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REVIEW

Arbuscular mycorrhizal association in coffee

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SUMMARY

Despite previous research on mycorrhizal association with plants, the data on associations with coffee (*Coffea* species) are very sparse despite the great economic importance of this crop for many tropical developing countries. The present paper reviews the main aspects of the association between arbuscular mycorrhizal fungi (AMF) and coffee plants. This review includes topics on mycorrhizal effects on coffee nutritional status, pathogen–AMF interactions and responses to several environmental stresses. It also summarizes findings about the natural occurrence of AMF in different soils in which coffee is cultivated, some ecological aspects of this specific association and outlines trends for future investigations, which must elucidate the real benefits of mycorrhizae to coffee plants.

INTRODUCTION

Coffee (*Coffea* spp.) has worldwide economic importance, is cultivated in more than 70 countries and represents one of the most heavily traded commodities in the modern world, second only to oil. Coffee represents a significant source of income for several developing countries in Africa, Asia and Latin America, directly or indirectly generating millions of jobs in those countries. The genus *Coffea* belongs to the Rubiaceae family, which includes about 100 species, all native to Africa, Madagascar and Mascarenes (Davis *et al.* 2006). However, only two species are commercially cultivated, *Coffea arabica* L. and *Coffea canephora* Pierre. In some African countries, the wild species *Coffea racemosa* Lour and *Coffea liberica* Hiern are cultivated at a small scale for local consumption.

On the other hand, mycorrhizae are the most common symbiotic species on earth, with arbuscular mycorrhizae (AM) being the most frequent type, occurring in about 0·80 of plant species and in almost all ecosystems (Strack *et al.* 2003). There are more than 150 species of arbuscular mycorrhizal fungi (AMF), which belong to the phylum Glomeromycota (Schüßler *et al.* 2001). The main AM benefit for plants is an increase in nutrient uptake, particularly phosphorus. This effect is a consequence of higher soil volume exploration by mycorrhizal roots via fungal extra-radical hyphae (Scheneiger & Jakobsen 2000). The AM association also has a positive effect on plants under drought conditions (Augé 2001, 2004) and it may protect plants against several pathogens (Vaast *et al.* 1998; Elsen *et al.* 2003). In addition, AMF improve soil structure and aggregation through the mechanical effects exerted by extra-radical hyphae or through hyphal glycoprotein exudation (Rillig & Mummey 2006). Thus, in spite of the fact that conventional agriculture regards AMF as biological agents of minor importance (Ryan & Graham 2002), AM association is considered an important component in sustainable agriculture (Jeffries & Barea 2001).

The presence of AM in coffee plants was first observed by Janse (1897), who found highly mycorrhizal coffee roots from Java Island. Since then, several studies have verified the occurrence and importance of AM symbiosis in coffee, especially in highly weathered and low-fertility soils (Siqueira *et al.* 1998) such as those from many tropical regions where this crop is cultivated. In addition, coffee has often been considered to have high mycorrhizal dependency, especially during the seedling formation stage (Sieverding & Toro 1986; Siqueira *et al.* 1993; Habte & Bittenbender 1999). Thus, the positive effects of

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AM on coffee seedling development and its prolonged benefits after transplantation in fields have received particular attention (Siqueira *et al.* 1998).

Most of the studies carried out on AM in coffee have been published in scientific journals that are often not easily accessible to the scientific community interested in this subject. Therefore, the aim of the present review is to outline the knowledge regarding AM in coffee. This includes topics on mycorrhizal effects on coffee nutritional status, pathogen–AMF interactions and responses to several environmental stresses, findings about the natural occurrence of AMF in different soils cultivated with coffee and some ecological aspects of this specific association. Trends for future investigations are also outlined, which must elucidate the real benefits of mycorrhizae to coffee plants.

