Latest advances and future perspectives in *Armillaria* research

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R. Heinzelmann et al. Advances and perspectives in Armillaria *research*

2

Abstract: The basidiomycete genus *Armillaria* s.l. (*Armillaria* s.s. and *Desarmillaria*) has a worldwide distribution and plays a central role in the dynamics of numerous woody ecosystems, including natural forests, tree plantations for timber production, orchards, vineyards, and gardens. Early studies have shown that all *Armillaria* species are capable of degrading dead woody substrates causing white rot. Moreover, most species exhibit a parasitic ability, and can be considered as facultative necrotrophs. Although over the years extensive research has been conducted on the phylogeny, biology, and ecology of different *Armillaria* species, numerous theoretical and applied questions remain open. Recently published studies have provided new perspectives, the most significant of which we present in this review. First, new investigations have highlighted the importance of a multilocus approach for depicting the phylogeny of the genus *Armillaria*. Second, the importance of clonality and sexuality for the different species is now better described, enabling a more accurate prediction of population dynamics in various environments. Third, genome sequencing has provided new insights into genome evolution and the genetic basis of pathogenicity and wood degradation ability. Fourth, several new studies have pointed out the possible influence of climate change on *Armillaria* distribution, biology and ecology, raising questions regarding the future evolution of *Armillaria* species and their effect on ecosystems. In this review, we also give a state-of-the-art overview of the control possibilities of parasitic *Armillaria* species. Finally, we outline some still open questions in *Armillaria* research, the investigation of which will strongly benefit from recent methodological advances.

Keywords: fungal forest pathogen, root and butt rot, population dynamics, basidiospores, host, global changes, disease management, -omics

Résumé: Le genre basidiomycète *Armillaria* s.l. (*Armillaria* s.s. et *Desarmillaria*) a une distribution mondiale et joue un rôle central dans la dynamique de nombreux écosystèmes, notamment en forêts naturelles, dans les plantations forestières, vergers, vignobles, et parcs arborés. Les premières études ont montré que toutes les espèces d'Armillaire étaient capables de dégrader les substrats ligneux morts provoquant une pourriture blanche. La plupart des espèces présentent aussi une capacité parasitaire, et peuvent être considérées comme des nécrotrophes facultatifs. Bien qu'au fil des années, des recherches approfondies aient été menées sur la phylogénie, la biologie, et l'écologie des différentes espèces d'Armillaire, de nombreuses questions théoriques et appliquées demeurent. Des études récemment publiées ont apporté de nouvelles perspectives, dont les plus importantes sont présentées dans cette revue. Premièrement, de nouveaux travaux ont mis en évidence l'importance d'une approche multi-gène pour décrire la phylogénie du genre *Armillaria*. Deuxièmement, l'importance de la clonalité et de la sexualité des différentes espèces est aujourd'hui mieux estimée, permettant une prédiction plus précise de la dynamique des populations dans divers environnements. Troisièmement, le séquençage du génome a permis de mieux comprendre l'évolution du génome et la base génétique du pouvoir pathogène et de la capacité de dégradation du bois. Quatrièmement, ces études ont mis en évidence la possible influence du changement climatique sur la distribution, la biologie et l'écologie du genre *Armillaria*, questionnant la future évolution des espèces d'Armillaire et leurs effets sur les écosystèmes. Dans cette revue, nous donnons également un aperçu de l'état de l'art des méthodes de contrôle des espèces d'Armillaire parasites. Enfin, nous identifions plusieurs questions encore ouvertes concernant le genre *Armillaria* dont la résolution devrait bénéficier fortement des progrès méthodologiques récents.

Mots clés: agent pathogène des arbres forestiers, pourriture de la souche ou des racines,

dynamique des populations, basidiospores, hôte, changements globaux, gestion des maladies, -

omique.

General introduction

The genus *Armillaria* s.l. (including its closely related genus *Desarmillaria*; Koch et al. 2017), which currently includes more than 40 officially described species worldwide, plays a central role in the dynamics of numerous woody ecosystems (Shaw III & Kile 1991). Its high success in terms of species diversity, as well as broad geographical distribution, likely relies on a combination of several factors:

(1) High adaptability to changing environments along geological timeframes. Species radiation in *Armillaria* s.s. began about 33 million years ago and to date a large number of species with long evolutionary histories exist (Koch et al. 2017). *Armillaria* species are found from the sea level to high elevations and in different types of soil (Guillaumin et al. 1993). High plasticity to environmental conditions also exists within *Armillaria* species and may represent an evolutionary advantage for this genus in the face of climate change and adaptation to new hosts (Labbé et al. 2017b);

(2) *Armillaria* species can acquire new food resources by adopting different strategies. All species exhibit a saprotrophic ability and can exploit all kinds of dead wood (e.g. roots, stumps, debris) from different woody species present in natural and artificial ecosystems (Cruickshank et al. 1997; Prospero et al. 2003a). In this process, both cellulose and lignin are degraded, resulting in a white rot. In this way, *Armillaria* genets (i.e. mycelium belonging to the same genotype) can persist over several tree generations on the same site and reach estimated ages of more than thousands of years (Ferguson et al. 2003; Bendel et al. 2006a). Some *Armillaria* species are also polyphagous facultative parasites, able to infect not only a broad range of already weakened (opportunistic or secondary parasites), but also healthy (primary parasites) woody plants (Gregory et al. 1991). Host infection results in a white rot of the roots and root collar which may

lead to the death of the tree (Fig. 1). Although the ecological strategy is species dependent, it can be influenced by the specific environmental context (Tsykun et al. 2012);

(3) *Armillaria* species employ an efficient dispersal strategy combining different modes of spread. Local spread (i.e. within a stand) occurs both via rhizomorphs and root contacts. Rhizomorphs are genus-specific (Koch et al. 2017), subterranean, melanized cord-like structures (1-5 mm in diameter), through which *Armillaria* species occupy the surrounding soil and reach and colonize new food resources (Fig. 1). Preferentially saprotrophic species can develop particularly dense networks of rhizomorphs, both in undisturbed and managed forests (Prospero et al. 2003a; Tsykun et al. 2012). The production of rhizomorphs is considered to be an adaptation strategy to harsh environments that has allowed *Armillaria* species to acquire a competitive advantage (Koch et al. 2017). In addition to rhizomorphs, direct contact between roots allows the fungal mycelium present in infected roots to infect healthy roots of neighboring trees (Rishbeth 1985; Lung-Escarmant & Guyon 2004) (Fig. 1). Dispersal over longer distances is assumed to occur via sexual basidiospores, whose role in the *Armillaria* life cycle is, however, still not completely clear. Effectively, little is known about the substrates on which basidiospores germinate, and at which distance basidiospores may be dispersed (Baumgartner et al. 2011). Recent studies (presented in this review) have evidenced considerable differences among *Armillaria* species or populations of the same species in the importance of the two ways of dispersal. These differences are most likely related to variations in environmental contexts.

From an anthropocentric point of view, the ecological success of *Armillaria* species has both positive and negative aspects. As saprotrophs, they are important wood decomposers that contribute to carbon and mineral cycling in forest ecosystems. This seems to be the main role of *Armillaria* in unmanaged forests (Kim & Klopfenstein 2011; Tsykun et al. 2012). Here, the selective killing of trees contributes to the replacement of individual trees or even tree species,

favoring biodiversity and regeneration (Dettman & van der Kamp 2001a; Bendel et al. 2006b). The parasitic activity of *Armillaria* is, however, of great concern in artificial and semi-artificial, managed ecosystems, including tree plantations for timber production, orchards, vineyards, parks, and gardens (Baumgartner & Rizzo 2002; Aguin-Casal et al. 2004; Morrison 2011; Drakulic et al. 2017). Here, tree mortality may lead to severe economic consequences.

Given the dual life style of most *Armillaria* species, their successful control is still problematic. In particular, removing all possible inoculum sources below ground (e.g. mycelium on residual roots or woody debris) represents a challenging task. Moreover, the broad host range of most parasitic *Armillaria* species limits the possibilities of selecting less susceptible tree species. Despite several attempts, the use of antagonistic fungi for the biocontrol of *Armillaria* does not yet represent an effective solution (Baumgartner et al. 2011).

In this review, we aim to highlight the latest research on *Armillaria* while presenting future perspectives for research. In particular, we show that in order to developing effective management strategies, a holistic approach that better accounts for all three actors (i.e. pathogen, host, and environment) involved in the interaction is necessary. Given recent advances in -omics (particularly genomics, transcriptomics and proteomics) and computational informatics, new conceptual avenues are now possible to better investigate, for example, host-pathogen interactions and population dynamics of *Armillaria* species. For basic information about the biology and epidemiology of *Armillaria,* we refer to previous comprehensive publications which are still current (Shaw III & Kile 1991; Guillaumin et al. 2005; Baumgartner et al. 2011; Guillaumin & Legrand 2013).

Phylogeny and species diversity

Species recognition

Species definition is challenging and over the years several different theoretical and operational species concepts have been developed (Mayden 1997; Taylor et al. 2000). For *Armillaria* systematics, although three operational species concepts are mainly used (morphological, biological, and phylogenetic species recognition), all have their limitations, and may give conflicting results.

