON, A. J.; RISBETH, J., 1988: Effect of suppression and felling on infection of oak and Scots pine by *Armillaria*. Eur. J. Forest Pathol. **18,** 161-168.

DELONG, R. L.; LEWIS, K. J.; SIMARD, S. W.; GIBSON, S., 2002: Fluorescent pseudomonad population sizes baited from soils under pure birch, pure Douglas-fir, and mixed forest stands and their antagonism toward *Armillaria ostoyae in vitro*. Can. J. For. Res. **32,** 2146-2159.

DETTMAN, J. R.; VAN DER KAMP, B. J., 2001a: The population structure of *Armillaria ostoyae* and *Armillaria sinapina* in the central interior of British Columbia. Can. J. Bot. **79,** 600-611.

DETTMAN, J. R.; VAN DER KAMP, B. J., 2001b: The population structure of *Armillaria ostoyae* in the southern interior of British Columbia. Can. J. Bot. **79,** 612-620.

DHARMANANDA, S., 2005: Modernizing Chinese medicine: The case of *Armillaria* as *Gastrodia substitute*. Institute for traditional medicine, Portland, OR. [Online] Available http://www.itmonline.org/arts/gastrodia.htm [Last accessed 10, February 2005]

DUMAS, M. T., 1988: Biological species of *Armillaria* in the mixed wood forest of Northern Ontario. Can. J. For. Res. **18,** 872-874.

ENTRY, J. A.; CROMACK, K. JR.; KELSEY, R. G.; MARTIN, N. E., 1991: Response of Douglas-fir to infection by *Armillaria ostoyae* after thinning or thinning plus fertilization. Phytopathology **81,** 682-689.

FERGUSON, B. A.; DREISBACH, T. A.; PARKS, C. G.; FILIP, G. M.; SCHMITT, C. L., 2003: Coarse-scale population structure of pathogenic *Armillaria* species in a mixed-conifer forest in the Blue Mountains of northeast Oregon. Can. J. For. Res. **33,** 612-623.

FILIP, G. M.; GOHEEN, D. J.; JOHNSON, D. W.; THOMPSON, J. H., 1989: Precommercial thinning in a ponderosa pine stand affected by Armillaria root disease: 20 years of growth and mortality in central Oregon. West J. Appl. For. **4,** 58-59.

FILIP, G. M.; GANIO, L. M.; OESTER, P. T.; MASON, R. R.; WICKMAN, B. E., 2002: Ten-year effect of fertilization on tree growth and mortality associated with Armillaria root disease, fir engravers, dwarf mistletoe, and western spruce budworm in northeastern Oregon. West. J. Appl. For. **17,** 122-128.

FINS, L.; BYLER, J. W.; FERGUSON, D. E.; HARVEY, A. E.; MAHALOVICH, M. F.; MCDONALD, G. I.; MILLER, D. L.; SCHWANDT, J. W.; ZACK, A., 2002: Return of the giants: restoring western white pine to the inland Northwest. J. For. **100**, 20-26.

FOX, R. T. V., 2000: Biology and life cycle. In: *Armillaria* Root Rot: Biology and Control of Honey Fungus. Ed. by FOX, R.T.V. Gosport, Hampshire, UK: Ashford Colour Press Limited. pp. 3-44. Chapter 1.

FRONTZ, T. M.; DAVIS, D. D.; BUNYARD, B. A.; ROYSE, D. J., 1998: Identification of *Armillaria* species isolated from bigtooth aspen based on rDNA RFLP analysis. Can. J. For. Res. **28,** 141-149.

FUKUDA, M.; NAKASHIMA, E.; HAYASHI, K.; NAGASAWA, E., 2003: Identification of the biological species of *Armillaria* associated with *Wynnea* and *Entoloma abortivum* using PCR-RFLP analysis of the intergenic region (IGR) of ribosomal DNA. Mycol. Res. **107,** 1435-1441.

GARRAWAY, M. O.; HUTTERMANN, A.; WARGO, P. M., 1991: Ontogeny and Physiology. In: Armillaria Root Disease. Agriculture Handbook No. 169. Ed. by SHAW, C. G. III.; KILE, G. A., Washington, DC: United States Dept. of Agriculture, Forest Service, pp. 21-47.

GREGORY, S. C., 1989: *Armillaria* species in northern Britain. Plant Pathology **38,** 93-97.

GREIG, B. J. W.; STROUTS, R. G., 1983: Honey fungus. Arboricult. Leafl. For. Commn 2. 16 pp.

GRUELL, G. E.; SCHMIT, W. C.; ARNO, S. F.; REICH, W. J., 1982: Seventy years of vegetative change in a managed ponderosa pine forest in western Montana - implications for resource management. Gen. Tech. Rep. INT-130. Odgen, UT, U.S.A.: U.S. Department of Agriculture, Forest Service, Intermountain Research Station, 42 pp.

GUILLAUMIN, J.-J.; BETHELAY, S., 1981: Détermination spécifique des armillaires par la méthode des groupes de compatibilité sexuelle. Spécialisation écologique des espéces françaises. Agronomie **1,** 897-908.

GUILLAUMIN, J.-J.; ANDERSON, J. B.; KORHONEN, K., 1991: Life Cycle, Interfertility, and Biological Species. In: Armillaria Root Disease. Agriculture Handbook No. 169. Ed. by SHAW, C. G. III.; KILE, G. A. Washington, DC: United States Dept. of Agriculture, Forest Service, pp. 10-20.

HADFIELD, J. S.; GOHEEN, D. J.; FILIP. G. M.; SCHMITT, C. L.; HARVEY, R. D., 1986: Root Diseases in Oregon and Washington Conifers. Portland, OR, U.S.A.: USDA Forest Service Publication R6-FPM-250-86. 27 pp.

HAGLE, S. K.; PERRY, K.; WILES, R., 2004: Jerry Johnson Campground Hazard Tree Evaluation. Missoula, MT, U.S.A.: United States Department of Agriculture, Forest Service, Northern Region. Forest Health protection Report 04-02. 14 pp.

HAGLE, S. K.; GOHEEN, D. J., 1988: Root disease response to stand culture. In: Proceedings of the future forests of the intermountain west: a stand culture symposium. Gen.Tech. Rep. INT-243. Ogden, UT, U.S.A.: U.S. Department of Agriculture, Forest Service, Intermountain Forest and Range Experiment Station. pp. 303-309.

HALMSCHLAGER, E.; KOWALSKI, T., 2004: The mycobiota in nonmycorrhizal roots of healthy and declining oaks. Can. J. Bot. **82,** 1446-1458.

HANNA, J. W.; KIM, M.-S.; KLOPFENSTEIN, N. B.; ZAMBINO, P. J.; MCDONALD, G. I., 2003a: Characterization of North American *Armillaria* species: Phylogenetic relationships from IGS, ITS, and nuclear large subunit ribosomal DNA sequences. Phytopathology **93,** S33.

HANNA, J. W.; KLOPFENSTEIN, N. B.; KIM, M.-S.; MCDONALD, G. I.; MOORE, J. A., 2003b: Intraspecific and intragenomic variation of *Armillaria ostoyae* within the western United States. Phytopathology **93,** S33.

HANNA, J. W.; KLOPFENSTEIN, N. B.; KIM, M.-S.; MCDONALD, G. I.; MOORE, J. A., 2004: Phylogeography of *Armillaria ostoyae* in the western United States. Phytopathology **94,** S38.

HARRINGTON, T. C., 1987: Compatibility of New Hampshire spruce and fir isolates of *Armillaria* with *A. obscura*. Phytopathology **77,** 117.

HARRINGTON, T. C.; WORRALL, J. J.; RIZZO, D. M., 1989: Root and butt rots and other disturbance agents in montane spruce-fir forests of New Hampshire. In: Proc. 7th Int. Conf. Root and Butt Rots, 9-16 August 1988; Vernon and Victoria, BC. Ed. by Morrison, D. J. Victoria, BC: International Union of Forestry Research Organizations, pp. 257-266.

HARRINGTON, T. C.; WINGFIELD, B. D., 1995: A PCR-based identification method for species of *Armillaria*. Mycologia **87,** 280-288.

HARVEY, A. E., 1994: Integrated roles for insects, diseases and decomposers in fire dominated forests of the Inland Western United States: past, present and future forest health. J. Sust. For. **2,** 211-220.

HINCE, E. C.; ROBBINS, E. I., 2003: Probing an Underground Acid-Mine drainage ecosystem. Geotimes, 3pp. [Online] Available http://www.geotimes.org/dec03/feature_AMD.html [Last accessed 10, February 2005]

HINTIKKA, V. 1973: A note on the polarity of *Armillariella mellea*. Karstenia **13,** 32-39.

HOLSINGER, J. R., 1981: *Stygobromus canadensis*, a troglobitic amphipod crusteacean from Castleguard Cave, with remarks on the concept of cave glacial refugia. 8th Proc. Internat. Speleological Congress, Bowling Green, KY, U.S.A. pp. 93-95.

HOOD, I. A.; MORRISON, D. J., 1984: Incompatibility testing of *Armillaria* isolates in a wood substrate. Can. For. Serv. Res. Notes **4,** 8-9.

HOOD, I. A.; REDFERN, D. B.; KILE, G. A., 1991: *Armillaria* in planted hosts. In: Armillaria Root Disease. Agriculture Handbook No. 169. Ed. by SHAW, C. G. III.; KILE, G. A. Washington, DC: United States Dept. of Agriculture, Forest Service, pp. 122-149.

HUGHES, M. B., 2000: North American Species of *Armillaria*: a Molecular Phylogenetic Study and an Analysis of Interactions with *Megacollybia platyphylla*. MS Thesis, State University of New York College of Environmental Science and Forestry, Syracuse, NY, U.S.A. 167 pp.

INTINI, M. G., 1989: Observations on the occurrence of *Armillaria ostoyae* on *Abies alba* (silver fir) in Italy. Proceedings of the 7th International Conference on Root and Butt Rots, Vernon and Victoria 1988. pp. 252-256.

JACQUES-FELIX, M., 1977: Le complexe de l'Armillaire. Trav. Ded. G. Viennot-Bourgin 1977. pp. 143-157.

JOHNSON, A. L. S.; WALLIS, G. W.; FOSTER, R. E., 1972: Impact of root rot and other diseases in young Douglas-fir plantations. Forestry Chronicle **48,** 316-319.

JOHNSON, D. W.; THOMPSON, J. H., 1975: Effect of precommercial thinning on ponderosa pine, *Pinus ponderosa*, infected with *Armillaria mellea*. Plant Dis. Rep. **59,** 308-309.

KALLAS, M. A., 1997: Armillaria Root Disease in the Black Hills of South Dakota. MS Thesis, Colorado State Univ., Fort Collins, CO, U.S.A. 172 pp.

KALLAS, M. A.; REICH, R. M.; JACOBI, W. R.; LUNDQUIST, J. E., 2003: Modeling the probability of observing Armillaria root disease in the Black Hills. For. Path. **33,** 241-252.

KECA, N.; BODLES, W. J. A.; WOODWARD, S.; KARADZIC, D., 2004: Identification of *Armillaria* spp. in Serbia. 11th International Conference on Root and Butt Rots, IUFRO working party 7.02.01. 16-22 August 2004. Poznań, Poland.

KILE, G. A., 1983: Identification of genotypes and the clonal development of *Armillaria luteobubalina* Watling & Kile in eucalypt forests. Aust. J. Bot. **31,** 657-671.

KILE, G. A.; WATLING, R., 1988: Identification and occurrence of Australian *Armillaria* species, including *A. pallidula sp. nov*. and comparative studies between them and non-Australian tropical and Indian *Armillaria*. Transactions of the Britsh Mycological Society. **91,** 305-315.

KILE, G. A.; MCDONALD, G. I.; BYLER, J. W., 1991: Ecology and disease in natural forests. In: Armillaria Root Disease. Agriculture Handbook No. 169. Ed. by SHAW, C. G. III; KILE, G. A. Washington, DC: United States Dept. of Agriculture, Forest Service, pp. 62-75.

KILE, G. A.; GUILLAUMIN, J.-J.; MOHAMMED, C.; WATLING, R.: 1994: Biogeography and pathology of *Armillaria*. In: Proceedings of the Eight International Conference on Root and Butt Rots, Wik, Sweden and Haikko, Finland, August 9-16, 1993. Ed. by JOHANSSON, M.; STENLID, J. Uppsala, Sweden: Swedish University of Agricultural Sciences. pp. 411-436.

KIM, H.-Y.; SONG, H.-G., 2000: Transformation of 2,4,6-trinitroluene by white rot fungus *Irpex lacteus*. Biotechnology Letters **22,** 969-975.

KIM, M.-S., 1999: Molecular genetic studies of *Armillaria* species: Application of molecular genetic tools toward understanding ecological relationships in forest ecosystems. PhD Dissertation, University of Nebraska, Lincoln, NE, U.S.A. 155 pp.

KIM, M.-S.; KLOPFENSTEIN, N. B.; MCDONALD, G. I.; ARUMUGANATHAN, K.; VIDAVER, A. K., 2000: Characterization of North American *Armillaria* species by nuclear DNA content and RFLP analysis. Mycologia **92,** 874-883.

KOENIGS, J. W., 1969: Root rot and chlorosis of released thinned western red-cedar. J. Forestry **67,** 312-315.

KORHONEN, K., 1978: Interfertility and clonal size in the *Armillariella mellea* complex. Karstenia **18,** 31-42.

KORHONEN, K., 1980: The origin of clamped and clampless basidia in *Armillariella ostoyae*. Karstenia **20,** 23-27.

KROMROY, K. W., 1999: Studies on the identification and ecology of *Armillaria* species in Minnesota and Wisconsin. PhD Dissertation, Univeristy of Minnesota, St. Paul, MN, U.S.A. 186 pp.

KROMROY, K. W., 2004: Identification of *Armillaria* Species in the Chequamegon-Nicolet National Forest. Research Note NC-388. St. Paul, MN, U.S.A.: United States Department of Agriculture, Forest Service, North Central Research Station. 10 pp.

KWAŚNA, H., 2002: Changes in microfungal communities in roots of *Quercus robur* stumps and their possible effect on colonization by *Armillaria*. J. Phytopathology **150,** 403-411.

KWAŚNA, H.; KOTYŃSKA, U.; ŁAKOMY, P.; MALLETT, K., 2001: Stimulation of *Armillaria* rhizomorph growth by oak root fungi. Acta Mycologia **36,** 257-272.

KWAŚNA, H.; ŁAKOMY, P.; MALLETT, K., 2004: Reaction of *Armillaria ostoyae* to forest soil microfungi. For. Path. **34,** 147-162.

ŁAKOMY, P., 1996: Zdolność produkcji ryzomorf przez różne genotypy *Armillaria ostoyae* (Romagn.) Herink (Rhizomorph production by *Armillaria ostoyae* different genotypes) In: choroby roslin a środowisko. Ed. by MAŃKA, M., Poznań: Polskie Towarzystwo Fitopatologiczne, pp. 145-153.

ŁAKOMY, P., 1998: Monitoring huby korzeni I opieńkowej zgnilizny korzeni w wybranych uprawach sosnowych Krainy Wielkopolsko-Pomorskiej. (Monitoring of *Heterobasidion annosum* and Armillaria root and butt rot in a few Scots pine plantations in central and northern Poland). Roczn. A.R. w Poznaniu, Rozp. Nauk. **283,** 1-82.

LANGRELL, S. R. H.; LUNG-ESCARMENT, B.; DECROOCQ, S., 2001: Isolation and characterization of polymorphic simple sequence repeat loci in *Armillaria ostoyae*. Mol. Ecol. Notes **1,** 305-307.

LANGSTON, N., 1995: Forest Dreams, Forest Nightmares: The Paradox of Old-Growth in the Inland West. Seattle, WA, U.S.A.: University of Washington Press, 368 pp.

LARSON, J.; BANIK, M. T.; BURDSALL, H. H. JR., 1992: Clamp Connections in North American *Armillaria* species: occurrence and potential application for delimiting species. Mycologia **84,** 214-218.

LIVINGSTON, W. H., 1990: *Armillaria ostoyae* in young spruce plantations. Can J. For. Res. **20,** 1773-1778.

LOCHMAN, J.; SERÝ, O.; JANKOVSKÝ, L.; MIKES, V., 2004: Variations in rDNA ITS of Czech *Armillaria* species determined by PCR and HPLC. Mycol. Res. **108,** 1153-1161.

LUNG-ESCARMANT, B.; GUYON, G., 2004: Temporal and spatial dynamics of primary and secondary infection by *Armillaria ostoyae* in a *Pinus pinaster* plantation. Phytopath. **94,** 125-131.

LUNG-ESCARMANT, B.; MOHAMMED, C.; DUNEZ, J., 1985: Nouvelles méthodes de détermination des Armillaires européens: immunology et électrophorése en gel de polycrylamide. European J. For. Path. **15,** 278-288.

LUSHAJ, B. M.; INTINI, M.; GUPE, E., 2003: Investigations on the distribution and ecology of *Armillaria* species in Albania. In: Root and Butt Rots of Forest Trees, 10th International Conference on Root and Butt Rots: Proc. of the IUFRO WP 7.02.01 Meeting. Québec City, Canada, September 16-22, 2001. Ed. by LAFLAMME, G.; Bérubé, J. A.; BUSSIÉRES, G. Natural Resources Canada, Canadian Forest Service, Laurential Forestry Centre, Sainte-Foy, Québec. Information Report LAU-X-126. pp. 93-104.

MALLETT, K. I.; HIRATSUKA, Y., 1987: Inoculation studies of lodgepole pine with Alberta isolates of the *Armillaria mellea* complex. Can. J. For. Res. **18,** 292-296.

MALLETT, K. I., 1990: Host range and geographic distribution of Armillaria root rot pathogens in the Canadian prairie provinces. Can. J. For. Res. **20,** 1859-1863.

MALLETT, K. I., 1992: Armillaria root rot in the Canadian prairie provinces. For. Con., Northwest Reg., North. For. Cent., Edmonton, Alberta. Info. Rep. NOR-X-329. 22 pp.

MALLETT, K. I.; MAYNARD, D. G., 1998: Armillaria root disease, stand characteristics, and soil properties in young lodgepole pine. Forest Ecol. Manag. **105,** 37-44.

MALLETT, K. I.; VOLNEY, W. J. A., 1999: The effect of Armillaria root disease on lodgepole pine tree growth. Can. J. For. Res. **29,** 252-259.

MARXMÜLLER, H., 1982: Etude morphologique des *Armillaria ss*. str. à anneau. Bull. Trimmest. Soc. Mycol. Fr. **98,** 87-124.

MARXMÜLLER, H.; PRINTZ, P., 1982: Honningsvampe. Svampe **5,** 1-10, 59-60.

MCDONALD, G. I., 1991a: Connecting forest productivity to behavior of soil-borne disease. In: Proceedings-Management and productivity of western-montane forest soils. General Technical Report INT-280, U.S. Comp. by HARVEY, A. E.; NEUENSHWANDER, L. F. Ogden, UT, U.S.A.: Department of Agriculture, Forest Service, Intermountain Research Station. pp. 129-144.

MCDONALD, G. I., 1991b: Relationships among site quality, stand structure, and Armillaria root rot in Douglas-fir forests. In: Symposium proceedings, interior Douglas-fir: the species and its management. 1990 February 27- March 1, Spokane, WA. Ed. by BAUMGARTNER, D. M.; LOTAN, J. E. Pullman, WA, U.S.A.: Cooperative Extension Service, Washington State University,. pp. 141-145

MCDONALD, G. I., 1998: Preliminary report on the ecology of *Armillaria* in Utah and the Inland West. In: Proceedings of the 46th Western International Forest Disease Work Conference. Reno, Nevada, Sept 28- Oct 2, 1998. Anchorage, AK. U.S.A.: USDA Forest Service R-10. pp. 85-92.

MCDONALD, G. I., 2000: Fungi: Indicators of ecosystems and ecosystem status. In: Proceedings of the Fifth Joint Meeting of the Western International Forest Disease Work Conference and Western Forest Insect Work Conference. Breckenridge, Colorado, Sept 13- 17, 1999. Comp. by GOHEEN, E. M. Central Point, OR, U.S.A.: Southwest Oregon Forest Insect and Disease Service Center. pp.18-23.

MCDONALD, G. I.; MARTIN, N. E.; HARVEY, A. E., 1987a: Occurrence of *Armillaria* spp. in forests of the northern Rocky Mountains. Research Paper INT-381. Ogden, UT, U.S.A.: U.S. Department of Agriculture, Forest Service, Intermountain Research Station,. 5 pp.

MCDONALD, G. I.; MARTIN, N. E.; HARVEY, A. E., 1987b: *Armillaria* in the northern Rocky Mountains: pathogenicity and host susceptibility on pristine and disturbed sites. Research Note INT-371. Ogden, UT, U.S.A.: U.S. Department of Agriculture, Forest Service, Intermountain Research Station. 5 pp.

MCDONALD, G. I.; KLOPFENSTEIN, N. B.; KIM, M.-S., 1998: Ecology of *Armillaria* in a 16-year-old northern Idaho white pine plantation. Phytopath. **88,** S60.

MCDONALD, G. I.; HARVEY, A. E.; TONN, J. R., 2000: Fire, competition and forest pests: landscape treatment to sustain ecosystem function. In: Proceedings from the Joint Fire Science conference and workshop: Crossing the millennium: integrating spatial technologies and ecological principles for a new age in fire management. Volume 2. Tech. Ed. by NEUENSCHWANDER, L. F.; RYAN, K. C. Moscow, ID, U.S.A.: University of Idaho and the International Association of Wildland Fire. pp. 195-211.

MCDONALD, G. I.; EVANS, J. S.; MOEUR, M.; RICE, T. M.; STRAND, E. K., 2003: Using digital terrain modeling and satellite imagery to map interactions among fire and forest microbes. In: Proceedings of Fire Conference 2000: The First National Congress on Fire Ecology, Prevention, and Management. Miscellaneous Publication No. 13. Ed. by GALLEY, K. E. M.; KLINGER, R. C.; SUGIHARA, N. G. Tallahassee, FL, U.S.A.: Tall Timbers Research Station. pp. 100-110.

MCLAUGHLIN, J. A., 2001: Distribution, hosts, and site relationships of *Armillaria* spp. in central and southern Ontario. Can. J. For. Res. **31,** 1481-1490.

MICHELI, P. A., 1729: *Nova Plantarum genera*. Florentiae.

MOHAMMED, C.; GUILLAUMIN, J.-J.; BERTHELAY, S., 1994: *Armillaria* species in China and Japan. Mycol. Res. **98,** 607-613.

MOORE, J. A.; MIKA, P. G.; SHAW, T. M., 2000: Root Chemistry of Mature Douglas-Fir Differs by Habitat Type in the Interior Northwestern United States. For. Sci. **46,** 531-536.

MORRISON, D. J., 1981: Armillaria root disease. A guide to disease diagnosis, development and management in British Columbia. Information Report. BC-X-203. Environment Canada, Canadian Forest Service.16 pp.

