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A SURVEY OF VESICULAR ARBUSCULAR

MYCORRHIZAE (VAM) WITH SPECIAL

REFERENCE TO TAXONOMY, PHYSIOLOGY

AND ECONOMICAL IMPORTANCE


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# A SURVEY OF VESICULAR ARBUSCULAR MYCORRHIZAE (VAM) WITH SPECIAL REFERENCE TO TAXONOMY, PHYSIOLOGY AND ECONOMICAL IMPORTANCE

BY

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# CHAPTER 1 : Introduction

## 1.1 General aspects on VAM

In 1885 the term mycorrhiza was used for the first time to describe the symbiosis of plant roots and fungi. Mycorrhiza literally means fungus root. Barea (1991) and Janerette (1991) classify mycorrhizae into five types:

i) **Ectomycorrhizae** where the fungal associate does not penetrate the root cells of the host, but only develop a Hartig net around the root cells;

ii) **Ectendomycorrhizae** or arbitoid mycorrhizae where the hyphae of the fungus form a Hartig net around the root cells as well as penetrating the root cells;

iii) **Ericoid endomycorrhizae** where septated hyphae of the fungus penetrate the host root to colonize the cortex cells intracellularly by forming coils. These mycorrhizae are confined to the Ericaceae;

iv) **Orchid endomycorrhizae** where septated hyphae also penetrate the host cortex cells by the formation of coils, but these mycorrhizae are confined to the Orchidaceae only, and

v) **Vesicular-arbuscular mycorrhizae (VAM)** where non-septate hyphae of the fungal partner penetrate the host cortex to form characteristic intercellular and intracellular vesicles and intracellular arbuscules. An external hyphal system which extends into the soil is also formed. No Hartig net is formed.

VAM are considered to be the most common mycorrhizal association in nature (Powell & Bagyaraj 1986; Barea 1991). These mycorrhizal associations are widely spread throughout the vascular plant kingdom, including major crops (Hayman 1982; Safir 1987). These VAM infected plants occur in natural soils over a broad ecological range, from aquatic to desert environments (Hayman 1981). However, they are rare or totally absent in the Brassicaceae, Cyperaceae, Commelinaceae, Juncaceae and Proteaceae, as well as in some members of the Capparaceae, Polygonaceae, Resedaceae, Urticaceae and herbaceous members of the Caryophyllales (Amaranthaceae, Caryophyllaceae, Chenopadiaceae and Portulacaceae). VAM

are absent in most of the ± 2000 woody species that are ectomycorrhizal (Kendrick 1992; Newman & Reddell 1987).

VAM-fungi (VAMF) are considered to be nonseptate zygomycetous fungi belonging to the genera *Glomus, Gigaspora, Sclerocystis, Entrophospora, Scutellospora* and *Acaulospora* in the family Glomaceae (Kendrick 1992; Barea 1991; Morton & Benny 1990). They are all obligate symbionts and have not been grown as pure cultures yet. Although VAMF are not particularly host specific, there is evidence that some would prefer to associate with certain host plants (Klein *et al*. 1992; Powell & Bagyaraj 1986).

The beneficial effects of VAM on enhanced nutrient uptake is important to those plants which have a coarse and poorly branched root system which lack root hairs (Howeler *et al*. 1987). VAM infected roots do not usually show visible morphological characteristics and can only be detected microscopically after clearing and staining the host roots.

## 1.2 History of VAM research

VAMF are beneficial to the plants for their ability to enhance nutrient absorption, especially in low fertility soils (Boerner 1990, Klein *et al*. 1992; Howeler *et al*. 1987). Classic studies into the fossil record of earlier plants showed VAM-like fungal colonizations of lycopsid and rhyniophyte rhizomes. These fossil plants are some of the most ancient of vascular plants, and their rhizomes are considered to be the oldest known from all fossil records. The fungal "spores" or the vesicles in these rhizomes look the same as vesicles formed by *Glomus* spp. in recent plants (Malloch 1987). These recent VAM forming fungi are evident in one of the most primitive orders of the recent Dicotyledonae, the Magnoliales (Safir 1987). Nicolson (1975) concluded that VAM probably developed with the vascular plants during evolution and that may be the reason why they are ubiquitous and have no host specificity.

Early studies on VAM was done only on the anatomy and to determine on which plants they occur. During these studies VAM was ignored by soil and plant scientists (Powell & Bagyaraj 1986). During the last two decades researchers became more interested in VAM,

since the discovery that these mycorrhizae play an important role in the phosphorus nutrition of the host plant. In this symbiotic association the fungus utilizes carbohydrates produced by the host plant, while the plant benefits from the increased absorption of nutrients like phosphorus and water through the extramatrical hyphae (Berch *et al.* 1991). These hyphae extend from the root surface into the soil as far as 8 cm or more away from the roots. They can absorb nutrients from much larger soil volumes than the zone surrounding a non-mycorrhizal root. This is particularly important for the absorption of nutrients of low mobility in the soil such as phosphorus, zinc and copper (Howeler *et al.* 1987).

Since researchers found that VAM could increase the phosphorus uptake from the soil by plants, they have been trying to manipulate this phosphorus-sparing effect in agriculture (Fitter 1987 and Cooper & Tinker 1978). The possible role of VAM in the biological control of root pathogens, biological nitrogen-fixation, hormone production and drought resistance, stimulated researchers to investigate also other disciplines of VAM. Today there is a broad interest in VAM research. The increase in reports on VAM studies and many conferences already held on the subject, prove that VAM can play a key role in plant nutrition and the cycling of nutrients in the ecosystem (Powell & Bagyaraj 1986; Barea 1991 & Mosse 1973).

Today most of the researchers are interested mainly in the prediction and measurement of plant growth reactions, inoculum production and the inoculation of agricultural crops with VAMF, thus the manipulation of this symbiosis to benefit man (Howeler *et al.* 1987). Great progress has already been made on the anatomy, taxonomy, physiology, phosphorus and mineral uptake, carbon cost, water relations, hormone production, ecology and biological interactions (Powell & Bagyaraj 1986). These disciplines contribute to research projects in trying to use VAM successfully in agriculture, horticulture, pomology and afforestation.

## 1.3 Purpose of this research

The continuous administration of fertilizers and pesticides to make the soil more fertile and pest free for agricultural use, is expensive, energy consuming and not always effective to enhance plant growth and health. Recently people are more aware of the conservation of nature, and much research is done to find biological methods to control pests and soil

impoverishment and degradation. Such biological control practices may reduce high costs of fertilizer and pesticides, especially where provision of food to the fast growing world population is of great importance. This phenomenon can also benefit the fast growing horticultural and forestry industries.

South Africa is considered as a third world country with a fast growing human population and a fluctuating economy. A great majority of the human population, as in the rest of Africa, are poor and practise agriculture for self-support. Most of the South African soils are exposed to long dry seasons and hot summers and most of its soils contain low levels of phosphorus. The winter climate is usually mild with occasional cold periods. The South African ecosystem is often under stress from severe droughts for several years. Other problems that contribute to the disturbance of the ecological regime in South Africa, is agricultural and industrial malpractice as well as erosion and the over exploitation of the already scarce water resources. South Africa has vast areas which are exposed to arid and semi-arid conditions. These areas are increasing in size, because of all the above mentioned factors.

The South African government spends huge amounts of money to subsidise the farming industry and to import certain foods to prevent starvation. The high costs of fertilizer and pesticides to increase food production, also contributes to this high expenditure. There is thus a great need to farm more cost effectively and to reduce the expenditure on fertilizer and pesticides. Large sums of money have already been spent to restore the former ecological regime, but the country is in great need of restoration practices that are more cost effective and less money consuming, and therefore agricultural and industrial malpractices should also be avoided. The question is: Can VAM become another effective biological control method against pests and soil impoverishment?

The main objective of this thesis is to relate the beneficial characteristics and aspects of VAM as identified from a wide range of research data to the enhancement of plant growth and productivity in the agriculture, horticulture and forestry enterprizes. More spesifically, this thesis will describe the manipulation of the beneficial characteristics and aspects of VAM such as for example the increase of nutrient absorption, and drought resistance to enhance

plant growth and crop production in the farming industries. Emphasis will be put on the anatomical, morphological, physiological and ecological aspects of this symbiotic relationship. The taxonomy of the fungal associate will, however, not be discussed intensively.

Articles, which are written on the beneficial effects of VAM, will be critically studied as far as possible to find whether the implementation of VAM in above mentioned practices, will indeed render the economy of third world countries such as South Africa more viable.

## 1.3.1 Research methods

This thesis is theoretically based and therefore the research into the subject will only be based on literature available on the subject. Most of the literature consulted were traced by using database programs such as Agricola, Science Citation, Biosis, Current Contents and Biological Abstracts. These programmes cite abstracts of articles written in most of the scientific magazines from over the whole world and are updated weekly or every second week. However, some books have been written on the subject of which some of the data are already outdated. Therefore, the referrence articles are mostly from 1980 onwards.

Most of the articles written, were published in overseas scientific magazines. Some of these magazines are also available in South Africa, thus most of the articles were collected from South African libraries. Others, not available in South Africa, were ordered from overseas libraries through an international-library service.

About 400 articles on the subject, were collected and studied. In addition six books were also studied. Of these articles 235 articles with relevant information are acknoledged in this thesis.

## 1.3.2 Hypothesis

There is evidence that some biological methods to control certain pests in South Africa are already successfully applied. The interest in VAM as a possible biological control to reduce fertilizer and pesticide application, should also stimulate South African researchers to

investigate the importance of VAM in agricultural, horticultural and forestry practices. Research on VAM in other countries showed that the benefits of VAM can be utilized as biological control methods in crop production as mentioned previously. It would appear that VAM can be manipulated to be successful substitutes for pesticides and fertilizer in agricultural, horticultural and forestry practices in future.

Hypothetically it can be stated that all the beneficial effects of VAM can be manipulated to enhance crop yield, tolerance to plant disease and drought stress, as well as a more efficient increase in the nutrient absorption of the plant. These fungi can thus be successfully introduced in areas with low fertility soil. This hypothesis will be tested at the hand of research data to be elaborated in this thesis.

# CHAPTER 2 : Taxonomy of VAM fungi (VAMF)

## 2.1 Introduction.

The taxonomy of VAMF will, for the purpose of this thesis, not be discussed in detail. Only a few general aspects which will help to classify and identify VAMF will be discussed in this chapter.

Researchers find it difficult to identify VAMF, because there is still much controversy on the taxonomy of these fungi. To make the task even more difficult is the fact that the different VAMF differ in their effectiveness to promote growth with changes in soil fertility, pH and and soil moisture. A specific species or strain of VAMF can even act differently in different host plants (Morton 1988). When researchers experiment with VAMF it is thus necessary that they know with which VAMF species they are working (Trappe 1982). Untill recently there was a great need for a thorough revision of the taxonomy of VAMF but Morton & Benny (1990) recently revised the taxonomy of this group of fungi.

The fact that VAM has not yet been isolated in a pure culture contributes to this problem referred to above (Powell & Bagyaraj 1986). Today researchers can make use of dichotomous keys such as that of Hall & Fish (1979) or synoptic keys such as that of Trappe (1982) and Hall (1986) to identify the different species of VAMF. These keys can constantly be updated or rewritten if new information of a specific species becomes available.

When the experiments of previous researches on VAM aspects are repeated, it often fails due to a lack of available living VAMF cultures or because the fungus used was not properly identified or described. Today only a few experimental cultures of VAMF are being maintained or preserved in personal or institutional herbariums. The identity and more information on the biology of VAMF have now become a necessity before experiments can be done successfully (Morton 1988).

On the positive side it can be said that 130 species of VAMF have already been identified,
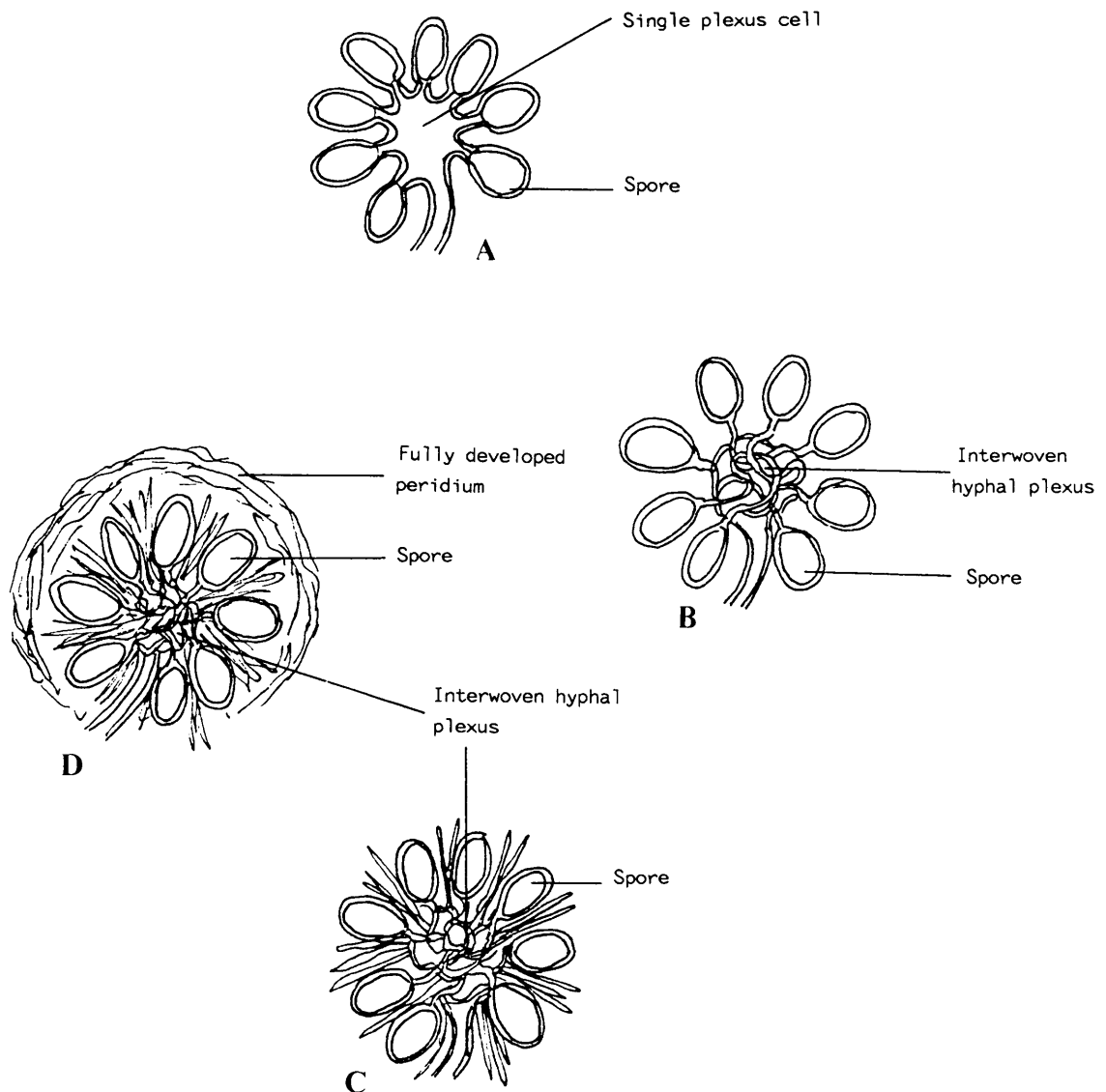
Fig. 2.1  The evolution of the sporocarps of *Sclerocystis* species:

A. The naked sporocarp of the most ancient species, *S. rubiformes*
B. The naked sporocarp of *S. taiwanensis*
C. The sporocarp of *S. liquidambris*
D. The sporocarp with a peridium of the most developed species, *S. ceremoides*

described and classified up to 1991 (Kendrick 1992; Simon *et al.* 1991).

## 2.2 Notes on the evolution of VAMF

The oldest specimens of VAM dates from as early as the Devonian period where plants such as species of *Asteroxylon* (Lycopsida), *Rhynia* (Rhyniopsida) and *Aglaophyton* were already infected by VAM-like fungi. These fungal fossils seem to be the same as modern VAMF but lack arbuscules and are estimated to be 370 years old (Malloch 1987; Barea 1991; Simon *et al.* 1993; Nicolson 1975). Nicolson (1975) and Stubblefield *et al.* (1987) suggest that there might have been collateral evolution between VAMF and land plants. According to Malloch (1987) and Herrera *et al.* (1993) VAMF might have been obligatory mycotrophs of the first land plants and Simon *et al.* (1993) suggest that VAMF were "instrumentals" to the colonization of land plants on earth.

Simon *et al.* (1993) pointed out that *Gigaspora* species diverged from *Scutellospora* species 353-462 million years ago and the family Acaulosporaceae and the family Gigasporaceae may have diverged from each other from a common ancester during the late palaeozoic era .

Morton & Benny (1990) imply that spores of *Glomus* and *Sclerocystis* species are absent in the fossil records because they did not form intramatrical spores. The lack of extramatrical spore fossils may also be due to decomposition. There is, however, evidence of fossils of sporocarps which resemble that of modern *Sclerocystis* species. In studies done by Stubblefield *et al.* (1987) VAMF-like structures and even arbuscule-like structures were also discovered in Triassic cycads such as *Antarcticycas* of Antarctica. These VAMF structures resemble that of VAMF in modern cycads. According to Morton & Benny (1990) it is accepted today that the Acaulosporaceae diverted from the Glomaceae. *Entrophospora* is considered to be the most advanced VAMF genus due to formation of spores in the saccule neck.

Interesting studies done by Wu (1993) on the ontogeny of the sporocarps of *Sclerocystis* species (Fig 2.1) showed that in the most primitive species the sporocarps were very simple. These sporocarps lacked a peridium and the spores were arranged around a single plexus

cell. In more advanced species the single plexus cell was replaced by interwoven hyphae with the spores arranged around these hyphae. At later stages of sporocarp evolution species showed that hyphal branches developed between the spores and that these hyphal branchlets later formed a peridium around the spores in the more recent species. Thus the evolutionary development of the sporocarp was from a simple, naked sporocarp to a sporocarp with spores arranged between complex interwoven hyphae and enclosed by a peridium.

## 2.3 Nomenclature

As for other fungi, the naming of VAMF is no easy task. The rules of the International Code of Botanical Nomenclature is also used to name and describe VAMF species. This concept will, however, not be discussed.

Most of the **holotypes** of VAMF today, are one or more of the following: one or more spores on microscope slides, preserved spores, dried or preserved sporocarps or, in some cases, illustrations and photographs of sporocarps and spores. These holotypes are kept in a few herbariums from over the world (Morton 1988). Some of the oldest holotypes are not suitable for taxonomic studies any more. This is because the description of the holotype was based on material that was either parasitized or was taken from the digestive system of rodents and which are now in a very poor condition (Morton 1988).

**Isotypes** (duplicate of the holotypes) are found mostly in other herbariums than those where the holotypes are kept. **Lectotypes** and **neotypes** of some VAMF are also available in some herbariums (Morton 1988).

Another problem with VAMF nomenclature is that some holotypes collected many years ago, consist of more than one VAMF species. Nowadays it is required that a type specimen of VAMF may only be of one single spore on a microscope slide to prevent confusion in future. A vial of spores of the same VAMF must accompany the holotype as isotypes. Preserving holotype spores in a permanent mountant can change the shape and size of the spores. To overcome this problem a black and white photograph of a fresh spore should accompany the holotype (Morton 1988). According to Morton (1988) a holotype may not be a living culture.

Valid names of newly described species of VAMF must be accompanied by a latin description. This description or prologue must then be published in a scientific journal. Descriptions of VAMF should be revised from time to time as new facts on these fungi become known (Morton 1988). The revision of previously described species led to the segregation of species of *Scutellospora* from those of *Gigaspora* (Walker & Sanders 1986; Simon *et al.* 1993). This was done because of the difference of germination characteristics, spore wall structures and auxiliary cell morphology. The discovery of a VAM fungus with vesicles that resembled those of *Acaulospora* but where most of the other characteristics differed, led to the description of a new genus, *Entrophospora* (Ames & Schneider 1979). A few studies showed synonymy between two taxa of VAMF when previous descriptions of these fungi were revised (Morton 1988).

## 2.4 Classification

The classification of organisms involves the grouping of organisms which appear to be similar, into taxonomic groups. Characteristics and relationships of organisms are used to put them into taxonomic groups. Changes in the classification often occur when new species or information are discovered.

At first all VAM forming species were classified in the order Endogonales, family Endogonaceae and genus *Endogone*, because it was accepted that all *Endogone* species form VAM associations with plants. However, today there is no evidence that any *Endogone* species form VAM (Morton 1988; Richards 1987). Morton & Benny (1990) divided the order Glomales into two suborders: the suborder Glomineae with the families Glomaceae (type family) and Acaulosporaceae, and the suborder Gigasporineae with the family Gigasporaceae. This division of the order Glomales is now generally accepted by researchers (Simon *et al.* 1993). The Glomaceae includes the genera *Glomus* (type genus) and *Sclerocystis*. The Acaulosporaceae includes the genera *Acaulospora* (type genus) and *Entrophospora*. The Gigasporaceae includes the genera *Gigaspora* (type genus) and *Scutellospora*. The only family under the Endogonales, the Endogonaceae (in rare occasions ectomycorrhizal), which at this stage are known to be saprobic and free-living, has only one genus, *Endogone*. Today

**1**
A
Sporiferous saccule
Budding spore
B
Spore
Parent hypha

**2**
A  B
C  D
Occlusion of spore wall

**3**
Spore
B
A
Bulbous suspensor-like hypha
Bulbous hypha
Projection

**4**
Peridium
Spore
Hyphal plexus

**5**
Spore
Projection
Germination shield
Bulbous suspensor-like hypha

**6**
Spore
Sporiferous saccule
Parent hypha
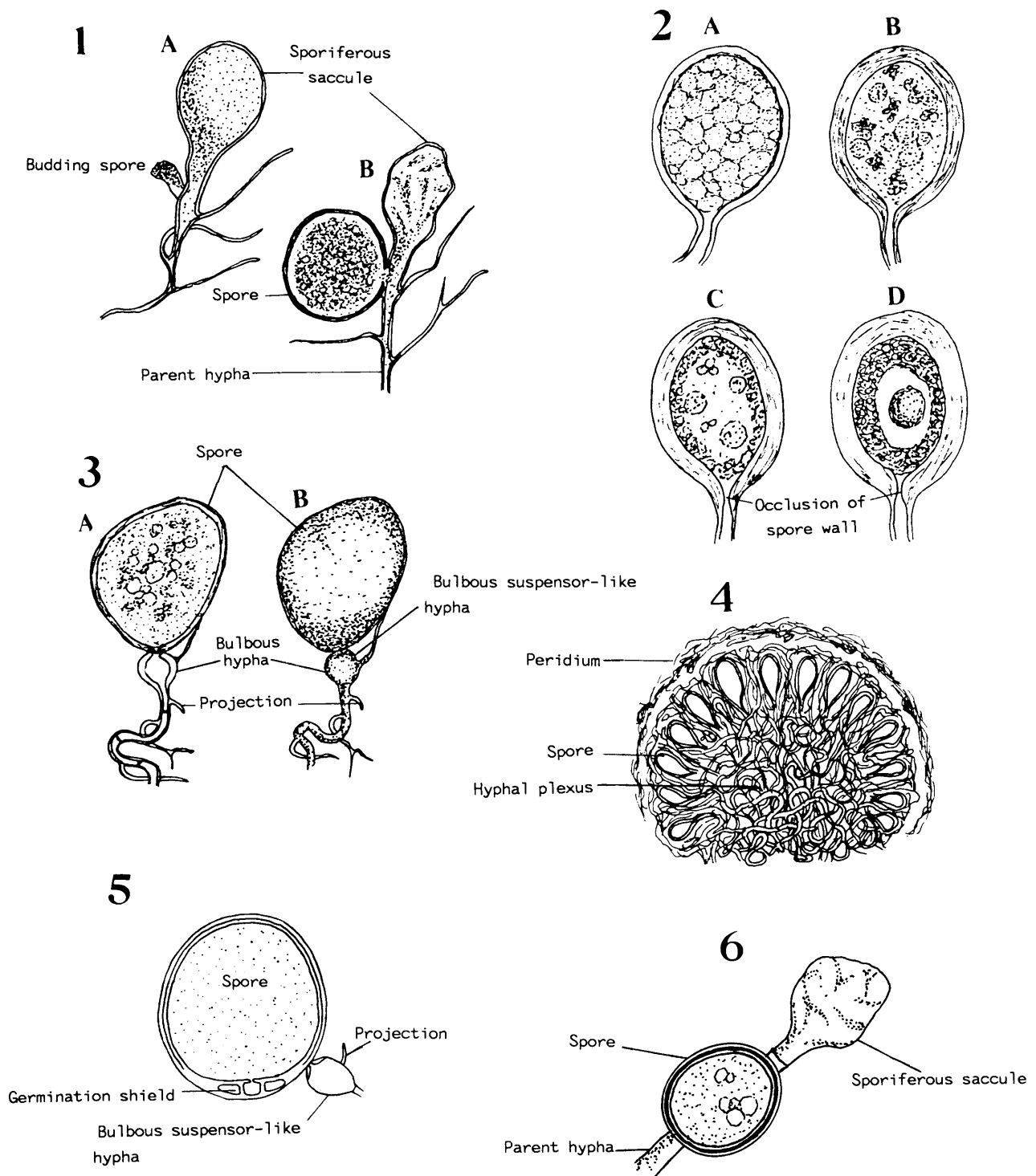
Fig. 2.2  Representative spores of VAM fungal genera
1. *Acaulospora*: A. Sporiferous saccule on hyphal tip with budding spore
            B. Collapsing saccule with maturing spore. Saccule contents migrate into spore
2. *Glomus*: A-D. Cross sections of maturing spores, showing the occlusion of the spore wall
3. *Gigaspora*: Spore on inflated bulbous suspensor-like hypha with lateral projections
            A. section; B. external view
4. *Sclerocystis* sporocarp in section
5. *Entrophospora*: Collapsed sporiferous saccule on hyphal tip, with mature spore which developed
            within the hypha
6. *Scutellospora*: Section of matured spore on bulbous suspensor-like hypha with projections. The
            germination shield can be seen on the bottom of the spore.—

we accept that VAMF are classified as follows:

Kingdom: Eumycota

Division: Zygomycotina

Class: Zygomycetes

Order: Glomales

Suborder: Glomineae                     Suborder: Gigasporineae

Family: Glomaceae          Family: Acaulosporaceae          Family: Gigasporaceae

Genera: *Glomus*              Genera:      *Acaulospora*              Genera: *Gigaspora*

*Sclerocystis*                    *Entrophospora*                    *Scutellospora*
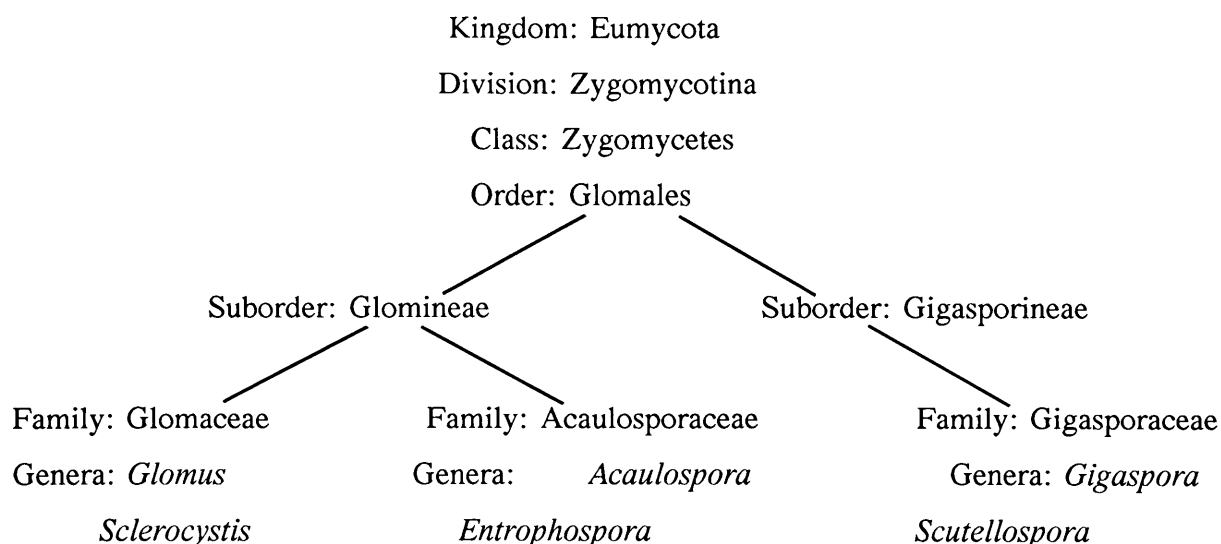
## 2.4.1 VAM fungal features for classification purposes

## 2.4.1.1 Spores

The spore features of VAMF are the main features used during taxonomic studies to classify and identifiy the fungi. VAMF usually form azygospores and large, thick walled chlamydospores in the soil. Azygospores may be derivatives of zygospores where the sexual reproduction process was not completed (Kendrick 1992; Morton 1988). Fig 2.2 show the differences between the spores of the six VAM fungal genera. Spore diameter and wall thickness as well as the way in which the spores are borne on suntending hyphae are also used during these studies.

