Secrets of the subterranean pathosystem of *Armillaria*

Kendra Baumgartner[1], Martin P. A. Coetzee[2] and Dirk Hoffmeister[3]

(1) United States Department of Agriculture-Agricultural Research Service (USDA-ARS), University of California, Davis, CA 95616, USA.
(2) Department of Genetics, Forestry and Agricultural Biotechnology (FABI), University of Pretoria, Pretoria 0002, South Africa.
(3) Friedrich-Schiller-Universität, Department of Pharmaceutical Biology, Beutenbergstr 11A, D-07745 Jena, Germany.

Kendra Baumgartner
Email: kbaumgartner@ucdavis.edu

Abstract

Armillaria root disease affects fruit and nut crops, timber trees and ornamentals in boreal, temperate and tropical regions of the world. The causal pathogens are members of the genus *Armillaria* (Basidiomycota, Physalacriaceae). This review summarizes the state of knowledge and highlights recent advances in Armillaria research.

Taxonomy: *Armillaria* includes more than 40 morphological species. However, the identification and delineation of species on the basis of morphological characters are problematic, resulting in many species being undetected. Implementation of the biological species' concept and DNA sequence comparisons in the contemporary taxonomy of *Armillaria* have led to the discovery of a number of new species that are not linked to described morphological species.

Host range: *Armillaria* exhibits a range of symbioses with both plants and fungi. As plant pathogens, *Armillaria* species have broad host ranges, infecting mostly woody species. *Armillaria* can also colonize orchids *Galeola* and *Gastrodia* but, in this case, the fungus is the host and the plant is the parasite. Similar to its contrasting relationships with plants, *Armillaria* acts as either host or parasite in its interactions with other fungi.

Disease control: Recent research on post-infection controls has revealed promising alternatives to the former pre-plant eradication attempts with soil fumigants, which are now being regulated more heavily or banned outright because of their negative effects on the environment. New study tools for genetic manipulation of the pathogen and characterization of the molecular basis of the host response will greatly advance the development of resistant rootstocks in a new stage of research. The depth of the research, regardless of whether traditional or genomic approaches are used, will depend on a clear understanding of where the different propagules of *Armillaria* attack a root system, which of the pathogen's diverse biolymer-degrading enzymes and secondary metabolites facilitate infection, and how the course of infection differs between resistant and susceptible hosts.

Introduction

Armillaria is one of the most important genera of fungal root pathogens worldwide. It attacks hundreds of tree species in both timber (e.g. *Abies*, *Picea*, *Pinus*, *Pseudotsuga*) (Entry *et al*., 1991; Wargo and Shaw, 1985) and agronomic (e.g. *Citrus*, *Juglans*, *Malus*, *Prunus*, *Vitis*) (Baumgartner

and Rizzo, 2001a; Guillaumin, 1977; Guillaumin *et al*., 1989a; Proffer *et al*., 1987) systems in both hemispheres and in a range of climates (Hood *et al*., 1991). *Armillaria* root disease affects agronomic and timber plantations established on land previously occupied by infected fruit/nut crops or infected forest stands. After clearing infected trees, mycelium surviving saprophytically in woody residual roots can remain buried in the soil, and serves as inoculum for infection of the next crop (Redfern and Filip, 1991). The persistence of such inoculum for years to decades (Baumgartner and Rizzo, 2002), combined with the lack of efficient methods to either effectively prevent (Bliss, 1951; Gubler, 1992; Munnecke *et al*., 1981) or cure (Adaskaveg *et al*., 1999; Aguin-Casal *et al*., 2006) infections, contributes to significantly reduced yields throughout the life of an infected plantation (Baumgartner, 2004) and that of successive plantings.

Armillaria possesses genetic adaptations to a range of symbioses with plants and other fungi. As the causal agent of *Armillaria* root disease, *Armillaria* is a facultative necrotroph; it colonizes living roots, kills root tissue and then utilizes the dead tissue as its source of nutrition. After the plant dies, *Armillaria* persists as a saprophytic white-rotter on infected portions of the root system (Redfern and Filip, 1991). In a very different type of plant symbiosis, *Armillaria* exhibits a rare form of mycorrhizal relationship, known as 'myco-heterotrophy', with the achlorophyllous orchids *Galeola* and *Gastrodia* (Kikuchi *et al*., 2008), in which the plant is thought to be the parasite and *Armillaria* is the host. Similar contrasting roles as either parasite or host are exhibited by *Armillaria* in symbioses with other fungi. For example, *Armillaria* is parasitized by *Entoloma abortivum* (Basidiomycota, Entolomataceae), which causes misshapen *Armillaria* fruiting bodies (carpophoroids) (Czederpiltz *et al*., 2001). In contrast, *Armillaria* is thought to be a mycoparasite of *Wynnea* (Ascomycota, Sarcoscyphaceae) (Fukuda *et al*., 2003).

Study tools for rapid plant infection (Baumgartner *et al*., 2010a) and genetic transformation of *Armillaria* (Baumgartner et al., 2010c), in combination with ongoing progress towards sequencing of the *A. mellea* genome (Joint Genome Institute, United States Department of Energy, Walnut Creek, CA, USA), are bringing new opportunities to *Armillaria* research. *Armillaria* does not have all the 'perks' of other basidiomycete systems (e.g. *Coprinopsis*). For example, fruiting in culture is possible for a few *Armillaria* species and only in the hands of a few researchers (Grillo *et al*., 2000). However, *Armillaria* does have both economic and ecological significance. *Armillaria* root disease affects many high-value crops and is one of the most serious diseases of boreal and temperate forestry. Furthermore, the most effective pesticide to prevent *Armillaria* root disease, methyl bromide, is to be banned from use as a soil fumigant worldwide. Researchers are tasked with developing alternatives. Discovering how *A. mellea* can persist for decades as mycelium in residual roots and can function as a parasite of some plants and as a host of others may lead to effective controls for *Armillaria* root disease.

Hartig (1874) was the first to suggest that the basidiocarps (Fig. 1a) of what was then referred to as '*Agaricus melleus*' were associated with the subcortical mycelial fans on nearby symptomatic trees (Fig. 1b) and with rhizomorphs found in surrounding soil (Fig. 1c). Indeed, *Armillaria* rhizomorphs are among the most morphologically and functionally complex of all mycelial cord-forming basidiomycetes, with distinct layers of cells (Fig. 1d) that function in apical or lateral growth (Motta, 1969) and with pores specialized for gas exchange (Pareek *et al*., 2006). This review summarizes topics from monographs on *Armillaria* (Fox, 2000; Shaw and Kile, 1991) as background information on the pathogen, and highlights recent advances in research on the biological secrets of this subterranean fungus, which glows in the dark (Mihail and Bruhn, 2007), is one of the largest and oldest organisms on Earth (Smith et al., 1992) and possesses edible basidiocarps ('honey mushrooms').

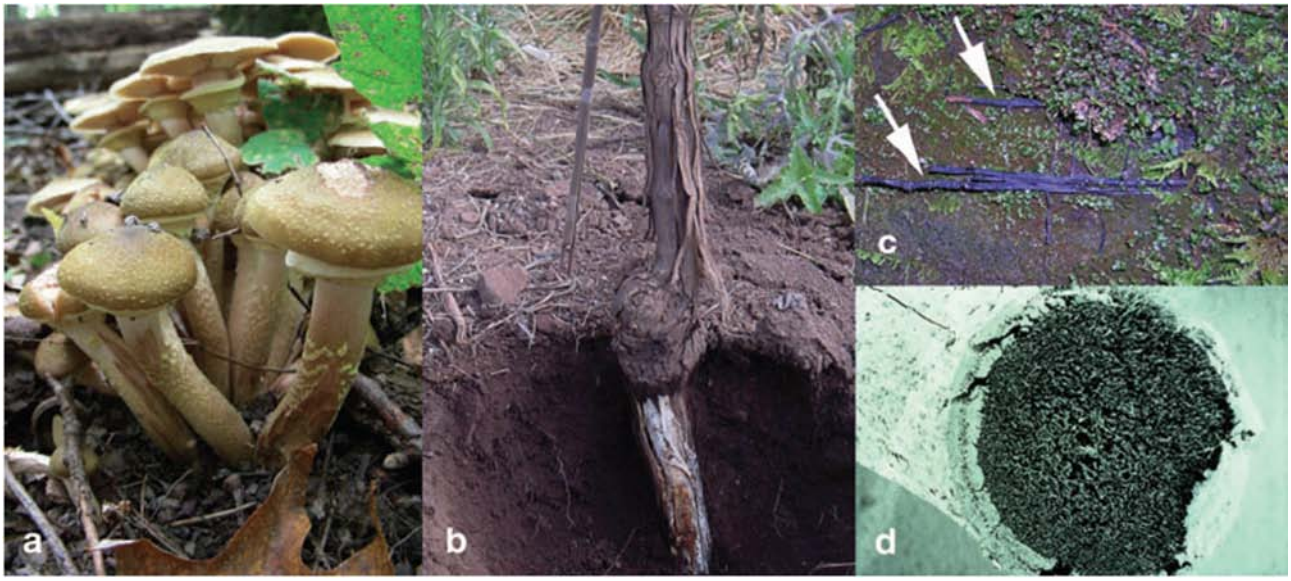


Figure 1. Signs of *Armillaria* root disease: (a) basidiocarps of *Armillaria mellea* from a mixed-hardwood forest in Northfield, MN, USA; (b) mycelial fans found beneath the bark at the root collar of *Vitis vinifera* (grapevine) infected with *A. mellea*; (c) rhizomorphs (see arrows) growing under the bark of dead, fallen *Abies alba* (European silver fir) in Oberjoch, Bavaria; (d) a rhizomorph in cross-section, showing the outer layers of cells (photograph by David M. Rizzo).

Taxonomy and life-cycle

The taxonomy of *Armillaria* dates back to the 1700s when Danish botanist Martin Vahl made reference to *Agaricus melleus* in his Flora Danica (Vahl, 1787), a species that is now accepted as *Armillaria mellea* (Vahl:Fr.) P. Kummer and is the type of the genus. The genus currently includes more than 40 morphological species as well as unique biological and phylogenetic species that are not equated with morphological species. The majority of these species have a tetrapolar heterothallic mating system and only a few species are homothallic. *Armillaria* species are unique in that their vegetative state is diploid, rather than dikaryotic as is the general nuclear state for other basidiomycetes.

Taxonomy

Until the 1980s, the classification of *Armillaria* species was dominated by the morphological species' concept. Following the criteria set by this concept *Armillaria* includes agarics with white spores, decurrent to adnate gills and diploid vegetative mycelium, that are wood inhabiting (parasitic or saprophytic) and produce black to reddish-brown rhizomorphs either in the field or in culture (Volk and Burdsall, 1995; Watling *et al*., 1991). Morphological characters useful for the delineation of *Armillaria* species include the ornamentation and structure of the stipe and pileus, location of the pigments, basidiospore size and ornamentation, and the presence or absence of clamp connections (Bérubé and Dessureault, 1988; Watling et al., 1991). Presently, the genus includes at least 40 morphological species (Pildain *et al*., 2010; Volk and Burdsall, 1995).

The recognition of *Armillaria* species on the basis of basidiocarp morphology has several limitations. Basidiocarps are ephemeral and irregularly produced; they are thus not readily available during field surveys. It is difficult to induce fruiting in culture and, when basidiocarps are produced, their morphological characteristics often do not correlate with those generated under natural conditions. Environmental factors may affect the characteristics of basidiocarps, for example meteorological conditions have been found to influence the dimensions and colour of basidiocarps belonging to *A. luteobubalina* (Kile and Watling, 1988). Finally, some species (e.g. *A. ostoyae*, *A.*

gemina) produce basidiocarps with identical morphology and are therefore indistinguishable (Bérubé and Dessureault, 1989).

As a result of the limitations of species' recognition strictly on the basis of basidiocarp morphology, a repertoire of additional phenotypic characters has been used in combination or as an alternative to basidiocarp morphology to delineate *Armillaria* species. Phenotypic characteristics include the morphology of the mycelium and rhizomorphs (e.g. Bérubé and Dessureault, 1989; Shaw *et al*., 1981), response to temperature (e.g. Mohammed *et al*., 1994; Rishbeth, 1986), response to phenolic acids and terpenes (e.g. Rishbeth, 1986), isozyme and protein profiles (e.g. Coetzee *et al*., 2009; Morrison *et al*., 1985; Mwenje and Ride, 1996; Mwenje *et al*., 2006) and the reaction of mono- and polyclonal antibodies (e.g. Fox and Hahne, 1989; Lung-Escarmant and Dunez, 1979; Lung-Escarmant *et al*., 1985). In many cases, these phenotypic characters are not unique for a specific species, but can be used to differentiate between species with similar morphologies.

Species' recognition on the basis of the biological species' concept was introduced into the taxonomy of *Armillaria* during the late 1970s. Researchers from Europe (Korhonen, 1978) and North America (Anderson and Ullrich, 1979) were the first to employ this concept in delineating biological species of *A. mellea sensu lato*. This species was considered in earlier literature to be highly polymorphic with variable rhizomorph production and pathogenicity, and a broad host range. Using mating tests for species' recognition, it was discovered that *A. mellea sensu lato* represented a species' complex that includes a number of intersterility groups or biological species. This discovery resulted in the extensive use of the biological species' concept in the taxonomy of *Armillaria*. Consequently, a number of biological species were identified from different regions of the world (Tables 1 and 2).

Table 1. *Armillaria* species and their equivalent biological species occurring in the Holarctic Floral Kingdom (Anderson, 1986; Anderson and Ullrich, 1979; Guillaumin *et al*., 1985; Korhonen, 1978; Motta and Korhonen, 1986; Ota *et al*., 1998b; Qin *et al*., 2007; Roll Hansen, 1985; Termorshuizen and Arnolds, 1987; Volk and Burdsall, 1995; Volk *et al*., 1996b; Watling *et al*., 1991; Zolciak *et al*., 1997).

Species	Western North America	Eastern North America	Europe	China	Japan
A. borealis			A	CBS M	
A. calvescens		NABS III			
A. cepistipes	NABS XI	NABS XI	B		NAG D
A. ectypa			*		*
A. gallica	NABS VII	NABS VII	E	CBS B	NAG A
A. gemina		NABS II			
A. jezoensis					H
A. mellea	NABS VI	NABS VI	D	CBS K	
A. mellea ssp. *nipponica*				CBS G	NAG Am
A. nabsnona	NABS IX				NAG B
A. ostoyae	NABS I	NABS I	C	CBS D	NAG C
A. sinapina	NABS V	NABS V		CBS A	F
A. singula					G
A. tabescens	*	*	*	CBS I	T
Undescribed species					
NABS X	*				
CBS C				*	
CBS F				*	
CBS H				*	
CBS J				*	
CBS L				*	
CBS N				*	
CBS O				*	
NAG E					*

Asterisks denote the presence of a species.
CBS, Chinese Biological Species; NABS, North American Biological Species; NAG, Nagasawa.

Table 2. *Armillaria* species and their equivalent biological species occurring in the non-Holarctic Floral Kingdoms (Abomo-Ndongo and Guillaumin, 1997; Coetzee *et al*., 2003a, 2000a; Lima *et al*., 2008; Pildain *et al*., 2010; Volk and Burdsall, 1995; Watling *et al*., 1991).