MORRISON, D. J., 2004: Rhizomorph growth habit, saprophytic ability and virulence of 15 *Armillaria* species. For. Path. **34,** 15-26.

MORRISON, D. J.; CHU, D.; JOHNSON, A. L. S., 1985a: Species of *Armillaria* in British Columbia. Can. J. Plant Path. **7,** 242-246.

MORRISON, D. J.; THOMSON, A. J.; CHU, D.; PEET, F.; SAHOTA, T. S., 1985b: Isozyme patterns of *Armillaria* intersterility groups occurring in British Columbia. Canadian J. Microbiol. **31,** 651-653.

MORRISON, D. J.; MALLET, K., 1996: Silvicultural management of Armillaria root disease in western Canadian forests. Can. J. Plant Path. **18**, 194-199.

MORRISON, D. J.; MERLER, H.; NORRIS, D., 1991: Detection, recognition and management of *Armillaria* and *Phellinus* root diseases in the southern interior of British Columbia. Canada – British Columbia Partnership Agreement on Forest Development: FRDA Rep. 179. 25 pp.

MORRISON, D. J.; PELLOW, K. W.; NORRIS, D. J.; NEMEC, A. F. L., 2000: Visible versus actual incidence of Armillaria root disease in juvenile coniferous stands in the southern interior of British Columbia. Can. J. For. Res. **30,** 405-414.

MORRISON, D. J.; PELLOW, K. W.; NEMEC, A. F. L.; NORRIS, D. J.; SEMENOFF, P., 2001: Effects of selective cutting on the epidemiology of Armillaria rot disease in the southern interior of British Columbia. Can. J. For. Res. **31,** 59-70.

MORRISON, D. J.; PELLOW, K. W., 2002: Variation in virulence among isolates of *Armillaria ostoyae*. For. Path. **32,** 99-107.

MUGALA, M. S.; BLENIS, P. V.; HIRATSUKA, Y.; MALLETT, K. I., 1989: Infection of lodgepole pine and white spruce by Alberta isolates of *Armillaria*. Can. J. For. Res. **19**, 685-689.

MUNDA, A., 1997: Research on honey fungus (*Armillaria* [Fr. Fr.] Staude) in Slovenia *(in Slovenian)*. In: Proceedings on the Occasion of 50 Years of the Slovenian Forestry Institute, Ljubljiana, Slovenia. pp. 211-220.

MYSZEWSKI, J. H.; FINS, L.; MOORE, J. A.; RUST, M.; MIKA, P. G., 2002: Variation in the root bark phenolics/sugar ratio of Douglas-fir grown in two plantations in northern Idaho. Can. J. For. Res. **32,** 556-560.

NAGASAWA, E., 1991: Taxonomic reassessment of *Armillaria mellea* in Japan. [Report for a Grant-in Aid for Scientific Research, No. 63560155.] Ministry of Education, Science and Culture of Japan, Tokyo. [In Japanese]

OLIVER, C. D.; IRWIN, L. L.; KNAPP, W. H., 1994: Eastside forest management practices: historical overview, extent of their applications, and their effects on sustainability of ecosystems. General technical report PNW-324. Portland, OR, U.S.A.: U.S. Forest Service, Pacific Northwest Research Station.73 pp**.**

OMDAL, D. W.; SHAW, C. G. III.; JACOBI, W. R.; WAGER, T. C., 1995: Variation in pathogenicity and virulence of isolates of *Armillaria ostoyae* on eight tree species. Plant Dis. **79,** 939-944.

OMDAL, D. W.; SHAW, C. G. III.; JACOBI, W. R., 2004: Symptom expression in conifers infected with *Armillaria ostoyae* and *Heterobasidion annosum*. Can. J. For. Res. **34,** 1210-1219.

OTA, Y.; MATSUSHITA, N.; NAGASAWA, E.; TERASHITA, T.; FUKUDA, K.; SUZUKI, K., 1998: Biological Species of *Armillaria* in Japan. Plant Dis. **82,** 537-543.

PANKUCH, J. M.; BLENIS, P. V.; LIEFFERS, V. J.; MALLETT, K. I., 2003: Fungal colonization of aspen roots following mechanical site preparation. Can. J. For. Res. **33,** 2372-2379.

PAREEK, M.; COLE, L.; ASHFORD, A. E., 2001: Variations in structure of aerial and submerged rhizomorphs of *Armillaria luteobubalina* indicate that they may be organs of absorption. Mycol. Res. **105,** 1377-1387.

PEET, F. G.; MORRISON, D. J.; PELLOW, K. W., 1996: Rate of spread of *Armillaria ostoyae* in two Douglas-fir plantations in the southern interior of British Columbia. Can. J. For. Res. **26,** 148-151.

PÈREZ SIERRA, A.; WHITEHEAD, D. S.; WHITEHEAD, M. P., 1999: Investigation of a PCR-based method for the routine identification of British *Armillaria* species. Mycol. Res. **103,** 1631-1636.

PÈREZ SIERRA, A.; WHITEHEAD, D. S.; WHITEHEAD, M. P., 2000: Molecular methods used for the detection and identification of *Armillaria*. In: *Armillaria* Root Rot: Biology and Control of Honey Fungus. Ed. by FOX, R. T. V. Gosport, Hampshire, UK: Ashford Colour Press Limited. pp. 95-110. Chapter 5.

PÈREZ-SIERRA, A.; HENRICOT, B., 2002: Identification of fungal species beyond morphology. Mycologist **16,** 42-46.

PEKŞEN, A.; KARACA, G., 2003: Macrofungi of Samsun province. Turk. J. Bot. **27,** 173-184.

PIELOU, E. C., 1991: After the Ice Age: the return of Life to Glaciated North America. The University of Chicago Press, Chicago, IL, U.S.A. 366 pp.

PIELOU, E. C.; FOSTER, R. E., 1962: A test to compare the incidence of disease in isolated and crowded trees. Can. J. Bot. **40,** 1176-1179.

PIERCEY-NORMORE, M. D.; EGGER, K. N.; BÉRUBÉ, J. A., 1998: Molecular phylogeny and evolutionary divergence of North American biological species of *Armillaria*. Mol. Phylogenet. Evol. **10,** 49-66.

PIERCEY-NORMORE, M. D.; BÉRUBÉ, J. A., 2000: Artificial inoculation with *Armillaria ostoyae* in established conifers stressed by defoliation, planting, and thinning in Newfoundland. Can. J. For. Res. **30,** 1758-1765.

PROFFER, T. J.; JONES, A. L.; EHRET, G. R., 1987: Biological species of *Armillaria* isolated from sour cherry orchards in Michigan. Phytopath. **77,** 941-943.

PROSPERO, S.; HOLDENRIEDER, O.; RIGLING, D., 2003a: Primary resource capture in two sympatric *Armillaria* species in managed Norway spruce forests. Mycol. Res. **107,** 329-338.

PROSPERO, S.; RIGLING, D.; HOLDENRIEDER, O., 2003b: Population structure of *Armillaria* species in managed Norway spruce stands in the Alps. New Phytol. **158,** 365-373.

PROSPERO, S.; HOLDENRIEDER, O.; RIGLING, D., 2004: Comparison of the virulence of *Armillaria cepistipes* and *Armillaria ostoyae* on four Norway spruce provenances. For. Path. **34,** 1-14.

RAMA, R.; MOUGIN, C.; MALOSSE, C.; CHAPLAIN, V.; SIGOILLOT, J.-C.; ASTHER, M., 2000: Biotransformation of PAHs by the white Rot Fungus *Trametes versicolor*. In: Bioremediation of contaminated soils (Environmental Science and pollution control series22). Eds. by WISE, D. L.; TRANTOLO, D. J.; CICHON, E. J.; INYANG, H. I.; STOTTMEISTER, U. New York: Marcel Dekker Inc. pp. 365-372.

RAY, J., 1704: *Historia Plantarum*, vol. 3. London.

REAVES, J. L.; SHAW, C. G. III.; MAYFIELD, J. E., 1990: The effects of *Trichoderma* spp. isolated from burned and non-burned forest soils on the growth and development of *Armillaria ostoyae* in culture. Northwest Sci. **64,** 39-44.

REAVES, J. L.; MCWILLIAMS, M., 1991: *In vitro* fruiting of *Armillaria* species. Res. Note PNW-RN-505. Portland, OR, U.S.A.: U.S. Department of Agriculture, Pacific Northwest Research Station. 7 pp.

REDFERN, D. B., 1975: The influence of food base on rhizomorph growth and pathogenicity of *Armillaria mellea* isolates. In: Biology and Control of Soil-borne Plant Pathogens. Ed. by BRUEHL, G. W. St. Paul, MN, U.S.A.: The American Phytopathological Society. pp. 69-73.

REDFERN, D. B.; FILIP, G. M., 1991: Inoculum and infection. In: Armillaria Root Disease. Agriculture Handbook No. 169. Ed. by SHAW, C. G. III.; KILE, G. A. Washington, DC: United States Dept. of Agriculture, Forest Service, pp. 48-60.

RHO, D.; HODGSON, J.; THIBOUTOT, S.; AMPLEMAN, G.; HAWARI, J., 2001: Transformation of 2,4,6-trinitrotoluene (TNT) by immobilized *Phanerochaete chrysosporium* under fed-batch and continuous TNT feeding conditions. Biotechnol. Bioeng. **73,** 271-281.

RIGLING, D.; LAWRENZ, P.; BLAUENSTEIN, H.; HEINIGER, U., 2003: An experimental study of the effects of ozone on tree-*Armillaria* interactions. In: Root and Butt Rots of Forest Trees, 10th International Conference on Root and Butt Rots: Proc. of the IUFRO WP 7.02.01 Meeting. Québec City, Canada, September 16-22, 2001. Ed. by LAFLAMME, G., BÉRUBÉ, J. A., and BUSSIÉRES, G. Natural Resources Canada, Canadian Forest Service, Laurential Forestry Centre, Sainte-Foy, Québec. Information Report LAU-X-126. pp. 24-26.

RISBETH, J., 1982: Species of *Armillaria* in southern England. Pl. Path. **31,** 9-17.

RISBETH, J., 1983: The Importance of honey fungeus (*Armillaria*) in urban forestry. Arboricultural J. **7,** 217-225.

RIZZO, D. M.; HARRINGTON, T. C., 1988: Root and butt rot fungi on red spruce and balsam fir in the White Mountains, New Hampshire. Plant Dis. **72,** 329-331.

RIZZO, D. M.; HARRINGTON, T. C., 1992: Nuclear migration in diploid-haploid pairings of *Armillaria ostoyae*. Mycologia **84,** 863-869.

RIZZO, D. M.; HARRINGTON, T. C., 1993: Delineation and biology of clones of *Armillaria ostoyae*, *A. gemina*, and *A. calvescens*. Mycologia **85,** 164-174.

RIZZO, D. M.; BLANCHETTE, R. A.; MAY, G., 1995: Distribution of *Armillaria ostoyae* genets in a *Pinus resinosa – Pinus banksiana* forest. Can. J. Bot. **73,** 776-787.

ROBINSON, R. M.; MORRISON, D. J., 2001: Lesion formation and host response to infection by *Armillaria ostoyae* in the roots of western larch and Douglas-fir. For. Path. **31,** 371-385.

ROBINSON, R. M.; MORRISON, D. J.; JENSEN, G. D., 2004: Necrophylactic periderm formation in the roots of western larch and Douglas-fir trees infected with *Armillaria ostoyae*. II. The response to the pathogen. For. Path. **34**, 119-129.

ROLL-HANSEN, F., 1985: The *Armillaria* species in Europe. A literature review. Eur. J. For. Path. **15,** 22-31.

ROMAGNESI, H., 1970: Observations sur les *Armillariella* (I). Bull. trimest. Soc. Mycol. Fr. **86,** 257-256.

ROMAGNESI, H., 1973: Observations sur les *Armillariella* (II). Bull. trimest. Soc. Mycol. Fr. **89,** 195-206.

ROTH, L. F.; SHAW, C.G. III.; ROLPH, L., 2000: Inoculum reduction measures to control Armillaria root disease in a severely infested stand of ponderosa pine in south-central Washington: 20 year results. West. J. Appl. For. **15,** 92-100.

SACKVILLE HAMILTON, N. R., 2001: Is local provenance important in habitat creation? A reply. J. Appl. Ecol. **38,** 1374-1376.

SCHAECHTER, E., 1997: In the company of mushrooms: A biologist's tale Cambridge, MA, U.S.A.: Harvard University Press. 280 pp.

SCHLOSSER, D.; GREY, R.; HÖFER, C.; FAHR, K., 2000: Degradation of chlorophenols by Basidiomycetes. In: Bioremediation of Contaminated Soils (Environmental Science and Pollution Control Series 22) Eds. by Wise, D. L.; Trantolo, D. J.; Cichon, E. J.; Inyang, H. I.; Stottmeister, U. New York: Marcel Dekker Inc. pp. 393-408.

SCHMITT, C. L., 2001: Root diseases in conifer forested communities in the Blue Mountains of northeastern Oregon and southeastern Washington – Detection and

management, values and impacts. USDA Forest Service Wallowa-Whitman Nat. For. Rep. BMPMSC-02-01. 44 pp.

SCHULZE, S.; BAHNWEG, G.; MÖLLER, E. M.; SANDERMANN, H. JR., 1997: Identification of the genus *Armillaria* by specific amplification of an rDNA-ITS fragment and evaluation of genetic variation within *A. ostoyae* by rDNA-RFLP and RAPD analysis. Eur. J. For. Path. **27,** 225-239.

SELOCHNIK, N.; PRZBYŁ, K.; UFNALSKI, K., 2004: Distribution and species diversity of the *Armillaria* complex in Russia. 11th International Conference on Root and Butt Rots, IUFRO working party 7.02.01. 16-22 August 2004. Poznań, Poland.

SHAW, C. G. III., 1975: Epidemiological insights into *Armillaria mellea* root rot in a managed ponderosa pine forest. Ph.D. dissertation. Oregon State University, Corvallis, OR, U.S.A. 201 pp.

SHAW, C. G. III.; ROTH, L. F., 1976: Persistence and distribution of a clone of *Armillaria mellea* in a ponderosa pine forest. Phytopathology, **66,** 1210-1213.

SHAW, C. G. III.; LOOPSTRA, E. M., 1988: Identification and pathogenicity of some Alaskan isolates of *Armillaria*. Phytopath. **78,** 971-974.

SHAW, C. G. III., 1989: *Armillaria ostoyae* associated with mortality of new hosts in Chihuahua, Mexico. Plant Dis. **73,** 775.

SHIGO, A. L., 2000: Armillaria root rots, predisposition and poor Sorauer. [Online] Available http://www.chesco.com/~treeman/SHIGO/ARM.html [Last accessed 10, February 2005]

SHIELDS, W. J.; HOBBS, S. D., 1979: Soil nutrient levels and pH associated with *Armillariella mellea* on conifers in northern Idaho. Can. J. For. Res. **9,** 45-48.

SIEPMANN, R., 1987: Kriterien zur beurteilung der reaction haploider tester mit diploiden *Armillaria*-isolierungen. Eur. J. Forest. Pathol. **17**, 308-311.

SICOLI, G.; ANNESE, V.; DE GIOIA, T.; LUISI, N., 2002: *Armillaria* pathogenicity tests on oaks in southern Italy. J. Plant Path. **84,** 107-111.

SICOLI, G.; FATEHI, J.; STENLID, J., 2003: Development of species-specific PCR primers on rDNA for the identification of European *Armillaria* species. For. Path. **33,** 287-297.

SINGH, P.; RICHARDSON, J., 1973: Armillaria root rot in seeded and planted areas in Newfoundland. For. Chron. **49,** 180-182.

SINGH, P., 1983: Armillaria root rot: Influence of soil nutrients and pH on the susceptibility of conifer species to the disease. Eur. J. For. Path. **13,** 92-101.

SMITH, M. L.; DUCHESNE, L. C.; BRUHN, J. N.; ANDERSON, J. B., 1990: Mitochondrial genetics in a natural population of the plant pathogen. Genetics **126,** 575-582.

SMITH, M. L.; BRUHN, J. N.; ANDERSON, J. B., 1994: Relatedness and spatial distribution of *Armillaria* genets in infected red pine seedlings. Phytopath. **84,** 822-829.

SMITH, R. B.; SIEGEL, L., 2000: Windows into the Earth: The Geologic Story of Yellowstone and Grand Teton National Parks. New York: Oxford University Press. 242 pp.

SOLLA, A.; TOMLINSON, F.; WOODWARD, S., 2002: Penetration of *Picea sitchensis* root bark by *Armillaria mellea*, *Armillaria ostoyae* and *Heterobasidion annosum*. For. Path. **32,** 55-77.

STANOSZ, G. R.; PATTON, R. F., 1987: Armillaria root rot in Wisconsin aspen sucker stands. Can. J. Forest Res. **17,** 995-1000.

STUART, J. D., 1998: Effects of fire suppression on ecosystems and diversity. In: Status and Trends of the Nation's Biological Resources. Ed. by MAC, M. J.; OPLER, P. A.; HAECKER, C. E. P.; DORAN, P. D. Reston, VA, U.S.A.: U.S. Department of the Interior, U.S. Geological Survey. 986 pp.

STURROCK, R. N., 2000: Management of root disease by stumping and push-falling. Natural Resources Canada, Canadian Forest Service, Pacific Forestry Centre, Victoria, B.C. Technology Transfer Note 16. 8 pp.

SUNG, J.-M.; PARK, Y.-J.; KIM, H.-J.; HARRINGTON, T. C., 1991: Biology and taxonomic concepts of *Armillaria* in pine forests in Korea. Kor. J. Plant Path. **7**, 6-16.

SZYNKIEWICZ, A.; KWAŚNA, H., 2004: The susceptibility of forest trees to Armillaria root rot. Sylwan **8,** 25-33. (abstract)

TARRY, J. C.; SHAW, C. G., 1966: association of *Armillaria mellea* with the dieback of *Ceanothus* in the Pacific Northwest. Plant Dis. Rep. **50,** 399-400.

TERASHIMA, K.; CHA, J. Y.; YAJIMA, T.; IGARASHI, T.; MIURA, K., 1998: Phylogenetic analysis of Japanese *Armillaria* based on the intergenic spacer (IGS) sequences of their ribosomal DNA. Eur. J. For. Path. **28,** 11-19.

TOSA, T.; MATSUSHITA, N.; INAGAWA, H.; SUZUKI, K., 2004: The virulence of *Armillaria mellea* and *A. ostoyae* in Japan. 11th International Conference on Root and Butt Rots, IUFRO working party 7.02.01. 16-22 August 2004. Poznań, Poland.

TSOPELAS, P., 1999: Distribution and ecology of *Armillaria* species in Greece. Eur. J. For. Path. **29,** 103-116.

ULLRICH, R. C.; ANDERSON, J. B., 1978: Sex and diploidy in *Armillaria mellea*. Exp. Mycol. **2,** 119-129.

VAN DER KAMP, B. J., 1993: Rate of spread of *Armillaria ostoyae* in the central interior of British Columbia. Can. J. For. Res. **23,** 1239-1241.

VAN DER KAMP, B. J., 1995: The spatial distribution of Armillaria root disease in an uneven-aged, spatially clumped Douglas-fir stand. Can. J. For. Res. **25,** 1008-1016.

VAN DER STAR, T.; BÉRBEÉ, M. L.; INDERBITZIN, P.; FISCHER, A. L., 2003: Direct GenBank Submission AY228342: Sequencing performed as class project Biology, 323, Submitted (03-FEB-2003). Archived at UBC herbarium F14310 Botany, University of British Columbia, Vancouver, BC.

VOLK, T. J.; BURDSALL, H. H. Jr., 1995: Nomenclatural study of *Armillaria* and *Armillariella* species (Basidiomycotina, Tricholomataceae) Fungiflora, Oslo, Norway: SYNOPSIS FUNGORUM **8**, 121 pp. ISBN 82-90724-14-4

VOLK, T. J.; BURDSALL, H. H. Jr., Banik, M. T., 1996: *Armillaria nabsnona*, a new species from Western North America. Mycologia **88,** 484-491.

WARGO, P. M., 1989: Elevation and *Armillaria* species relationships in spruce-fir forests of northeastern United States. In: Proc. 7th Int. Conf. Root and Butt Rots, 9-16 August 1988; Vernon and Victoria, B.C. Ed. by Morrison, D. J. Victoria, BC: International Union of Forestry Research Organizations, pp. 340-346.

WARGO, P. M.; SHAW, C. G. III., 1985: Armillaria root rot: the puzzle is being solved. Plant Dis. **69**, 826-832.

WARGO, P. M.; CAREY, A. C., 2001: Effects of metals and pH on *in vitro* growth of *Armillaria ostoyae* and other root and butt rot fungi of red spruce. For. Path. **31,** 5-24.

WATLING, R.; GREGORY, N. M., 1980: Larger fungi from Kashmir. Nova Hedwiga **32,** 493-564.

WATLING, R.; KILE, G. A.; GREGORY, N. M., 1982: The genus *Armillaria*-Nomenclature, typification, The Identity of *Armillaria mellea* and species differentiation. Trans. Br. Mycol. Soc. **78,** 271-285.

WATLING, R., KILE, G. A.; BURDSALL, H. H. JR., 1991: Nomenclature, Taxonomy, and Identification. Agriculture Handbook No. 169. Ed. by SHAW, C. G. III.; KILE, G. A. Washington, DC: United States Dept. of Agriculture, Forest Service. pp. 1-9.

WEIN, R. W., 1999: ENCS/BOT 204: Lecture 2 – Glaciation and vegetation dynamics [Online]. Available http://www.rr.ualberta.ca/courses/encs204/contents/ch2.htm [Last accessed 10, February 2005]

WHITE, E. E.; DUBETZ, C. P.; CRUICKSHANK, M. G.; MORRISON, D. J., 1998: DNA diagnostic for *Armillaria* species in British Columbia: within and between species variation in the IGS-1 and IGS-2 regions. Mycologia **90,** 125-131.

WHITE, E. E.; MORRISON, D. J., 1999: DNA Testing: An application to Armillaria root disease. Technology Transfer Note, Forestry Research applications, Pacific Forestry Centre, N. 18, 3 pp.

WHITNEY, R. D., 1988: Armillaria root rot damage in softwood plantations in Ontario. For. Cron. **64,** 345-351.

WHITNEY, R. D., 1997: Relationship between decayed rots and aboveground decay in three conifers in Ontario. Can. J. For. Res. **27,** 1217-1221.

WIENSCZYK, A. M.; DUMAS, M. T.; IRWIN, R. N., 1997: Predicting *Armillaria ostoyae* infection levels in black spruce plantations as a function of environmental factors. Can. J. For. Res. **27,** 1630-1634.

WILKINSON, D. M., 2001: Is local provenance important in habitat creation? J. Appl. Ecol. **38,** 1371-1373.

WILLIAMS, R. E.; MARSDEN, M. A., 1982: Modeling probability of root disease center occurrence in northern Idaho forests. Can. J. For. Res. **12,** 876-882.