## 2.4.1.2 Germination of spores

The observation of the development and germination of VAMF spores with the light microscope and electron microscope is confined to only a few studies. These studies showed that the germination of VAM fungal spores differs between genera. Features that can be used are for example the formation of a germination shield or not, and the way and position where

the germination tube penetrates the spore wall. There is thus a need for more information on this aspect before it can be effectively used during taxonomic studies. The fixation and subsequent sectioning of VAMF spores today, however, should lead to new research on these aspects. Changes in the characteristics of the spore wall and of subtending hyphae should receive more careful attention during further taxonomic studies (Morton 1988).

### 2.4.1.3 Vegetative Structures

Until recently morphological characteristics of the intramatrical phase of VAMF were not really used during taxonomical studies, due to the diversity of significant morphological characteristics of a specific VAM fungus in different hosts and growth media. Insufficient information of variations of intramatrical and extramatrical structures such as the diameter of the hyphae and wall thickness, the formation of vesicles and arbuscules and the formation of coils, limits the taxonomic value of these structures (Morton 1988). According to Morton (1988) some aspects of intramatrical hyphal morphology are already being used to distinguish between genera of VAMF. These characteristics include:

  i) the way in which hyphae branch;

 ii) vesicle occurrence and density as well as their formation pattern;

iii) projections and constrictions which appear on intramatrical hyphae;

 iv) the intensity in which intramatrical hyphae stain with certain staining and mounting
     media, and

  v) the diameter of intramatrical hyphae.

These characteristics of a specific VAMF species appear to stay the same in different environmental and host conditions.

### 2.4.2 Methods to study VAMF taxonomically

### 2.4.2.1 Collection of VAMF spores and other material

When one wants to do a taxonomic study of VAMF in a certain area, as many as possible spores should be collected from the soil around all the plants in that area. One must also

remember that certain VAMF species sporulate at different times of the year and that there will be a fluctuation in spore numbers during the year. This implies that the collection of the spores must be done throughout the year (Hall 1986). The factors which influence VAMF sporulation and spore numbers will be discussed in chapter 5.

Some VAMF such as species of *Glomus* and *Sclerocystis* can form epigeous sporocarps which are sometimes brightly coloured or hypogeous sporocarps which are usually difficult to observe. Most of these sporocarps are large enough to be seen with the naked eye. Larger sporocarps can be picked up by hand by thorough examination of large quantities of litter and topsoil. Smaller ones (smaller than 1 mm) as well as spores can be obtained by the wet sieving and decanting method described by Gerdemann & Nicolson (1963). With this method a slurry of soil is washed through a graded series of soil sieves with pores ranging in diameter from 2000 $\mu$m to 60 $\mu$m. The retained material can then be further processed by centrifugation or flotation to isolate the spores from most of the debris. The individual spores are then picked up from the remaining debris by using a Pasteur pipette or other devices while looking through a microscope (Hall 1986). According to St. John & Koske (1988) spore containing soil samples must be collected randomly.

The scanning electron microscope can also be used to obtain useful information about the surface of VAMF spores and other VAMF structures. Yao *et al*. (1992) and Koske & Walker (1985) described methods to prepare VAMF material for SEM studies.

## 2.4.2.2 Storage of spores and sporocarps.

Large sporocarps can be stored dry in herbariums or in small vials with lactophenol. Small sporocarps, portions of sporocarps and single spores can be mounted on microscope slides before storing it. Miracle mounting fluid, polyvinyl lactic acid or polyvinyl lactophenol and plain lactophenol are suitable mountants for VAMF. When lactophenol is used the edges of the cover slip should be sealed with nail varnish after mounting the VAMF spore to prevent it from drying out. The size and colour of VAMF spores may change in the mounting fluids, thus notes on these aspects of the spores should be obtained before mounting. There is also evidence that lactophenol acts as a good preservative against pathogens and is therefore an

excellent medium to mail spores and sporocarps to other countries (Hall 1986).

## 2.4.2.3 Sectioning of VAM fungal spores

Useful information can be obtained from thin sections of VAMFspores for transmission electron microscopy and light microscopy. According to Hall (1986) spores are usually embedded in glycolmethacrylate resin after using 3 % glutaraldehyde or 5 % acrolein as fixatives (24 h at 0°C). For obtaining good infiltration of the resin into the VAMF spores the spores can be punctured with a thin needle. However, when the spores are punctured the contents of the spores become somewhat disrupted. After sections are with an ultramicrotome and air drying them, it can be stained with 0,5 % aqueous Toluidine Blue (buffered to a pH of 4,4) and 0,05% Tripan Blue. The sections can then be mounted on microscope slides in Euparol or "miracle" mounting fluid.

## 2.5 Identification of VAMF

Morphological characteristics of spores are the most important for the identification of VAMF. Spores can either be compared with holotypes, isotypes, lectotypes and neotypes or they can be identified by matching the collected specimen with the original descriptions. Keys and illustrations are also helpful to identify different species. Several keys to identify VAMF up to the species level can be used, such as those published by Hall & Fish (1979), Trappe (1982) and Hall (1986). Keys to identify VAMF up to the genus level are more manageable and combine information such as the one published by Kendrick (1992).

The keys to identify VAM fungi are based mainly on the features of spores and subtending hyphae. According to Morton (1988), the following characteristics are most important:
A. Organization of spores:
    1. Aggregation of spores in host roots
    2. Aggregation of spores in sporocarps
B. Sporocarp morphology:
    1. Arrangement of spores
    2. Size of sporocarps

    3. Colour of sporocarps

    4. Shape of sporocarps

    5. Surface texture of sporocarps

    6. Peridium of sporocarps

    7. Glebal hyphae of sporocarps

    8. Substances exuding from sporocarps when cut

C. Morphology of intact spores:

    1. Colour of spores

    2. Size of spores

    3. Sinking or floating ability of spores in solvents such as sucrose

    4. Shape of spores

D. Spore wall structure:

    1. Wall type

    2. Wall ornamentation

    3. Number of wall layers

    4. Grouping of wall layers

    5. Position of wall layers in the group of wall layers

    6. Reaction of walls to Melzer's reagent

E. Factors modifying spore morphology:

    1. Chemical effects

    2. Mechanical effects.

F. Morphology of subtending (sporogenous) hyphae:

    1. Number of hyphae per spore

    2. Colour of hyphae

    3. Shape of hyphae

    4. Number of wall layers in the hyphal walls

    5. Diameter of hyphae or suspensor cells

    6. Features of the pedicel in *Acaulospora*

G. Occlusion of spore walls when the spores part from the contents of the subtending hypha

H. Morphology of the sporiferous saccule:

    1. Colour of saccule

    2. Shape of saccule

Table 2.1  The main features which distinguish the genera of VAMF from each other.

| Genus | Gigaspora | Acaulospora | Sclerocystis | Glomus | Scutellospora | Entrophospora |
|---|---|---|---|---|---|---|
| Spore type | Azygospores | Azygospores | Chlamydospores | Chlamydospores | Azygospores | Azygospores |
| Single or in sporocarps | Singly | Singly or rarely in sporocarps | Always in sporocarps | Singly or in sporocarps | Singly | Singly; rarely in sporocarps |
| How spore is borne | Bulbous subtending hyphae with projections | Pedicel-like hyphal branch; main hypha with saccule on tip | Single subtending hypha | Single subtending hypha | Bulbous subtending hypha | Spore form within hypha with saccule on hyphal tip |
| Spore colour | From hyaline to black | From hyaline to red-brown | Shades of brown | Hyaline, yellow, brown, black | Orange, red or dark brown | Mostly hyaline, sometimes yellow or orange-brown |
| Spore shape | Globose or sub-globose ellipsoid | Globose ellipsoid rarely elongated | Oblong to clavate or sub-globose; rarely ellipsoid | Globose, ellipsoid or irregular | Obovoid, ovoid, ellipsoid, oblong, fusiform and clavate | Globose, sub-globose or ellipsoid |
| Number of layers in spore wall | 2 or more layers | 2 or more layers | 1 or 2 layers | 1 to many layers | 2 to 3 layers | 2 or more layers |
| Spore size | 200-600$\mu$m long | 100-400$\mu$m long | Up to 125$\mu$m long | 20-400$\mu$m long | 100-300$\mu$m long | 50-150$\mu$m long |
| Vesicles and arbuscules within host plant | arbuscules only | Arbuscules and vesicles | Arbuscules and vesicles | Arbuscules and vesicles | Arbuscules and vesicles | Arbuscules and vesicles |

3. Diameter of saccule

4. Walls of saccule.

I. The hyphal distance between spore and sporiferous saccule

J. Non-morphological characteristics:

1. Biochemical characteristics

2. Immunological characteristics

3. Genetic characteristics.


## 2.6 Features of the Genera

The important features of each VAMF genus were extracted from published papers of Hall (1986), Kendrick (1992), Gerdemann & Trappe (1975) and Morton (1988) and combined to give the following short descriptions (Table 2.1).


### 2.6.1 *Glomus*

*Glomus* is the commonest genus of all VAMF. More than 50 species have already been described.

The species produce globose, ellipsoid or irregular chlamydospores, 20 - 400 $\mu$m in diameter. It is usually formed in the soil near the plant roots or at the soil surface but in very rare occasions within host roots. The spore can be hyaline, yellow, brown or black.

Single spores are formed on single subtending hyphae, which can be constricted at the point of attachment of the spore.

The spore walls are usually very thick (up to 55$\mu$m) and consist of one to many layers. The wall ornamentation is not very complex.

The spores can also be produced in large sporocarps of 1 - 2 mm across. In only a few species the sporocarps are less than 1 mm across. The sporocarp diameter rarely varies within the same species. The sporocarps are usually irregularly-shaped, although they are

usually rounded on the upper surface. The spores can be arranged randomly or arranged in discrete clusters between interwoven glebal hyphae. In some species the spores are radially arranged from the base of the sporocarp. In a few species the sporocarps have distinctive features. In some sporocarps the spores are exposed. In a few other species the surface could be covered with irregular-shaped thin walled swollen cells. The interwoven hyphae around the sporocarp (peridium) may give a cotton or felt texture to the sporocarp.

A peridium may be present or absent or may appear as patches over the sporocarp surface. The hyphae of the peridium can be tightly or loosely woven. Sometimes erect hyphal tips can be observed amongst the tangled hyphae of the peridium. The sporocarps are formed mostly hypogeous in the soil or in leaf litter and seldom on the soil surface.

The germination of spores can either be via old subtending hyphae or more rarely through the spore wall.

All species form endomycorrhizae with both arbuscules and vesicles in the host root.

### 2.6.2 *Gigaspora*

Individual azygospores with a range of 200 - 600 $\mu$m diameter are formed. Spores can be globose, sub-globose or ellipsoid, but a few species form elongated or irregular spores. The spore colour can vary from hyaline to black.

The spore walls can be up to 20 $\mu$m thick and are usually formed by two or more layers. The outer layer may be ornamented.

Spores are formed singly on the end of a bulbous subtending hypha which can be pyriform in shape and bearing one or more short lateral projections (possible remains of collapsed hyphal branches of unknown function).

Species are never sporocarpic.

Germination can take place in two ways. Firstly germ tubes can arise from compartments that develop between two inner wall layers and then penetrate the outer spore wall. Secondly the germ tube arise from between warty material laid down on the inner layers of the spore wall and then penetrate the spore wall (no compartments are formed).

The majority of species form endomycorrhizae with arbuscules, but no vesicles are formed in the host root.

Most species form highly ornamented vesicles, 20 - 50 $\mu$m in diameter, borne singly or in clusters of 12 or more on single coiled hyphae in the soil outside the host root.

### 2.6.3 *Acaulospora*

Spores are globose or ellipsoid and occasionally elongated, are 100 - 400 $\mu$m long with a maximum breadth of 250$\mu$m. The spore colour ranges from hyaline to red-brown.

Spores are borne singly in the soil and are usually formed on the side of a hypha. On the tip of the hypha there is a thin walled, terminal vesicle or sporiferous saccule. The saccule later collapse as the contents of the saccule migrate into the maturing spore. The saccule remains as a non-functional remnant on the subtending hypha of the spore. The colour and shape of the sporiferous saccule are not taxonomically important.

The spores are borne on a pedicel-like branch of the parent hypha. There appears to be a pore between the spore and the subtending hypha. This may be due to the occlusion (closing) of the spore walls where the spore is attached to the subtending hypha after spore maturation.

The spore wall is amorphous, is two or more layered and can be up to 12 $\mu$m thick. In some species the outer layer of the spore wall can have a very complex ornamentation.

Sporocarps are formed by only two known species.

Germination takes place via peripheral compartments similar to that of *Gigaspora.*

Endomyccorhizae with both arbuscules and vesicles are formed in the host root. The vesicles are lobed and taxonomically important.

### 2.6.4 *Sclerocystis*

The chlamydospores are always formed in sporocarps. The spores can be up to 125 $\mu$m long and up to 50 $\mu$m wide. The colour of the chlamydospores are mostly in shades of brown and are oblong to clavate in shape. A few species have sub-globose spores and are in rare occasions ellipsoid, ovoid or obovoid.

One to two wall layers are present.

The sporocarps are usually less than 1 mm across and their shape can be globose, sub-globose to ellipsoid and in some cases pulvinate or cushion-shaped. The spores are arranged in a single layer around a single plexus of glebal hyphae. The glebal hyphae are very orderly arranged within the sporocarp. The central core of thin hyphae contains no spores. The epigeous sporocarps can be produced in masses of up to several centimetres in diameter on the surface of the soil or on leaves, twigs or mosses.

A peridium may be present around the sporocarps of some species.

Endomycorrhizae with arbuscules and vesicles are formed in the host root.

### 2.6.5 *Entrophospora*

The mostly globose or sub-globose (sometimes ellipsoid) spores are mostly yellow or orange brown and may be hyaline in rare occasions. Spore diameter ranges from 50 - 150 $\mu$m.

The spores are produced singly on a hypha. A terminal sporiferous saccule is formed at the tip of the parent hypha and then the spore is formed inside the parent hypha just below the sporiferous saccule. The contents of the saccule migrate into the maturing spore. The sporiferous saccule can be ellipsoid or obovoid. The saccule wall can be 2,5-10 $\mu$m thick and

is of taxonomic value.

The spore wall is usually amorphous and formed by a thick ornamented outer and one or more thin inner layers. The wall of the parent hypha usually stays intact to the developing spore wall. When the spore mature it appears as a thick colourless outer layer.

In very rare occasions the spores are formed in small sporocarps.

True arbuscules and vesicles are formed within the host root.

### 2.6.6 *Scutellospora*

Spores can be from 100 - 300 $\mu$m long and may be orange, red or dark brown to black. The spores are usually obovoid, ovoid or ellipsoid but some species have oblong, fusiform and clavate spores.

One newly described species has unusually large globose to sub-globose spores with a diameter of 350-700 $\mu$m and a prominent germination shield (Walker & Diederichs 1989).

Spores are borne singly on bulbous "suspensor" cells which can have a pyriform shape. The wall of the "suspensor" cells are continuous with the thin hyaline outer layers of the spore wall.

The spore wall is amorphous and usually has two or three layers but up to six layers have been observed in some species.

True arbuscules and vesicles are formed within the host root.

### 2.7 Conclusion

One must keep in mind that it is necessary to know the VAMF species to be used in experimental studies. Different VAMF behave differently in certain host-fungus relationships

and it is therefore necessary to use the same VAMF species when the experiments are repeated. Today many experiments fail or the results are not accepted because of inaccurate identification or descriptions of VAMF.

# CHAPTER 3 : The Morphology of VAMF in host plants

## 3.1 Introduction

Vesicular-arbuscular mycorrhizae (VAM) are formed when the host roots are penetrated by hyphae from germinating spores or viable propagules of the fungal associate. The spores are the fungal reproductive structures which usually consist of one or more than one cell.

As already mentioned, there is evidence that VAMF are able to support the host plant in better nutrient and water absorption as well as enhancing host plant growth and health. This is also the case with many major crops. The incorporation of the VAM symbiosis in agriculture, horticulture and afforestation, requires a thorough understanding of the events that occur during the host-fungal relationship. This knowledge can be used to determine the effectivity of the host-fungus relationship which is important for the selection of VAMF to enhance growth responses in the host plant. The rate of infection and colonization of VAMF in the host plant is important means to determine the effctiveness of a VAM realtionship. Mathematical models are often used to provide information on the rates of VAM infection and development under certain environmental changes which may affect VAM (Buwalda *et al.* 1982; Bécard & Fortin 1988). Brundrett *et al.* (1985) and Toth *et al.* (1991) also developed procedures for studying the early stages of VAM infection and VAM formation.

There is no macroscopic alterations of the normal morphology of the host root when VAMF invade the host root, such as those alterations which accompany other types of mycorrhizae for example as the case with ectomycorrhizas. The only way to be sure that the host root is infected by VAMF, is by microscopic examination. Superficially they resemble uninfected roots. However, VAM infection does show a yellow pigmentation in the roots of onions, other species of the Liliaceae and maize (Carling & Brown 1982; Powell & Bagyaraj 1986; Janerette 1991).

Those plant species which lack fine root systems and intense root hair development, are more mycorrhizal dependent and therefore more frequently infected. It is also accepted that VAMF

replace certain root hair functions such as mineral nutrient absorption (Bonfante-fasolo 1986).

According to Carling & Brown (1982) and Cooke *et al*. (1992) the structures that are produced by VAMF within host roots include:

  i) an intramatrical hyphal system in the root continuous with a extramatrical hyphal network extending into the soil;

 ii) intracellular arbuscules - generally accepted as the place of nutrient transfer between the symbionts, and

iii) intercalary or terminal vesicles accepted as VAMF storage organs.

Berch *et al*. (1991) identify two types of VAMF depending on the morphological features of the hyphae: The more common coarse VAMF which form coarse extra- and intramatrical hyphae and the finer VAMF with fine hyphae which can cope better under stressing environmental conditions.

Mycorrhizal infections occur only in the epidermis or ectodermis as well as in the cortical parenchyma of young host roots. The hyphae of VAMF do not penetrate the endodermis and are therefore not present in the vascular cylinder of the host plant. These hyphae are also absent in the meristematic regions and older root tissues where secondary growth has already taken place (Bonfante-Fasolo 1986).

Studies to determine the length of extramatrical hyphae compared to the length of the infected host root were conducted by Abbott & Robson (1985). They found that the length of hyphae differed for each fungus-host relationship and can be influenced by environmental conditions. These extramatrical hyphae are considered as root hair extensions. The differences in the distribution and amount of hyphal material of different species of VAMF in the soil may be important when selecting VAMF for agriculture and forestry. The amount of extramatrical hyphal material is sometimes a mean of the effectiveness of the fungi for nutrient uptake. Thus the more or longer external hyphae are, the better the nutrient uptake.

## 3.2 Methods for studying the morphology of VAM.

As already mentioned earlier in this chapter, the VAMF structures can, in most cases, only be detected through microscopical investigation after the staining of the fungal material within the host plant root.

The most popular method to stain roots for the examination of vesicles and arbuscules of VAMF is the method described by Phillips & Hayman (1970). According to their method root segments can be fixed in FAA or 50% ethanol. The infected roots are cleared by heating it in 10% KOH for 1 hour at 90°C. This procedure removes the host cytoplasm and nuclei, and the vascular cylinder becomes visible. The roots are then rinsed in water and acidified with diluted HCl. The infected root pieces are then stained by simmering it for 5 minutes in 0,05% trypan blue in lactophenol. The excessive stain is removed with clear lactophenol. The root segments are mounted on microscopic slides, temporarily in lactophenol or permanently in PVA- (polyvinyl alcohol resin) lactophenol. Then it can be studied with a light microscope. Heavily pigmented roots can also be cleared by using KOH and hydrogen peroxide prior to staining. Sodium hypochlorite or nitric acid are also suitable for clearing roots.

Koske & Gemma (1989) aimed to eliminate as many as possible of the toxic and offensive compounds from the fixative and staining solutions. Their modified procedures exclude the use of FAA as a fixative and reduce the percentage of diluted KOH from 10% to 2,5%. They also shortend the time for KOH treatment to a maximum of 30 minutes and used household ammonia as a substitute for hydrogen peroxide. The lactophenol used during the staining procedures can be substituted with acidic glycerol. The stained root pieces can then be stored in acidic glycerol for up to 16 months.

Most of the techniques presently used to detect VAMF structures within the host root, are destructive to the root samples and time consuming. Fluorescein diacetate (FDA) is often used as an indicator of acetylesterase and some proteolitic enzyme activities in metabolically active hyphae of certain fungi. FDA is a non-polar molecule which becomes hydrolysed in the presence of certain fungal enzymes. The fluorescein which accumulates in the cell
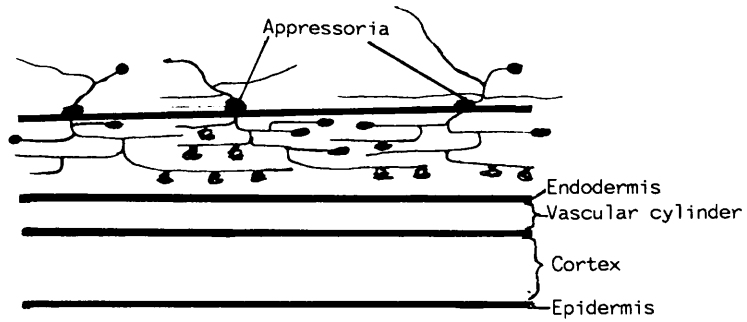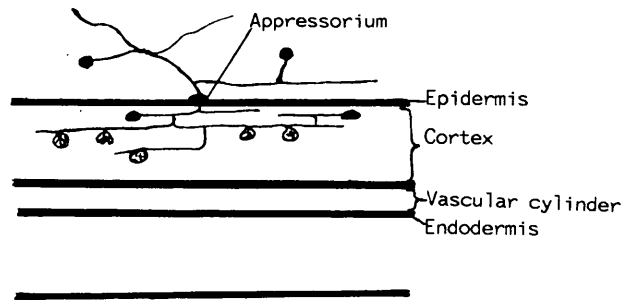
Fig. 3.1  A diagram of the infection units within the host root
        A. Single infection unit
        B. Infection units overlapping longitudinally

becomes fluorescent when excited with ultraviolet (UV) light. The hyphal tips, arbuscules and sporophores autofluoresce brightly with FDA treatment. All the other metabolic active VAMF structures do not fluoresce (Ames *et al.* 1982).

