

Pathogen profile

Secrets of the subterranean pathosystem of *Armillaria*KENDRA BAUMGARTNER^{1,*}, MARTIN P. A. COETZEE² AND DIRK HOFFMEISTER³¹United States Department of Agriculture-Agricultural Research Service (USDA-ARS), University of California, Davis, CA 95616, USA²Department of Genetics, Forestry and Agricultural Biotechnology (FABI), University of Pretoria, Pretoria 0002, South Africa³Friedrich-Schiller-Universität, Department of Pharmaceutical Biology, Beutenbergstr 11A, D-07745 Jena, Germany**SUMMARY**

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; Munneke *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).

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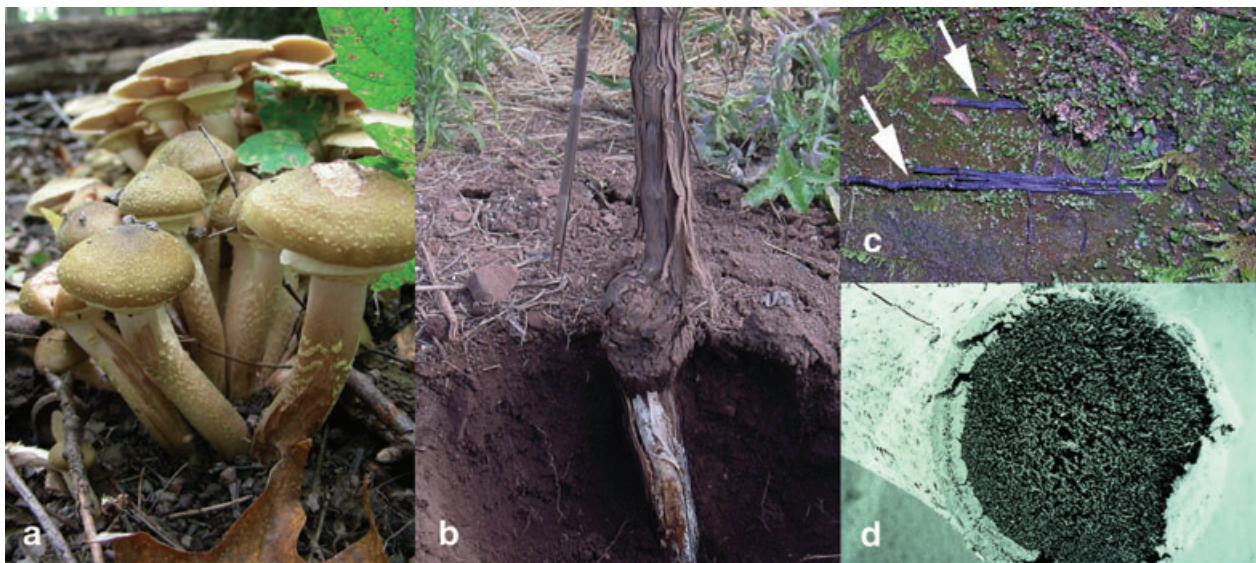


Fig. 1 Signs of *Armillaria* root disease: (a) basidiocarps of *Armillaria mellea* from a mixed-hardwood forest in Northfield, MN, USA; (b) mycelial fans found beneath the bark at the root collar of *Vitis vinifera* (grapevine) infected with *A. mellea*; (c) rhizomorphs (see arrows) growing under the 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).

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').

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).

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).

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
<i>A. borealis</i>			A	CBS M	
<i>A. calvescens</i>		NABS III			
<i>A. cepistipes</i>	NABS XI	NABS XI	B		NAG D
<i>A. ectypa</i>			*		*
<i>A. gallica</i>	NABS VII	NABS VII	E	CBS B	NAG A
<i>A. gemina</i>		NABS II			
<i>A. jezoensis</i>					H
<i>A. mellea</i>	NABS VI	NABS VI	D	CBS K	
<i>A. mellea</i> ssp. <i>nipponica</i>				CBS G	NAG Am
<i>A. nabsnana</i>	NABS IX				NAG B
<i>A. ostoyae</i>	NABS I	NABS I	C	CBS D	NAG C
<i>A. sinapina</i>	NABS V	NABS V		CBS A	F
<i>A. singula</i>					G
<i>A. tabescens</i>	*	*	*	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.

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-1 α) 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.