ARBUSCULAR MYCORRHIZA OCCURRENCE IN *COFFEA*

Like many crops, coffee associates symbiotically with AMF (Sieverding 1991). Numerous studies have shown the natural occurrence of AMF in the soils of coffee orchards, as well as the presence of mycorrhizal structures in coffee roots (Lopes *et al.* 1983; Balota & Lopes 1996; Pavan *et al.* 1999; Colozzi-Filho & Cardoso 2000; Theodoro *et al.* 2003; Muleta *et al.* 2007). Lopes *et al.* (1983) identified 22 AMF species in coffee rhizosphere soil from a Brazilian coffee production region; the most frequent AMF genera found were *Acaulospora* and *Glomus*. These were also the predominant genera found in other coffee-cultivated soils in Venezuela, Colombia and Mexico (Riess & Sanvito 1985; Toro-Garcia 1987; Cruz 1989). Other AMF genera, such as *Scutellospora*, *Gigaspora* and *Sclerocystis*, have also been described in different coffee orchard soils (Colozzi-Filho & Cardoso 2000). Recently, in a native forest in Ethiopia, where coffee coexists with other trees in its original ecosystem, high AMF species richness has been reported, with representatives of five genera of AMF. *Glomus* was the dominant genera, followed by *Gigaspora*, *Acaulospora*, *Entrophospora* and *Scutellospora* (Muleta *et al.* 2007).

Nevertheless, it is difficult to carry out taxonomic identification in ecological studies because of the low number of researchers in this area and the long training and experience necessary to be able to distinguish different AMF species. However, molecular techniques have helped to identify some AMF species occurring in soils or in plant roots (Rivillas & Dodd 1996; Tisserant *et al.* 1998; Colozzi-Filho & Cardoso 2000). Specific polymerase chain reaction (PCR) primers are being designed for different AMF to facilitate detection of symbionts within roots (Redecker 2000). In addition, the determination of some isozymes that occur only when AMF colonize roots has allowed for the identification of AMF in coffee, as well as some tropical legume mycorrhizae (Rivillas & Dodd 1996; Tisserant *et al.* 1998). Even in poorly colonized coffee roots, a mycorrhiza-specific isozyme of *Acaulospora tuberculata* BEG41 has been found, indicating the high sensitivity of this technique in discriminating specific mycorrhizal associations (Dodd *et al.* 2000).

The occurrence of different AMF genera and species in coffee soils and roots varies depending on several factors, such as edapho-climatic conditions and culture practices. For example, in some tropical and subtropical regions, climatic seasons are divided into rainy and dry seasons, as the amount of precipitation may vary dramatically. These changes may influence mycorrhizal colonization, as observed in coffee orchards in Brazil, with low colonization rates in the dry season that increase at the beginning of the rainy season (Saggin-Junior & Siqueira 1996). However, the great range in the proportion of adult coffee plants with mycorrhizae, varying from proportions as low as 0·04 to more than 0·80, indicates the existence of different environmental factors that influence colonization in the field: including different native AMF populations, soil inocula and cultural practices (Saggin-Junior & Siqueira 1996).

Crop age may also influence coffee mycorrhizal colonization, both positively, as already observed in coffee-shaded orchards in Colombia, or negatively, as in monocropping orchards in Brazil, where this response was related to crop sustainability (Saggin-Junior & Siqueira 1996) and to P requirements of coffee plants of different ages (Siqueira *et al.* 1998; see below). Crop management and culture practices, such as liming, legume intercropping, organic management and monocropping, among many others, can alter a soil's physicochemical and biological characteristics and may thus greatly influence AMF diversity and abundance in soils. Liming, for example, usually influences AM colonization positively because of its effects on spore germination by eliminating fungistatic factors and by influencing the composition of AMF populations (Siqueira *et al.* 1984).

In some areas of Brazil, coffee has been cultivated with high-density populations per unit area, increasing productivity and avoiding soil erosion (Pavan *et al.* 1999). This cultivation system has been compared with traditional low-density cultivation, which favours soil erosion and contributes to organic matter and nutrient losses. Thus, it has been verified that high densities increase exchangeable Ca, Mg, K, extractable P, pH, moisture content and AM colonization of roots, thereby improving soil fertility (Pavan *et al.* 1999).