Another method to detect VAM structures is by using fluorescence microscopy on root samples stained with acid fuchsin and observed through certain filter combinations. After clearing and acid fuchsin staining, the root samples are mounted on a microscope slide. The roots are then examined through a Zeiss Jenamed 2 microscope with tungsten and UV lamps or other microscopes of the same magnitude capabilities and functions. For most investigations, particularly to determine if infection had occurred or to determine the intensity of VAMF structures, the examination of the acid fuchsin stained roots is satisfactory. Detailed structures of entry points (appressoria), coils and the mycelium are seen through focal levels. Vesicles show up very well but arbuscules are discernable only in the thinnest preparations with intensive infections (Merryweather & Fitter 1991).

## 3.3 The infection of VAM in angiosperm roots

According to Bonfante-Fasolo (1986) the infection of VAM develops in two stages:
i) the extramatrical phase with extramatrical hyphae and external vesicles or spores in the soil surrounding the host roots, and
ii) an intramatrical (intraradical) phase with intracellular hyphae, intercellular hyphae, arbuscules and vesicles and, in rare occasions, internal spores.

Intramatrical VAMF infection in host plant roots develops into discrete units which are connected to the extramatrical mycelium (Fig. 3.1 A en B). The infection units may or may not overlap longitudinally (Richards 1987; Sanders & Sheikh 1983; Barea 1991). The fungal structures in one of these infection units are in developmental or age sequences with the oldest structures nearest to the entry point or appressorium and the youngest structures near the hyphal apex. The intamatrical VAMF hyphae branch frequently to ensure uniform distribution of the fungus mycelium in the host root (Brundrett *et al.* 1985).

● Spore

——————→ Host roots

— — — — — VAM fungal mycelium

1mm



Fig. 3.2 Extent of the growth of extramatrical mycelium after VAM fungal spore germination, before the hyphae infect the host root

The hyphae of VAMF are usually nonseptate but degenerating hyphae may form septa which close off the dying parts. In some cases, however, thin septate lateral hyphal branches develop from the larger hyphae (Bonfante-Fasolo 1986).

### 3.3.1 The extramatrical phase

VAMF spores form extramatrical hyphae radially from the spore, after germination (Fig. 3.2) (Bonfante-Fasolo 1986). Other infective soil borne propagules of VAMF, which can also form hyphae, includes vesicles and still viable hyphal pieces of previously formed hyphal systems and infected host root pieces in the soil (Carling & Brown 1982; Barea 1991).

According to Bonfant-Fasolo (1986) the extramatrical mycelium of VAMF is continuous with the intramatrical hyphae. The diameter of the extramatrical hyphae can vary from $2 - 27\mu m$, and hyphal walls can be thin or thick. These hyphae usually have a yellow colour and can also have typical unilateral angular projections.

Later, when the VAM fungus is established within the host root, the intramatrical mycelium induces more mycelium development outside the root. This extramatrical mycelium will continue to grow to explore greater volumes of soil for the absorption of nutrients and water. If the intramatrical mycelium is disturbed by conditions which alter the physiology of the host plant, the growth of extramatrical mycelium will subsequently decrease.

The extramatrical mycelium can produce reproductive structures such as spores and vesicles. These spores are normally borne terminally on short lateral branches, depending on the VAMF species. The external vesicles are usually thick-walled with dense cytoplasmic contents of oil globules (Bonfante-Fasolo 1986).

When an extramatrical hypha of a VAM fungus comes into contact with the host root surface, the hypha swells apically to form an appressorium-like structure. One extramatrical hypha may form several appressoria along the host root (Carling & Brown 1982; Sanders & Sheikh 1983). In some cases the hyphae can penetrate the host root without forming an appressorium (Richards 1987). The penetration of the VAMF hyphae occurs from 0,5 - 1cm

behind the root tip of active "feeder" roots where differentiation and elongation of root cells have not taken place (Carling & Brown 1982; Cooke *et al.* 1992).

The hyphae of VAMF can penetrate the host root in more than one way depending on the anatomy of the host root. According to Bonfante-Fasolo (1986) and Sanders & Sheikh (1983) the infecting hyphae can:

i) directly penetrate the wall of root hairs or epidermal cells of the host. The diameter of the hyphae decreases during the penetration process;

ii) pass through the intercellular spaces between neighbouring epidermal cells of the root. The hyphae then enter the first layer of cortical cells to form intracellular loops or coils, or

iii) penetrate the host root between epidermis cells, to spread further from the entry point through intercellular spaces.

Studies on the ultrastructural contents of the extramatrical hyphae showed that these hyphae contain cytoplasm with nuclei, mitochondria, endoplasmic reticulum and ribosomes. The cytoplasm is highly vacuolated and smaller vacuoles  contain electron-dense polyphosphate granules. The extramatrical hyphal walls are complex and are usually formed by two layers. The outer layer is electron-transparent and the inner layer electron-dense with organized alternate light and dark stacked lamellae. The extramatrical hyphal walls are formed by proteins and polysaccharides such as chitin (Bonfante-Fasolo & Grippiolo 1982; Gianinazzi-Pearson *et al.* 1981; Bonfante-Fasolo *et al.* 1981).

### 3.3.2 The Intramatrical phase

During the infection process by VAMF, the spores and hyphae secrete certain enzymes (cellulase) which dissolve small portions of the wall of the host cell. The hyphae then penetrate host roots after appressorium formation through the root hairs or epidermal cells (Janerette 1991; Mosse & Hepper 1975).

Inter- and intracellular hyphae develop from the appressorium growing longitudinally along the length of the host root instead of growing radially or circumficially (Fig. 3.1). When

Host plasmalemma

Fungal wall

Fungus

Interfacial matrix

Peripheral plasmalemma
of the host cell

Middlelamella

Host cell wall

CELL 1

CELL 2

Fusion of host plasmalemma around the
fungus and peripheral plasmalemma of
against the host cell wall

CELL 1

CELL 2

Hypha penetrates host cell walls

Constricted hyphal tip

CELL 1

CELL 2

Hypha reverts to original diameter
after cell wall penetration

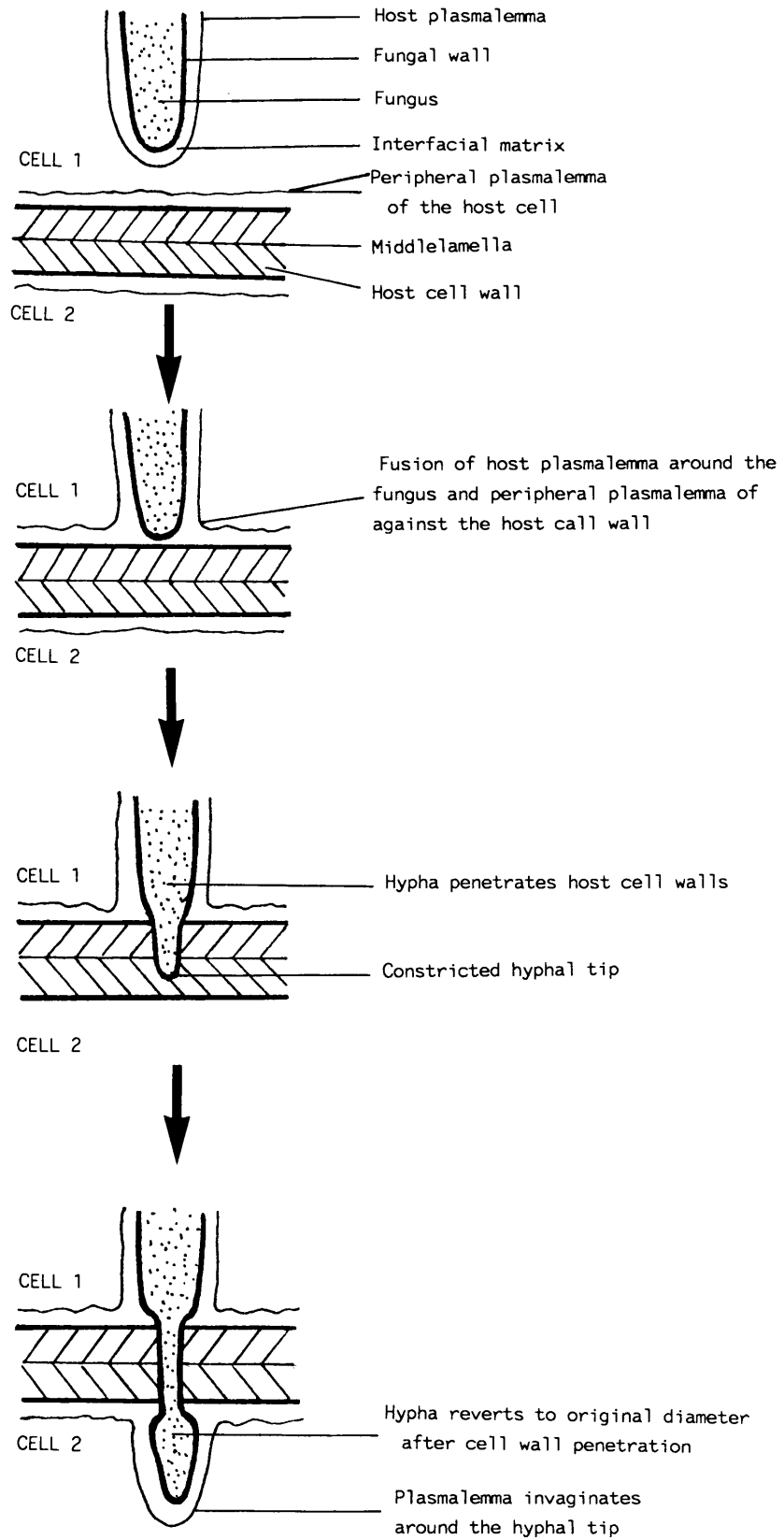Plasmalemma invaginates
around the hyphal tip

CELL 1

CELL 2

Fig. 3.3  A diagrammatic representation of sequential events when an intramatrical hypha grows from one host
cortical cell to another showing the hyphal penetration through the cell walls and the invagination
of the host plasmalemma around the hyphal tip

VAMF further colonize the host root, the fungi do not disturb the integrity of the host cells. There is also evidence that the host does not initiate dramatic responses against the invasion of the fungus. When the intracellular hyphae penetrate the host cells, they are usually enclosed by the host plasmalemma as well a thin layer of host cytoplasm. An interfacial zone forms between the intracellular hyphal wall and the host plasmalemma which contain matrix material. This material appears to be the same composition as that of the host cell walls (Carling & Brown 1982).

### 3.3.2.1 Intracellular hyphae in the outer cortex layers of the root

Once the hyphae of VAMF have penetrated the host root, the pattern in which the hyphae spreads, depends on the species of host plant and VAM fungus involved. The further development of the intramatrical mycelium is restricted to the cortex of the host root.

Coil formation is common in the mycorrhizal roots of many plants. Where roots do not form many air spaces, the hyphae spread intracellularly by means of intracellular coils and loops. After penetration, the infecting hyphae can form coils before penetrating neighbouring cortex cells. In some cases coils are not formed and the hyphae spread further through the intercellular air spaces between the cortical cells. (Bonfante-Fasolo 1986; Brundrett et al. 1985).

The hyphal diameter decreases when an infecting hypha grows from one host cell to another through the cell walls and reverts to its original size in the next cell (Fig 3.3). The host cell plasmalemma also appears to be a continuous membrane when the hyphae pass from one cell to another (Bonfante-Fasolo 1986). As the intracellular hypha, growing through the cytoplasm of one cell, reaches the periphery of the cell, the host plasmalemma around the hypha becomes continuous with the peripheral plasmalemma of the host cell. The matrix material in the interfacial zone becomes continuous with the primary wall of the host cell. The constricted hyphal tip then passes through this wall and the middlelamella between the neighbouring host cells by mechanical and probably enzymatic mechanisms. In the same way the hypha then grows through the wall of the next cortical cell and the host plasmalemma invaginates around the penetrating hyphal tip again as the hyphal tip reverts to its original

Direction of hyphal growth

A

Original hypha

Hyphal side branch

Hyphal branch bifurcates to form
parallel hyphae growing in
opposite directions

B

Original hypha bifurcates to form
parallel hyphae growing in the
same direction
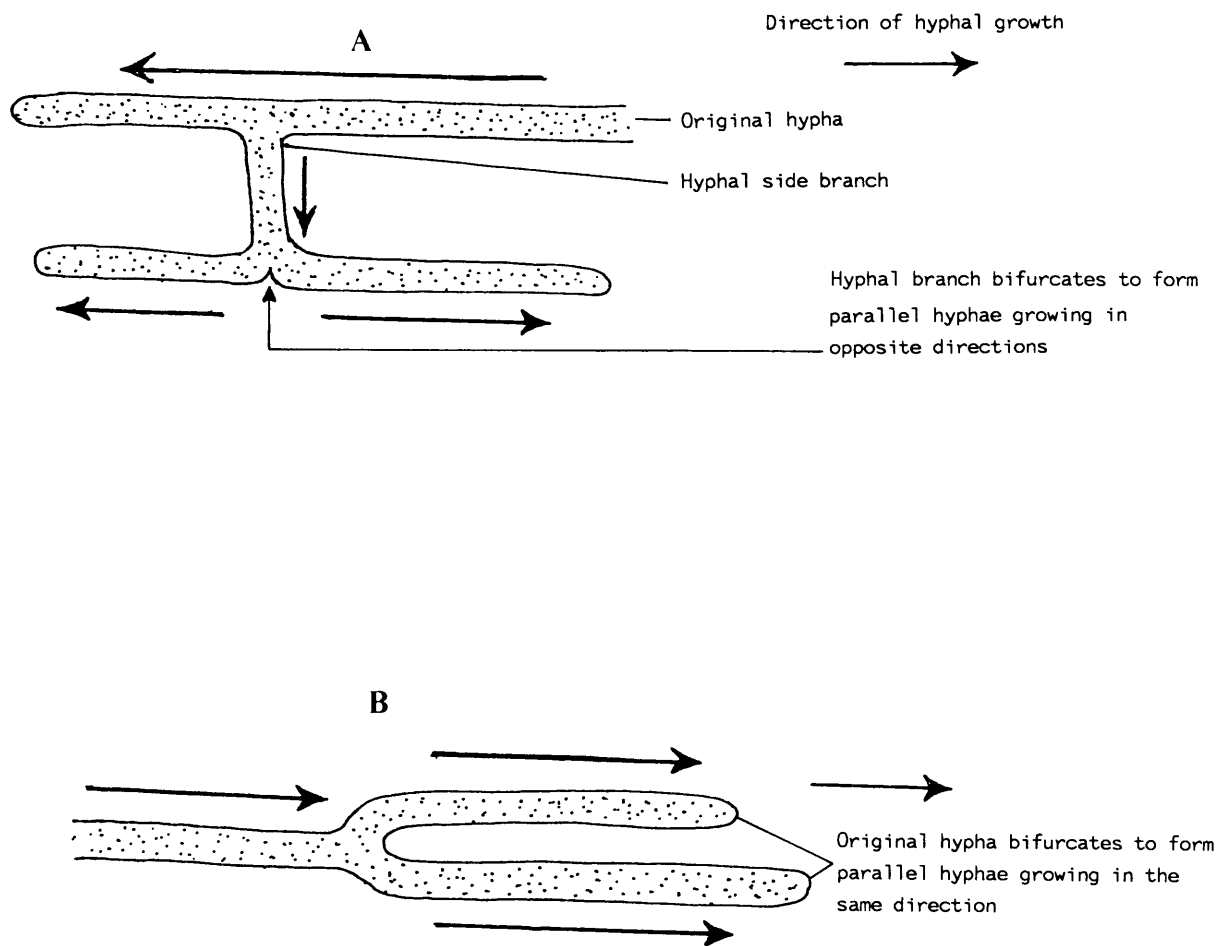
Fig. 3.4  A. H-furcation and B. Y-furcation of VAM fungal hyphae

diameter.

The contents of intracellular hyphae consist of small nuclei, cytoplasm with vacuoles enclosing electron-dense phosphate granules, lipid droplets and glycogen granules. These hyphae usually have thick, osmophilic walls (Bonfante-Fasolo 1986).

### 3.3.2.2 Intercellular hyphae in the host root

The intercellular hyphae are usually found in the inner layers of the cortical parenchyma and grow through the intercellular spaces between the cortical cells (Brundrett *et al*. 1985). These hyphae show a wavy pattern due to the outline of the host cell walls. Sometimes these intercellular hyphae seem to form H-connections when hyphae grow parallel to each other. This occurs when a branch, arising from a longitudinal hypha bifurcates to form two new hyphae which grow in opposite directions parallel to the original hypha (Fig 3.4 A). Y-junctions or furcations can also form when a longitudinal hypha bifurcates to form hyphae which then grow parallel to each other (Fig 3.4 B) (Bonfante-Fasolo 1986).

The ultrastructural contents of intercellular hyphae show small nuclei, mitochondria, globose electron-dense granules within larger vacuoles, glycogen granules and lipid droplets. Large numbers of phosphorus granules also occur in these hyphae (White & Brown 1979). The walls of VAMF hyphae inside the host root usually have one layer but in rare occasions two layers can be seen (Bonfante-Fasolo 1986).

### 3.3.2.3 The arbuscules

Typical arbuscules are formed when some of the side branches of intercellular or intracellular VAMF hyphae penetrate the host cortical cells. After penetration the hyphae repeatedly branch dichotomously within the lumen of the host cell (Fig 3.5). During the early colonization stages formation of arbuscules occur in cortical cells near the entry point. Later during the colonization of the fungus the arbuscules are formed acropetally away from the initial entry point (Sanders & Sheikh 1983).
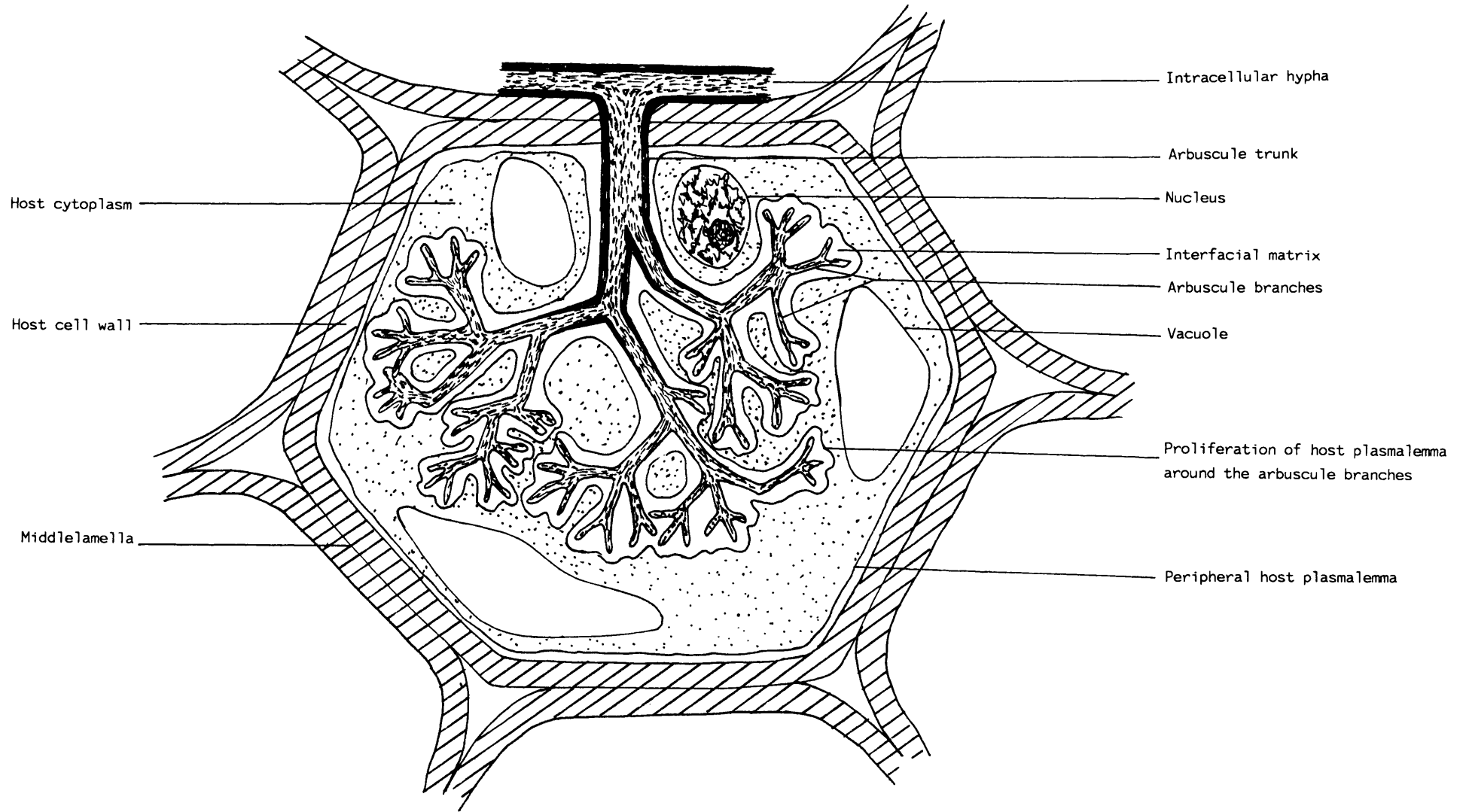
Fig. 3.5 A representation of a fully developed arbuscule within the host cortex cell

Labels:
- Intracellular hypha
- Arbuscule trunk
- Nucleus
- Interfacial matrix
- Arbuscule branches
- Vacuole
- Proliferation of host plasmalemma around the arbuscule branches
- Peripheral host plasmalemma
- Host cytoplasm
- Host cell wall
- Middlelamella

Active intercellular hypha

Host cell wall

Host plasmalemma around clump

Host cytoplasm

Collapsed intercellular hypha

Encasement material

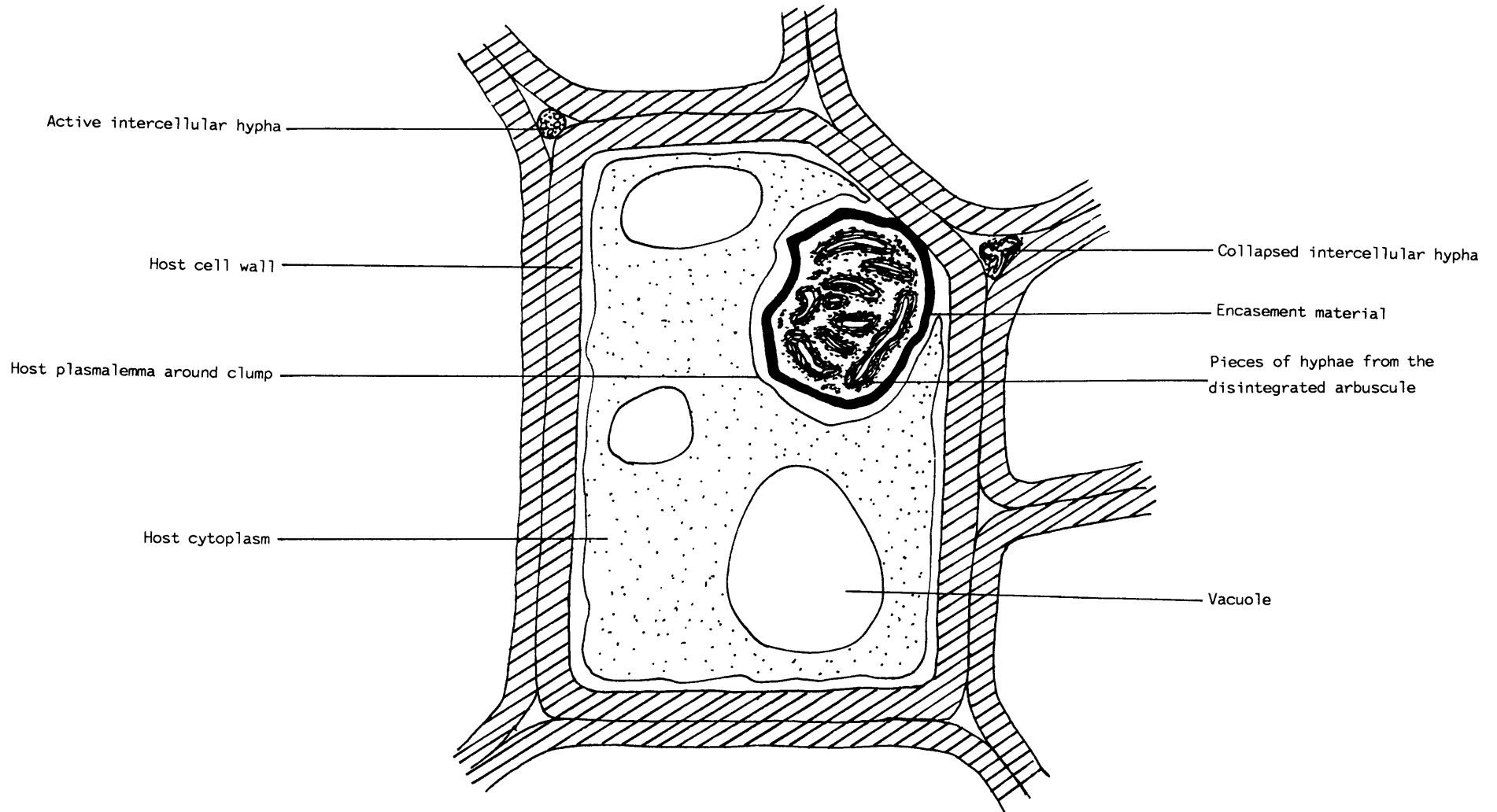Pieces of hyphae from the disintegrated arbuscule

Vacuole

Fig. 3.6    A representation of a clump with surrounded encasement material after arbuscule degeneration

The wall of the arbuscule forming hypha is modified and there is a significant decrease in diameter and thickness of the wall when the finer branches of the arbuscule are formed. During arbuscule formation the host plasmalemma does not undergo any cytochemical changes due to the invagination or proliferation around the developing arbuscules but only increase in size and altering its form (Bonfante-Fasolo *et al. 1981*).

