

8. Endomycorrhizae in the tropics

V. GIANINAZZI-PEARSON and H. G. DIEM

1. Introduction

The term 'endomycorrhiza' is used to describe mutualistic symbiotic associations between certain fungi and plant roots, in which the fungal partner grows mainly inside the root cortex and penetrates the cells of the host root.

Endomycorrhizae include three groups: ericoid mycorrhizae, orchidaceous mycorrhizae and vesicular-arbuscular (VA) mycorrhizae. This review is restricted to VA mycorrhizae which are widely distributed in the world and potentially very important both economically and ecologically.

Although VA mycorrhizae were first observed before the turn of the 20th century [59, 103], research on the fungal symbionts began much later with the development of techniques for maintaining these fungi in pot cultures [136]. Considerable advances were made following extraction of their spores from soil [65] and after the discovery that plants inoculated with VA fungi grew better due to increased phosphate uptake [24, 66]. Increased absorption of potassium, sulphate, zinc and strontium-90 by mycorrhizal plants has also been shown experimentally [33, 68].

General information concerning VA mycorrhizae may be found in the recent excellent review by Hayman [90]. Some aspects of the biochemistry of VA mycorrhizae were recently discussed by Gianinazzi-Pearson and Gianinazzi [73] and the relevance of VA mycorrhizae to plant nutrition in agriculture has been reviewed by Tinker [214].

At the international workshop held in Kumasi (1978), different aspects concerning the role of VA mycorrhizae in tropical ecosystems and in tropical agriculture were discussed by Bowen [33], Black [31] and Mosse and Hayman [143]. Since a detailed review on the ecology of VA mycorrhizae in relation to tropical environments is lacking, the aim of this chapter is to summarize recent advances in our knowledge in this field. Some considerations are also given to the possible use of VA mycorrhizae in tropical agriculture with the hope that their utilization for improving plant production can become a practical reality for many tropical countries.

2. Morphology of VA Mycorrhizae

VA infection does not apparently change the external morphology of roots; the internal morphology can be readily observed after clearing and staining the

infected roots [160]. Although the morphology of the infection can vary slightly depending on both the host plant and the VA fungus [2, 67], certain features are generally observed. Development of a VA infection begins with formation of an ill-defined appressorium (Fig. 2) on the root surface by external hyphae originating from spores or other infected roots in the soil. Like pathogenic fungi, VA fungal hyphae penetrate within or between the epidermal cells from the appressorium then spread inter- and intra-cellularly within the cortex along the root length, sometimes forming coils within the outer cortical cells. Apart from the mycelium, two structures are typically formed by VA fungi within roots:

- arbuscules (Fig. 3), which are formed intra-cellularly by repeated bifurcation of an infecting hypha and where nutrient exchanges most probably occur between the host and the symbiont. The arbuscules are relatively short-lived and they degenerate to form a granular mass of fungal wall material within the host cell;
- vesicles (Figs. 1 and 3), which are most often ovoid swellings with lipidic contents usually formed terminally on hyphae and which are thought to act as temporary storage organs.

Little is known about the factors controlling the formation and the degeneration of these two fungal structures.

Outside the mycorrhizal roots a loose network of external hyphae, continuous with the internal mycelium, invades and explores the soil. These external hyphae take up nutrients, especially phosphorus, from the soil and translocate them to the internal mycelium where they are released from the fungal structures into the root cells [73, 214]. VA fungi generally form resting spores in soil, either singly, or in hypogeous or epigeous sporocarps. However, sometimes true spores of the fungal symbiont also occur within the root cortex [150]. Figure 4 shows the dead cortex of Sudan grass roots completely occupied by numerous spores of *Glomus mosseae* after being inoculated with this fungus. Present-day taxonomy of VA fungi is based entirely on the morphology of these spores and sporocarps.

3. The ecology of VA mycorrhizae in the tropics

3.1. The occurrence of VA mycorrhizae

As reported in what are probably the first records of VA mycorrhizae in the tropics by Janse [103] in Java and by Johnston [109] in Trinidad, the VA association is by far the most common kind of mycorrhiza. VA mycorrhizae are in fact widely distributed geographically and throughout the plant kingdom [90], but not all plant families form them. In temperate regions members of the Chenopodiaceae, Cyperaceae and Cruciferae are generally non-mycorrhizal [96] and in the tropics

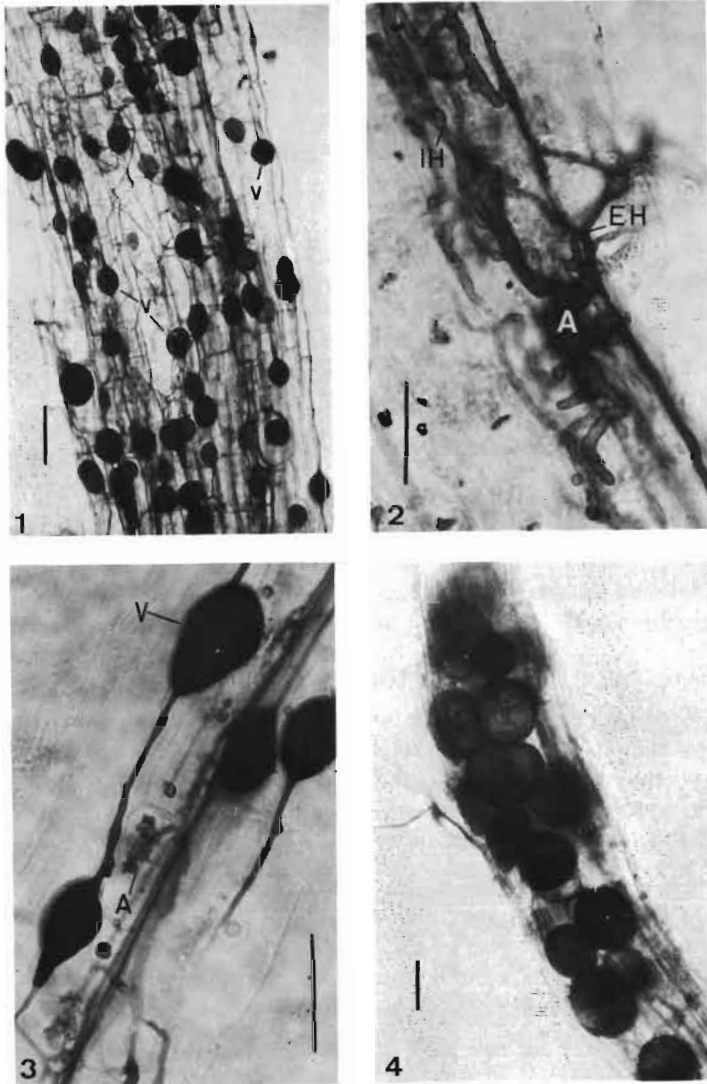


Plate 1. Morphology of VA mycorrhizae.

(Fig. 1) General view of *Vigna unguiculata* root infected with *Glomus mosseae* and showing a network of internal hyphae with vesicles (V); bar = 100 μ m; (Fig. 2) Part of cortex of *V. unguiculata* root showing early stage of infection, appressorium (A), external (EH) and internal (IH) hyphae; bar = 50 μ m; (Fig. 3) Part of cortex of *V. unguiculata* root showing vesicles (V) and arbuscules (A) within host cells; bar = 50 μ m; (Fig. 4) Segment of Sudan grass root showing internal spores of *Glomus mosseae*; bar = 100 μ m.

VA mycorrhizae are sometimes lacking in natural and semi-natural vegetation, for example in a number of plant families belonging to the xerophytic groups [111].

3.1.1. Forest tree species. Many species of forest trees in the tropics can be infected by VA fungi [177]. Under lowland rain forest condition in Nigeria [173, 177], in Sri Lanka [52], in Java [181], in Costa Rica [101, 102], in Cuba [95], in Philippines [217] and in Brazil [212], a wide range of forest tree species form VA mycorrhizae. Most attention has been focused on forest trees in the humid tropics and there remain many gaps in our knowledge concerning VA mycorrhizae associated with trees growing in the arid or semi-arid tropics. Studies carried out in Senegal on mycorrhizal associations of *Azadirachta indica*, *Casuarina equisetifolia* and *Acacia senegal* revealed variabilities in the VA infections of trees growing under dry conditions [53].

3.1.2. Perennial and annual crop plants. VA mycorrhizae occur on almost all perennial crops of economic importance in the tropics, such as avocado [75], cocoa [118, 148], citrus [123, 125, 213], rubber [222], cassava [97, 218, 227], papaya [172], finger millet [12], tea and pineapple [225], oil palm, litchi, sugarcane, coffee and coconut (see Janos [102] and Redhead [176]) and diverse tropical legumes [163] (see Section 4.3.2).

3.1.3. Host susceptibility to VA infection. It is generally admitted that there is a marked lack of host specificity amongst the different VA fungal isolates or species. However, Mosse [140], Jehne [104] and Cornet and Diem (unpublished data) found that certain VA fungi may be preferentially associated with a particular plant species.

