Fungal Biology

Marcela C. Pagano *Editor*

Recent Advances on Mycorrhizal Fungi

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Fungal biology has an integral role to play in the development of the biotechnology and biomedical sectors. It has become a subject of increasing importance as new fungi and their associated biomolecules are identified. The interaction between fungi and their environment is central to many natural processes that occur in the biosphere. The hosts and habitats of these eukaryotic microorganisms are very diverse; fungi are present in every ecosystem on Earth. The fungal kingdom is equally diverse consisting of seven different known phyla. Yet detailed knowledge is limited to relatively few species. The relationship between fungi and humans has been characterized by the juxtaposed viewpoints of fungi as infectious agents of much dread and their exploitation as highly versatile systems for a range of economically important biotechnological applications. Understanding the biology of different fungi in diverse ecosystems as well as their interactions with living and nonliving is essential to underpin effective and innovative technological developments. This series will provide a detailed compendium of methods and information used to investigate different aspects of mycology, including fungal biology and biochemistry, genetics, phylogenetics, genomics, proteomics, molecular enzymology, and biotechnological applications in a manner that reflects the many recent developments of relevance to researchers and scientists investigating the Kingdom Fungi. Rapid screening techniques based on screening specific regions in the DNA of fungi have been used in species comparison and identification, and are now being extended across fungal phyla. The majorities of fungi are multicellular eukaryotic systems and, therefore, may be excellent model systems by which to answer fundamental biological questions. A greater understanding of the cell biology of these versatile eukaryotes will underpin efforts to engineer certain fungal species to provide novel cell factories for production of proteins for pharmaceutical applications. Renewed interest in all aspects of the biology and biotechnology of fungi may also enable the development of “one pot” microbial cell factories to meet consumer energy needs in the 21st century. To realize this potential and to truly understand the diversity and biology of these eukaryotes, continued development of scientific tools and techniques is essential. As a professional reference, this series will be very helpful to all people who work with fungi and useful both to academic institutions and research teams, as well as to teachers, and graduate and postgraduate students with its information on the continuous developments in fungal biology with the publication of each volume.

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Chapter 1
Overview of the Recent Advances in Mycorrhizal Fungi

Marcela C. Pagano and Vijai K. Gupta

1.1 Introduction

Since 1999 researchers have noted the increasing decline of populations and loss of species in all ecosystems (Spellerberg and Sawyer 1999). Today, in spite of more financial resources available to conservation, there is a need of prioritization of good decisions by decision makers and how to make better choices. It is known the urgency in increasing knowledge on geography and ecology of present world biodiversity in order to do the best uses of limited resources for conservation (Spellerberg and Sawyer 1999) is known. This will help to reduce human impact on ecosystems and to improve sustainable managements.

Agropecuary activity, lumber extraction, and urban development are factors that act in forestry fragmentation, particularly in margin of rivers, and coast. Only at this time we are truly recognizing the importance of conservation of river forest cover and that volume of water stored in soil and organisms living in the biosphere is bigger than superficial and aquifer recharge waters (Pinto-Coelho and Havens 2015). Shifts in available water together with climatic change have become constraints not only in countries with arid climate but also in countries such as Brazil, which suffer limitations in water supply (Pinto-Coelho and Havens 2015). Thus, governmental decisions in this country focus, for example, on agroecosystems less dependent on rainfall. Moreover, there is increased interest to study the tropical riparian forests that have been extremely impacted in their important ecological functions (Ribeiro et al. 2009; Pagano and Cabello 2012a). The use of native species for restoration of riparian environments is increasingly accepted and initial management studies

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revealed that native flora present Arbuscular mycorrhizal fungi (AMF) in their roots as well as high spore number in the different associated soils (Stürmer et al. 2012; Pagano and Cabello 2012a). For example, recent studies by Mardhiah et al. (2014) in islands along a 40-year chronosequence of the Tagliamento River, Italy, showed increased water stable aggregates with site age confirming the essential role of soil biota and plant roots in aggregating soils subjected to physical disturbances.

Nowadays a whole management approach to soils is mandatory as undisturbed soils are necessary for food but also for clean air and water (drinking water depends on maintaining soil properties) and to attain better human health (Wall and Six 2015). As part of the soil biota and colonizing plant roots, the arbuscular mycorrhizas (AM) link the biotic and geochemical components of the ecosystems providing more important ecosystem services. Research on Mycorrhizas has gone through several stages (Stürmer 2012); however, the present period has revolutionized research on these fungi.