Nuclei, mitochondria, glycogen particles, lipid globules and electrondense granules within the vacuoles can also be seen in the hyphae of the arbuscule (Bonfante-Fasolo 1986).

Most researchers consider the arbuscule to be the main site for nutrient transfer between host and fungus. Therefore an infection unit of VAMF becomes self-supporting only when arbuscules are formed to extract carbon compounds from the host cells. Before arbuscule development, the unit is sustained by compounds from the extramatrical mycelium or from reserves of the spores and other infecting propagules. (Sanders & Sheikh 1983; Bonfante-Fasolo 1986).

The arbuscule is functioning as a feeding structures for only a few days whereafter they start to degenerate. Arbuscule trunk development and degeneration happen gradually and the rate depends on the fungus-host association (Alexander *et al*. 1988). During the degeneration process of the arbuscule the finest branches deteriorates first. The deteriorating process progresses towards the main trunk of the arbuscule. Septa are frequently formed to close off the deteriorating hyphae from the rest of the arbuscule. This process goes on until the whole arbuscule collapse to form a dense, irregular clump within the host cell (Carling & Brown 1982; Bonfante-Fasolo 1986; Alexander *et al*. 1988). The host then forms an encasement around the clump (Fig 3.6). This encasement material appears to be of the same composition as that of host cell walls (Alexander *et al*. 1988). Later the host cell returns to normal after the break down and subsequent disappearance of the arbuscule clump (Bonfante-Fasolo 1986; Alexander *et al*. 1989).

The arbuscule is considered to be the functional unit of the VAM symbiosis, thus the place where the host plant and the fungus really interact with each other symbiotically to transfer "food" between each other. The host-fungus relationship in the VAM association is complex
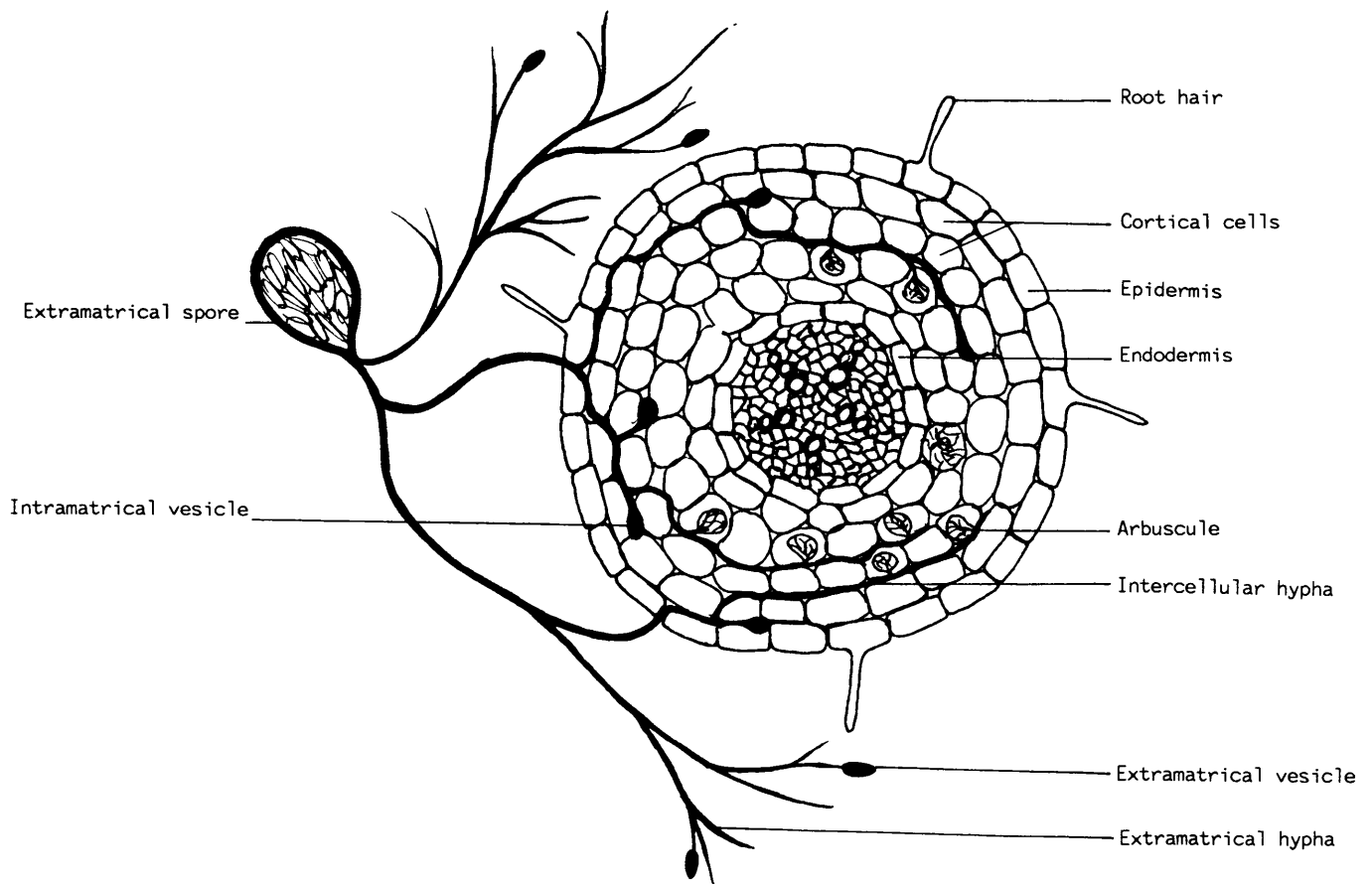
Fig. 3.7  A representation of a transverse section of a root colonized by VAMF showing the intramatrical and extramatrical vesicles
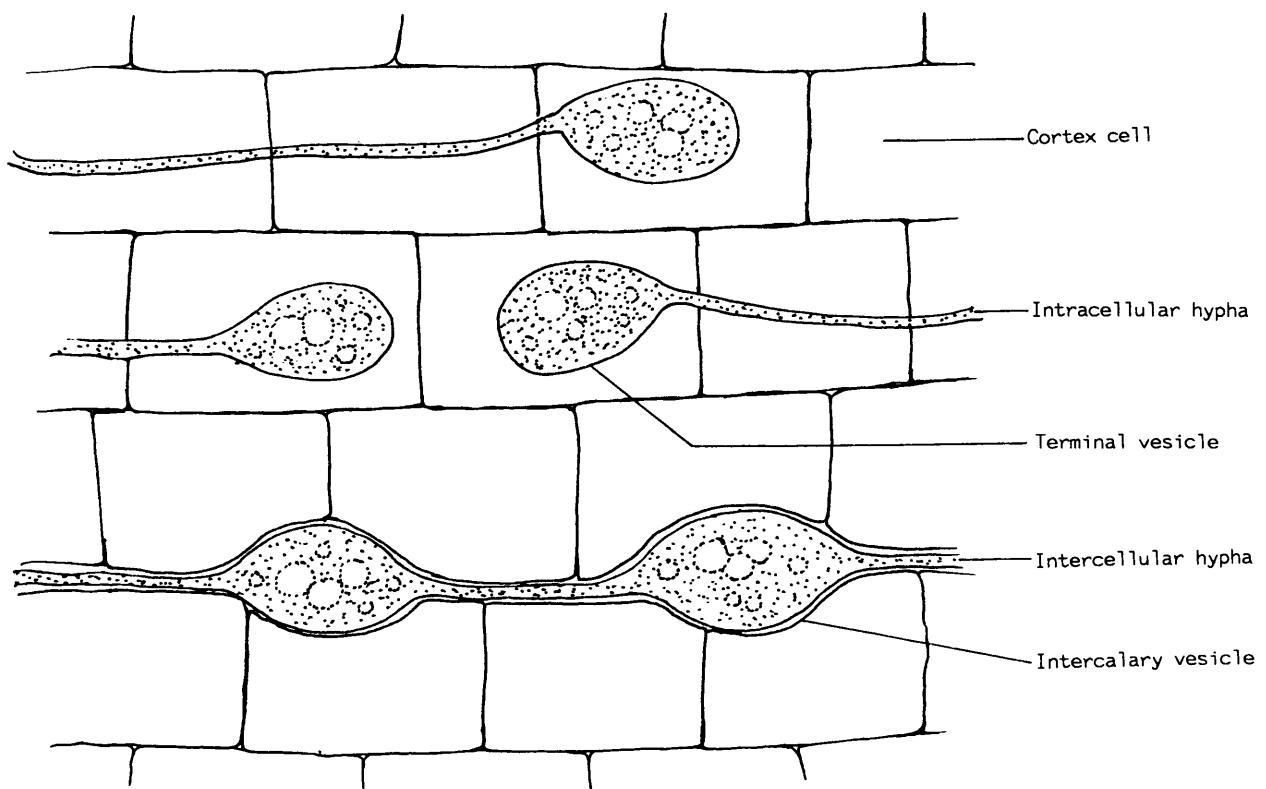


Fig. 3.8  A representation of intramatrical vesicles

and dynamic and can be influenced by several environmental conditions. To understand this relationship better, one must try to determine which stages during the development of the arbuscule are controlled by which partner. Alexander *et al.* (1988) and Alexander *et al.* (1989) report that during the development of the arbuscule it appears that the formation of the trunk and the branches are under the control of the fungus. The size of the arbuscule is also under the control of the fungus.

The increase in host plasmalemma due to the invagination of the plasmalemma around the arbuscule branches is, however, under the control of both the fungus and the host. The degeneration of the arbuscule appears to be under the control of the fungus but the formation of encasement material around the collapsed arbuscule is under the control of the host plant. The degeneration of encasement material and "digestion" of the collapsed arbuscule material are under the control of the host plant (Alexander *et al.* 1989).

### 3.3.2.4 Vesicles

The globose vesicles of VAMF are usually intercalary or terminal swellings of a hypha and can sometimes function as soil borne spores (Brundrett *et al.* 1985; Bonfante-Fasolo 1986). The vesicles can be formed in the soil or within the tissue of the host root (Fig. 3.7). Inter- or intracellular vesicles are usually formed within the host root (Fig 3.8) and the size of these vesicles differ significantly within the same root (Bonfante-Fasolo 1986). *Gigaspora* species, however, do not form vesicles in the host root and are therefore not considered as real VAMF (Morton 1988; Bonfante-Fasolo 1986; Carling & Brown 1982).

Many nuclei, glycogen granules and small vacuoles with electron-dense granules can be seen in the protoplasm of young vesicles but when these vesicles are mature only large lipid droplets can usually be identified (Brundrett *et al.* 1985; Bonfante-Fasolo 1986).

The vesicle wall usually have more than one layer. The outer layer appears to be smooth and have no ornamentation. According to Bonfante-Fasolo (1986) the vesicle walls show a trilaminated pattern due to the arrangement of different electron-dense layers. Intercellular vesicles are in contact with the cell walls of the host and intracellular vesicles are, as

arbuscules, enclosed by the host plasmalemma. The vesicles function mainly as VAM fungal storage organs or as resting reproductive propagules to infect another suitable host (Bonfante-Fasolo 1986; Barea 1991; Brundrett *et al.* 1985; Berch *et al.* 1991).

## 3.4 Ultrastructural and cytochemical composition of VAMF

The ultrastructural composition of the walls of VAMF changes during the invasion of the host root. Bonfante-Fasolo & Grippiolo (1982) report the following changes: The diameter of the hyphae and the thickness of the hyphal walls decrease progressively from the extramatrical phase towards the finest arbuscule branches. The texture of the hyphae walls changes from lamellar to amorphous. There is also a progressive simplification of the wall components of the hyphae from the extramatrical phase towards the arbuscule. Extramatrical hyphae usually have two layers while the intramatrical hyphae are usually monolayered.

Extramatrical hyphal walls are sometimes microfibrillar with complex stratifications. Young hyphae walls are usually thin and monolayered compared to the thickened and older hyphal walls which consist of two layers. Arbuscular hyphal walls, however, are thin and monostratified and are considered to be the area where nutrient exchange take place. Chitin and chitosan are common compounds in the hyphal walls of Zygomycetes and are also found in the hyphal walls of VAMF (Bonfante-Fasolo & Grippiolo 1982).

The cytochemical ingredients of the matrix in the interfacial zone, as already mentioned, appears to be of host origin. Bonfante-Fasolo (1981) and Bonfante-Fasolo (1986) indicate that the material is mainly composed of glycoprotein (polysaccharides and protein). Scannerini & Bonfante-Fasolo (1979) report that pectin is the most abundant compound in the interfacial zone and that chitin is usually absent. There is also evidence of a fibrillar cellulose compound which appears around the larger branches of the arbuscule as well as around the collapsed arbuscule. This compound may also have a host plant origin.

## 3.5 Changes in the host due to the invasion of VAMF

It is important to determine if there is any change in the host cells during the invasion of
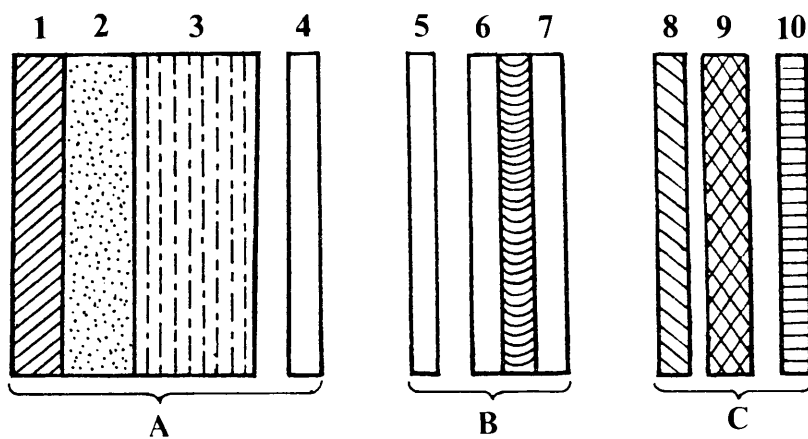
Fig 3.9   A murograph of the wall layers of VAM fungal spores showing the type and grouping of the different
          wall layers
          1. Expanding wall layer
          2. Evanescent wall layer
          3. Laminated wall layer
          4-7. Unit wall layers
          8. Membranous wall layer
          9. Coriaceous wall layer
          10. Amorphous wall layer
A, B and C: Wall layer groups. A group represent the layers which stay intact when spore is crushed

VAMF. This will also contribute to the better understanding of the physiology of the host due to VAM infection. Berta *et al.* (1990) studied the infected host cells with microfluorometric and flow-cytometric analysis. This method revealed that there are indeed changes in the nuclei during the VAM symbiosis. The host nuclei undergo hypertrophy but there are no changes in the DNA contents in comparison to non-infected cells. There is, however, an increase in the fluorescence capabilities of the infected host cell nuclei when stained with DAPI (6-diamidino-2-phenylindole). This may be due to a lower degree of chromatin condensation in the host nuclei during VAM infection (Berta *et al.* 1990; Balestrini *et al.* 1992).

Balestrini *et al.* (1992). indicate that in uninfected host cells the nuclei are usually found near the cell wall. In infected cells with coils and unbranched intracellular hyphae, the nuclei remain peripheral in the host cells or the nuclei migrate to the centre during arbuscule formation. There is also evidence of re-organization of the other host cell organelles in arbuscule containing cells, changing the cytoskeleton of the cell. After the degeneration of the arbuscule, however, the cytoskeleton of the host cell reverts to normal.

Changes in the host due to VAM infection, other than enhanced plant growth, is an increase in the amount of vascular tissue, the lignification of the xylem, and the number of vascular bundles. There are also noticeable cytological changes such as the enlargement of intercellular spaces due to the disappearance of the middlelamella (Gianinazzi-Pearson *et al.* 1981). The disappearance of starch in arbuscule containing host cells, is different from normal host cells, where usually large amounts of starch are found, is observed (Bonfante-Fasolo 1986; Gianinazzi-Pearson *et al.* 1981).

Other changes to the morphology of the plant apart from the changes in the host root, are an increase in the size of the leaves as well as an increase in the thickness of the leaves (Carling & Brown 1982). The size of the midrib as well as the size and the number of plastids in the cells of the leaves increase due to VAM infection (Krishna *et al.* 1981; Carling & Brown 1982). These changes are proof of the beneficial effects of VAM on the physiology of the host plant and will not be discussed here.

According to Dexheimer & Gerard (1989) the invagination of the host plasmalemma around the developing arbuscule results in the formation of plasmalemmasomes, which then further increase the surface area of the plasmalemma. They also found that ATP-ase activity, which is normally absent in the plasmalemma of mature cortical cells, was evident in the plasmalemma around the arbuscule. This extraordinary activity of ATP-ase may be caused by the formation of new wall material in the interfacial zone (Bonfante-Fasolo *et al*. 1981). According to Dexheimer & Gerard (1989) there is evidence that the host cytoplasm around the arbuscule forms large quantities of endoplasmic reticulum. The endoplasmic reticulum is in contact with the host plasmalemma around the interfacial zone. This may result in the transfer of polysaccharides directly towards the plasmalemma and therefore short-circuiting the golgi-apparatus during the transfer of these compounds to the interfacial zone.

## 3.6 Spore morphology

The spores of different VAMF species show very distinctive morphological features and in most cases are used for taxonomic studies. The taxonomical aspects were already thoroughly discussed in Chapter 2. On the other hand, the basics of spore cytochemistry and morphology have still to be discussed in the context of this chapter.

The greater part of the walls of VAMF spores (80 - 90%) consists of polysaccharides and the remaining part (10-20%) of proteins and lipids. Chitin and cellulose form the basic structure of the spore walls. As already mentioned chitosan is also found in VAMF (Morton 1988).

To examine spore wall structure, spores must be isolated from the soil by methods such as wet sieving and decanting (Gerdemann & Nicolson (1963). Spores which are free from VAMF parasites are then mounted on microscope slides, broken under pressure and observed with a light microscope (Morton 1988).

The basic characteristics of the spore wall morphology are used to identify or classify VAMF. In most cases more than one of the following wall layers, usually represented by murographs (Fig. 3.9) can be distinguished (Morton 1988; Morton & Benny 1990):

I. The **laminated layer** which is a rigid layer composed of fused multiple layers or lamellae in mature spores, can break easily when pressure is applied to the spore. The laminated layers are usually coloured in pigmented spores and provide the spore its specific form.

II. The **unit layer** is rigid and single layered and breaks easily under pressure. So far all VAMF have more than one unit layer and is in some cases pigmented.

III. The **evanescent layer** looks almost the same as the unit layers of young spores, but when the spore matures the layer breaks down and disappear. The transitory nature of this layer makes the taxonomic evaluation of spores difficult.

IV. The **membranous layer** which is usually hyaline, thin, flexible and single-layered. It becomes wrinkled when the spore is crushed. In some VAMF spores it does not break easily when the spore is crushed. This layer is found in all VAMF except species of *Sclerocystis*.

V. The **coriaceous layer** which is thick, flexible and usually hyaline, forms as a single layer and does not break easily when the spores are lightly crushed. This layer has a leathery appearance.

VI. The **expanding layer** is also hyaline and expands when exposed to certain acidic mountants like lactophenol and PVL. Distinctive hyaline to yellow radial columns form in the expanded halo surrounding the spore in above mentioned mountants.

VII. The usually transparent **amorphous layer**, is not easily observed, due to its change of characteristics in different chemical substances. When the pH of a mountant is less then 2.0 it becomes plastic but in a non-acidic mountant such as water or glycerol it appears to be rigid. It does not break when the spore is lightly crushed. The inner surface is also highly wrinkled. This layer is found in species of *Acaulospora, Scutellospora* and *Entrophospora.*
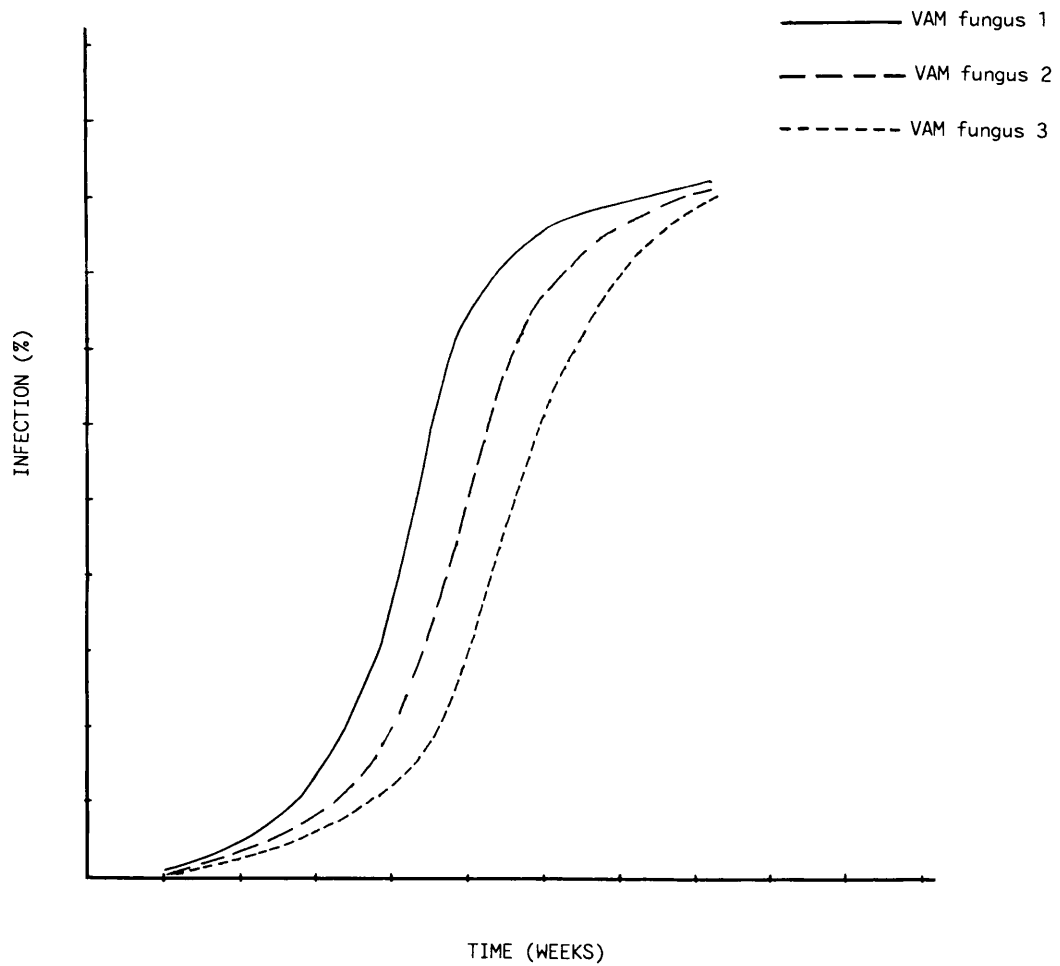
Fig. 4.1  A typical sigmoidal pattern of the colonization of three different VAMF reaching the same plateau
phase at the end but differ in their pattern of colonization over a period of time

According to Morton (1988) and Morton & Benny (1990) the laminated, unit and membranous spore wall layers may have an ornamented appearance. These ornamentations can be rounded, tendril-like, colliculate or tooth-like projections or crowded spines, tubercules, hemispherical or patch-like warts and pustules, depending on the species and are mostly seen as taxonomic important features.

Not all the VAMF have all the layers as described above. The number of layers and the type of layers differ between each VAMF species but, however, appear to be stable in a specific VAMF species. The spore wall layers are also arranged into different layer groups as seen in Fig. 3.9. The layers that stay intact when the spore is crushed are considered to be a layer group (Morton 1988).

## 3.7 The life cycle of VAMF

The life cycle of VAMF is diagrammatically illustrated in Fig. 3.10. One or more hyphae, branching from the germination tube of a spore comes into contact with the host root and penetrate the host root if the host plant is susceptible to VAMF (Richards 1987). Hyphae branching from other propagules of VAMF such as pieces of extramatrical mycelium or VAM host root pieces can also start infection in the host in the same way (Barea 1991). When a host plant is infected by VAMF for the first time it is known as primary infection (Bowen 1987; Sanders & Sheikh 1983). After penetration of the host root, the hyphae form an intramatrical mycelium with arbuscules (Barea 1991). Together with arbuscule formation the fungus also forms an extramatrical mycelium which penetrates the soil around the host root (Bowen 1987). Both the intramatrical and extramatrical mycelium can form vesicles and spores which can infect a new host plant or neighbouring roots of the same host plant (Bonfante-Fasolo 1986). When infective propagules of VAMF infect roots of already infected host plants, it is called secondary infection (Sanders & Sheikh 1983).