WORRALL, J. J., 1994: Population structure of *Armillaria* species in several forest types. Mycologia **86,** 401-407.

WORRALL, J. J.; SULLIVAN, K. F.; HARRINGTON, T. C.; STEIMEL, J. P., 2004: Incidence, host relations and population structure of *Armillaria ostoyae* in Colorado campgrounds. Forest Ecol. Manag. **192,** 191-206.

WU, Y.; JOHNSON, D. W.; ANGWIN, P. A., 1996: Identification of *Armillaria* species in the Rocky Mountain Region. Renewable Resources, Rocky Mountain Region, USDA Forest Service Tech. Rep. R2-58. 26 pp.

ŻÓŁCIAK, A., 2004: Identification of *Armillaria* species in Norway spruce stands in Poland. 11th International Conference on Root and Butt Rots, IUFRO working party 7.02.01. 16-22 August 2004. Poznań, Poland.

CHAPTER 2:

Phylogeography of *Armillaria ostoyae* in the western United States

Summary

Sequences of nuclear ribosomal DNA regions (i.e., large subunit, internal transcribed spacer, 5.8S, and intergenic spacer) were obtained using a direct-PCR method from *Armillaria ostoyae* genets collected from the western United States. Many of the *A. ostoyae* genets contained heterogeneity among rDNA repeats, indicating intragenomic variation and likely intraspecific hybridization. Intragenomic variation was verified by visually editing base sequence offsets in regions with insertions/deletions, and using sequence-specific internal primers to resequence heterogeneous regions. Phylogenetic analyses using Bayesian Inference methods defined groups within *A. ostoyae*. Analysis of *A. ostoyae* from outside the western USA indicates the presence of a Circumboreal group of *A. ostoyae* that also occurs in Utah, USA; two other phylogeographic groups were associated with the Rocky Mountain and Pacific Northwest regions of the USA. Mixed sequences, an indication of intraspecific hybrids, were common in some geographic regions. Hypothetically, groups may have physically converged after long-term geographic isolation. Subsequent hybridization events may have influenced species evolution and could also have contributed to variation in pathogenicity and virulence. The occurrence of these groups and intraspecific hybrids also allows further conjecture that paleogeography and paleoclimate have influenced the phylogeography of *A. ostoyae*. In addition, other *Armillaria* species were examined for evolutionary relationships with the groups of *A. ostoyae*. These findings will provide a basis for future research relating ecological function to genetic diversity within *A. ostoyae*.

1 Introduction

Throughout its circumboreal distribution, *Armillaria ostoyae* (Romag.) Herink is a principle cause of Armillaria root rot disease (GUILLAUMIN et al. 1989; MORRISON and PELLOW 2002). In western North America, it adversely impacts commercial timber production by causing significant tree mortality and a reduction in tree growth (WILLIAMS et al. 1989). The effects of root disease in general are often underestimated, and losses due to *A. ostoyae* are often difficult to detect because signs of infection may not be readily observable (PARTRIDGE et al. 1977; CRUICKSHANK 2000). For these reasons, total losses due to *A. ostoyae* across western North America are largely unknown or underestimated; however, studies have shown volume loss as high as 40% over 4-8 years in a 18-year-old Douglas-fir [*Pseudotsuga menziezii* (Mirb.) Franco] plantation (CRUICKSHANK 2000). Total annual losses due to Armillaria root disease are estimated to be between 1.4 and 3.8 million m^3 in British Columbia (WHITE and MORRISON 1999).

During the last century, many forest stands within the western USA (particularly the Inland West) have experienced a shift in tree species composition (GRUELL et al. 1982; HARVEY 1994; LANGSTON 1995; STUART 1998). This shift in species composition is largely due to 1) fire suppression, 2) silvicultural practices, and 3) white pine blister rust (caused by *Cronartium ribicola* Fisch.) introduction. It is now apparent that this tree species shift has increased the occurrence and intensity of Armillaria root disease (MCDONALD et al. 1987). Fire suppression has led to increased density and overcrowding of shade-tolerant, potentially climax species [e.g., Douglas-fir and true firs (*Abies* spp.)] and displaced much of the fire-dependant seral species [e.g., larch (*Larix* spp.) and pines (*Pinus* spp.)] that previously existed within park-like stands. In general, Douglas-fir and

grand fir (*Abies grandis* Mill.) are known to be highly susceptible to *A. ostoyae*, especially in areas of the interior western USA, where they are often ecophysiologically maladptated or unfit for their environment (KILE et al. 1991; MCDONALD et al. 2003). In contrast, the seral species that once occupied these sites are generally less susceptible to *Armillaria* disease. An exception to the general tendency that serals are less susceptible is when trees are not locally adapted to the environment in which they are grown (e.g., trees derived from nursery-grown seedlings or off-site seed sources). Many of these nursery-grown seedlings may become more susceptible at planting if their root systems are damaged (SCHMITT 2001). In addition, such trees may also be ecophysiologically maladapted; and thus become predisposed by environmental stress to render them more susceptible to infection/damage by pathogens such as *A. ostoyae* (MCDONALD 1990). Selective harvesting practices may also increase risk of damage due to *A. ostoyae*. The harvesting process creates root wounds through which *A. ostoyae* can infect, while also producing stumps that can serve as a food base for increasing the inoculum potential. The introduction of *C. ribicola*, further exacerbated a host species shift by contributing to a reduction of the white pine cover type to less than 10% of its original 2 million ha (5 million acres) (FINS et al. 2002). Although Armillaria root rot has become a primary concern and shifts in tree species compositions have been recognized, fire suppression continues, and Douglas-fir continues to be planted in favor of serals because of its perceived higher economic value.

An individual genet of *A. ostoyae* can range in size from a small patch that occurs on a single tree to one of the largest organisms on earth (SMITH et al. 1992). In northeastern Oregon, one individual (genet) was identified that occupied nearly 900 ha,

with an estimated age between 1,900 and 8,650 years old (FERGUSON et al. 2003). The advanced age of this individual shows an ability to survive through a changing environment spanning several forest generations that likely included diverse species compositions. Other studies have shown that *A. ostoyae* genets can show varying levels of pathogenicity (OMDAL et al. 1995) and virulence (OMDAL et al. 1995; MORRISON and PELLOW 2002). While *A. ostoyae* is generally thought of as highly pathogenic, one notable study reported that most isolates of *A. ostoyae* appeared to be merely saprophytic with only occasional mild pathogenicity on declining trees (BÉRUBÉ and DESSUREAULT 1988). Also, distinct differences of *A. ostoyae* epidemiology have also been noted among coastal and interior regions of western North America (MCDONALD 1990; GOHEEN and OTROSINA 1998; MORRISON and PELLOW 2002). A variety of ecophysiological factors related to both host and pathogen have been hypothesized as contributing agents to differences in pathogenicity and epidemiology of *A. ostoyae* (MCDONALD 1990; MORRISON and PELLOW 2002).

Armillaria ostoyae can be identified by *in vitro* mating tests (KORHONEN 1978). Using these tests, *A. ostoyae*, which was previously classified as European Biological Species C (EBS C) and North American Biological Species I (NABS I), can be distinguished from four other *Armillaria* species in Europe, nine other *Armillaria* species in North America, and nine other *Armillaria* species in Asia (KORHONEN 1978; ANDERSON and ULLRICH 1979; OTA et al. 1998). PCR-based methods are now commonly used to discern rDNA differences among *Armillaria* species (ANDERSON and STASOVSKI 1992; HARRINGTON and WINGFIELD 1995; VOLK et al. 1996; BANIK et al. 1996; WHITE et al. 1998; KIM et al. 2000; and others as reviewed by PEREZ-SIERRA et al. 2000). These

differences in DNA can be used for identification, phylogenetic analyses, and assessments of genetic variability.

Genetic variability within *A. ostoyae* has been shown to exist within multiple regions of the ribosomal DNA (rDNA) from various geographic locations. Genetic variation has been observed within the intergenic spacer region 1 (IGS-1) (Anderson and Stasovski 1992), within the internal transcribed spacers (ITS-1 and ITS-2) and IGS-1 of European isolates (CHILLALI et al. 1998; SICOLI et al. 2003), and within the IGS-1 of Asian isolates (TERASHIMA et al. 1998). However, most previous studies on genetic variability of *A. ostoyae* have only examined a relatively small number (e.g., three to five) of isolates. Among other studies, the intergenic spacer region 2 (IGS-2) was used to show genetic variation among 24 Canadian isolates and two European isolates of *A. ostoyae* (WHITE et al. 1998). In addition, Random Amplified Polymorphic DNA (RAPD) analysis was used to show high genetic variability among 20 European *A. ostoyae* isolates (SCHULZE et al. 1997), and anonymous nucleotide sequences provided evidence of genetic variability among North American *A. ostoyae* genets (PIERCEY-NORMORE et al. 1997).

Genetic techniques applied to *A. ostoyae* have been primarily aimed at distinguishing it from other *Armillaria* species. Although genetic variability has been observed in *A. ostoyae*, this variation has not been studied in depth. Using PCR-based methods, studies of other *Armillaria* species have shown a high degree of intraspecific genetic variability (COETZEE et al. 2000; DUNNE et al. 2002). In these previous studies, genetic variability was used to investigate phylogeographic relationships within *Armillaria* species, including *A. mellea sensu stricto* (Vahl:Fr.), a species having

circumboreal distribution (COETZEE et al. 2000), and *A. luteobubalina* Watling & Kile, a species with partial circumaustral distribution that occurs in Australia and South America, but not in Africa (COETZEE et al. 2003). The purpose of this study is to identify genetic differences among genets of *A. ostoyae*, and examine intra- and interspecific phylogeographic relationships. Investigation of these differences is important toward understanding 1) varying levels of pathogenicity and virulence within *A. ostoyae*, 2) phylogeographic relationships among *A. ostoyae* genets and genets of other *Armillaria* species, and 3) adaptation to diverse environmental factors.

2 Materials and methods

2.1 Genet selection

Genets of *A. ostoyae* (Table 2-1) and other *Armillaria* species (Table 2-2) of the northern hemisphere were obtained for use in this study from an archived collection at the USDA Forest Service, Rocky Mountain Research Station, Forestry Sciences Laboratory (Moscow, Idaho, USA). Isolates from this collection had previously been identified to genets with the use of somatic incompatibility pairing tests (MCDONALD and MARTIN 1988; GUILLAUMIN et al. 1991; WU et al. 1996). These genets were further identified to species using haploid x haploid mating, haploid x diploid pairing tests, diploid x diploid paring tests (KORHONEN 1978; MALLETT et al. 1989; MCDONALD and MARTIN 1988), and/or restriction fragment length polymorphism (RFLP) analysis of the IGS-1 region of rDNA (HARRINGTON and WINGFIELD 1995; WHITE et al. 1998; KIM et al. 2000). Species identification was later verified by nucleotide similarity with sequences within GenBank by using the BLAST search function.

2.2 Polymerase chain reaction (PCR) and sequencing

In preparation for PCR, the genets were grown on malt agar medium (0.75% malt extract, 0.75% dextrose, 0.5% peptone, and 1.5% agar) within sealed polystyrene Petri dishes (60 mm x 15 mm) at 21 C in the dark for 2 weeks. PCR products from rDNA including nuclear large ribosomal subunit (LSU), internal transcribed spacers (ITS-1 and ITS-2), 5.8S rDNA, and IGS-1 were obtained by direct PCR method (i.e., mycelium was scraped from pure culture and added directly to the PCR reaction mixture to serve as DNA template). The following primer sets were used for initial amplification of the following specified rDNA regions: 1) LSU: 5.8SR (5'-TCG ATG AAG AAC GCA GCG-3') and LR7 (5'-TAC TAC CAC CAA GAT CT-3') (MONCALVO et al. 2000);
2) ITS: ITS-1F (5'-CTT GGT CAT TTA GAG GAA GTA A-3') (GARDES and BRUNS 1993) and ITS4 (5'- TCC TCC GCT TAT TGA TAT GC-3') (WHITE et al. 1990); and 3) IGS-1: LR12R (5'-CTG AAC GCC TCT AAG TCA GAA-3') (VELDMAN et al. 1981), paired with O-1 (5'-AGT CCT ATG GCC GTG GAT-3') (DUCHESNE and ANDERSON 1990) and/or A5SR1 (5'-AAC CAC AGC ACC CAG GAT T-3'), a primer specifically designed for this project based on the 5S rRNA gene of 29 Basidiomycotina species (HWANG and KIM 1995).

The PCR reaction mixtures included 2.5 units Ampli*Taq*® DNA polymerase (Applied Biosystems, Foster City, California, USA) per reaction along with 200 μM dNTPs, 4 mM $MgCl_2$, 5 μl 10X PCR Buffer, and 0.5 μM of each primer for a final reaction volume of 50 μl. Reaction mixtures were incubated in a MJ Research (Reno, Nevada, USA) PTC-200 peltier thermal cycler under the following conditions for specified rDNA regions: 1) LSU: initial denaturation at 94 C for 3 min, followed by 35

cycles of 94 C for 60 sec (denaturation), 55 C for 30 sec (annealing), and 72 C for 2 min (extension) followed by a final extension for 5 min at 72 C; 2) ITS+5.8S: initial denaturation at 94 C for 2 min 30 sec, followed by 36 cycles of 94 C for 60 sec (denaturation), 48 C for 60 sec (annealing) and 72 C for 1 min 30 sec (extension) followed by a final extension for 10 min at 72 C; or 3) IGS-1: initial denaturation at 95 C for 1 min 35 sec, followed by 35 cycles of 90 C for 30 sec (denaturation), 60 C for 1 min (annealing), and 72 C for 2 min (extension), followed by a final extension for 10 min at 72 C. PCR products were prepared for sequencing using ExoSAP-IT™ (USB Corporation, Cleveland, Ohio, USA) and sequenced at Davis Sequencing, Inc. (Davis, California, USA). IGS-1 and ITS+5.8S regions were sequenced with the same primers used for initial amplification, while the LSU region was sequenced using the LR0R (5'-ACC CGC TGA ACT TAA GC-3'), LR5 (5'-TCC TGA GGG AAA CTT CG-3), and LR15 (5'-TAA ATT ACA ACT CGG AC-3') primers (MONCALVO et al. 2000; VILGALYS 24 June, 2004).

2.3 Sequence editing

The sequence chromatograms for each genet were edited by hand/eye with BioEdit software (HALL 1999). Two separate researchers performed independent editing of sequence chromatograms in order to check for errors. While some of the chromatograms indicated homogeneous rDNA repeats (one peak for each nucleotide position), most of the chromatograms showed heterogeneity among the rDNA repeats. Careful attention was given to these chromatograms showing heterogeneity because heterogeneous rDNA can represent inter- and/or intraspecific genetic variation within an individual (see first

section of Discussion). In this study, heterogeneity was detected when a chromatogram contained either one or more single nucleotide polymorphism(s) (SNP; represented by a double peak occurring at a single nucleotide position) or a "frame-shift" (overlapping peaks due to length variation among rDNA repeat) was apparent (Figs. 2-1, 2-2, and 2-3). When possible, heterogeneous sequences were deciphered into homogeneous sequence representations by one of the three methods described in Figs. 2-1, 2-2, and 2-3 before phylogenetic analyses. The first method shown represents the manual hand/eye editing of a frame-shift (Fig. 2-1). The second method is similar to that of the mismatch amplification mutation assay (MAMA) method (Fig. 2-2) (CHA et al. 1992; RAUSCHER et al. 2002). This second method was used within the IGS-1, for which reverse complementary primers were created and applied to product shown to contain a SNP at base pair position 683 of the IGS-1 region. The primers applied to this position were AOHR1T (5'-TGC CGT TCA AAA-3'), AOHR1G (5'-TGC CGT TCA AAC-3'), and AOHR1C (5'-TGC CGT TCA AAG-3'). The third method simply splits a chromatogram containing a single SNP into two predicted sequences (Fig. 2-3). If predicted sequences deciphered by these three methods showed heterogeneity among rDNA repeats from a single genet, the different sequences were assigned a letter code (e.g., A or B) after the genet names. Remaining polymorphisms were coded with the IUPAC codes for Ambiguous Nucleotides; however, these polymorphisms were certainly not regarded as ambiguous but rather the result of heterogeneous product. These sequences with two or more ambiguous sites were then eliminated from further analyses to minimize uncertainty within phylogenetic analyses (see first section of Results). All sequences used in

phylogenetic analysis have been deposited into GenBank (as shown in Tables A3-1 and A3-2).

Table 2-1. Armillaria ostoyae isolates used in this study

Isolate	Alternate number(s)	Origin	Collector(s) or Reference	LSU Sequnece Type(s)	ITS Sequnece Type(s)	IGS Sequnece Type(s)
BC18F		Washington, USA	M.-S. Kim	LSUOS7, LSUOS10	ITSOS6	IGSOS3
FF4	91058 / T1	Eastern, Finland	Unknown	LSUOS1	ITSOS35*, ITSOS36*	IGSOS32*, IGSOS33*
MNF4	118	Oregon, USA	Ferguson et al. 2003	LSUOS10	ITSOS6*, ITSOS8*	ISGOS9, IGSOS15
NA142		Washington, USA	G.I. McDonald	LSUOS10, LSUOS11	ITSOS15	IGSOS16
NA144		Washington, USA	G.I. McDonald	LSUOS25*	ITSOS21, ITSOS22	IGSOS10, IGSOS17
NA150		Washington, USA	G.I. McDonald	LSUOS8, LSUOS10	ITSOS26*, ITSOS27*	IGSOS2
NA212		Washington, USA	G.I. McDonald	LSUOS10, LSUOS11	ITSOS8	IGSOS2, IGSOS15
NA254		Washington, USA	G.I. McDonald	LSUOS22*	ITSOS10, ITSOS11	IGSOS2, IGSOS3
NA260		Washington, USA	G.I. McDonald	LSUOS6, LSUOS10	ITSOS14	ISGOS2
NC53		Idaho, USA	IFTNC**	LSUOS10, LSUOS11	ITSOS10, ITSOS11	IGSOS3, IGSOS16
NC94		Washington, USA	IFTNC**	LSUOS10	ITSOS6	IGSOS2
NC164		Idaho, USA	IFTNC**	LSUOS7, LSUOS10	ITSOS26*	IGSOS2, IGSOS3
NC436		Idaho, USA	IFTNC**	LSUOS9, LSUOS10	ITSOS6	IGSOS5, IGSOS15
NC491		Washington, USA	IFTNC**	LSUOS6, LSUOS10	ITSOS37*	IGSOS2, IGSOS6
NC580		Washington, USA	IFTNC**	LSUOS10	ITSOS6	IGSOS2, IGSOS4
NC671		Idaho, USA	IFTNC**	LSUOS7, LSUOS10	ITSOS12, ITSOS13	IGSOS3, IGSOS16
NC765		Washington, USA	IFTNC**	LSUOS6, LSUOS10	ITSOS34*	IGSOS2
NC837		Washington, USA	IFTNC**	LSUOS19*	ITSOS28*, ITSOS29*	IGSOS9, IGSOS23
NC863		Idaho, USA	IFTNC**	LSUOS10	ITSOS16	IGSOS3, IGSOS15
NC887		Idaho, USA	IFTNC**	LSUOS10	ITSOS10, ITSOS11	IGSOS2, IGSOS5
NC895		Idaho, USA	IFTNC**	LSUOS10	ITSOS6	IGSOS2, IGSOS11
NC905		Washington, USA	IFTNC**	LSUOS6	ITSOS24, ITSOS25	IGSOS2
NC911		Washington, USA	IFTNC**	LSUOS10, LSUOS11	ITSOS38*, ITSOS39*	IGSOS2, IGSOS16
NC1070		Idaho, USA	IFTNC**	LSUOS23*, LSUOS24*	ITSOS40*, ITSOS41*	IGSOS2, IGSOS7
NC1091		Idaho, USA	IFTNC**	LSUOS10	ITSOS62*, ITSOS63*	IGSOS2
NC1126		Idaho, USA	IFTNC**	LSUOS11	ITSOS6	IGSOS16
NC1180		Idaho, USA	IFTNC**	LSUOS19*	ITSOS28*, ITSOS29*	IGSOS2, IGSOS23
NC1187		Idaho, USA	IFTNC**	LSUOS10, LSUOS11	ITSOS10, ITSOS23	IGSOS2, IGSOS16
NC1245		Idaho, USA	IFTNC**	LSUOS10, LSUOS11	ITSOS6	IGSOS2, IGSOS16
NM115		New Mexico, USA	G.I. McDonald	LSUOS3	ITSOS42*	IGSOS18
NM120		New Mexico, USA	G.I. McDonald	LSUOS3	ITSOS2	IGSOS18, IGSOS19

Table 2-1. Armillaria ostoyae isolates used in this study **(continued)**

Isolate	Alternate number(s)	Origin	Collector(s) or Reference	LSU Sequnece Type(s)	ITS Sequnece Type(s)	IGS Sequnece Type(s)
NM235	BS2	New Mexico, USA	Omdal et al. 1995	LSUOS3	ITSOS2	IGSOS18, IGSOS22
NM236	PP1	New Mexico, USA	Omdal et al. 1995	LSUOS14*	ITSOS2, ITSOS4	IGSOS26*
NM238	BS1	New Mexico, USA	Omdal et al. 1995	LSUOS1, LSUOS3	ITSOS2, ITSOS3	IGSOS27*
NM239	WF2	New Mexico, USA	Omdal et al. 1995	LSUOS3	ITSOS2	IGSOS18
NM241	DF1	New Mexico, USA	Omdal et al. 1995	LSUOS3	ITSOS2	IGSOS18
NM242	A2	New Mexico, USA	Omdal et al. 1995	LSUOS3	ITSOS43*	IGSOS18
NM244	A3	New Mexico, USA	Omdal et al. 1995	LSUOS3	ITSOS2	IGSOS18
NM245	WP2	New Mexico, USA	Omdal et al. 1995	LSUOS12*	ITSOS44*	IGSOS26*
NM246	WF1	New Mexico, USA	Omdal et al. 1995	LSUOS3	ITSOS30*	IGSOS18
NM248	PP3	New Mexico, USA	Omdal et al. 1995	LSUOS3	ITSOS30*	IGSOS18
NM249	A4	New Mexico, USA	Omdal et al. 1995	LSUOS3	ITSOS31*	IGSOS18, IGSOS21
NM250	PP4	New Mexico, USA	Omdal et al. 1995	LSUOS13*	ITSOS2, ITSOS3	IGSOS26*
OR10		Oregon, USA	G.I. McDonald	LSUOS15*	ITSOS45*, ITSOS46*	IGSOS2
OR22		Oregon, USA	G.I. McDonald	LSUOS6	ITSOS59*, ITSOS61*	IGSOS2
P255		Idaho, USA	G.I. McDonald	LSUOS3	ITSOS5	IGSOS18, IGSOS20
P1401		Idaho, USA	G.I. McDonald	LSUOS6	ITSOS24	IGSOS2
P1404		Idaho, USA	G.I. McDonald	LSUOS6	ITSOS24	IGSOS2
P2003		Idaho, USA	G.I. McDonald	LSUOS10	ITSOS6	IGSOS2
P4352		Idaho, USA	G.I. McDonald	LSUOS10	ITSOS12	IGSOS16
P4661		Idaho, USA	G.I. McDonald	LSUOS10	ITSOS12	IGSOS16
PC514		Idaho, USA	G.I. McDonald	LSUOS16*	ITSOS56*, ITSOS57*	IGSOS2, IGSOS18
R957		Washington, USA	G.I. McDonald	LSUOS6, LSUOS10	ITSOS17	IGSOS2
R959		Washington, USA	G.I. McDonald	LSUOS10	ITSOS18	IGSOS2
R1075		Idaho, USA	G.I. McDonald	LSUOS18*	ITSOS32*, ITSOS33*	IGSOS24*, IGSOS25*
R1083		Idaho, USA	G.I. McDonald	LSUOS10	ITSOS6	IGSOS2, IGSOS12
R1140		Oregon, USA	G.I. McDonald	LSUOS10	ITSOS6	IGSOS2, IGSOS14
R1202		Oregon, USA	G.I. McDonald	LSUOS8	ITSOS14	IGSOS2
R1237		Washington, USA	G.I. McDonald	LSUOS6, LSUOS10	ITSOS58*, ITSOS60*	IGSOS2, IGSOS13
R1283		Idaho, USA	G.I. McDonald	LSUOS10, LSUOS11	ITSOS47*, ITSOS48*	IGSOS8, IGSOS16
R1329		Montana, USA	G.I. McDonald	LSUOS10, LSUOS11	ITSOS6	IGSOS2, IGSOS16
R1334		Montana, USA	G.I. McDonald	LSUOS10	ITSOS6	IGSOS2, IGSOS16