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
<i>A. affinis</i>	*					
<i>A. camerunensis</i>			*			
<i>A. duplicata</i>						India
<i>A. fellea</i>						New Guinea
<i>A. fumosa</i>				*		
<i>A. fuscipes</i>			*			
<i>A. griseomellea</i>		*				
<i>A. heimii</i>			SIG II			
<i>A. hinnulea</i>				*	*	
<i>A. limonea</i>					*	
<i>A. luteobubalina</i>				*		
<i>A. mellea</i>			SIG I			
<i>A. mellea</i> ssp. <i>nipponica</i>						Bhutan
<i>A. melleo-rubens</i>	*					
<i>A. montagnei</i>		*				
<i>A. novae-zelandiae</i>		*		*	*	New Guinea, Sumatra, Malaysia, Fiji
<i>A. omniuens</i>						India
<i>A. pallidula</i>				*		
<i>A. paulensis</i>		*				
<i>A. pelliculata</i>			*			
<i>A. procera</i>		*				
<i>A. puiggarii</i>		*				
<i>A. sparrei</i>		*				
<i>A. tabescens</i>	*					
<i>A. tigrensis</i>		*				
<i>A. umbrinobrunnea</i>		*				
<i>A. viridiflava</i>		*				
<i>A. yungensis</i>		*				
Undescribed species						
BPS I						Bhutan
NZ <i>Armillaria</i> sp.					*	

Asterisks denote the presence of a species.

BPS, Bhutan Phylogenetic Species; NZ, New Zealand; SIG, Somatic Incompatibility Group.

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 homo-heteromixis (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 nucleate 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

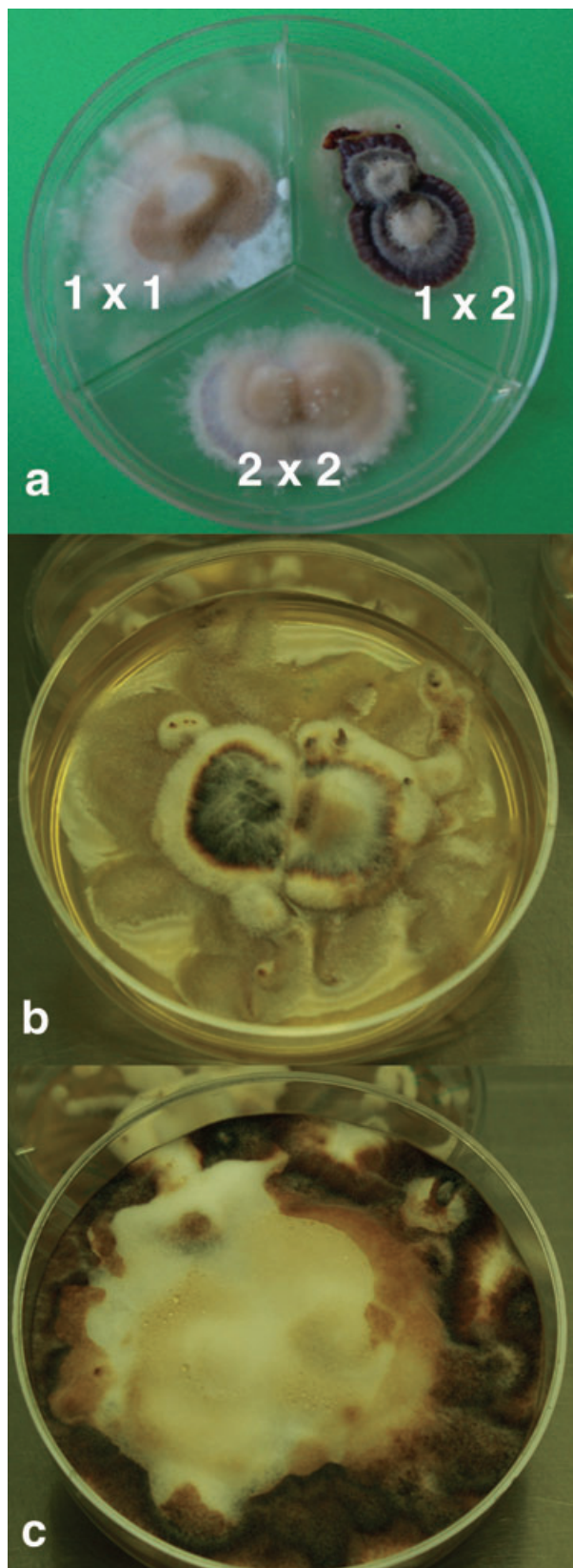


Fig. 2 Mycelial interactions in *Armillaria*: (a) self-pairings of haploid strains 1×1 and 2×2 show the characteristically fluffy morphology of haploid mycelia, which is in contrast with the flattened morphology of the diploid mycelium formed by mating strains 1 and 2; (b) two diploid strains that are 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.