## 3.8 VAM in plants other than the Angiosperms

Most of the morphological studies are done on VAM fungal relationships with Angiosperm host plants. As the most ancient VAMF were observed in fossils of early land plants, their

morphology should also be investigated in host plants other than angiosperms. Angiosperms, today, are considered to be the most developed plants on earth and according to evolutionists, developed from the more ancient plants such as the bryophytes, pteridophytes and gymnosperms.

The association of VAMF and bryophyte hosts are called mycothalli. Studies were already done on a few liverworts under the genera *Pellia, Monoclea* and *Marchantia.* The penetrating hyphae form the typical arbuscules, coils and intracellular vesicles of VAMF inside the rhizoids and in the lowermost layer of the thallus of the bryophyte host. Later the arbuscules collapse to form so-called sporangioles. In these bryophyte hosts VAMF are present in cells with true chloroplasts. Large starch grains are also absent in cells infected with arbuscules (Bonfante-Fasolo 1986).

Certain reports give evidence of VAMF in recent pteridophytes. The formation of typical VAM structures can be observed in the cortical regions of the roots but are absent in the stelé, the root tips and the endodermis of the sporophyte. The infection of the fungus is usually in areas where root hairs are absent. The gametophyte with gametangia are not infected by VAMF. Starch grains are absent in arbuscule containing host cells but reappear after arbuscule degeneration (Bonfante-Fasolo 1986). Iqbal *et al.* (1980) and Ponton *et al.* (1990) also report that typical VAMF structures can be observed within the roots of pteridophyte hosts.

VAMF in gymnosperms such as species of Podocarpus and cycads form typical appressoria when infecting the host roots and typical coils, arbuscules and vesicles are formed within the cortex of the host root. However, intercellular hyphae are not formed in the gymnosperm hosts, for the penetrating hyphae spread intracellular. The ultrastructural and cytochemical characteristics of this host-fungus relationship is the same as that of VAM in angiosperms (Bonfante-Fasolo 1986). Richards (1987) also stipulates that some gymnosperm species contain both ecto- as well as VAM, as previously reported.

## 3.9 Conclusion

The study of the morphology of organisms provides necessary information which forms the basis on which other disciplines, for example physiology and genetics are studied. However, the morphology is also an important basis for taxonomical and ecological studies of organisms.

In the case of VAM and its associates, it is therefore also necessary to investigate the morphology of the VAM relationship. This includes the changes within the host due to VAMF invasion as well as the morphology of the fungal associate. This will lead to the better understanding of the structure-function relationship of typical VAMF structures. If this relationship is thoroughly understood, it could be used or manipulated by man to the advantage of agricultural, horticultural and afforestation development.

# CHAPTER 4 : The Physiology of VAM

## 4.1 Introduction

VAMF are generally associated with enhanced growth and nutrient absorption of the host plant especially those nutrients which are transported through the soil by diffusion (Johansen *et al.* 1992). There is evidence of other beneficial effects of VAM to host plants as already mentioned in the introductory chapter. It is thus important to investigate the physiology of these beneficial effects before they can be manipulated for agricultural uses.

In comparison to other classes of fungi, very little work has been done to demonstrate the physiology of VAM, for there are several problems related to the studies on the physiology of VAM. According to Cooper (1986) some of the problems are as follows:

1. VAM fungi has not been grown as an axenic culture yet, which makes studies without the availability of a host difficult. The extramatrical mycelium can be stripped from the host root to be studied but the physiological characteristics of the extramatrical mycelium might be different to that of the intramatrical mycelium. Another method is to degrade the host tissue by enzymatic activity, leaving the intramatrical mycelium to be studied.

2. The overwhelming response by the host plant to phosphorus uptake makes it difficult to distinguish other host responses apart from the generally enhanced phosphorus-uptake.

3. When comparing mycorrhizal plants with non-mycorrhizal plants, the plants should be of the same physiological age and should have the same phosphorus content. This can only be achieved by amending the phosphorus content of the soil where the non-mycorrhizal plant grows. This will cause the non-mycorrhizal plant to have the same phosphorus content as the mycorrhizal plant.

4. It is also difficult to estimate the optimal percentage of the fungus which should be in the host root to maintain sufficient nutrient uptake.

5. The experiments to obtain physiological information of the developmental stages of VAM are extensive and time consuming. After initiating infection in the host root, colonization of the fungus goes through an initial lag-phase due to slow establishment of the VAM fungus, a period where the colonization expands rapidly, and a plateau phase
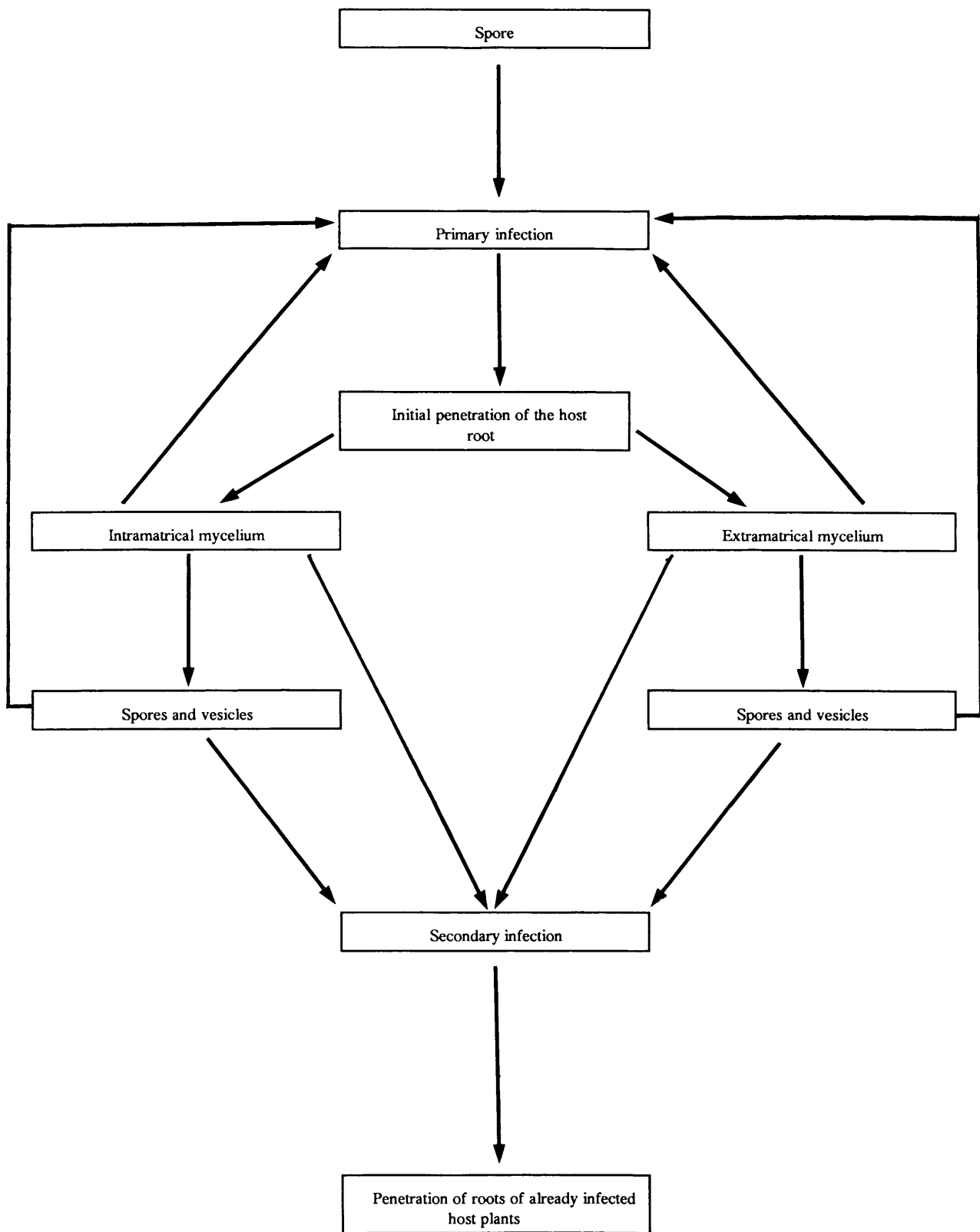
Fig. 3.10 A schematic diagram of the life cycle of VAMF

where there is a balanced relationship between the host and the fungus (Fig. 4.1.).

Barea (1991) and Schwab *et al.* (1983) conclude that VAM infection and colonization follow a sigmoidal pattern.

## 4.2 Factors influencing VAM development.

**Environmental factors** play a major role in the physiology of VAM. These factors can influence VAM directly or indirectly by influencing the physiology of the host plant which then subsequently influence the symbiosis. Most of these factors will be thoroughly discussed in chapter 7 which deals with the ecology of VAM.

Bowen (1987) indicates that there are five **physiological stages** during VAM infection and colonization:

  i) pre-infection stage where the recognition of the fungus and the host takes place and the further encounters of the two associates;

 ii) the penetration of the root ("primary infection");

iii) inter- and intracellular growth of the fungal hyphae in the host root cortex;

 iv) the development and functioning of the arbuscule, and

  v) the growth of the fungus outside the host root and the formation of extramatrical hyphae.

Herrera *et al.* (1993) suggest that mycorrhizal responsiveness is a function of: i) the age and the species of the host; ii) the VAMF species; and iii) the physical and biological features of the soil.

There is reason to believe that environmental factors such as light and temperature play a definite role play in both the effectiveness of VAM infection and the functioning of it, for these factors have an effect on host vigour and the production of carbon compounds available to the fungal component. There is also an indication that at very low temperatures, VAM can be harmful to the plant due to the carbon cost of the fungus (Hayman 1982).

**Soil factors** such as pH and fertility also influence the physiology of VAM indirectly by influencing the physiology of the host plant as well as the germination of the spores.

Other **soil organisms** also interact with VAMF and can therefore have an influence on the physiology of VAM. A good example of such organisms is *Rhizobium*-like bacteria in legumes which can increase the initial establishment of VAMF, because of polysaccharide production which further leads to the production of polygalacturonase which in turn stimulates VAMF penetration at the infection point (Hayman 1982). Other soil bacteria can also stimulate VAM infection (Bagyaraj 1986; Manjunath *et al.* 1981). There is even evidence of bacterium-like organisms within the spores of some VAMF species which may play a role in the germination of spores of the fungi (Sward 1981).

**Host-fungus factors** can also play a major role in influencing the physiology of VAM. Hayman (1982) suggests that:

  i) closely related plants act differently to VAM infection;

 ii) VAM fungi which form the most abundant extramatrical hyphae, are not necessarily the most effective, and

iii) the amount of infection in one host plant, which appear to be the same as in another host plant, can differ in their effectiveness towards the symbiosis.

**Pesticides** also have an effect on VAMF. According to Hayman (1982) there is evidence that compounds such as PCNB, Benomyl, Triademefon, Drazaxolon, Aldrin and Chlorferinphos inhibit VAM infection. Nematicides such as DBCP can, on the other hand, stimulate infection. These are all factors which will be considered in chapter 6 in relation to crop production, horticulture and afforestation.

Another significant fact is that the host plant does not use its metabolic defence mechanisms to reject the invasion of VAMF as in the case of pathogens. This may be due to the physiological changes in the roots of the host plant infected by VAM (Gianinazzi-Pearson & Gianinazzi 1983).

A

± 0,2mm   ± 1mm   ± 50mm

Root hair

spores

Vesicles

Appressorium

absorbed and transported
through VAM fungal hyphae
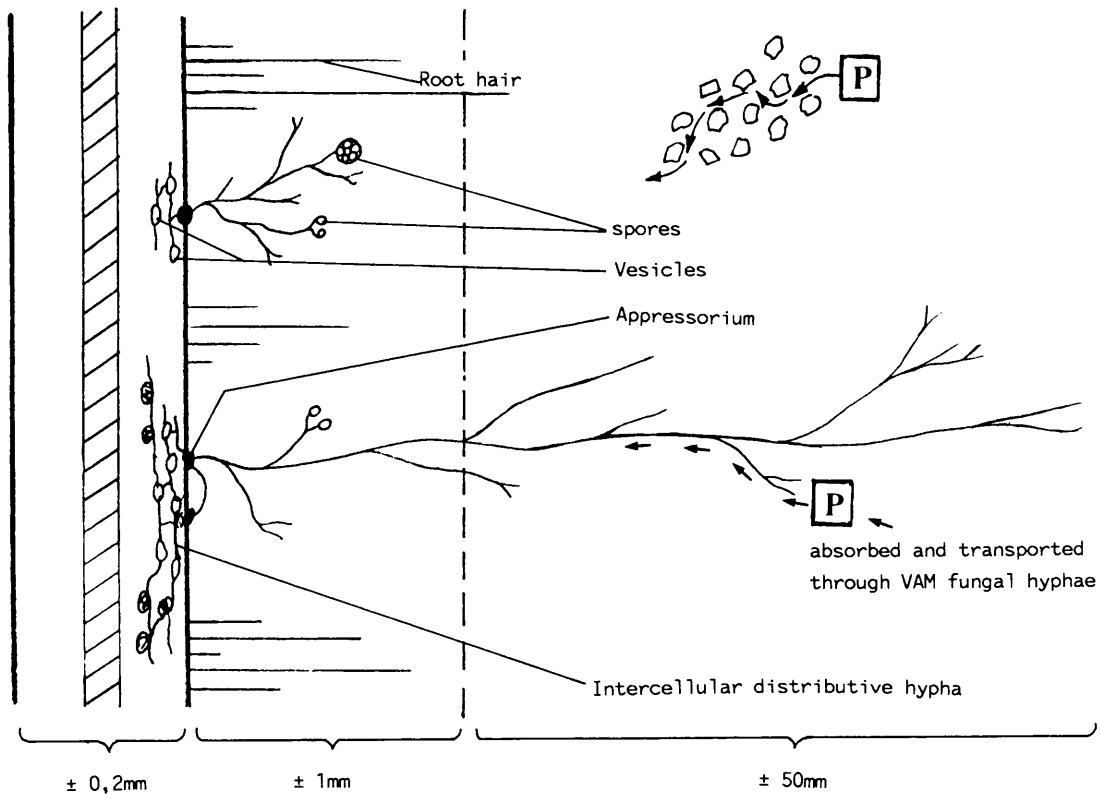
Intercellular distributive hypha

P = Phosphorus

Tp = Transport compound

1   2
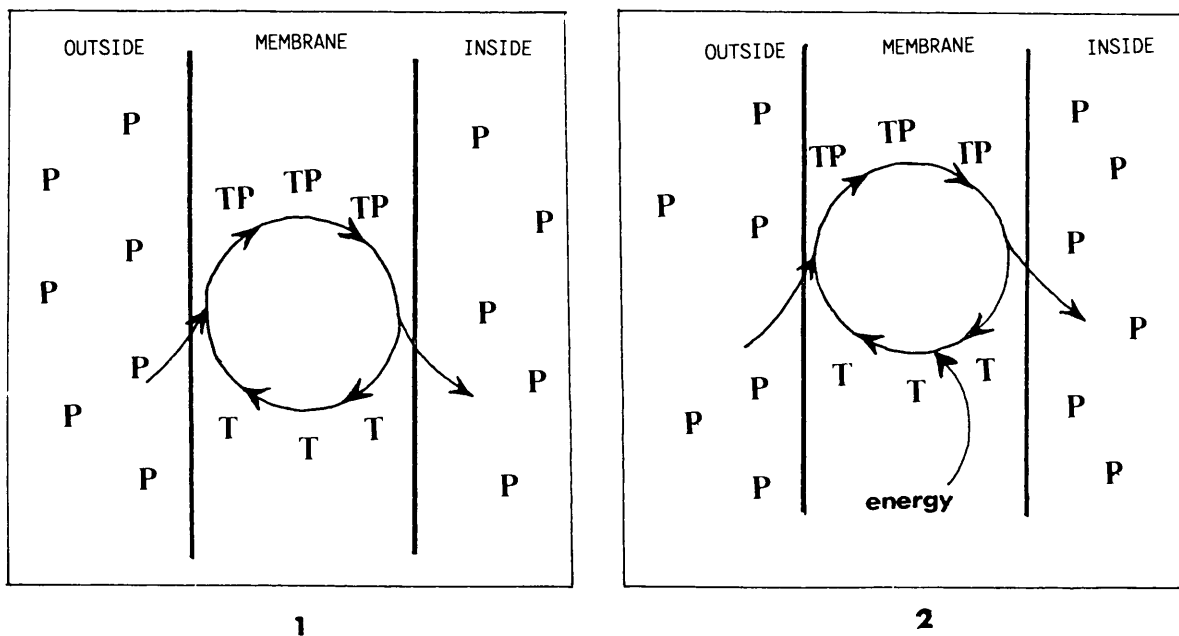
B

Fig. 4.2  A. A diagrammatic representation of a VAMF infected host root showing the absorption of phosphorus
from the surrounding soil through the extensive extramatrical hyphal system
B. A schematic representation of the passive (1) and active (2) system of phosphorus transfer from
the soil to the fungus

According to Hayman (1982) the effectiveness of the initial infection of VAM fungi depends on factors such as:

i) the type of inoculum (propagules);

ii) the amount of propagules available to successfully infect the host plant, and

iii) and the number of germination tubes formed by the spores, for the spores of different species form different number of germination tubes.

The host also plays an important role in the effectiveness of VAM infection. According to Hayman (1982), Hetrick (1986) and Bagyaraj (1986) some non-mycorrhizal plant roots exudate toxic compounds into the soil which inhibit VAM infection or their epidermis are incompatible towards VAM fungi, which also inhibits VAMF to infect the host.

## 4.3 The symbiotic relationship between the fungus and the host

The major importance of VAM symbiosis is the mutualistic exchange of nutrients between the plant associate and the fungal associate. This indicates that both associates benefit from this association.

As already mentioned, VAMF are beneficial to their hosts due to their contribution to the increased absorption of phosphorus and other nutrients and subsequent plant growth enhancement. On the other hand, the host provides carbohydrate compounds to the fungus.

### 4.3.1 Phosphorus physiology and biochemistry

Phosphorus is one of the most essential elements absorbed in large amounts by plants from the soil. This element plays major roles in several metabolic activities as the "energy carriers", ATP, in the plant. If phosphorus is therefore not available in adequate quantities in the soil the plant would soon show disease-like symptoms. Phosphorus is very important to the photosynthesis process and it is also an integral part of the membranes of plant cells. Low levels of phosphorus in the leaves will cause a slow down in the photosynthesis process. This will subsequently reduce the amount of sugars produced in the leaves. This reduction of sugars can retard plant growth (Foyer & Spencer 1986).

**4.3.1.1 Phosphorus absorption mechanisms of VAMF**

The absorption of nutrients by plants depends on the mobility of these nutrient ions in the soil. The limiting factor will then be the total surface area of the roots. The extramatrical hyphae of VAM fungi are considered to be extensions of the root hair, enlarging the absorption surface of the root for increased absorption of phosphorus and other nutrients (Fig.4.2). Gianinazzi-Pearson & Gianinazzi (1983) tried to estimate the total hyphal length per root length and found approximately 0,8 to 1,34m of mycelium per centimetre host root.

Phosphorus absorbtion is known to be temperature sensitive. Less phosphorus is absorbed at low temperatures than at higher temperatures (Gianinazzi-Pearson & Gianinazzi 1983). This will also have an effect on the VAM relationship.

Phosphorus can be present in the soil in three forms:
  i) soluble inorganic phosphorus;
 ii) insoluble inorganic phosphorus, and
iii) organic phosphorus compounds such as phytate.

When the concentration of phosphorus ions is low in the soil, it can become depleted from the soil at a fast rate because of its relative immobility and movement by diffusion only (Cooper 1986). These conditions can cause a low level of phosphorus in the leaves.

VAM are considered to be the cause of enhanced growth and biomass production in soil with low phosphorus levels as compared to non-mycorrhizal plants which will not show the same improvement in growth (Gupta *et al.* 1990). When phosphorus levels are low in the soil, VAMF will increase the absorption of phosphorus from the soil by shortening the distance that phosphorus ions must diffuse through the soil to the plant roots (Bolan *et al.* 1987). Extramatrical hyphae also explores the soil more thoroughly for phosphorus (Fig. 4.2 A).

VAM fungal hyphae absorb soluble phosphorus directly from the phosphorus pools beyond the depletion zone of the host root and then translocate it to the host (Cooper 1986; Hayman 1982). The amount of nutrients absorbed by the hyphae depends on the development of the

extramatrical mycelium and this can be linked to the growth response of the host (Cooper 1986).

According to Hayman (1982) VAMF infected roots can absorb more phosphorus than non-mycorrhizal roots in soils with low phosphorus levels because VAM fungal infected roots:

i) stay functional for a longer period;

ii) can absorb phosphorus beyond the threshold levels for phosphorus absorption for normal plants;

iii) have more ATP-ase activity for the absorption of phosphorus against the concentration gradient, and

iv) will still grow vigorously and absorb phosphorus and water when the host plant grow in fumigated soil while non-mycorrhizal plants become stunted in fumigated soil.

Cooper (1986) suggests that VAMF can solubilize and mobilize insoluble phosphorus for absorption. Usually this phosphorus source is unavailable to non-mycorrhizal plants. VAMF are also efficient (i) in soils which bind or "fix" phosphorus to compounds in the soil at fast rates so that it unavailable for plant use and (ii) in soils containing high levels of insoluble iron and aluminium phosphates. VAM can utilize organic phosphorus compounds, due to the more effective hydrolysis of these compounds which is not possible in non-mycorrhizal plants.

VAMF, like most other fungi, might obtain phosphorus by active absorbtion or by passive absorption (Gianinazzi-Pearson *et al.* 1991). Thomson *et al.* (1990) mention two transport systems which are most likely to play a role in nutrient uptake:

i) An active high affinity system driven by ATP-ase. This system is dominant in soils where the phosphorus levels are low.

ii) A passive low affinity system which is driven by diffusion of nutrients because of an electrochemical gradient between the outside and the inside of the fungal hyphae (high phosphorus concentration outside and low phosphorus concentration inside). Usually these two systems (Fig. 4.2 B) act together during the process of phosphorus absorption by the VAMF hyphae (Thomson *et al.* 1990).

Thomson *et al.* (1990) point out that different VAM fungal species will differ in their efficiency of phosphorus uptake. Thus VAMF with the ability to take up phosphorus more efficiently will take up phosphorus much faster from the soil than those without this ability.

Cooper (1986) identified three possible mechanisms for the more efficient uptake of insoluble phosphorus by mycorrhizal plants:

i) the presence of the enzyme phosphatase in the soil due to the activities of microorganisms, active growing extramatrical hyphae and root surfaces caused by VAM fungal infection can enhance the amount of available phosphorus from organic compounds;

ii) the presence of phosphorus solubilizing bacteria and fungi can add phosphorus to the soil. The population numbers of these organisms are increased when VAMF are present, and

iii) organic acids, such as oxalic acid, exudated from fungi, can also solubilize inorganic phosphorus and phosphate attached to iron and aluminium hydroxides to release phosphorus for absorption, but according to Cooper (1986) this phenomenon has not yet been confirmed with VAMF.

High levels of phosphorus in the hyphae of VAMF can cause a serious disturbance in the normal host cell metabolism but to overcome this problem, VAMF translocate phosphorus in their vacuoles as polyphosphate (Gianinazzi-Pearson & Gianinazzi 1983).

Phosphorus is stored in VAMF mostly in a granular polyphosphate form in the hyphae and in developing arbuscules but is absent in collapsed arbuscules. Enzymes such as alkaline phosphatase, are present in the external and internal VAM fungal hyphae but are absent in non-mycorrhizal root tissues (Cooper 1986). Alkaline phosphatase enzymes are also present in VAM fungal vacuoles. These enzymes play an important role in the synthesis and degradation of polyphosphates. Polyphosphate activity occurs only in the intramatrical hyphae and disappear from the vacuoles in the finer branches of the arbuscule (Gianinazzi-Pearson & Gianinazzi 1983).

Polyphosphate granules are stored in vacuoles only for a short while after which it is rapidly transferred to the host. According to Cooper (1986) and Hayman (1982) the polyphosphate

granules are most likely transported by cytoplasmic streaming.

## 4.3.1.2 Transfer of phosphorus to the host cells

The surface of the finest arbuscule branches, which are metabolic highly active, is regarded as the main location where the exchange of nutrients takes place, and thus phosphorus too (Kendrick 1992). The transfer of phosphorus takes place through living membranes and not because of arbuscule collapse and degeneration. Polyphosphate granules are translocated through the hyphae to the arbuscule branches (Cooper 1986). The enzymes for polyphosphate metabolism are present in greater amounts in mature arbuscules than in intercellular and intracellular hyphae. In the host plasmalemma, which proliferates around the arbuscule wall, there is an intense activity of ATP-ase which indicates that phosphorus is actively transferred from the arbuscule to the host by active absorption. ATP-ase activity is absent in the host plasmalemma near very young developing or collapsed arbuscules (Gianinazzi-pearson *et al.* 1991).

## 4.3.1.3 The effect of phosphorus levels on phosphate physiology

After several studies of the effect of phosphorus on VAM, it was found that it is rather the high levels of phosphorus within the plant itself that inhibit VAM infection than phosphorus levels in the soil. Studies by Koide & Li (1990) showed that it is the phosphorus levels in the host root rather than the host shoot that regulate VAM infection. De Miranda *et al.* (1989), Anderson (1988) and Krikun *et al.* (1990) state that low levels of phosphorus in the plant change the phospholipid composition of the host cell membranes. This change then increases the permeability of the membrane which will lead to a subsequent leakage of root exudate. The increase in the leakage of root exudate increases VAM infection. However, high levels of soluble phosphorus in the soil contribute to higher phosphorus levels in the plant. Schwab *et al.* (1983) indicate that the major effect of phosphorus on VAM occurs during the rapid growth phase of VAMF colonization (Fig. 4.1). With high levels of phosphorus in the plant, there is a decrease of mycorrhiza-specific phosphatase enzymes of the fungus which suppress VAMF infection.