Since *Armillaria* undergoes sexual reproduction, species recognition based on the morphological characteristics of fruiting bodies (basidiocarps) is reasonable. However, fruiting bodies are rarely available in nature because they are short-lived and irregularly produced over time. Moreover, in some species, fruiting bodies are difficult to induce *in vitro* and their characteristics do not necessarily resemble those of the fruiting bodies collected in nature (Guillaumin et al. 1985). In addition, the macro- and microcharacteristics of fruiting bodies widely overlap between related species, such as *A. cepistipes*, *A. gallica* and *A. nabsnona* (Antonin et al. 2009; Park et al. 2018) or *A. ostoyae* and *A. gemina* (Bérubé & Dessureault 1989).

Most *Armillaria* species have a heterothallic mating system (only two haploid mycelia with opposite mating types can fuse) and reproductive barriers exist between species (Guillaumin 2005). Therefore, biological species recognition has been intensively used in the genus *Armillaria* since the 1970s (Korhonen 1978; Anderson & Ullrich 1979). Consequently, most of the currently known *Armillaria* species were first described as biological species. Biological species recognition, however, has some serious limitations. Allopatric species may show incomplete reproductive barriers and be, at least partially, interfertile (e.g. *A. cepistipes* and *A. sinapina*; Bérubé et al. 1996; Banik & Burdsall 1998). Moreover, mating tests for species identification in which haploid (single spore) testers of known species are paired with diploid strains of unknown species sometimes provide ambiguous results (e.g. Hasegawa et al. 2011; Keca et al. 2015). Single spore testers for the complete list of *Armillaria* species are difficult to

obtain and they may degenerate over time, changing culture morphology independently of the mating process (Harrington et al. 1992).

In recent years, due to rapid progress in DNA-based techniques and the increased availability of DNA sequences, genealogical concordance phylogenetic species recognition, which relies on the concordance of more than one gene genealogy (Taylor et al. 2000), has gained wide popularity. Nevertheless, it also has some constraints for *Armillaria* systematics. One major drawback is that single gene phylogenies do not necessarily reflect the species' evolutionary history and relationship, eg. because of incomplete lineage sorting, homoplasy, or horizontal gene transfer. As a result, the topology of phylogenetic trees may be incongruent among genes (Kim et al. 2006; Hasegawa et al. 2010; Tsykun et al. 2013; Coetzee et al. 2015; Koch et al. 2017). This issue can largely be avoided by using a multilocus approach (Tsykun et al. 2013; Koch et al. 2017). Intraspecific variation of genes may also be a problem and sometimes blur a species' phylogenetic tree topology, especially if only one gene is considered. For example, *A. borealis* seems to possess two rather distinct versions of the translation elongation factor 1-α (EF-1α) gene, which appear as unrelated clusters in EF-1α phylogenies (Tsykun et al. 2013; 2016; Klopfenstein et al. 2017). However, in multilocus phylogenies, *A. borealis* is usually represented as a single cluster (Tsykun et al. 2013; Guo et al. 2016). Reconstructing species phylogenies with a multitude of genes or even genomic data would be required to circumvent the biases of single gene phylogenies and to further unravel the mechanisms behind speciation in this large genus.

Species delimitation based on the phylogenetic approach should also be biologically meaningful and criteria have to be developed to distinguish between intraspecific genetic lineages, representing intraspecific variation, and new taxonomic entities, representing new species. A polyphasic approach should be followed to equate genetic lineages with biological (reproductive), morphological, and functional differentiation. Recent studies by Brazee et al.

(2012b), Hood & Ramsfield (2016) and Elías-Román et al. (2018) which describe three new species (*A. altimontana*, *A. aotearoa* and *A. mexicana*, respectively) represent good examples of a successful co-application of different species recognition approaches. For example, *A. altimontana* was for a long-time recognized as a distinct biological species in North America (NABS X) (Anderson & Ullrich 1979; Morrison et al. 1985; Banik & Burdsall 1998). Later on, its species rank was confirmed using different molecular techniques (Kim et al. 2000; Kim et al. 2001; Ross-Davis et al. 2012). Consequently, *A. altimontana* was recently formally described as a new species, including its morphological characteristics (Brazee et al. 2012b). Furthermore, *A. altimontana* appears to be specifically associated with *Abies*-dominated forest types at mesic, higher elevation sites of the western interior of North America (Brazee et al. 2012b). A similar case is represented by *A. aotearoa* in New Zealand (Hood & Ramsfield 2016). In contrast, *A. mexicana* was discovered as a distinct phylogenetic species in Mexico (Elías-Román et al. 2013). Extensive analysis of its morphological features confirmed its separation from other North American species (Elías-Román et al. 2018).

An interesting case where biological species recognition is questioned by phylogenetic species recognition is *A. ostoyae*. This species is widespread in the Northern Hemisphere and isolates from Eurasia and North America show interfertility (Anderson et al. 1980). However, recent phylogenetic analyses suggest that North American *A. ostoyae* and Eurasian *A. ostoyae* represent closely related, but clearly distinct genetic lineages (Klopfenstein et al. 2017), raising the question of whether these lineages are actually different (sub)species. Although for now the official name of the species, including both lineages, is still *A. ostoyae* (on July 29 2017, the recommendation of the Nomenclature Committee for Fungi to conserve *A. ostoyae* over *A. solidipes* (May 2017), was accepted by the International Botanical Congress (Turland et al.

2017)), further taxonomic and phylogenetic investigations, including multiple genes or genomic

data, would be necessary to clarify this question.

Phylogeny

Since the 1990s, the phylogeny of the genus *Armillaria* has been explored based on molecular

variations in different genes. Initially, mainly the tandemly repeated multicopy ribosomal DNA

(rDNA) operons (Duchesne & Anderson 1990; Anderson & Stasovski 1992), in particular the

intergenic spacer 1 (IGS-1) between the 28S and 5S gene and the internal transcribed spacers

(ITSs) between 18S and 5.8S and 5.8S and 28S, were studied (Harrington & Wingfield 1995;

Kim et al. 2000; Coetzee et al. 2003; Kim et al. 2006). Subsequently, Maphosa et al. (2006)

demonstrated the utility of the single copy EF-1α gene for constructing phylogenies in

Armillaria. In comparison to rDNA sequences, the EF-1α gene appears to better resolve closely

related *Armillaria* species, such as *A. gallica* and *A. cepistipes* and other related species

(Maphosa et al. 2006; Hasegawa et al. 2010; Ross-Davis et al. 2012; Tsykun et al. 2013; Coetzee

et al. 2015; Guo et al. 2016; Klopfenstein et al. 2017). Hence, this gene represents a valuable

diagnostic tool for the genus.

Multilocus phylogenies have recently begun to replace single gene phylogenies (Tsykun et

al. 2013; Guo et al. 2016; Koch et al. 2017), as they describe the evolutionary history of the

genus more accurately. The reconstruction of the phylogeny of the genus based on genomic data

is currently possible only for very few species (Sipos et al. 2017) because of the limited number

of available genomes.

The phylogenetic studies conducted in recent years consistently attribute the genus

Armillaria to the Agaricales superclade within the Basidiomycota, where it forms, together with

other genera, the family Physalacriaceae (Matheny et al. 2006; Collins et al. 2013). A recent

phylogenomic analysis, based on 835 conserved single copy genes, confirmed the phylogenetic

position of the genus *Armillaria* in this family, and identified *Guyanagaster* and

Cylindrobasidium as the most closely related sister genera (Sipos et al. 2017). In agreement with

each other, the studies by Koch et al. (2017) and Sipos et al. (2017) date the split of *Armillaria*

from its sister genus *Guyanagaster* to 40-50 million years ago. Since recently, two separate

genera are recognized within *Armillaria*, the genus *Desarmillaria*, composed of the ex-annulated

species *D. ectypa* and *D. tabescens* (formerly *A. ectypa* and *A. tabescens*), and the genus

Armillaria s. s., which contains all species characterized by annulated fruiting bodies (Koch et al.

2017).

The Northern and Southern Hemisphere harbor distinct sets of *Armillaria* species (Fig. 3)

which constitute clearly separated clades in the phylogeny of *Armillaria* (Klopfenstein et al.

2017; Koch et al. 2017), thereby confirming previous studies with similar findings based on

analyses of fewer isolates (Coetzee et al. 2011). The few *Armillaria* species that the Southern

Hemisphere shares with the Northern Hemisphere (Fig. 3) were most likely introduced

accidentally by humans through the transport of infected plant material (e.g. *A. mellea* was

introduced to South Africa from Europe by early Dutch settlers, Coetzee et al. 2001b; Coetzee et

al. 2018).

To date, phylogenetic analyses of Northern Hemisphere species including various DNA

regions (e.g. translation elongation factor 1-α gene, ribosomal IGS-1 and ITS regions) have

generally been consistent in depicting the following superclades: (1) "*Desarmillaria*" clade

including *D. ectypa* and *D. tabescens*; (2) "*Ostoyae*" clade including *A. ostoyae*, *A. borealis* and

A. gemina; (3) "*Gallica*" clade including *A. gallica*, *A. calvescens*, *A. cepistipes*, *A. altimontana*,

A. sinapina, *A. nabsnona*; and the undescribed biological species Nag. E from Japan, and (4)

"*Mellea*" clade including *A. mellea, A. mellea* ssp. *nipponica* and *A. mexicana* (Hasegawa et al.