Thus, the importance of AMF and ectomycorrhizas (ECM) for soil organic matter and soil carbon conservation is nowadays more recognized (Bradford 2014) and the association of trees with different mycorrhizal fungi is highlighted to understand the biotic interactions in carbon dynamics (Averill et al. 2014). Therefore, their preservation is highly recommended (Lalaymia et al. 2014). Moreover, some analysis pointed mycorrhizal and dark septate endophyte fungal symbioses as critical to predicting ecosystem response to global change (Kivlin et al. 2013).

This chapter discusses advances on mycorrhizal fungi potential drawing on recent results of research worldwide and with special attention to developments in Latin America. Studies in mycorrhizas have developed largely; however, research on ecology and biogeography is still limited. Moreover, the applications of mycorrhizas in environmental issues are still incipient. These limitations are also discussed.

1.2 The Mycorrhizal Symbioses

Microorganisms are increasingly investigated for novel compounds from saprophytic terrestrial fungi to marine habitats and living plants with their endophytes (Schueffler and Anke 2014). A growing worldwide attention on fungi is noticed, as of 100,000 known fungal species more than one million are predictable to exist (Schueffler and Anke 2014).

Among soil microorganisms, AMF perform symbioses with more than 80 % of all terrestrial plant species (Smith and Read 2008; Pagano 2012). Additionally, some aquatic and wetland plants can associate with AMF (see Pagano 2012). The mycorrhizal symbioses can consist in the internalization of one of the partners into the plant host but also in changing the symbiotic partner when a variation in environmental conditions occurs (Buscot 2015). Mycorrhizas are related to most environmental issues facing us today: deforestation, climate change, pollution, and introduced or invasive species (Pagano and Cabello 2012b; Bellgard and Williams 2011; Pagano 2013; Yang et al. 2014).

Arbuscular mycorrhizas link the biotic and geochemical components of the ecosystem providing more than six ecosystem services: increasing plant/soil adherence and stability (the extra radical mycelial web improves rooting and plant establishment), promoting plant growth (reducing fertilizer requirement), increasing plant resistance to stresses (drought, salinity, heavy metals, and mineral nutrient exhaustion), increasing soil stability, structure, and water retention (through the secretion of glomalin and growth of the mycelial web), enhanced plant resistance against biotic stresses, bioregulation of vegetal development and better plant quality for human health (Modification of plant physiology), and increasing plant community diversity (van der Heijden et al. 1998; Gianinazzi et al. 2010).

It could be said that many regions remain unsampled for AMF and characterization of AMF life history and the community dynamics is yet little studied (Morton et al. 2004). For example, the consequences of AMF in the progress of plant invasions were poorly studied. Some works (Yang et al. 2014) tested the mycorrhization of invasive plants (*Solidago Canadensis*), showing changes in AMF species composition associated with native and exotic plant species and shifts in plant biomass and nutrient uptake. The authors discard allelopathic effects and highlight that altered AMF facilitated the invasion of this plant species. Lekberg et al. (2013) also noted that exotic mycotrophic plants can replace poorer native flora and suggested that AMF can respond more to plant functional group than plant provenance.

Some fungi are very attractive for use in mycoremediation due to their lignolytic enzymes that can degrade chemical pollutants (fossil fuels, wood, solid wastes) in the environment (Karaman et al. 2012). Various projects aiming at the restoration of degraded ecosystems or afforestation in semiarid areas and that have used mycorrhizal associations were located in Mexico (Allen et al. 1998; Carrillo-Garcia et al. 1999; Corkidi and Rincón 1997; Monroy-Ata et al. 2007) and in Spain (Lozano et al. 2014). For ecological restoration, the effect of climate change on species distribution must be taken into account (Gastón and García-Viñas 2013; Harris et al. 2006). If the selected species cannot tolerate the environmental modifications predicted under climate change, the restoration strategy will not work in the mid and long term. To date, only historical and current climatic conditions are taken into consideration in establishing the necessary requirements for restoration projects (Ravenscroft et al. 2010). Recently, Gelviz-Gelvez et al. (2015) showed eight potential plant species for use in ecological restoration projects in the semiarid region of Central Mexico. They based on suitable ecological attributes for restoration (cover, abundance, sociability, mycorrhization) and tolerance to projected future climate changes. Several species that would not tolerate environmental modifications caused by climate change will be inappropriate for use in long-term ecological restoration plans.