Different causes have been proposed to explain the susceptibility of a plant to VA infection:

- physiological: the principal effect of VA mycorrhizae on plant growth is phosphate mediated and plant species or cultivars which are highly P-dependent tend to be strongly susceptible to VA infection although some plant species or cultivars which are less P-dependent can also be strongly infected. Recent observations by Janos [102] indicate that in some tropical forest tree growth responses to mycorrhizae are correlated to seed dry weight, and Hall [81] suggested that plant growth rates may influence P absorption and therefore responses to mycorrhizae.
- anatomical: according to Baylis [26] mycotrophy is largely a feature of woody and herbaceous plants lacking root hairs (magnolioid roots). In their survey on tropical forest trees, Janos [101] in Costa-Rica and St John [208] in Brazil have shown a significant relationship between magnolioid root characteristics and VA infection. Tropical herbage legumes such as *Centrosema* and *Stylosanthes* are sparse in root hairs and so potentially sensitive to VA infection [41]. *Leucaena leucocephala* [147] and many species of *Acacia* (Cornet and Diem, unpublished

data) which have few root hairs are strongly mycorrhiza-dependent in P-deficient soils.

3.2. The VA fungi observed

Most attention has been devoted to the survey of VA fungi in temperate zones of both hemispheres and it is only during the past few years that attempts have been made to record VA mycorrhizal species found in the tropics. There is now evidence that species of four genera of the Endogonaceae (*Glomus*, *Gigaspora*, *Acaulospora*, *Sclerocystis*) form VA mycorrhizae in the tropics.

Principal records of different species of VA mycorrhizal fungi have been made in the humid tropics [176, 177]. Waidyanatha [219] working on *Hevea* plantations in Sri Lanka observed a wide range of spore types; most belonged to the genus *Glomus*, several to *Sclerocystis* species and some were species of *Acaulospora* and *Gigaspora*. In an extensive survey, Herrera and Ferrer [95] reported a similar distribution of endogonaceous genera in Cuban soils where the number of species of Endogonaceae per soil sample was higher than that recorded in other countries. Nadarajah [148] found that in Malaysian soils most spores also belonged to *Glomus* species and very few to other genera.

In the few studies on the taxonomic distribution of VA fungi in the dry tropics, the endogonaceous spores that have been isolated from arid or semi-arid soils belong to the three genera *Glomus*, *Gigaspora* and *Sclerocystis* [53, 56, 175]; typical spores of *Acaulospora* species have not been reported in these soils. Bulbous-based spores (*Gigaspora* spp.) are amongst the most common and spores closely resembling *Glomus mosseae* are also abundant. In conclusion, the current surveys of endogonaceous spores seem to indicate the world-wide distribution of several species and genera [177].

According to Diem *et al.* [53], it is impossible to indicate whether the taxonomic distribution of VA fungi varies widely in terms of environmental factors encountered in different tropical regions but Herrera and Ferrer [95] hypothesized that indigenous VA fungi may be strongly affected by local conditions. In general, *Glomus* species seem to be predominant both in the humid tropics and in the semi-arid areas, and certain *Gigaspora* species appear to be well adapted to dry and hot conditions [197, 198]. Herrera and Ferrer [95] have suggested that *Sclerocystis* may be considered as a tropical genus whilst *Acaulospora* species are apparently less common in semi-arid zones [53].

The taxonomical study of VA fungi in the tropics remains a vast area for research; new species of VA fungi have already been described in Nigeria [157], India [69], and probably several VA fungi found in the Ivory Coast [182], Sri Lanka [52] and Cuba [95] are new. Recently Nicolson and Schenck [151] have compiled an inventory of endogonaceous fungi including new species from the region of Florida which to some extent may be considered a subtropical zone.

4. Major factors affecting VA mycorrhizae

4.1. Climatic factors

Although VA mycorrhizae are formed in the soil and special attention should therefore be given to soil factors, climatic factors may also be important since they can act on soil characteristics, control the physiology of the host plant and consequently influence the relationships between the plant and its endophyte.

4.1.1. Light. Microorganisms living closely in symbiosis with plant roots obtain their carbon energy source from the host plant and thus rely on both the photosynthetic ability of the plant and the translocation of photosynthates to the root. For such systems, light is obviously a limiting factor [55]. The stimulatory effect of light on the development of VA mycorrhizae has been shown by Furlan and Fortin [62] and Hayman [87]. Shading not only reduces root infection and spore production [68] but also the plant response to VA mycorrhiza [132, 152]. This is probably due to a reduced spread of internal hyphae within root tissues, and consequently restricted growth of external hyphae in the soil. Redhead [174] postulated that day length may play an important role in VA mycorrhiza development and this seemed to be confirmed by Daft and El-Giahmi [44]. However, shading did not have a negative effect on the development of mycorrhizae of *Khaya grandifolia* [174] which suggests that the effect of light on VA mycorrhizae depends on the photosensitivity of the species of host plant. In fact, using plant species which exhibit different light requirements, Johnson [106] showed that infection level of *Coprosma leptospermum* and *Microlaena* sp. did not decrease with shading whilst in *Griselinia* sp. and *Parsonsia* sp., they were significantly lower in heavy shade.

Because of the beneficial effect of light, it could be expected that under the high solar radiation generally occurring in the tropics, VA infection levels would be high and growth responses marked. However, the favourable effect of light may be reduced in some cases, e.g. plants growing beneath forest canopies or in certain tropical regions subjected to variable sunshine due to the monsoons.

4.1.2. Temperature. Photosynthesis and translocation through the plant are affected by air temperature but the influence of this factor on VA mycorrhizae has received little attention. Furlan and Fortin [60] and Hayman [87] showed that infection levels in onion roots increased with increasing ambient temperature up to 26 °C and this was sometimes associated with increased plant growth response, especially with alternating day/night temperatures. However, in these studies it is difficult to distinguish the effects of air temperature from those of the soil since no effort was made to independently control the temperature of the latter.

4.2. Physical-chemical soil factors

4.2.1. Soil temperature. The common characteristic of tropical soils is the high temperatures found both in humid regions and in semi-arid regions. In the lowland humid tropics, soil temperature at the beginning of the growing season can be 45–50 °C at a depth of 5 cm [156]. Daily mean temperatures in the semi-arid zone of West Africa are also high (40 °C) and their seasonal variation is relatively small [39]. It is therefore necessary to consider the role of high temperatures rather than that of low temperatures when discussing the development and the eco-physiology of VA mycorrhizae in the tropics; very little information, however, is available on this subject.

Effect on VA infection. VA mycorrhiza establishment consists of three distinct phases: spore germination in soil, hyphal penetration of root cells and development within the root cortex. From the few studies made, it appears that optimum temperatures for spore germination can vary widely between different VA fungi. Certain *Gigaspora* species isolated from soils in Florida, in a subtropical zone, germinated best at a temperature (34 °C) which was considerably higher than that for optimum germination of *Glomus* species (20 °C) from cooler climates [49, 198]. Fungal penetration and development in roots are similarly sensitive to variations in soil temperature. Smith and Bowen [205] showed that infection by fungi in soil from a temperate climate in southern Australia increased with increasing soil temperature and reached a maximum at 16–25 °C, whilst Schenck and Schroder [197] reported that maximum infection by a *Gigaspora* species isolated from a Florida soil occurred at 30–33 °C.

Under field conditions precocious infections by indigenous VA fungi have been observed in peanuts sown during the hot season in Senegal, where infection reached 53% of roots on 16-day old seedlings [71], and similar observations have been also made on cowpea (Bertheau, personal communication).

These observations deserve further investigation for they suggest that VA fungi from different climates may be adapted to different soil temperatures, a point to be considered before introducing efficient VA fungi from temperate climates into tropical soils.

Effect on VA mycorrhizal function. For *Eupatorium odoratum*, *Guizotia abyssinica* and *Sorghum bicolor* better growth and enhanced P uptake by mycorrhizal plants occurs at 30 °C rather than at 25 °C [132, 152]. High temperature (ca. 35 °C) during the day is not harmful to the development and physiological activity of the mycorrhizae if night temperatures are only 25 or 30 °C [132]. The stimulatory effect of mycorrhizal infection only decreases at 40 °C. In the tropics, day-time soil temperatures are rarely below 15 °C or above 35 °C beneath plant canopy, although that of bare soil can reach 40–45 °C. According to Moawad's data, these temperatures are probably not a limiting factor for the activity of VA mycorrhizae.

Effect on survival of VA fungi. There is no report on the effect of high temperature on the survival of VA fungal structures in soil, although it may be

assumed that high temperature existing in the bare soil after the host plant is harvested or has died could dramatically affect the survival of different VA fungal structures especially spores. Bowen [33] has suggested the necessity of selecting species or strains highly resistant to high temperatures. Temperature responses of different species or isolates of VA fungi, however, may also depend on other soil features, e.g. soil texture. Rhizobia, for example, are particularly sensitive to high temperatures in sandy soil but not to the same extent in clay soil because in the latter the clay envelope which surrounds the bacterial cells confers an increased resistance to high temperature and desiccation [122]. The role of clay soil in the storage of fungal cultures has been demonstrated [19], and it could be that high temperature tolerance of VA fungi is more marked in heavy textured soils than in sandy soils.

4.2.2. Soil water content. Tropical soils are quite different from one another in water content due to the wide range of soil textures and climates in the tropics.

Although VA mycorrhizae can occur in aquatic plants [15, 206], it is generally admitted that their development is adversely affected by waterlogged soil conditions [111, 124]. Vast regions in the tropics are governed by an arid or semi-arid climate, and water relations of VA mycorrhizae could be of particular importance here [33]. Knowledge of the ecophysiology of VA mycorrhizae in relation to soil water potential is essential for evaluation of the role of VA mycorrhizae in these regions.