2010; Tsykun et al. 2013; Coetzee et al. 2015; Guo et al. 2016; Klopfenstein et al. 2017; Koch et al. 2017). Phylogenetic studies of *Armillaria* species from the Southern Hemisphere have thus far outlined the existence of: (1) "Australasian-South American" superclade containing *A. novae-zelandiae*, *A. affinis*, *A. limonea*, *A. paulensis*, *A. hinnulea*, *A. pallidula*, *A. fumosa*, and *A. luteobubalina*, and (2) "African superclade" containing *A. camerunensis*, *A. heimii* and *A. fuscipes* (Coetzee et al. 2001a; Coetzee et al. 2003; Coetzee et al. 2005a; Coetzee et al. 2005b; Maphosa et al. 2006; Lima et al. 2008; Pildain et al. 2009; Koch et al. 2017).

Besides these well-known and well-characterized *Armillaria* species and subspecies, numerous new phylogenetic lineages have been discovered in recent years (e.g. Coetzee et al. 2005b; Guo et al. 2016) whose taxonomic rank is sometimes still not clear.

Biogeography

The first study on the biogeography of *Armillaria* by Kile et al. (1994), which was based on species distribution patterns, suggested that ancestral forms of *Armillaria* already existed before continents started to separate and that the present-day distribution of the genus resulted from adaptive radiation mediated by the break-up of these landmasses. More recent studies, however, relying upon molecular clock analyses, have estimated the genus to be much younger, rejecting the previous hypothesis (Coetzee et al. 2011; Koch et al. 2017). According to Koch et al. (2017), the armillarioid clade, which currently includes the genera *Armillaria* s.s., *Desarmillaria* and *Guyanagaster*, arose approximately 51 million years ago in Eurasia, which is well after continental break-up. *Armillaria* s.s. separated from *Desarmillaria* approximately 41 million years ago, and started to diversify approximately 33 million years ago. The ancestral range analyses conducted by Koch et al. (2017) suggest that *Armillaria* migrated from Eurasia to Africa and Australasia, and was introduced multiple times from Eurasia to North America. The authors

propose that *Armillaria* migrated as a pathogen of existing plants at that time, and that species started to diverge after available pathways disappeared and migration was no longer possible. Interestingly, the predicted origin of *Armillaria* in South America is Australasia and not North America. Koch et al. (2017) hypothesize that *Armillaria* may have travelled from Australasia to South America along an ancient dispersal route via the Antarctic, or alternatively by long-distance dispersals of basidiospores.

Species diversity and distribution

Even though the Index Fungorum (http://www.indexfungorum.org/) and Mycobank (http://www.mycobank.org/) currently list about 70 *Armillaria* species, only approximately 40 of them are well-characterized (Fig. 3). The other taxa mainly represent morphological species which were described in the early days of *Armillaria* research but were never equated with biological or phylogenetic species. Although, to date, we have a general overview of the global distribution of the different *Armillaria* species (Fig. 3), most publications on the topic involve isolates from Europe and North America, whereas collections from Asia, South America, and Africa are still relatively sparse. Moreover, detailed knowledge of the regional incidence and ecology of many of the reported species is frequently lacking, as most reports are limited to host and host status (dead or alive).

Sympatric species interaction

Field investigations conducted mainly in North America and Europe have shown that several *Armillaria* species can occur sympatrically in the same forest stand (e.g. Legrand et al. 1996; Cruickshank et al. 1997; Bruhn et al. 2000; Dettman & van der Kamp 2001a; Prospero et al. 2003b; Bendel et al. 2006a; Szewczyk et al. 2016; Mesanza et al. 2017). The co-occurrence of

preferentially saprotrophic and parasitic species appears to be particularly frequent, as in the case with *A. cepistipes* or *A. sinapina* with *A. ostoyae* and *A. mellea* with *A. gallica*. This may reflect evolution of different ecological strategies because of competition for the same resources.

Since the mentioned studies only provide a "snapshot" for the evolution of *Armillaria* species, repeated sampling and analyses over time in the same stand, combined with experimental studies, would provide more information on the outcome of interspecific interactions, such as the long-term co-existence of different species or replacement of one species by another. For instance, a study conducted by Prospero et al. (2006) involving potted Norway spruce (*Picea abies*) seedlings suggested a rather neutralistic co-existence of *A. cepistipes* and *A. ostoyae* as rhizomorphs in the soil. Regarding their ability to colonize stumps, however, the two species appeared to experience a mutually negative effect from the interaction, likely because of interspecific competition.

Population structure and dynamics

Vegetative spread

One important biological trait of *Armillaria* species is their ability to spread in the soil via rhizomorphs (Rishbeth 1988; Guillaumin et al. 1993; Prospero et al. 2003a; Tsykun et al. 2012). This form of vegetative propagation combined with mycelial transfer from root to root can under certain circumstances result in the formation of very large clonal individuals (Smith et al. 1992; Ferguson et al. 2003; Bendel et al. 2006a). More often, however, the estimated size of an *Armillaria* genet measures less than one hectare (Brazee et al. 2012a; Travadon et al. 2012; Dutech et al. 2017; Lehtijarvi et al. 2017; Tsykun et al. 2017).

The observed variation in genet size is partly due to differences among *Armillaria* species in their ecological strategies. Some species (e.g. *A. gallica, A. cepistipes)* intensively produce

perennial rhizomorphs through which they rapidly expand in the soil and capture new food

resources (Guillaumin et al. 1993; Prospero et al. 2006). For instance, Tsykun et al. (2012)

estimated that the rich organic soils of Ukrainian primeval beech (*Fagus sylvatica*) forests harbor

approximately 512 kg of rhizomorphs (dry weight) per hectare, mainly by *A. cepistipes*. For other

species (e.g. *A. mellea, A. ostoyae*), rhizomorphs are less abundant and spread in the soil mainly

occurs via root contact (Baumgartner & Rizzo 2001).

Intraspecific genet size differences

Important differences in genet size have also been observed among populations of the same

species, such as in *A. ostoyae* (Dettman & van der Kamp 2001b; Ferguson et al. 2003). Several

authors have assumed that these intraspecific differences might be related to the frequency and

intensity at which sexual reproduction occurs in the specific population (Ferguson et al. 2003;

Bendel et al. 2006a). Humid and mild climatic conditions are assumed to favor fructification and

consequently the production of sexual basidiospores (Ferguson et al. 2003). This could lead to

higher rates of establishment for new genotypes of *Armillaria* spp. and to stronger competition

for host resources among genotypes, which may result in more numerous, discrete and small

genets. Other factors such as composition, distribution and density of tree species, soil properties

(e.g. the amount of organic material in the litter, temperature, water saturation), or anthropogenic

disturbances (e.g. logging, stump removal) may also affect the density and size of *Armillaria*

genets (Guillaumin & Legrand 2005; Szewczyk et al. 2016; Kubiak et al. 2017).

   An elegant method for comparing the spatial genetic structure of *Armillaria* populations

would be the molecular characterization of the clonal structure by conducting random sampling

over several tens of hectares and estimating the mean clonal range for each population. This

would determine the minimum distance at which clonality has no further effect on population

genetic structure as performed in Reusch et al. (1999). Such estimates have recently been

obtained for an *A. mellea* population in California, USA (Travadon et al. 2012) and an *A. ostoyae*

population in France (Dutech et al. 2017). Both studies reported small mean clonal ranges (130 m

and < 150 m, respectively). This is consistent with small genet sizes expected in geographical

areas that have warm climates and humid winters, thus stimulating fructification.

Spread by basidiospores

The importance of basidiospores for population dynamics of *Armillaria* species has been debated

for a considerable time, mostly because, for the majority of species, the substrate on which

basidiospores germinate is still not clearly identified (Baumgartner et al. 2011). Although some

studies report evidence of successful germination on fresh dead wood (stumps and buried wood

segments; e.g. Hood et al. 2008), artificial inoculations of the same types of substrates have failed

or obtained very low success rates (Rishbeth 1970; Kile 1983). These results specifically question

the importance and frequency of the establishment of new genotypes in natural populations via

basidiospores and generally highlight the difficulty of *Armillaria* inoculations.

The frequent occurrence of a large number of small genets in newly planted forests suggests

that germination of basidiospores may be efficient under some specific environmental conditions

(Rishbeth 1970, 1988; Legrand et al. 1996). Recent population genetic studies have confirmed

that local populations (i.e. at the scale of tens or hundreds of hectares) are generally composed of

numerous genets with low or no linkage disequilibrium between alleles (after removing the clonal

effect), suggesting that sexual reproduction is a major evolutionary process in *Armillaria*

populations (Travadon et al. 2012; Dutech et al. 2017). Using spatial genetic structure analysis

and isolation-by-distance models (Wright 1969; Rousset 1997), these studies have also estimated

that spatial dispersal of basidiospores occurs mainly within a few hundred meters (Travadon et al.