Species	Central America	South America	Africa	Australia	New Zealand	Other regions
A. affinis	*					
A. camerunensis			*			
A. duplicata						India
A. fellea						New Guinea
A. fumosa				*		
A. fuscipes			*			
A. griseomellea		*				
A. heimii			SIG II			
A. hinnulea				*	*	
A. limonea					*	
A. luteobubalina				*		
A. mellea			SIG I			
A. mellea ssp. *nipponica*						Bhutan
A. melleo-rubens	*					
A. montagnei		*				
A. novae-zelandiae		*		*	*	New Guinea, Sumatra, Malaysia, Fiji
A. omnituens						India
A. pallidula				*		
A. paulensis		*				
A. pelliculata			*			
A. procera		*				
A. puiggarii		*				
A. sparrei		*				
A. tabescens	*					
A. tigrensis		*				
A. umbrinobrunnea		*				
A. viridiflava		*				
A. yungensis		*				
Undescribed species						
BPS I						Bhutan
NZ *Armillaria* sp.					*	

Asterisks denote the presence of a species.
BPS, Bhutan Phylogenetic Species; NZ, New Zealand; SIG, Somatic Incompatibility Group.

The application of the biological species' concept in the taxonomy of *Armillaria* is complicated by some practical limitations. Mating tests to determine identity yield the best results when they are performed between a monosporous isolate from a known species (i.e. a haploid 'tester' strain) and a monosporous isolate from the unknown field strain. During a sexually compatible interaction, haploid strains are diploidized, resulting in a change from white fluffy mycelium (haploid morphology) to a dark crustose mycelium (diploid morphology) (Hintikka, 1973). Identity can also be determined in pairings between a haploid tester strain and a diploid field strain, the latter of which is recovered from mycelial fans or rhizomorphs on symptomatic plants. However, diploidization of haploid isolates (a process analogous to the Buller phenomenon in dikaryotic basidiomycetes) is slow, and diploid–haploid interactions are sometimes ambiguous (Korhonen, 1978, 1983; Siepmann, 1987). Tester strains may, however, degrade over time, becoming similar in morphology to diploid cultures; consequently, these strains are not suitable for mating tests (Harrington *et al*., 1992). Furthermore, reproductive barriers between two allopatric intersterility groups may be incomplete and will therefore make the assignment of anonymous isolates to a biological species difficult. For example, European Biological Species (EBS) B (*A. cepistipes*) is interfertile with the North America Biological Species (NABS) XI, and is therefore conspecific with this species (Banik and Burdsall, 1998; Morrison et al., 1985). However, EBS B is partially interfertile with NABS V (*A. sinapina*) and NABS X (Anderson, 1986; Bérubé *et al*., 1996). Despite these limitations, the biological species' concept still forms the basis of many contemporary taxonomic studies, especially in Europe, where biological species correspond to morphological species.

Since the first DNA sequences of Northern Hemisphere *Armillaria* species were published by Anderson and Stasovski (1992), DNA sequence comparisons and phylogenetic analyses of DNA sequence data have increasingly been used to discover new species and identify isolates of *Armillaria* to the species' level. Other DNA-based methods that have been employed for this purpose include DNA–DNA hybridization (Jahnke *et al*., 1987), DNA base composition (Motta *et al*., 1986), amplified fragment length polymorphisms (AFLPs) (Pérez-Sierra *et al*., 2004; Wingfield *et al*., 2009) and restriction fragment length polymorphisms (RFLPs) (Anderson *et al*., 1987; Smith and Anderson, 1989) of mitochondrial DNA, whole cell nuclear DNA (Anderson *et al*., 1987), the complete ribosomal intron (Anderson and Bailey, 1989; Schulze *et al*., 1995) and RFLPs of PCR-amplified regions of the rDNA intergenic spacer I (IGS-I) and the internally transcribed spacer (ITS) (Chillali *et al*., 1997; Chillali *et al*., 1998; Coetzee *et al*., 2000b; Harrington and Wingfield, 1995; Mwenje *et al*., 2003).

Since the early 1990s, DNA sequencing has become routine for the species'-level identification of *Armillaria* collections, which frequently consist entirely of isolates recovered from mycelial fans, rhizomorphs and decayed wood (and not basidiocarps). Phylogenetic analyses of IGS-I, ITS and the nuclear gene elongation factor 1-α (EF-1α) support the existence of the biological and morphological species earlier discovered within the genus *Armillaria* (Anderson and Stasovski, 1992; Coetzee *et al*., 2001a; Maphosa *et al*., 2006). This approach has been used in recent surveys of previously uninvestigated regions of the world. As a result, it has revealed the presence of distinct taxonomic entities for which a morphological description is not possible because of the absence of basidiocarps, and for which mating tests cannot be performed as a consequence of unavailable tester strains (Coetzee *et al*., 2003a; Coetzee *et al*., 2005; Mwenje *et al*., 2003). Moreover, recent studies have utilized DNA-based analyses as a foundation for further taxonomic studies to equate lineages with morphological species on the basis of the morphological characteristics of their basidiocarps (Pildain *et al*., 2009; Pildain *et al*., 2010).

Life cycle

Armillaria is unique among basidiomycetes in that its long-lived vegetative stage is diploid rather than dikaryotic (Korhonen and Hintikka, 1974). The basidiocarp gives rise to sexual basidiospores which, in turn, germinate to form haploid, uninucleate, primary mycelia. Mating is controlled by a bifactorial mating system for most *Armillaria* species (Ullrich and Anderson, 1978), with the exception of *A. heimii*, which has a unifactorial mating system (Abomo-Ndongo *et al*., 1997). In a compatible mating, the hyphal cells at the interaction zone of the two haploid colonies are briefly dikaryotic, but quickly diploidize to produce a clampless secondary mycelium with one diploid nucleus per cell and, eventually, the mitochondrial genome of only one of the haploid strains predominates (Anderson and Ullrich, 1982). Haploid and diploid strains of *Armillaria* can be distinguished in culture on the basis of their morphology (Fig. 2a) (Hintikka, 1973). Many *Armillaria* species (with the exception of *A. mellea*; Ota *et al*., 1998a) have a second, brief dikaryotic stage during fruiting. A recent advance in the knowledge of the *Armillaria* life cycle comes from the characterization of the nuclear behaviour during this second dikaryotic stage. Diploid hyphae in the subhymenium haploidize, by unknown means, and the two resulting haploid nuclei per cell then migrate into the basidium via a clamp connection (Grillo *et al*., 2000). Nuclei resulting from such somatic haploidization in the subhymenium can have either parental or recombinant genotypes, the latter of which would make this pre-meiotic reduction stage a means of increasing recombination during meiosis.

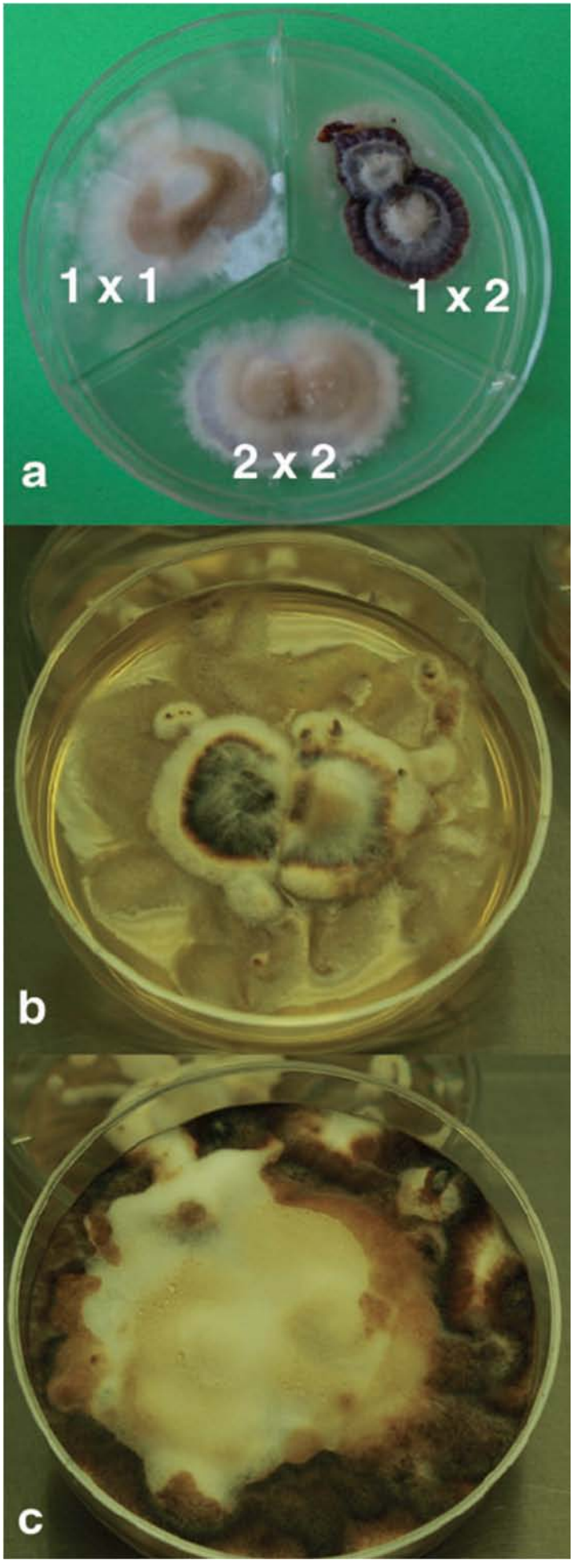
Most *Armillaria* species are heterothallic. In their basidia, the diploid nucleus undergoes meiosis, and the four resulting haploid nuclei migrate to four uninucleate basidiospores (Hintikka, 1973). Monosporous isolates are haploid, and must mate to produce a fertile, diploid mycelium (Ullrich and Anderson, 1978). Research over the past 20 years on homothallic *Armillaria* has greatly

improved the knowledge of where homothallic populations of *A. ectypa*, *A. heimii*, *A. mellea* and *A. puiggarii* (Abomo-Ndongo *et al*., 1997; Guillaumin, 1973) occur, and the understanding of the nature of homothallism. The nuclear and mating behaviour of the few homothallic strains that have been examined in detail suggests that they exhibit a type of homoheteromixis (secondary homothallism). In the basidia of homothallic strains, the four haploid products of meiosis fuse into two pairs, and the two resulting diploid nuclei migrate to two of four basidiospores (Abomo-Ndongo *et al*., 1997; Ota *et al*., 1998a). In the two occupied basidiospores, the diploid nuclei undergo post-meiotic mitosis and, with occasional back-migration of one nucleus to the basidium, basidiocarps of homothallic strains yield a mixture of uninucleate, binucleate and anucleate basidiospores. These stages of basidiospore development in homothallic strains most closely resemble those of other secondary homothallic basidiomycetes (e.g. Stereum; Calderoni *et al*., 2003), except that, in homothallic *Armillaria*, the haploid nuclei mate in the basidia immediately after meiosis and the nuclei in the basidiospores are diploid. Monosporous isolates of homothallic strains are fertile (Abomo-Ndongo *et al*., 1997; Ota *et al*., 1998a; Qin *et al*., 2007). In addition, there is 100% compatibility among sibling monosporous isolates of homothallic *Armillaria* (Abomo-Ndongo *et al*., 1997), which distinguishes them from heterothallic strains, which have 25% or 50% compatibility for those with bifactorial or unifactorial mating systems, respectively.

Diploid–diploid interactions

Plasmogamy between diploid mycelia is controlled by the somatic incompatibility (SI) system (Shaw and Roth, 1976). Although the number of loci controlling this reaction in *Armillaria* is not known, characterization of such loci in other basidiomycetes dictates that secondary mycelia must share the same alleles at all SI loci in order to be compatible (Worrall, 1997). Investigations of population structure in *Armillaria* were once limited to pairings between diploid isolates (SI tests; Fig. 2b,c). Because of the 1-month time period required for completion of an SI test, the scale of study was typically limited to a collection of isolates from within a single group of dead and dying plants (‘disease center’; Fig. 3a). Delineation of field isolates into SI groups and the mapping of their spatial distribution within a disease centre revealed patterns of dispersal (Shaw and Roth, 1976). The presence of multiple SI groups among multiple trees, for example, suggests infection by basidiospores. In contrast, a single SI group recovered from multiple, adjacent trees suggests infection by mycelium. However, SI tests do not distinguish all closely related individuals (Guillaumin *et al*., 1996; Kile, 1983) and more accurate genotyping emerged with the eventual development of molecular markers (e.g. Smith *et al*., 1994). Individual genotypes have since been found to extend far beyond a single disease centre (e.g. 500 m for *A. ostoyae*, Dettman and van der Kamp, 2001; 800 m for *A. gallica*, Smith *et al*., 1992).

Figure 2. Mycelial interactions in *Armillaria*: (a) self-pairings of haploid strains 1 × 1 and 2 × 2 show the characteristically fluffy morphology of haploid mycelia, which is in contrast with the flattened morphology of the diploid mycelium formed by mating strains 1 and 2; (b) two diploid strains that are somatically incompatible, as seen by their different colony coloration and the dark dividing line; (c) two diploid strains that are somatically compatible, based on their ability to merge into a single colony.


1 x 1
1 x 2
2 x 2
a
b
c

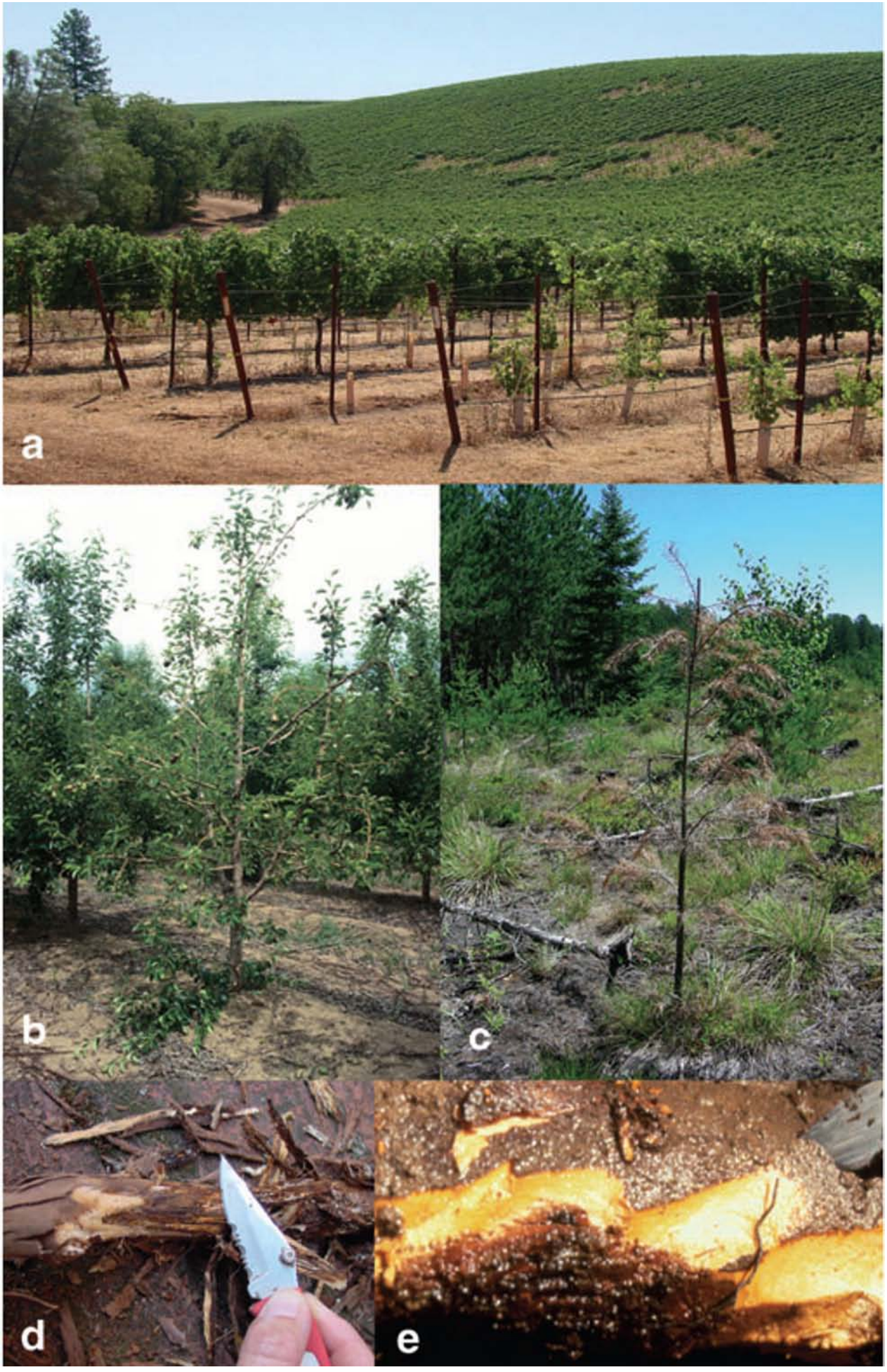

a
b
c
d
e

Figure 3. Symptoms and indicators of *Armillaria* root disease: (a) gaps in the vineyard canopy are disease centres caused by *Armillaria mellea* inoculum from residual roots of infected forest trees that previously inhabited the site (note remnant forest stand at left); (b) symptomatic *Pyrus communis* (pear) with a very sparse canopy (photograph by David M. Rizzo); (c) dead *Pinus resinosa* (red pine) in a reforested conifer stand in Cloquet, MN, USA; (d) partially decayed root collar of a grapevine; (e) necrotic lesion on a pear root 'sprouting' a rhizomorph (photograph by David M. Rizzo).