Table 2-1. Armillaria ostoyae isolates used in this study **(continued)**

Isolate	Alternate number(s)	Origin	Collector(s) or Reference	LSU Sequnece Type(s)	ITS Sequnece Type(s)	IGS Sequnece Type(s)
R1348		Montana, USA	G.I. McDonald	LSUOS6, LSUOS10	ITSOS34*	IGSOS2
R1362		Montana, USA	G.I. McDonald	LSUOS4, LSUOS5	ITSOS2	IGSOS23
R1366		Montana, USA	G.I. McDonald	LSUOS10	ITSOS6, ITSOS9	IGSOS2, IGSOS15
R1374		Montana, USA	G.I. McDonald	LSUOS20*	ITSOS49*, ITSOS50*	IGSOS16, IGSOS23
R1424		Oregon, USA	G.I. McDonald	LSUOS21*	ITSOS51*, ITSOS52*	IGSOS28*, IGSOS29*
SSF4		Montana, USA	B.A. Ferguson	LSUOS10, LSUOS11	ITSOS6, ITSOS7	IGSOS2, IGSOS16
SSF6		Montana, USA	B.A. Ferguson	LSUOS10	ITSOS19, ITSOS20	IGSOS3, IGSOS16
ST1		New Hampshire, USA	T.C. Harrington	LSUOS1	ITSOS1	IGSOS1
ST2		Washington, USA	J.F. Ammiratti	LSUOS10	ITSOS6, ITSOS7	IGSOS2, IGSOS16
TS7		Chihuahua, Mexico	C.G. Shaw III	LSUOS3	ITSOS2	IGSOS18
U5		Utah, USA	G.I. McDonald	LSUOS1	ITSOS53*	IGSOS34*, IGSOS35*
U16		Utah, USA	G.I. McDonald	LSUOS1	ITSOS54*	IGSOS34*, IGSOS35*
U73		Utah, USA	G.I. McDonald	LSUOS3	ITSOS31*	IGSOS18
USSR		Primorye, Russia	G.M. Filip	LSUOS1, LSUOS2	ITSOS55*	IGSOS30*, IGSOS31*
WA6		Washington, USA	G.I. McDonald	LSUOS17*	ITSOS32*, ITSOS33*	IGSOS2, IGSOS23

* Represents a heterogenous genotype

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Table 2-2. Armillaria isolates used in this study

Species	Isolate[a]	Alternate number	Origin	Collector(s) or Reference	LSU Sequnece Type(s)
A. calvescens	ST3	JB56A	Québec, Canada	J.A. Bérubé	LSUCA3
	ST17	PR-3	Michigan, USA	M.T. Banik	LSUCA2
	ST18	FFC-7	Michigan, USA	M.T. Banik	LSUCA1
A. cepistipes	M110	SP82-14	British Columbia, Canada	D.J. Morrison	LSUCE2
	S20	SP83-07	British Columbia, Canada	D.J. Morrison	LSUCE1, LSUCE2
A. gallica	M70	SP81-29	British Columbia, Canada	D.J. Morrison	LSUGA1
	ST22	EL-1	Michigan, USA	M.T. Banik	LSUGA1, LSUGA2
	ST23	MA-1	Wisconsin, USA	M.T. Banik	LSUGA3, LSUGA4
A. gemina	ST8[b]	JJW153	New York, USA	J.J. Worrall	LSUGE1
	ST9[c]	JJW64	New York, USA	J.J. Worrall	LSUGE1
	ST11[d]	MIELKE	West Virginia, USA	M.E. Mielke	LSUGE1, LSUGE2
A. mellea	ST5	GB934	Virginia, USA	G.F. Bills	LSUME4*
	ST20	A3	Wisconsin, USA	M.T. Banik	LSUME1, LSUME2
	ST21	TCH-2	New Hampshire, USA	T.C. Harrington	LSUME1, LSUME3
A. nabsnona	C21		Idaho, USA	G.I. McDonald	LSUNA1
	M90		British Columbia, Canada	D.J. Morrison	LSUNA1
	ST16	SHAW,C	Alaska, USA	C.G. Shaw	LSUNA2
A. sinapina	M50	SP81-1	British Columbia, Canada	D.J. Morrison	LSUSI2
	ST12	AMM9065	Washington, USA	J.F. Ammirati	LSUSI1
	ST13	CF-2	Michigan, USA	M.T. Banik	LSUSI1, LSUSI2
A. tabescens	AtMuS2		South Carolina, USA	G. Schnabel	LSUTA1, LSUTA2
	OOi99		Georgia, USA	G. Schnabel	LSUTA1
	OOi210		Georgia, USA	G. Schnabel	LSUTA1
NABS[e] X	837		Idaho, USA	G.I. McDonald	LSUX1, LSUX2
	D82		Idaho, USA	G.I. McDonald	LSUX1, LSUX3
	POR100		Idaho, USA	G.I. McDonald	LSUX1, LSUX4

* Represents a heterogenous genotype not included in analysis

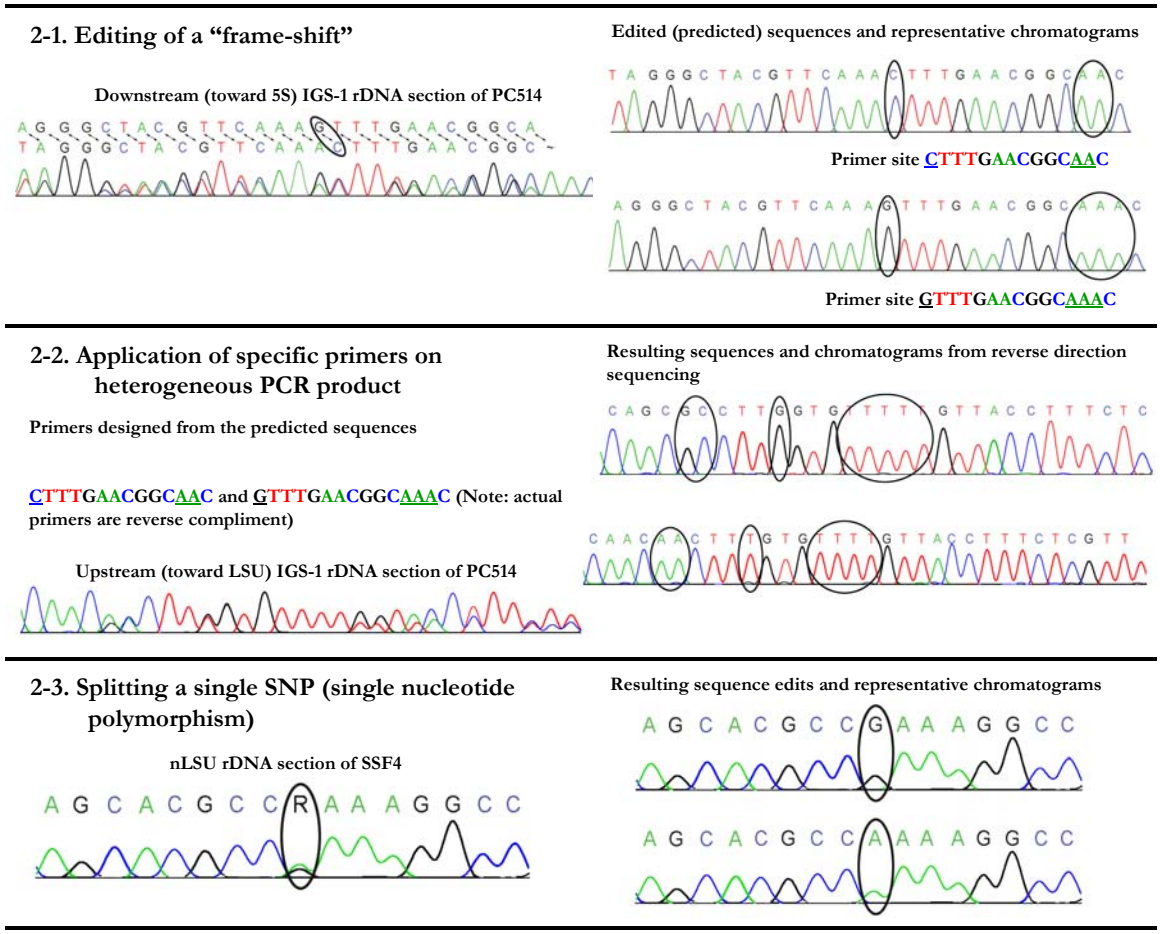
[a] Each isolate represents a distinct genet

[b] Also represents genotypes ITSGE1, IGSGE3, and IGSGE4

[c] Also represents genotypes ITSGE2*, ITSGE3*, IGSGE5, and IGSGE6

[d] Also represents genotypes ITSGE1, IGSGE1, and IGSGE2

[e] North American Biological Species



Figs. 2-1, 2-2, and 2-3. Several methods for editing heterogeneous product into homogenous sequence types.

2.4 Sequence alignments

Sequences were manually aligned for each of three regions (LSU – both ends, proximal to ITS-2 and IGS-1; ITS + 5.8S; and IGS-1). Duplicate sequence types were eliminated from the sequence alignments so that only unique genotypes were compared. For the LSU region, genets from both Table 2-1 and Table 2-2 were included in the alignment for interspecific comparison, while sequence alignments for ITS and IGS regions contained only genets in Table 2-1 for intraspecific comparisons.

2.5 Phylogenetic analysis

Phylogenetic analyses were performed for each dataset using neighbor-joining (NJ) (SAITOU and NEI 1987), Parsimony, and Bayesian analysis methods.

Any gaps in sequence alignments were treated as missing and coded using a simple gap-coding method (SIMMONS and OCHOTERENA 2000). Neighbor-joining analysis was performed using the Tamura-Nei model for estimation of evolutionary distance in MEGA (version 2.1, KUMAR et al. 2001). Relative support for nodes in resulting trees was evaluated using 1,000 bootstrap replicates (FELSENSTEIN 1985). Parsimony analysis was performed using PAUP* (4.0b10) (SWOFFORD 2001). Multistate taxa were interpreted as polymorphisms, starting trees were obtained via stepwise addition with random addition sequence of 10 replicates, one tree was held at each step during stepwise addition, tree-bisection-reconnection was used, and the steepest descent option was not in effect. The analysis of the LSU ends was done with MaxTrees set to auto increase while the ITS + 5.8S and IGS-1 regions were set to a maximum of 10,000 trees. A bootstrap method with heuristic search was used with 1,000 bootstrap replicates on each dataset to obtain 50% bootstrap majority-rule consensus trees (FELSENSTEIN 1985).

Bayesian analysis was performed by MrBayes v3.0B4 (HUELSENBECK and RANQUIST 2001). Bayesian inference of phylogeny calculates the posterior probability of phylogenetic trees. To select appropriate evolutionary models for use in Bayesian analysis, MrModeltest 1.0b (NYLANDER 2003) was used. Four chains were run for 3×10^6 generations generating files with 30,001 trees, the first 6,000 of these trees were discarded as the "burnin" of the chains. The remaining 24,001 trees were used to make 95% majority-rule consensus trees using PAUP* (4.0b10).

2.6 GenBank comparison

All sequences were compared to those in GenBank hosted by the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) using the Nucleotide-nuleotide BLAST (blastn) function (ALTSCHUL et al. 1997). Sequences having identical nucleotide compositions were noted to obtain additional insight from phylogenetic analyses.

3 Results

3.1 Heterogeneity (intra-individual variation)

Heterogeneous rDNA products, an indication of intraspecific and intragenomic variation, were common in all regions analyzed. Many of these rDNA heterogeneous products were deciphered into two predicted sequences using the methods described in Figs. 2-1, 2-2, and 2-3. In this situation, the deciphering process could result in two genotypes per individual for a single rDNA region. When heterogeneous rDNA products remained undecipherable, the remaining heterogeneous rDNA product was represented by two or more ambiguously coded nucleotide positions per individual within a single rDNA region (noted by an asterisks in Tables 2-1 and 2-2). Sequences containing ambiguous nucleotides at multiple positions were excluded from analyses. Out of 77 individuals of *A. ostoyae*, heterogeneity was detected in 37 (48%) individuals within the LSU region, 45 (58%) within the ITS, and 46 (60%) within the IGS-1. Only 14 (16%) of the 77 individuals were homogeneous in all three rDNA regions analyzed. Using the sequence editing techniques (Figs. 2-1, 2-2, and 2-3), we were able to decipher heterogeneous product into homogenous sequence representations to be used in phylogenetic analyses

for 23 (30% of total) individuals of the LSU, 14 (18% of total) of the ITS, and 36 (47% of total) of the IGS-1.

3.2 Sequence data

Many of the genets of this study contained identical sequence types; however, duplicate sequence types were removed from the dataset for phylogenetic analyses. Sequence types derived from each genet are shown in Tables 2-1 and 2-2.

3.2.1 LSU

The LSU dataset produced 11 unique sequence types of *A. ostoyae* (LSUOS1-LSUOS11) for comparison to sequences representing the nine other North American *Armillaria* species (*A. calvescens* Bérubé & Dessur. - LSUCA1 and LSUCA2; *A. cepestipes* Velen.- LSUCE1 and LSUCE2; *A. gallica* Marxmüller & Romagn. - LSUGA1, LSUGA2, LSUGA3, and LSUGA4; *A. gemina* Bérubé & Dessur. - LSUGE1 and LSUGE2; *A. mellea sensu stricto* - LSUME1 and LSUME2; *A. nabsnona* Volk & Burdsall - LSUNA1 and LSUNA2; *A. sinapina* Bérubé & Dessur. - LSUSI1 and LSUSI2; *A. tabescens* (Scop.) Emel - LSUTA1 and LSUTA2; and North American Biological Species X - LSUX1, LSUX2, LSUX3, and LSUX4). Each sequence contained 976 total characters including simple-gap coded indels. Of these characters, 920 were constant and 16 variable characters were parsimony uninformative; however, 40 characters were parsimony informative. A single most-parsimonious tree was found with optimality criterion set to parsimony in PAUP* (4.0b10). This single tree yielded a total length of 60 steps, CI = 0.950, RI = 0.967, RC = 0.919, HI = 0.050, and a G-fit score of -39.250.

3.2.2 ITS + 5.8S

The ITS dataset consisted of 25 unique sequence types of *A. ostoyae* (ITSOS1-ITSOS25) and one *A. gemina* outgroup sequence (ITSGE1). Each sequence contained 793 total characters including simple-gap coded indels. Of these characters, 770 were constant and 13 variable characters were parsimony uninformative, and 10 characters were parsimony informative. With optimality criterion set to parsimony, 120 equally parsimonious trees were revealed. An optimum sample tree from the heuristic search yielded a total length of 27 steps, CI = 0.852, RI = 0.857, RC = 0.730, HI = 0.148, and a G-fit score of –9.000.

3.2.3 IGS-1

The IGS-1 dataset consisted of 23 unique sequence types of *A. ostoyae* (IGSOS1-IGSOS23), and six sequences of the *A. gemina* (IGSOS1-IGSOS6) were used as an outgroup. Each sequence contained 583 total characters including simple-gap coded indels. Of these characters, 537 were constant, 15 variable characters were parsimony uninformative, and 31 characters were parsimony informative. With optimality criterion set to parsimony, 805 equally parsimonious trees were revealed. An optimum sample tree from the heuristic search yielded a total length of 54 steps, CI = 0.889, RI = 0.955, RC = 0.848, HI = 0.111, and a G-fit score of -29.600.

3.3 Phylogeographic analyses

Nearly identical congruency for LSU, ITS+5.8S, and IGS-1 datasets was shown by 50% majority-rule bootstrap-consensus trees from the parsimony analyses (as illustrated by Figs A2-1, A2-3, A2-4), 50% majority-rule consensus trees from neighbor-joining

analyses (as illustrated by Figs A2-2, A2-4, A2-6), and the 90% majority-rule consensus trees created for the Bayesian analysis (Figs. 2-4, 2-5, and 2-6).

3.3.1 LSU

Phylogenetic trees of the LSU region using three different inference methods showed three phylogeographically distinct groups of *A. ostoyae*, as shown in the Bayesian radial 90% majority-rule consensus tree and corresponding map (Fig. 2-4). We refer to these groups as the Circumboreal, Rockies, and Northwest groups. A group with circumboreal distribution consisted of two clades represented by sequence types LSUOS1 and LSUOS2, which were derived from genets collected in Utah (USA), New Hampshire (USA), Russia, and Finland. The Rockies group consisted of sequence types LSUOS3-LSUOS5, which were distributed among genets from Idaho (USA), Montana (USA), Utah (USA), and New Mexico (USA). The Northwest group consisted of sequence types LSUOS6-LSUOS11, with associated genets collected from Idaho (USA), Montana (USA), Oregon (USA), and Washington (USA). A hybrid individual from New Mexico (USA) was found to contain both Circumboreal (LSUOS1) and Rockies (LSUOS3) sequence types. Rockies and Northwest groups were separated from the root of the Circumboreal groups branches with posterior probabilities of 93% and 92%, respectively. The LSU region was also used to compare relationships of North American *Armillaria* species to *A. ostoyae* groups. The LSU was the only rDNA region studied that allowed unambiguous alignment of *A. mellea sensu stricto* and *A. tabescens* sequences with sequences of *A. ostoyae* and the other seven North American *Armillaria* species. The

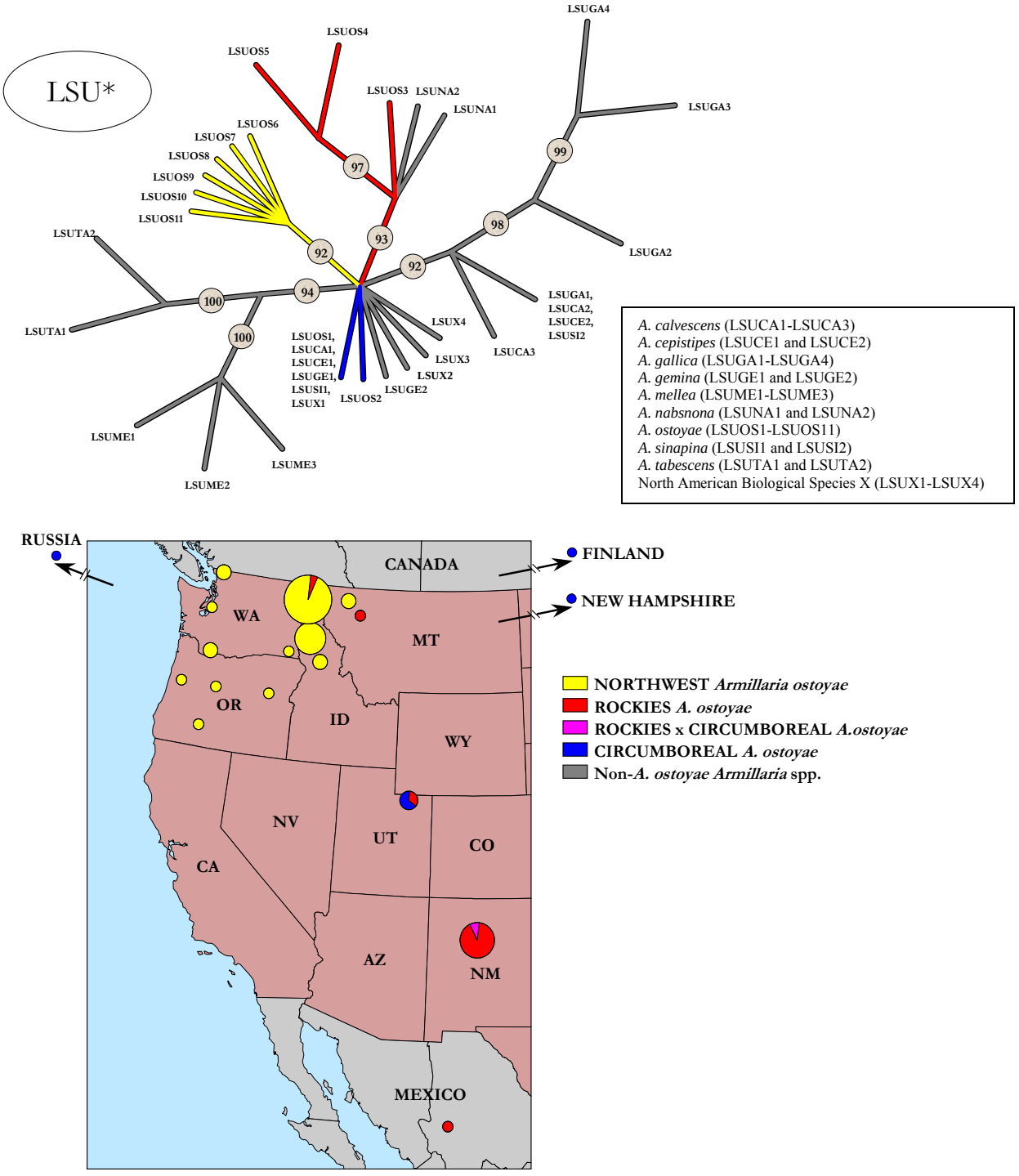


Fig. 2-4. Phylogeographic distribution of *Armillaria ostoyae* based on the nuclear large ribosomal subunit (LSU). Radial 90% majority-rule consensus tree of *Armillaria* species based on 24,001 trees from Bayesian inference analysis of the LSU region and corresponding phylogeographic distribution of *A. ostoyae* genets based on major clades. Numbers between clades indicate estimated posterior probability. *Ambiguous sequences from 14 heterogeneous *A. ostoyae* isolates were excluded from analysis.