2012; Dutech et al. 2017), which is consistent with previous direct estimates of spore dispersal
(Worrall et al. 2004; Power et al. 2008). This limited dispersal of most basidiospores could
explain the significant inbreeding rate estimated within several populations (Prospero et al. 2008;
Baumgartner et al. 2011; Tsykun et al. 2017). These results should, however, be confirmed by
additional studies as they appear to be influenced by the type of molecular markers considered,
having been mainly obtained by means of microsatellite markers (Labbé et al. 2017a; Tsykun et
al. 2017) which in the presence of null alleles may create a spurious signal of inbreeding
(Chybicki & Burczyk 2009). The relatedness among genotypes could for example be verified
using single nucleotide polymorphisms (SNPs) or markers targeting mating type alleles.

Despite the progress made in population genetic analyses, questions remain regarding the
timing and conditions required in nature for the germination and fusion of sexually compatible
basidiospores to produce a new diploid mycelium. Recently, Heinzelmann et al. (2018) suggested
that basidiospores may be present in nature at high density. This would enable the rapid
diploidization of a haploid mycelium, leading to successful colonization of an organic substratum
and subsequent infection of a new host. The establishment of numerous new diploid genotypes
could, therefore, occur over short time periods, when local environmental conditions are
favorable (Legrand et al. 1996), and assuming that many spores with compatible (different)
mating-type alleles are germinating on the same substrate at the same time.

Another mycelial fusion frequently assumed to be important in the population dynamics of
Armillaria species is the so-called Buller phenomenon (Korhonen 1978). It is well described for
in vitro pairings of haploid and diploid mycelium, and was widely used for biological species
identification (e.g. Rizzo & May 1994) before molecular tools started to gain importance.
However, the Buller phenomenon has not yet been observed in nature. Moreover, it should have a
limited effect on the genetic population structure, since, at least *in vitro*, it generally results in the

replacement of the haploid nucleus by the diploid nucleus with very rare recombination events among nuclei (Rizzo & May 1994; Carvalho et al. 1995). A recent genomic study performed on a large genet of *A. gallica* (Anderson & Catona 2014) did not detect such recombination events, confirming their rarity in natural populations.

Estimation of gene flow

The estimation of the restricted dispersal of most basidiospores within a few hundred meters seems to contradict several studies performed at larger spatial scales (i.e. the order of hundreds to thousands of hectares) at which estimates of genetic differentiation among populations were low or not significantly different from zero (Prospero et al. 2008; Baumgartner et al. 2010c; Brazee et al. 2012a; Heinzelmann et al. 2012; Tsykun et al. 2017). The rare long-distance spore dispersal events described in other fungal species (Edman et al. 2004) may also occur in *Armillaria* and mitigate spatial genetic structure at large spatial scales.

Inconsistent results among population genetic studies performed at different spatial scales are, indeed, not uncommon (e.g. Puebla et al. 2012 for marine organisms), and not necessarily associated with significant gene flow among populations. Quantification of current gene flow using genetic differentiation estimates is problematic because such estimates depend on theoretical models with assumptions that are often biologically unrealistic (Whitlock & McCauley 1999). Moreover, when an isolation-by-distance model is assumed and the spatial scale of the study is not in accordance with the dispersal range of the species, gene flow estimates may become unlinked to the estimated genetic differentiation among populations (Rousset 1997). All these factors may explain why only a weak signal of genetic differentiation was detected at the landscape scale in Switzerland in *A. cepistipes* (Heinzelmann et al. 2012), or in Eastern North

America in *A. gallica* (Brazee et al. 2012a), despite the estimation of the restricted dispersal of

most basidiospores at the local scale (Travadon et al. 2012; Dutech et al. 2017).

Historical effects on genetic diversity

In contrast to studies that have shown low F_{ST} estimates, Bayesian genetic analysis and principal

component analysis have demonstrated the existence of several differentiated genetic clusters for

A. mellea in North America (Baumgartner et al. 2010c), *A. cepistipes* in Europe (Tsykun et al.

2017), and *A. luteo-virens* in Asia (Xing et al. 2014). As for their host tree populations in the

Northern Hemisphere (Hewitt 2000), past climatic changes associated with periods of glaciation

may have affected the geographical distribution of most *Armillaria* species, leading to genetic

isolation between surviving populations in different geographical refuges. It is noteworthy that

different genetic clusters appear to co-exist in the same geographical region (e.g. Eastern North

America) suggesting that recolonization originated from different genetically divergent refuges

(Baumgartner et al. 2010c). The historical signal of recolonization is strong in *A. mellea* in North

America, and has been confirmed by phylogenetic studies (Baumgartner et al. 2012). In contrast,

no such signal was detected in any *Armillaria* species in Europe (Tsykun et al. 2013), indicating

the minor effect of past glaciation on the genetic diversity of *Armillaria* species within this

continent. However, this absence of a phylogeographical signal could also be due to insufficient

sample sizes in continental studies. Approximate Bayesian computation analyses of an *A. ostoyae*

population in France found the signature of a genetic bottleneck that may have occurred more

than 10,000 years ago, which would correspond with the end of the last glacial period (Labbé et

al. 2017a). These contrasting results suggest that the impact of past climatic changes is rather

different among *Armillaria* species and geographical regions. Additional phylogeographical

studies, combining new statistical and genomic methods (e.g. Boitard et al. 2016) with more

intense sampling over continental areas, should be performed to better understand the evolutionary history of *Armillaria* species.

Methodological advances

Since the advent of molecular biology, both the number and type of markers available for population genetic analyses have rapidly increased. This also holds true for non-model organisms such as *Armillaria* species. Some authors have pointed out that the nature of molecular markers itself may also affect the outcome of population genetic studies, in addition to the sampling strategy adopted. For instance, microsatellite (single sequence repeats, SSR) and single nucleotide polymorphism (SNP) markers have different rates and mechanisms of mutation, genome-wide distribution patterns, and biological functions (Guichoux et al. 2011; Fischer et al. 2017). In a recent study by Tsykun et al. (2017), these two types of markers showed contrasting sensitivity when used for depicting the population structure of *A. cepistipes* at different spatial scales. SSRs were best suited to detect genetic structure at a smaller geographic scale under a systematic sampling in interconnected forests. In contrast, SNPs in conserved single copy genes were less sensitive to sampling design and revealed ancient divergence between distant populations. Therefore, the molecular markers to be used should be carefully selected based on the specific goals of the particular study.

Genomics and beyond

Genomes

The start of genomics in *Armillaria* was marked with the release of the *A. mellea* genome in 2013 (Collins et al. 2013). More recently, Sipos et al. (2017) sequenced, assembled, and analyzed four other *Armillaria* genomes, including those of *A. cepistipes*, *A. gallica*, as well as those of a

European and a North American *A. ostoyae* strain. In addition, the draft genome sequence of *A. fuscipes*, the first *Armillaria* species from the Southern Hemisphere to be sequenced, is also available (Wingfield et al. 2016). The genomes of *A. mellea* and *A. fuscipes* are still highly fragmented, whereas the genomes of the other species are assembled to a high degree (Table 1). To date, however, none of these genomes have been assembled to chromosome-scale sequences.

In contrast to most other white rot fungi, *Armillaria* species have large genomes ranging from 53 to 85 Mb, and are predicted to contain 14,473 to 25,704 genes (Collins et al. 2013; Wingfield et al. 2016; Sipos et al. 2017; Table 1). Pairwise comparisons of the genomes of different *Armillaria* species have revealed a high degree of synteny (Sipos et al. 2017). Moreover, the comparison of five of these *Armillaria* genomes with the genomes of 22 other Agaricomycetes has revealed a significant genome expansion in the genus *Armillaria* (Sipos et al. 2017). Interestingly, this expansion is driven by gene family diversification, and not by transposon proliferation as observed for other plant pathogenic fungi (Raffaele & Kamoun 2012). The expanded gene families include pathogenicity-related genes, genes involved in lignocellulose-degradation, and *Armillaria*-specific genes with mostly unknown functions, several of which are important for rhizomorph development (Sipos et al. 2017). The expansion of transposable elements in *Armillaria* genomes is only modest compared with other members of the Agaricomycetes. In addition, it appears that weakly pathogenic species, such as *A. cepistipes* and *A. gallica*, tend to have a greater number of repetitive sequences (i.e. transposons) than more pathogenic species, such as *A. ostoyae* and *A. mellea* (Sipos et al. 2017).

Wood degrading genes

The versatility of *Armillaria* species as white-rot fungi to degrade all structural components of plant cell walls is well reflected in their genomes which possess a large repertoire of genes

associated with plant cell wall degradation, including lignin-, cellulose-, hemicellulose- and pectin-degrading enzymes (Collins et al. 2013; Sipos et al. 2017). In contrast with other white-rot fungi, the genomes of *Armillaria* species (albeit those examined to date) show an underrepresentation of ligninolytic gene families and an overrepresentation of pectinolytic gene families (Sipos et al. 2017). The *in vivo* activity of some plant cell wall degrading genes has been confirmed by the transcriptomic analysis of a mycelial fan invading a grand fir (*Abies grandis*) sapling (Ross-Davis et al. 2013), and the proteomic and transcriptomic analysis of different tissues and developmental stages of *in vitro* cultures (Collins et al. 2013; Sipos et al. 2017). The secretion, presence, and activity of a very limited number of ligninolytic enzymes in infected host tissue had been previously demonstrated (Robene-Soustrade et al. 1998; Stoytchev & Nerud 2000).