Novel modes of genetic exchange

The genetic structure of natural populations of *Armillaria* is shaped primarily by sexual reproduction (Baumgartner *et al*., 2010b; Prospero *et al*., 2008; Saville et al., 1996). *Armillaria* also exhibits other types of genetic exchange. First, diploid and haploid mycelia can fuse to form a diploid mycelium with a recombinant nuclear genome (Carvalho *et al*., 1995). Such somatic recombination is rare in diploid–haploid interactions of *Armillaria* in the laboratory, which typically result in replacement of the haploid nucleus by the diploid nucleus (Rizzo and May, 1994). Nonetheless, evidence of somatic recombination in natural populations of *A. mellea* and *A. ostoyae* (Baumgartner *et al*., 2010b; Prospero *et al*., 2008) suggests that this process has a role in shaping *Armillaria* populations, as is the case for other root-pathogenic homobasidiomycetes (e.g. *Heterobasidion annosum*; Johannesson and Stenlid, 2004). Second, mitochondrial recombination has been documented in laboratory matings of compatible haploid mycelia of *A. gallica*, albeit at very low frequency (Saville *et al*., 1996), and also in natural populations of *A. gallica* (Saville *et al*., 1998). The genetic basis of this phenomenon in *Armillaria* is not known. In other study systems, for example the protist *Physarum*, mating-type alleles interact with mitochondrial plasmids to either permit or prevent mitochondrial recombination after the fusion of gametes (Takano *et al*., 2010). It is possible that mitochondrial recombination helps to restore function to a mitochondrial genome degraded over the many mitotic divisions expected during the long-lived diploid stage of *Armillaria*, a hypothesis that has been postulated to explain an increased rate of mitochondrial recombination among older individuals of the filamentous ascomycete *Podospora anserina* (van Diepeningen *et al*., 2010).

Geographical distribution, host plant range and disease symptoms

The distribution and host range of *Armillaria* species are best known from surveys of North America (Baumgartner and Rizzo, 2001a; Bérubé, 2000; Bruhn *et al*., 2000; Morrison *et al*., 1985; Schnabel *et al*., 2005; Volk *et al*., 1996a), the UK (Rishbeth, 1982), continental Europe (Aguin-Casal *et al*., 2004; Antonín *et al*., 2009; Guillaumin *et al*., 1993; Keca *et al*., 2009) and Scandinavia (Johannesson and Stenlid, 1999). More recent surveys from Japan (Matsushita and Suzuki, 2005; Ota *et al*., 1998b), China (Qin *et al*., 2007) and Bhutan (Coetzee *et al*., 2005) have revealed new, undescribed species that do not mate with and have low sequence similarity to known species. The distributions of species from the southern parts of the world are best known from surveys in Australia and New Zealand (Kile and Watling, 1988), with more recent investigations from parts of South America (Pildain *et al*., 2010). Relatively recent surveys from Africa (Coetzee *et al*., 2000a; Mwenje *et al*., 2006; Wingfield *et al*., 2009) have revealed new, undescribed species. In general, these studies, together with studies focusing specifically on pathogenicity, show that species of *Armillaria* have broad host ranges and the majority survive as generalists.

Geographical distribution

The genus *Armillaria* has a worldwide distribution (Kile *et al*., 1994). However, species occurring naturally in either the Holarctic or non-Holarctic (Palaeotropical, Neotropical, Australian, South African and Antarctic) floral kingdoms (geographical areas with a relatively uniform composition of plant species) are, with exception, restricted to the respective regions (Tables 1 and 2). Within the floral kingdoms, some species have a transcontinental distribution, whereas other species are confined to specific continents (Tables 1 and 2). Phylogenetic analyses based on combined rDNA ITS and large subunit, and EF-1α, grouped representatives from North America, Europe, South America, Australia, New Zealand and Africa in clusters that reflected the Holarctic—non-Holarctic dichotomy (M.P.A. Coetzee *et al*., unpublished data). All species from the Holarctic floral kingdom grouped within a single cluster, species from Australia, South America and New Zealand formed a separate group, and species from Africa resided in a third group.

Armillaria as a generalist pathogen and saprobe

Armillaria species are known primarily as pathogens of a broad range of mostly woody, dicotyledonous hosts (e.g. *A. mellea* with >500 host species; Raabe, 1962). The majority of *Armillaria* species are considered to be facultative nectrotrophs; they have a parasitic phase and a saprophytic phase (Rishbeth, 1985). First, *Armillaria* colonizes the cambium of living roots (parasitic phase). Second, the fungus kills the cambium, causing a necrotic lesion beneath the root bark. Lastly, the fungus feeds on the dead tissue (saprophytic phase). It is this saprophytic capability that makes *Armillaria* root disease so difficult to prevent. Mycelium can persist for years in residual roots left buried in the soil after clearing infected hosts. In contrast, the ability of *Armillaria* species to function as saprophytes, white-rot fungi specifically, is considered to be beneficial in natural ecosystems, because they degrade lignin and thus have an important role in carbon cycling. *Armillaria* species vary considerably in virulence. All species have the ability to colonize living roots, but some species are found to be the primary cause of plant death (e.g. *A. ostoyae*), whereas others typically attack hosts already stressed by insect pests or some other predisposing factor (e.g. *A. cepistipes*) (Prospero *et al*., 2004). This is not to say that there is no variation in virulence within a species; intraspecies' variation has been identified among strains of some *Armillaria* species (e.g. *A. ostoyae*; Morrison and Pellow, 2002). Among the more virulent species, some are reported from timber trees (e.g. *A. ostoyae*) more often than from agronomic crops (e.g. *A. mellea*) (Gregory *et al*., 1991). However, such differences may reflect geographical distribution rather than host preference.

Armillaria as a mycorrhiza

With two genera of monocotyledonous hosts, orchids *Galeola* and *Gastrodia*, several species of *Armillaria* form a unique type of mycorrhizal relationship, in which the plant parasitizes the fungus (myco-heterotrophy). The orchid lacks chlorophyll and is thought to draw carbon from the mycelium. As the orchid exhibits no foliar or root symptoms of infection (Zhou *et al*., 1987), it is assumed that the orchid is not a source of nutrition for *Armillaria*. Instead, the fungus may gain its nutrition from a second host plant, with which it remains connected via rhizomorphs and has a typical pathogenic relationship. The orchid populations are endangered in China and Japan, and cultivation of their corms, which are important in eastern traditional medicine, is hindered by a poor understanding of the relationship between *Armillaria* and the orchid. The interaction is poorly characterized with respect to *Armillaria*, aside from species' identity (Cha and Igarashi, 1995; Sekizaki *et al*., 2008), but has been better characterized with respect to the orchid over the past 20 years. In fact, the first attempts to examine the molecular basis of an *Armillaria*–host interaction were based on the orchid. Such efforts have been focused primarily on the development of methods to produce gastrodianin, which is thought to be responsible for the plant's medicinal properties (Jin and Tian, 2000). The novel antifungal protein gastrodianin has been characterized from orchid

roots, and the gene encoding for the protein (*gafp*) has been cloned (Wang *et al*., 2001; Xu *et al*., 1998). Gastrodianin inhibits *A. mellea* in vitro (Hu *et al*., 1999) and its promoter is fungal inducible by the ascomycete *Trichoderma viride* (Sa *et al*., 2003), but the mechanism by which gastrodianin prevents *Armillaria* from decomposing orchid corms is not known. As *gafp* transcripts are found at higher concentrations in orchid flowers than in the roots, it seems that gastrodianin may have other roles in addition to the inhibition of *Armillaria* (Wang *et al*., 2007).

Symptoms and signs of *Armillaria* root disease

The most apparent indicator of *Armillaria* root disease, which is visible even at a distance from an infected field, is the disease centre (Fig. 3a). The presence of dead plants is common among *Armillaria* root disease centres, and this is in contrast with other root parasites that primarily weaken, but do not typically kill, their hosts (e.g. *Meloidogyne incognita*, root-knot nematode; Gubler *et al*., 2004). Individual symptomatic plants within a disease centre display varying degrees of severity of stunted shoots, dwarfed foliage, wilting, premature defoliation, resinosis in the case of conifers and dwarfed fruit in the case of fruit and nut crops (Fig. 3b,c). *Armillaria* forms thick, white mats of vegetative fungal tissue (mycelial fans)—a diagnostic feature—beneath the bark of infected roots (Fig. 1b). Rhizomorphs (Fig. 1c) are sometimes found on infected plants, and are either interwoven within the mycelial fans and root bark or are found extending into the soil.

The most common course of symptom development is a gradual, multiyear reduction in shoot growth and yield. This results in death of the host, once it can no longer survive on the remaining functional vascular tissue in the roots. Less often, and on a few hosts, symptoms develop rapidly, followed by plant death, which occurs near the time of fruit ripening (e.g. grapevine; Baumgartner and Rizzo, 2002). The onset of foliar symptoms is typically associated with the presence of subcortical mycelial fans at both the root collar and on the majority of main, lateral roots. Partially decayed wood beneath a mycelial fan has a water-soaked appearance (Fig. 3d). *Armillaria* is easily cultured from such partially decayed wood, using water agar amended with benomyl and streptomycin to select against contaminating, soil-borne ascomycete fungi and bacteria (Harrington et al., 1992). *Armillaria* cultures recovered from these sources can be distinguished from other basidiomycetes primarily by the absence of clamp connections, the absence of reproductive structures and the presence of rhizomorphs (Watling *et al*., 1982).

In vitro and *in planta* pathogen physiology

Research advances in *Armillaria* physiology have been primarily in the characterization of biopolymer-degrading enzymes, secondary metabolites and natural products that are produced in vitro. Biopolymer-degrading enzymes are clearly required for the decomposition of root wood (e.g. cellulase, laccase, peroxidase). The enzymes allow *Armillaria* to first penetrate root bark and then to decompose the underlying cambium and secondary xylem. Some genes with a putative role in secondary metabolism have been identified in *A. mellea* (Misiek and Hoffmeister, 2008). The roles of *Armillaria* secondary metabolites in the evasion/triggering of a host's defence system, the establishment of new genotypes, and the initiation of morphological differentiation and competition are under investigation.

In comparison with the knowledge of the in vitro physiology of *Armillaria*, details of its *in planta* physiology are relatively limited. This is a result in part of the fact that *Armillaria* infects plants via roots in the form of rhizomorphs or hyphae originating from inoculum sources. These below-ground interactions can therefore not be observed. Furthermore, researchers have relied on infection assays carried out in the glasshouse, which have only recently been replaced by more rapid and reliable methods (Baumgartner *et al*., 2010a). Our understanding of the infection process is based on a coarse level of investigation of the distribution of rhizomorphs on individual woody roots, and

microscopy of tissues adjacent to contact points with rhizomorphs (Thomas, 1934). However, not all *Armillaria* species make rhizomorphs in the field, and *Armillaria* has been detected in fine roots in the field (Bergemann and Garbelotto, 2006). It is currently not known whether infections can be initiated by hyphae on woody roots because they do not seem to have the capacity for the mechanical penetration of root bark, as has been documented for rhizomorphs.

Secondary metabolism

Sesquiterpene aryl esters constitute the main group of secondary metabolites of the genus *Armillaria*. Armillylorsellinate (Donnelly *et al*., 1982) and melleolide (Midland *et al*., 1982) represent the two prototypical structures, which differ from each other only in the position of a double bond. A combination of biosynthetic processes (e.g. hydroxylation, methylation, chlorination, oxidation/reduction) generates in excess of 50 sesquiterpene aryl ester derivatives (Misiek and Hoffmeister, 2011), which makes them one of the most diverse groups of natural products known among fungi. Given this immense structural diversity, *Armillaria* exemplifies the 'screening hypothesis' (Firn and Jones, 2003). In this hypothesis, bioactivity is considered to be an infrequent property of the majority of natural products, and so fungi capable of producing a high diversity of natural products are more likely to evolve bioactive ones (e.g. antibiotics), thereby enhancing their ecological fitness. Indeed, antimicrobial activity has been reported for some of *Armillaria*'s aryl esters (Arnone *et al*., 1986; Donnelly *et al*., 1982; Momose *et al*., 2000).

Recent advances in revealing the ecological roles of *Armillaria* natural products suggest that aryl esters are involved in the activation of the host defence response. This is based on the exposure of human cancer cells (Jurkat T cells) to the aryl ester arnamial, and findings of caspase-3 activation and induction of apoptotic cell death (Misiek *et al*., 2009). Another possible role of aryl esters is in interspecific communication, based on their ability to inhibit both fungal and host cells (Peipp and Sonnenbichler, 1992; Sonnenbichler *et al*., 1997). Alternatively, aryl esters may function in competition, based on the fact that they have greater inhibitory effects on other wood-rotting fungi [e.g. *Tapinella panuoides* (Basidiomycota, Tapinellaceae), *Omphalotus illudens* (Basidiomycota, Marasmiaceae)] than on nonwood-rotting fungi [e.g. *Aspergillus* (Ascomycota, Trichocomaceae); Misiek and Hoffmeister, 2011]. In this way, aryl esters may have an important role in the persistence of *Armillaria* mycelium within residual roots by preventing colonization by rhizomorphs of other saprophytic fungi. As the mycoparasite *Trichoderma* (Ascomycota, Hypocreaceae) is completely resistant to inhibition by *Armillaria* aryl esters (Misiek and Hoffmeister, 2011), screening for resistance to these natural products may be an efficient means of identifying antagonistic strains for biological control.

Other classes of small-molecule natural products have also been described from *Armillaria*. For example, diatretol, a modified diketopiperazine composed of l-leucine and l-phenylalanine, reported from *A. ectypa* shows some activity against the bacterium *Bacillus* and the Brassicaceous plant *Lepidium* (peppercress) (Arnone *et al*., 1996). Ayer and Macaulay (1987) reported a series of abietane- or pimarane-derived diterpene carboxylic acids, which are 'scaffolds' not known from other fungi, but primarily from conifers. Finally, armillaramide, a novel sphingolipid, was isolated from basidiocarps of a Chinese *A. mellea* isolate (Gao *et al*., 2001).