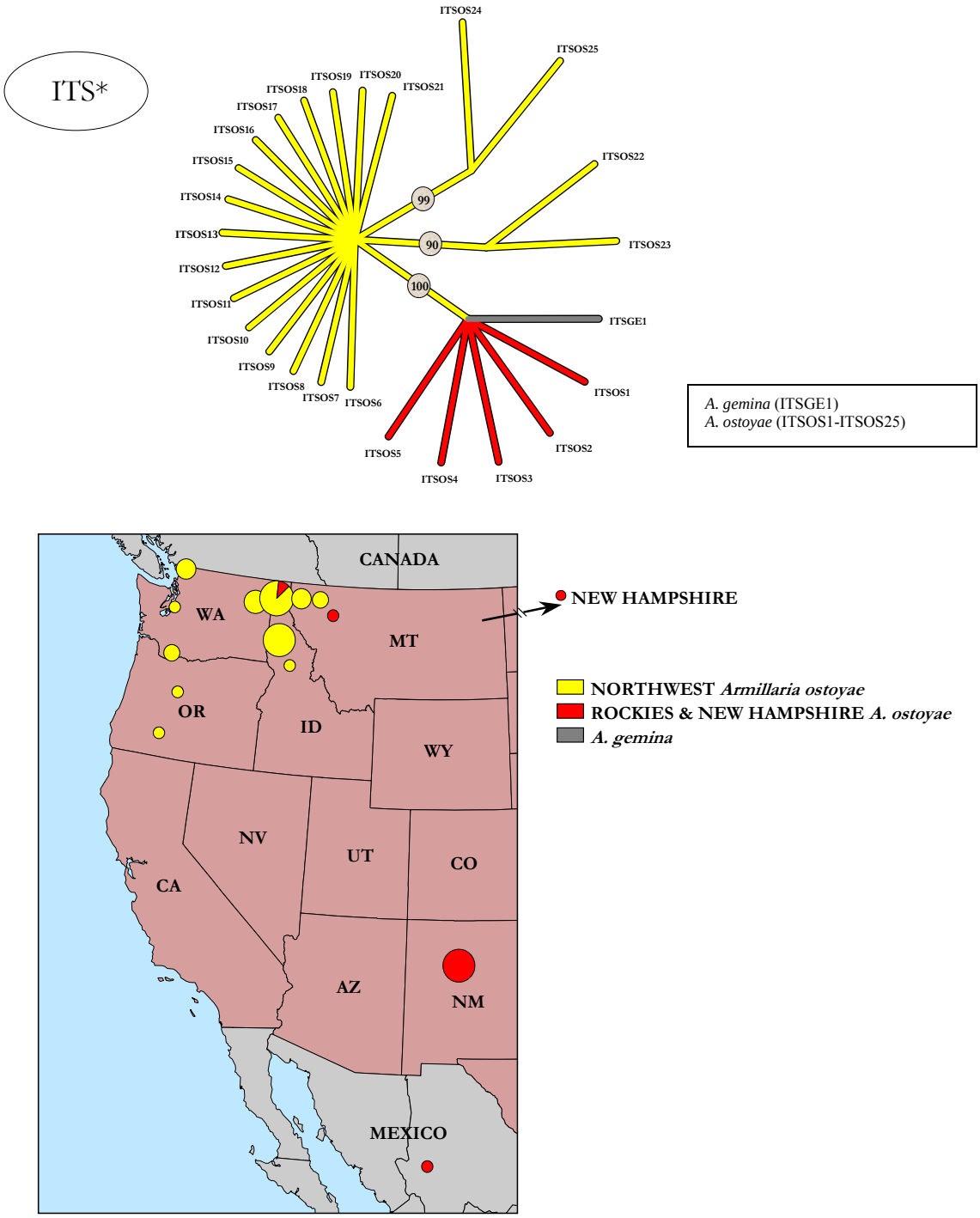


Fig. 2-5. Phylogeographic distribution of *Armillaria ostoyae* based on the internal transcribed spacer and 5.8S rDNA (ITS). Radial 90% majority-rule consensus tree of *Armillaria* species based on 24,001 trees from Bayesian inference analysis of the ITS and corresponding phylogeographic distribution of *Armillaria ostoyae* genets based on major clades. Numbers between clades indicate estimated posterior probability. *Ambiguous sequences from 31 heterogeneous *A. ostoyae* isolates were excluded from analysis.

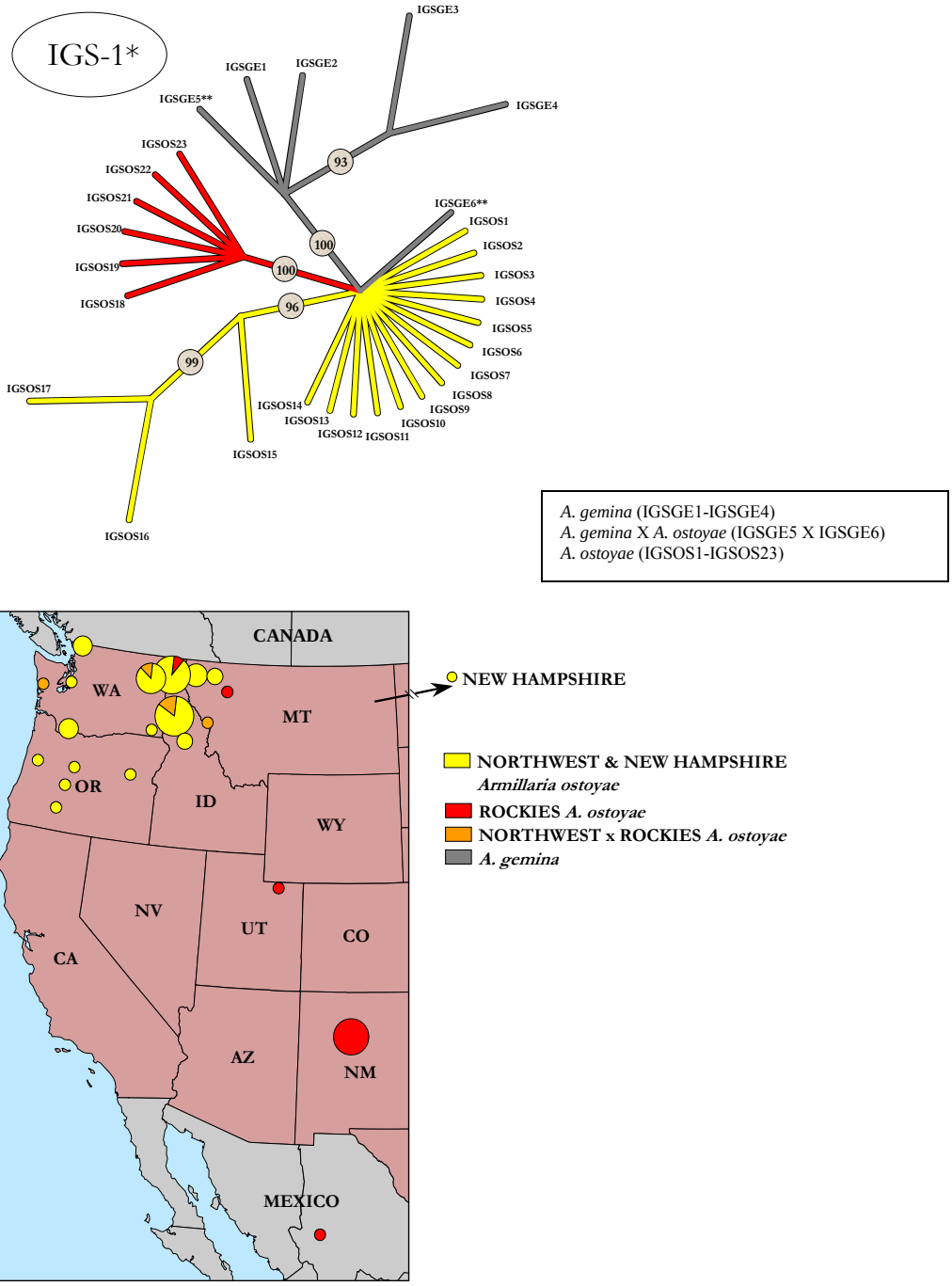


Fig. 2-6. Phylogeographic distribution of *Armillaria ostoyae* based on the intergenic spacer one rDNA (IGS-1). Radial 90% majority-rule consensus tree of *Armillaria* species based on 24,001 trees from Bayesian inference analysis of the IGS-1 and corresponding phylogeographic distribution of *Armillaria ostoyae* genets based on major clades. Numbers between clades indicate estimated posterior probability. *Ambiguous sequences from 10 heterogeneous *A. ostoyae* isolates were excluded from analysis. **A. ostoyae* X *A. gemina* interspecific hybrid (IGSGE5 X IGSGE6)

Circumboreal (LSUOS1) sequence type is identical to and shares a terminal node with sequence types of five other species [*A. calvescens* (LSUCA1), *A. cepestipes* (LSUCE1), *A. gemina* (LSUGE1), *A. sinapina* (LSUSI1), and NABS X (LSUX1)]. This branch forms a "starburst-like" structure (polytomy) with other sequence types of *A. gemina* (LSUGE2), *A. ostoyae* (LSUOS2), and NABS X (LSUX2, LSUX3, and LSUX4). The branches of this polytomy are rooted by an ancestral node from which several of the North American *Armillaria* species (i.e., *A. calvescens*, *A. cepistipes*, *A. gallica*, *A. gemina*, *A. nabsnona, A. ostoyae, A. sinapina*, and NABX) may have been derived. *Armillaria mellea* and *A. tabescens* were separated from this root by a Bayesian posterior probability of 94% and separated from each other by a posterior probability of 100%. *Armillaria nabsnona* was another species showing sequence similarity to *A. ostoyae*, with two sequence types (LSUNA1 and LSUNA2) showing close relationship to (forms a multifurcation with) the sequence type LSUOS3 of the *A. ostoyae* Rockies group.

3.3.2 ITS + 5.8S

Phylogenetic analyses of the ITS region using three different inference methods showed two phylogeographically distinct groups (Rockies/New Hampshire and Northwest) of *A. ostoyae*, as shown in the Bayesian radial 90% majority-rule consensus tree and corresponding map (Fig. 2-5). The two groups were separated by a posterior probability of 100%. The Northwest group consisted of a large polytomy containing 16 sequence types (ITSOS6-ITSOS21) and two subgroups; each subgroup was comprised of two sequence types that radiated from the nodal root of the polytomy with posterior probabilities of 90% (ITSOS22 and ITSOS23) and 99% (ITSOS24 and ITSOS25).

Although ITS analysis clearly shows that these Northwest subgroups are distinct from the large polytomy, non-hybrid genets containing these sequence types are co-defined by their LSU sequence types that show a monophyletic Northwest group (Fig. 2-4). The *A. gemina* outgroup, represented by a single sequence type (ITSGE1), grouped with that of the *A. ostoyae* Rockies/New Hampshire group (ITSOS1-ITSOS5).

3.3.3 IGS-1

Phylogenetic analyses of the IGS-1 region using three different inference methods showed two phylogeographically distinct groups (Rockies and Northwest/New Hampshire) of *A. ostoyae* as shown in the Bayesian radial 90% majority-rule consensus tree and corresponding map (Fig. 2-6). The Rockies group (IGSOS18-IGSOS23) was separated from the polytomous Northwest/New Hampshire group sequences (IGSOS1-IGSOS14) by a posterior probability of 100%. Several Northwest sequence types branched from the internal node of the Northwest/New Hampshire polytomy. The first of these sequence types (IGSOS15) split from this internal node with a posterior probability of 96%, whereas two other sequence types (IGSOS16 and IGSOS17) split from the basal node of the former by a posterior probability of 99% ending in bifurcation. Similar to the ITS region, non-hybrid genets with these sequence types are codefined to the Northwest group by the LSU analysis. Five *A. gemina* sequence types (IGSGE1-IGSGE5) were separated from the Northwest/New Hampshire group by a 100% posterior probability; however, one *A. gemina* sequence type (IGSGE6) grouped with that of the Northwest/New Hampshire polytomy. One genet (ST9) of *A. gemina* was found to harbor both IGSGE5 and IGSGE6 sequence types. This individual may represent an interspecific

hybrid between *A. ostoyae* and *A. gemina*, as previously shown by M.-S. Kim (per. comm.).

3.3.4 Phylogeographic congruency of rDNA regions

The Rockies and Northwest groups were observed in all three rDNA regions analyzed. The only inconsistency is the representative sequences from the New Hampshire isolate (ST1), which grouped with the Circumboreal sequence types for the LSU, the Rockies sequence types for the ITS, and the Northwest sequence types for the IGS-1. The three other Circumboreal group representatives from the LSU analysis could not be included in the ITS or IGS-1 analysis due to heterogeneity in their PCR products. The genet (USSR) from Primorye, Russia, consisted of two different sequence types (LSUOS1 and LSUOS2) in the LSU region suggesting that a greater sample size in regions outside the western USA may yield more regionally defined groups of *A. ostoyae* throughout the northern hemisphere.

3.4 Genbank similarities

As of September 23, 2004, GenBank contained only 20 *A. ostoyae* sequences that covered some part of the LSU, ITS, or IGS-1 rDNA regions. The only submission identical to sequences from this study was that of GenBank accession AY228342 (VAN DER STAR et al. 2003). The collection material from which this sequence had been derived was collected near Vancouver, British Columbia, Canada. The 627 basepairs of the LSU rDNA region from this sequence are identical to LSU Northwest group sequence types LSUOS9, LSUOS10, and LSUOS11; while the complete ITS sequence was

identical to ITS Northwest group sequence type ITSOS6. Another notable GenBank entry is that of accession D89924 derived from an *A. ostoyae* isolate from Asahikawa, Japan and is noted as being mycorrhizal with orchid (Terashima et al. 1998). This 590-basepair sequence only differed from that of the IGS-1 Rockies group sequence type IGSOS18 by a single basepair and a single 20-basepair indel.

4 Discussion

4.1 Circumboreal group

It has been hypothesized that the origin of southern hemisphere *Armillaria* species, *A. novae-zelandiae* (G.Stev.) Herink and *A. luteobubalina,* may precede the breakup of the supercontinent Gondwanaland (COETZEE et al. 2003). This concept raises considerations about the influence of historical paleogeographic and paleoclimatic events on modern day distribution of phylogeographically distinct groups of *A. ostoyae* and other *Armillaria* species of the northern hemisphere. In the northern hemisphere, the Circumboreal group of *A. ostoyae* occurs on three continents, and this group shares identical LSU sequence types with several other *Armillaria* species. This pattern may indicate sequence conservation since the Jurassic period, with an origin that may precede Pangea. Although this postulated date of origin is earlier than that of current estimates for the divergence of *Armillaria* (PIERCEY-NORMORE et al. 2000), current trends in the estimation of fungal divergence times have been pushing back earlier estimates (TAYLOR 2004).

4.2 Rockies group

This study provides evidence that the Rockies group of *A. ostoyae* may be ancestral to that of *A. nabsnona*. *Armillaria nabsnona* sequence types are similar to *A. ostoyae* Rockies sequence types in the LSU. As shown by the Circumboreal *A. ostoyae* group, relationships based on the LSU may date back hundreds of millions of years. A similar relationship between *A. nabsnona* and *A. ostoyae* was not observed for the more variable rDNA repeat regions. These relative differences may reflect different evolutionary rates for various rDNA regions. Similarly, the single *A. ostoyae* isolate from eastern USA used in this study clusters with either the Rockies group using ITS data or the Northwest group using IGS-1 data. This discrepancy perhaps represents an anomaly derived from the small sample size from the eastern USA. However, it must also be considered that these "non-coding" rDNA regions may evolve at different rates and / or may have been subjected to selection pressure. Also, local ecological factors may influence rates of evolution, so that organisms of the same species may evolve at different rates even within the same rDNA regions. This premise, opposing neutral theory (KIMURA 1983), supports evidence that evolutionary rates are not always constant across lineages (BRITTEN 1986; AVISE 1994; LI 1997; SANDERSON 1997). Although the potential function of these regions remains cryptic, growing evidence suggests that these "non-coding" regions may have functions, such as influencing growth rates (ELSER et al. 2000; GOROKHOVA et al 2002).

The similarity of the Rockies group sequence types to that of a sequence found in Japan indicates a possible evolutionary link between *A. ostoyae* of North America and Asia. One possible avenue for the intercontinental movement of *A. ostoyae* was an ancient land bridge that connected North America with Asia over 65 million years ago

(TIFFNEY 1985a, 1985b; WEN 1999; XIANG and SOLTIS 2001). At that time, Alaska apparently had a temperate climate that could have supported *Armillaria* species for several million years (SPICER and PARRISH 1986; BROUWERS et al. 1987; WOLFE and UPCHURCH 1987).

4.3 Northwest group

The distribution of Northwest group of *A. ostoyae* is similar to a well-known distribution pattern shared by over 100 species known as the mesic forest disjunct (BRUNSFELD et al. 2001). Our study does not provide definitive evidence as to when this group diverged from the Rockies and Circumboreal groups, but it does show a high variability and relatively large polytomies for each of the three rDNA regions analyzed. These polytomies show sequence types having equal interrelatedness, which can result from a sample size that is too small to resolve differences (soft polytomy) or from adaptive radiation (hard polytomy) (MADDISON 1989). Adaptive radiation occurs when a single lineage produces descendants with a wide variety of adaptive forms. The history of the Northwest is filled with events that may have favored adaptive radiation. The history of the ever-changing environment of the Pacific Northwest has been filled with insect outbreaks, catastrophic fires, volcanic activity, glacial events, and some of the largest floods known in the history of earth. Heterogeneous environments created by such events may have favored diverse genotypes within this group. Further study of this group with DNA analysis techniques with greater sensitivity may reveal phylogenies related to these events.

4.4 Heterogeneity (intra-individual variation)

Direct PCR has been shown to detect 90% of the heterogeneous rDNA products in an individual and the relative peak height seems to reflect relative copy number (RAUSCHER et al. 2002). In this study, heterogeneous products indicating intraspecific and intragenomic variation within *A. ostoyae* were common in all regions analyzed. Several genotype arrangements are possible when two or more ambiguously coded nucleotide positions exist for an individual adding uncertainty to genotyping (PRESA et al. 2002). For example, product representation containing two ambiguous nucleotides (R = A/G) and (Y = C/T) has four possible genotype combinations. The individual may contain all four combinations but may only contain two of the four combinations making any of the four combinations uncertain. This uncertainty grows as the number of ambiguous nucleotides increase. For example, three ambiguous nucleotides have eight possible combinations of which two to eight may actually occur, four ambiguous nucleotides have sixteen possible combinations, and so forth. Often these heterogeneous individuals appear to be hybrids among known sequence types (having ambiguous nucleotide positions at locations that are polymorphic among known clean sequences). Including these suspect hybrids not only adds uncertainty of genotypes but also can potentially reduce phylogenetic signal by collapsing clades between that of its parental origins.

The process of concerted evolution is thought to homogenize rDNA repeats throughout the genome of most individual eukaryotes (ELDER AND TURNER 1995). However in *Armillaria* species heterogeneity within rDNA repeats seems to be the rule rather than the exception. Over time, concerted evolution may homogenize rDNA

repeats; however, evidence within *Armillaria* species shows that individuals can retain rDNA sequences from more than one divergent parent following mating in culture (KIM et al. 2001). The direct-PCR results of this study suggest that this phenomenon happens in nature as well. The rate and mechanisms that control homogenization within *Armillaria* species remain unknown, perhaps further mating tests coupled with sequencing techniques that probe for differences among repeats may help address these questions. Phylogenetic analysis based on sequences derived from few-several rDNA clones may produce erroneous results because scores of clones may be needed to sufficiently detect variation among rDNA repeats (RAUSCHER et al. 2002); however, this problem may be circumvented by using sequences derived from direct PCR. A single clone from an individual may represent a rare genotype from ancient parental heritage and several clones may not be enough to detect multiple genotypes present within a heterogeneous individual. Although we were successful in showing heterogeneity within individuals using direct PCR, our deciphered genotypes may in fact represent a consensus of sequence types, each containing minor variations. If a single individual is able to retain rare ancestral genotypes due to incomplete homogenization from concerted evolution, it is possible that heterogeneous sequences from individuals can be used to produce phylogenies that show parental evolutionary history of those individuals.

4.5 Protein coding versus rDNA genes

Current trends in evolutionary studies of fungi have encouraged many researchers to switch from using rDNA genes to sequences that encode protein, such as β–tubulin and elongation factor 1-α (BRUNS and SHEFFERSON 2004). These protein-coding sequences

have been favored because they do not contain as many indels (insertions and deletions) that are found in the rDNA genes. Thus, protein-coding sequences are more easily aligned and variation among repeats is not a problem when sequences are present as a single copy. However, variation among repeat types can provide significant phylogenetic insights that are not available with single-copy genes, if highly ambiguous alignments (i.e. attempting to align *A. mellea sensu stricto* with that of *A. ostoyae* in the ITS and IGS-1) or ambiguous sequences are excluded from analysis. Through vigilant sequence editing, techniques to decipher heterogeneity within an individual (Fig. 2-1, 2-2, and 2-3), and elimination of ambiguity within the dataset, we were able to show rDNA genes can harbor phylogenetic information that has previously been unavailable or used inappropriately. With this approach, rDNA sequences may provide more powerful information toward understanding evolutionary events, because the divergent parental histories should be represented within heterogeneous rDNA from a single organism.

4.6 Implications of hybridization

Although once thought to be very rare, hybridization has now been recognized in most fungal phyla (BRASIER 2000; SCHARDL and CRAVEN 2003) and families of organisms (DE SOUZA et al. 2004). Furthermore, most individuals in this study showed hybridization at some level. Three different levels of hybridization are observed in this study: 1) interspecific hybridization between two species (e.g., *A. gemina* x *A. ostoyae* within the IGS-1), 2) intraspecific hybridization between divergent groups of the same species (e.g., Rockies *A. ostoyae* x Circumboreal *A. ostoyae* in the LSU), and 3) intraspecific hybridization within the same group of the same species (detected in all three regions

analyzed). In theory, a hybrid may have a greater ability to adapt to diverse environmental niches than that either of its parents, thereby allowing hybrids to occupy new or changing niches from which speciation events may subsequently occur (FOWLER and LEVIN 1984; RIESEBERG et al., 1990, 2003; RIESEBERG, 1991; ARNOLD, 1997; GOLDMAN, 2004). In addition, hybrids often show increased vigor and ability to exploit resources (INGVARSSON and WHITLOCK 2000; EBERT et al. 2002; KAYE and LAWRENCE 2003). Differences in virulence, pathogenicity, and epidemiology are often most notable among hybrid fungal pathogens; however, fungal mutualists with increased vigor may better adapt and exploit resources for their hosts as well (SCHARDL and CRAVEN 2003).