The rich machinery of the plant cell wall degrading enzymes of *Armillaria* likely also aids pathogenicity by, for example allowing *Armillaria* early access to dead wood, thereby potentially bypassing competing microbes as suggested by Sipos et al. (2017).

Pathogenicity-related genes

The genomes of *Armillaria* encode a range of genes more specifically associated with pathogenicity (Sipos et al. 2017). Some examples include expansins, which help loosen plant cell walls, cerato-platanin genes, which induce cell death in the host, salicylate hydroxylases, which may help to develop tolerance against salicylic acid that plays an important role in plant defense, or LysM (CBM50) domains. The latter are particularly overrepresented in *Armillaria* (Sipos et al. 2017), and may play a role in the suppression of chitin-triggered host immunity and the protection of the fungal cell wall from hydrolysis through chitinases and other hydrolytic enzymes secreted by the host or competing microbes (Kombrink & Thomma 2013; Akcapinar et

al. 2015; Sánchez-Vallet et al. 2015). Further, *Armillaria* encodes many pathogenicity associated secondary metabolites (e.g. terpene cyclases, nonribosomal peptide synthetases-like, prenyl transferases, halogenases, and polyprenyl synthases), small secreted proteins, as well as other genes that may assist in pathogenicity, such as genes involved in competitive abilities against microbes (e.g. Cys-2 peroxiredoxin, thioredoxin, class II chitinase, prohibitins, and many other redox-active, degradative and attack-type proteins) (Collins et al. 2013; Sipos et al. 2017).

Secondary metabolites

Secondary metabolites, often also called natural products, are small molecules produced by fungi and other organisms which do not have an essential function in normal growth and development. The genomes of *Armillaria* species encode several genes involved in the biosynthesis of secondary metabolites (Sipos et al. 2017). Among other functions, secondary metabolites are associated with pathogenicity and competition with other microbes. Misiek & Hoffmeister (2012) demonstrated the inhibitory effect of sesquiterpene aryl esters, the main group of secondary metabolites produced by *Armillaria*, against other wood-degrading Basidiomycetes. In line with this, a wide array of genes involved in secondary metabolism is expressed in rhizomorphs (Sipos et al. 2017). In addition, the cytotoxicity of secondary metabolites of *Armillaria* against human cancer and non-cancer cells has been demonstrated (Misiek et al. 2009; Bohnert et al. 2011; Bohnert et al. 2014b). To date, however, the mechanisms behind these antifungal and cytotoxic properties of secondary metabolites in *Armillaria* are barely understood (Bohnert et al. 2014a; Bohnert et al. 2014b). Interestingly, the two properties appear to be unlinked and follow a dissimilar structure-activity relationship (Bohnert et al. 2014a).

Validation of gene functions

The functional validation of genes in *Armillaria* is still lacking, and new genetic tools need to be developed to attain this goal. To date, the only established tools are a transformation system that makes use of *Agrobacterium tumefaciens* and permits the transformation of basidiospores and a system for heterologous gene expression (Baumgartner et al. 2010b; Ford et al. 2015, 2016). Currently, efforts are underway to establish CRISPR-Cas9 technology for gene editing in *Armillaria* (Ford & Bourquin 2018), which, once operational, may facilitate the study of gene function in *Armillaria* considerably.

Genetic mapping

In contrast to a genome sequence, which is a physical representation of the genome and gives the absolute positions of loci on a chromosome, a genetic map shows the relative positions of loci along a chromosome, with the distance between loci being proportional to the observed recombination rate between them. Recently, Heinzelmann et al. (2017a) published the first high-density genetic map for an *Armillaria* species, namely *A. ostoyae*, which contains 11 linkage groups and has a total length of 1,007.5 cM. This map anchors 61 of the 109 scaffolds (≈ 93% of the total sequence length) of the *A. ostoyae* genome assembly published by Sipos et al. (2017) and shows a high collinearity with that genome, indicating the accuracy of both, the genome assembly and the linkage map for *A. ostoyae*.

A genetic map also enables the study of the genetic basis of variation in morphological characteristics. For example, conspecific *Armillaria* strains may substantially differ in their phenotypic characteristics such as virulence, rhizomorph production, and growth rate (Prospero et al. 2004; Heinzelmann & Rigling 2016; Heinzelmann et al. 2017b; Labbé et al. 2017b). A quantitative trait locus (QTL) analysis conducted by Heinzelmann et al. (2017a) identified a single, gene dense region of 87 kb linked to a severe defect in hyphal growth in *A. ostoyae*, which

segregated as a natural mutation in the mapping population. Although further analyses are needed to determine which candidate gene is the cause of the dramatically altered growth phenotype, the successful application of forward genetics in *Armillaria* highlights that genetic mapping combined with high-quality genomic data can be used to elucidate the genetic basis underlying natural variation in phenotypic traits.

Impact of climatic and environmental changes

General considerations

Climate change, invasive species and changes in forest management not only have an impact on forest ecosystems, but also on the pathogens inhabiting them (Desprez-Loustau et al. 2016). However, how climatic and environmental changes are affecting *Armillaria*, its hosts, and their co-existence is poorly understood, and research over the last few years on the pathogen side has not brought many new insights (see the recent review of Kubiak et al. 2017). Predicting the effect of climate change on the incidence of Armillaria root disease, for example, is challenging because different stages of lifecycle, including dispersal, infection, and reproduction, may be affected directly and indirectly, and effects may be completely different (La Porta et al. 2008; Dukes et al. 2009; Kubiak et al. 2017). In addition, the lack of long-term studies on tree mortality associated with *Armillaria* and its multiple and complex interactions with environmental changes induced by human activities (Cienciala et al. 2017) limit our understanding of the evolution of *Armillaria* in a changing environment.

Species distribution

Climate change is generally expected to cause shifts in the geographic distribution of species, including *Armillaria* and its hosts (Klopfenstein et al. 2009; Hanewinkel et al. 2012; Dyderski et

al. 2018). Such shifts are expected to be more pronounced at the species' Northern and Southern distribution range margins, and could strongly affect the dynamics of ecosystems by changing the composition of the resident *Armillaria* community. Because interactions between *Armillaria* species, as well as the climatic niches occupied by different species, are still poorly characterized (Cruickshank et al. 1997; Prospero et al. 2006), it is difficult to accurately predict the outcome of the geographical shifts. In theory, different *Armillaria* species could co-exist and occupy different ecological niches, or some species could be replaced by others.

A promising way to investigate these questions is illustrated in the study of Klopfenstein et al. (2009). The authors modeled the current and prospective distribution of *A. ostoyae* in western North America along with that of Douglas-fir (*Pseudotsuga menziesii*), a frequent host species in this region, using bioclimatic models. The predicted shifts in suitable climate space for *A. ostoyae* were minor, whereas the suitable climate space of Douglas-fir was markedly reduced, suggesting that *Armillaria* may cope better with a changing climate than its host species.

Armillaria *survival and incidence*

Even though the geographic distribution of *Armillaria* species may not change significantly within the next few decades, higher annual temperatures may directly affect the activity of the fungus. For example, in regions with temperate climate, warmer winters may lead to an extension of the activity period of rhizomorphs (Rishbeth 1978). In addition, higher precipitation and temperatures in autumn may favor *Armillaria* fructification, especially in Boreal regions, thereby increasing the importance of dispersal by basidiospores as suggested by Ferguson et al. (2003). In contrast, higher summer temperatures, predicted by different climatic scenarios, may lead locally to overheated and dry soils, whose impact on survival and growth of *Armillaria* inoculum and rhizomorphs is uncertain. The few studies that have investigated the effects of soil moisture on

rhizomorph growth have found evidence of the negative impact of drought on some *Armillaria* species (e.g. *A. luteobubalina*; Pearce & Malajczuk 1990).

Another aspect which has been partially neglected, is the survival of *Armillaria* in wood during the dry season and times of drought. Although several studies have investigated the importance of soil depth, tree species, or the size of wood fragments for *Armillaria* conservation (e.g. Bruhn et al. 1998; Kromroy et al. 2005), to our knowledge, no exhaustive data is available on the effects of drought on this possibility of survival. *Armillaria* species typically produce a pseudosclerotial plate (visible as dark zone lines) around the colonized wood, which is thought to protect the mycelium against antagonistic interactions with other microbes (Guillaumin & Botton 2005). This structure, comparable to the melanized outer cortex of rhizomorphs, could also help in case of unfavorable abiotic conditions in uncolonized sections of the neighboring substrate. Thus far, these presumed seasonal and interactive effects of climate change, together with the quality of wood resources, and soil properties have only been studied with respect to a few *Armillaria* species.