Bioluminescence

The genus *Armillaria* represents one phylogenetic lineage in which fungal bioluminescence occurs. Perhaps the oldest accounts of 'glowing wood' can be attributed to the luminous effects of *Armillaria* hyphae or rhizomorphs (Desjardin *et al*., 2008). The significance of fungal bioluminescence is little understood. Several hypotheses for its ecological basis have been proposed and include the attraction of spore-dispersing invertebrates or predators of fungivores (Desjardin *et*

al., 2008; Weitz, 2004). Bioluminescence may have no ecological value and, instead, the release of light (rather than heat) may be a by-product associated with detoxification of peroxides formed during lignin degradation (Bermudes *et al*., 1992). Mihail and Bruhn (2007) identified complex temporal dynamics of luminescence in *A. gallica*, *A. mellea* and *A. tabescens*, but could not confirm previous reports on diurnal oscillation. The biochemical mechanism of bioluminescence in basidiomycetes remains obscure; both enzymatic and nonenzymatic reaction cascades have been proposed (Airth and Foerster, 1962; Shimomura *et al*., 1993).

Biopolymer-degrading enzymes

Examples of biopolymer-degrading enzymes characterized from *Armillaria* include manganese-dependent peroxidases, pectin lyases, pectin methylesterases, polygalacturonases, phenol oxidases, proteinases and metalloproteases (Barry *et al*., 1981; Lee *et al*., 2005; Mwenje and Ride, 1997, 1999; Robene-Soustrade *et al*., 1992; Wahlstrom *et al*., 1991). Here, we highlight laccases (E.C. 1.10.3.2), as they play, in concert with peroxidases, a critical role in the decomposition of the highly recalcitrant substrate lignin, a process achieved only by white-rot fungi (Baldrian, 2006). In addition to their role in wood decay, laccases may also be essential for morphological differentiation, based on the relationship between the onset of laccase I activity and the formation of rhizomorph initials (Worrall *et al*., 1986). Two dissimilar extracellular laccases of approximately 60 kDa have been purified from *A. mellea*, and their expression is dependent on the growth medium (Billal and Thurston, 1996; Rehman and Thurston, 1992). Curir *et al*. (1997) independently reported the purification of an 80-kDa laccase from *A. mellea*. Laccase isoenzymes in European *Armillaria* species were investigated by isoelectric focusing gel electrophoresis, which revealed one common enzyme across all isolates, and additional laccases whose formation was a function of cultivation parameters and species (Robene-Soustrade and Lung-Escarmant, 1997).

Root infection

Armillaria produces wind-dispersed basidiospores, but the role of these propagules in root infection is thought to be limited. Instead, infections are initiated primarily by the vegetative stage of the fungus. *Armillaria* can grow in contact with a host in the form of a rhizomorph, which originates from an inoculum source (i.e. a residual root or an infected root of a neighbouring host) and extends through the soil (Fig. 3e). Nutrients are translocated from the mycelium within the substrate to the rhizomorph's growing tip, in a respiration-dependent manner and by a process more rapid than diffusion (Eamus *et al*., 1985). Most of what is known about the infection process is based on observations of *Armillaria* rhizomorphs at their infection points on woody root tissue. To penetrate root bark, rhizomorphs employ a combination of enzymatic degradation and mechanical force (Thomas, 1934; Zeller, 1926). The mycelium subsequently colonizes the underlying cambium. A small, mycelial fan forms beneath the bark at the infection site and then expands either in the direction of the root tip or the base of the trunk (root collar). The mycelial fan decomposes the cambium and the secondary xylem.

Alternatively, infection can occur in the absence of a rhizomorph when a susceptible root grows in direct contact with an inoculum source. It is not known whether such an infection initiated by hyphae occurs on the same woody portions of the root system as those involving rhizomorphs. This is an important research question because some of the most virulent *Armillaria* species (e.g. *A. luteobubalina*, *A. mellea*, *A. tabescens*) rarely form rhizomorphs in the field (Baumgartner and Rizzo, 2001a; Kile, 1981; Rishbeth, 1985). The ability of *Armillaria* to colonize fine roots in the laboratory (Fig. 4a) (Baumgartner *et al*., 2010a) and reports of *Armillaria* detection from fine roots in the field (Bergemann and Garbelotto, 2006) make it clear that the pathogen is not confined to woody portions of the root system. The absence of penetration pegs or other structures specialized for mechanical cell penetration suggests that hyphal penetration of the surface of fine, non woody

roots is a result of secretion of biopolymer-degrading enzymes (Fig. 4b). Colonization of the root cortex follows (Fig. 4c), but it is not clear whether the pathogen then kills these fine roots or, instead, establishes a quiescent infection that becomes active when the root matures or spreads from fine roots to more mature roots. Documenting these stages of infection and linking them with the enzymes and secondary metabolites produced by *Armillaria* in vitro are directions of *Armillaria* research that are now within reach.

Defining the biochemical and molecular bases of *Armillaria*–host interactions is an area in *Armillaria* research that is in its infancy. For example, the gene encoding the protein gastrodianin (*gafp*), which originates from the orchid Gastrodia with which *Armillaria* forms a myco-heterotrophic relationship (Zhou *et al*., 1987), has only been introduced recently into *Prunus domestica* (plum). This is currently one of only a few *Armillaria* hosts for which an efficient genetic transformation system is available. In glasshouse trials, transformants containing the *gafp* gene showed greater tolerance than wild-type plums to the root-knot nematode *Meloidogyne incognita* and the root-pathogenic oomycete *Phytophthora cinnamomi* (Nagel *et al*., 2008). Further experiments are in progress to evaluate the response of these transformants to *A. mellea*, *A. tabescens* and *A. ostoyae* (K. Baumgartner and G. Schnabel, unpublished research), all of which are virulent on *Prunus* crops (e.g. almond, cherry, peach, plum) in different parts of North America and Europe (Baumgartner and Rizzo, 2001a; Guillaumin *et al*., 1989b; Proffer *et al*., 1987; Schnabel *et al*., 2005). If gastrodianin inhibits *Armillaria in planta*, such a finding would advance our understanding of the biochemical basis by which the orchid *Gastrodia* circumvents symptom expression.

Epidemiology and control of *Armillaria* root disease

The epidemiology of *Armillaria* root disease differs to some extent between stands of planted hosts (e.g. fruit crops, timber trees) and natural forest stands, and this is thought to be a result of a greater proportion of inoculum originating from residual roots in the former. *Armillaria* infection of planted hosts is thought to occur primarily below ground on the roots and root collars of living trees. Infection of planted hosts by wind-dispersed basidiospores is thought to be insignificant, based in part on indirect evidence, such as the localized (rather than random) distribution of symptomatic plants in disease centres and the presence of residual roots found among such disease centres (Baumgartner and Rizzo, 2002; Rizzo *et al*., 1998).

Research on the control of *Armillaria* root disease has shifted from focusing only on pre-plant eradication of soil-borne inoculum to new, post-infection methods that limit the growth of or mitigate yield losses from an existing infection. This change in approach for the control of *Armillaria* root disease is concomitant with that of other root parasites (e.g. root-knot nematode, *Verticillium* wilt). This shift in disease control methods is primarily a result of the increased regulation of soil fumigants, which are toxic biocides that threaten worker safety, deplete the ozone layer and kill beneficial organisms.

Planted stands

In the field among planted hosts, the distribution of *Armillaria* root disease is localized, consisting of disease centres (Fig. 1d), the locations of which reflect the positions of infected forest trees that formerly occupied the site, where inoculum is concentrated below ground. A disease centre in an orchard, vineyard or timber plantation is often occupied by a single, diploid individual of *Armillaria* (e.g. Baumgartner and Rizzo, 2002; Dettman and van der Kamp, 2001; Prospero *et al*., 2008; Rizzo *et al*., 1998). This is a consistent finding among numerous planted hosts infected by different *Armillaria* species and in different regions of the world, as cited above for example. Such evidence, coupled with the fact that inoculation attempts with basidiospores very rarely result in infection

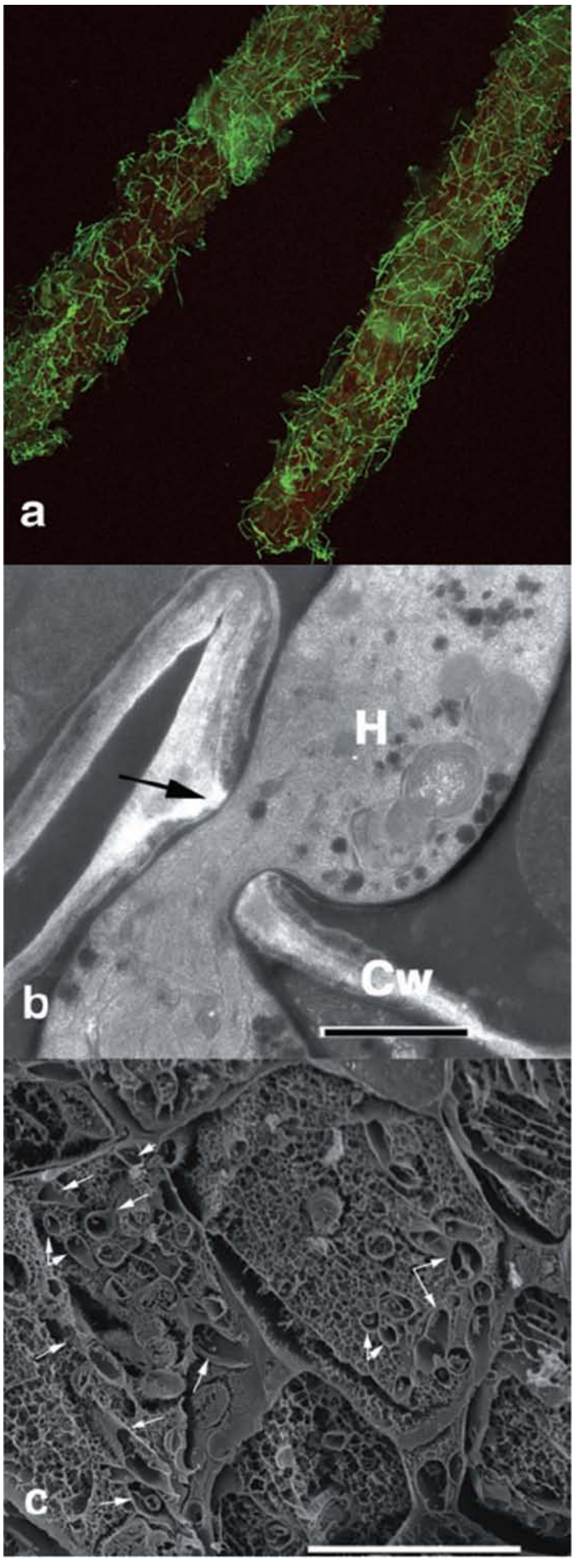

a
H
Cw
b
c

Figure 4. *In planta* growth of *Armillaria*: (a) grapevine roots (5 µm in diameter) colonized by *A. mellea* hyphae; (b) penetration of the root epidermis of a *Lupinus albus* cv. 'Tifblue' (white lupine) seedling by *A. tabescens* hyphae (bar, 1 µm; Cw, cell wall; H, hypha) (photograph by Kerik Cox); (c) intracellular growth of *A. mellea* hyphae (see arrows) in cryo-prepared grapevine root (bar, 20 µm) (photograph by Dennis Margosan).

(Kile, 1983; Rishbeth, 1970), suggests that basidiospores may have a very minor role in infection of planted hosts. Instead, a resident diploid mycelium colonizes planted hosts when roots grow in contact with the residual roots of infected trees that were cleared prior to planting. Mycelium can persist saprophytically for months to years in such residual roots buried in the soil, which thus serve as the source of inoculum (Baumgartner and Rizzo, 2002; Rizzo *et al*., 1998). Once established, the expansion of disease centres is a result of vegetative spread of the fungus from one host to the next via rhizomorph growth through the soil and/or as mycelium through susceptible roots, rather than through spore dispersal. This is in spite of the fact that basidiocarps form on planted hosts.

Natural stands

Among naturally established hosts, disease centres typically occur in association with forest management activities (e.g. logging, planting of susceptible timber species) and the presence of virulent *Armillaria* species (e.g. *A. luteobubalina*, *A. ostoyae*) (Wargo and Shaw, 1985). One diploid individual often occupies these disease centres. However, the presence of multiple genotypes per disease centre, reported by various researchers, is indicative of infections occurring also by means of basidiospores (Baumgartner and Rizzo, 2001b; Kile, 1983; Prospero *et al*., 2003; Rizzo *et al*., 1995; Rizzo and Harrington, 1993; Worrall, 1994). The larger number of such reports from naturally established hosts than from planted hosts suggests that basidiospores may have a more important role in the spread of *Armillaria* root disease in natural ecosystems than in agronomic systems.

Role of basidiospores

There have been several recent discoveries that have improved our understanding of the role of basidiospores in the population structure of *Armillaria*. Investigations with microsatellite markers have revealed that *Armillaria* populations are panmictic, and evidence of gene flow between populations separated by 1000–3000 km suggests that there is unrestricted spore dispersal across such distances (Baumgartner *et al*., 2009; LeFrancois *et al*., 2002; Prospero *et al*., 2010; Worrall *et al*., 2004). This lack of population subdivision is surprising, given that the fruiting season is limited and basidiocarps deteriorate within days of formation. Nonetheless, it is consistent with the observation that basidiospores can remain dormant for months and under relatively harsh environmental conditions (Shaw, 1981). Haploid mycelia are very rarely encountered in natural populations of *Armillaria* (Peabody *et al*., 2000). The rarity of these mycelia is probably a result of a very brief period between the time of spore germination and mating of haploid mycelia to form diploid mycelia, as is the case with primary mycelia of other wood decay homobasidiomycetes (e.g. *Heterobasidion annosum*; Garbelotto *et al*., 1997).

Further research is needed to identify the substrate colonized by basidiospores. As the basidiospores are wind dispersed, it is possible that they germinate on woody debris on the soil surface (e.g. tree stumps), and that the resulting haploid mycelium persists saprophytically. Evidence in support of this hypothesis has been provided by the recovery of *A. novae-zelandiae* from pieces of *Pinus radiata* wood acting as traps for wind-dispersed spores (Hood *et al*., 2002), and the recovery of *A. ostoyae* from naturally occurring woody debris in *P. resinosa* stands (Kromroy *et al*., 2005). As roots frequently proliferate into rotted tree stumps, it is possible that the colonization of such substrates by spores provides an opportunity for the infection of living roots. Alternatively,

Armillaria spores may percolate through the soil profile to germinate directly on living roots. Indeed, haploid strains of *A. mellea* are as virulent as diploid strains (Baumgartner *et al*., 2010c).

Role of diploid–haploid interactions

The role of genetic exchange between haploid and diploid mycelia in the epidemiology of *Armillaria* is unclear. A haploid mycelium is compatible with its diploid parent because at least one of its mating-type alleles at both mating-type loci is different from that of the parent. Therefore, all spores from a given basidiocarp can mate with their diploid parent (Anderson and Ullrich, 1982). In the laboratory, the diploid nucleus often displaces the haploid nucleus (Carvalho *et al*., 1995; Rizzo and May, 1994) and, in the case of a diploid–haploid interaction between a diploid parent and one of its haploid gametes, displacement of the haploid nucleus by the diploid nucleus would result simply in expansion of the parent's mycelium. Such an interaction would be undetectable in the field, once the haploid mycelium is diploidized, as both parent and gamete share the same mitochondrial genome. This type of reproduction is functionally clonal, and such a reproductive mode can explain both the rarity of haploid mycelium from natural populations of *Armillaria* and the existence of large, persistent diploid genotypes spanning the root systems of many adjacent hosts.