Influence of drought on VA mycorrhiza development in soil. Many plants growing in desert and semi-arid regions are normally mycorrhizal showing that VA infections can develop under water-stressed conditions. The influence of drought conditions on the development of VA fungi inside and outside the root can be different. Daniels and Trappe [49] showed that spore germination was favoured in soil at or above field capacity. In experiments with *Khaya grandifolia* Redhead [174] found that whilst extra-matrical mycorrhizal spores and fungal development may be drastically affected by drought, mycorrhizal infection levels can be high, presumably because water content within the roots remains sufficiently high for continued fungal spread in the host tissues.

Role of VA mycorrhizae in plant growth under drought conditions. In dry climates plants are often subjected to relatively long periods of water stress, and an interesting question is whether soil water supplies to the plant can be improved by VA mycorrhizae. Although, as previously mentioned, many plants from desert and semi-arid regions are mycorrhizal, little is known of the significance of this association for growth and survival of these species.

Recently, Menge *et al.* [127] reported that mycorrhizal infection enabled avocado seedlings to resist transplant shock because VA mycorrhizae could improve the water absorption capacity of the host plant. Figure 5 illustrates the behaviour of mycorrhizal and non-mycorrhizal seedlings of *Acacia raddiana* grown in the same soil with the same water potential. In the middle of the day, when air humidity is low, leaflets of mycorrhizal seedlings remained open whereas those of the controls

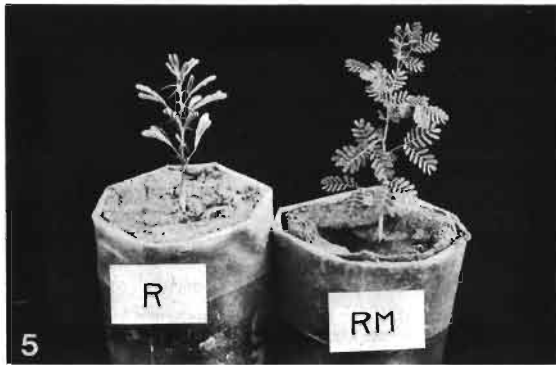


Fig. 5. Effect of VA infection on the growth and the behaviour of *Acacia raddiana*; non-mycorrhizal seedling with closed leaflets (R) and mycorrhizal seedling with open leaflets (RM) observed when air humidity is low; 60-day old seedlings (photography by courtesy of F. Cornet).

are closed suggesting that evapotranspiration is higher on non-mycorrhizal plants than on mycorrhizal ones. According to Janos [102], not only do mycorrhizal perennial trees grow more rapidly than uninfected seedlings but also mycorrhizae improve the survival of some tree species. *Carica papaya* control seedlings died after wilting in hot, sunny days whereas inoculated plants survived.

It has been suggested that drought resistance of mycorrhizal plants may be due to a decreased resistance of roots to water flow and therefore an enhanced water transport into roots [190]. P-deficient plants are more susceptible to drought [7] and Safir *et al.* [191] reported that better P nutrition in mycorrhizal plants could enhance water transport in soybeans. Another possible mechanism would be the absorption of water by the extensive external mycelium of mycorrhizal roots. However, in either case water content in the soil would be quickly exhausted. A recent interesting finding on the relationship between soil water potential and mycorrhizal activity is that the amount of water used to produce 1 g dry matter was much lower in mycorrhizal than in non-mycorrhizal plants growing in dry soil fertilized with $\text{Ca}_5(\text{PO}_4)_3\text{OH}$ [202]. Consequently, the greater drought resistance of mycorrhizal plants may be simply due to a more economic utilization of water by plants growing in P-deficient soils [132].

How mycorrhizal plants could economize on water consumption remains to be determined. In recent work on citrus seedlings, stomatal conductance (which provides information on the resistance to water flow in the leaf–air interface) and transpiration flux density were slightly but not significantly influenced by mycorrhizae during a period of water stress whilst when plants were rewatered leaf conductance, transpiration flux density and photosynthesis were consistently higher in mycorrhizal plants [120]. In arid and semi-arid regions it seems therefore probable that mycorrhizae play an important role in the growth and drought

resistance of a number of plants because of their ability to regulate uptake of soil nutrients and water.

4.2.3. Soil pH. VA fungi often show adaptation to soil pH and this can be a determining factor for endophyte efficiency. Both spore germination and mycorrhiza development by different fungal species can be significantly affected by variations in soil pH [49, 79, 137]. Hayman and Mosse [92] obtained infection and growth stimulation of *Coprosma robusta* by *Glomus mosseae* in two soils of pH 5.6 and 7.0 but not in acid soils of pH 3.3 to 4.4, whilst after liming to pH 6.5 infection occurred in all and growth stimulation in most of the soils. Similar results have also been obtained with *Paspalum notatum* growing in acid tropical soils [138] and with soybean which responded positively to both *Gigaspora gigantea* and *G. mosseae* in limed soil (pH. 6.2) but only to *Gigaspora gigantea* in acid soil (pH 5.1) even though infection levels with both fungi were comparable [204].

On the contrary, Cornet and Diem (unpublished data) found that *Acacia raddiana* responded positively to both *Glomus mosseae* and *Glomus* E₃ in an acid soil from Senegal (pH 4.5) but only to *Glomus mosseae* in the same soil after liming to pH 6.9.

The relationship between soil pH and VA mycorrhizal effect is complex and depends not only on the fungal species, soil type or forms of P but also on the plant species. In the case of *Guizotia abyssinica* VA mycorrhizae strongly depressed plant growth at pH 4.3 in the presence of different fertilizers whereas mycorrhizal *Tagetes minuta* was not affected at the same pH [77].

4.2.4. Organic matter and root residues. The important role of organic matter in the environment is evident, especially in the tropics where continuously high temperatures favour rapid rates of decay of plant residues in soil. Organic matter influences soil structure, pH, nutrient and water-holding capacity, all of which may directly and/or indirectly influence VA mycorrhizal development and efficiency. According to Sheikh *et al.* [201], endogonaceous spore population seems to be closely correlated with the level of organic matter content in Pakistan soils. Maximum spore numbers were recovered from soils containing 1–2% organic matter and spores were sparse in soils with below 0.5% organic matter. No such correlation has been observed in temperate soils with higher (2–13%) organic matter contents (Gianinazzi-Pearson, unpublished data) although organic manures often enhance mycorrhizal development in tropical soils [109].

An aspect of the study of organic matter which deserves attention is the impact of mycorrhizal root residues themselves on the ecology of VA fungi in soil. Numerous mycorrhizal plants are annuals and mycorrhizal root systems are thus continuously being incorporated into soils and degraded by soil microorganisms. Almost nothing is known regarding the fate of the endophyte mycelia outside and inside the root tissues.

Redhead [175] suggested that seasonal die-back of Sudan and Sahel savanna grasses could stimulate endogonaceous spore production thus increasing spore

populations, as observed when arable crops such as maize, barley and wheat are harvested. Mycorrhizal root debris in soil can also be an important reservoir of inoculum. Daft *et al.* [45] claimed that most infections of *Endymion non-scriptus* in nature arose directly from the decaying old root systems through which the new roots grew and Warner and Mosse [221] indicated some saprophytic ability of VA fungi in soil that would enable them to establish a base (possibly in particles of organic material) from which they could infect a host plant.

Many authors have emphasized that spores are not important for maintaining infection when infected roots are present especially in the case of natural plant communities when VA fungi may be non-sporing or poorly-sporing types [25, 207]. Rives *et al.* [183] also suggested that in areas with low annual rainfall contact between infected root debris and roots of uninfected plants may constitute the most efficient mode of VA mycorrhizal spread.

4.2.5. Chemical soil factors. The relationship between VA mycorrhizae and chemical factors have been little studied in tropical soils. Sheikh *et al.* [210] reported that chemical factors and soil chemical treatments may directly influence the occurrence of VA fungi in Pakistan soils and that low P supply which limits plant growth favoured spore production. In Californian soils, P content in soil was inversely correlated with mycorrhizal spore numbers [126]. Studies in temperate climates have shown that high P concentrations in soils reduce VA infection, probably due to the high internal P concentrations developing within the host tissues [5, 130, 192].

The effect of N and P fertilizers on VA mycorrhizae have been extensively studied in temperate soils by Hayman [88]. He showed that N fertilizer (188 kg N ha⁻¹ as 'Nitro-Chalk') had a large negative effect on the mycorrhizal population and plots not given N contained 2 to 7 times more endogonaceous spores and 2 to 4 times more VA infection than plots given N. Porter and Beute [162] also found that mycorrhizal peanuts growing in soils containing little N produced more spores than in soils containing much N. Hayman [88] found that the effects of N fertilizer were more marked than those of P fertilizer whilst Jensen and Jakobsen [105] observed that these two fertilizers generally affected mycorrhiza formation equally.

In spite of the observed effects of fertilizers, VA fungi can be very abundant in fertile soils [93] (Trouvelot, personal communication) and this is probably because VA populations are influenced not only by fertilizers but also by different crop plants, soils, management practices etc. [115].

As well as the extensive use of chemical fertilizers for plant production, there has been increased development and use of soil pesticides for crop protection. The majority of these chemicals, and especially fungicides, can greatly reduce both the development and sporulation of VA fungi [57, 129, 149]. The nematicide DBCP*, however, frequently increases VA infection and/or sporulation [30, 71].

It is not known whether VA mycorrhizae can affect chemical characteristics of soils. Bowen [33] hypothesized that VA fungi could counteract some forms of
*1-2 dibromo-3-chloropropane

soil toxicity by absorbing elements harmful to plants or assisting plant tolerance of high alkalinity or high salinity in tropical soils. These questions deserve further investigations.