Host predisposition

Armillaria will be indirectly affected by climatic and environmental changes through the predisposition of its hosts to disease because of chronic stress due to climatic maladaptation (Desprez-Loustau et al. 2006; Dukes et al. 2009; Sturrock 2012; Dempster 2017). Furthermore, tree susceptibility to disease agents will be temporarily increased by more frequent extreme weather events such as droughts, storms, temperature extremes, and floods (Woods et al. 2010; Seneviratne et al. 2012). *Armillaria* may specifically benefit in regions where mild and humid seasons (promoting growth and dispersal of the pathogen) alternate with dry and hot seasons, increasing host stress and allowing the pathogen to overcome the host's defenses. Some extreme

events, such as periodic floods, besides increasing host stress and susceptibility, may also reduce inoculum potential by reducing rhizomorph abundance in the soil (Szewczyk et al. 2016).

Climatic stressors often interact with biotic stressors, such as foliar and other pathogens or pests, leading to complex tree decline syndromes. *Armillaria* is regularly reported to be involved in such declines occurring after droughts and/or severe outbreaks of foliar pathogens (Wargo & Harrington 1991; Clinton et al. 1993; Burrill et al. 1999; Horsley et al. 2002; Marçais & Breda 2006; Haavik et al. 2015; Pavlov 2015; Holuša et al. 2018). The specific role of *Armillaria* in such declines has, however, rarely been assessed in the past and is often difficult to disentangle from the effects of other contributing agents.

Recently, Aslam & Magel (2018) demonstrated that drought-stressed saplings of black locust (*Robinia pseudoacacia*) that were wound-inoculated with *A. mellea* during dormancy were unable to restrict the spread of the pathogen. In contrast, drought stressed black locust saplings that were wound-inoculated during the growing season were able to prevent the establishment of *A. mellea*, as observed in the well-watered saplings. This suggests that not only abiotic stress may have an effect on host susceptibility to *Armillaria*, but also the time of year when such stress occurs.

Some time ago, Wargo (1972, 1996) hypothesized that specifically the depletion of starch reserves and the excess of available glucose, fructose and nitrogen in the bark and outerwood of roots, in response to drought stress or defoliation may promote the growth of *Armillaria* and reduce host ability to compartmentalize *Armillaria* infections. Interestingly, screening for resistance against Armillaria root disease in Douglas-fir reveals that trees with greater levels of *Armillaria* resistance also have a higher drought tolerance (Cruickshank & Filipescu 2017), suggesting that more frequent drought associated with climate warming, over the long-term, may select for trees with higher resistance to *Armillaria* infection.

Interactions with exotic pathogens

Non-native fungal pathogens that are accidentally introduced into a new area can be drivers of ecological change (e.g. Loo 2009). They may, in turn, interact in the new environment with native pathogens, such as *Armillaria* species, thereby exacerbating tree decline. In Europe, native *Armillaria* species have been shown to interact with the invasive pathogen *Hymenoscyphus fraxineus*, the causal agent of ash dieback (Gross et al. 2014). This fungus may cause, among other symptoms, necrotic collar lesions at the stem base of European ash (*Fraxinus excelsior*) and narrow-leafed ash (*Fraxinus angustifolia*) (Husson et al. 2012; Chandelier et al. 2016). Such lesions may be secondarily invaded by *Armillaria* species, which may reduce tree stability and accelerate tree decline (Lenz et al. 2016; Marçais et al. 2016; Enderle et al. 2017; Timmermann et al. 2017). Remarkably, *A. gallica* and *A. cepistipes* usually prevail in the collar necrosis, suggesting that less pathogenic and preferentially saprotrophic *Armillaria* species actually benefit from the interaction with *H. fraxineus* (Skovsgaard et al. 2010; Bakys et al. 2011; Husson et al. 2012; Marçais et al. 2016; Enderle et al. 2017).

Control of Armillaria root disease

General considerations

The control of Armillaria root disease in forest and agricultural systems is challenging because of the saprophytic ability of *Armillaria* and its hidden growth below ground (Redfern & Filip 1991; Guillaumin et al. 1993). The potential of *Armillaria* to persist for decades in dead wood (e.g. stumps, woody debris or root fragments in the soil) results in an increased risk of tree mortality in subsequent tree plantations on infected sites (e.g. Lung-Escarmant & Guyon 2004; Kromroy et al. 2005). The choice of disease management strategies strongly depends on the targeted ecosystems

and the economic value of the host species (Bogdanski et al. 2018). Until now, control strategies

for Armillaria root disease have primarily been aimed at reducing the amount of *Armillaria*

inoculum in the soil, either by removing infected plants and woody debris after tree harvesting or

by treating the soil of infected stands with highly toxic fumigants (Baumgartner et al. 2011). In

recent years, due to the worldwide ban of the fumigant methyl bromide, fumigant research has

generally stopped and more environmentally-friendly control methods, such as post-infection

treatments, the mitigation of yield losses, or the planting of less susceptible or resistant hosts,

have been proposed as possible alternatives (Baumgartner et al. 2011). The development of new

biological control methods and the improved characterization of microbiological soil

communities (Vayssier-Taussat et al. 2014) will provide new promising options to control

Armillaria root disease.

Mechanical stump removal

The practice of mechanically removing stumps and as much of the root system as possible after

logging (to reduce the amount of inoculum) is a procedure that is often followed and is highly

recommended for managing Armillaria root disease (Morrison & Mallett 1996). Recent studies

have investigated the long-term effects (i.e. ranging from 21 to 50 years) of stump and root

removal on the incidence of Armillaria root disease in subsequent plantations of various

coniferous species in Canada and Scandinavia (Shaw III et al. 2012; Cleary et al. 2013; Morrison

et al. 2014, Cruickshank et al. 2018). Except for Shaw III et al. (2012), all studies concluded that

this method is effective in reducing the incidence of Armillaria root disease, especially during the

first years after the establishment of a new plantation. Shaw III et al. (2012), however, found that

stump removal alone has poor long-term benefits and that only more intensive treatments (i.e.

maximum removal of roots by machine ripping and removal of visible remaining roots by hand)

are effective. Thus, it is advisable to consider extensive stump and root removal only in areas with high mortality due to Armillaria root rot, given the costs involved.

Besides disease incidence, several other factors should be evaluated before undertaking stump removal. First, stumps in disease-free areas may also be at risk as they could become colonized by *Armillaria* inoculum coming from neighboring, infected stands. Some studies have indeed shown that new *Armillaria* genotypes can become established in first plantation areas where disease symptoms were previously not observed (Rishbeth 1988; Legrand et al. 1996). Cases like this may occur due to colonization by basidiospores originating from other stands. Second, mechanical stump removal is expensive, and likely only cost-effective for trees of high economic value. It should be said that stump harvesting for bioenergy offers an additional financial benefit which may help minimize treatment costs while also reducing the disease impact in the next rotation of the stand (Bogdanski et al. 2018). Third, stump removal may have negative ecological consequences (e.g. loss of organic compounds and nutrients in the soil) and increase the susceptibility of soil to erosion, both of which, in turn, affect the productivity of trees (Norris et al. 2014; Achat et al. 2015). Thus, this measure cannot always be considered as an efficient practical solution for Armillaria root disease management.

An alternative or complementary measure to stump removal could be the ring barking of trees before felling to reduce the amount of carbohydrate stored in the roots and, thus, potentially making them less attractive to *Armillaria* (Guillaumin & Legrand 2013). This practice, however, has not proven to be efficient in the presence of abundant rhizomorphs in the soil.

Chemical and biological control

Other frequently used pre-planting methods to control Armillaria root disease include the treatment of infected soil or plants with biological or chemical agents (reviewed in Baumgartner

et al. 2011). Chemical treatment consists mainly of soil fumigation using methyl bromide or carbon disulfide which are able to kill fungal mycelium in infected roots. This method has been applied, with variable success, especially in orchards or vineyards. To date, fumigants are generally prohibited due to their high toxicity. Less damaging for the environment, biological control is based on the antagonistic effect of other microbes, especially bacteria and fungi, against *Armillaria*. This approach aims at decreasing *Armillaria* incidence and its ability to infect new hosts or colonize dead wood (Dumas 1992; Chapman & Xiao 2000). Unfortunately, except in some rare cases (Chapman et al. 2004), biological control has proven difficult to apply and has had little success in the field (Raziq and Fox 2003; Baumgartner & Warnock 2006). Even the new antagonists described in recent studies have so far failed to demonstrate their efficiency at a large scale in the field (Szwajkowska-Michalek et al. 2012; Mesanza et al. 2016; Kwasna & Szynkiewicz-Wronek 2018).

Tomalak (2017) recently discovered nematodes of the species *Bursaphelenchus fraudulentus* parasiting *Armillaria*. *In vitro* and *in vivo* artificial inoculations showed that this mycetophagous wood nematode is able to feed, disperse and reproduce in the mycelium of *A. ostoyae,* quickly killing the entire colony. In addition, *B. fraudulentus* can reproduce within *A. ostoyae* rhizomorphs, suggesting that these structures could facilitate its spread. The presence of hyperparasites such as this nematode species in nature*,* might explain the observation of disease foci without further spatial expansion after a first period of high mortality in some forest ecosystems (Durrieu et al. 1985), and, thus, open new opportunities in Armillaria root disease management.