Role of rhizomorphs

Armillaria rhizomorphs are not overwintering propagules like the sclerotia of *Rhizoctonia solani* (Basidiomycota, Ceratobasidiaceae) or the chlamydospores of *Fusarium oxysporum* (Ascomycota, Nectriaceae), which become detached from infected roots and remain dormant in the soil for months in the absence of a host. Once disconnected from their substrate, rhizomorph viability is on the order of days. Nonetheless, just as the sclerotia and chlamydospores of the above-mentioned fungi, *Armillaria* rhizomorphs are infectious. Field reports of extensive rhizomorph networks for some species (e.g. *A. gallica*; Morrison, 2004; Prospero *et al*., 2006; Redfern and Filip, 1991) suggest that *Armillaria* species may vary in their relative modes of below-ground spread. As it is not clear whether the patterns and/or stages of root infection are the same for infections initiated by rhizomorphs versus hyphae, it is difficult to draw conclusions about the relationship between the capacity to form rhizomorphs and virulence. Furthermore, there are field reports of intraspecies' variation from crop to crop (Baumgartner and Rizzo, 2002; Rizzo *et al*., 1998). Indeed, the ability to manipulate rhizomorph growth with various amendments to the growth medium (Weinhold, 1963), or with various temperature and moisture combinations (Pearce and Malajczuk, 1990), suggests that rhizomorph formation is influenced by a complex set of factors in addition to species' identity. It is possible that rhizomorphs have a role in *Armillaria* physiology, in addition to their epidemiological importance, based on a recent discovery that rhizomorphs contain pores specialized for gas exchange (Pareek *et al*., 2006). Given that rhizomorphs have been shown to grow towards rather than away from the soil surface (Morrison, 1976), they may help mycelium to respire in the low oxygen and high carbon dioxide environment beneath the root bark.

***Armillaria* introductions**

Few *Armillaria* introductions have been reported. We highlight such cases here because they have had contrasting epidemiological consequences. Heterothallic strains of the Northern Hemisphere species *A. mellea* have been recovered from planted hosts in South Africa, and are likely to have originated from Europe based on phylogenetic comparisons with strains representing the geographical range of *A. mellea* (Coetzee *et al*., 2001b, 2003b). The spread of *Armillaria* root disease following the above-cited introductions appears to have been spatially restricted; strains have spread to neighbouring plants within the urban gardens in which they were introduced, but not to distant agronomic crops. Homothallic strains of *A. mellea* in Africa are thought to have been

introduced from Asia, where the only other homothallic populations are known (Abomo-Ndongo and Guillaumin, 1997; Ota *et al*., 2000). Hosts from which African strains have been reported are all native to Asia (e.g. *Camellia sinensis* (Chinese tea); Abomo-Ndongo and Guillaumin, 1997). More importantly, African strains are all vegetatively compatible with each other (Abomo-Ndongo and Guillaumin, 1997; Ota *et al*., 2000), and are also vegetatively compatible with many strains from Japan (Ota *et al*., 2000). Within Africa, the homothallic strains have been reported from locations separated by a maximum distance of 4000 km (Ethiopia, Kenya, Tanzania and Sao Tome). Assuming they are siblings, based on their vegetative compatibility, and given that homothallic strains have spores that function as zygotes (Abomo-Ndongo *et al*., 1997; Ota *et al*., 1998a; Qin *et al*., 2007), it is possible that homothallic *A. mellea* was introduced to one location via the transport of infected plant material from Asia, and then spread to other locations in Africa as basidiospores.

Disease control

Most research on the control of *Armillaria* root disease has focused on the prevention of infection of agronomic crops. As the inoculum for such hosts originates below ground, in the form of mycelium in residual roots, the control tactic examined most has been soil fumigation (Adaskaveg *et al*., 1999; Bliss, 1951; Munnecke *et al*., 1981, 1970). For example, the fumigants methyl bromide and carbon disulphide can kill mycelium in partially decayed tree roots to a soil depth of approximately 1 m. This tactic, which is one of the few effective control treatments for *Armillaria* root disease, is typically used before the establishment of high-value crops (e.g. wine grapes, walnuts) on previously forested sites or sites on which a former crop has been diagnosed with *Armillaria* root disease (Gubler *et al*., 2004). Efficacy is variable, depending on the preponderance of the soil characteristics that limit penetration of the fumigant (e.g. clay, soil organic matter, moisture) and the size of the infected residual roots (Bliss, 1951). As such soil characteristics are almost impossible to minimize, many farmers choose, instead, to manually remove as many residual roots as possible, typically after deep tilling of the soil to bring roots to the surface. Given that the most potent fumigant against inoculum of *Armillaria*, methyl bromide, will eventually be banned from use in the USA, an alternative control method is needed.

Another pre-plant treatment that has been examined is soil inoculation with antagonistic fungi for the biological control of *Armillaria* root disease. This approach was pursued on the basis of findings that sublethal doses of fumigants weaken mycelium, thereby predisposing it to attack by indigenous populations of myco-parasitic, soil-borne fungi such as *Trichoderma* (Garrett, 1957; Munnecke *et al*., 1973, 1981; Ohr *et al*., 1973), a hypothesis that was first proposed by Bliss (1951). Although some *Trichoderma* strains show excellent *in vitro* and *in planta* inhibition of *A. mellea* growth (Raziq and Fox, 2003), it is difficult to achieve the necessary concentrations of this antagonistic fungus in field soil (Shaw and Roth, 1978), especially at depths of greater than 0.3 m (Otieno *et al*., 2003). Saprobic wood decay fungi (e.g. *Ganoderma lucidum*) have been found to overtake *Armillaria* mycelium in wood, suggesting that the inoculation of planted hosts scheduled for eventual removal may bring about some level of eradication of the pathogen (Chapman and Ziao, 2000; Cox and Scherm, 2006; Pearce *et al*., 1995).

Recent advances in research on the control of *Armillaria* root disease have shown a shift in focus from futile attempts at the eradication from the soil of inoculum to post-infection treatments that mitigate yield losses. Farmers may choose this approach, instead of replanting, for crops that take several years to reach productive maturity (e.g. walnut). One such post-infection treatment is the cultural practice of root collar excavation, which involves the permanent removal of soil from the base of a plant's trunk, using either a shovel or, for the treatment of many plants, a high-pressure air hose. Root collar excavation has been shown to increase the yield of wine grapes and to cause mycelial fans to recede from the root collar, thereby improving the function of vascular tissue at the base of the trunk (Baumgartner, 2004). This cultural practice is used commercially for the control of

Armillaria root disease on grapevine in California, which is the top producer of grapes in North America, and is being evaluated on peach in the southeastern USA (Guido Schnabel, Clemson University, SC, USA, personal communication). A second post-infection treatment, also demonstrated to improve yields of grapevine, is the application of commercial inoculants that contain antagonistic bacteria (e.g. *Bacillus subtilis*) via the drip-irrigation system (Baumgartner and Warnock, 2006). The mechanism by which such bacteria may bring about yield increases of symptomatic plants may include one or more of the general mechanisms of biocontrol: parasitism/antibiosis, competitive exclusion of the pathogen and/or the promotion of plant growth. *Armillaria mellea*, *A. ostoyae* and *A. luteobubalina*, three of the most virulent *Armillaria* species, are inhibited in vitro by common soil-borne bacteria (e.g. *Bacillus* spp., fluorescent pseudomonads) and actinobacteria (e.g. *Streptomyces* spp.) (Baumgartner and Warnock, 2006; de Vasconcellos and Cardoso, 2009; Delong *et al*., 2002; Dumas, 1991). This biological practice has only been used experimentally; commercial-scale adoption is restricted by the limited production and short viability period of commercial inoculants.

Post-infection chemical control with fungicides has been evaluated on an experimental basis. Two sterol demethylation inhibitors (DMIs), cyproconazole and propiconazole, have been shown to reduce the severity of foliar symptoms and to decrease mortality in grapevines (Aguin-Casal *et al*., 2006) and almonds (Adaskaveg *et al*., 1999), respectively. Such fungicides can be applied to the soil or injected into the trunk of infected plants as a means of treating an infected host. This approach is not used on a commercial scale in part because such fungicides are not widely registered for the control of *Armillaria* root disease or for soil/wood injection. Nonetheless, this approach has promise as a curative method for use on moderately symptomatic plants, just as the cultural and biological treatments summarized above.

Future prospects: resistant plant material

Resistance to *Armillaria* root disease varies amongst agronomic crops. For example, replanting an infected peach orchard with walnut trees (Fig. 5a), which are more tolerant than peach and other stone fruits (Thomas *et al*., 1948), may be a feasible control option. This is especially effective when conducted in combination with the thorough removal of residual peach roots. However, not all farmers have the flexibility to change crops, as alternative crops often require different equipment or climates. Given this reality of modern agriculture, a logical solution is to graft susceptible fruiting cultivars to resistant rootstocks, an approach used to control many root parasites (e.g. crown gall bacterium *Agrobacterium tumefaciens* in walnut). *Armillaria* resistance exists among commercial rootstocks of grapevine (Baumgartner and Rizzo, 2006), stone fruits (Guillaumin *et al*., 1989b; Wilkins *et al*., 2002) and walnut (Reil, 1997). Experiments are in place to identify additional sources of *Armillaria* resistance from a diverse germplasm for these crops (Fig. 5b).

Gaps in the knowledge of the *Armillaria* infection process (e.g. patterns of root infection between rhizomorphs versus hyphae) limit the identification of measures of *Armillaria* resistance. Macro- and microscopic observations of the host response to *Armillaria* infection of woody roots are relatively consistent among species of fruit and nut crops, timber species and hardwood trees. For example, these include the formation of additional cell layers (secondary periderm, callus) adjacent to an infection site, resinosis (in the case of conifers), increased phenolic concentration and compartmentalization of the pathogen within the secondary xylem (Morrison *et al*., 1991). In some hosts, the onset of such responses corresponds with field observations of resistance (e.g. *Picea*; Entry *et al*., 1992) and the length of *Armillaria* mycelial fans (e.g. *Prunus*; Guillaumin *et al*., 1989b), and therefore may be measures of resistance. The adaptation of such measures for the identification of resistant germplasm, however, is not feasible given the large numbers of plants requiring screening and the multiple years taken for such symptoms/signs to develop.

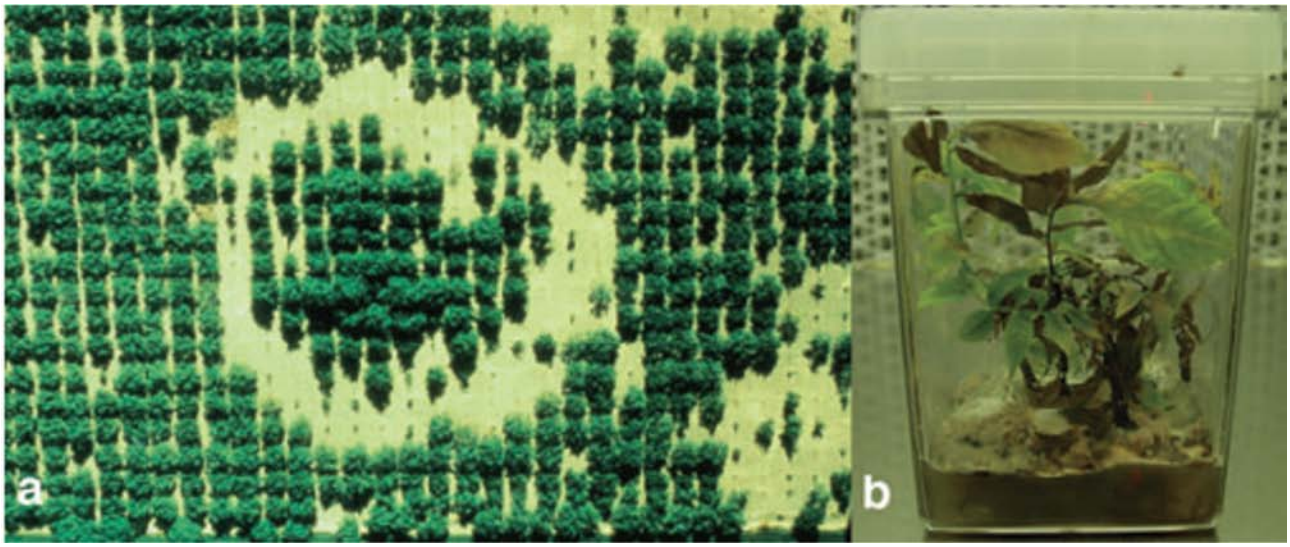


Figure 5. Resistance to *Armillaria* root disease: (a) *Juglans regia* (walnut) trees replanted within the *Armillaria* root disease centre of this *Prunus amygdalus* (almond) orchard are more tolerant of *Armillaria mellea* infection than are almonds; (b) screening micropropagated walnut rootstock (paradox type 'Vlach') for resistance.

Genomic resources for grapevine (three public genome sequences, Affymetrix microarrays) have fostered the first examination of the molecular basis of an *Armillaria*–host interaction to identify the genes expressed in response to infection using suppression subtractive hybridization (Perazzolli *et al*., 2010). Grapevines express a homologue of a phase change-related protein from *Quercus* in response to infection by *A. mellea*, and this protein inhibits colony expansion in vitro. In addition, *Armillaria* induces the expression of several genes in the ethylene and jasmonic acid signalling pathways, which have widely recognized involvement in host defence responses to a broad range of unrelated pathogens (e.g. *Phymatotrichopsis omnivora*; Uppalapati *et al*., 2009). When used to characterize differential gene expression in resistant versus susceptible hosts, a genomic approach, such as that of Perazzolli *et al*. (2010), could bring about the discovery of molecular markers of resistance. This would assist in the identification of resistant progeny from crosses between *Armillaria*-resistant and *Armillaria*-susceptible parents. A classical breeding approach could progress rapidly with such DNA marker-assisted selection.

For crops with no resistant germplasm, however, management options other than resistant rootstocks are needed. The identification of mycoparasites that are 'superpathogens' of *Armillaria* would advance the development of biological control for *Armillaria* root disease. The soil-borne fungus *Trichoderma* has already been shown to be an effective parasite of *Armillaria* (Raziq and Fox, 2005). Antagonistic strains of rhizosphere bacteria are also a promising area of research (Baumgartner and Warnock, 2006; Perazzolli et al., 2007), especially given the ease of their application through drip-irrigation systems. Of course, a knowledge of exactly which parts of the root system are targeted by different infectious propagules of *Armillaria* is a critical—and still unclear—detail that is needed to pinpoint applications of antagonistic strains. Indeed, such knowledge would benefit the development of chemical and cultural strategies.

The genome of *A. mellea* will soon be available as an additional resource for the investigation of unanswered research questions relevant to *Armillaria*. It is foreseen that the genome will shed light on questions pertinent to the life cycle of *Armillaria*, nonmeiotic mechanisms of genetic exchange, the mechanism of mitochondrial recombination and the genetic basis of homothallism, to name a few. It is also believed that genes and metabolic pathways will be discovered that will aid in our understanding of *Armillaria* as a phytopathogen. In this way, it is possible to imagine that *Armillaria* may yet turn out to be a model study system.

Acknowledgments

We thank G. T. Browne (United States Department of Agriculture-Agricultural Research Service, Davis, CA, USA) and T. Kasuga (United States Department of Agriculture-Agricultural Research Service, Davis, CA, USA) for helpful comments on the manuscript. We also thank D. M. Rizzo (University of California, Davis, CA, USA) for direction, guidance and many discussions of Armillaria biology. Some photographs were kindly provided by D. M. Rizzo, K. Cox (Cornell University, Geneva, NY, USA) and D. Margosan (United States Department of Agriculture-Agricultural Research Service, Parlier, CA, USA).

Literature Cited

Abomo-Ndongo, S. and Guillaumin, J.J. (1997) Somatic incompatibility among African *Armillaria* isolates. *Eur. J. Forest Pathol.* **27**, 201–206.

Abomo-Ndongo, S., Mohammed, C. and Guillaumin, J.J. (1997) Sexual behavior of *Armillaria heimii* and *A. mellea* isolates from Africa. *Eur. J. Forest Pathol.* **27**, 207–224.

Adaskaveg, J.E., Forster, H., Wade, L., Thompson, D.F. and Connell, J.H. (1999) Efficacy of sodium tetrathiocarbonate and propiconazole in managing *Armillaria* root rot of almond on peach rootstock. *Plant Dis.* **83**, 240–246.