4.3. Biological factors: interactions with soil microorganisms

Large populations of microorganisms live in the soil and an intense microbial activity exists around plant roots. Saprophytic soil-inhabiting microorganisms in the rhizosphere interact with the plant and more specialized parasitic or symbiotic organisms infect the living roots. There are, however, major gaps in our knowledge of the interactions that VA mycorrhizae may have with these other soil microorganisms, especially in tropical soils.

4.3.1. Phosphate-solubilizing bacteria. Many experiments (quoted by Mosse *et al.* [146]) have shown that rock phosphates only become available to plants in acid soils but not in neutral or alkaline soils, and VA infections did not alter these relationships. VA mycorrhizae do not mobilize insoluble soil phosphate but only increase the absorption of that which is already available to roots. Experiments with ^{32}P confirm that both mycorrhizal and non-mycorrhizal plants utilize the same pool of available soil P [74, 145, 161, 193]. The possible processes involved in P absorption by VA mycorrhizae have been reviewed by Tinker [214]. Mosse [139] discussed different mechanisms involved in the solubilization of organic and inorganic phosphate in the soil and in the rhizosphere. It is well known that two groups of bacteria, chemo-organotrophs such as some *Pseudomonas* and *Bacillus* species and chemo-lithotrophs such as thiobacilli, are able to solubilize insoluble phosphate.

The possibility of synergistic interactions between VA fungi and phosphate-solubilizing chemo-organotroph bacteria has been investigated in alkaline soils by Barea *et al.* [21] and Azcon *et al.* [8] using lavender and maize. In some cases the combined inoculation of a *Glomus* species (E_3 type) with these bacteria significantly increased plant growth above that achieved with either microorganism separately. However, the effect of *G. mosseae* on plant growth was not enhanced by the same bacteria. Azcon *et al.* [8] attributed this difference to an improvement in the efficiency of *Glomus* E_3 due to a lowering of soil pH by the introduced bacteria. In fact, *Glomus* E_3 is more tolerant of acid than alkaline soils whereas *G. mosseae* is not favoured by a lowered pH. It was also suggested that some solubilization of rock phosphate by the bacteria did occur and that inoculation with VA fungi favoured the early establishment of phosphate-solubilizing bacteria in the rhizosphere, although after 2 months of plant growth bacterial populations declined in the usual way.

Recently, the influence of inoculation with *Glomus fasciculatus* and *Bacillus circulans* on growth of finger millet and P uptake from ^{32}P -labelled tricalcium phos-

phate was studied [171]. In the treatment receiving both inocula a synergistic effect was also recorded with increased P uptake and growth in mycorrhizal plants.

Thiobacilli are well known for their ability to solubilize insoluble rock phosphate in soil when they are introduced together with S; they oxidize S to H_2SO_4 which in turn partially dissolves the rock phosphate. Swaby [210] claimed that this principle could be exploited in agriculture and that it had given promising results in field tests. The ability of thiobacilli to solubilize rock phosphate in the presence of S is used commercially in the Lipman process. Hayman [89] found that this process was as cheap or as efficient as the use of standard P fertilizers only in warm, wet soils and Swaby [210] in fact considers that this method to solubilize rock phosphate could be used economically in the wetter tropics.

Table 1 shows how inoculation of a soil from Senegal with thiobacilli can improve growth of *Vigna unguiculata* in the presence of a supplement of rock phosphate. The effect of an interaction between the thiobacilli and VA fungi on growth and nodulation seems to vary in terms of the species of fungus used. A synergistic effect was only recorded with the combined inoculum *Glomus* E₃ and thiobacilli as far as nodule dry weight is concerned.

Edaphic factors in humid tropical regions or in irrigated tropical soils are certainly very favourable to the activity of thiobacilli. With the continuously increasing cost of P fertilizers, the possible use of phosphate-solubilizing thiobacilli alone or in combination with appropriate VA fungi deserves more attention. P-deficient tropical soils often harbour high populations of indigenous VA fungi and exploitation of combined activities of VA mycorrhizae and thiobacilli may contribute to a better use of rock phosphate in agriculture, especially in developing countries where rock phosphate is a natural resource.

4.3.2. *N*₂-fixing microorganisms

Free-living bacteria. There are few publications concerning interactions between VA mycorrhizae and free-living *N*₂-fixing bacteria. From three studies that have dealt with *Azotobacter* [14, 22, 34] and one with *Azospirillum* [28] the effect of interactions between VA mycorrhizae and these free-living bacteria can be summarized under four main aspects:

- (1) *Populations of N₂-fixing bacteria and total soil microflora.* The common finding of studies on *Azotobacter* is that VA infections favourably affect bacterial populations in the rhizosphere of host plants [14, 22]. *Azotobacter* numbers decrease more slowly in the rhizosphere of mycorrhizal plants than in the rhizosphere of non-mycorrhizal plants and total soil bacteria populations have been found to increase in the presence of *Azotobacter* and VA mycorrhiza together.
- (2) *VA infection.* VA infection is increased by the presence of *Azotobacter* in dual inoculation experiments [14, 34] and there is some evidence that this is due to the production of growth-promoting substances by the bacterium [9]. *Azospirillum*, on the contrary, does not appear to have any appreciable effect on VA infection levels [28].

Table 1. Effect of double inoculation with thiobacilli and VA fungi on growth and nodulation of *Vigna unguiculata* cv 58-185 in sterile soil. (Ollivier and Diem, unpublished data)

	Average shoot dry wt. (g plant ⁻¹)	Average nodule dry wt. (g plant ⁻¹)	Root segments infected (%)	Shoot N (%)	Shoot P (%)
Control	2.78 ^a	46 ^a	0	1.4	0.080
Thiobacilli	3.90 ^b	64 ^b	0	1.4	0.080
<i>G. mosseae</i>	5.88 ^c	191 ^c	100 ^a	1.8	0.095
<i>Glomus</i> E ₃	4.98 ^{bc}	148 ^d	99 ^a	1.6	0.105
Thiobacilli +					
<i>G. mosseae</i>	5.68 ^c	189 ^c	99 ^a	1.7	0.090
Thiobacilli +					
<i>Glomus</i> E ₃	5.22 ^c	178 ^c	99 ^a	1.7	0.095

60-day old plants all inoculated with *Rhizobium* strains CB 756

Soil: pH (KC1): 6.2; total P (ppm): 79; available P, Olsen (ppm): 10 and supplemented with rock phosphate (0.25 g kg⁻¹ soil)

Values followed by the same letters in columns are not significantly different ($P = 0.05$)

- (3) *N₂ fixation*. There is no conclusive information concerning the effect of VA mycorrhizae on N_2 fixation by free-living bacteria.
- (4) *Plant growth*. Growth stimulation is greater in plants inoculated with both *Azotobacter* and VA fungi than either microorganism alone [14, 34]. Bertheau [28] also found some synergistic effects of dual inoculation of wheat with *G. mosseae* and an *Azospirillum* species; although these were negative for a first crop, they greatly improved growth of a consecutive crop on the same soil.

Symbiotic bacteria: Rhizobium. With the exception of certain *Lupinus* species which are immune to VA infection [135, 216], legumes are relatively poor foragers of P and generally very responsive to VA infection. Much of the information regarding mycorrhizal effects on legume growth has been obtained from studies on soybeans cultivated in more temperate climates (see for example Ross and Harper [188], Schenck and Hinson [194], Bagyaraj *et al.* [16], Carling and Brown [37] and Asimi *et al.* [5]).

Crush [41] speculated that apart from effects on the host's P supply VA mycorrhizae could influence the legume-*Rhizobium* by altering the root and/or rhizosphere environment for rhizobia. There are many reports concerning the nutritional effects of VA mycorrhizae in legumes (see Munns and Mosse [147]), but virtually nothing is known of direct interactions between VA mycorrhizae and rhizobia. VA fungi do not appear to penetrate nodule tissues [5, 41] and Carling *et al.* [38] concluded, from observations that P fertilizer produces similar plant growth responses to mycorrhiza in soybean, that the VA fungi probably have no direct effect on the symbiotic bacterium. However, the more detailed investigations by Asimi *et al.* [5] indicate that nodulation and nitrogenase activities of *R. japonicum* can still be significantly enhanced by mycorrhiza at high P fertilizer levels and this subject deserves further attention.

Tropical legumes associated with VA mycorrhizae. Annual and perennial tropical legumes can be strongly mycotrophic and although much information comes from soybean studies the influence of VA mycorrhizae has been investigated in some strictly tropical plant species: peanut [43, 71, 78, 162], *Stylosanthes guyanensis*, *Centrosema pubescens* [41, 146], *Pueraria phaseolides* [220] and *Vigna unguiculata* [12, 76, 80, 99, 227] (see also Tables 1, 3, 5, 6). Among legume trees of economic importance, species belonging to only two genera have been studied: *Leucaena leucocephala* [147, 227] and *Acacia senegal* [53], *A. farnesiana* [108], *A. holosericea* and *A. raddiana* (Cornet and Diem, p. 224).

Effect of VA mycorrhizae on N₂ fixation and growth of tropical legumes. Asai [4] first demonstrated that several legumes grew poorly and failed to nodulate in sterilized soil if they were not mycorrhizal. Many investigations have since confirmed this early finding (see Munns and Mosse [147]), and considerable attention is now being given to the tripartite association between plants, rhizobia and VA fungi. As an adequate P supply is necessary not only for plant growth but also for a satisfactory nodulation and N_2 fixation, VA mycorrhizae, in increasing P uptake by the plant, are obviously an important factor in the tripartite association.