Agroforestry systems, based on the cultivation of crops of interest under a diversity of shade trees, are recognized to reduce land degradation and increase nutrient availability (Young 1997). Additional advantages of these systems are derived from a suppression of coffee pests, such as couch grass (*Digitaria salarum*), temperature regulation and maintenance of diversity (Muschler 2001; DaMatta 2004). Agroforestry coffee-growing systems are, in fact, a culture practice adopted in several countries, such as Colombia, Mexico and several countries in Central America. Cardoso *et al.* (2003) investigated the vertical distribution of AMF spores under agroforestry (shaded) and monoculture coffee (unshaded) systems. Greater numbers of AMF spores were found in the deep soil layers of agroforestry systems than in monocultural coffee plantation soils. Cardoso *et al.* (2003) suggested that the higher concentration of AMF spores was related to higher mycorrhiza occurrence at those soil depths, which may be important to increase P availability to plants and to improve the efficiency of P cycling processes in these coffee systems.

In a natural coffee forest in Ethiopia, the diversity of AMF spores in relation to the composition of shade tree species has been investigated recently and markedly higher densities of AMF spores were observed under leguminous shade trees compared with non-leguminous ones (Muleta *et al.* 2007). The species of shade tree also had an influence on the distribution of AMF genera in coffee rhizospheres, with *Acaulospora* occurring more frequently under leguminous trees than under other shade tree families. The same authors found that nearly 0·95 of the collected coffee roots under the influence of different shade tree species were associated with two or more AMF genera, with *Glomus*, *Gigaspora* and *Acaulospora* being predominant.

NUTRITIONAL EFFECTS OF ARBUSCULAR MYCORRHIZA IN COFFEE

As with many other agronomic crops, AM growth promotion in coffee plants has been mainly attributed to the nutritional effects of the symbiosis (Siqueira *et al.* 1998; Bhattacharya & Bagyaraj 2002; Sanchez *et al.* 2005). Improvements in plant mineral nutrition are mainly related to uptake by extra-radical hyphae from the non-rhizosphere soil region and nutrient transport to the plant root (Schweiger & Jakobsen 2000). After N, P is the most frequently limiting macronutrient for plant growth and is needed in millimolar concentrations in the cellular environment. In order to meet this requirement, and considering the soil P-depleted areas that form around the roots, plants rely upon several mechanisms, such as high affinity transporters, the release of phosphatases and organic acids and association with AMF (Requena 2005), which involves a highly regulated route for P exchange between plants and fungal symbionts (Poulsen *et al.* 2005). Thus, P is undoubtedly the most important nutrient taken up by AMF, which is reflected in the large number of reports focused on the improved P status of AM plants; this situation is no different for coffee. However, the uptake of nutrients other than P, such as N, Ca, Mg, Fe, Mn, Zn and Cu, is also influenced by AM (Clark & Zeto 2000). Sanchez *et al.* (2005) found a positive effect of AMF inoculation on coffee P concentrations, which resulted in higher growth when compared with non-inoculated plants. However, depending on the AMF species inoculated, different responses were observed, with *Glomus fasciculatum*, *Glomus mosseae* and *Glomus intraradices* being the most efficient symbionts. In three typical coffee-cultivated soils in Costa Rica, Sanchez *et al.* (2000) observed that as soil fertility decreased, AMF inoculation increased efficiency in terms of growth. In this context, AM growth promotion was inversely related to soil P availability, with diminished positive effects as P availability increased. However, plant mycorrhizal dependency and symbiotic efficiency are directly influenced by soil P availability (Colozzi-Filho *et al.* 1994; Saggin-Junior & Siqueira 1995), making the degree of availability of adequate soil P a very important factor in the establishment of efficient mycorrhizal associations.

Osório *et al.* (2002) evaluated the effects of mycorrhizal inoculation and organic amendment on *C. arabica* seedling growth. Plant growth was improved by organic amendment and was further increased by mycorrhizal inoculation, but it was severely reduced in the unamended soil, which had low organic matter content, low pH, high extractable Al and low availability of P, B, Ca, Mg and K. Vaast & Zasoski (1992) observed higher N, Ca and Mg accumulations in mycorrhizal coffee plants, which as a result showed better growth than non-mycorrhizal plants.