Aguin-Casal, O., Sainz-Oses, M.J. and Mansilla-Vazquez, J.P. (2004) *Armillaria* species infesting vineyards in northwestern Spain. *Eur. J. Plant Pathol.* **110**, 683–687.

Aguin-Casal, O., Mansilla-Vazquez, J.P. and Sainz-Oses, M.J. (2006) *In vitro* selection of an effective fungicide against *Armillaria mellea* and control of white root rot of grapevine in the field. *Pest Manag. Sci.* **62**, 223–228.

Airth, R.L. and Foerster, G.E. (1962) Isolation of catalytic components required for cell-free fungal bioluminescence. *Arch. Biochem. Biophys.* **97**, 567–569.

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*, **8**, 837–839.

Anderson, J.B. and Bailey, S.S. (1989) Variation in ribosomal DNA among biological species of *Armillaria*, a genus of root-infecting fungi. *Evolution*, **43**, 1652–1662.

Anderson, J.B. and Stasovski, E. (1992) Molecular phylogeny of Northern hemisphere species of *Armillaria*. *Mycologia*, **84**, 505–516.

Anderson, J.B. and Ullrich, R.C. (1979) Biological species of *Armillaria mellea* in North America. *Mycologia*, **71**, 402–414.

Anderson, J.B. and Ullrich, R.C. (1982) Diploids of *Armillaria mellea*: synthesis, stability, and mating behavior. *Can. J. Bot.* **60**, 432–439.

Anderson, J.B., Petsche, D.M. and Smith, M.L. (1987) Restriction fragment polymorphisms in biological species of *Armillaria mellea*. *Mycologia*, **79**, 69–76.

Antonín, V., Tomsovsky, M., Sedlák, P., Májek, T. and Jankovsky, L. (2009) Morphological and molecular characterization of the *Armillaria cepistipes–A. gallica* complex in the Czech Republic and Slovakia. *Mycol. Prog.* **8**, 259–271.

Arnone, A., Cardillo, R. and Nasini, G. (1986) Secondary mold metabolites. 14. Structures of mellolides-B-D, 3 antibacterial sesquiterpenoids from *Armillaria mellea*. *Phytochemistry*, **25**, 471–474.

Arnone, A., Capelli, S., Nasini, G., Meille, S.V. and dePava, O.V. (1996) Secondary mould metabolites. 52. Structure elucidation of diatretol—a new diketopiperazine metabolite from the fungus *Clitocybe diatreta*. *Liebigs Ann.* **11**, 1875–1877.

Ayer, W.A. and Macaulay, J.B. (1987) Metabolites of the honey mushroom, *Armillaria mellea*. *Can. J. Chem.* **65**, 7–14.

Baldrian, P. (2006) Fungal laccases—occurrence and properties. *FEMS Microbiol. Rev.* **30**, 215–242.

Banik, M.T. and Burdsall, H.H., Jr (1998) Assessment of compatibility among *Armillaria cepistipes*, *A. sinapina*, and North American biological species X and XI, using culture morphology and molecular biology. *Mycologia*, **90**, 798–805.

Barry, F.P., Doonan, S. and Ross, C.A. (1981) Cleavage by trypsin and by the proteinase from *Armillaria mellea* at epsilon-N-formyl-lysine residues. *Biochem. J.* **193**, 737–742.

Baumgartner, K. (2004) Root collar excavation for postinfection control of *Armillaria* root disease of grapevine. *Plant Dis.* **88**, 1235–1240.

Baumgartner, K. and Rizzo, D.M. (2001a) Distribution of *Armillaria* species in California. *Mycologia*, **93**, 821–830.

Baumgartner, K. and Rizzo, D.M. (2001b) Ecology of *Armillaria* species in mixed-hardwood forests of California. *Plant Dis.* **85**, 947–951.

Baumgartner, K. and Rizzo, D.M. (2002) Spread of *Armillaria* root disease in a California vineyard. *Am. J. Enol. Vitic.* **53**, 197–203.

Baumgartner, K. and Rizzo, D.M. (2006) Relative resistance of grapevine rootstocks to *Armillaria* root disease. *Am. J. Enol. Vitic.* **57**, 408–414.

Baumgartner, K. and Warnock, A.E. (2006) A soil inoculant inhibits *Armillaria mellea in vitro* and improves productivity of grapevines with root disease. *Plant Dis.* **90**, 439–444.

Baumgartner, K., Grubisha, L., Fujiyoshi, P., Garbelotto, M. and Bergemann, S.E. (2009) Microsatellite markers for the diploid Basidiomycete fungus, *Armillaria mellea*. *Mol. Ecol. Res.* **9**, 943–946.

Baumgartner, K., Bhat, R. and Fujiyoshi, P. (2010a) A rapid infection assay for *Armillaria* and real-time PCR quantitation of the fungal biomass *in planta*. *Fungal Biol.* **114**, 107–119.

Baumgartner, K., Bruhn, J., Travadon, R. and Bergemann, S.E. (2010b) Contrasting patterns of genetic diversity and population structure of *Armillaria mellea sensu stricto* in the eastern and western United States. *Phytopathology*, **100**, 708–718.

Baumgartner, K., Fujiyoshi, P., Foster, G.D. and Bailey, A.M. (2010c) *Agrobacterium*-mediated transformation for investigation of somatic recombination in the fungal pathogen *Armillaria mellea*. *Appl. Environ. Microbiol.* **76**, 7990–7996.

Bergemann, S.E. and Garbelotto, M. (2006) High diversity of fungi recovered from the roots of mature tanoak (*Lithocarpus densiflorus*) in northern California. *Can. J. Bot.* **84**, 1380–1394.

Bermudes, D., Petersen, R.H. and Nealson, K.H. (1992) Low-level bioluminescence detected in *Mycena haematopus* basidiocarps. *Mycologia*, **84**, 799–802.

Bérubé, J.A. (2000) *Armillaria* species in Newfoundland. *Can. J. Forest Res.* **30**, 507–512.

Bérubé, J.A. and Dessureault, M. (1988) Morphological characterization of *Armillaria ostoyae* and *Armillaria sinapina* sp. nov. *Can. J. Bot.* **66**, 2027–2034.

Bérubé, J.A. and Dessureault, M. (1989) Morphological studies of the *Armillaria mellea* complex: two new species, *A. gemina* and *A. calvescens*. *Mycologia*, **81**, 216–225.

Bérubé, J.A., Dessureault, M., Berthelay, S. and Guillaumin, J.J. (1996) Interfertility between *Armillaria cepistipes* and *A. sinapina*. *Phytoprotection*, **77**, 67–74.

Billal, F. and Thurston, C.F. (1996) Purification of laccase II from *Armillaria mellea* and comparison of its properties with those of laccase I. *Mycol. Res.* **100**, 1099–1105.

Bliss, D.E. (1951) The destruction of *Armillaria mellea* in citrus soils. *Phytopathology*, **41**, 665–683.

Bruhn, J.N., Wetteroff, J.J., Mihail, J.D., Kabrick, J.M. and Pickens, J.B. (2000) Distribution of *Armillaria* species in upland Ozark Mountain forests with respect to site, overstory species composition and oak decline. *Forest Pathol.* **30**, 43–60.

Calderoni, M., Sieber, T.N. and Holdenrieder, O. (2003) *Stereum sanguinolentum*: is it an amphithallic basidiomycete. *Mycologia*, **95**, 232–238.

Carvalho, D.B., Smith, M.L. and Anderson, J.B. (1995) Genetic exchange between diploid and haploid mycelia of *Armillaria gallica*. *Mycol. Res.* **6**, 641–647.

Cha, J.Y. and Igarashi, T. (1995) *Armillaria* species associated with *Gastrodia elata* in Japan. *Eur. J. Forest Pathol.* **25**, 319–326.

Chapman, B. and Ziao, G. (2000) Inoculation of stumps with *Hypholoma fasciculare* as a possible means to control *Armillaria* root disease. *Can. J. Bot.* **78**, 129–134.

Chillali, M., Idder-Ighili, H., Agustian, A., Guillaumin, J.-J., Mohammed, C. and Botton, B. (1997) Species delimitation in the African *Armillaria* complex by analysis of the ribosomal DNA spacers. *J. Gen. Appl. Microbiol.* **43**, 23–29.

Chillali, M., Idder-Ighili, H., Guilaumin, J.-J., Mohammed, C., Lung Escarmant, B. and Botton, B. (1998) Variation in the ITS and IGS regions of ribosomal DNA among the biological species of European *Armillaria*. *Mycol. Res.* **102**, 533–540.

Coetzee, M.P.A., Wingfield, B.D., Coutinho, T.A. and Wingfield, M.J. (2000a) Identification of the causal agent of *Armillaria* root rot of *Pinus* species in South Africa. *Mycologia*, **92**, 777–785.

Coetzee, M.P.A., Wingfield, B.D., Harrington, T.C., Dalevi, D., Coutinho, T.A. and Wingfield, M.J. (2000b) Geographical diversity of *Armillaria mellea s. s.* based on phylogenetic analysis. *Mycologia*, **92**, 105–113.

Coetzee, M.P.A., Wingfield, B.D., Bloomer, P., Ridley, G.S., Kile, G.A. and Wingfield, M.J. (2001a) Phylogenetic relationships of Australian and New Zealand *Armillaria* species. *Mycologia*, **93**, 887–896.

Coetzee, M.P.A., Wingfield, B.D., Harrington, T.C., Steimel, J., Coutinho, T.A. and Wingfield, M.J. (2001b) The root rot fungus *Armillaria mellea* introduced into South Africa by early Dutch settlers. *Mol. Ecol.* **10**, 387–396.

Coetzee, M.P.A., Wingfield, B.D., Bloomer, P., Ridley, G.S. and Wingfield, M.J. (2003a) Molecular identification and phylogeny of Armillaria isolates from South America and Indo-Malaysia. *Mycologia*, **95**, 285–293.

Coetzee, M.P.A., Wingfield, B.D., Roux, J., Crous, P.W., Denman, S. and Wingfield, M.J. (2003b) Discovery of two northern hemisphere *Armillaria* species on Proteaceae in South Africa. *Plant Pathol.* **52**, 604–612.

Coetzee, M.P.A., Wingfield, B.D., Kirisits, T., Chhetri, D.B., Bloomer, P. and Wingfield, M.J. (2005) Identification of *Armillaria* isolates from Bhutan based on DNA sequence comparisons. *Plant Pathol.* **54**, 36–45.

Coetzee, M.P.A., Maphosa, L., Mwenje, E., Wingfield, M. and Wingfield, B. (2009) Characterisation of *Armillaria* species based on pectic isozyme analyses. *Fungal Divers.* **36**, 9–16.

Cox, K.D. and Scherm, H. (2006) Interaction dynamics between saprobic lignicolous fungi and *Armillaria* in controlled environments: exploring the potential for competitive exclusion of *Armillaria* on peach. *Biol. Control* **37**, 291–300.

Curir, P., Thurston, C.F., Daquila, F., Pasini, C. and Marchesini, A. (1997) Characterization of a laccase secreted by *Armillaria mellea* pathogenic for Genista. *Plant Physiol. Biochem.* **35**, 147–153.

Czederpiltz, D.L.L., Volk, T.J. and Burdsall, H.H., Jr (2001) Field observations and inoculation experiments to determine the nature of the carpophoroids associated with *Entoloma abortivum* and *Armillaria*. *Mycologia*, **93**, 841–851.

Delong, R.L., Lewis, K.J., Simard, S.W. and 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. Forest Res.* **32**, 2146–2159.

Desjardin, D.E., Oliveira, A.G. and Stevani, C.V. (2008) Fungi bioluminescence revisited. *Photochem. Photobiol. Sci.* **7**, 170–182.

Dettman, J.R. and van der Kamp, B.J. (2001) The population structure of *Armillaria ostoyae* and *Armillaria sinapina* in the central interior of British Columbia. *Can. J. Bot.* **79**, 600–611.

van Diepeningen, A.D., Goedbloed, D.J., Slakhorst, S.M., Koopmanschap, A.B., Maas, M., Hoekstra, R.F. and Debets, A.J.M. (2010) Mitochondrial recombination increases with age in *Podospora anserina*. *Mech. Ageing Dev.* **131**, 315–322.

Donnelly, D., Sanada, S., O'Reilly, J., Polonsky, J., Prange, T. and Pascard, C. (1982) Isolation and structure (X-ray analysis) of the orsellinate of armillol, a new antibacterial metabolite from *Armillaria mellea*. *J. Chem. Soc. Chem. Commun.* **2**, 135–137.

Dumas, M.T. (1991) Inhibition of *Armillaria* by bacteria isolated from soils of the Boreal Mixedwood Forest of Ontario. *Eur. J. Forest Pathol.* **22**, 11–18.

Eamus, D., Thompson, W., Cairney, J.W.G. and Jennings, D.H. (1985) Internal structure and hydraulic conductivity of Basidiomycete translocating organs. *J. Exp. Bot.* **36**, 1110–1116.

Entry, J.A., Cromack, K.J., Hansen, E. and Waring, R. (1991) Response of western coniferous seedlings to infection by *Armillaria ostoyae* under limited light and nitrogen. *Phytopathology*, **81**, 89–94.

Entry, J.A., Martin, N.E., Kelsey, R.G. and Cromack, K.J. (1992) Chemical constituents in root bark on five species of western conifer saplings and infection by *Armillaria ostoyae*. *Phytopathology*, **82**, 393–397.

Firn, R.D. and Jones, C.G. (2003) Natural products—a simple model to explain chemical diversity. *Nat. Prod. Rep.* **20**, 382–391.

Fox, R.T.V. (2000) Armillaria *Root Rot: Biology and Control of Honey Fungus*. Andover, UK: Intercept Limited.

Fox, R.T.V. and Hahne, K. (1989) Prospects for the rapid diagnosis of *Armillaria* by monoclonal antibody ELISA. In: *Proceedings of the Seventh International Conference on Root and Butt Rots* (Morrison, D.J., ed.), pp. 458–468. Victoria, BC: Forestry Canada.

Fukuda, M., Nakashima, E., Hayashi, K. and 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.

Gao, J.M., Yang, X., Wang, C.Y. and Liu, J.K. (2001) Armillaramide, a new sphingolipid from the fungus *Armillaria mellea*. *Fitoterapia*, **72**, 858–864.

Garbelotto, M.M., Lee, H.K., Slaughter, G., Popenuck, T., Cobb, F.W. and Bruns, T.D. (1997) Heterokaryosis is not required for virulence of *Heterobasidion annosum*. *Mycologia*, **89**, 92–102.

Garrett, S. (1957) Effect of a soil microflora selected by carbon disulphide fumigation on survival of *Armillaria mellea* in woody host tissues. *Can. J. Microbiol.* **3**, 135–149.

Gregory, S.T., Rishbeth, J. and Shaw, C.G., III (1991) Pathogenicity and virulence. In: Armillaria *Root Disease Agriculture Handbook No. 691* (Shaw, C.G., III and Kile, G.A., eds), pp. 76–87. Washington, DC: United States Department of Agriculture Forest Service.

Grillo, R., Korhonen, K., Hantula, J. and Hietala, A.M. (2000) Genetic evidence for somatic haploidization in developing fruit bodies of *Armillaria tabescens*. *Fungal Genet. Biol.* **30**, 135–145.

Gubler, W.D. (1992) Armillaria root rot. In: *Grape Pest Management Edition 2 Publication #3343* (Flaherty, D.L., Christensen, L., Lanini, W.T., Marois, J.J., Phillips, P.A. and Wilson, L.T., eds), pp. 92–93. Oakland, CA: University of California, Division of Agriculture and Natural Resources.