There is a vast amount of literature concerning the effect of VA mycorrhizae on nodulation, N_2 fixation and growth of legumes; this section has been confined to a few examples related to strictly tropical legume species. Interactions between VA mycorrhizae and symbiotic N_2 -fixing bacteria have been extensively studied in *Centrosema pubescens* and *Stylosanthes guyanensis*, an important forage legume in the tropics, in a large number of both temperate and tropical soils [41, 141, 146]. VA mycorrhizae exerted stimulatory effects on nodulation and N_2 fixation of these two legumes in all the P-deficient soils tested and nodulation and growth were improved in both sterile and non-sterile soils. Similar mycorrhizal effects have been obtained with *Vigna unguiculata* in Nigeria [99] and Senegal (Ollivier and Diem, unpublished data), and with *Pueraria phaseolides* and *Stylosanthes guyanensis* in Sri Lanka [220]. It is highly probable that the most important factor involved in the mycorrhizal responses of tropical legumes growing in P-deficient soils is the improved P nutrition of the host plant.

Three main factors can determine the extent to which VA mycorrhizae assist P uptake and therefore their effect on legumes production: plant species, mycorrhizal fungal species and available soil P.

Influence of the host species. Legumes can differ widely in their growth responses to VA infection. Growth and nodulation of *Astragalus sinensis*, *Glycine max*, and *Ornithopus* sp. are greatly improved by VA mycorrhiza whereas in *Vicia sativa* and *Lupinus luteus* this is less marked [4]; *Stylosanthes* sp. and *Centrosema* sp. are much more dependent on VA mycorrhizae than temperate legumes such as *Trifolium* and *Lotus* sp. [41]. The rapidity with which legumes respond to VA infection can also vary between species of the same genus; *A. holosericea* responds to VA infection 5 weeks after inoculation whereas 8 weeks are necessary before mycorrhizal effects are visible in *A. raddiana* growing under the same conditions and inoculated with the same VA fungus (Cornet and Diem, unpublished data). These variations in mycorrhizal dependency are probably related to the ability of a given legume species to forage for P in the soil. Differences in mycorrhiza development and effects on plant growth can even differ between cultivars of the same host species and this topic is fully discussed in Section 4.4.

Influence of the VA fungal species. VA fungi are not host specific but the efficiency of a mycorrhizal system will partly depend on the physiological characteristics of the fungal symbiont (ability to translocate and transfer nutrients), the amount and distribution of the soil mycelium and on interactions between the fungal species and the environment [141]. Comparisons between *Glomus* and *Gigaspora* species in soybean [37], *Glomus* and *Acaulospora* species in alfalfa [155] and *Glomus*, *Gigaspora* and *Acaulospora* species in cowpea [99] have confirmed that VA fungi, and even isolates of the same fungal species [1], often vary in their effects on growth of the host plant even when infection levels are similar. However, such comparison between effectiveness of different VA fungi are only valuable in a given soil and should not be generalized to another environment because effectiveness of a fungal strain can greatly vary in terms of soil characteristics and fertility [37] (see also Table 2).

Table 2. Effect of soluble phosphate additions to soil on yield responses of soybeans infected by two VA fungi in sterile soil (Gianinazzi-Pearson and Gianinazzi, unpublished data)

	ppm P added to soil			
	0	57	114	228
Control	1.2 ^a	4.2 ^a	7.6 ^a	7.0 ^a
<i>G. mosseae</i>	2.9 ^b	5.4 ^b	8.3 ^a	7.5 ^a
<i>G. fasciculatus</i>	5.0 ^c	6.3 ^b	9.5 ^b	10.1 ^b

P added in form of KH_2PO_4

Results expressed as pod dry wt, g plant⁻¹

Values followed by the same letters in columns are not significantly different ($P = 0.05$)

Soil: pH (H_2O): 7.1; total P (ppm): 698; available, P, Olsen (ppm): 56

Influence of added soluble phosphate and insoluble rock phosphate. Applications of increasing amounts of soluble phosphate to soil considerably influence the incidence and the efficiency of the VA infection [5], although the extent of this effect also depends on the fungal species involved (Table 2). However, in very poor soils addition of soluble phosphate up to an appropriate level can enhance mycorrhizal effects on plant growth [97, 168] (Table 3). Increased utilization of soluble and sparingly soluble phosphate fertilizers by mycorrhizal plants [143] will depend not only on the amount of available P already in the soil but also on the soil's P-fixing capacity and water content. The diffusion coefficient of phosphate decreases with decreasing soil humidity and available P content [3] so that in many arid and semi-arid P-deficient tropical soils, inoculation with VA fungi alone may be insufficient to improve yields greatly and should be accompanied by irrigation and/or applications of appropriate levels of P fertilizers in order to obtain optimum plant productivity.

Interactions between VA fungi, nodulation and rock phosphate have been studied in several tropical legumes [99, 141, 146, 220]. Utilization of rock phosphate by mycorrhizal legumes for growth and nodulation depends on soil pH (see Section 4.3.1). In neutral or alkaline soils, added rock phosphate remains unavailable to both mycorrhizal and non-mycorrhizal plants.

Symbiotic actinomycetes: Frankia. Many VA fungi have been observed in close association with different non-leguminous N_2 -fixing plants [56, 185, 186, 224] but there are few reports that VA mycorrhizae influence N_2 fixation in these actinomycete-nodulated plants although they could exert a similar influence to that observed in legumes. Amongst actinomycete-nodulated plants growing in tropical climates, *Casuarina* is of great economic interest because of its use for afforestation in semi-arid and nutrient-poor soils. Apart from the presence of proteoid roots usually observed on seedlings raised in nurseries [53], roots of *Casuarina* can be heavily infected by VA fungi [53, 56, 185] and preliminary experiments have shown that double inoculation of *C. equisetifolia* with *G. mosseae* and crushed nodules significantly improves plant growth and nodulation (Table 4). In *Ceanothus velutinus*, Rose and Youngberg [187] also found that plant dry weight, number

Table 3. Growth responses of *Vigna unguiculata* cv 58-185 to triple superphosphate, rock phosphate and inoculation with *Glomus mosseae* in sterile soil (Ollivier and Diem, unpublished data)

	Average shoot dry wt. (g plant ⁻¹)	Average nodule dry wt. (mg plant ⁻¹)	Root segments infected (%)	Shoot N (%)	Shoot P (%)
Control	3.14 ^a	54 ^a	0	2.12	0.055
Triple superphosphate	4.27 ^b	119 ^b	0	2.46	0.055
Rock phosphate	2.68 ^a	51 ^a	0	2.54	0.082
<i>G. mosseae</i>	4.30 ^b	107 ^b	100 ^a	2.67	0.085
<i>G. mosseae</i> +					
Triple superphosphate	5.10 ^c	147 ^c	99 ^a	3.38	0.142
<i>G. mosseae</i> +					
Rock phosphate	5.03 ^c	109 ^b	95 ^a	2.67	0.085

Triple superphosphate added: 10 ppm P; rock phosphate added: 40 ppm P

54-day old plants, all inoculated with *Rhizobium* strain CB 756

Soil: pH (KCl): 6.2; total P (ppm): 79; available P, Olsen (ppm): 10

Values followed by the same letters in columns are not significantly different ($P = 0.05$)

Table 4. Effect of soluble phosphate, crushed nodules and *Glomus mosseae* on growth and nodulation of *Casuarina equisetifolia* (Diem and Gauthier, unpublished data)

	Average shoot dry wt. (g plant ⁻¹)	Average nodule dry wt. (mg plant ⁻¹)	Shoot N (%)	Root segments infected (%)
Control	2.69 ^a	0 ^a	0.82	0
Crushed nodules	4.23 ^b	57.2 ^b	1.20	0
Crushed nodules + <i>G. mosseae</i>	7.69 ^c	132.2 ^c	1.25	47
Crushed nodules + soluble phosphate	7.49 ^c	106.6 ^c	1.61	0

Soluble phosphate added as K₂HPO₄, [0.5 g kg⁻¹ soil]

6-month old plants

Soil: pH (KCl): 6.2; total P (ppm): 79; available P, Olsen (ppm): 10

Values followed by the same letters in columns are not significantly different (*P* = 0.05)

and weight of nodules and N and P content were greater in mycorrhizal nodulated plants than in nodulated-only plants. The recent isolation of many strains of *Frankia* from nodules of *Comptonia* [36], *Alnus* [18, 27, 116], *Elaeagnus* [18] *Casuarina* [64] and *Hippophaë* (Gauthier, Diem and Dommergues, unpublished data) will no doubt contribute to the development of studies on the effect of VA mycorrhizae on *Frankia*-nodulated plants.

4.3.3. Phytohormone-producing bacteria. Gunze and Hennessy [80] suggested that indole-3-acetic acid application could influence arbuscule formation in VA mycorrhiza. Phytohormones synthesized by certain bacteria (*Rhizobium*, *Azotobacter*, *Pseudomonas*) can significantly increase VA infection [9]. A large proportion of rhizosphere bacteria are able to produce phytohormones but how and to what extent they influence VA infection is not known.