With the continued progress in community analyses using DNA-based methods (e.g. Vayssier-Taussat et al. 2014), it is likely that the network of antagonists interacting with *Armillaria* species will be better described soon, increasing the options for biological control. The

identification of the factors that maintain optimal diversity and structure in the microbial soil community, thereby enabling the highest resilience of ecosystems infested by *Armillaria,* could represent a valid alternative to curative treatment using biological agents that are often difficult to apply (Baumgartner & Warnock 2006).

Silvicultural measures

Another possibility for management relies on the use of resistant (i.e. resisting to the pathogen) or tolerant (i.e. coping with the pathogen by surviving and growing) varieties or tree species. Variations in quantitative resistance to *Armillaria* species have been investigated both among (e.g. Ferguson et al. 2003; Baumgartner et al. 2011; Vyse et al. 2013), and within (e.g. Baumgartner & Rizzo 2006; Solla et al. 2011; Cruickshank & Jaquish 2014) host species. Recent advances in genomics could help breeding programs to select traits for resistance in host species (e.g. Plomion et al. 2016), although phenotyping for resistance to Armillaria root disease is a time-consuming process. According to Cruickshank & Jaquish (2014), breeding programs should focus not only on resistance, but also on tolerance, as both traits have potentially positive effects.

To reduce disease incidence, as well as the risk of rapid adaptation of *Armillaria* to more resistant tree species or varieties, mixtures of species or varieties should be considered. The use of stand diversification for the control of Armillaria root disease has been studied for a considerable length of time (Gerlach et al. 1997; Fleming et al. 2005). Results have been, however, contradictory, likely also because of the polyphagous nature of the pathogenic *Armillaria* species considered. Morrison et al. (2014) showed that admixing resistant and susceptible species by the artificial establishment of a stand in British Columbia did not significantly reduce disease impact on the susceptible species over a period of 40 years. Similar findings were reported by Cruickshank et al. (2018). In contrast, a study by Baleshta et al. (2015)

did reveal how the presence of naturally regenerated broadleaf trees (specifically *Betula papyrifera*) can reduce mortality due to *A. ostoyae* and increase productivity of conifer plantations (specifically *Pseudotsuga menziesii* var. *glauca*).

The mechanisms that account for how interspersed resistant hosts protect susceptible species have not been clearly identified yet (Iason et al. 2018). On one hand, it could be due to a barrier effect provided by the resistant species and its associated fungal community, reducing the soil-borne spread of *Armillaria* to the more susceptible host species (i.e. associational resistance, Barbosa et al. 2009). On the other hand, the reduced density of the susceptible species may limit root contacts and the transmission of the pathogen (i.e. dilution effect; Prospero & Cleary 2017). Complex responses were obtained when different densities of resistant and susceptible species were tested (Baleshta et al. 2015; Metslaid et al. 2018). In both studies, an optimal density of resistant or susceptible species was found, suggesting an equilibrium between the effect of competition among susceptible and resistant species and the probability of disease spread. It is likely that these optimal densities vary along with factors such as the type of admixture, the particular *Armillaria* species, and climatic factors (Nguyen et al. 2016). Thus, additional studies are required in order to draw firm conclusions on the real benefit of admixture.

Integrated management

Given the ability of *Armillaria* to disperse among stands, Armillaria root disease management should instead be considered at a regional scale, and not merely at the stand level. For instance, recent epidemiological and population genetic approaches have suggested that the rapid, massive and dense plantation of maritime pine (*Pinus pinaster*) in south-western France in the 19th century may have offered an opportunity for *A. ostoyae* to adapt to this intensive monoculture

forest, and thus, to rapidly expand from the pre-existing forest areas (Labbé et al. 2015; Labbé et al. 2017a).

Considering the modern context of agricultural intensification (i.e. monocultures in large areas without cultural rotations) and the variability in virulence among *Armillaria* genotypes and species (Prospero et al. 2004; Heinzelmann et al. 2017b; Labbé et al. 2017b), the evolutionary potential for higher virulence in the genus *Armillaria* should receive more attention. In this regard, an important role could also be played by the reservoir of inoculum in dead woody substrate (Soularue et al. 2017). Because most problems associated with Armillaria root disease are observed in plantations or intensively managed forests, a complementary management strategy could be to foster natural regeneration in stands with low mortality. Although natural regeneration is sometimes difficult to achieve, for example because of browsing pressure by ungulates, it has at least two major benefits. First, this process favors the selection of resistant individuals and likely the establishment of a less favorable microbial community for *Armillaria.* Second, natural stand regeneration has lower economic costs relative to plantations. However, the efficiency of this strategy needs to be investigated further in stands with high mortality, because contrasting results are reported (Bace et al. 2012; Nevalainen 2017).

Further research perspectives

In the near future, next generation sequencing and its numerous applications will most likely continue to gain importance for *Armillaria* research and infiltrate its various disciplines. The amount of data generated with these recent technologies will provide ample opportunities to tackle old questions from a new perspective. For example, generating a collection of high-quality genomes of different *Armillaria* species may help to resolve the genomic specificities of *Armillaria* species with different ecological strategies (e.g. parasitic vs. saprotrophic).

Furthermore, comparing *Armillaria* genomes with those of other root-rot pathogens (e.g. *Heterobasidion* or *Phellinus* spp.) and purely saprotrophic fungi may permit the identification of genes involved in pathogenesis and wood degradation (e.g. Suzuki et al. 2012; Kües et al. 2015). Further, establishing transcriptomic data sets that cover the entire infection process from epiphytic growth of rhizomorphs on roots, to root penetration, and its establishment in the root, may help to uncover the molecular processes taking place at the pathogen-host interface.

Genomic data may also be used to infer the phylogeny of the genus in more detail and help to resolve current taxonomic issues and the evolutionary processes at the foundation of the fascinating radiation of *Armillaria* species around the world. Moreover, using a genome-based approach will enable the assessment of population dynamics and factors that impact the variability of populations at much higher resolution than in current population genetic studies. A more comprehensive reconstruction of population dynamics will, for example, lead to a better understanding of how increased host availability (e.g. large plantations) may lead to population expansion and higher virulence, thus improving the risk prediction of future large outbreaks (Labbé et al. 2017a; Labbé et al. 2017b). Genomic studies may also greatly help identify genomic regions under selection by comparing *Armillaria* populations in different climatic and host contexts using the so-called "reverse ecology" approach (Ellison et al. 2011) as a complement to the transcriptomic approach discussed above.

A key issue for an improved understanding of the evolutionary dynamics of *Armillaria* populations is to link genomic and phenotypic variation, and to understand the importance of the phenotypic variation for the pathogen and its interaction with hosts. However, phenotyping larger sets of isolates, for example, for virulence, one of the most studied traits in *Armillaria*, is still a considerable challenge because the currently used method (i.e. inoculation of potted seedlings; Morrison 2004; Prospero et al. 2004; Heinzelmann et al. 2017b; Labbé et al. 2017b) is time

consuming, in terms of preparation and experiment duration. Recent attempts to simplify the virulence screening of *Armillaria* isolates (Baumgartner et al. 2010a; Ford et al. 2017) are only partially conclusive. In addition, it will be important to develop tools for functional gene validation, which would finally allow us to demonstrate the causative role of candidate genes for the targeted phenotypes.

Overall, it will be crucial for upcoming research to consider a holistic approach that considers the pathogen, the host, and the environment, as well as the complex interactions among them. As outlined in this review, the ecological strategy and disease outcome of *Armillaria* species varies depending on the environmental context. Extending recently developed models by integrating the different stages of the life cycle of *Armillaria*, information about pathogen-host interactions, climatic and environmental variables, and simulating long evolutionary timeframes (Soularue et al. 2017), may be a promising approach to predict the outcomes of different environmental and climatic scenarios. Here also, however, field investigations and experimental research will be necessary to obtain baseline data to feed and optimize the models. For example, as outlined in this review, several aspects of the *Armillaria* life cycle are still poorly known (e.g. conditions and substrate for basidiospore germination), and resolving these questions would significantly improve model accuracy.

Finally, the major challenge will be to translate the insights gained in the various fields of *Armillaria* research into control strategies to mitigate the impact of Armillaria root disease in current and future woody ecosystems. Tree species admixture and natural regeneration appear to be promising strategies to increase host diversity and promote a more diverse microbial soil community, thereby reducing the impact of Armillaria root disease. However, many questions remain unanswered. As several recent studies presented in this review have shown, the interaction between *Armillaria*, its hosts, and the environment is complex and difficult to predict.

Acknowledgements

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Table 1. Summary statistics of the *Armillaria* genomes reported in Collins et al. (2013),

Wingfield et al. (2016) and Sipos et al. (2017).