Gubler,W.D., Baumgartner, K., Browne, G.T., Eskalen, A., Rooney Latham, S., Petit, E. and Bayramian, L.A. (2004) Root diseases of grapevines in California and their control. *Austral. Plant Pathol.* **33**, 157–165.

Guillaumin, J.J. (1973) Etude du cycle caryologique de deux especes appartenant au genre *Armillariella*. *Ann. Phytopathol.* **5**, 317.

Guillaumin, J.J. (1977) Apricot root rot, *Armillariella mellea* (Vahl) Karst. *EPPO Bull.* **7**, 125–135.

Guillaumin, J.-J., Lung, B., Romagnesi, H. and Marxmüeller, H. (1985) Systematique des Armillaires du groupe Mellea. Consequences phytopathologiques. *Eur. J. Forest Pathol.* **15**, 268–277.

Guillaumin, J.J., Mohammed, C. and Berthelay, S. (1989a) *Armillaria* species in the Northern temperate hemisphere. In: *Proceedings of the Seventh International Conference on Root and Butt Rots* (Morrison, D.J., ed.), pp. 27–43. Victoria, BC: Forestry Canada.

Guillaumin, J.J., Pierson, J. and Grassely, C. (1989b) The susceptibility of different *Prunus* species used as stone fruit rootstocks to *Armillaria mellea* (*sensu stricto*). In: *Proceedings of the Seventh International Conference on Root and Butt Rots* (Morrison, D.J., ed.), pp. 197–207. Victoria, BC: Forestry Canada.

Guillaumin, J.J., Mohammed, C., Anselmi, N., Courtecuisse, R., Gregory, S.C., Holdenrieder, O., Intini, M., Lung, B., Marxmuller, H., Morrison, D., Rishbeth, J., Termorshuizen, A.J., Tirro, A. and Vandam, B. (1993) Geographical distribution and ecology of the *Armillaria* species in western Europe. *Eur. J. Forest Pathol.* **23**, 321–341.

Guillaumin, J.J., Anderson, J.B., Legrand, P., Ghahari, S. and Berthelay, S. (1996) A comparison of different methods for the identification of genets of *Armillaria* spp. *New Phytol.* **133**, 333–343.

Harrington, T.C. and Wingfield, B.D. (1995) A PCR-based identification method for species of Armillaria. *Mycologia*, **87**, 280–288.

Harrington, T.C., Worrall, J.J. and Baker, F.A. (1992) *Armillaria*. In: *Methods for Research on Soilborne Phytopathogenic Fungi* (Singleton, L.L., Mihail, J.D. and Rush, C.M., eds), pp. 81–85. St. Paul, MN: American Phytopathological Society.

Hartig, R. (1874) *Important Diseases of Forest Trees. Contributions to Mycology and Phytopathology for Botanists and Foresters*. St. Paul, MN: American Phytopathological Society.

Hintikka, V. (1973) A note on the polarity of *Armillariella mellea*. *Karstenia*, **13**, 32–39.

Hood, I.A., Redfern, D.B. and Kile, G.A. (1991) *Armillaria* in planted hosts. In: Armillaria *Root Disease Agriculture Handbook No. 691* (Shaw, C.G., III and Kile, G.A., eds), pp. 122–149. Washington, DC: United States Department of Agriculture Forest Service.

Hood, I.A., Horner, I.J., Gardner, J.F. and Sandberg, C.J. (2002) *Armillaria* root disease of *Pinus radiata* in New Zealand. 1: Basidiospore dispersal. *N. Z. J. Forest Sci.* **32**, 94–102.

Hu, Z., Huang, Q.-Z., Liu, X.-Z. and Yang, J.-B. (1999) Primary structure and cDNA cloning of the antifungal protein GAFP-I from *Gastrodia elata*. *Acta Bot. Yunnan*, **21**, 131–138.

Jahnke, K.-D., Bahnweg, G. and Worral, J.J. (1987) Species delimitation in the *Armillaria mellea* complex by analysis of nuclear and mitochondrial DNAs. *Trans. Br. Mycol. Soc.* **88**, 572–575.

Jin, W.S. and Tian, D.Q. (2000) Studies of the chemistry and pharmacology of *Gastrodia elata*. *Chin. Trad. Drug Technol.* **2**, 21–30.

Johannesson, H. and Stenlid, J. (1999) Molecular identification of wood inhabiting fungi in an unmanaged *Picea abies* forest in Sweden. *Forest Ecol. Manag.* **115**, 203–211.

Johannesson, H. and Stenlid, J. (2004) Nuclear reassortment between vegetation mycelia in natural populations of the basidiomycete *Heterobasidion annosum*. *Fungal Genet. Biol.* **41**, 563–570.

Keca, N., Karadzic, D. and Woodward, S. (2009) Ecology of *Armillaria* species in managed forests and plantations in Serbia. *Forest Pathol.* **39**, 217–231.

Kikuchi, G., Higuchi, M., Yoshimura, H., Morota, T. and Suzuki, A. (2008) In vitro symbiosis between *Gastrodia elata* Blume (Orchidaceae) and *Armillaria* Kummer (Tricholomataceae) species isolates from the orchid tuber. *J. Jpn. Bot.* **83**, 77–87.

Kile, G.A. (1981) *Armillaria luteobubalina*: a primary cause of decline and death of trees in mixed species eucalypt forests in central Victoria. *Aust. Forest Res.* **11**, 63–77.

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. and 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*. *Trans. Br. Mycol. Soc.* **91**, 305–315.

Kile, G.A., Guillaumin, J.J., Mohammed, C. and Watling, R. (1994) Biogeography and pathology of *Armillaria*. In: *Proceedings of the 8th International Conference on Root and Butt Rots* (Johannson, M. and Stenlid, J., eds), pp. 411–436. Uppsala: Swedish University of Agricultural Sciences.

Korhonen, K. (1978) Interfertility and clonal size in the *Armillariella mellea* complex. Karstenia, **18**, 31–42.

Korhonen, K. (1983) Observations on nuclear migration and heterokaryotization in *Armillaria*. *Crypt. Mycol.* **4**, 79–86.

Korhonen, K. and Hintikka, V. (1974) Cytological evidence for somatic diploidization in dikaryotic cells of *Armillariella mellea*. *Arch. Microbiol.* **95**, 187–192.

Kromroy, K.W., Blanchette, R.A. and Grigal, D.F. (2005) *Armillaria* species on small woody plants, small woody debris, and root fragments in red pine stands. *Can. J. Forest Res.* **35**, 1487–1495.

Lee, S.Y., Kim, J.S., Kim, J.E., Sapkota, K., Shen, M.H., Kim, S., Chun, H.S., Yoo, J.C., Choi, H.S., Kim, M.K. and Kim, S.J. (2005) Purification and characterization of fibrinolytic enzyme from cultured mycelia of *Armillaria mellea*. *Protein Expr. Purif.* **43**, 10–17.

LeFrancois, J.-B., Lung-Escarmant, B. and Langrell, S.R.H. (2002) Development of novel polymerase chain reaction (PCR) based microsatellite markers in *Armillaria gallica* by cross-species amplification and species-specific cloning. *Mol. Ecol. Notes*, **2**, 182–184.

Lima, M.L.A., Asai, T. and Capelari, M. (2008) *Armillaria paulensis*: a new South American species. *Mycol. Res.* **112**, 1122–1128.

Lung-Escarmant, B. and Dunez, J. (1979) Differentiation of *Armillariella* and *Clitocybe* species by the use of immuno-enzymatic ELISA procedure. *Ann. Phytopathol.* **11**, 515–518.

Lung-Escarmant, B., Mohammed, C. and Dunez, J. (1985) Nouvelles méthodes de détermination des Armillairés européens: immunologie et electrophorèse en gel de polycrylamide. *Eur. J. Forest Pathol.* **15**, 278–288.

Maphosa, L., Wingfield, B., Coetzee, M., Mwenje, E. and Wingfield, M.J. (2006) Phylogenetic relationships among *Armillaria* species inferred from partial elongation factor 1-alpha DNA sequence data. *Aust. Plant Pathol.* **35**, 513–520.

Matsushita, N. and Suzuki, K. (2005) Identification of *Armillaria* species in Japan using PCR-RFLP analysis of rDNA intergenic spacer region and comparisons of *Armillaria* species in the world. *J. Forest Res.* **10**, 173–179.

Midland, S.L., Izac, R.R., Wing, R.M., Zaki, A.I., Munnecke, D.E. and Sims, J.J. (1982) Melleolide, a new antibiotic from *Armillaria mellea*. *Tetrahedron Lett.* **23**, 2515–2518.

Mihail, J.D. and Bruhn, J.N. (2007) Dynamics of bioluminescence by *Armillaria gallica*, *A. mellea* and *A. tabescens*. *Mycologia*, **99**, 341–350.

Misiek, M. and Hoffmeister, D. (2008) Processing sites involved in intron splicing of *Armillaria* natural product genes. *Mycol. Res.* **112**, 216–224.

Misiek, M. and Hoffmeister, D. (2011) Sesquiterpene-aryl ester natural products in North American *Armillaria* species. *Mycol. Prog.* DOI: 10.1007/s11557-010-0720-3 (in press).

Misiek, M.,Williams, J., Schmich, K., Huttel,W., Merfort, I., Salomon, C.E., Aldrich, C.C. and Hoffmeister, D. (2009) Structure and cytotoxicity of arnamial and related fungal sesquiterpene aryl esters. *J. Nat. Prod.* **72**, 1888–1891.

Mohammed, C., Guillaumin, J.-J., Botton, B. and Intini, M. (1994) Species of *Armillaria* in tropical Africa. In: *Proceedings of the 8th International Conference on Root and Butt Rots* (Johannson, M. and Stenlid, J., eds), pp. 402–410. Uppsala: Swedish University of Agricultural

Sciences.

Momose, I., Sekizawa, R., Hosokawa, N., Iinuma, H., Matsui, S., Nakamura, H., Naganawa, H., Hamada, M. and Takeuchi, T. (2000) Melleolides K, L and M, new melleolides from *Armillariella mellea*. *J. Antibiot.* **53**, 137–143.

Morrison, D.J. (1976) Vertical distribution of *Armillaria mellea* rhizomorphs in the soil. *Trans. Br. Mycol. Soc.* **66**, 393–399.

Morrison, D.J. (2004) Rhizomorph growth habit, saprophytic ability and virulence of 15 *Armillaria* species. *Forest Pathol.* **34**, 15–26.

Morrison, D.J. and Pellow, K.W. (2002) Variation in virulence among isolates of *Armillaria ostoyae*. *Forest Pathol.* **32**, 99–107.

Morrison, D.J., Chu, D. and Johnson, A.L.S. (1985) Species of *Armillaria* in British Columbia. *Can. J. Plant Pathol.* **7**, 242–246.

Morrison, D.,Williams, R. and Whitney, R.D. (1991) Infection, disease development, diagnosis, and detection. In: Armillaria *Root Disease Agriculture Handbook No. 691* (Shaw, C.G., III and Kile, G.A., eds), pp. 62–75. Washington, DC: United States Department of Agriculture Forest Service.

Motta, J.J. (1969) Cytology and morphogenesis in the rhizomorph of *Armillaria mellea*. *Am. J. Bot.* **56**, 610–619.

Motta, J.J. and Korhonen, K. (1986) A note on *Armilaria mellea* and *Armillaria bulbosa* from the middle Atlantic states. *Mycologia*, **78**, 471–474.

Motta, J.J., Peabody, D.C. and Peabody, R.B. (1986) Quantitative differences in nuclear DNA content between *Armillaria mellea* and *Armillaria bulbosa*. *Mycologia*, **78**, 963–965.

Munnecke, D.E.,Wilbur,W.D. and Kolbezen, M.J. (1970) Dosage response of *Armillaria mellea* to methyl bromide. *Phytopathology*, **60**, 992–993.

Munnecke, D.E., Kolbezen, M.J. and Wilbur, W.D. (1973) Effect of methyl bromide or carbon disulfide on *Armillaria* and *Trichoderma* growing on agar medium and relation to survival of *Armillaria* in soil following fumigation. *Phytopathology*, **63**, 1352–1357.

Munnecke, D.E., Kolbezen, M.J., Wilbur, W.D. and Ohr, H.D. (1981) Interactions involved in controlling *Armillaria mellea*. *Plant Dis.* **65**, 384–389.

Mwenje, E. and Ride, J.P. (1996) Morphological and biochemical characterization of *Armillaria* isolates from Zimbabwe. *Plant Pathol.* **45**, 1036–1051.

Mwenje, E. and Ride, J.P. (1997) The use of pectic enzymes in the characterization of *Armillaria* isolates from Africa. *Plant Pathol.* **46**, 341–354.

Mwenje, E. and Ride, J.P. (1999) Purification and characterization of an endo-polygalacturonase (PG1) from a Zimbabwean species of *Armillaria*. *Physiol. Mol. Plant Pathol.* **55**, 131–139.

Mwenje, E., Wingfield, B.D., Coetzee, M.P.A. and Wingfield, M.J. (2003) Molecular characterisation of *Armillaria* species from Zimbabwe. *Mycol. Res.* **107**, 291–296.

Mwenje, E., Wingfield, B.D., Coetzee, M.P.A., Nemato, H. and Wingfield, M.J. (2006) *Armillaria* species on tea in Kenya identified using isozyme and DNA sequence comparisons. *Plant Pathol.* **55**, 343–350.

Nagel, A.K., Schnabel, G., Petri, C. and Scorza, R. (2008) Generation and characterization of transgenic plum lines expressing the *Gastrodia* antifungal protein. *HortScience*, **43**, 1514–1521.

Ohr, H.D., Munnecke, D.E. and Bricker, J.L. (1973) The interaction of *Armillaria mellea* and *Trichoderma* spp. as modified by methyl bromide. *Phytopathology*, **63**, 965–973.

Ota, Y., Fukuda, K. and Suzuki, K. (1998a) The nonheterothallic life cycle of Japanese *Armillaria mellea*. *Mycologia*, **90**, 396–405.

Ota, Y., Matsushita, N., Nagasawa, E., Terashita, T., Fukuda, K. and Suzuki, K. (1998b) Biological species of *Armillaria* in Japan. *Plant Dis.* **82**, 537–543.

Ota, Y., Intini, M. and Hattori, T. (2000) Genetic characterization of heterothallic and non-heterothallic *Armillaria mellea sensu stricto*. *Mycol. Res.* **104**, 1046–1054.

Otieno,W., Termorshuizen, A., Jeger, M. and Othieno, C. (2003) Efficacy of soil solarization, *Trichoderma harzianum*, and coffee pulp amendment against *Armillaria* sp. *Crop Protect.* **22**, 325–331.

Pareek, M., Allaway,W.G. and Ashford, A.E. (2006) *Armillaria luteobubalina* mycelium develops air pores that conduct oxygen to rhizomorph clusters. *Mycol. Res.* **110**, 38–50.

Peabody, R.B., Peabody, D.C. and Sicard, K.M. (2000) A genetic mosaic in the fruiting stage of *Armillaria gallica*. *Fungal Genet. Biol.* **29**, 72–80.

Pearce, M.H. and Malajczuk, N. (1990) Factors affecting the growth of *Armillaria luteobubalina* rhizomorphs in soil. *Mycol. Res.* **94**, 38–48.

Pearce, M.H., Nelson, E.E. and Malajczuk, N. (1995) Effects of the cordforming saprotrophs *Hypholoma australe* and *Phanerochaete filamentosa* and of ammonium sulphamate on establishment of *Armillaria luteobubalina* on stumps of *Eucalyptus diversicolor. Mycol. Res.* **99**, 951–956.

Peipp, H. and Sonnenbichler, J. (1992) Occurrence of antibiotic compounds in cultures of *Armillaria ostoyae* growing in the presence of an antagonistic fungus or host plant cells. *Biol. Chem.* **373**, 675–683.