As previously mentioned, phosphorus plays a major role in the photosynthesis process. If the phosphorus absorption increase due to VAMF infection, the carbohydrate contents of the host plant increase. Infection by the fungus which is dependant on the carbohydrates from the host is positvely affected (Harris *et al*. 1985). Different VAMF reacts differently to a certain phosphorus status of host plants and according to Cooper (1986) there are some VAMF which can tolerate high phosphorus levels.

### 4.3.2 Nitrogen nutrition and metabolism

There is experimental evidence that VAM fungi increase the nitrogen absorption capacity of the host plant (Cooper 1986). According to Hayman (1982) VAM infected nitrogen-fixing plants can absorb more nitrogen as a result of increased phosphorus absorption. Cooper (1986) states that nitrogen absorption can be enhanced by VAM fungi in two ways:

i) VAMF stimulate other free living nitrogen-fixing microorganisms in the soil to increase in number and activity, thus increasing the fixed nitrogen which is available for plant use, and

ii) they stimulate the increase in nodulation of nitrogen fixing organisms in legume and actinorhizal hosts resulting in more nitrogen for plant use.

VAMF infected plants usually contain lower nitrogen levels in their tissue due to the vigorous growth of the plant which consume all the available nitrogen for the growth process as compared to non-mycorrhizal plants. This increased absorption of nitrogen and phosphorus will subsequently increase nodulation and nitrogen fixation by *Rhizobium*-like bacteria in some plant species. VAMF are sometimes a necessity for proper growth in nitrogen-fixing host plants, especially where they are in competition with other plants for soil nitrogen and also with soil microorganisms which use the same nitrogen source. VAM can also play a role in the growth and ecology of soil microorganisms by reducing the nitrogen pool in the soil (Johansen *et al*. 1992).

Nitrogen is usually absorbed by plants in an ammonium or nitrate form. Ammonium is relatively immobile compared with the highly mobile nitrate (Johansen *et al*. 1992). Cooper (1986) states that VAMF have more effect on increasing the absorption of nitrogen from

ammonium sources than from nitrate sources, because ammonium is directly incorporated into the cell for amino acid synthesis (Barea 1991). Nitrate, on the other hand, must first be reduced to nitrite and then to ammonium. Nitrate is usually transported in the hyphae by mass flow. According to Johansen *et al.* (1992) VAMF appear to have enzymes for the assimilation of ammonium. There is evidence that spores of some species of VAMF are able to reduce nitrate to ammonium (Sundaresan *et al.* 1988). There is also evidence that VAMF prefer to incorporate ammonium to the hyphae, especially when ammonium is the dominant compound, because less energy is necessary to assimilate ammonium than to assimilate nitrate. Further evidence shows that high levels of ammonium and nitrate in the soil can reduce VAM infection (Johansen *et al.* 1992).

This information could be of great significance to the plant production industries and will be further elicited in chapter 6.

### 4.3.3 Absorption of other nutrients.

The increased permeability of the membranes of the host root due to low phosphorus levels in the plant causes other important nutrients for example potassium, calcium, magnesium, iron, copper, zinc, sodium and boron to be absorbed together with phosphorus. On the other hand, with higher levels of phosphorus in the plant, VAM infection decreases and a micronutrient deficiency might occur (Cooper 1986). The enhanced absorption of high levels of some of these nutrients, however, can be toxic to the plant.

Copper and zinc are not very mobile in the soil and therefore VAMF contribute to the increased absorption of these nutrients by plants (Hayman 1982). Zinc can be absorbed directly by VAMF without the influence of phosphorus levels in the plant and be translocated via hyphae and arbuscules to the host (Cooper 1986). Faber *et al.* (1990) indicate that zinc moves to the plant root by diffusion and mass flow and the plant roots affect the diffusion gradients of the zinc in the soil. As a result the zinc can later become depleted in the rhizosphere of the host root. VAMF, however, can enhance zinc absorption due to the extramatrical hyphae that explore the soil more efficiently. Cooper (1986) points out that VAM plants absorb sulphur much more rapidly than non-mycorrhizal plants because of the

greater absorbing power of the extramatrical hyphae. The absorption of sulphur might also be linked to the increase in the phosphorus absorption. The manganese levels of mycorrhizal plants are usually less than in non-mycorrhizal plants (Cooper 1986; Syvertsen & Graham 1990). It is important to note that the greater absorption of nutrients is directly related to the specific absorptive quantities of the hyphae, a factor which need to be further investigated in relation to agricultural and horticultural production.

Different VAMF react differently to high levels of heavy metals such as copper and zinc. There is evidence that some species of VAMF are more tolerant to these heavy metals but this will be discussed in chapter 5.

Hyphae of VAMF are known to translocate small amounts of calcium to the host roots, because of higher transpiration rates in VAM infected plants (Huang *et al*. 1984). The role of calcium in VAM plants is that of being a secondary constituent of polyphosphate granules and it is also involved in the transfer of phosphorus to the host by stimulating certain phosphatase enzyme activity. Calcium is also necessary to maintain the integrity of the membranes (Cooper 1986).

## 4.3.4 Carbon physiology and biochemistry.

VAM infection is definitely affected by the factors which influence photosynthesis such as light intensity, day length and defoliation. During winter times the low light intensity, short photoperiod and defoliation inhibit photosynthesis and consequently less carbohydrate compounds are synthesized. With less carbohydrates available to the fungus, infection decreases (Gianinazzi-Pearson & Gianinazzi 1983). According to Cooper (1986) there is also a decrease in the number of vesicles that are formed under these conditions.

Douds & Schenck (1990) state that the nitrogen:phosphorus ratios can also affect VAMF colonization, for these ratios affect the carbohydrates available to the fungus. They also indicate that some species form spores within the host root cortex because of the shorter route for the transport of carbohydrates from the host to the fungus.

Gianinazzi-Pearson & Gianinazzi (1983) report that the host plant tolerates the flow of carbon to the fungus during the symbiosis because the fungal associate compensate for the loss of carbohydrates by creating circumstances for an increased nutrient absorption by the host plant. VAM also contributes to better water relations in the plants (especially under dry conditions).

## 4.3.4.1 Carbon transfer between the host and the fungus

The transfer of carbohydrates to the fungus happens when the arbuscule is mature, prior to arbuscule degeneration. The fungus obtain most of the carbon compounds in a soluble form and then converts it to an insoluble form such as glycogen and lipids within the fungus, rendering it unobtainable for the host (Gianinazzi-Pearson & Gianinazzi 1983; Cooper 1986). The accumulation of carbohydrates as lipids occurs in the older arbuscules and hyphae but are absent in very young arbuscules and hyphae. The storage of glycogen is always associated with large concentrations of lipids in hyphae and arbuscules (Cooper 1986). Host cells with arbuscules lack starch and this absence of starch is associated with the accumulation of glycogen in arbuscules (Gianinazzi-Pearson & Gianinazzi 1983).

According to Cooper (1986) and Gianinazzi-Pearson & Gianinazzi (1983) the carbohydrates are transferred to the fungus by osmosis by maintaining a concentration gradient for soluble carbohydrates between the host and the fungus. This is achieved as the fungus continually converts the carbohydrates into insoluble glycogen and subsequently into lipids. Thus, a gradient of carbohydrate concentration develops between the interfacial matrix and the host plasmalemma. The carbohydrates are therefore passively transferred into the interfacial matrix between the arbuscule and host plasmalemma and then "picked" up by the fungus by active absorbtion.

Cooper (1986) also refers to evidence of the movement of carbohydrates back from the fungus to the host. Sometimes these carbohydrates return to the host as skeletal material where it is changed into amino acids which in turn can be transferred from the host to the fungus.

Lipids are generally the storage product of carbon compounds from the host. Infected roots have more lipids than uninfected roots. Spores usually contain a vast amount of lipid which can increase as the spore germinates. According to Jabaji-Hare (1988) and Cooper (1986) triglycerides are the most abundant lipids in the mycelium and spores of VAM fungi. Cooper (1986) further indicates that there is an increase of phospholipids in infected roots due to the proliferation of the host cytoplasmic membrane during arbuscule development. Phospholipids are also present in the fungal hyphae, but this type of phospholipids differ from that of the host tissue. The arbuscule walls contain glycolipids, which are absent in the chitinous hyphal and vesicle walls.

VAMF contain fatty acids that are very much the same as those found in other Zygomycetes fungi but the presence of non-adecanoic acid in the hyphae of VAMF relate more to those produced in Chytridiomycetes (protocstisan) fungi (Jabaji-Hare 1988). VAM fungal spores also contain a poly-unsaturized fatty acid, which is rare in other fungi (Cooper 1986).

Peroxidase and catalase activities are present in degenerating arbuscules which might play a role in $\alpha$ and $\beta$ oxidation of fatty acids in the arbuscule (Cooper 1986). Spanu & Bonfante-Fasolo (1988) show that the cell-wall-bound peroxidase activity in VAMF infected host roots is higher compared to non-mycorrhizal host roots. This activity is also present during the initial infection stage of VAMF, especially in the time when the hyphae penetrate the host root.

There are more sterols in infected than in uninfected host roots. These sterols are basically 24-methylcholesterol, cholesterol and campesterol (Cooper 1986). An increase in sterol metabolism also occurs which might induce enhanced growth response of the host. These sterols play an important role in changing the permeability of the host membranes during infection, thus increasing the ability of the host plant to take up more nutrients and water. According to Cooper (1986) sterols exhibit hormonal activities.

It is necessary to take cognition of the "gains" which the VAMF obtain from the symbiotic association with the host plant, although it may not appear to be relevant for the purpose of this thesis. The existence of VAM depends on the carbohydrates and other carbon compounds

which are found in the host plant.

## 4.3.4.2 Carbon cost of the symbiosis

Most of the carbon compounds obtained by the fungus is used for respiration and only a small part is incorporated into fungal material (Cooper 1986). The demand for carbon from the host depends on the stage of fungal development. Young host plants with a developing mycelium demand more carbon than non-mycorrhizal plants of the same age. This might be due to the demand of carbon for the developing mycelium as well as for the high demand for the developing plant itself. (Syvertsen & Graham 1990; Cooper 1986). However, at later stages of infection the demand for carbon is more or less the same in older host plants and in non-mycorrhizal plants (Douds et al. 1988).

Sometimes the inoculation of plants with VAMF may result in growth depression rather than growth enhancement. The carbon demand of the fungus results in less carbon for the host. This decrease in carbon will subsequently lead to less amino acid and protein synthesis. This will cause the existence of free $NH_4^+$ within the plant which cause the pH to rise. The higher pH will suppress host plant growth (Buwalda & Goh 1982). Cooper (1986) formulated reasons for this growth depression and pointed out that it can be caused by:

i) the pathogenic phase of infection of the endophyte where the demand for carbon compounds is high;

ii) competition for soil phosphorus between the fungus and the host;

iii) phosphorus toxification, because of too high levels of phosphorus in the host plant;

iv) the increase of host cytoplasm, due to the proliferation of host membranes around the developing arbuscules;

v) the disturbance of protein synthesis in the host due to reduced carbon sources, and

vi) the alteration of hormonal levels in the host plant due to VAM infection which can cause a change in pH or mobilization of carbohydrates.

In most cases the host plants compensate for the carbon loss to the fungus in several ways. They adapt to better utilization of nutrients and to higher photosynthesis rates by forming more chlorophyll (thus more photosynthetic units). This may result in the development of

thicker leaves and larger stomatal openings or longer stomatal opening times during photoperiod (Hayman 1982; Cooper 1986). In experiments conducted by Potty & Indira (1990) they showed that there is higher absorption of $CO_2$ in VAM plants compared to non-mycorrhizal plants and also a greater leaf area and dry matter. These adaptations lead to the synthesis of enough carbon compounds to balance the demand by the fungus for carbon and the need for enough carbon for the host plant to grow effectively.

Also this aspect of VAMF infection might have to be considered when VAMF are manipulated to benefit plant growth industries.

## 4.4 Hormonal effects

There is evidence that VAM infection enhance plant growth due to their ability to influence growth hormone production in the host plant roots, such as auxins, gibberellins and cytokinines. Studies by Barea & Azcón-Anguilar (1982) showed that these growth hormones can increase VAM formation due to better host plant growth. VAM fungi can also increase cytokinin activity in the leaves of host plants (Cooper 1986; Hayman 1982; Gianinazzi-Pearson & Gianinazzi 1983). Danneberg et al. (1992) and Allem et al. (1982) showed that abscisic acid and indole-acetic acid levels also increase in VAMF infected host plants.

## 4.5 Water relations

VAMF are found in a wide range of habitats, from desert environments to aquatic environments. VAM infection has several implications for plants concerning their water relations. According to Fitter (1988) the mycorrhizal effects on water relations are more of secondary consequence with regard to changes in phosphorus nutrition. VAM infected plants have lower water resistance (higher hydraulic conductivity), higher transpiration rates, higher water potentials and lower stomatal resistance to opening than is the case in non-mycorrhizal plants (Cooper 1986). Huang et al. (1985) found that the transpiration rates of mycorrhizal plants are two times faster than that of non-mycorrhizal plants.

Extramatrical hyphae, as already mentioned, are considered to be root hair extensions, by which the absorbing surface for water is increased. These hyphae can even bypass dry zones for several centimetres, during dry periods. The absorbed water then moves through the hyphae to the host, probably by mass flow. Another indirect contribution of VAM infection is that the host have longer and thicker roots. The presence of VAMF in the roots of the host plant also increases root branching, thus increasing the absorbtion surface of the host root itself (Huang *et al*. 1985; Cooper 1986). VAM infection also enhance the permeability of the host membranes due to the greater absorption of phosphorus, thus causing better water transport through the membranes of the host plant (Cooper 1986).

According to Hayman (1982) VAMF infected plants can cope better to water stress than non-infected plants and can also reduce the wilting and shock effects when plants are transplanted. VAMF infected plants may even tolerate continuous drought better than non-infected plants. These contributions to plants during drought conditions are due to VAM causing the lowering of the water potential of the plants, although the stomatal resistance to open remained low. Physiological water stress in plants causes the formation of a substance called proline and this substance is relatively lower in VAMF infected plants than in non-infected plants (Cooper 1986).

According to Huang *et al*. (1985) the leaf temperatures of VAM plants are usually lower due to their higher transpiration rates. They also found stomas of mycorrhizal plants adjust more rapidly to ambient humidity when the soil water potential is low.

## 4.6 Conclusion

Today the main aim in VAM research is to manipulate VAMF for agricultural, horticultural and forestry uses. Thus the physiology of VAM must be thoroughly understood, more specially their beneficial effects on nutrient and water absorption resulting in vigorous host plant growth and ultimately in higher crop production.

However, one must remember that growth depression in some host- fungus relationships due to VAMF infection does occur. The researcher must not turn a blind eye on these non-

beneficial aspects of VAMF infections, because even this can be manipulated to serve the interests of the farmer, the horticulturalist or the forester. In this research, however, the main focus will be upon the beneficial aspects of VAMF infections.

Although much research has already been done on the physiology of VAM, there is still a need for more realistic research in this field (Hayman 1982).

# CHAPTER 5 : The Ecology of VAMF

## 5.1 Introduction

VAMF are geographically ubiquitous and occur over a broad range of ecological conditions (Bagyaraj 1991). These fungi are present in almost all undisturbed soil the world over, where they play an important role in the health of most plants. The abundance of VAMF throughout the soils of the world, is an indication of their ability to survive in extreme environmental conditions for relatively long periods of time (Bagyaraj 1991). To use these attributes of VAM fungi in agriculture, one must understand the ecology of VAM, because the life cycle of VAMF is greatly influenced by most ecological factors such as season, edaphic and environmental factors (Hetrick 1986). On the other hand, VAM has an enormous influence on the success of ecological and agronomic systems (Herrera *et al.* 1993).

As already mentioned in chapter 4, VAMF are beneficial to host plants for their ability to absorp nutrients from low fertile soils and subsequently increasing host growth. VAMF are also able to increase the population of soil organisms useful to plants, to increase nitrogen-fixation in legume and non-legume plants and to make the plant more resistant to disease (Bagyaraj 1991; Hetrick 1986; Linderman 1991; Herrera *et al.* 1993). VAMF are obligate symbionts and cannot grow apart from the host plant (Bagyaraj 1991).

Every organism in the rhizosphere is influenced by other organisms such as plants, and edaphic factors such as temperature, pH, nutrient content, organic matter, and other physical properties of the soil. Other environmental and climatic conditions, as well as by several activities of the human being can influence the characteristics of the soil. According to Linderman (1991) there is a modification of plant root morphology, physiology and root exudation due to VAMF infection which subsequently influences the other microorganisms in the rhizosphere.

Fitter (1985) reports that several studies show that VAMF are less effective under field than under laboratory conditions, because of:

i) the competition with other soil organisms;

ii) other organisms feeding on extramatrical hyphae, and

iii) fluctuations in the soil water contents which influence the diffusibility of nutrients in the soil.

According to Bagyaraj (1991) VAMF survive on a very wide range of hosts and are associated with more than 90% of all vascular plants. These fungi have already been observed in at least a thousand genera which represent about two hundred families. Bagyaraj (1991) estimates that there are over 300 000 susceptible host species for the 120 different VAMF already described.

*Glomus* species are most commonly found the world over. *Gigaspora* and *Sclerocystis* species are more confined to tropical areas. *Scutellospora, Acaulospora* and *Entrophospora* species are also distributed in most soils of the world, but are not as common as *Glomus* (Bagyaraj 1991).

VAMF are usually non-specific towards host plants, but some host-fungus relationships are more successful than others (Herrera *et al.* 1993). Newman & Reddell (1987) and Bagyaraj (1991) report that there is a great difference within plant families in the dependency on different VAMF. On the other hand, there is also an indication that non-mycorrhizal plants usually are taxonomically related. This may be due to morphological and physiological similarities between non-mycorrhizal plants such as the amount of root hair (Kim *et al.* 1989).

## 5.2 Dispersal of VAM fungal propagules

The fungal species of VAM occur in many countries including South Africa and their dispersal all over continents was probably before continental drifting. According to fossil records VAM-like structures were already found in plant roots 370 million years ago (Hetrick 1986). Bagyaraj (1991) reports that VAM fungi spreads from one living root to another through hyphal growth, and the spread of spores and other viable propagules by vectors like the wind, water and animals. The dispersal of VAM fungi can be divided into two categories: passive dispersal of propagules and active dispersal of spores and hyphae.

## 5.2.1 Active dispersal

This type of dispersal is by means of the fungus symbiont itself. According to Hetrick (1986) hyphae of the fungus grow through the soil at a rate of about 0,43 m per year. Other investigators such as Mosse *et al.* (1982) found that the growth of hyphae through the soil could be much faster. Hetrick (1986) also states that different VAMF differ in the rate of hyphal growth through the soil. The viability of the hyphae can influence the hyphal growth rate. Plant species, their age and root densities play a very important role in the rate of spread of VAMF. Bagyaraj (1991) indicates that the active dispersal is not very important for the spread of VAMF. The physical and biological features of the soil also play an important role on the spread rate of hyphae (Herrera *et al.* 1993; Bagyaraj 1991; Hetrick 1986).

The distance of hyphal growth before sporulation from the initial point of inoculation can be a means to determine the rate of spread of VAMF. Mosse *et al.* (1982) found that within 15 months the spores of inoculated fungi were spread irregularly up to 4,5 m from the original point of inoculation. After 21 months the spores were distributed all over a plot of 22,5 x 1,8 m. They found that the spread of VAMF through the soil was relatively fast and the rate was dependent on the fungal species and the susceptibility of the host. They also found that if susceptible hosts were available to the VAMF for a long period of time, the fungi would still be available to the second year crop and that reinoculation was therefore not necessary. Hetrick (1986) indicates that the rate of spread is faster in non-sterile soils than in sterile soil.

Bagyaraj (1991) found that the rate of hyphal growth is much faster if other species of VAMF are already present in the soil and that fumigants like formalin retard the rate of spread. There is much controversy as to whether hyphae of VAMF are chemotactically attracted towards the host plant or not (Hetrick 1986).

## 5.2.2 Passive dispersal

This type of dispersal is aided by other factors, so called vectors. Bagyaraj (1991) and Warner *et al.* (1987) show that single spores, epigeous or hypogeous sporocarps, or pieces

of infected host roots with viable hyphal pieces, can be dispersed by either abiotic vectors such as wind or water or by biotic vectors such as animals. Certain rodents and small birds are particularly fond of eating epigeous and sometimes hypogeous sporocarps. The spores are not digested by these animals and are still infective when deposited through their faeces on the soil near plant roots (Hetrick 1986). There is also evidence of single spores found in the guts and casts of several soil burrowing organisms like earthworms, millipedes, ants, wasps, gophers and termites. These spores can stay infective for up to eleven months (Hetrick 1986; Bagyaraj 1991).

Other burrowing animals bring the hypogeous spores to the soil surface which can then be further dispersed by other vectors such as the wind or water (Bagyaraj 1991). There is evidence of viable spores found in swallow nests. These birds collect mud which can contain VAM fungal spores to build their nests with. (Bagyaraj 1991; Hetrick 1986).

Spores are sometimes also found in the digestive ducts and faeces of grasshoppers, crickets and rabbits. These animals do not feed on VAMF, but eat leaves on which spores could have been deposited by the wind (Hetrick 1986; Bagyaraj 1991; Linderman 1991). Bagyaraj (1991) also states that the bodies of small animals that feed on sporocarps can be "dusted" with spores which can be carried away great distances and deposited over a broad area. Man can also be an important vector for dispersing spores by transplanting plants infected by VAMF, to other areas.

The wind can disperse spores onto seeds such as mucilaginous seeds of plants which are then dispersed further by wind or other vectors. Because VAMF spores are relatively small and very light. Wind can therefore carry spores over vast distances before depositing it (Bagyaraj 1991; Warner et al. 1987).

Water can also be an important vector in dispersing VAM fungal spores. It causes the mass flow of spore containing soils to other areas or erode topsoil with spores to other places (Bagyaraj 1991). Studies by Koske & Gemma (1990) on Hawaiian islands showed that VAM fungal propagules and host plant seeds had probably been dispersed by sea birds or by tidal movements of the sea. Then they were washed ashore, to vegetate newly formed shores.

They also studied the effect of sea water on the viability of VAM fungal propagules and found that VAM fungal propagules could withstand sea water conditions for up to 12 days or even more and still be infective.

## 5.3 Influence of environmental conditions on the life cycle of VAMF

During the symbiotic relationship between the VAM fungus and the host plant, the fungus benefits the host by enhancing the nutrient and water absorption of the host as well as helping the host to be more resistant against disease. On the other hand, the host plant will supply carbon compounds to the fungus as well as giving it a safe environment to stay, away from antagonistic organisms. However, this symbiotic association is greatly influenced by environmental conditions (Bagyaraj 1986; Hayman 1982).

The colonization of host plants by VAMF and the subsequent sporulation of these fungi are influenced by a wide range of environmental, host and fungal conditions. These factors are divided into two categories: biotic and abiotic factors.

## 5.3.1 Biotic factors

## 5.3.1.1 VAM fungal factors

The success of VAMF in the soil is shown by their abundant and ubiquitous distribution in most types of soils and environmental conditions. Their success can also be contributed to their ability to adapt to mild environmental changes. However, there is evidence that some of these fungi can adapt to extreme environmental conditions.

Indigenous VAMF can adapt better to environmental stresses than introduced VAMF and can therefore survive better under abnormal environmental conditions (Hetrick 1986). On the other hand, indigenous VAMF are not always the most effective to promote an increase in plant growth. These fungi concentrate more on survival than to maintain their effectiveness in the host plant (Barea 1991). More effective VAM fungal strains should be selected for agricultural, horticultural, forestry practices and the revegetation of disturbed areas (Herrera

*et al.* 1993; Mullahey & Speed 1991). In some cases introduced or non-indigenous VAM fungal species are more effective for enhancement of host growth than indigenous species. This indicates that introduced VAM fungal species are better competitors to invade the host plant and to increase the absorption of nutrients than indigenous species (Hetrick 1986).

The germination of VAMF spores does not always depend on certain environmental conditions but also depends on the species of VAMF itself. There is evidence that the germination of spores of some species of VAMF are blocked by a dormancy factor for a certain period of time depending on the species of VAMF (Bowen 1987; Bagyaraj 1991). Bowen (1987) found that this dormancy period is longer in dry soils than in wet soils. Several studies showed that VAM fungal spores will not germinate in sterile soil and that this dormancy factor should be broken before the spores will germinate. The presence of certain soil microflora has been shown to break this dormancy and subsequently stimulates the germination of spores (Hetrick 1986; Bowen 1987). According to Bagyaraj (1991) this dormancy can also be broken by cold treatments or desiccation in the laboratory. Linderman (1991) and Watrud *et al.* (1978) indicate that the germination of VAM fungal spores is influenced by several factors including self-inhibitors within the spores.

Different VAMF compete with each other to infect susceptible host plants. This competition will, however, result in the most effective VAMF to infect the host plants at the end. Hetrick (1986) states that multiple inoculation of a host with several VAM fungal species will result in less infection and colonization than with only one species at a time. Different fungi will compete differently with other species (Bagyaraj 1991). According to Hetrick (1986) it is difficult to distinguish between the species once they colonize the root. Only a few VAM fungal species have distinctive characteristics within the root.