4.7 Future Studies

Continued studies are underway at the USDA Forest Service - RMRS, Forestry Sciences Laboratory in Moscow, Idaho to: 1) understand rates and mechanisms of concerted evolution within *Armillaria* species; 2) understand relationships among *Armillaria* species, groups, and individuals; 3) analyze possible contribution of phylogeographically distinct groups to differences in pathogenicity and epidemiology; and 4) determine phylogenetic relationships to historical paleogeographic and paleoclimatic events.

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References

ALTSCHUL, S. F.; MADDEN, T. L.; ALEJANDRO, A.; SCHÄFFER, A. A.; ZHANG, J.; ZHANG, Z.; MILLER, W.; LIPMAN, D. J., 1997: Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucl. Acids Res. **25,** 3389-3402.

ANDERSON, J. B.; ULLRICH, R. C., 1979: Biological species of *Armillaria mellea* in North America. Mycologia **71,** 402-414.

ANDERSON, J. B.; STASOVSKI, E., 1992: Molecular phylogeny of Northern Hemisphere species of *Armillaria*. Mycologia **84,** 505-516.

ARNOLD, M. L., 1997: Natural hybridization and evolution. Oxford University Press, Oxford, UK.

AVISE, J. C., 1994: Molecular markers, natural history and evolution. Chapman and Hall, New York.

BANIK, M. T.; VOLK, T. J.; BURDSALL, H. H. JR., 1996: *Armillaria* species of the Olympic Peninsula of Washington state, including confirmation of North American biological species XI. Mycologia **88**, 492-496.

BÉRUBÉ, J. A.; DESSUREAULT, M., 1988: Morphological characterization of *Armillaria ostoyae* and *Armillaria sinapina sp. nov*. Can. J. Bot. **66,** 2027-2034.

BRASIER, C. M., 2000: The rise of hybrid fungi. Nature **405**, 134-135.

BRITTEN, R. J., 1986: Rates of DNA sequence evolution differ between taxonomic groups. Science **231**, 1393-1398.

BROUWERS, E. M.; CLEMENS, W. A.; SPICER, R. A.; AGER, T. A.; CARTER, L. D., SLITER, W. V., 1987: Dinosaurs on the North Slope, Alaska: High latitude, latest cretaceous environments. Science **237**, 1608-1610

BRUNS, T. D.; SHEFFERSON, R. P., 2004: Evolutionary studies of ectomycorrhizal fungi: recent advances and future directions. Can. J. Bot. **82**, 1122-1132.

BRUNSFELD, S. J.; SULLIVAN, J.; SOLTIS, D. E.; SOLTIS, P. S., 2001: Comparative phylogeography of northwestern North America: A synthesis. In: Integrating Ecology and Evolutionary Processes in a Spatial Context. Ed. by SILVERTOWN, J.; ANTONOVI, J., Oxford, UK: Blackwell Science. pp. 319-339.

CHA, R. S.; ZARBL, H.; KEOHAVONG, P.; THILLY, W. G., 1992: Mismatch amplification mutation assay (MAMA); application to the c-H-*ras* gene. PCR Methods and Applications **2**, 14-20.

CHILLALI, M.; WIPF, D.; GUILLAUMIN, J.-J.; MOHAMMED, C.; BOTTON, B., 1998: Phylogenetic analysis of the European *Armillaria* species based on the sequences of the Internal Transcribed Spacer (ITS) of ribosomal DNA. New Phytologist **138,** 553-561.

COETZEE, M. P. A.; WINGFIELD, B. D.; HARRINGTON, T. C., 2000: Geographical diversity of *Armillaria mellea s.s.* based on phylogenetic analysis. Mycologia **92,** 105-113.

COETZEE, M. P. A.; WINGFIELD, B. D.; RIDLEY, G. S.; WINGFIELD, M. J., 2003: Molecular identification and phylogeny of *Armillaria* isolates from South America and Indo-Malaysia. Mycologia **95,** 285-293.

CRUICKSHANK, M., 2000: Volume loss of Douglas-fir infected with *Armillaria ostoyae*. In: Proceedings, From Science to Management and Back: A Science Forum for Southern Interior Ecosystems of British Columbia. Ed. by HOLLSTEDT, C.; SUTHERLAND, K.; INNES, T. Kamloops, B.C., Canada: Southern Interior Forest Extention and Research Partnership, pp. 127-129.

DE SOUZA, F. A.; KOWALCHUK, G. A.; LEEFLANG, P.; VAN VEEN, J. A.; SMIT, E., 2004: PCR-denaturing gradient gel electrophoresis profiling of inter- and intraspecies 18S rRNA gene sequence heterogeneity is an accurate and sensitive method to assess species diversity of arbuscular mycorrhizal fungi of the genus *Gigaspora*. Appl. Environ. Microbiol. **70**, 1413-1424.

DUCHESNE, L. C.; ANDERSON, J. B., 1990: Location and direction of transcription of the 5S rRNA gene in *Armillaria*. Mycol. Res. **94**, 266-269.

DUNNE, C. P.; GLEN, M.; TOMMERUP, I. C.; SHEARER, B. L.; HARDY, G. E. S. J., 2002: Sequence variation in the rDNA ITS of Australian *Armillaria* species and intra-specific variation in *A. luteobubalina*. Australasian Plant Path. **31,** 241-251.

EBERT, D.; HAAG, C.; KIRKPATRICK, M.; RIEK, M.; HOTTINGER, J. W.; PAJUNEN, V. I., 2002: A selective advantage to immigrant genes in a *Daphnia* metapopulation. Science 295, 485-488.

ELDER, J. F. JR.; TURNER, B. J., 1995: Concerted evolution of repetitive DNA sequences in eukaryotes. Q. Rev. Biol. **70**, 297-320.

ELSER, J. J.; STERNER, R. W.; GOROKHOVA, E.; FAGEN, W. F.; MARKOW, T. A.; COTNER, J. B.; HARRISON, J. F.; HOBBIE, S. E.; ODELL, G. M.; WEIDER, L. J., 2000: Biological stoichiometry from genes to ecosystems. Ecol. Letters **3**, 540-550.

FELSENSTEIN, J., 1985: Confidence limits on phylogenies: An approach using the bootstrap. Evolution **39**, 783-791.

FERGUSON, B. A.; DREISBACH, T. A.; PARKS, C. G.; FILIP, G. M.; SCHMITT, C. L., 2003: Coarse-scale population structure of pathogenic *Armillaria* species in a mixed-conifer forest in the Blue Mountains of northeast Oregon. Can. J. For. Res. **33,** 612-623.

FINS, L.; BYLER, J. W.; FERGUSON, D. E.; HARVEY, A. E.; MAHALOVICH, M. F.; MCDONALD, G. I.; MILLER, D. L.; SCHWANDT, J. W.; ZACK, A., 2002: Return of the giants: restoring western white pine to the inland Northwest. J. For. **100**, 20-26.

FOWLER, N. L.; LEVIN, D. A., 1984: Ecological constraints on the establishment of a novel polyploid in competition with its diploid progenitor. Amer. Nat. **12**, 703-711.

GARDES, M.; BRUNS, T. D., 1993: ITS primers with enhanced specificity of basidiomycetes: application to the identification of mycorrhizae and rusts. Mol. Ecol. **2**, 113-118.

GOHEEN, D. J.; OTROSINA, W. J., 1998: Characteristics and consequences of root diseases in forests of western North America. In: User's Guide to the Western Root Disease Model, Version 3.0. Tech. coord. by FRANKEL, S. J. Gen. Tech. Rep. PSW-GTR 165. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Station, pp. 3-8.

GOLDMAN, D. H.; JANSEN, R. K.; VAN DEN BERG, C.; LEITCH, I. J.; FAY, M. F.; CHASE, M.W., 2004: Molecular and cytological examination of Calopogon (Orchidaceae, Epidendroideae): circumscription, phylogeny, polyploidy, and possible hybrid speciation. Amer. J. of Bot. **91**, 707-723.

GOROKHOVA, E.; DOWLING, T. E.; WEIDER, L. J.; CREASE, T. J.; ELSER, J. J., 2002: Functional and ecological significance of rDNA intergenic spacer variation in a clonal organism under divergent selection for production rate. Proc. R. Soc. Lond. **269**, 2373-2379.

GRUELL, G. E.; SCHMIT, W. C.; ARNO, S. F.; REICH, W. J., 1982: Seventy years of vegetative change in a managed ponderosa pine forest in western Montana - implications for resource management. Gen. Tech. Rep. INT-130. Odgen, UT, USA: U.S. Department of Agriculture, Forest Service, Intermountain Research Station, 42 pp.

GUILLAUMIN, J.-J.; MOHAMMED, C.; BERTHELAY, S., 1989: *Armillaria* species in the northern temperate hemisphere. In: Proceedings of the 7th International Conference on Root and Butt Rots, Victoria and Vernon, British Columbia, Canada, 9-16 August 1998 Ed. by MORRISON, D.J. Victoria, BC: Forestry Canada, pp. 27-43.

GUILLAUMIN, J.-J.; ANDERSON, J. B.; KORHONEN, K., 1991: Life cycle, interfertility, and biological species. In: Armillaria Root Disease. Ed. by SHAW, C.G. III.; KILE, G.A. Agriculture Handbook No. 691. Washington, DC: USDA, Forest Service, pp. 10-20.

HALL, T. A., 1999: BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids. Symp. Ser. **41**, 95-98.

HARRINGTON, T. C.; WINGFIELD, B. D., 1995: A PCR-based identification method for species of *Armillaria*. Mycologia **87**, 280-288.

HARVEY, A. E., 1994: Integrated roles for insects, diseases and decomposers in fire dominated forests of the Inland Western United States: past, present and future forest health. J. Sust. For. **2,** 211-220.

HUSELSENBECK, J. P.; RONQUIST, F., 2001: MrBayes: Bayesian inference of phylogenetic trees. Biometrics **17**, 754-755.

HWANG, S.-K.; KIM, J.-G., 1995: Nucleotide sequence analysis of the 5S ribosomal RNA gene of the mushroom *Tricholoma matsutake*. Jour. Microbiol. **33**, 136-141.

INGVARSSON, P. K.; WHITLOCK, M. C., 2000: Heterosis increase the effective migration rate. Proc. R. Soc. London **B267**, 1321.

KAY, T. N.; LAWRENCE, B., 2003: Fitness effects of inbreeding and outbreeding on golden paintbrush (*Castilleja levisecta*): Implications for recovery and reintroduction. Institute for Applied Ecology, Corvallis, Oregon and Washington Department of Natural Resources, Olympia, Washington.

KILE, G. A.; MCDONALD, G. I.; BYLER, J. W., 1991: Ecology and disease in natural forests. In: Armillaria Root Disease. Agriculture Handbook No. 169. Ed. by SHAW, C.G. III.; KILE, G.A., Washington, DC: United States Dept. of Agriculture, Forest Service, pp. 62-75.

KIM, M.-S.; KLOPFENSTEIN, N. B.; MCDONALD, G. I.; ARUMUGANATHAN, K.; VIDAVER, A. K., 2000: Characterization of North American *Armillaria* species by nuclear DNA content and RFLP analysis. Mycologia **92**, 874-883.

KIM, M.-S.; KLOPFENSTEIN, N. B.; MCDONALD, G. I.; ARUMUGANATHAN, K.; VIDAVER, A. K., 2001: Use of flow cytometry, fluorescence microscopy, and PCR-based techniques to assess intraspecific and interspecific matings of *Armillaria* species. Mycol. Res. **105**, 153-163.

KIMURA, M., 1983: The neutral theory of molecular evolution. Cambridge University Press. Cambridge.

KORHONEN, K., 1978: Interfertility and clonal size in the *Armillariella mellea* complex. Karstenia **18,** 31-42.

KUMAR, S.; TAMURA, K.; JAKOBSEN, I. B; NEI, M., 2001: MEGA2: Molecular Evolutionary Genetics Analysis software. Bioinformatics 2001 Dec; **17,** 1244-1245.

LANGSTON, N., 1995: Forest Dreams, Forest Nightmares: The Paradox of Old-Growth in the Inland West. Seattle, WA: University of Washington Press, 368 pp.

LI, W.-H., 1997: Molecular evolution. Sinauer, Sunderland, Mass.

MADDISON, W. P., 1989: Reconstructing character evolution on polytomous cladograms. Cladistics **5**, 365-377.

MALLETT, K. I.; HOPKIN, A. A.; BLENIS, P. V., 1989: Vegetative incompatibility in diploid isolates of *Armillaria* North American biological species I and V. Can. J. Bot. **67**, 3083-3089.

MCDONALD, G. I.; MARTIN N. E.; HARVEY A. E., 1987: Occurrence of *Armillaria* spp. in forests of the northern Rocky Mountains. Res. Pap. INT-381. Ogden, UT, USA: U.S. Department of Agriculture, Intermountain Research Station. 7 p.

MCDONALD, G. I.; MARTIN, N. E., 1988: *Armillaria* in the northern Rockies: Delineation of isolates into clones. USDA For. Serv. Res. Pap. INT-RP-385. Ogden, UT, USA; U.S. Dept. of Agriculture, Forest Service, Intermountain Research Station. 13 p.

MCDONALD, G. I., 1990. Connecting forest productivity to behavior of soil-borne diseases. In: Proceedings-Management and Productivity of Western Montane forest Soils. Comp. by HARVEY A. E.; NEUENSCHWANDER, L. F. Ogden, UT: U.S. Department of Agriculture, Forest Service, Intermountain Research Station, pp. 129-144.

MCDONALD, G. I.; EVANS, J. S.; MOEUR, M.; RICE, T. M.; STRAND, E. K., 2003: Using digital terrain modeling and satellite imagery to map interactions among fire and forest microbes. In: Proceedings of Fire Conference 2000: The First National Congress on Fire Ecology, Prevention, and Management. Miscellaneous Publication No. 13. Ed. by GALLEY, K. E. M.; KLINGER, R. C.; SUGIHARA, N. G. Tallahassee, FL, U.S.A.: Tall Timbers Research Station. pp. 100-110.

MONCALVO, J.-M.; LUTZONI, F. M.; REHNER, S. A.; JOHNSON, J.; VILGALYS, R., 2000: Phylogenetic relationships of agaric fungi based on nuclear large subunit ribosomal DNA sequences. Syst. Biol. **49**, 278-305.

MORRISON, D. J.; PELLOW, K. W., 2002: Variation in virulence among isolates of *Armillaria ostoyae.* For. Path. **32,** 99-107.

NYLANDER, J. A. A., 2003. MrModeltest 1.0b A simplified version of David Posada's "Modeltest 3.06". Department of Systematic Zoology. Uppsala, Sweden. [Online] Available http://www.ebc.uu.se/systzoo/staff/nylander.html [Last accessed 10, February 2005]

OMDAL, D. W.; SHAW, C. G. III.; JACOBI, W. R.; WAGER, T. C., 1995: Variation in pathogenicity and virulence of isolates of *Armillaria ostoyae* on eight tree species. Plant Dis. **79,** 939-944.

OTA, Y.; MATSUSHITA, N.; NAGASAWA, E.; TERASHITA, T.; FUKUDA, K.; SUZUKI, K., 1998: Biological Species of *Armillaria* in Japan. Plant Disease **85,** 537-543.

PARTRIDGE, A. D.; CANFIELD, E. R.; CHACKO, R. J., 1977: Forest Pathology Outline. University of Idaho Forest, Wildlife and Range Experiment Station, Moscow, ID, USA Contribution No. 49. 236 p.

PEREZ-SIERRA, A.; WHITEHEAD, D.; WHITEHEAD, M., 2000: Molecular methods used for the detection and identification of *Armillaria*. In: Armillaria Root Rot: Biology and Control of Honey Fungus. Ed. by FOX, R. T. F. Andover, UK: Intercept, pp. 95-110.

PIERCEY-NORMORE, M. D.; EGGER, K. N.; BÉRUBÉ, J. A., 1997: Molecular phylogeny and evolutionary divergence of North American Biological species of *Armillaria*. Mol. Phylo. Evol. **10,** 49-66.

PRESA, B. G.; PARDO, B. G.; MARTÍNEZ, P.; BERNATCHEZ, L., 2002: Phylogeographic congruence between mtDNA and rDNA ITS markers in brown trout. Mol. Biol. Evol. **19**, 2161-2175.

RAUSCHER, J. T.; DOYLE, J. J.; BROWN, H. D., 2002: Internal transcribed spacer repeat-specific primers and the analysis of hybridization in the *Glycine tomentella* (Leguminosae) polyploid complex. Mol. Ecol. **11**, 2691-2702.

RIESEBERG, L. H., 1991: Homoploid reticulate evolution in *Helianthus* (Asteraceae): evidence from ribosomal genes. Amer. J. Bot. **78**, 1218-1237.

RIESEBERG, L. H.; CARTER, R.; ZONA, S., 1990: Molecular tests of the hypothesized hybrid origin of two diploid *Helianthus* species (Asteraceae). Evolution **44**, 1498-1511.

RIESEBERG, L. H.; RAYMOND, O.; ROSENTHAL, D. M.; LAI, Z.; LIVINGSTONE, K.; NAKAZATO, T.; MURPHY, J. L.; SCHWARZBACH, A. E.; DONOVAN, L. A.; LEXER, C., 2003: Major ecological transitions in wild sunflowers facilitated by hybridization. Science **301**, 1211-1216.

SAITOU, N; NEI, M., 1987: The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4**, 406-425.

SANDERSON, M. J., 1997: A nonparametric approach to estimating divergence times in the absence of rate constancy. Mol. Biol. Evol. **14**, 1218-1231.

SCHARDL, C. L.; CRAVEN, D., 2003: Interspecific hybridization in plant-associated fungi and oomycetes: a review. Mol. Ecol. **12**, 2861-2873.

SCHMITT, C. L., 2001: Root Diseases in Conifer Forested Communities in the Blue Mountains of Northeastern Oregon and Southeastern Washington – Detection and Management, Values and Impacts. USDA Forest Service, Wallowa-Whitman National Forest, Report BMPMSC-02-01, 44 p.

SCHULZE, S.; BAHNWEG, G.; MÖLLER, E. M.; SANDERMANN, H. JR., 1997: Identification of the genus *Armillaria* by specific amplification of an rDNA-ITS fragment and evaluation of genetic variation within *A. ostoyae* by rDNA-RFLP and RAPD analysis. Eur. J. For. Path. **27,** 225-239.

SICOLI, G.; FATEHI, J.; STENLID, J., 2003: Development of species-specific PCR primers on rDNA for the identification of European *Armillaria* species. For. Path. **33,** 287-297.

SIMMONS, M. P.; OCHOTERENA, H., 2000: Gaps as characters in sequence-based phylogenetic analyses. Syst. Biol. **49**, 369-381.

SMITH, M.; BRUHN, J.; ANDERSON, J., 1992: The fungus *Armillaria bulbosa* is among the largest and oldest living organisms. Nature **356**, 428-431.

SPICER, R. A.; PARRISH, J. T., 1986: Paleobotanical evidence for cool north polar climates in middle Cretaceous (Albian-Cenomanian) time. Geology **14**, 703-706.

STUART, J. D., 1998: Effects of fire suppression on ecosystems and diversity. In: Status and Trends of the Nation's Biological Resources. Ed. by MAC, M. J.; OPLER, P. A.; HAECKER, C. E. P.; DORAN, P. D. Reston, VA, USA: U.S. Department of the Interior, U.S. Geological Survey, 986 pp.

SWOFFORD, D. L., 2001: PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sunderland, MA, USA: Sinauer Associates.

TAYLOR, J. W., 2004: Geological time, evolutionary rates, and the history of fungi. University of California, Berkeley, CA. Presented at: Annual meeting of the American Phytopathological Society, August 2nd 2004, Anaheim, CA.

TERASHIMA, K.; CHA, J. Y.; YAJIMA, T.; IGARASHI, T.; MIURA, K., 1998: Phylogenetic analysis of Japanese *Armillaria* based on the intergenic spacer (IGS) sequences of their ribosomal DNA. Eur. J. For. Path. **28,** 11-19.

TIFFNEY, B. H., 1985a: The Eocene North Atlantic Land Bridge: its importance in tertiary and modern phytogeography of the Northern Hemisphere. J. Arnold Arbor Harv. Univ. **66**, 243-273.

TIFFNEY, B. H., 1985b: Perspectives on the origin of the floristic similarity between eastern Asia and eastern North America. J. Arnold Arbor Harv. Univ. **66**, 73-94.

VAN DER STAR, T.; BERBEE, M. L.; INDERBITZIN, P.; FISCHER, A. L., 2003: Direct GenBank Submission AY228342: Sequencing performed as class project Biology, 323, Submitted (03-FEB-2003). Archived at UBC herbarium F14310 Botany, University of British Columbia, Vancouver, BC.

VELDMAN, T.J.; KLOOTWIJK, J.; DE REGT, V. C. H. F.; RUDI, R. J., 1981: The primary and secondary structure of yeast 26S rRNA. Nucl. Acids Res. **9**, 6935-6952.

VILGALYS, R., 24 June 2004: Conserved primer sequences for PCR amplification and sequencing from nuclear ribosomal RNA. Vilgalys lab, Duke University. [Online] Available http://www.biology.duke.edu/fungi/mycolab/primers.htm [Last accessed 10, February 2005].

VOLK, T. J.; BURDSALL, H. H. JR.; BANIK, M. T., 1996: *Armillaria nabsnona*, a new species from western North America. Mycologia **88**, 484-491.

WEN, J., 1999: Evolution of eastern Asian and eastern North American disjunct distributions in flowering plants. Annu. Rev. Ecol. Syst. **30**, 421-455.

WHITE, E. E.; DUBETZ, C. P.; CRUICKSHANK, M. G.; MORRISON, D. J., 1998: DNA diagnostic for *Armillaria* species in British Columbia: within and between species variation in the IGS-1 and IGS-2 regions. Mycologia **90,** 125-131.

WHITE, E.; MORRISON, D., 1999: DNA Testing: An application to Armillaria root disease. Technology Transfer Note 18. Victoria, BC, Canada: Canadian Forest Service, Pacific Forestry Centre.

WHITE, T. J.; BRUNS, T.; TAILOR, J., 1990: Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR protocols: a guide to methods and applications. Eds. by INNIS, M. A.; GELFAND, D. H.; SNINSKY, J. J.; WHITE, T. J., San Diego: Academic Press. pp. 315-322.

WILLIAMS, R. E.; SHAW, C. G. III.; WARGO, P. M.; SITES, W. H., 1989: Armillaria Root Disease. Forest Insect and Disease Leaflet 78 (rev.). U.S. Department of Agriculture, Forest Service. 8 p.

WOLFE, J. A.; UPCHURCH, G. R. Jr., 1987: North American non-marine climates and vegetation during the Late Cretaceous. Palaeogeogr. Palaeoclim. Palaeoecol. **61**, 33-77.

WU, Y.; JOHNSON, D. W.; ANGWIN, P. A., 1996: Identification of *Armillaria* species in the Rocky Mountain Region. Renewable Resources, Rocky Mountain Region, USDA Forest Service Tech. Rep. R2-58. 26 pp.

XIANG, Q.-Y.; SOLTIS D. E., 2001: Dispersal-Vicariance analyses of intercontinental disjuncts: historical biogeographical implications for angiosperms in the Northern Hemisphere. Int. J. Plant Sci. **162**: S29-S39.