Perazzolli, M., Faccin, S., Schwarz, F., Gatto, P., Pertot, I., Gessler, C. and Moser, C. (2007) Functional characterization of grape defence genes to improve the biocontrol activity of *Pseudomonas fluorescens* against *Armillaria mellea*. *IOBC/WPRS Bull.* **30**, 293–296.

Perazzolli, M., Bampi, F., Faccin, S., Moser, M., De Luca, F., Ciccotti, A.M., Velasco, R., Gessler, C., Pertot, I. and Moser, C. (2010) *Armillaria mellea* induces a set of defense genes in grapevine roots and one of them codifies a protein with antifungal activity. *Mol. Plant–Microbe Interact.* **23**, 485–496.

Pérez-Sierra, A., Guillaumin, J.-J., Spooner, B.M. and Bridge, P.D. (2004) Characterization of *Armillaria heimii* from Africa. *Plant Pathol.* **53**, 220–230.

Pildain, M., Coetzee, M., Rajchenberg, M., Petersen, R.,Wingfield, M. and Wingfield, B. (2009) Molecular phylogeny of *Armillaria* from the Patagonian Andes. *Mycol. Prog.* **8**, 181–194.

Pildain, M.B., Coetzee, M.P.A., Wingfield, B.D., Wingfield, M.J. and Rajchenberg, M. (2010) Taxonomy of *Armillaria* in the Patagonian forests of Argentina. *Mycologia*, **102**, 392–403.

Proffer, T.J., Jones, A.L. and Ehret, G.R. (1987) Biological species of *Armillaria* isolated from sour cherry orchards in Michigan. *Phytopathology*, **77**, 941–943.

Prospero, S., Rigling, D. and Holdenrieder, O. (2003) Population structure of *Armillaria* species in managed Norway spruce stands in the Alps. *New Phytol.* **158**, 365–373.

Prospero, S., Holdenrieder, O. and Rigling, D. (2004) Comparison of the virulence of *Armillaria cepistipes* and *Armillaria ostoyae* on four Norway spruce provenances. *Forest Pathol.* **34**, 1–14.

Prospero, S., Holdenrieder, O. and Rigling, D. (2006) Rhizomorph production and stump colonization by co-occurring *Armillaria cepistipes* and *Armillaria ostoyae*: an experimental study. *Forest Pathol.* **36**, 21–31.

Prospero, S., Lung-Escarmant, B. and Dutech, C. (2008) Genetic structure of an expanding *Armillaria* root rot fungus (*Armillaria ostoyae*) population in a managed pine forest in southwestern France. *Mol. Ecol.* **17**, 3366–3378.

Prospero, S., Jung, E., Tsykun, T. and Rigling, D. (2010) Eight microsatellite markers for *Armillaria cepistipes* and their transferability to other *Armillaria* species. *Eur. J. Plant Pathol.* **127**, 165–170.

Qin, G.F., Zhao, J. and Korhonen, K. (2007) A study on intersterility groups of *Armillaria* in China. *Mycologia*, **99**, 430–441.

Raabe, R.D. (1962) Host list of the root rot fungus *Armillaria mellea*. *Hilgardia*, **33**, 25–88.

Raziq, F. and Fox, R.T.V. (2003) Comparisons between the in vitro and in vivo efficacies of potential fungal antagonists of *Armillaria mellea*. *Biol. Agric. Hortic.* **21**, 263–276.

Raziq, F. and Fox, R.T.V. (2005) Combinations of fungal antagonists for biological control of *Armillaria* root rot of strawberry plants. *Biol. Agric. Hortic.* **23**, 45–57.

Redfern, D.B. and Filip, G.M. (1991) Inoculum and infection. In: Armillaria *Root Disease Agriculture Handbook No. 691* (Shaw, C.G., III and Kile, G.A., eds), pp. 48–61. Washington, DC: United States Department of Agriculture Forest Service.

Rehman, A.U. and Thurston, C.F. (1992) Purification of laccase-I from *Armillaria mellea*. *J. Gen. Microbiol.* **138**, 1251–1257.

Reil, W. (1997) *Walnut Rootstock Evaluation for Resistance to* Armillaria *Root Rot*. Modesto, CA: California Walnut Marketing Board.

Rishbeth, J. (1970) The role of basidiospores in stump infection by *Armillaria mellea*. In: *Root Diseases and Soil-Borne Pathogens* (Tousson, T.A., Bega, R.V. and Nelson, P.E., eds), pp. 141–146. Berkeley, CA: University of California Press.

Rishbeth, J. (1982) Species of *Armillaria* in southern England. *Plant Pathol.* **31**, 9–17.

Rishbeth, J. (1985) Infection cycle of *Armillaria* and host response. *Eur. J. Forest Pathol.* **15**, 332–341.

Rishbeth, J. (1986) Some characteristics of English *Armillaria* species in culture. *Trans. Br. Mycol. Soc.* **86**, 213–218.

Rizzo, D.M. and Harrington, T.C. (1993) Delineation and biology of clones of *Armillaria ostoyae*, *A. gemina*, and *A. calvescens*. *Mycologia*, **85**, 164–174.

Rizzo, D.M. and May, G. (1994) Nuclear replacement during mating in *Armillaria ostoyae*. *Microbiology*, **140**, 2115–2124.

Rizzo, D.M., Blanchette, R.A. and May, G. (1995) Distribution of *Armillaria ostoyae* genets in a *Pinus resinosa–Pinus banksiana* forest. *Can. J. Bot.* **73**, 776–787.

Rizzo, D.M., Whiting, E.C. and Elkins, R.B. (1998) Spatial distribution of *Armillaria mellea* in pear orchards. *Plant Dis.* **82**, 1226–1231.

Robene-Soustrade, I. and Lung-Escarmant, B. (1997) Laccase isoenzyme patterns of European *Armillaria* species from culture filtrates and infected woody plant tissues. *Eur. J. Forest Pathol.* **27**, 105–114.

Robene-Soustrade, I., Lung-Escarmant, B., Bono, J.J. and Taris, B. (1992) Identification and partial characterization of an extracellular manganese dependent peroxidase in *Armillaria ostoyae* and *Armillaria mellea*. *Eur. J. Forest Pathol.* **22**, 227–236.

Roll Hansen, F. (1985) The *Armillaria* species in Europe: a literature review. *Eur. J. Forest Pathol.* 15, 22–31.

Sa, Q., Wang, Y., Li, W., Zhang, L. and Sun, Y. (2003) The promoter of an antifungal protein gene from *Gastrodia elata* confers tissue-specific and fungus-inducible expression patterns and responds to both salicylic acid and jasmonic acid. *Plant Cell Rep.* **22**, 79–84.

Saville, B.J., Yoell, H. and Anderson, J.B. (1996) Genetic exchange and recombination in populations of the root-infecting fungus *Armillaria gallica*. *Mol. Ecol.* **5**, 485–497.

Saville, B.J., Kohll, Y. and Anderson, J.B. (1998) mtDNA recombination in a natural population. *Proc. Natl. Acad. Sci. USA*, **95**, 1331–1335.

Schnabel, G., Ash, J.S. and Bryson, P.K. (2005) Identification and characterization of *Armillaria tabescens* from the southeastern United States. *Mycol. Res.* **109**, 1208–1222.

Schulze, S., Bahnweg, G., Tesche, M. and Sandermann, H. (1995) Identification of European *Armillaria* species by restriction-fragment length polymorphisms of ribosomal DNA. *Eur. J. Forest Pathol.* **25**, 214–223.

Sekizaki, H., Kuninaga, S., Yamamoto, M., Asazu, S.N., Sawa, S., Kojoma, M., Yokosawa, R. and Yoshida, N. (2008) Identification of *Armillaria nabsnona* in *Gastrodia* tubers. *Biol. Pharm. Bull.* **31**, 1410–1414.

Shaw, C.G., III (1981) Basidiospores of *Armillaria mellea* survive an Alaskan winter on tree bark. *Plant Dis.* **65**, 972–974.

Shaw, C.G., III and Kile, G.A. (1991) *Armillaria* Root Disease. *United States Department of Agriculture-Forest Service Handbook No. 691*. Washington, DC: United States Department of Agriculture-Forest Service.

Shaw, C.G., III and Roth, L.F. (1976) Persistence and distribution of *Armillaria mellea* in a ponderosa pine forest. *Phytopathology*, **66**, 1210–1213.

Shaw, C.G., III and Roth, L.F. (1978) Control of *Armillaria* root rot in managed coniferous forests. *Eur. J. Forest Pathol.* **8**, 163–174.

Shaw, C.G., III, MacKenzie, M., Toes, E.H.A. and Hood, I.A. (1981) Cultural characteristics and pathogenicity to *Pinus radiata* of *Armillaria novaezelandiae* and *A. limonea*. *N. Z. J. Forest. Sci.* **11**, 65–70.

Shimomura, O., Satoh, S. and Kishi, Y. (1993) Structure and nonenzymatic light emission of two luciferin precursors isolated from the luminous mushroom *Panellus stipticus*. *J. Biolumin. Chemilumin*. **8**, 201–205.

Siepmann, R. (1987) Criterion for the valuation of matings between diploid *Armillaria* isolates and haploid testers. *Eur. J. Forest Pathol.* **17**, 308–311.

Smith, M.L. and Anderson, J.B. (1989) Restriction fragment length polymorphisms in mitochondrial DNAs of *Armillaria*: identification of North American biological species. *Mycol. Res.* **93**, 247–256.

Smith, M.L., Bruhn, J.N. and Anderson, J.B. (1992) The fungus *Armillaria bulbosa* is among the largest and oldest living organisms. *Nature*, **356**, 428–431.

Smith, M.L., Bruhn, J.N. and Anderson, J.B. (1994) Relatedness and spatial distribution of *Armillaria* genets infecting red pine seedlings. *Phytopathology*, **84**, 822–829.

Sonnenbichler, J., Guillaumin, J.J., Peipp, H. and Schwarz, D. (1997) Secondary metabolites from dual cultures of genetically different *Armillaria* isolates. *Eur. J. Forest Pathol.* **27**, 241–249.

Takano, H., Onoue, K. and Kawano, S. (2010) Mitochondrial fusion and inheritance of the mitochondrial genome. *J. Plant Res.* **123**, 131–138.

Termorshuizen, A. and Arnolds, E. (1987) On the nomenclature of the European species of the *Armillaria mellea* group. *Mycotaxon*, **30**, 101–116.

Thomas, H.E. (1934) Studies on *Armillaria mellea* (Vahl) Quelet: infection, parasitism, and host resistance. *J. Agric. Res.* **48**, 187–218.

Thomas, H.E., Roberts, C. and Amstutz, A. (1948) Rootstock susceptibility to *Armillaria mellea*. *Phytopathology*, **38**, 152–154.

Ullrich, R.C. and Anderson, J.B. (1978) Sex and diploidy in *Armillaria mellea*. *Exp. Mycol.* **2**, 119–129.

Uppalapati, S.R., Marek, S.M., Lee, H.K., Nakashima, J., Tang, Y., Sledge, M.K., Dixon, R.A. and Mysore, K.S. (2009) Global gene expression profiling during *Medicago truncatula–Phymatotrichopsis omnivora* interaction reveals a role for jasmonic acid, ethylene, and the flavonoid pathway in disease development. *Mol. Plant–Microbe Interact.* **22**, 7–17.

Vahl, M. (1787) *Flora Danica*.

de Vasconcellos, R.L.F. and Cardoso, E.J.B.N. (2009) Rhizospheric streptomycetes as potential biocontrol agents of *Fusarium* and *Armillaria* pine rot and as PGPR for *Pinus taeda*. *BioControl*, **54**, 807–816.

Volk, T.J. and Burdsall, H.H., Jr (1995) *A Nomenclatural Study of* Armillaria *and* Armillariella *Species*. Oslo: Synopsis Fungorum.

Volk, T.J., Burdsall, H.H., Jr and and Banik, M.T. (1996a) *Armillaria nabsnona*, a new species from western North America. *Mycologia*, **88**, 484–491.

Wahlstrom, K., Karlsson, J., Holdenrieder, O. and Stenlid, J. (1991) Pectinolytic activity and isozymes in European *Armillaria* species. *Can. J. Bot.* **69**, 2732–2739.

Wang, H.X., Yang, T., Zeng, Y. and Hu, Z. (2007) Expression analysis of the gastrodianin gene ga4B in an achlorophyllous plant *Gastrodia elata* Bl. *Plant Cell Rep.* **26**, 253–259.

Wang, X., Bauw, G., Van Damme, E.J.M., Peumans, W.J., Chen, Z.-L., Van Montagu, M., Angenon, G. and Dillen, W. (2001) Gastrodianin-like mannose-binding proteins: a novel class of plant proteins with antifungal properties. *Plant J.* **25**, 651–661.

Wargo, P.M. and Shaw, C.G., III (1985) *Armillaria* root rot: the puzzle is being solved. *Plant Dis.* **69**, 826–832.

Watling, R., Kile, G.A. and Burdsall, H.H., Jr (1991) Nomenclature, taxonomy, and identification. In: Armillaria *Root Disease Agriculture Handbook No. 691* (Shaw, C.G., III and Kile, G.A., eds), pp. 1–9. Washington, DC: United States Department of Agriculture-Forest Service.

Watling, R., Kile, G.A. and Gregory, N.M. (1982) The genus *Armillaria*—nomenclature, typification, the identity of *Armillaria mellea* and species differentiation. *Trans. Br. Mycol. Soc.* **78**, 271–285.

Weinhold, A.R. (1963) Rhizomorph production by *Armillaria mellea* induced by ethanol and related compounds. *Science*, **142**, 1065–1066.

Weitz, H.J. (2004) Naturally bioluminescent fungi. *Mycologist*, **18**, 4–5.

Wilkins, B.S., Ebel, R.C., Dozier, W.A., Pitts, J., Eakes, D.J., Himelrick, D.G., Beckman, T. and Nyczepir, A. (2002) Field performance of Guardian™ peach rootstock selections. *HortScience*, **37**, 1049–1052.

Wingfield, B.D., Maphosa, L., Coetzee, M.P.A., Mwenje, E. and Wingfield, M.J. (2009) Characterisation of Zimbabwean *Armillaria* using IGS-1 sequences and AFLP analysis. *Fungal Divers.* **34**, 187–196.

Worrall, J.J. (1994) Population structure of *Armillaria* species in several forest types. *Mycologia*, **86**, 401–407.

Worrall, J.J. (1997) Somatic incompatibility in basidiomycetes. *Mycologia*, **89**, 24–36.

Worrall, J.J., Chet, I. and Huttermann, A. (1986) Association of rhizomorph formation with laccase activity in *Armillaria* spp. *J. Gen. Microbiol.* **132**, 2527–2533.

Worrall, J.J., Sullivan, K.F., Harrington, T.C. and Steimel, J.P. (2004) Incidence, host relations and population structure of *Armillaria ostoyae* in Colorado campgrounds. *Forest Ecol. Manag.* **192**, 191–206.

Xu, Q., Liu, Y., Wang, X., Gu, H. and Chen, Z. (1998) Purification and characterization of a novel anti-fungal protein from *Gastrodia elata*. *Plant Physiol. Biochem.* **36**, 899–905.

Zeller, S.M. (1926) Observations on infections of apple and prune roots by *Armillaria mellea* Vahl. *Phytopathology*, **16**, 479–484.

Zhou, X., Yang, X.H., Liang, H.X. and Liu, C.Y. (1987) *Gastrodia Morphology*. Beijing: Science Press.

Zolciak, A., Bouteville, R.-J., Tourvieille, J., Roeckel-Drevet, P., Nicolas, P. and Guillaumin, J.-J. (1997) Occurrence of *Armillaria* ecotype (Fr.) Lamoure in peat bogs of the Auvergne—the reproduction system of the species. *Crypt. Mycol.* **18**, 299–313.