There is a certain optimal threshold density of VAM fungal propagules which will cause successful infection and subsequent colonization of the host root. If sufficient inoculum is not available, no infection will take place (Herrera *et al.* 1993). This threshold differs with each species of VAMF, as well as in different host-fungus combinations. An increase in propagule density will subsequently increase the rate and percentage of colonization up to a certain level where further inoculation will have no effect (Hetrick 1986). Host plants with a short

growing season therefore need high propagule levels so that the infection and colonization can be rapid enough for maximum host growth before the end of the growing season (Hertick 1986). This phenomenon will play a major role in the production of crops with short growing seasons, thus it is necessary to inoculate these crops with concentrated or large volumes of VAM inoculum.

The inoculum potential or infectiveness of VAMF varies between different species. This could also affect the rate and percentage of colonization in the host root. The inoculum potential of a VAMF is the potential of a specific amount of inoculum to cause successful host root infection under certain environmental and host conditions (Menge 1986). VAMF with the highest inoculum potential will produce the highest percentage of infection and colonization (Daniels *et al.* 1981).

### 5.3.1.2 Host and non-host factors

There are many host and non-host factors which can influence the life cycle of VAMF and the possibility of host root infection. This will also influence the diversity and distribution of VAMF in the soil.

As already mentioned in chapter 4, it is the phosphorus levels in the plant that control the amount of root exudate due to the influence of this nutrient on the permeability of root cell membranes. The lower the phosphorus content the higher the permeability of the membranes and leakage of root exudates. These exudates have an effect on VAMF, especially for initiating VAM fungal infection (Graham *et al.* 1982).

The exudates from host roots are the trigger for the germination of spores of many types of fungi. There is, however, much controversy whether this phenomenon is also applicable to VAM fungal spores. Several studies have shown that VAMF spores germinate equally well in the presence of host and non-host roots (Bagyaraj 1991; Daniels *et al.* 1981; Hetrick 1986). However, some studies show that the root exudates of certain host plants do stimulate the germination of spores and that VAM fungal species that are host specific, might need a stimulus from the host plant before spores will germinate (Hetrick 1986). Hetrick (1986)

concluded that there must be other factors which trigger the germination of VAMF spores. She also states that other soil microorganisms and the chemical and physical conditions of the soil, are much more important to spore germination than the absence or presence of a host plant. There is also a correlation between the environmental conditions necessary for seed germination, and spore germination such as temperature, soil moisture and photoperiod (Bagyaraj 1991; Hetrick 1986). There is evidence that root exudates influence the hyphal growth in the vicinity of the host root. This will lead to successful penetration and infection of the host root (Dixon et al. 1989; Millner 1988).

Sporulation and spore numbers are good indications of the percentage of colonization but there is evidence that a few species of VAMF seldomly sporulate under favourable fungal-host conditions (Hetrick 1986).

Azcón & Ocampo (1981) and Koide and Mooney (1987) found that the amount of sugar components in the host root exudate gives an implication of the percentage of colonization of VAMF. This implies that the higher the percentage of VAMF colonization in the host plant the less sugar will be present in the root exudate. This is due to the utilization of host synthesized sugar by VAMF within the host root.

The level of organic matter in the soil influences VAM colonization and sporulation due to its effect on the texture, pH, nutrient content and water holding capacity of the soil. These conditions can influence the occurrence and the effectivity of VAMF in the host plant. High levels of organic matter in tropical soils usually contribute to higher colonization of VAMF in tropical plants. Roots of annual plants infected with VAMF can subsequently form part of organic matter and are thus available to other nutrient cycling organisms (Bagyaraj 1991).

According to Hetrick (1986) and Bagyaraj (1991) the presence of certain non-hosts between hosts can reduce colonization and sporulation of VAMF. This is due to probable toxic compounds in their root exudates or toxic components from degenerating seed coats of non-host plants after seed germination. However, there is evidence of higher colonization of VAMF in host plants when planted together with other non-hosts (Bagyaraj 1991). This may be due to the competition of of hosts and non-hosts for nutrients and the host favours VAMF

infection to increase the nutrient absorption.

The difference in the dependence of host plants to VAMF infection can also influence the percentage of colonization and subsequent sporulation of VAMF in the host roots (Bagyaraj 1991; Koide & Mooney 1987). In contradiction to this Hetrick (1986) implies that high percentages of VAMF colonization in a host root do not always result in an increase in host plant growth and health. The dependency of a host plant on VAMF infection is also not directly correlated to the nutrient status of the soil. Azcón & Ocampo (1981) and Koide & Mooney (1987) conclude that different VAMF causes different dependencies by the host plant. Some VAM fungal species prove to be more efficient in nutrient absorption than others. They also indicate that the densities of the roots can influence the dependency of the host to VAM infection. Host plants which only form a few roots are more dependant on VAMF than plants which form an abundance of roots in the soil.

Other host plant factors which can influence the percentage of VAM colonization is defoliation and pruning. According to Hetrick (1986) pruning of host plants does not significantly affect root colonization of VAMF, but play a major role in the frequency of sporulation. As already mentioned in chapter 4 defoliation of host plants during winter times can decrease VAMF colonization and sporulation severely due to the lack of photosynthate available to the fungus (Bagyaraj 1991; Hetrick 1986).

There is a horizontal variation in the occurrence of VAM inoculum due to the distribution of roots of host and non-host plants. Vertical variations of VAM inoculum also occur due to differences in host root densities and other edaphic conditions (Koide & Mooney 1987).

VAMF differ in their effectiveness in different hosts. The effectiveness of VAMF is often measured by their ability to form extensive extramatrical hyphae. The extent of these hyphae influence the ability of VAMF to absorb nutrients from nutrient deficient soils (Bagyaraj 1991).

### 5.3.1.3 VAM and nitrogen-fixing organisms

**Rhizosphere** is the term for the soil surrounding the roots of the plant. In this rhizosphere there are a great variety of soil organisms, each playing an important role in the fertility of the soil and probably influencing plant roots. The majority of these organisms are bacteria and to a lesser degree are fungi such as VAMF. Most of these organisms contribute to the availability of different nutrients in the soil necessary for optimal plant growth. This rhizosphere is the part of the soil that is most adversely altered by man by adding fertilizers and toxic compounds to enhance plant growth and kill pathogenic organisms. Man is also able to manipulate the soil organisms in such a way that it will positively influence the plant.

According to Bagyaraj (1986) VAMF are unique because of their location, partly inside the host plant and partly in the surrounding rhizosphere. The internal part of VAMF is therefore protected from other pathogenic and antagonistic organisms in the soil, as mentioned in chapter 4. This means that VAMF can usually colonize the host root without disturbance, thus increasing their effect in the host plant.

VAMF in the host root and as part of the rhizosphere, interact with other soil microorganisms. There is evidence that VAMF have a positive influence on those bacteria and actinomycetes which are useful to plant growth, by increasing their population numbers. This increase will then have a much greater influence on plant growth (Linderman 1991).

There are many reports on tripartite symbiosis in plants where the plant forms a symbiotic association with ecto- or endomycorrhizal and a nitrogen-fixing associate. Examples of such plants are species of *Alnus, Casuarina, Myrica, Comptonia, Elaeagnus, Hippophae, Shepherdia, Purshia, Dryas, Cercocarpus, Cycas, Ceanothus, Colletia, Discaria, Rubus, Parasponia* and *Coriaria* (Herrera *et al.* 1993; Quispel *et al.* 1993). *Alnus* species can also form a tetrapartite association with ecto- and endomycorrhizae and a nitrogen fixing associate (Herrera *et al.* 1993). Tripartite symbiosis of VAMF and nitrogen-fixing organisms with plants, is a great advantage to the plants, especially in nutrient poor soils of tropical forests (Berliner & Torrey 1989). Most herbaceous legumes are infected with rhizobium-like bacteria as well as VAMF. This tripartite association functions better than with either one of the two

plant associates alone (Herrera *et al.* 1993; Linderman 1991). VAM can increase the formation of nodules of these bacteria and thus increase nitrogen-fixation and subsequently enhance plant growth (Bagyaraj 1991; Bagyaraj 1986; Kawai & Yamamoto 1986).

Diederichs (1990) stipulated that a dual symbiosis is a very dynamic association which can be altered by different environmental conditions. Low soil phosphorus, for example, inhibits nodulation and VAMF will increase the absorption of phosphorus in these conditions. In turn it will increase nodulation and nitrogen-fixation and subsequently enhance plant growth. The dependency of certain legumes on VAM is influenced by the availability of nutrients, especially phosphorus, in the soil. A legume such as *Leucaena leucocephala* is extremely dependent on VAM in low phosphorus soils (Herrera *et al.* 1993).

Herrera *et al.* (1993), Diederichs (1990) and Barea (1991) indicate that nitrogen-fixing plants require a high phosphorus status within the host plant, thus VAM infection is extremely important in soils with low levels of phosphorus to improve phosphorus absorption. Linderman (1991) and Bagyaraj (1991) state that VAM can also supply all the other nutrients required for nitrogen-fixing, for example copper and zinc. The absorption of other nutrients necessary for plant growth such as magnesium and calcium in low fertile soils are also enhanced by VAMF in the tripartite association (Kawai & Yamamoto 1986). VAMF can also contribute to nitrogen-fixation by absorbing $NO_3^-$ and $NH_4^+$, which are usually not easily absorbed by most plants (Herrera *et al.* 1993).

According to Bagyaraj (1986) VAMF will still increase the nodulation and nitrogen fixing of plants when the phosphorus content of the host plant and the soil is high enough to eliminate the effect of VAMF on plant growth. VAMF will, however, be totally eliminated under very high phosphorus conditions.

There is evidence that in tripartite associations the two symbiotic associates of the host plant will compete for carbon compounds or photosynthate, but the host adapts to this situation by pushing up its photosynthetic rate and use their nutrients more efficiently than non-mycorrhizal plants (Herrera et al. 1993; Linderman 1991).

Herrera *et al.* (1993) indicate that the species diversity of a tropical rain forest is due to the

diversity of VAMF which reduces the competition between the different plants of the forest.

According to Herrera *et al.* (1993) woody legumes and plants infected with nitrogen-fixing actinomycete fungi, play an important role in the restoration of the soil which is depleted at about 0,7% per year. According to Linderman (1991) and Herrera *et al.* (1993) VAMF can also enhance the nodulation and nitrogen-fixation of certain actinomycetous fungi in non-legume host plants, especially those in tropical and arid areas. These plants can then adapt better to arid conditions when infected with VAMF. Plants infected with VAMF can also adapt to high levels of pollutants from mining sites (Herrera et al. 1993; Bagyaraj 1986).

Herrera *et al.* (1993) report that *Rhizobium*-like bacteria are quite tolerant to salinity and with the selection of strains of VAMF that can withstand salinity, the consequent dual inoculation of legumes, can be very successful.

Species of *Comptonia* and *Myrica* are dependant on the nitrogen-fixing actinomycete fungi, such as species of *Frankia,* even in nitrogen efficient soils. If there is, however, a lack of sufficient amounts of nitrogen in the soil, VAMF can play an important role in the absorption of nitrogen compounds to avoid plant degradation (Berliner & Torrey 1989).

The physiological activities of both VAMF and nitrogen-fixing bacteria in the host plant are regulated by genetic and biochemical interactions between the symbionts (Herrera *et al.* 1993). Herrera *et al.* (1993) reports that VAMF structures usually occur around the nodules of nitrogen-fixing organisms, but can also penetrate the nodules of actinorhizal plants. VAMF never penetrate the rhizobial nodules of legumes, though.

Some of these bacteria are living very close to the extramatrical hyphae and spores of VAMF and even get carbon compounds from the VAM fungus. The fungus will then transport the fixed nitrogen to the host plant (Linderman 1991).

There is a synergistic association between VAMF and free-living nitrogen-fixing bacteria such as *Azotobacter, Beijerinkia, Derxia* and *Clostridium species.* There is evidence that VAM can stimulate these bacteria to increase their population numbers and subsequently

increase their nitrogen-fixing ability. These bacteria are also known to stimulate VAMF to produce more spores and to enhance VAM infection in the host plant (Bagyaraj 1986; Manjunath *et al.* 1981).

### 5.3.1.4 VAM and other soil organisms

VAMF do not only interact with nitrogen-fixing organisms but also with other soil organisms which play a role in host plant growth.

VAM can increase the population numbers of phosphorus-solubilizing bacteria. These bacteria, such as *Agrobacterium* and *Pseudomonas* species, are able to solubilize phosphorus forms such as rock phosphate, which can not be utilized by plants (Bagyaraj 1986; Raj *et al.* 1981). It is also known that these bacteria can produce plant growth hormones such as cytokinin, gibberellin and auxin and vitamins such as vitamin B12, niacin, riboflavin, biotin and pantothenate. These vitamins are absorbed by VAMF for better growth within the host root. The beneficial effect of VAMF on plant growth enhancement can even be better when the numbers of these phosphorus-solubilizing bacteria are increased (Bagyaraj 1986; Young 1990).

Phosphorus-solubilizing fungi such as *Aspergillus niger* and *Penicillium funiculosum* can further form a synergistic association with some VAMF (Bagyaraj 1991; Linderman 1991). They both increase each other's growth, which then increases the absorption of phosphorus, potassium, zinc and magnesium. This enhanced absorption of nutrients will then increase host plant growth (Bagyaraj 1991; Linderman 1991; Manjunath *et al.* 1981).

Actinomycetes fungi such as *Streptomyces* species are known to promote plant growth. Some VAMF can increase the population numbers of these actinomycetes, thus enhancing plant growth even better. Unfortunately there is also evidence of an antagonistic association between VAMF and these beneficial actinomycetes, so that together they inhibit each others growth and subsequently suppress plant growth instead (Bagyaraj 1986). Bagyaraj (1986) indicates that this might be due to the antibiotic effect of *Streptomyces* species to VAMF.

Other soil organisms also play a very important role in the survival of VAM inoculum. VAMF spores and extramatrical hyphae become food for mites, worms, nematodes or collembola insects (Linderman 1991). Bacterial parasites can penetrate VAM fungal spores by degrading the spore wall with specific enzymes (Hetrick 1986). All the organisms that can decrease the formation of VAMF propagules will be further discussed in Chapter 6. Hetrick (1986) and Linderman (1991) indicate that a certain *Amoeba* species is able to penetrate VAMF hyphae and spores and subsequently reducing VAM fungal colonization of host roots. Mature VAMF spores are more resistant to parasitism than young spores, because of more melanin present in the spore walls (Hetrick 1986).

Some soil microflora can also inhibit the growth of VAMF hyphae and sporulation, however, these microflora do not eliminate VAMF propagules (Linderman 1991). This might be because of competition between these organisms and VAMF for nutrients.

There is also evidence of some soil bacteria and fungi which can stimulate the germination of VAM fungal spores as well as hyphal growth and the subsequent production of spores (Linderman 1991).

## 5.3.1.5 Survival of VAM fungal propagules

The longevity of VAM fungal propagules under natural ecological conditions plays an important role in the survival of VAMF as symbiotic associates of host plants. The survival of these propagules are, however, greatly influenced by environmental and host plant conditions. The factors which can destroy VAM fungal propagules can in some severe cases result in the total devastation of VAMF from a certain environment.

Herrera *et al.* (1993) give six factors which can reduce or destroy VAMF in natural and agricultural ecosystems:

  i) the mechanical disturbance of the soil;

 ii) drought;

iii) erosion;

 iv) the formation of soil mounds due to the activities of burrowing animals such as gophers

and moles;

v) fallow of soil for long periods without susceptible hosts, and

vi) the planting of monoculture non-host for too long periods without susceptible host cultures in between.

Koide & Mooney (1987) also found that the formation of gophers' mounds can decrease VAMF propagules in the soil. On the other hand, this activity of gophers can contribute to more nutritious soil to be brought to the surface thus more nutrients are available for absorption by the remaining VAMF for plant use. Bagyaraj (1991) pointed out that continuous mono-culture practices in certain areas will definitely reduce VAMF propagule numbers.

The survival of VAM fungal propagules depends on the type of propagules. Spores are considered to survive extreme environmental conditions for long periods (up to three years) (Bowen 1987). Colonized root pieces and extramatrical hyphae will colonize new host plants much faster than VAM fungal spores can, but are less infective after long periods of time.

The time of sporulation by VAMF can also have an influence on the survival of these fungi. Sporulation sometimes occurs only during unfavourable environmental and host plant conditions, for example, when crops are harvested or are at the end of their growing season. In some cases there is no need for VAMF to waste the energy on sporulation when everlasting crops such as certain grass species, are highly infected by them. (Bagyaraj 1991).

Large spores store enough energy to germinate and form germ tubes and hyphae in search of a susceptible host (Hetrick 1986). If the germ tubes and hyphae do not come into contact with a susceptible host, the spore will still be viable to germinate for several times until a susceptible host is found (Bagyaraj 1991). In fields where there is a monoculture of non-hosts the unsuccessful attempts of the spores to germinate will after a period be detrimental to the survival of the particular VAM fungal species in the soil. Attempts of spores to germinate prior to favourable seasonal conditions will also reduce propagule quantity (Hetrick 1986; Miller *et al.* 1985; Bagyaraj 1991). The dormancy of the spores of some species of VAMF may be a precaution against the germination attempts of spores during unfavourable

conditions (Bagyaraj 1991).

The inoculum potential of hyphal fragments and infected root pieces decreases when the top part of the soil becomes very dry, but spores are adapted to survive very dry conditions. However, propagules of VAMF will very soon become less infective in moist conditions (Bagyaraj 1986). The survival of VAM propagules are also greatly reduced by parasitism as already mentioned earlier (see 5.3.1.4.).

According to Herrera *et al.* (1993) and Jasper *et al.* (1989) extramatrical hyphae can survive the dry conditions of arid areas for up to a month after the host plant has died. However, if the soil is disturbed, the viability of these hyphae will decrease extremely. Jasper *et al.* (1989) also indicate that in some parts of the world the soil can be dry for up to six months prior to a rainy season while VAM fungal propagules can still be infective.

According to Hetrick (1986) and Bagyaraj (1991) some VAMF sporulate in protected areas, for example within old seed coats and nematode cysts or even within the host root itself. This may be a protection method against VAMF parasites and will also contribute to the survival of the spores.

Other factors which can have a negative influence on the survival of VAM fungal propagules are the storage of topsoil for very long periods and the fallow of agricultural soil. This will prevent VAMF propagules to infect host plants and therefore the viability of the propagules decrease and may later not be infective any more (Herrera *et al.* 1993). There is evidence that even non-VAM plants such as species from the Cruciferae and Chenopodiaceae, will become minimally infected by VAMF just for the benefit of survival if susceptible host plants are not available (Hetrick 1986).

Herrera *et al.* (1993) also point out that there are definite seasonal fluctuations in the sporulation of VAMF. When certain plants, for example grass species, stop growing during unfavourable seasonal conditions and most parts of the plants die VAMF will sporulate. This will increase spore numbers of VAMF to infect the host plants again during the next growing season (Bagyaraj 1991).

The grazing of animals on VAM infected grasses will reduce plant size and plant growth rate especially during dry seasons. These practices will not significantly decrease the percentage of root colonization of VAMF (Wallace 1987). Mullahey & Speed (1991) report that this activity can even lead to a slight increase in VAMF colonization of the host plant if the host plants are not exposed to stressful environmental conditions.

## 5.3.2 Abiotic factors

### 5.3.2.1 Nutrients in the soil

As already mentioned previously in this chapter the fertility of the soil and other edaphic conditions play a major role in the functioning of VAM. It was also mentioned that certain VAMF can adapt to altered soil conditions and still be beneficial to the host.

According to Bagyaraj (1991) there is less competition between plants for nutrients when the plants are colonized by VAMF. This is because VAMF utilize the available nutrients more effectively and they have the ability to bridge nutrient deficient and depletion zones.

The nutrient contents of the soil can play a very important role in the infectivity and subsequent colonization of VAMF in host plants. Most studies show maximum VAMF colonization of host roots in low fertile soils. According to Bagyaraj (1991) long term fertilization of soils will change the populations and species composition of VAMF in these soils.

High levels of phosphorus and nitrogen together decrease VAMF colonization and subsequent sporulation. However, if the phosphorus contents low and the zinc and nitrogen content is high, there will be an increase in colonization (Herrera *et al.* 1993; Bagyaraj 1991). The germination of VAM fungal spores is not really influenced by the fertility of the soil. When phosphorus levels are increased, there is a slight increase in the germination of spores, but the increase of potassium and nitrogen has no effect (Hetrick 1986). Manganese and zinc, on the other hand, inhibit spore germination and there is an indication that zinc and copper can inhibit VAMF colonization in some plants (Bagyaraj 1991).

The addition of glucose to the soil lead to an increase in the activity of other microorganisms and parasites which results in the suppression of spore germination. Sugar compounds can also decrease germ tube length, as well as the number of spores formed by VAMF (Hetrick 1986).

Sodium and chloride in saline soils inhibit spore germination and also hyphal growth. The salinity of the soil in arid areas is usually high and can thus be detrimental to most VAMF, because VAMF are sensitive to the high levels of sodium in the soil. In contradiction to this there is evidence that some VAMF in halophytes can tolerate relatively high levels of salinity in spite of high levels of sodium (Herrera et al. 1993; Bagyaraj 1991; Lioi & Giovannetti 1989; Hirrel 1981; Pond et al. 1984). There is evidence that some species of VAMF can even reduce the chloride contents of the host plant (Barea 1991).

There is also evidence of high VAM colonization in drought stressed plants that grow in fertile soils. Moisture deficit decreases the diffusibility of nutrients in the soil and so less nutrients can be taken up (Hetrick 1986).

Interplant hyphal bridges of VAMF can occur between neighbouring host plants and there is evidence that these hyphae are able to transfer nutrients between the host plants. The connected plants can be intraspecific or interspecific, one to be the donor and the other the receiver plant (Whittingham & Read 1982; Haystead et al. 1988; Francis et al. 1986). The effectiveness of these hyphal bridges depends on the species and their susceptibility to VAM infection as well as to their level of nutrient demand (Francis et al. 1986; Cooper 1986; Hayman 1982).

VAM plants and non-VAM plants get their phosphorus from the same pool. The diffusability of phosphorus in the soil can limit the absorption of phosphorus from the soil. Some soils fix phosphorus at a fast rate and then the available phosphorus becomes depleted for plant use. If the phosphorus pool becomes depleted VAM is extremely important for their contribution to the absorption of phosphorus by exploring the soil more thoroughly (Abbott & Robson 1986).

## 5.3.2.2 Water potential of the soil

Changes of the water potential in the soil can change the physiology of the plant and this could have a major influence on the life cycle of VAMF. However, VAM play a significant role in the absorption of extra water for the plant, especially under drought stressed conditions (already mentioned in chapter 4).

Spores germinate best between field water capacity and soil saturated with water. According to Bowen (1987) the spores of VAMF must become hydrated before germination can occur. Therefore they will not germinate during dry seasons. He also found the water logging of soils inhibit germination. The main issue here may be the lack of oxygen available for the germination process. Such limitations of oxygen can also decrease VAM colonization. There is evidence of some VAMF species which can adapt to low oxygen supply (Bagyaraj 1991; Barea 1991).

Too dry conditions inhibit spore germination and hyphal growth (Bagyaraj 1991; Wallace 1987). When host growth is very slow and the rainfall is very low, for example during drought conditions VAMF spores can stay dormant until more favourable conditions prevail (Herrera *et al.* 1993; Hetrick 1986). According to Herrera *et al.* (1993) VAMF help to utilize limited supplies of water and nutrients, especially in arid areas.

Studies by Wallace (1987) show that in very dry conditions VAMF, although present in the host roots, will have no effect on the uptake of water and other nutrients. This may be due to less carbon compounds available to the fungus which will inhibit further colonozation of the fungus or will cause VAMF infection to decrease. The fungus may also lack enough energy sources to absorb water and nutrients against concentration gradients.

According to Bagyaraj (1991), Ragupathy *et al.* (1990) and Farmer (1985) some species of VAMF are found to infect marsh plants, free floating and submerged hydrophytes. Tanner & Clayton (1985) indicate that the same VAMF species can infect aquatic and terrestrial plants but VAM development is slower in aquatic plants. This phenomenon proves the adaptability of certain species of VAMF to extreme conditions.

## 5.3.2.3 Temperature and light

Temperature and light are two of the main factors changing within a season and are very important in the development of VAMF in host roots. Koske (1987) showed that VAMF can be directly influenced by temperature or indirectly by the influence of the temperature to the host.

The seasonal changes of light and temperature play a major role in the timing of colonization and especially sporulation of most species of VAMF due to the seasonal changes in host plant growth. For most VAM fungal species the higher temperatures of summer result in higher root colonization and sporulation. There are reports that indicate that in general mycelial growth is maximum at temperatures between 28 and 34°C, has maximum arbuscular development at 30°C and maximum sporulation and vesicle development at 35°C. Spore germination is best at temperatures between 20 to 25°C (Hetrick 1986; Bagyaraj 1991; Schenck *et al.* 1975). Cold periods will inhibit colonization and sporulation of VAMF (Hetrick 1986). Bagyaraj (1991) indicates that spores of the same VAM fungal species will germinate at different temperatures, depending on other environmental conditions. Land & Schönbeck (1991) showed that the germination of spores will not occur under 5°C.

VAMF will colonize the host root and sporulate more readily in the growth season of summer host plants which is usually from early spring to the beginning of autumn. During this time enough spores will be produced to effectively colonize hosts during the next growing season. A significant decline in colonization and sporulation occurs during the winter season (Hetrick 1986; Bentivenga & Hetrick 1992; Cooke *et al.* 1992; Van Duin *et al.* 1990).