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).

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

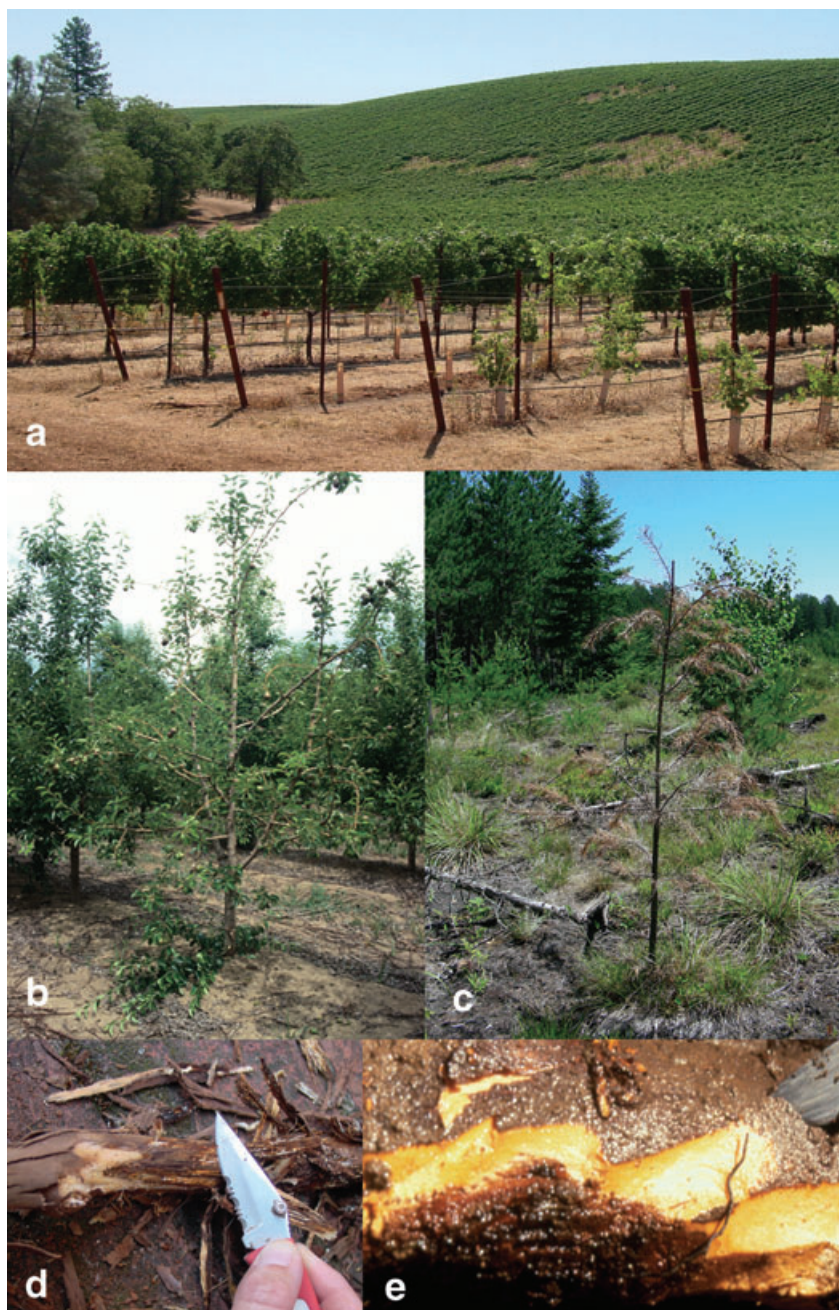


Fig. 3 Symptoms and indicators of *Armillaria* root disease: (a) gaps in the vineyard canopy are disease centres caused by *Armillaria mellea* inoculum from residual roots of infected forest trees that previously inhabited the site (note remnant forest stand at left); (b) symptomatic *Pyrus communis* (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).