The beneficial effects of AM association on plant nutrition have also been observed in coffee plants propagated *in vitro* (Vaast *et al.* 1996), where the association enhanced root and shoot growth of seedlings and plant P status, resulting in a lower root-to-shoot ratio when compared with non-mycorrhizal plants. Vaast *et al.* (1996) concluded that different AMF species presented different tolerances to increasing soil P availability, which were related to host foliar P status. The same happened for AMF pre-colonized coffee seedlings, which showed better development and a higher bean yield when transplanted into a low-fertility soil when compared with plants that were not pre-colonized (Siqueira *et al.* 1998). In this highly weathered soil, mycorrhizal inoculation and P addition were complementary. However, the consistent effects of mycorrhiza on plant development and productivity diminished with crop age. These variable responses complicate the understanding of mycorrhizal effects on field crops, as many biological and chemical factors influence the coffee–mycorrhizal association. For example, as commented upon by Siqueira *et al.* (1998), young perennial plants have a high P requirement but this diminishes with plant age, explaining the importance of AMF inoculation during the nursery stage and in recently transplanted plants. In contrast, adult crops do not have the same requirement for P supplies as they do for other mineral nutrients that may represent limiting factors for coffee productivity.

Long-term P addition or high P input into soils has been related to the suppression of AM development, which may in turn reduce the uptake of Zn ions and of other nutrients by plants (Alloway 2004), decreasing the benefits of AM association. Phosphorus-induced Zn deficiency due to decreases in AM colonization has already been observed in coffee plants (Vaast 1995; Vaast *et al.* 1996). On the other hand, increases in AM colonization resulted in higher foliar Zn concentrations, suggesting a correlation between AM colonization rates and Zn nutrition in coffee plants.

NON-NUTRITIONAL EFFECTS OF MYCORRHIZA

Besides positive nutritional effects, AM are known to increase resistance to drought and salinity and to increase tolerance to pathogens. It is recognized that AMF can influence the water balance of highly watered and dehydrated plants (Augé 2001). The increase in root water uptake in mycorrhizal plants has been associated with an improvement in root conductance of water flow (Augé 2001), including increases in the root length and alterations of the root morphology (Davies *et al.* 1996). In addition, when compared with non-mycorrhizal plants, mycorrhizal ones had higher stomatal conductance and transpiration rates, in addition to higher amounts of carbon fixed during water stress (Duan *et al.* 1996), resulting in changes in leaf physiology and in intrinsic hydraulic or biochemical properties (Augé 2001). More recently, it has been shown that root colonization by AMF enhances gene expression of tonoplast-localized aquaporins, transmembrane proteins that facilitate the membrane water potential gradient, thus improving the efficiency of mycorrhizal plants under drought conditions (Ruiz-Lozano 2003; Porcel *et al.* 2005). While these studies were not performed with coffee plants, similar mechanisms might be suggested to enhance mycorrhizal coffee resistance under water deficit conditions.

In addition to the well-addressed AMF effects on plant physiology, other ecological processes have been attributed to mycorrhizal fungi, including their contributions to soil structure (reviewed by Rillig & Mummey 2006), which is of great relevance for agroecosystem sustainability. AMF influence soil particle aggregation through biochemical, biophysical and biological processes, which include the mechanical action of fungal hyphae, the excretion of glycoproteins and other extracellular compounds, and AMF interactions with soil biota.

COFFEE PERFORMANCE UNDER DIFFERENT ENVIRONMENTAL STRESSES: THE ROLE OF MYCORRHIZAE

Coffee plants, as with other species and organisms, are subjected to numerous environmental stresses that may affect physiological processes. However, several plant mechanisms exist to counteract any possible negative effects on their metabolism. It is largely recognized that AM association is crucial in the ecology and physiology of many terrestrial plants, helping them to cope with several environmental stresses, such as drought, high metal concentrations, salinity and temperature stresses.

According to DaMatta (2004), world production of coffee beans is predicted to decrease dramatically, mainly due to unfavourable climatic conditions; in particular, drought is considered one of the main environmental stresses affecting coffee yield. Water limitation in coffee may cause physiological injuries, leading to the accumulation of different metabolites, such as proline (Mazzafera & Teixeira 1989).

The spread of coffee cultivation to marginal lands with unfavourable climatic conditions, together with global climatic changes, may constrain coffee yields and quality (Silva *et al.* 2004); as such, drought and extreme temperatures are the main climatic limitations for expected commercial coffee production, as reviewed by DaMatta & Ramalho (2006). Those authors examined the importance and consequences of drought and temperature stress on coffee physiology and production. In this context, what is the role of mycorrhizae? The present authors found no reports that have studied the influence of mycorrhiza on coffee performance under different water availability or temperature conditions. Nevertheless, it can be inferred that efficient AMF inoculation may improve coffee development since mycorrhizal symbioses have resulted in better water relations for other important crop species, which in turn led to increased plant growth (Augé 2001; Porcel *et al.* 2005).