Increased interest on global change has led to a higher number of reports. With regard to climate change, it has been demonstrated that reduction in precipitation reduces crop production (Sanchez et al. 2001), increases weed invasibility (Kreyling

Table 1.1 Number of reports dealing with AMF/ECM worldwide

Topic	Number of reports
AMF	7573
ECM	2815
AMF and climate change	104
AMF and pesticides	93
AMF and invasive species	58
AMF and ECM	7

Database survey conducted on April 2015 (SCOPUS); *AMF*, arbuscular mycorrhizal fungi; *ECM*, ectomycorrhizas

et al. 2008), and reduces the mycorrhization rates (Augé 2001; Smith and Read 2008). Moreover, different AMF communities can be found in degraded vs. native forest as well as in different tree plantations, crops, and vegetation types (see Pagano and Dhar 2014), as generally these fungi can occur in preferred associations with plant species (Helgason et al. 2002; Li et al. 2010; Torrecillas et al. 2012). Moreover, the impacts of herbicide-resistant weeds on soil biota of crop production fields are not well known (Kremer 2014).

Studies of the interactions between biological, physical, and chemical soil attributes and mycorrhizas remain scarce and are especially lacking in the context of long-term forest recovery and ecological restoration. Also studies on ecology and biogeography and the applications of mycorrhizas in agriculture and environmental issues are limited. Increasingly studies compare seminatural savanna, recovered savanna, and agroecosystem, in both dry and wet periods (Furrazola et al. 2015).

Of the over 7573 published references for AMF found in our Google Scholar search (Table 1.1), only 104, less than 2 %, investigated the effect of climate change on these fungi or the relations with pesticides, and less than 1 % regarded invasive plant species. Of over 2815 reports on ECM less than 3 % were related to climate change. Moreover, only seven reports included both AMF and ECM.

Additionally, with few exceptions, most studies assessed mycorrhizas in only one ecosystem type and generally at two seasons. Many plant species and different vegetational types in tropical regions including semiarid environments were found associated with AMF (Pagano et al. 2013; Silva et al. 2014) and this symbiosis requires to be understood under global change. Increasing interest on crops, specially corn, citrus, and coffee, and the soil biota enhancing agricultural sustainability is noted.

Among recent reviews, only three included ECM (Table 1.2). However, increased interest on ECM due to the use of nitrogen from soil organic matter that is otherwise unavailable to plants, whereas AM fungi, like roots, primarily take up inorganic nitrogen from the soil (Bradford 2014) is noted. This is due to the fact that studies have revealed that ecosystems dominated by trees that associate with

Table 1.2 Some recent reviews and book chapters dealing with AMF and ECM

Reports	References
Evolution and diversity in AMF and ECM	Buscot (2015)
Microbial inoculants in agriculture	Velivelli et al. (2015)
Ectomycorrhizal fungi as potential organic matter decomposers	Lindahl and Tunlid (2015)
Function in AMF symbioses	Bucher et al. (2014)
Mycorrhizal fungi and ecosystem responses to Global change	Mohan et al. (2014)
Implications of herbicide resistance and AMF	Kremer (2014)
Mycorrhizal and dark septate endophyte fungi and Global change	Kivlin et al. (2013)
Ecology of AMF	Willis et al. (2013)
Mycorrhiza as a biotechnological tool for improving orchid growth	Hossain et al. (2013)
Maintenance and preservation of ECM and AMF	Lalaymia et al. (2014)
Diversity of AMF in special habitats	Li et al. (2013)
AMF-induced tolerance to drought stress in citrus	Wu et al. (2013)
History of the taxonomy and systematics of AMF	Stürmer (2012)
Role of AMF in ecosystem services and Agroecology	Gianinazzi et al. (2010)
AMF technology in floriculture	Koltai (2010)
AMF association in coffee	Andrade et al. (2009)
Summary on AMF	Morton et al. (2004)

ECM store more carbon per unit of nitrogen than plants associated with AMF (Averill et al. 2014).

The specific benefic role of AMF has received attention for cultivation of floricultural species. Some efforts concentrating towards the integration of AMF technology in floriculture practices to increase plant yield and the employment of commercial inocula in floriculture (with little success) were compiled by Koltai (2010).

1.3 Natural Environments

1.3.1 Spore Abundance and Diversity

AMF biodiversity in natural environments is typically higher than in agricultural systems, because of more plant diversity and a more complex habitat that can support a wider variety of organisms. However, it depends on the biome, vegetation type, and site (Douds and Millner 1999). Usually a universal pattern of AMF distribution (some abundant AMF species and rare species) is found, but dominant AMF can change with time (seasonal variations and succession) (Buscot 2015).