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-1 α , 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 necrotrophs; 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; intraspecific 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); Misiak 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 (Misiak 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, diatretole, 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* (peppergrass) (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;

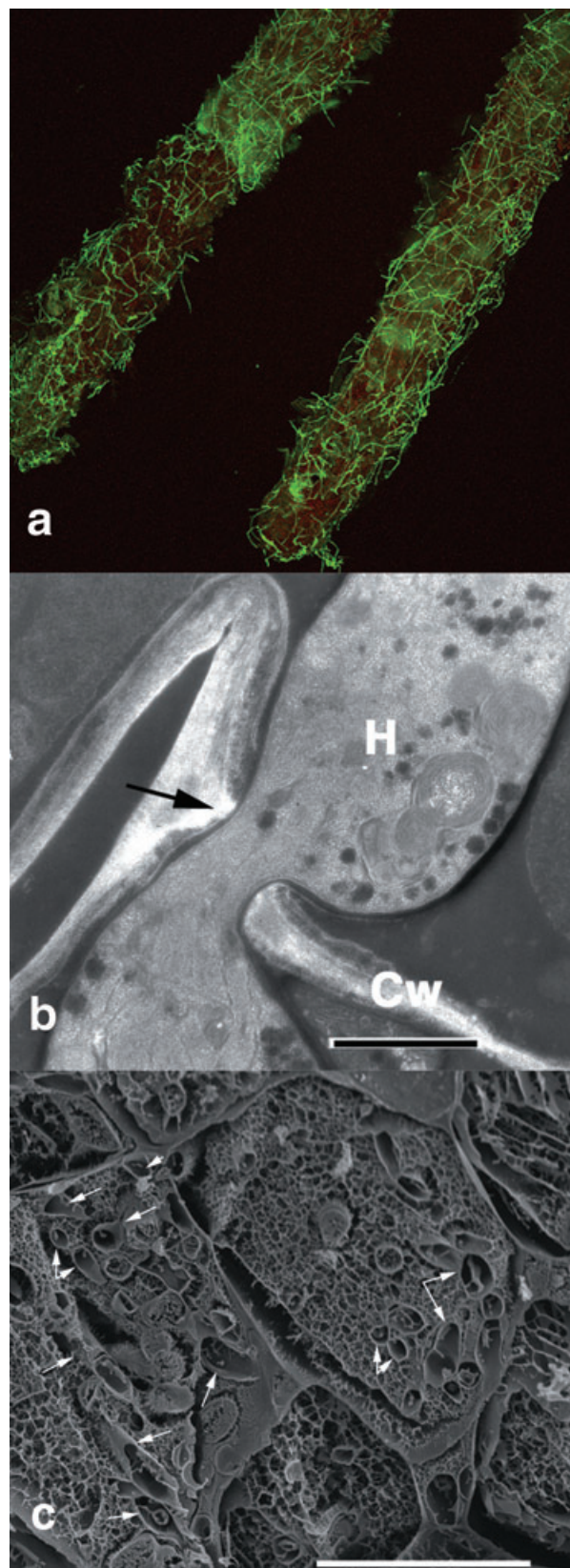
Fig. 4 *In planta* growth of *Armillaria*: (a) grapevine roots (5 μm in diameter) colonized by *A. mellea* hyphae; (b) penetration of the root epidermis of a *Lupinus albus* cv. 'Tifblue' (white lupine) seedling by *A. tabescens* hyphae (bar, 1 μm ; Cw, cell wall; H, hypha) (photograph by Kerik Cox);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

Kile, 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, nonwoody 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 infection 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 (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. Alterna-

tively, *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; Munneke *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; Munneke *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

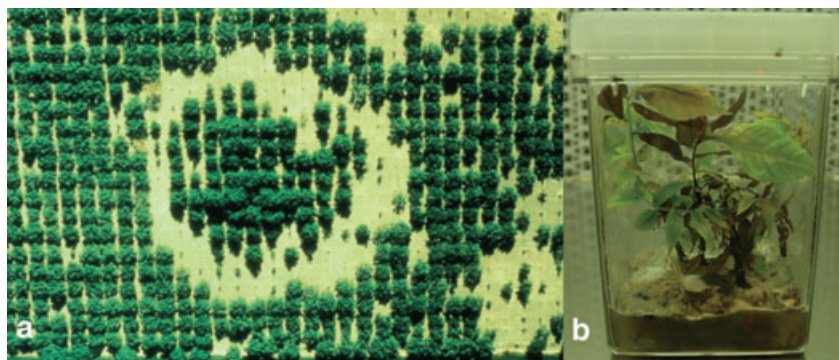
being evaluated on peach in the southeastern USA (Guido Schnabel, Clemson University, SC, USA, personal communication). A second post-infection treatment, also demonstrated to improve yields of grapevine, is the application of commercial inoculants that contain antagonistic bacteria (e.g. *Bacillus subtilis*) 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

Fig. 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.



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.

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.

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