Species	Strain	Origin	Total length (Mbp)	Number of scaffolds	N50 (Mbp)[1]	Number of genes	Reference
A. cepistipes	B5	Europe	75.50	182	3.29	23,461	Sipos et al. (2017)
A. gallica	Ar21-1	North America	85.34	319	1.04	25,704	Sipos et al. (2017)
A. mellea[2]	DSM 3731	Europe	58.36	4,377	0.37	14,473	Collins et al. (2013)
A. ostoyae	C18/9	Europe	60.10	106	2.28	22,705	Sipos et al. (2017)
A. ostoyae	28-4	North America	58.01	229	0.72	20,811	Sipos et al. (2017)
A. fuscipes	CMW 2740	Africa	53.00	24,436	0.01	14,515	Wingfield et al. (2016)

[1] N50 is the shortest sequence length at 50% of the genome assembly.

[2] Summary statistics for the *A. mellea* genome reported in Collins et al. (2013) are different from

those reported in the JGI genome portal (i.e. total length: 79.55 Mbp; number of scaffolds:

29,300; N50: 0.02).
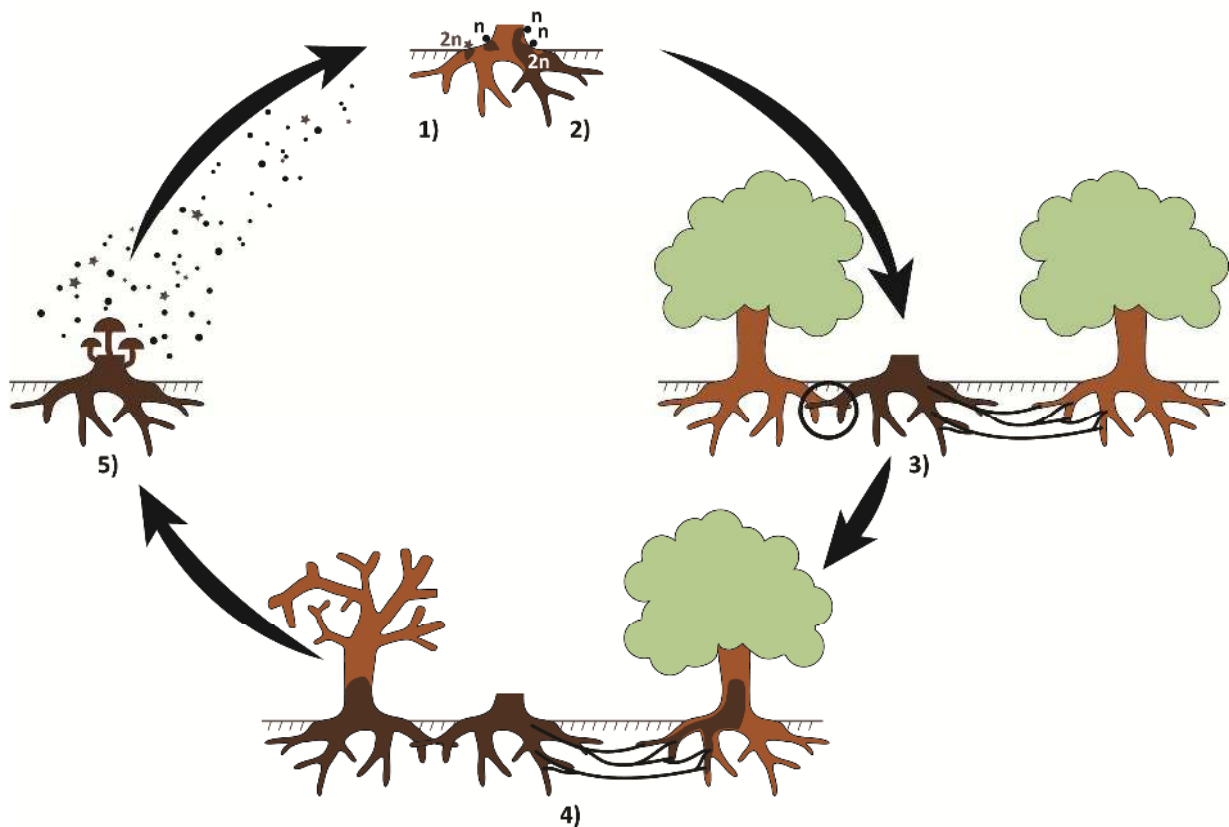
Figure 1. Schematic representation of the life cycle of *Armillaria* species. (1) Basidiospores germinate on woody substrate (e.g. wood fragments, possibly stumps) originating a haploid (n) mycelium (heterothallic species) or a diploid (2n) mycelium (homothallic species); (2) In heterothallic species, after mating between two compatible haploid mycelia, a diploid mycelium is formed. In both heterothallic and homothallic species, the diploid mycelium colonizes the woody substrate; (3) Healthy trees are infected either by root contacts with infected woody substrates or by soil rhizomorphs growing out from infected woody substrate; (4) *Armillaria* invades the root system and lower stem of the infected trees, killing the cambium or/and causing heart rot; (5) Fruiting bodies develop on dead/moribund woody substrate and release basidiospores into the environment.
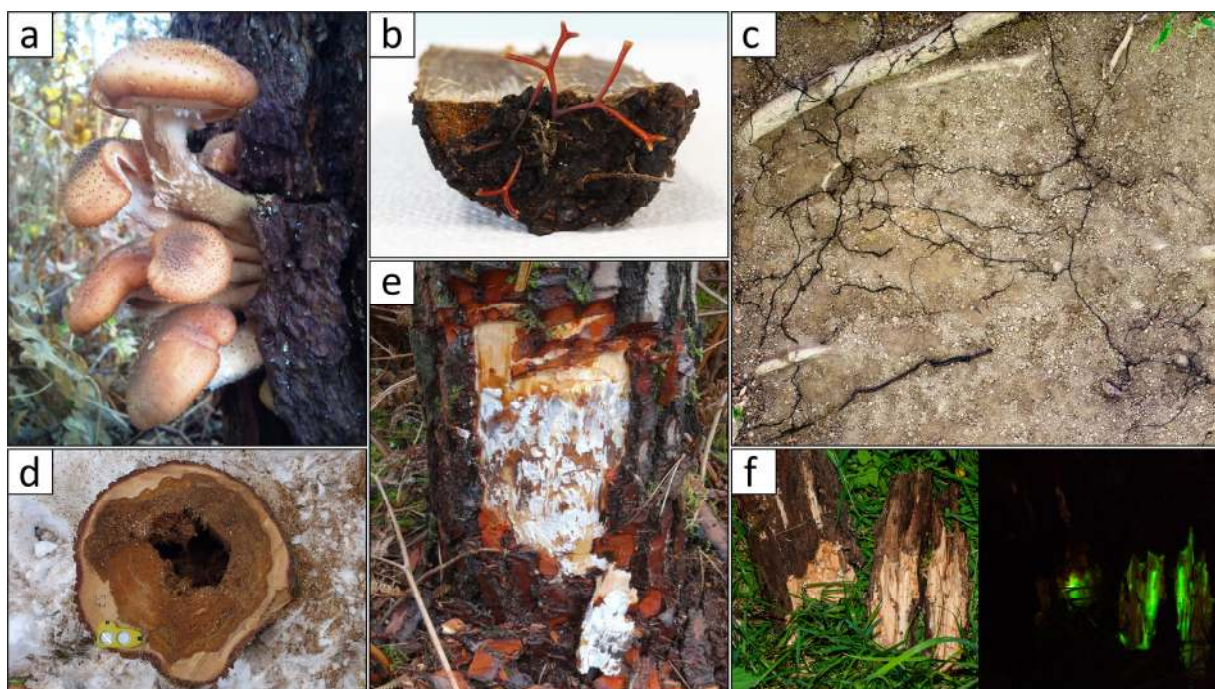
Figure 2. Main signs of the presence of *Armillaria* species. (a) Fruiting bodies of *A. ostoyae* on the trunk of a dead maritime pine (*Pinus pinaster*); (b) Rhizomorphs growing out of a wood segment colonized by *A. borealis*; (c) Network of rhizomorphs of *Armillaria* spp. in the soil. (d) Heart rot caused by *A. cepistipes* on Norway spruce (*Picea abies*); (e) Mycelial fans of *A. ostoyae* developing in the cambial area of an infected maritime pine; (f) *Armillaria* spp. colonized wood fragments showing typical bioluminescence. Photo credits: (a) F. Labbé; (b) and (e) R. Heinzelmann; (c) and (f), H. Baggenstos/A. Rudolf; (d) Phytopathology, WSL.

Figure 3. Geographical distribution of the currently known *Armillaria* s.s. and *Desarmillaria* species (Korhonen 1978; Anderson and Ullrich 1979; Roll-Hansen 1985; Anderson 1986; Motta and Korhonen 1986; Watling et al. 1991; Guillaumin et al. 1993; Termorshuizen and Arnolds 1994; Cha and Igarashi 1995; Volk and Burdsall 1995; Abomo-Ndongo and Guillaumin 1997; Ota et al. 1998; Coetzee et al. 2001b; Coetzee et al. 2003; Qin et al. 2007; Lima et al. 2008; Pildain et al. 2010; Brazee et al. 2012b; Hood and Ramsfield 2016; Elías-Román et al. 2018). * indicates species that is only reported in the specific geographic area. § indicates species putatively introduced into the specific geographic area. # indicates biological species awaiting formal description.