Soil salinity is a widespread problem restricting plant growth, especially in arid, semiarid and tropical areas. The coffee crop is expanding to new areas, until recently considered to be marginal or unsuitable for its cultivation, and it has frequently been necessary to provide additional irrigation in these regions. In addition, the lack of quality irrigation water in arid and semiarid areas, as well as in other areas with adequate climatic condition for coffee cultivation, sometimes contributes to saline/osmotic stress (Camargo 1987). On this topic, some studies have shown that coffee seedlings are highly susceptible to salinity, leading to death after exposure to water salinity levels from 1·5 to 6·0 dS/m (Karasawa *et al.* 2000). Nevertheless, older coffee seedlings coped well with salinity, since they are not subjected to excessive saline water (up to 2·0 dS/m) and adequate rain distribution occurs throughout the year (Matiello 1999). The present authors found no reports linking mycorrhizal effects to coffee performance under saline stress, but some workers have reported the presence of AM plants in salt stress environments (Ruiz-Lozano & Azcon 2000; Aliasgharzadeh *et al.* 2001). In those studies, AMF prevented leaf dehydration caused by salinity and drought stress (Aroca *et al.* 2007) and, under control conditions, root hydraulic conductance in AM plants was about half that in non-mycorrhizal plants. The reduction in Na uptake, together with concomitant increases in P, N and Mg absorption and higher chlorophyll content in mycorrhizal plants, was suggested as important salt-alleviating mechanisms for plants growing in saline soils (Giri & Mukerji 2004).

Some agricultural systems used for coffee cropping, such as monoculture, low planting density or the use of modern machinery in fields, may lead to soil compaction and soil degradation processes. The stressful effects of soil compaction result in the degradation of soil structure, reduced water potential, increased soil erosion and reduced root growth, which decrease plant growth and yields. AMF inoculation may to some extent improve plant growth (Miransari *et al.* 2007), mainly by increasing the root surface area through production of extra-radical hyphae.

The concentrations of heavy metals and other trace elements are increasing in soils due to human activities, threatening the correct functioning of the ecosystems. Metal toxicity in coffee, as is the case for other plant species, causes oxidative stress (Gomes-Junior *et al.* 2006*a*, *b*), which may lead to cellular damage and impaired function, resulting in growth and biochemical alterations (Mazzafera 1998) and excessive accumulation of metals in tissues. In the specific case of coffee soils, pesticides, fertilizers or organic amendments are the most frequent exogenous sources of metals or chemical toxicity. In addition, soils with naturally high acidity and aluminium concentrations are frequent in tropical and subtropical soils. In this respect, Al toxicity affects the normal growth of coffee shoots and roots and diminishes P, Ca and Mn foliar concentrations (Pavan & Bingham 1982). The protective effects of AM against excessive concentrations of Al have been shown by some authors (Cuenca *et al.* 2001; Konrad 2003), suggesting that the higher P uptake by mycorrhizal roots increases intracellular P concentrations, thus decreasing Al solubility by precipitation. Agricultural soils in Costa Rica (Wilcke *et al.* 2000) and Kenya (Lepp *et al.* 1984), and probably those from many other coffee-producing countries, have been successively amended with high application rates of Cu-containing fungicides, extensively used in coffee orchards for disease control, resulting in Cu accumulation in different soil horizons. Sewage sludge application in coffee orchards as a plant fertilizer and soil conditioner is becoming a frequent agricultural practice and a viable solution for sludge disposal (Martins *et al.* 2005). Unfortunately, depending on the origin of the sludge, it may be a source of metal accumulation in soils. Metal accumulation in soils may lead to the transfer of these heavy metals to coffee plants, causing physiological disorders or excessive metal accumulation (Dickinson *et al.* 1988; Loland & Singh 2004) that could enter the food chain via agricultural products. Several studies have addressed the role of AMF in altering the capacity of plants to accumulate heavy metals and trace elements, as well as the functioning of plants under contaminated soil conditions (Rivera-Becerril *et al.* 2002; Andrade *et al.* 2004, 2007).