4.3.4. Soil-borne phytopathogenic fungi. Biological control by VA mycorrhizae. Many reports in the literature indicate that plants previously inoculated with VA fungi show an increased resistance to certain fungal root diseases, for example *Fusarium* wilts, and root rots [195]. The mechanism of this mycorrhizal effect on pathogens is not known in most instances and several hypotheses have been proposed to explain the observed protection. As Schönbeck [200] points out, VA fungi are not thought to act directly on the pathogen but rather by causing changes in the host tissues. For example, they may stimulate lignification or the development of callosities or lignitubers in the host cells thus creating a physical barrier to penetration of the pathogen (Becker, quoted by Schenck and Kellam [195]). VA infections can also induce biochemical changes in host tissues which could render them unfavourable for pathogen development (Dehne and Schönbeck, quoted by Schönbeck [200]). Alternatively, prior occupation of the root tissues by VA fungi as first colonizers could simply physically exclude a pathogen competing for the same infection sites.

The influence of VA mycorrhizae on fungal root pathogens however appears to vary greatly with the disease complex studied. There are reports in which VA fungi do not show any stimulating effect on plant resistance to attack by fungal pathogens and in some cases the presence of pathogens reduces the beneficial effects of mycorrhizae, or disease is more severe in the mycorrhizal plants than in non-mycorrhizal plants [50, 51, 126, 195].

It is now evident that interactions between VA fungi, fungal root pathogens and host plants are complex and each combination should be considered individually; the effectiveness of VA mycorrhizae in protecting plants varies according to the species, strain or variety of the VA fungus and plant involved [23, 195]. However, as Wilhelm [223] pointed out, the evaluation of VA mycorrhizae as biocontrol agents remains one of the most challenging areas in plant pathology. In fact, if prior root colonization by VA fungi can reduce certain root diseases then this would be of great interest for many tropical plants which must be grown in nurseries before transplanting. Inoculation of these plants with selected VA fungi when growing

under nursery conditions could provide a good method for protecting plants against pathogen attacks which risk to subsequently occur in the field. Unfortunately, studies on this aspect of VA mycorrhizae are still lacking.

4.3.5. Plant parasitic nematodes. Most investigations of nematode–VA mycorrhizae interactions are related to plant species naturally cultivated under tropical or subtropical climates: tobacco [20, 58, 180], cotton [98, 184], citrus [153, 154], soybean [110, 196, 199] and cowpea (Table 5).

Effect of nematodes on VA infection. Data published by Fox and Spasoff [58] suggest that there is a competitive interaction between *Heterodera solanacearum* and *Gigaspora gigantea* in tobacco. Each organism adversely affects the reproduction of the other and this seems to be due to a competition for both space and food supply in the root system. Table 5 shows a slight but significant reduction in mycorrhizal infection of *V. unguiculata* by *Pratylenchus sefaensis* and O'Bannon and Nemeč [153] also found less vesicle formation and mycelium growth by *Glomus etunicatus* in nematode-infected citrus roots. The effect of nematodes on the sporulation of VA fungi seems to be variable; it may be detrimental [196, 199], indifferent [6, 98] or stimulative [17].

Effect of VA infection on nematodes. There are several reports of significantly reduced development of root-knot nematodes or reduced formation of root galls in different plants pre-inoculated with *G. mosseae*, *G. fasciculatus* and *G. macrocarpus* (respectively Sikora and Schönbeck [203]; Bagyaraj *et al.* [17]; Kellam and Schenck [110]).

Reduction in numbers of migratory endoparasitic nematodes by *Gigaspora margarita* in cotton roots have also been observed [98, 199]. Schenck *et al.* [199] indicated that nematode–VA mycorrhizae interactions can differ with the host cultivar and other workers have reported greater populations of root-knot larvae from mycorrhizal plants than from non-mycorrhizal plants [6, 179]. Thus, as in the case of protection against fungal pathogens, interactions between VA fungi, nematodes and plant roots appear complex and seem to vary with each combination [195].

Effect of nematode–VA mycorrhizae interactions on plant growth. In studies where parasitic nematodes that reduce plant growth have been used, it has generally been found that plants inoculated with both nematodes and VA fungi have intermediate yields between those inoculated with either microorganism alone, indicating that the beneficial effect of VA fungi does not completely compensate for the damage caused by the nematodes [6, 153, 154]. Such observations have, for example, been made on tropically grown *V. unguiculata* inoculated with both *G. mosseae* and *P. sefaensis* (Table 5). Although Germani *et al.* [72] found that the harmful effect of *Scutellonema cavenessi* on soybean can be totally suppressed by *G. mosseae*, growth of mycorrhizal plants infected with nematodes must generally be governed by an equilibrium between an inhibitory (nematodes) and a stimulative (VA fungi) factor, the state of this equilibrium depending on the host plant, the nematode and the VA fungus involved as well as the soil environment.

Table 5. Effect of single and combined inoculations with *Glomus mosseae* and *Pratylenchus sefaensis* on growth and nodulation of *Vigna unguiculata* cv TVX 7 – 5H in sterile soil (Ollivier, Almeida and Diem, unpublished data)

	Average pod dry wt. (g plant ⁻¹)	Average nodule dry wt. (mg plant ⁻¹)	Average total dry wt. (g plant ⁻¹)	Root segments infected by VA fungus (%)	Shoot N (%)	Shoot P (%)
Control	5.75 ^a	175 ^a	11.09 ^a	0	2.05	0.06
<i>P. sefaensis</i>	4.66 ^b	181 ^b	9.85 ^b	0	2.40	0.06
<i>G. mosseae</i>	6.50 ^c	248 ^b	15.22 ^c	93 ^a	2.40	0.09
<i>P. sefaensis</i> +						
<i>G. mosseae</i>	5.90 ^a	258 ^b	12.92 ^d	82 ^b	2.40	0.12

60-day old plants, all inoculated with *Rhizobium* strain CB 756

Values followed by the same letters in columns are not significantly different ($P = 0.05$)

Soil: pH (KCl): 6.9; total P (ppm): 172; available P, Olsen (ppm): 102

4.3.6. *Hyperparasitic fungi*. Spores of certain VA fungi can often be parasitized by such hyperparasitic fungi as *Anquillospora pseudolongissimi*, *Humicola fuscoatra* and a species of *Phlyctochytrium* [46, 189]. The presence of hyperparasitic fungi in soil can cause a decrease in the population density of VA fungal species and affect the physiological function of the mycorrhizae [189].

VA fungi seem to differ in their susceptibility to hyperparasities; for example, *Glomus macrocarpus* is more susceptible to parasitism than *Gigaspora gigantea* [189], whilst *Gigaspora constrictus* is more resistant than *Gigaspora margarita* to attack [46]. It has been suggested that hyperparasitic fungi may play a role in controlling VA fungal flora in the soil and, according to Daniels and Menge [46], the use of hyperparasitized VA mycorrhizal inoculum may explain some of the erratic results obtained in tests with VA fungi.

4.4. *Host genotype*

VA mycorrhizae are not always beneficial associations and this depends not only on the culture conditions but also on the species of fungus and host plant involved. Whilst VA fungi are known to vary in their ability to infect and transfer P to the plant, very little is known of the role of the host genotype in the expression of VA mycorrhizae. The efficiency of a same VA fungus can vary markedly between different species of host plant so that certain host–fungus associations are more effective than others [117, 169]. Skipper and Smith [204] suggested that the specific cultivar–fungal response was dependent on soil pH. Menge *et al.* [128] attributed these variations in mycorrhizal dependency to the differing ability of plants to absorb P from low P soils but other characteristics inherent to plants may also be determinant (Section 3.1.3). Response to VA mycorrhizae can also vary within a plant species; in screening experiments Bertheau *et al.* [29] obtained positive, negative or no response to VA infection in wheat depending on the host cultivar and irrespective of infection levels. Similar observations have been made on maize lines [83] and on *Vigna unguiculata* cultivars; Table 6 shows the effect of inoculation of three VA fungi on growth of two cultivars. Whilst growth is stimulated by all three fungi in one cultivar (58-185) the other only responds to infection by *Glomus* E₃ and *Glomus mosseae* but not *Glomus epigaeus*. O'Bannon *et al.* [155], on the contrary, did not find such varietal effects in alfalfa, despite the differences in dormancy, hardiness and area of adaptation of the different cultivars.

5. Agricultural significance of VA mycorrhizae

5.1. *Inoculation experiments*

Experimental studies on the effect of VA mycorrhizae on plant growth only began in the 1960's. These were generally confined to pot experiments in a small volume

Table 6. Growth responses of two cultivars of *Vigna unguiculata* to inoculation with *Glomus mosseae*, *Glomus epigaeus* and *Glomus E₃* in sterile soil (Ollivier, Bertheau, Diem and Gianinazzi-Pearson, unpublished data)

	cv 58-185		cv Bambeý 30	
	Average shoot dry wt. (g plant ⁻¹)	Root segments infected (%)	Average shoot dry wt. (g plant ⁻¹)	Root segments infected (%)
Control	0.75 ^a	0	1.10 ^a	0
<i>G. epigaeus</i>	1.47 ^{ab}	40 ^a	1.28 ^a	32 ^a
<i>Glomus E₃</i>	2.40 ^c	91 ^b	2.24 ^b	89 ^b
<i>G. mosseae</i>	1.71 ^{cb}	81 ^b	1.99 ^b	85 ^b

50-day old plants, all inoculated with *Rhizobium* strain CB 756

Soil: pH (KCl): 6.2; total P (ppm): 79; available P, Olsen (ppm): 10

Values followed by the same letters in columns are not significantly different ($P = 0.05$)

of sand or sterilized soil, so that during a decade little was known about the effect of an introduced VA fungus on plant growth in the presence of a natural soil microflora and competition from indigenous VA fungi. Knowledge is still very limited, in the absence of suitable techniques, of the competitive ability of introduced pre-selected VA fungi in natural, non-sterilized soils.