AMF spores (singly, in clusters or in sporocarps) are generally found in the soil and rhizosphere (some spores can be found within roots). Aggregate distribution is commonly found: thus some samples can present more spores than others. Therefore, sampling effort and strategy can affect our vision of AMF communities. For that reason Whitcomb and Stutz (2007) have suggested collecting 15 samples to detect ~80 % of AMF species present in one plot. They studied an experimental site associated with Central Arizona—Phoenix Long-Term Ecological Research at the Desert Botanical Garden in Phoenix, Arizona.

Nevertheless, sometimes it is very difficult to collect or analyze that amount of samples and the use of taxon accumulation curves is recommended to evaluate AMF diversity (Whitcomb and Stutz 2007). For example, if composite samples with little quantities of soils will be analyzed, we need to use less soil quantity (such as 10 or 20 g) to analyze the AMF abundances. Sometimes, few AMF species can be observed with one of them dominating, such as in one soil sample from Pará State, Brazil, where M. Pagano (unpublished) retrieved only one dominant species, *Acaulospora excavata*, at higher abundance (>4 spores g^{-1} soil). Examples are shown by Pagano et al. (2013) who distinguished 32 AMF in semiarid vegetation types in Brazil, recovering ~72 % of the estimated AMF species richness for woody caatinga vegetation (unpublished results). Accumulation curves of AMF species found in the studies and the estimated richness based on the first-order Jackknife index (Jackknife 1) are compared in order to indicate the number of analyzed samples sufficient to detect the majority of AMF species present in the area. Furthermore, Silva et al. (2014) recovered a similar percent of the AMF richness in semiarid region of Brazil. Coutinho et al. (2015) reported a higher recovery of the total estimated richness through an altitude gradient in the rocky soils of the Serra do Cipó, Brazil.

Distributions of AMF spores vary both spatially and temporally within and between host plants and with soil types. In natural vegetation spore numbers can vary from 1 to 20 g^{-1} dry soil (Lugo et al. 2008; Matias et al. 2009; Becerra et al. 2009; Velázquez and Cabello 2011; Velázquez et al. 2013; Carvalho et al. 2012; Soteras et al. 2012; Pagano et al. 2013; Freitas et al. 2014; Coutinho et al. 2015). However, some spores can be counted as fresh and be nonviable, especially those of *Glomus*.

Increasing works on pristine sites show higher AMF occurrence at intermediate compared to low altitudes while decreasing values at > 1100 m of altitude (Coutinho et al. 2015). Previous reports from Pampa de Achala (2250 m above sea level) in Sierras de Córdoba, Argentina, mentioned a total number of spores ranged between 0.08 and 40 spores mL^{-1} soil (Lugo and Cabello 2002). In general, at elevated altitudes, higher values of spore abundances are found in the dry season which decreased in the wet period (Lugo and Cabello 2002). Oehl et al. (2006) found new AMF *Acaulospora alpina* in grasslands of the Swiss Alps at altitudes between 1800 and 2700 m above sea level.

It was reported that more than 20–25 taxa reported in field studies rarely occur (Willis et al. 2013). However, recent advances in spore identification and AMF classifications (which have increased the number of AMF species to 270, http://glomeromycota.wix.com/lbmicorrizas) have changed this picture.

Moreover, new AM species are described continuously, such as *Acaulospora entreriana*, retrieved from rhizosphere of native plant species from scrublands, grassland, and palm forest dominated by *Butia yatay* in Argentina (Velazquez et al. 2008, 2010) or *Septoglomus furcatum*, from rhizosphere of native tree species in Brazil (Błaszkowski et al. 2013), to name just a few. Therefore, new reports show increased number of AMF species. For example, Freitas et al. (2014) have identified 41 spore morphotypes in rhizosphere of three legume trees in Amazonian forest. Carvalho et al. (2012) reported 49 AMF species for six different natural habitats (highland fields, bogs, cerrado, etc.) and Silva et al. (2014) 50 AMF species for three semiarid sites (a dry forest, a transitional zone, and a moist forest).

Spores of AMF show seasonal patterns of abundance in natural environments: spores are greatest in the autumn (for Northern hemisphere) in areas where there are marked warm/cold seasons and lowest during the growing season. Thus, collecting spores in the autumn (or dry period), after they are produced, helps their identification (Douds and Millner 1999).

In Amazonian forest, Freitas et al. (2014) studied spores in the rhizosphere of three mycotrophic tree Leguminosae. They found that soil characteristics and plant host identity were responsible for affecting AMF communities. They observed less variation of species composition, showing that dominant AMF species can be recruited by plant communities. In Argentina, earlier studies coordinated by M. Cabello (Velazquez et al. 2008, 2010, 2013) in preserved areas such as El Palmar National Park, Entre Ríos province, reported 46 AMF species for five vegetation types.