PATHOGEN–ARBUSCULAR MYCORRHIZA INTERACTIONS IN COFFEE

Plant-parasitic nematodes associated with coffee planting stocks in coffee nurseries and orchards include root-knot (*Meloidogyne* spp.) and root-lesion (*Pratilenchus* spp.). Coffee infection by nematodes causes reductions in plant growth and coffee bean yield, leading to important production losses ranging from 15 to 60% (Campos *et al.* 1985). Sustainable agriculture is characterized by low agro-chemical inputs for crop production systems, and plant–microbe interactions have been considered in order to promote plant nutrition and fitness. In this sense, mycorrhizal inoculation may benefit a plant's ability to resist biotic stressors by conferring a certain degree of tolerance and ability to withstand pathogenic attack (Azcón-Aguilar & Barea 1996). AMF association with plant roots may increase P uptake by plants and compete for penetration sites and nutrients with soil-borne pathogens, decreasing the deleterious effects of root pathogens (Azcón-Aguilar & Barea 1996).

Baumann (2006) speculated about the possible role of caffeine exudation by coffee roots in the establishment and 'condition' of an adequate seedling rhizosphere for plant development, facilitating mycorrhiza formation. In this way, coffee plants would be more prepared to withstand soil-borne pathogens, such as nematodes. The recently discovered decaffeinated coffee plant would be a suitable plant material to test this suggestion (Silvarolla *et al.* 2004), as caffeine is absent in the whole plant (Silvarolla & Mazzafera, unpublished results).

Some work has shown that *Gigaspora margarita* or P supply increased coffee tolerance to *Meloidogyne incognita*, diminishing damages in fine root production (Lopes *et al.* 1987) or, independently of the added P, promoting seedling growth and improving mineral nutrition (Lana *et al.* 1991; Vaast 1995). Coffee plants with well-established mycorrhizal symbioses and improved P status had enhanced tolerance to *Pratylenchus coffeae*, and it was observed that AM inoculation limited the extent of nematode lesions (Vaast *et al.* 1998). The protective effects of AMF inoculation against nematodes have been described in other important cultures, such as olive trees, banana and peach-almond (Calvet *et al.* 2001; Elsen *et al.* 2003; Castillo *et al.* 2006). In olive plants, mycorrhizal inoculation significantly reduced root galling severity resulting from root infections by *Meloidogyne incognita* and *Meloidogyne javanica* and also reduced nematode reproduction (Castillo *et al.* 2006). Several mechanisms have been suggested to explain AMF's protection against several pathogens, including increasing production of phenolic compounds, induction of some resistance mechanism and plant defence system activation (Benhamou *et al.* 1994; Gianinazzi-Pearson *et al.* 1996). Thus, mycorrhizal inoculation of coffee plants in nurseries may be a useful practice for minimizing the deleterious effects of nematode infection (Vaast *et al.* 1998).

OTHER ASPECTS OF THE COFFEE–AMF ASSOCIATION

Frequently, AMF are considered to be unable to decompose complex organic molecules due to their small production of hydrolytic enzymes and their obligatory biotrophism (Varma 1999), and have been excluded from studies of the degradation of organic substances. However, the importance of AMF for retention and mineral nutrient recycling in montane tropical ecosystems has been pointed out recently (Aristizabal *et al.* 2004), based on the occurrence of AMF spores, hyphae and vesicles within decomposing leaves of several montane plant species, including *C. arabica* L.

AMF may also influence the concentration and composition of secondary metabolites, such as essential oils (Khaosaad *et al.* 2006), apocarotenoids (Fester *et al.* 2002) and phenolic compounds (Devi & Reddy 2002), among others. It would be of interest to investigate the influence of these symbiotic fungi on coffee beans, as several secondary metabolites are involved in the quality of the beverage produced from these beans, including alkaloids (Ashihara 2006), chlorogenic acids (Farah & Donangelo 2006) and terpenes (Speer & Kölling-Speer 2006).