APPENDIX 1: *Armillaria* distribution and relationship maps

Fig. *A1-1*. Map of *Armillaria* species distribution at the Intermountain Forest Tree Nutrition Cooperative Huckleberry Creek study site.

Maps of figures A1-1, A1-2, and A1-3 are based on *Armillaria* collections taken by members of the Intermountain Forest Tree Nutrition Cooperative, University of Idaho, during the summers of 1999 and 2000. Somatic incompatibility pairings to determine *Armillaria* genets as well as species identification using molecular methods were performed at the USDA Forest Service, Rocky Mountain Research Station, Moscow, ID. The Huckleberry Creek study site shows a single genet of *Armillaria ostoyae* occupying the extent of the site. As shown by the black stars, indicating mycelial bark fans present on live trees, this genet is an active pathogen that has created a large mortality center characterized by patchy openings. However, plots 6 and 7 are dominated by North American Biological Species (NABS) X and *A. ostoyae* is not present. This may be due to a possible interaction among NABS X and *A. ostoyae*, or the ecology of these plots favors NABS X and is not suitable for *A. ostoyae*.

Figure A1-1 **(continued)**

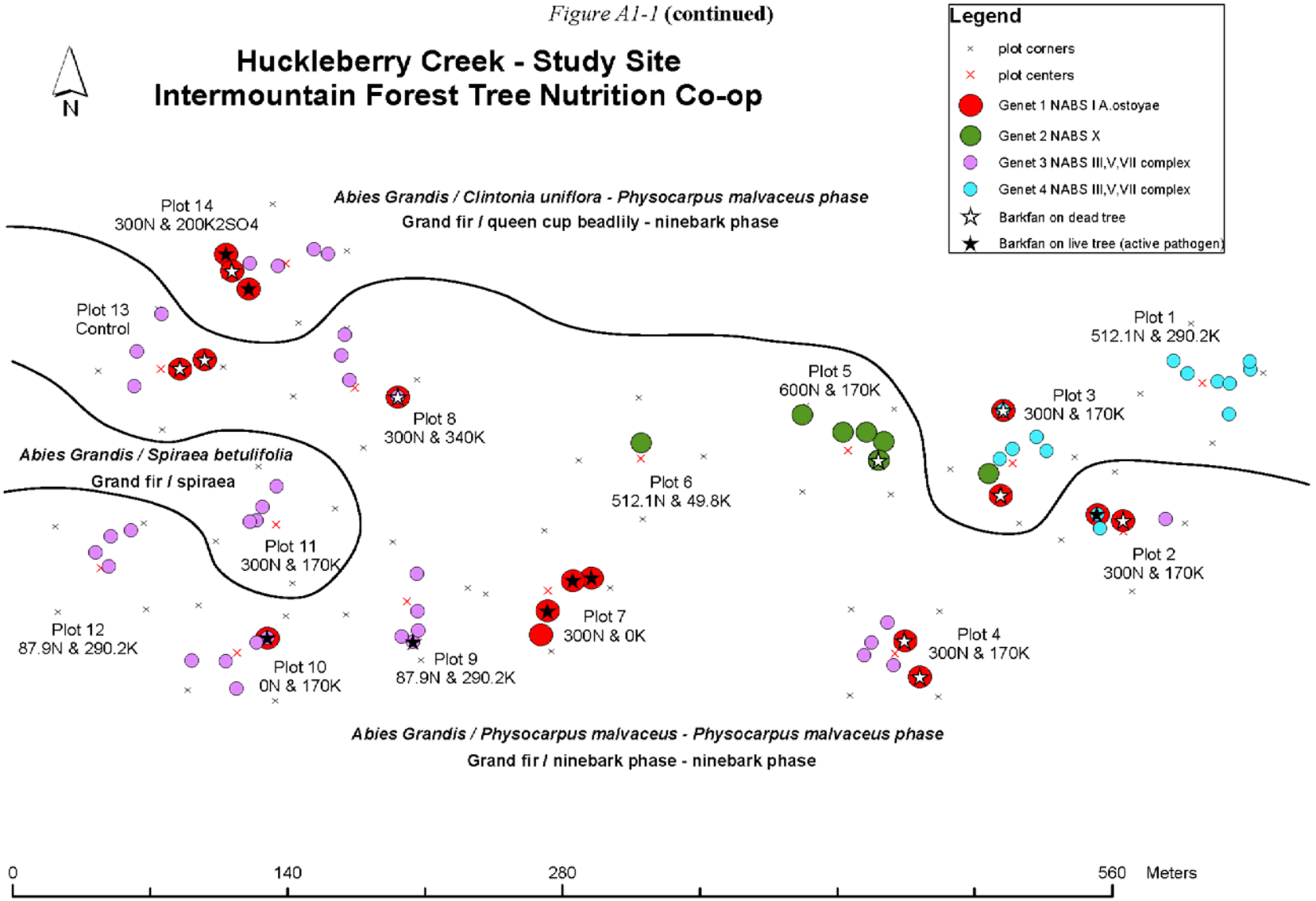


Fig. *A1-2*. Map of *Armillaria* species distribution at the Intermountain Forest Tree Nutrition Cooperative Spirit Lake study site.

The Spirit Lake study site shows three genets of *Armillaria ostoyae*. As indicated by mycelial bark fans on live trees (black stars on map) on plot 2, *A. ostoyae* genets one and two are active pathogens. A single genet of North American Biological Species (NABS) X is present on the site. On plots 3 and 4, genets of *A. ostoyae* overlap with the NABS X genet. *Armillaria ostoyae* genets on these plots show no sign of active pathogenicity. Pathogenicity of *A. ostoyae* may be limited by an interaction with NABS X or other ecological factors specific to these plots that favor growth of NABS X.

Figure A1-2 **(continued)**

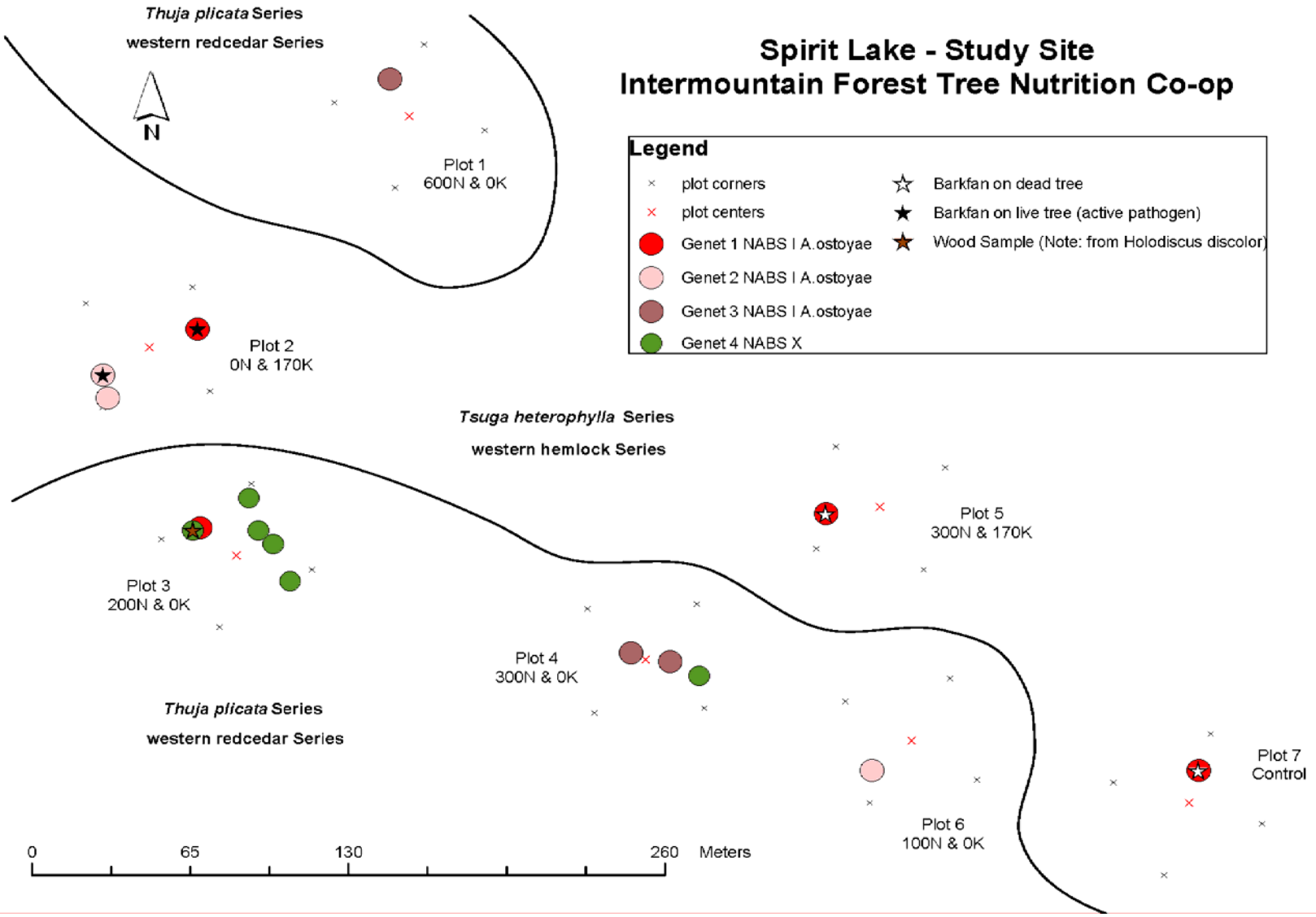
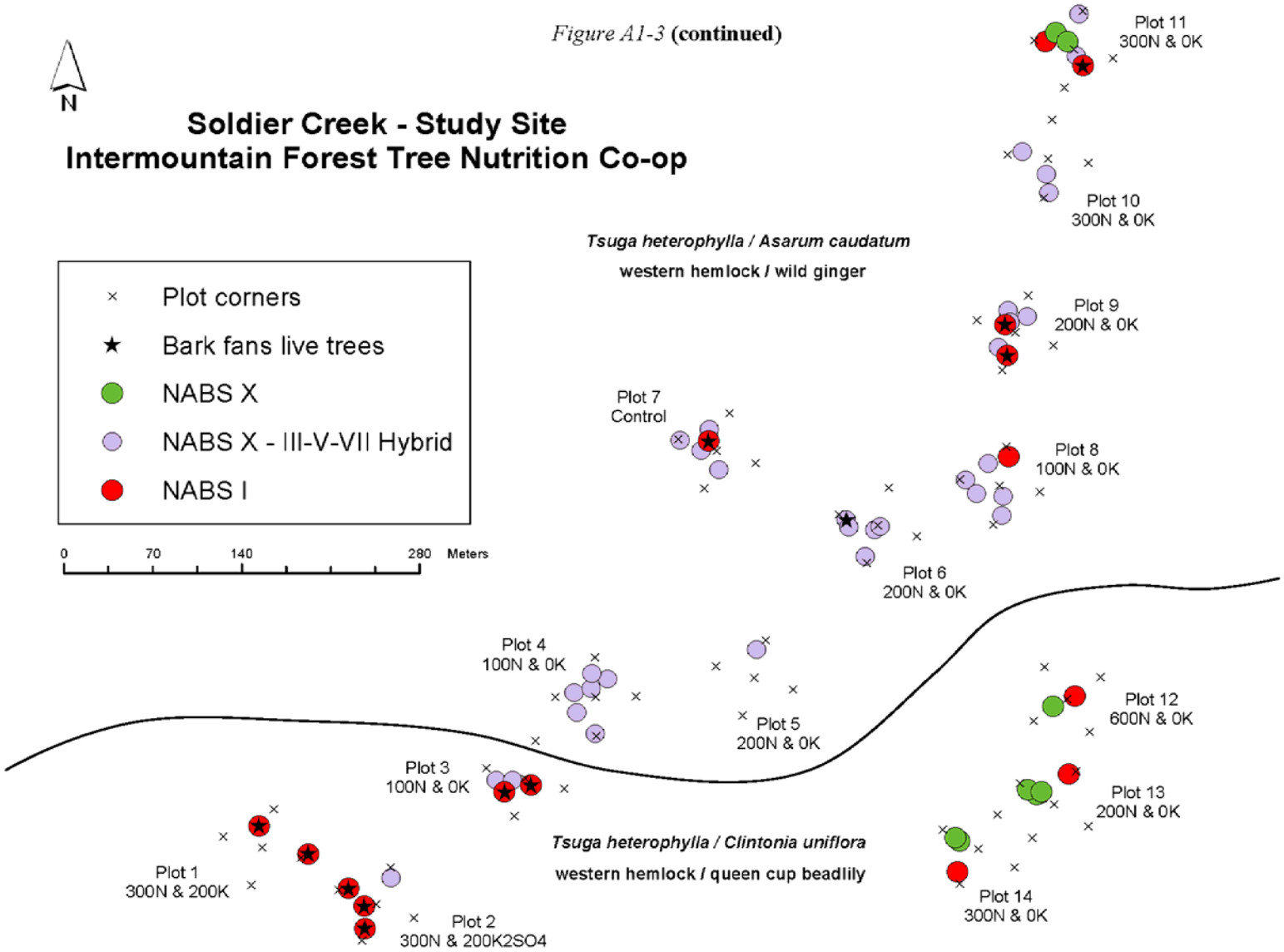


Fig. *A1-3*. Map of *Armillaria* species distribution at the Intermountain Forest Tree Nutrition Cooperative Soldier Creek study site.

The Soldier Creek study site shows a similar situation to that of the Spirit Lake study site (Fig. A1-2). *Armillaria ostoyae* is found in a non-pathogenic state where its distribution overlaps with North American Biological Species (NABS) X. Many of the plots where NABS X is not present, *A. ostoyae* collections indicate high pathogenicity. Limited pathogenicity of *A. ostoyae* may be due to *Armillaria* species interactions. Such interactions may be based on specific environmental conditions. Under these circumstances forest managers may want to manage for conditions that favor NABS X, as it is an indicator that mortality due to *A. ostoyae* will be limited.

Figure A1-3 **(continued)**



APPENDIX 2: Supplemental phylogenetic analysis

Table A2-1. Armillaria species key - Figs. A2-1, A2-2, A2-3, A2-4, A2-5, and A2-6

Species	**Sequence Types**
A. calvescens	LSUCA1-LSUCA3
A. cepistipes	LSUCE1 and LSUCE2
A. gallica	LSUGA1-LSUGA4
A. gemina	LSUGE1 and LSUGE2; ITSGE1; IGSGE1-IGSGE6
A. mellea	LSUME1-LSUME3
A. nabsnona	LSUNA1 and LSUNA2
A. ostoyae	LSUOS1-LSUOS11; ITSOS1-ITSOS25; IGSOS1-IGSOS23
A. sinapina	LSUSI1 and LSUSI2
A. tabescens	LSUTA1 and LSUTA2
NABS[1] X	LSUX1-LSUX4

[1] North American Biological Species

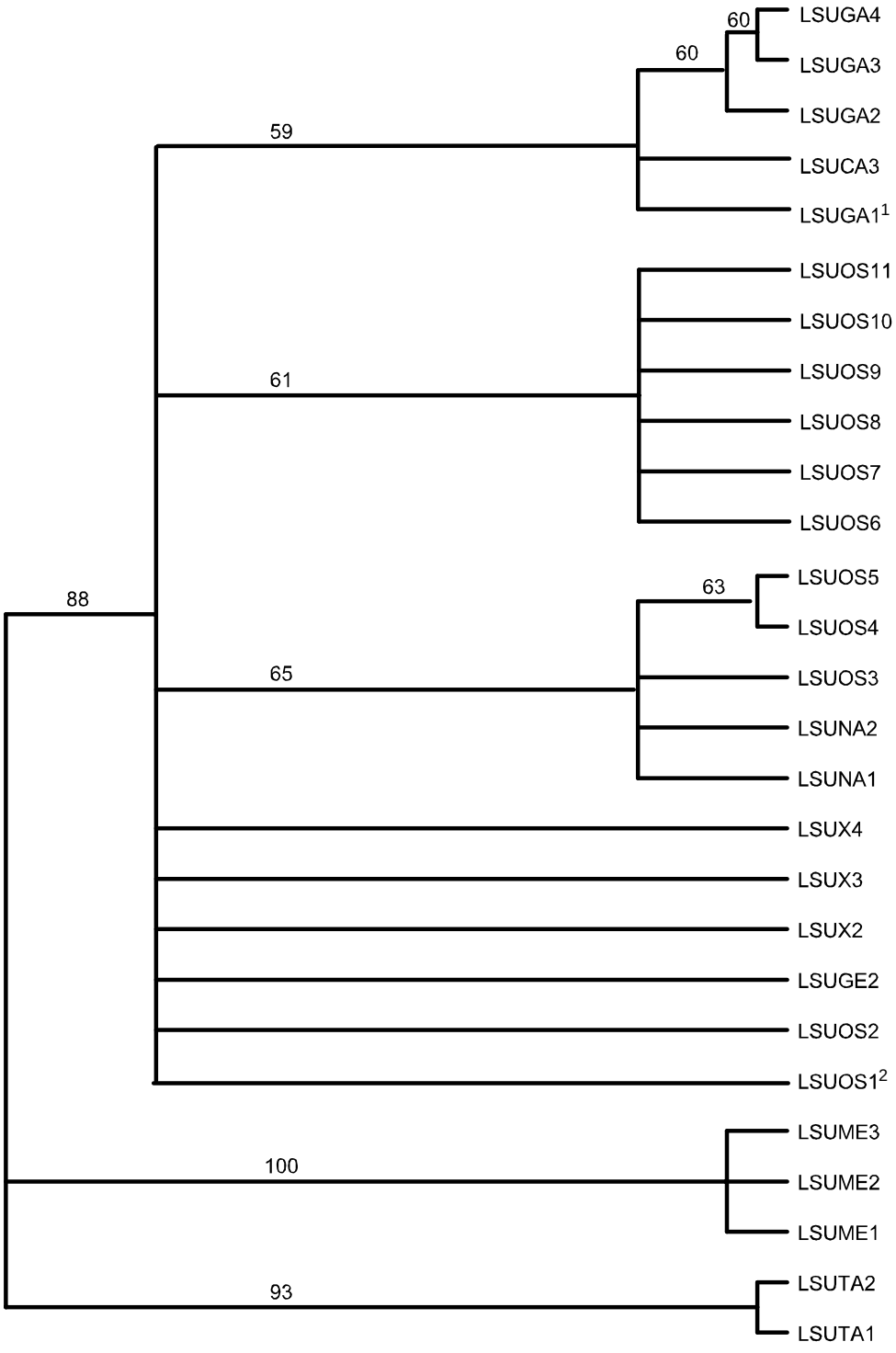


Fig. A2-1. Parsimony analysis of the nuclear large ribosomal subunit (LSU). Bootstrap 50% majority-rule consensus tree from parsimony analysis using heuristic search of the LSU sequence data. Bootstrap percentage values are indicated above branches based on 1,000 bootstrap replicates. [1]LSUGA1 also represents LSUCA2, LSUCE2, and LSUSI2. [2]LSUOS1 also represents LSUCA1, LSUCE1, LSUGE1, LSUSI1, and LSUX1.

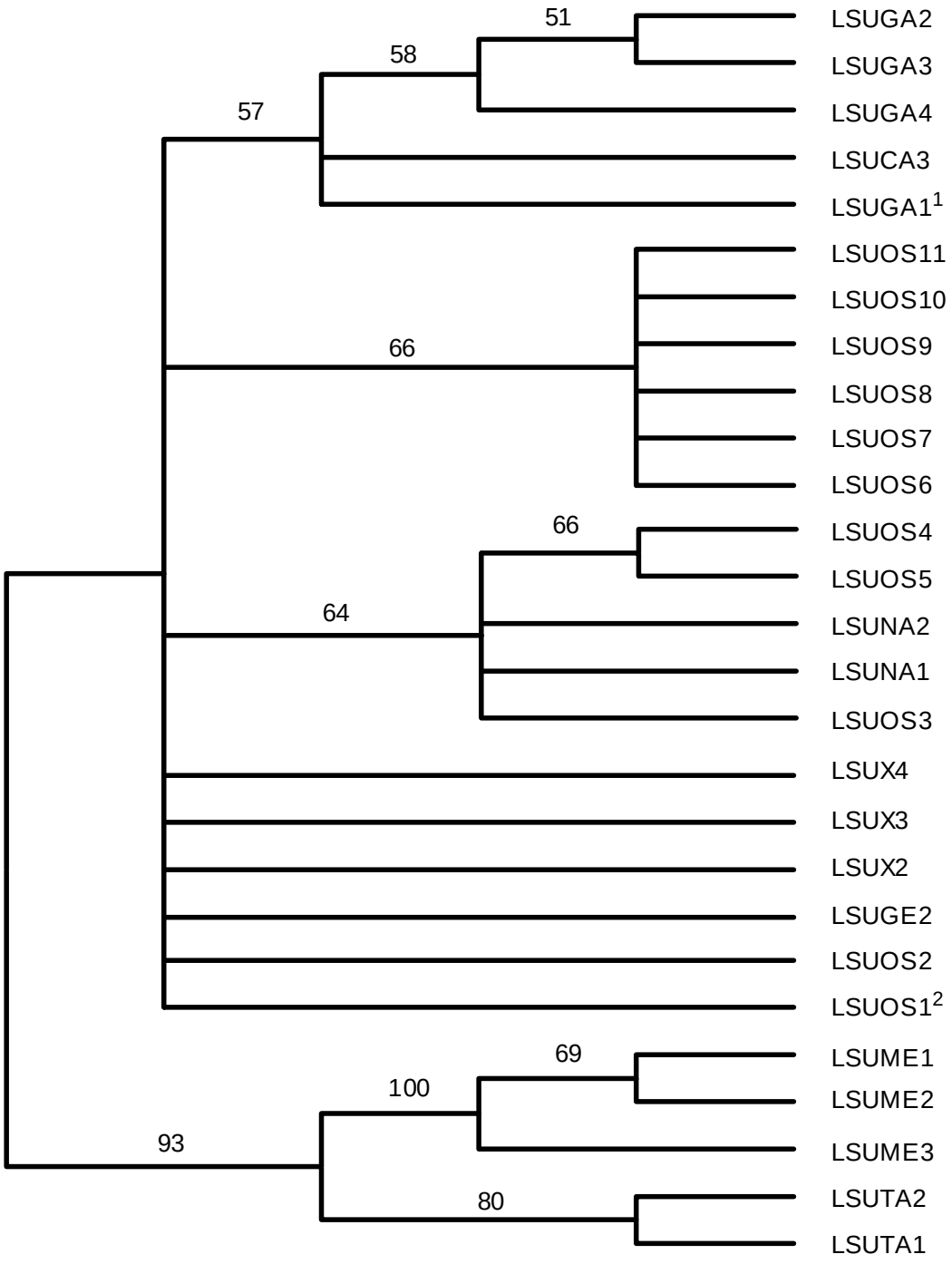


Fig. A2-2. Neighbor-joining analysis of the nuclear large ribosomal subunit (LSU). Bootstrap 50% majority-rule consensus tree from neighbor-joining analysis using the Tamura-Nei model on the LSU region sequence data. Bootstrap percentage values are indicated above branches based on 1,000 bootstrap replicates. [1]LSUGA1 also represents LSUCA2, LSUCE2, and LSUSI2. [2]LSUOS1 also represents LSUCA1, LSUCE1, LSUGE1, LSUSI1, and LSUX1.