In more stressed environments such as highlands and high altitude mountain ranges which have severe environmental conditions, we can find less AMF diversity. For example, Lugo and Cabello (2002) reported 17 AMF species from natural mountain grasslands at 2250 m above sea level, in Sierras de Córdoba, Argentina. In Natural saline soils of Central Argentina, Soteras et al. (2012) investigated the rhizosphere of the halophyte *Atriplex lampa* in two environments retrieving 18 AMF species. In Puna Grassland highland limited by the Cordillera Real to the east and the Andes to the west, varying in altitude from 2000 to 4400 m above sea level, Lugo et al. (2008) observed low species richness (10 AMF taxa). In rupestrian fields, Brazil, Pagano and Scotti (2009) and Pagano et al. (2010) also observed low AMF diversity (6 species) in some native plant species; however, few plant individuals were analyzed. However, 51 AMF species were reported (including 14 species possibly new to science and mostly from *Glomus* followed by *Acaulospora*) in four different vegetation types (Coutinho et al. 2015).

1.3.2 Root Colonization

In tropical climates, higher root colonization is generally found during the rainy season. Usually, the colonization rate increased in the rainy period (Allen et al. 1998; Silva Junior and Cardoso 2006; Pagano et al. 2009), which may be related to the improvement of soluble nutrients and to a higher root activity in that season. However, in some vegetation types, such as the thorny dry woody savanna (caatinga) in Brazilian tropical semiarid zone, this pattern is not clearly followed (Pagano et al. 2013). This can be due to the short and sporadic rainy season when leaves, flowers, and the herbaceous vegetation grow (Rizzini 1997). Moreover, rates of root colonization from 2 to 79 % can be found depending on the plant host species, such as in rupestrian fields, Brazil, where Pagano and Scotti (2009) and Pagano et al. (2010) also observed high root colonization (>25 %) in some native plant species.

Additionally, in different stages of forest succession spores can increase in the rainy period compared to native forest area where sporulation cannot differ (Bonfim et al. 2013). Pagano et al. (2012) found a higher sporulation in managed forest in the rainy period. In the ECM symbiosis colonization rates usually increase in the rainy period (Pagano and Scotti 2008; Pagano et al. 2009). ECM fungi are particularly dominant in ecosystems where plants are limited by N (Read 1991), but elevated levels of N from fertilizers inhibit growth of external hyphae and fruit bodies (Olsson et al. 2003).

In the studies by Pagano (2012) on the AM communities in the Brazilian dry forest and in riparian forest restoration experiments, the choice of tree species for restoration purposes seems to have great implications on the conservation of AMF species; thus, highly dependent tree hosts could be selected over mycorrhizal-independent hosts. Researches by Urcelay, dedicated to study the Chaco Forest (South America's dry seasonal forest), in Argentina, showed that fire occurrence negatively affects AMF communities mainly as direct effects of fire on soil fungi (Longo et al. 2014).

In most studies, the *Arum*-type pattern of colonization (Dickson et al. 2007) was seen to be dominant for woody trees (Pagano 2012); however, sometimes both *Arum* and *Paris* types can be found indistinctly (Rodríguez-Rodríguez et al. 2013), but it depends on the host species, as Fernández et al. (2012) reported only Paris type for mycorrhizal species of pteridophytes. It is well known that the *Arum* type is formed in most plants that usually grow in sunlight, and that the spreading rate of colonization is faster in this type of colonization (Brundrett and Kendrick 1990). However, other environmental factors such as temperature and soil moisture content may model AM morphology in roots (Cavagnaro et al. 2001). In general, vesicles and hyphae are the most common AM structures observed in the plant roots. It has been shown that storage vesicles appear at later stages of colonization, being important contributors to carbon drain from the plant (Jakobsen et al. 2003), as well as propagules (Smith and Read 2008). Commonly, septate hyphae and microsclerotium of dark septate endophytic fungi can be found during root examinations (M. Pagano, personal observations; Fernández et al. 2012).

Commonly, higher AM colonization rate of plants occurs in mixed plantation, compared to monocultures, and increased intensity of root colonization of native host can be attained even if the native species are mixed with exotic *Eucalyptus species* (Pagano et al. 2009, 2012).

Today, a higher number of reports informed about AMF diversity and gradually this information is able to support decisions for ecosystem conservation. Some studies did examine mycorrhizal and ecosystem responses in multiple locations within