MYCORRHIZAL TECHNOLOGY APPLICATION IN COFFEE

Commercial substrates for coffee seedling production found on the market are usually poor in nutrients and require fertilization. In this respect, the use of AMF is of special interest for improving the efficient use of fertilizers and diminishing the amount required for adequate coffee seedling growth, as observed by Tristão *et al.* (2006), who verified the positive effect of AMF inoculation in coffee seedlings growing in pine-bark- and coconut-fibre-based substrates.

Coffee seedlings are mainly produced by farmers from selected seeds. Seedlings at an appropriate stage are then transferred to the field. However, in coffee nurseries, in order to control soil diseases, seedlings are frequently produced in sterilized, fumigated soils or commercial substrates that are not conducive to mycorrhiza formation (Howeler *et al.* 1987). Inoculation of highly effective AMF is expected to greatly improve the nutritional state of seedlings and their establishment after transplantation, as already verified by some authors (Lopes *et al.* 1983; Sieverding & Toro 1986; Siqueira *et al.* 1987; Rivera *et al.* 2003). Thus, AMF inoculation may constitute a viable economic alternative for efficient seedling production, decreasing the use of fertilizers and pesticides, diminishing the time for field transplantation and producing more vigorous plants able to better withstand environmental stresses during the acclimatization period (Costa *et al.* 2003). Siqueira *et al.* (1998) calculated the benefit under Brazilian low-fertility soil conditions of mycorrhizal pre-colonization of coffee transplants with respect to the savings on P fertilizers, estimating the mycorrhizal effect to be equal to 254 kg/ha of P_2O_5 and equal to a cost of US$ 20·00 per ha of AMF inoculation.

Arbuscular mycorrhizal inoculation has also been found to improve the growth and nutrient uptake of several *in vitro* propagated perennial plants (Blal *et al.* 1990; Vidal *et al.* 1992; Aguín *et al.* 2004; Lovato *et al.* 2006), and also of Arabica coffee plants (Vaast et al. 1996). *In vitro* propagated *C. arabica* plants have relatively small root systems, which affects nutrient uptake efficiency and leads to a lack of benefit from fertilizer addition. AMF inoculation has been shown to improve P status and growth in coffee propagated *in vitro*, confirming the high AMF dependency of coffee seedlings (Vaast *et al.* 1996).

The selection process for formulating effective AMF inoculants for a target plant and/or variety should consider the physiological and ecological adaptations of native AMF to the target environment (Calvente *et al.* 2004). Those authors verified the higher effectiveness of native AMF for promoting growth during the commercial nursery production of olive varieties. Unfortunately, mycorrhizal technologies, such as AMF inoculants, are not always available in most coffee-producing countries, making it difficult to incorporate this sustainable technology where its use can be of highest relevance. However, commercially available mycorrhizal inoculum normally leads to an extremely high density of AMF propagules per volume of soil (Costa *et al.* 2003), thereby enabling a rapid mycorrhizal establishment of coffee seedlings and making AMF inoculation a highly recommended practice at the nursery stage. Therefore, AM fungi are gaining popularity as biofertilizers and biocontrol agents (Sylvia 1999) and the industry of mycorrhizal inoculum production is expanding around the world (Corkidi *et al.* 2004; Todd 2004).

CONCLUSION

In conclusion, the ecological and agricultural importance of AM symbiosis in coffee, mainly during the seedling formation stage which, under natural conditions, is highly dependent on mycorrhizae, has been discussed. The beneficial effects of mycorrhizae on the nutritional status of plants and on the ability of these plants to withstand different environmental stresses have made these mycosymbionts a key factor for sustainable agriculture. These benefits vary depending on the AMF strains or ecotypes used for inoculation, which makes the selection of an effective AMF for predetermined edapho-climatic conditions a prerequisite for achieving the desired positive response from the association. The use of efficient mycorrhizal inocula in coffee nurseries may be a promising technology for the production of healthy and vigorous coffee plantlets, thus increasing survival after field transplantation. Nevertheless, knowledge about the role and benefits of mycorrhizae in this important economic crop is still very sparse. The relevance of the symbioses in relation to environmental factors, such as drought, extreme temperatures and chemical toxicity stress conditions, is now of significant interest since agricultural lands are subject to changes in modern environmental conditions that threaten agricultural production and quality.

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