The success of VA inoculation under natural conditions depends on many factors including establishment of introduced VA mycorrhizae, crop management, production of inoculum and up to now greater success has been obtained with nursery-raised perennials, which are often produced in disinfected soils, than in field-sown annuals.

5.1.1. Nursery-raised perennial plants. A number of perennial plants particularly citrus and forest tree species cannot apparently develop normally unless the roots become mycorrhizal and it has been suggested that a large number of species of hardwood seedlings have an obligate dependence on VA mycorrhizae for normal growth. Stunting of citrus seedlings in fumigated nurseries soils can be corrected by VA inoculation [113, 125, 213] and some VA inoculation experiments have also been applied to improve growth of important forest tree species [35, 40, 108, 114, 119].

These plant species are normally raised in fumigated or steamed soil in nurseries, so that VA inoculation at this stage can ensure good infection before transplanting the seedlings in the field. Under these conditions, VA inoculations should have a great possibility to succeed. The importance of VA mycorrhizae for nursery production of seedlings has been reviewed by Menge *et al.* [125] for citrus and by Kormanik *et al.* [114] for hardwood species.

5.1.2. Field-sown annual plants. More than twenty VA inoculation experiments have been attempted up to now to study the effects of artificial inoculation on growth of annual crops growing in non-sterile soils. Mosse *et al.* [144] first

demonstrated that beneficial effects of VA mycorrhizae on pre-inoculated onions were maintained after transplanting into unsterilized field soil and almost simultaneously, increased onion growth and corn yield were reported after direct inoculation of unsterilized soil at the time of sowing [100, 142].

Important growth responses have also been obtained in wheat, maize and alfalfa seedlings inoculated with selected VA fungi before transplanting into field soils with low levels of available P [11, 112]. These and subsequent experiments raise the hope of practical applications; however, only limited extrapolations can be made of results from pot or transplant experiments to field conditions.

In spite of this and of the ubiquity of indigenous VA fungi in field soils, positive responses to inoculation of crops directly in the field have occasionally been obtained in non-disinfected soils. The increased growth and development of VA inoculated cotton obtained by Rich and Bird [179] is the first report of successful inoculation of a crop plant in the field. Black and Tinker [32] also increased potato yield in a field inoculation experiment using naturally infected soil which was applied in the furrows before planting.

Promising results have been obtained with soybeans in India [16], onion, barley and alfalfa in England [158] and alfalfa in Spain [10] using infected roots and soil as inoculum placed below seeds at the time of planting. Other successful field inoculation experiments have been reported with *Lotus pedunculatus* and clover in New Zealand using respectively soil pellets [85] or seeds pelleted with infested soil [165]. In each example indigenous VA fungal populations were either extremely low or very inefficient for plant growth. There is evidence that under natural conditions and in the presence of indigenous VA fungal flora, an introduced fungus can sometimes act as a regulator of plant productivity rather than as a stimulative factor for plant growth. Results obtained with inoculated field-grown soybeans in Senegal (Table 7) suggest a role of VA mycorrhizae in producing homogeneous rather than increased shoot and grain yields.

5.2. Mycorrhizal inoculum

One of the major obstacles to the establishment of pre-selected VA fungi in field-grown crops is to provide sufficient inoculum for large-scale operations. As long as

Table 7. Effect of field inoculation with *Glomus mosseae* on yield of soybean cv 44A-73 (Ganry and Diem, unpublished data)

	Yield (g m^{-2})		Coefficient of variation (%)		Root segments infected (%) ^a
	Grain	Shoot + pod	Grain	Shoot + pod	
Control	137	284	33	27	38
<i>G. mosseae</i>	138	339	30	14	48
Control + P	155	412	12	15	36
<i>G. mosseae</i> + P	185	416	4	8	42

^a 55-day old plants

P added as triple superphosphate: $60 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$

isolated VA fungi cannot be grown on synthetic medium, VA mycorrhizal inoculum has to be prepared by multiplication of the selected fungi in roots of susceptible host plants growing in sterilized substrates or soil. Pots or large containers are currently used to produce large quantities of inoculum under greenhouse conditions with maximum protection against contamination with other VA fungal species, pathogenic or hyperparasitic fungi.

5.2.1. Infectivity of inoculum. Plant growth responses appear to be related not only to the ability of VA fungi to translocate and transfer nutrients to the plant but also to the rapidity with which various inocula of the same fungus infect roots. Hall [82] found that an inoculum of root segments caused more rapid plant response to VA infection than spores, whilst Manjunath and Bagyaraj [121] reported that, on the contrary, mycorrhizal establishment was better with spores. According to Powell [164] and Johnson [107] hyphae from root segments would infect more rapidly since they infect immediately without forming pre-infection structures as germinating spores do. Differences in the extent of external hyphae developing from either root segments or spores may account for the large differences in inoculum infectivity ratings between different mycorrhizal fungi [167].

5.2.2. Types of inoculum. Inoculum can consist of infested soil, mycorrhizal roots with external mycelium and/or spores obtained from pot cultures. For certain fungi like *Glomus mosseae* pure suspensions of hyphae and spores can be collected from infected roots and soil. Several methods exist for recovering spores from soil: wet-sieving and decantation [70], flotation-adhesion [209] and the flotation-bubbling method [61]. For physiological studies, axenic mycorrhizal spores can be subsequently obtained by different sterilization treatments [131].

Different types of inoculum have been studied in order to facilitate manipulations and to maintain fungal infectivity (survival) during storage. Infested soil can be a highly infective type of inoculum, suitable for large-scale inoculations [32, 170, 179]. VA fungi have also been introduced into soil as soil pellets containing a mixture of infected root fragments, infested soil and clay [84]. Recently, in Senegal, Ganry and Diem (Table 7) successfully infected field-grown soybeans with an inoculum consisting of spores and infected roots of *Glomus mosseae* entrapped in beads of alginate gel [54].

There is almost no information on methods for long-term storage of mycorrhizal inoculum. Mosse and Hayman [143] claim that air-dried sievings of soil mycelium and infected root fragments can retain infectivity up to three years when stored at low temperatures. Jackson *et al.* [100] successfully used lyophilized mycorrhizal roots to inoculate soybean growing in non-sterile soil, but in more extensive tests Crush and Pattison [42] found that lyophilization led to greatly reduced infectivity of VA fungi. Recently preservation of VA spores by L-drying was proposed for routine long-term storage of mycorrhizal inoculum [215].

5.2.3. Methods to improve inoculum production. Different approaches have been

made to improve both qualitatively and quantitatively the production of inoculum:

Selection of the host plant. One way to massively produce inoculum is to grow a given VA fungus in roots of strongly mycotrophic plants. With this aim in mind, Bagyaraj and Manjunath [13] recently compared the mycotrophy of different host plants, and it appears from their screening that, under tropical conditions, *Panicum maximum* is a suitable host for culturing VA fungi in large quantities.

An original and potentially effective method for production of mycorrhizal inoculum has been recently suggested by Parke and Linderman [159] using the moss *Funaria hygrometrica* as a host for culturing VA fungi. The moss-mycorrhizal inoculum can easily be peeled off the soil surface so that the mycorrhizal pot culture may be left intact for successive crops of moss. Mosses have the advantage of supporting few pathogens that attack most higher plants and the merit of this method is to produce 'clean' rather than important amounts of mycorrhizal inoculum.

Host plant culture. Menge *et al.* [125] proposed a scheme for producing large amounts of inoculum for inoculation of citrus in nurseries. The pre-selected VA fungus, carefully maintained in pot culture in the greenhouse, is multiplied on a selected healthy host plant grown in sterilized soil in large containers. For phytosanitary reasons this host plant should have no diseases in common with the crop for which the inoculum (infected roots and soil) is intended. Another technique for mass production of inoculum which has recently been devised (Mosse and Thompson, quoted by Mosse and Hayman [143]) consists of growing an infected host plant in a nutrient flow culture system and harvesting the external mycelium and spores that are produced.

Selection of the VA fungus. This must take into account the efficiency of the VA fungus, its ability to produce large amounts of inoculum and the facility with which the latter can be manipulated. A fungus which seems to meet these requirements is *Glomus epigaeus* recently described by Daniels and Trappe [48]. Its efficiency on a wide range of host plants, the ability to produce abundant sporocarps on the soil surface, and the ease with which these can be harvested and stored make it a commercially interesting fungus [47]. However, *Glomus epigaeus* appears to be adapted to cooler climates [49] and research is needed for fungi or similar potential in tropical soils.

5.3. Inoculation techniques

Since annual crops are generally sown directly whilst perennial crops are often transplanted into the field, inoculation techniques will vary: perennials can be pre-infected in nursery soils whilst for annuals inoculum must be introduced into soil either directly or by pelleting of seeds.

5.3.1. Pre-inoculated transplants. A number of perennial tropical crops like coffee, tea, cocoa, rubber, oil palm and citrus are normally raised in nurseries. Seedlings

can be pre-inoculated in disinfected soils or substrates and transplanted into the field when the roots are heavily infected [125]. Pre-inoculation at the nursery stage may also be usefully applied to tree species used in arboriculture or for afforestation (see Kormanik *et al.* [114]). The transplants could act as centers of infection for adjacent plants if the VA fungi are able to successfully compete with indigenous populations. As nothing is known of this aspect of VA mycorrhizae, the necessity for repeated inoculation after transplant should be investigated.