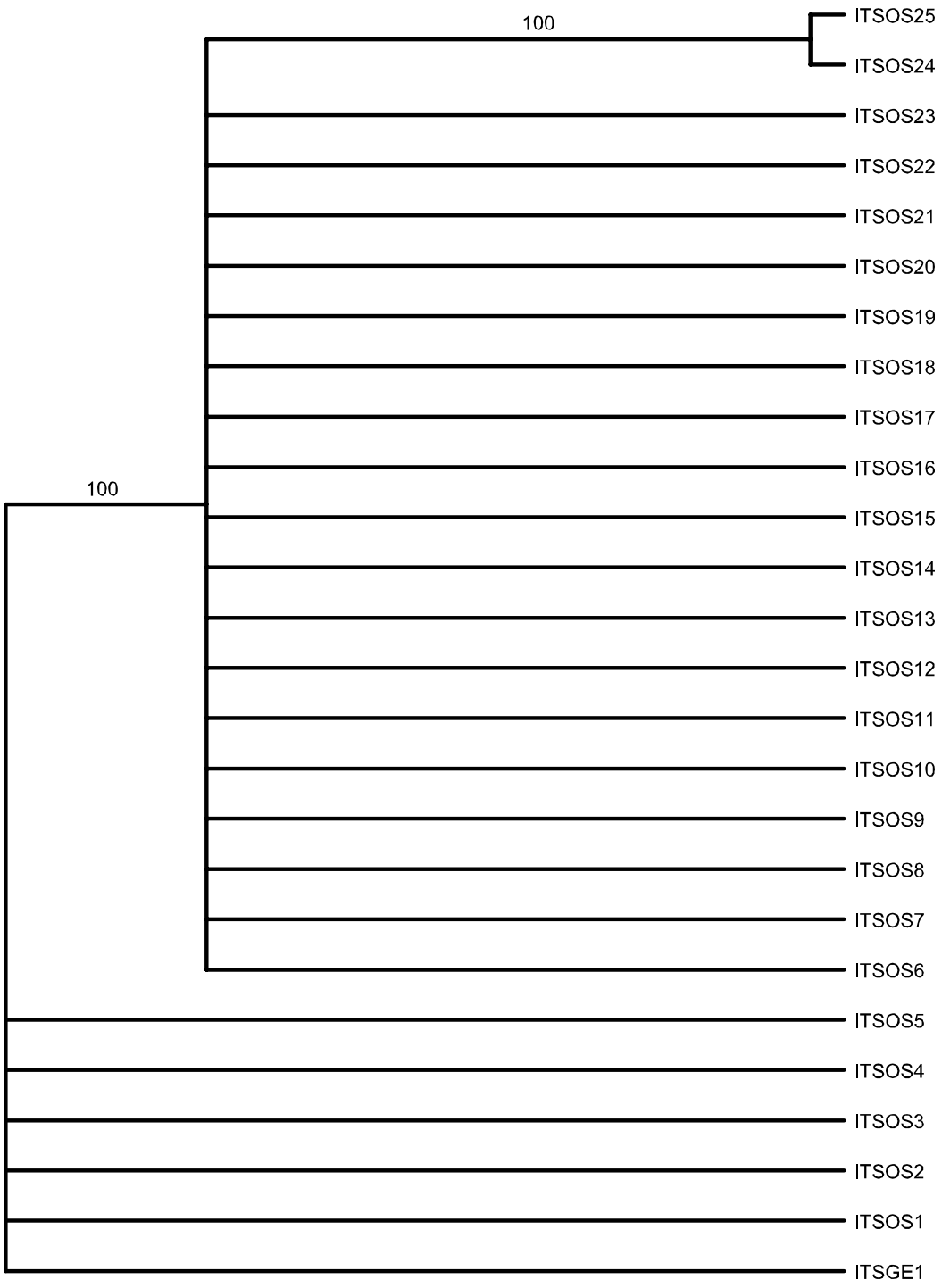


Fig. A2-3. Parsimony analysis of the internal transcribed spacer and 5.8S rDNA (ITS). Bootstrap 50% majority-rule consensus tree from parsimony analysis using heuristic search of the ITS + 5.8S region sequence data. Bootstrap percentage values are indicated above branches based on 1,000 bootstrap replicates.

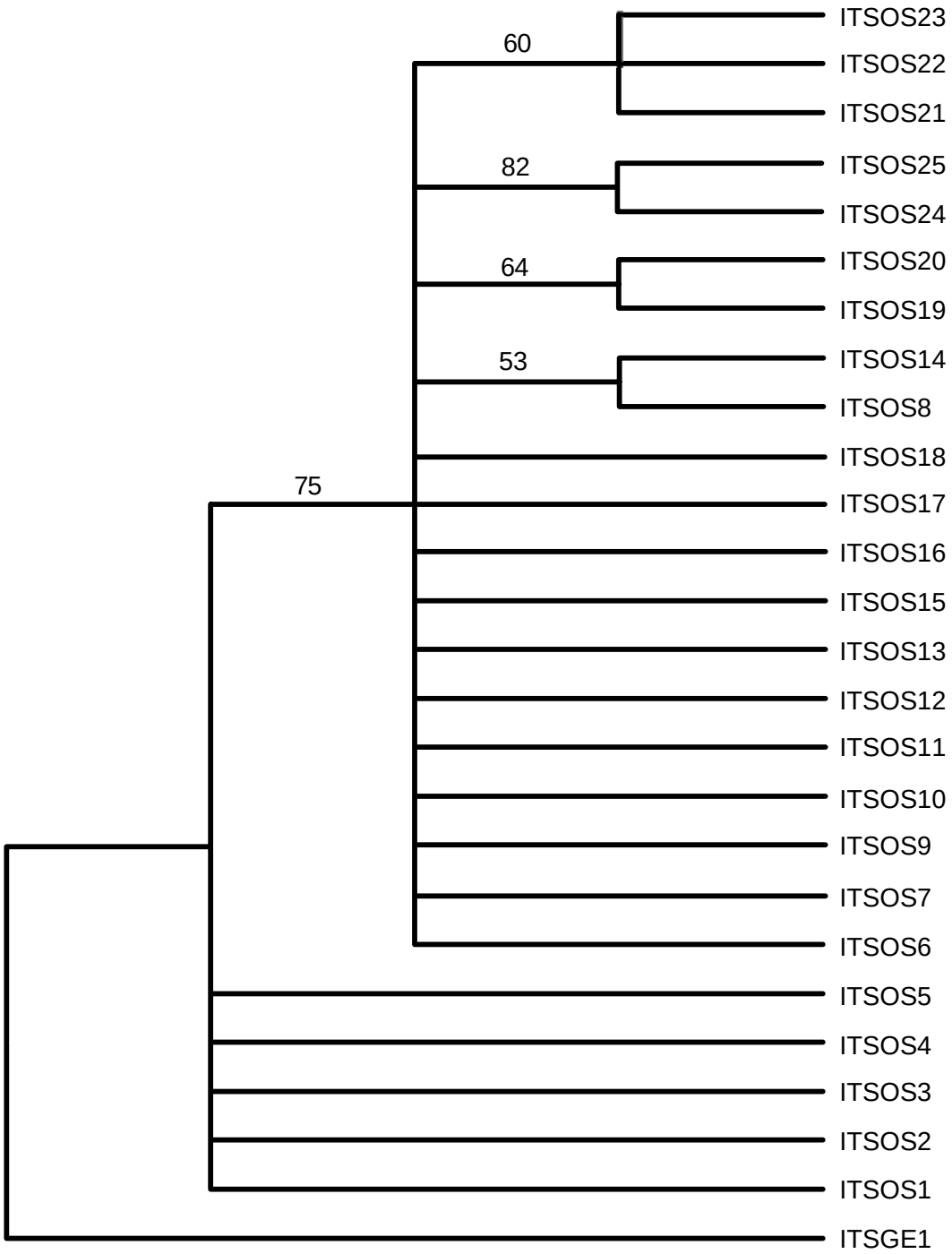


Fig. A2-4. Neighbor-joining analysis of the internal transcribed spacer and 5.8S rDNA (ITS). Bootstrap 50% majority-rule consensus tree from neighbor-joining analysis using the Tamura-Nei model on the ITS + 5.8S region sequence data. Bootstrap percentage values are indicated above branches based on 1,000 bootstrap replicates.

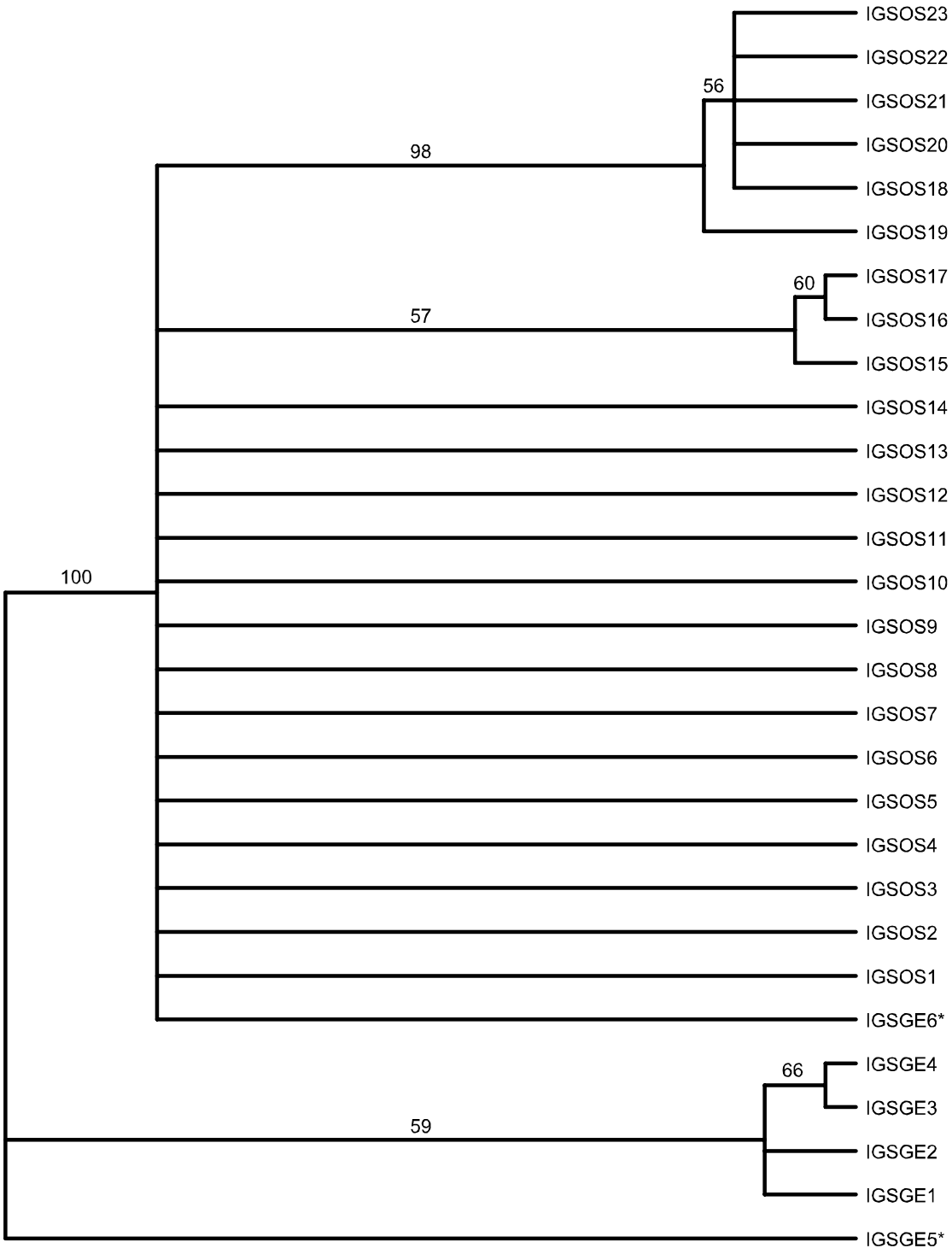


Fig. A2-5. Parsimony analysis of the intergenic spacer one rDNA (IGS-1). Bootstrap 50% majority-rule consensus tree from parsimony analysis using heuristic search of the IGS-1 region sequence data. Bootstrap percentage values are indicated above branches based on 1,000 bootstrap replicates. * *Armillaria ostoyae* x *A. gemina* interspecific hybrid (IGSGE5 x IGSGE6)

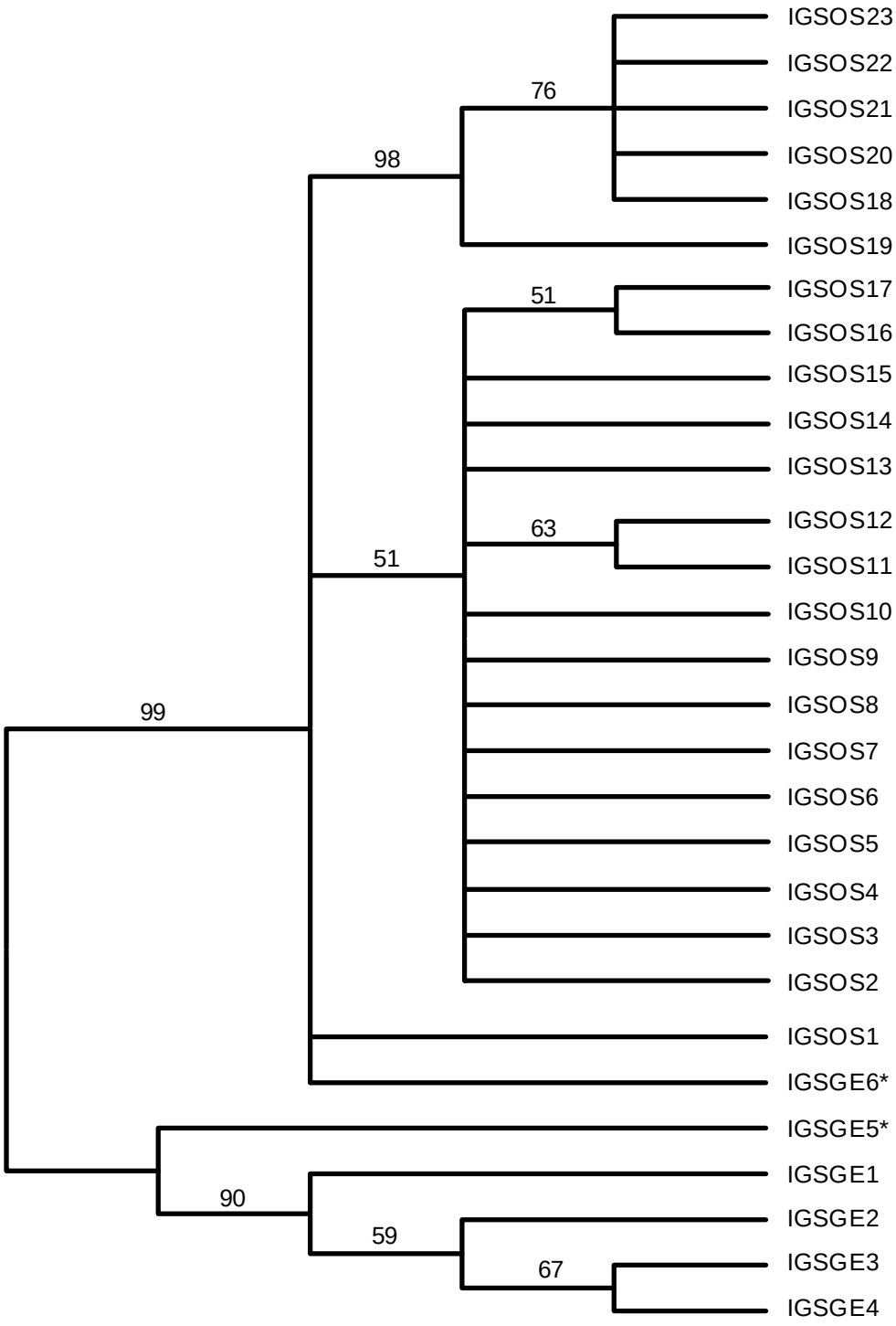


Fig. A2-6. Neighbor-joining analysis of the intergenic spacer one rDNA (IGS-1). Bootstrap 50% majority-rule consensus tree from neighbor-joining analysis using the Tamura-Nei model on the IGS-1region sequence data. Bootstrap percentage values are indicated above branches based on 1,000 bootstrap replicates. * *Armillaria ostoyae* x *A. gemina* interspecific hybrid (IGSGE5 x IGSGE6)

APPENDIX 3: GenBank accession numbers

Table A3-1. GenBank accession numbers of *Armillaria ostoyae* sequences used in this study

Isolate	LSU	ITS	IGS
BC18F	AY973686, AY973693	AY996630	AY968123
FF4	AY973655	AY996694, AY996695	AY968198, AY968199
MNF4	DQ011904	AY996631, AY996648	AY968136, AY968143
NA142	AY973694, AY973729	AY996664	AY968148
NA144	AY973752	AY996670, AY996671	AY968138, AY968164
NA150	AY973690, AY973695	AY996677, AY996679	AY968086
NA212	AY973696, AY973730	AY996649	AY968087, AY968144
NA254	AY973749	AY996651, AY996655	AY968088, AY968124
NA260	AY973687, AY973697	AY996662	AY968089
NC53	AY973698, AY973731	AY996652, AY996656	AY968125, AY968149
NC94	AY973699	AY996632	AY968090
NC164	AY973688, AY973700	AY996678	AY968091, AY968126
NC436	AY973692, AY973701	AY996633	AY968131, AY968145
NC491	AY973677, AY973702	AY996696	AY968092, AY968133
NC580	AY973703	AY996634	AY968093, AY968130
NC671	AY973689, AY973704	AY996658, AY996661	AY968127, AY968150
NC765	AY973678, AY973705	AY996692	AY968094
NC837	AY973745	AY996680, AY996682	AY968137, AY968183
NC863	AY973706	AY996665	AY968128, AY968146
NC887	AY973707	AY996653, AY996657	AY968095, AY968132
NC895	AY973708	AY996635	AY968096, AY968139
NC905	AY973679	AY996673, AY996676	AY968097
NC911	AY973709, AY973732	AY996697, AY996698	AY968098, AY968151
NC1070	AY973750, AY973751	AY996699, AY996700	AY968099, AY968134
NC1091	AY973710	AY996721, AY996722	AY968100
NC1126	AY973733	AY996636	AY968152
NC1180	AY973746	AY996681, AY996683	AY968101, AY968184
NC1187	AY973711, DQ011902	AY996654, AY996672	AY968102, AY968153
NC1245	AY973712	DQ011905	AY968103, AY968154
NM115	DQ011903	AY996701	AY968165
NM120	AY973662	AY996616	AY968166, AY968179
NM235	AY973663	AY996617	AY968167, AY968182
NM236	AY973740	AY996618, AY996628	AY968190
NM238	AY973656, AY973664	AY996619, AY996626	AY968193
NM239	AY973665	AY996620	AY968168
NM241	AY973666	AY996621	AY968169
NM242	AY973667	AY996702	AY968170
NM244	AY973668	AY996622	AY968171
NM245	AY973738	AY996703	AY968191
NM246	AY973669	AY996684	AY968172
NM248	AY973670	AY996685	AY968173
NM249	AY973671	AY996686	AY968174, AY968181
NM250	AY973739	AY996623, AY996627	AY968192
OR10	AY973741	AY996704, AY996705	AY968104
OR22	AY973680	AY996718, AY996720	AY968105
P255	AY973672	AY996629	AY968175, AY968180
P1401	AY973681	AY996674	AY968106
P1404	AY973682	AY996675	AY968107
P2003	AY973713	AY996638	AY968108

Table A3-1. GenBank accession numbers of *Armillaria ostoyae* sequences used in this study **(continued)**

Isolate	LSU	ITS	IGS
P4352	AY973714	AY996659	AY968155
P4661	AY973715	AY996660	AY968156
PC514	AY973742	AY996715, AY996716	AY968109, AY968176
R957	AY973683, AY973716	AY996666	AY968110
R959	AY973717	AY996667	AY968111
R1075	AY973744	AY996688, AY996690	AY968188, AY968189
R1083	AY973718	AY996639	AY968112, AY968140
R1140	AY973719	AY996640	AY968113, AY968142
R1202	AY973691	AY996663	AY968114
R1237	AY973684, AY973720	AY996717, AY996719	AY968115, AY968141
R1283	AY973721, AY973735	AY996706, AY996707	AY968135, AY968157
R1329	AY973722, AY973736	AY996641	AY968116, AY968158
R1334	AY973723	AY996642	AY968117, AY968159
R1348	AY973685, AY973724	AY996693	AY968118
R1362	AY973675, AY973676	AY996624	AY968185
R1366	AY973725	AY996643, AY996650	AY968119, AY968147
R1374	AY973747	AY996708, AY996709	AY968160, AY968186
R1424	AY973748	AY996710, AY996711	AY968194, AY968195
SSF4	AY973726, AY973737	AY996644, AY996646	AY968120, AY968161
SSF6	AY973727	AY996668, AY996669	AY968129, AY968162
ST1	AY973657	AY996615	AY968085
ST2	AY973728	AY996645, AY996647	AY968121, AY968163
TS7	AY973673	AY996625	AY968177
U5	AY973658	AY996712	AY968200, AY968202
U16	AY973659	AY996713	AY968201, AY968203
U73	AY973674	AY996687	AY968178
USSR	AY973660, AY973661	AY996714	AY968196, AY968197
WA6	AY973743	AY996689, AY996691	AY968122, AY968187

Table A3-2. GenBank accession numbers of Armillaria sequences used in this study

Species	**Isolate**	**LSU**
A. calvescens	ST3	AY213559
	ST17	AY213560, AY213561
	ST18	AY213562
A. cepistipes	M110	AY213581
	S20	AY213582
	W113	AY213583
A. gallica	M70	AY213568
	ST22	AY213569, AY213570
	ST23	AY213571
A. gemina	ST8	AY213555
	ST9	AY213556, AY213557
	ST11	AY213558
A. mellea	ST5	AY213584, AY213585
	ST20	AY213586
	ST21	AY213587
A. nabsnona	C21	AY213572
	M90	AY213573
	ST16	AY213574
A. sinapina	M50	AY213563, AY213564
	ST12	AY213565
	ST13	AY213566, AY213567
A. tabescens	AtMuS2	AY213588
	OOi99	AY213589
	OOi210	AY213590
NABS X	837	AY213575, AY213576
	D82	AY213577, AY213578
	POR100	AY213579, AY213580