5.3.2. Pelleting seeds with inoculum. Selected VA fungi have been successfully introduced into soil with annual crops by pelleting seeds with mycorrhizal inoculum [84, 165, 211]. Inoculum, consisting of root pieces and/or spores, can be coated onto seeds using inert additives, such as perlite or methyl cellulose [63, 86, 211]. The pelleted seed method would be a convenient method of inoculation in practice but according to certain reports [100, 125] the effectiveness of the inoculum is reduced because it is not in an area of root proliferation. Possible harmful effects of pelleting on seed germination should also be taken into account [84].

5.3.3. Direct inoculation of soil. Inocula in the form of soil pellets [84], lyophilized infected roots [100], infested soil and infected roots [1, 16, 32, 158, 170] or a slurry of infected sievings [226] are introduced into the soil either with or below seeds. These methods favour early infection of the actively growing main host roots by the introduced VA fungus rather than by the more dispersed propagules of indigenous VA fungi. Comparing different methods of direct inoculation of two VA fungi, Hayman *et al.* [94] found that plants inoculated in furrows with crude or fluid-drilled inoculum applied with the seed were most infected. They suggested that fluid drilling could be a suitable technique for field inoculation because smaller quantities of inoculum are needed and inocula such as rhizobia can also be incorporated for legumes.

5.3.4. Concluding remarks. The problem of establishment of selected VA fungi in the field is not only one of introduction of inoculum but also one of its survival and spread in the face of competition from indigenous VA fungi.

Based on results obtained in greenhouse experiments, Powell [166] calculated that even a mycorrhizal fungus having a fast rate of spread would only move 65 m in 150 years which suggests that natural spread of VA fungi through soil is probably slow. According to Powell the movement of topsoil (and VA fungi) by soil erosion, worm activities or agricultural machinery can contribute to the spread of VA fungi. The method used by Rich and Bird [179] in their successful field inoculation trials is an example to illustrate the high effectivity of the displacement of infested soil to soil lacking VA fungi.

An alternative field inoculation technique could therefore consist of creating a few reservoirs for infection in the same field and then spreading the infested topsoil over the surrounding areas. This proposed '*in situ* multiplication-propagation'

method is somewhat similar to that used for the multiplication of *Azolla* to fertilize rice fields (Watanabe, this volume).

The soil in the area to be infested can be previously fumigated to ensure good multiplication of the selected VA fungus, which would be cultured using a strongly mycotrophic host plant provided with an abundant fine root system (Sudan grass, Guinea grass . . .). When soil and roots are well infected, propagation of the fungus in the top soil can be carried out using agricultural machinery. The major advantages of this method are that *in situ* mass production eliminates problems of inoculum conservation and transport, inoculum should be highly infective due to the abundant freshly infected roots with extra-matrical hyphae and spores, and the adaptation of a given efficient VA fungus to characteristics of the field soil can be determined before its use.

6. Use of VA mycorrhizae as biofertilizers for tropical plants

The beneficial effect of VA mycorrhizae on plant growth is particularly spectacular in P-deficient soils and because of the generally low availability of P in tropical soils, the potential for the exploitation of VA mycorrhizae in agriculture seems to be much greater than in temperate soils. Cornet and Diem (unpublished data) found that seedlings of *Acacia raddiana* failed to grow in a sterilized soil from Senegal unless they were mycorrhizal or supplemented with a soluble phosphate source. In these conditions, they grew well even though they were not nodulated. This suggests that in some tropical soils P deficiency may be even more important than N deficiency as a factor limiting plant growth.

As Black [31] points out, VA mycorrhizae could be important factors for increasing plant productivity in developing countries for several reasons: the intrinsically low availability of P or high P-fixing ability of many tropical soils, the difficulties for locally manufacturing soluble phosphate fertilizers and the high cost of importation, and the mycotrophic habits of many tropical plant species of economic importance especially legumes. Jehne [104] examined some aspects of managing VA mycorrhizae for enhancing nutrition, growth and productivity of tropical pastures. However, as Hayman [91] emphasized, 'undue faith in mycorrhizae as an agriculture elixir should be tempered by a realistic appraisal of which aspects might best be exploited on a practical scale', the last sections of this review are therefore devoted to an assessment of the limitations, potentialities and research needs for applications of VA mycorrhizae in agricultural practices in the tropics.

6.1. Limitations

6.1.1. Lack of inoculum. Because of the difficulties in obtaining important quantities of inoculum, practical VA inoculation is at present provisionally

restricted to the nursery level. Two major problems remain for annual crops: the massive production of high quality inoculum and inoculation techniques applicable on a field scale [91].

Mosse and Hayman [143] calculated that the amount of inoculum required to inoculate 1 ha (2,500–8,000 kg) is impractical to produce, handle or transport. Thus as long as VA fungi cannot be cultured *in vitro* or suitable techniques for field inoculation have not been developed (Section 5.3.4), the application of VA mycorrhizae for improved production of annual crops will be severely limited.

6.1.2. Competition with indigenous VA fungi. The success of field inoculation with a given VA fungus depends not only on the nutrient status of a soil (e.g. P levels) but also on an ensemble of factors controlling infection and establishment of the introduced efficient fungus. Inoculation with specific VA fungi has in fact been successful in field soils where indigenous VA fungal populations were either extremely low or very inefficient for plant growth (Section 5.1.2).

Density and competitiveness of indigenous VA fungal populations in the soil are important limiting factors for the introduction of new species of VA fungi. In the situation that the indigenous fungi are efficient, their effect on plant growth would mask that induced by the introduced fungi, but even if the former are less or not efficient, their high population in the soil could be so important that they readily infect most of the host root system preventing subsequent infection by the more efficiently introduced fungi. Unfortunately very little is known concerning the competition between VA fungi for infection sites in roots.

In pot inoculation experiment with *Glomus monosporus* and *Glomus fasciculatus*, Abbott and Robson [1] found that there was no antagonistic effect by any of the introduced fungi on early stages of infection by the indigenous fungi and the VA fungi which were most efficient (*G. monosporus*) produced more mycorrhizae at an earlier stage in plant growth than the less efficient fungi. Generally, in the case of pot experiments with efficient VA fungi in non-sterile soil, it is probable that the quantity of inoculum introduced into the soil is so great that early infection of the root system occurs and its propagation through the host tissue is favoured by the pot-imposed restrictions on plant growth [99]. This is probably one of the reasons for which extrapolations of pot experiments to field trials can lead to discrepancies in the mycorrhizal effects obtained.

6.2. Potentialities

The potential benefits of VA inoculations concern a wide range of tropical plant species normally raised in nurseries before transplanting VA inoculations could be extended to industrial perennials of great importance in the tropics such as cocoa, coffee, tea, rubber or fruit trees. *In vitro* vegetative propagation techniques are being increasingly employed for the rapid reproduction of high quality, healthy plants of a wide variety of temperate and tropical species. The precocious

inoculation of such plants with efficient VA fungi during their multiplication *in vitro* is a recent promising perspective for the use of VA mycorrhizae [134].

Other situations where inoculation of VA fungi would most probably be highly beneficial is in the reclamation of marginal soils or of eroded, degraded or unstable habitats. Such soil situations are frequent in the tropics under both semi-arid and humid climates and investigations have recently begun into the potential role of VA mycorrhizae in revegetation practices in such severely disturbed habitats of semi-arid regions, where reduction of active inoculum in disturbed marginal soils seems to be an important ecological factor determining the subsequent colonizing plant species [133, 178]. Soil structure could be also controlled by external hyphae of VA fungi which contribute to stabilize e.g. dunes by aggregating sand grains [150].

7. Research needs and conclusion

Since interactions between the host plant, VA fungus, soil and climate will determine the mycorrhizal effect on plant growth, research for practical applications of VA mycorrhizae should bear in mind all these factors and therefore be directed towards finding appropriate host-VA fungus combinations adapted to well-defined soil types and climatic conditions.

Recent research has shown that varieties within a plant species can differ greatly in their response to inoculations with VA fungi, although the reasons for this genetical variation is yet to be determined. Screening of the most responsive varieties and species of crop plants is of utmost importance for mycorrhiza research in the tropics. Similar variations can be observed in the ability of different species or isolates of VA fungi to improve plant growth. However, a large number of inoculation experiments with tropical plants have employed VA fungi isolated from soils in temperate regions and not necessarily adapted to conditions occurring in tropical soils. There is therefore an urgent need to isolate VA fungi from different types of tropical soils and to assess their efficiency for plant growth, their ecological limits and their ability to develop and survive under adverse environmental conditions. This research for efficient tropical VA fungal isolates should also take into account their ability to produce large amounts of inoculum which can be easily manipulated and stored.

There are still large gaps in our knowledge concerning certain aspects of VA mycorrhizae which are relevant to temperate as well as tropical zones and which limit the advances that can be made towards practical applications. Amongst these are the inability to successfully culture isolated VA fungi, the lack of information concerning competition between VA fungi in soils and for infection sites, the relationship between inoculum potential and infectivity of VA fungi, and the influence of nutrients other than phosphate on mycorrhizal responses.

In spite of this and the need for further research into the more fundamental aspects of VA mycorrhizae (fungal physiology, host-fungus interactions, . . .), the utilization of this symbiotic association for improving production of certain, especially perennial crop plants and forest trees is coming to age as a potential practical reality.

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