Light is extremely important to the photosynthetic process and thus also to the production of carbohydrates as mentioned in chapter 4. High levels of carbohydrates are necessary to sustain VAMF. Low light intensities decrease the amount of root exudate, because of low photosynthate production. This will definitely decrease the colonization of VAMF and spore numbers during sporulation but does not have an influence on the sporulation process. (Bagyaraj 1991; Graham *et al.* 1982; Bentivenga & Hetrick 1992). This phenomenon proves

that the host plant control the percentage of VAM infection in their roots. Hetrick (1986) and Bagyaraj (1991) found that long photoperiods (more than 12 hours) and high light intensities enhance colonization of VAMF in the host plant.

Apart from this, light may also play a significant role in the germination of VAM fungal spores. Schenck *et al.* (1975) found that more spores germinate under dark conditions than during light conditions.

Topography and altitude affect temperature and moisture which result in host plant density and diversity. Due to this fact, these conditions can indirectly influence the diversity and density of VAMF (Gibson & Hetrick 1988; Blaschke 1991).

### 5.3.2.4 The pH of the soil

The pH of the soil has a great influence on the fertility of the soil, because changes in pH can change the diffusibility of nutrients in the soil. These changes can thus influence VAMF due to the physiological changes within the host plant. The pH of the soil can also directly influence VAM fungal propagules in the soil.

The pH of the soil has a definite influence on the germination of VAM fungal spores but the effect differ in different fungal species. Some germinate well in alkaline soil, others better in acid or neutral soils (Hetrick 1986; Bagyaraj 1991; Barea 1991; Bowen 1987). Some VAMF are more effective in enhancing host growth under low pH conditions, others at intermediate soil pH levels and some prefer alkaline soils to perform best. There is also evidence of some VAMF that can function effectively under a broad range of pH levels and others are more specific to pH (Hayman & Tavares 1985; Barea 1991). Acidic soils usually contain high levels of aluminium and low levels of calcium and magnesium. This phenomenon is common to tropical oxisols and ultisols and can definitely affect VAMF. However, there are VAM fungal species that are tolerant to these conditions and will still enhance plant growth if phosphorus levels are low (Howeler *et al.* 1987). Barea (1991) found that a change in the pH can change the solubility status and availability of certain nutrients in the soil, thus affecting the absorption of these nutrients from the soil by VAM infected

plants. (Lioi & Giovannetti 1989; Barea 1991).

The occurrence of certain acid forming gasses such as $SO_2$, $CO_2$ and certain toxic nitrogen containing gasses due to industrial activities, are in most cases responsible for acid rain. This can change the pH of the soil and subsequently have a negative influence on the host-fungus relationship of VAM as well as on the survival of VAMF propagules in the soil (Clapperton *et al.* 1990; Heijne *et al.* 1989)

### 5.3.2.5 Fire

Fire is considered to be one of the most deleterious environmental factors which can instantly kill all the plants growing in a specific area. High intensity fires can have a very negative influence on VAM population numbers due to the elimination of VAM propagules and host plants. These burnt fields will take very long to restore the natural plants and can easily become susceptible to weeds and to plants such as unwanted grasses and intruder plants (Wicklow & Howard 1989). However, according to Gibson & Hetrick (1988) and Berch *et al.* (1991) low intensity fires to improve plant growth during the next growing season (slash burning), will not harm VAMF and their percentage of colonization in host plants.

Klopatek *et al.* (1988) concluded that fires during dry seasons can reduce VAMF infection more than fires during the rainy seasons. They conclude that the reduction of VAM fungal infection and VAM fungal propagules depends on the intensity of the fire.

### 5.3.2.6 Pesticides

Pesticides and fumigation practices can influence the presence of VAMF but this will be broadly discussed in chapter 6 which handles the effect of VAM and agriculture. Some of these chemicals can be detrimental to VAMF resulting in a decrease in VAMF propagules in the soil. Subsequently less infection will occur in the host plants (Bagyaraj 1991; Herrera et al. 1993).

Linderman (1991) states that fumigation and sterilization of soil has the following influences on the VAM fungi:

i) It eliminates microbial activities in the soil;

ii) It eliminates competition between soil microbes, and

iii) in some cases it can even increase the availability of nutrients.

Kim et al. (1989) and Bentivenga & Hetrick (1992) indicates that VAM fungi are more abundant in non-fumigated soils than in fumigated or sterilized soil.

## 5.4 VAM in plant succession and the revegetation of disturbed areas

The benefit of VAMF in the early events of succession is still in an experimental phase but there are indications that they can play an important role to establish a diversity of plants during the succession process, although most pioneer plants are usually non-mycorrhizal (Cuenca & Lovera 1992; Gange et al. 1990). According to Malloch (1987) VAMF were "strict biotrophs" since the origin of land plants. This symbiotic association between the fungus and the host plant could be responsible for some of the diversity in plant communities today. VAMF are, however, extremely important to those pioneer plants that are obligatory mycotrophic in the restoration of extremely disturbed areas such as deforested tropical rain forests where low fertility is a characteristic of the soil (Bagyaraj 1991; Herrera et al. 1993; Pendleton & Smith 1983).

Herrera et al. (1993) and Bagyaraj (1991) indicate that a decrease of VAMF in the soil results in a decrease in the diversity of plant species growing in that area. On the other hand, if the dependency of host plants to VAMF is low, the inoculation of these plants with VAMF will not contribute to the successful rehabilitation of these plants in their natural habitats (Flessner & Stubbendieck 1992).

The use of nitrogen-fixing plants can play an important role as pioneers in the vegetation of areas with poor soil conditions such as arid areas or the revegetation of disturbed areas. These plants can do even better if they are also inoculated with VAMF to rehabilitate these areas. These nitrogen-fixing plants are also able to alter the physical properties of the soil,

Fig. 5.1 VAM in the rehabilitation and restoration practices of severely disturbed areas

such as the the trapping of windblown soil particles. (Herrera et al. 1993).

In arid areas the absence of water as well as low levels of available nitrogen and other nutrients to the host, are limiting factors to plant growth in these areas. These poor conditions can result in the desertification of the areas. Wind, denitrification and the volatilization of ammonia are also important contributors to the loss of nitrogen in desert areas.

VAMF can also inhabit the roots of trees such as species of *Acacia* and *Prosopis* which occur naturally in arid areas and can thus be successfully used to rehabilitate desertified areas. These trees can also be used as pioneers in the restoration of mining areas, and beneficial effects of VAMF can contribute to the establishment of these legumes in the disturbed mining soils (Fig. 5.1) (Herrera *et al*. 1993).

The dual inoculation of the legume, sweetvetch, with nitrogen-fixing bacteria and VAMF, will increase root and shoot dry weight, as well as the dry weight of leaf material compared to non-mycorrhizal plants. Today sweetvetch (*Hedysarum boreale*) is broadly used to revegetate extremely disturbed areas (Bagyaraj 1986; Redente & Reeves 1981).

Sidhu *et al*. (1990) stated that *Casuarina equisetifolia*, which is a hardy and fast growing plant, can successfully be used to revegetate wastelands. This plant is an important fuel wood plant and with the dual inoculation of these plants with VAMF and a *Frankia* species, the plant growth can be dramatically increased, especially in saline soils with low phosphorus levels.

The tolerance of rhizobium-like bacteria and VAMF towards the dry and harsh conditions can determine the success of the dual symbiosis with legumes in arid areas and other disturbed areas. Both rhizobium-like bacteria and VAMF should be able to withstand the harsh environmental conditions such as changes in pH, heavy metal pollution, lack of sufficient water and nutrient supplies and high temperatures. This will be very important in the selection of VAMF to infect the plants for rehabilitation practices (Herrera *et al*. 1993; Stahl *et al*. 1988; Jasper *et al*. 1988).

In sand dunes of coastel areas the availability of nutrients is low, because of the sand's lack in the ability to retain water and leaching of important nutrients occurs at fast rates. VAMF can therefore also play an important role in the establishment of susceptible pioneer plants to revegetate these dunes (Herrera et al. 1993).

In studies by Koske & Gemma (1990) it was found that indigenous plants are more heavily infected by VAMF than alien or intruder plants. This can affect the selection of suitable host plant-fungus combinations in the vegetation of newly formed sand dunes.

In a salt marsh plant environment some VAMF strains are able to adapt to salinity (already mentioned earlier in this chapter). Thus, VAMF can be useful to infect host plants used during the restoration of disturbed salt marsh areas (Cooke & Lefor 1990).

Michelsen (1993) found that the inoculation of certain *Acacia* species for revegetation purposes prior to establishing the plants under field conditions, is better than introducing selected VAMF to field soils where the plants are going to be used.

The colonization, spore production and propagule numbers of VAMF are reduced by severe disturbance of the soil, but the revegetation of such areas can increase it again. VAM are also important in the process of succession in disturbed areas by enhancing the number and diversity of plants over a period of time (Cuenca & Lovera 1992).

## 5.5 Conclusion

One of the most devastating practises of man is the degradation of many ecological systems. This is done by malpractices such as deforestation of natural forests, pollution of the environment, practices which promote erosion and agricultural malpractices which can lead to the desertification of vast areas. These practices which can reduce the quantity and quality of plants, as well as the diversity of the plants species, can also be detrimental to those soil organisms which are beneficial to plant growth and health. VAMF form a great part of these soil organisms. They do not benefit host plants only but are also beneficial to other soil organisms which enhance plant growth. VAMF are also natural enemies to some plant

pathogens. These beneficial characteristics of VAMF can thus be used to rehabilitate those degraded ecosystems and can also be effectively applied during agricultural, horticultural and forestry practises.

On the other hand, these above mentioned practices are greatly influenced by the ecological factors of a certain area. The crops that are planted should therefore adapt to these conditions. By inoculating plants with selected VAMF, the crops can do even better to withstand ecological changes which can be devastating to the crops.

Throughout this chapter the influence of certain ecological conditions on the VAM symbiosis was pointed out. Man is not able to manipulate all of these conditions and therefore the practices of man should adapt to these conditions to sustain natural ecosystems as well as maximum crop production.

From what is said so far concerning VAMF, it must be clear that there are two major areas for research regarding the significance of the data available on VAMF for the agricultural and other plant industries:

i) how the benficial aspects of VAMF could be utilized on a big enough scale to benefit farming and its related industries, and

ii) how significantly the ecological conditions conducive to optimal VAMF growth and colonization can be created and maintained to increase crop production.

It will be impossible to address these issues in full in this thesis. Some aspects related to the uses will, however, be discussed in the next two chapters in context of the aim of the thesis.

# CHAPTER 6 : The Importance of VAM in Agriculture, Horticulture and afforestation

## 6.1 Introduction

In most cases VAMF show very little host specificity and according to researchers they are ubiquitously found in soil all over the world. The interest in VAMF became significant when researchers found that VAMF can increase nutrient absorption in host plants and subsequently enhance plant growth. They realized their importance in natural ecosystems and that VAMF should be manipulated to improve the growth of agricultural crops and their use as commercial biotic "fertilizers" (Abbot & Robson 1986; Mosse 1973). Later research led to the discovery of their important role in the revegetation of severely disturbed areas such as mining sites (Abbot & Robson 1986; Cuenca & Lovera 1992). This was already discussed more intensively in chapter 5.

Until recently research on crops was mostly done under greenhouse conditions. Today the emphasis falls on studying VAM under field conditions. According to Abbot & Robson (1986) the beneficial effects of VAM in agriculture are:

  i) an increase in growth of the host;

  ii) an increase of nutrient absorption by the host;

  iii) more resistance to drought for the host, and

  iv) an increase in the tolerance of the host against pathogens.

According to Millner (1991) there are two main approaches to the use of VAM in agriculture. The one approach deals with the management of indigenous VAMF in agricultural soils and the other approach is to select the most suitable and most effective VAMF to inoculate crops. As much information as possible about anatomical, taxonomical, physiological and ecological aspects should therefore be obtained before suitable VAMF can be selected to inoculate a certain crop. Unfortunately only about 15% of all the VAM fungal species already described, have already been evaluated physiologically to determine their effectiveness in certain host plants. There is therefore still much room for extensive research and experimentation.

Millner (1991) suggested several factors to be taken into account when choosing a suitable VAM fungus for research purposes:

i) morphological and physiological characteristics of the different VAMF such as the arbuscules, vesicles and extramatrical mycelium which determines the effectivity of the fungus;

ii) water relations of the fungus-host relationship. VAM are known to make the plant more tolerant to short periods of water stress (the extramatrical hyphae of VAMF aggregate the soil particles and this enhance the water holding capacity of the soil);

iii) infection measurements which deal with determining the percentage of infection and the ratios between the extramatrical and intramatrical hyphae;

iv) host factors such as the dependancy of the host on VAM, the morphology of the roots and the host plant physiology, and

v) edaphic factors such as the fertility of the soil, as well as the presence of other soil microorganisms.

## 6.2 The importance of correct procedures for the study of the use of VAM in agriculture

As already mentioned VAM fungi has not yet been cultured axenically and much research is needed to succeed in this (Mosse 1973; Abbott & Robson 1986). This inability to culture VAMF axenically stands in the way of producing large quantities of inoculum which is free from other soil organisms. Today smaller amounts of inoculum can be produced in "pot cultures". The plants from which the inoculum is obtained are grown in sterilized soil, free from indigenous VAMF and other soil organisms (Abbott & Robson 1986; Mosse 1973). The procedures for producing inoculum in this way and the use of it, will be broadly discussed in chapter 7.

The research carried out by several investigators such as Millner (1991) and Howeler *et al.* (1987) led to a proposed  model for VAMF inoculum research and development which can contribute to the difficult task of selecting most suitable VAMF inoculum. This step by step

procedures can easily be used to inoculate host plants in nurseries before transplanting them into the field or to directly inoculate host plants which grow in the field (Millner 1991).

## Stage I

Single species VAMF spores must first be isolated from the soil and be used to inoculate host plants grown in sterilized soil to form single species "pot cultures".

## Stage II

The VAMF from the "pot cultures" should be tested for their effectiveness on certain crops. Changes in the dry matter of the VAMF infected host plants should be measured and compared to non-infected plants. The VAMF from the pot cultures should be evaluated for their competitative abilities with other VAMF and their ability to withstand certain edaphic conditions, such as pesticidal and fumigation practices in the soil better than for example indigenous VAMF can do. The interaction of VAMF with other soil microorganisms should also be investigated.

## Stage III

The isolate of VAMF must be monitored for their effectiveness under wide ranges of soil conditions such as soil pH, soil density, phosphorus fertility, temperature and moisture.

## Stage IV

If the VAM fungus seems to be effective enough to produce an increase in growth and nutrient absorption of certain crops as well as an increase in the tolerance of the crop to pathogens and water stress, researchers should then find methods to produce enough inoculum to use under field conditions.

## Stage V

The inoculum should be tested under field conditions for its effectiveness, adaptability, survival and dispersal and then be inoculated onto the crops in the field. The timing and method of inoculation should be determined as well as the need for reinoculation.

**Stage VI**

If the VAM inoculum proves to be effective under field conditions, it can be used to benefit agricultural practices.

When testing for or experimenting with the effects of VAMF on certain plants, one must ensure that proper control plants match the experimental plants. The soil of control plants must, for example, match the soil microorganisms and nutrient content of the soil of the experimental plants. The inoculum used for the experimental plants should also be free from pathogens. Control plants should therefore differ from experimental plants only in the phenomenon that is being tested. If the tests are between mycorrhizal and non-mycorrhizal plants for example, the controls should only differ in the absence of VAMF. There is also a need for comparing mycorrhizal to non-mycorrhizal plants under more natural conditions to see if the effects of VAM are beneficial to the agricultural industry (Abbott & Robson 1986; Koide & Li 1989).

Jakobsen & Anderson (1982) and Jakobsen (1984) state that when the soil must be sterilized to eliminate unwanted organisms during the experiments, it should be done properly. This would prevent experimental failure due to the intrusion of unwanted organisms.

The response of the host on VAM fungal infection can be measured in several ways. Kendrick (1992) suggests four ways to determine the symbiotic effectiveness:

i) Spore production of the fungal symbiont. One must bring into account, however, that these fungi produce spores sporadically and with time the spores can disappear, due to predators and pathogens which feed on them. Other problems encountered when determining spore production, are that very small spores are not that easily obtained from the soil, that some of VAM fungi produce spores in sporocarps and that there is also evidence that some VAM fungi only produce spores in very rare occasions.

ii) By calculating the percentage of infection or VAMF colonization by the gridline intersect method through which infected roots are cleared and stained.

iii) The extent and density of extramatrical hyphae can also be an indication of the symbiotic effectiveness.

iv) Host response can be measured by weighing the dry matter of the roots and the shoots

of the host plant. Other response data can be obtained by measuring the height of the plant, stem diameter, shoot volume, the number of leaves and their area, amount of root exudates, transplant survival, crop yield and host resistance to disease.

## 6.3 The effect of VAM on plant growth

### 6.3.1 VAMF and host plant nutrition

One of the most important beneficial effects of VAMF is that they are able to enhance plant growth under certain conditions. Since these fungi occur in most of the plants including crops as already mentioned, this benefit can be of major importance in the agricultural, horticultural and forestry practices. The way in which VAMF take up nutrients and the translocation and the transfer of nutrients to the host plant have been thoroughly discussed in Chapter 4.

The increase of VAMF infected host plant growth is usually due to the enhanced absorption of nutrients (mentioned in chapter 4). Abbott & Robson (1986) give four main reasons for the enhanced absorption of nutrients in VAM fungal infected plants compared to non-infected plants:

  i) the distance that nutrients must diffuse to the roots are shortened by the extramatrical hyphae of VAM fungi;

 ii) VAM may increase the rate of nutrient absorption of VAM infected plants increasing the concentration gradient of nutrients between the soil and the host root;

iii) evidence that VAMF are able to change the status of some nutrients so that it becomes more available for plant use, and

 iv) increase of absorbing surface by VAM fungal hyphae.

As already mentioned in chapter 3, VAM is very important to plants with coarse roots and few root hairs. These plants are dependent on VAMF especially in low fertile soils and soils with a low phosphorus content (Powell & Bagyaraj 1986; Abbott & Robson 1986; Howeler *et al.* 1987). Tropical soils have low levels phosphorus as well as low levels of other nutrients which are important to plants (Sulia & Shandranath 1991). VAMF are also

important for the absorption of nutrients which move by diffusion through the soil (Gianinazzi-Pearson *et al*. 1983).

Different cultivars of the same host plant species can differ in their dependency on VAMF (Barea 1991; Toth *et al*. 1990; Heckman & Angle 1987). Kapulnik & Kushnir (1991) experimented with this phenomenon on several wheat cultivars. They found that different wheat cultivars differ in their dependency on VAMF under the same nutritional treatment. Another finding in this regard was that the breeding of disease resistant wheat cultivars decrease VAM dependency. They also found that the percentage of VAMF colonization is dependent on the growth stages of the wheat plants. The VAMF reach their maximum colonization when the plant reaches maturity. They further found that VAM increase the number of grains per spike. Some plants such as *Manihot esculenta* and *Stylosanthes* spp. are considered as obligate VAM fungal infected plants (Abbott & Robson 1986).

Recent work done on micropropagated avocado plantlets by Vidal *et al*. (1992) showed that when these plantlets are inoculated with VAMF, there is an increase in the formation of well developed roots. There is also an improvement in the growth of the shoots when these plantlets were transplanted to pots. VAM also increase the phosphorus, nitrogen and potassium contents in the plantlets.

According to Schwab *et al*. (1983) and Shukla & Vanjare (1990) colonization of VAMF depends on the levels of phosphorus in the crop plant (already mentioned in chapter 4). Therefore, high phosphorus levels in the plant decrease VAM infection. The inhibiting VAM fungal infection in the root will also lead to a decrease in the formation of extramatrical hyphae. Koch *et al*. (1982) found that the addition of phosphorus initially causes less vigorous growth of apple seedlings inoculated with VAMF than non VAM seedlings. However, in the end maximum growth was greater in mycorrhizal seedlings compared to non-mycorrhizal seedlings with the addition of phosphorus. This is an indication of VAMF adaptation to higher phosphorus levels.

The application of high levels of phosphorus, nitrogen and potassium in the soil will decrease VAMF infection (Howeler *et al*. 1987). Barea (1991) point out that VAMF increase the

direct absoption of nitrogen from the soil. Nitrogen can also be transferred to neighbouring crop plants via hyphal bridges formed by VAMF between the crops. Furlan & Bernier-Cardou (1989) found that there is an optimal ratio between these three nutrients to stimulate VAM symbiosis and thus maximum host plant growth.

Hepper (1983) found that nitrate application enhanced VAM fungal infection in lettuce. She tested this phenomenon at different levels of phosphorus and found that high levels of phosphorus inhibited the infection of VAMF, but at low levels of phosphorus the percentage of VAM fungal infection was correlated with the nitrogen content of the host plant.

It can be concluded that VAMF cannot be a substitute for phosphorus fertilizer, but these fungi can utilize the added phosphorus more effectively. In this regard Grey et al. (1989) found that plant growth responses as a result of VAM fungal infection are the same as the growth responses of non-mycorrhizal plants with phosphorus addition.

Pheiffer & Bloss (1988) found that VAM increase the growth of guayule in soils with low phosphorus levels but with the addition of phosphorus to the soil the levels of zinc and copper decreased. In another experiment they tested the effect of salinity on the fungus-crop plant symbiosis and they found that VAM can decrease the accumulation of chloride in plants in saline soils as mentioned in chapter 5. Another finding was that salinity reduced sporulation of certain VAMF. This will also affect the availability of VAMF propagules to infect following crops.

Different VAM fungi may also differ in their tolerance to salinity which will play a role in the selection of the most suitable VAM fungus in these soils. According to Pheiffer & Bloss (1988) not much work has been done on this matter.

As already mentioned, VAM is also beneficial to plants for their enhancement of the absorption of nutrients other than phosphorus, especially those that move in the soil by diffusion. According to Abbott & Robson (1986) a decrease in infection by VAMF can subsequently cause a deficiency of micronutrients in the host plant. They also indicated that zinc and sulphur are absorbed much faster by mycorrhizal roots. Quispel et al. (1993) and

Barea (1991) found that the rate of the absorption of nutrients other than phosphorus, such as zinc, copper, iron and sulphur, is increased due to VAMF, especially in tropical soils where soil contains low levels of phosphorus and nitrogen.

Cobalt is an important micro nutrient in plants for their role in the formation of cyanocobaltamine (vitamen B 12). However, high levels of cobalt within the plant can be toxic to the plant. Cesium can be absorbed by plants although it has no significant role in the plant. When cesium is taken up by the plant in large quantities it can be harmful, because of their gamma-emitting radionuclides. Rogers & Williams (1986) give evidence that VAM are able to increase the absorption of cobalt and cesium and thus can be responsible for the toxification of the host plant. However, in cobalt deficient soils VAM will be beneficial to the absorption of this nutrient. Dueck et al. (1986) state that VAMF can reduce the toxicity of zinc in the host plant. This could also be the case for other heavy metals.

Faber et al. (1990) and Sharma et al. (1988) found that the absorption of zinc is not linked to the absorption of phosphorus and that because of this, VAM can prevent zinc deficiency, which is very common in crops. Sharma et al. (1988) concluded that VAM can thus prevent Khaira disease in rice due to zinc defficiency.

Foliar fertilization is sometimes better for mycorrhizal plants, because soil fertilization might have a negative influence on soil organisms, such as VAMF. Soil fertilizers could later end up in fixed compounds which are unavailable for plant use as well as the leaching of nutriens. Dixon et al. (1989) found that foliar fertilization of citrus seedlings with boron increased VAMF infection and subsequently stimulated their symbiotic effectiveness.

Different VAMF differ in their effectiveness to enhance plant growth due to their differences in the absorption of nutrients. According to Abbott & Robson (1986) the factors which can influence the effectiveness of VAMF, is their ability to form extensive extramatrical hyphae and the extensive colonisation of the host root system.

## 6.3.2 VAMF and Non-nutritional effects

### 6.3.2.1 Soil conditions to sustain VAMF for favourable farming practices

There are many ecological factors apart from the nutrient status of the soil which must be taken into account when trying to manipulate VAMF for agricultural uses or select the most suitable VAMF under certain conditions. These factors are already extensively discussed in chapter 5. One must also remember that the beneficial effect may change if the ecological conditions change drastically. However, some species of VAMF are able to adapt to extreme conditions.

One of the most important factors is the pH of the soil and VAMF are sensitive to pH changes in the soil as well as within the plant. Selected species of VAMF which are tolerant to mild changes of soil pH can, on the other hand, play a role in future agricultural practices in acidic or alkaline soils. To most VAMF the stability of the pH is important to be most effective. pH can also affect the germination of spores, thus will influence the chances of host infection. The effect of pH changes on VAMF was already discussed in chapter 5.

Soil temperature and moisture conditions affects VAMF, because it affects nutrient absorption. Different VAMF respond differently to certain temperatures, but most of them function optimal in temperatures from 20 to 30°C. However, there is evidence of some VAM fungi that can tolerate cooler temperatures and thus will play a role in the selection of VAMF for these cooler conditions (Howeler *et al.* 1987). As already mentioned in chapter 4 the temperature, light intensity and moisture affects the physiology of the host plant which could subsequently affect the effectiveness of the symbiosis between VAMF and crops.

Some VAMF are more tolerant to water stress than others. Very dry conditions reduce spore production of most VAMF (Howeler *et al.* 1987). If this is the case VAMF propagule numbers will decrease and the reinoculation of crops should be considered. As already mentioned (Chapter 4) VAMF reduces water stress in host plants by bridging dry zones in the soil. Millner (1991) and Barea (1991) emphisized the importance of the extramatrical hyphae of VAMF to improve the ability of the soil to retain water. However, the disturbance
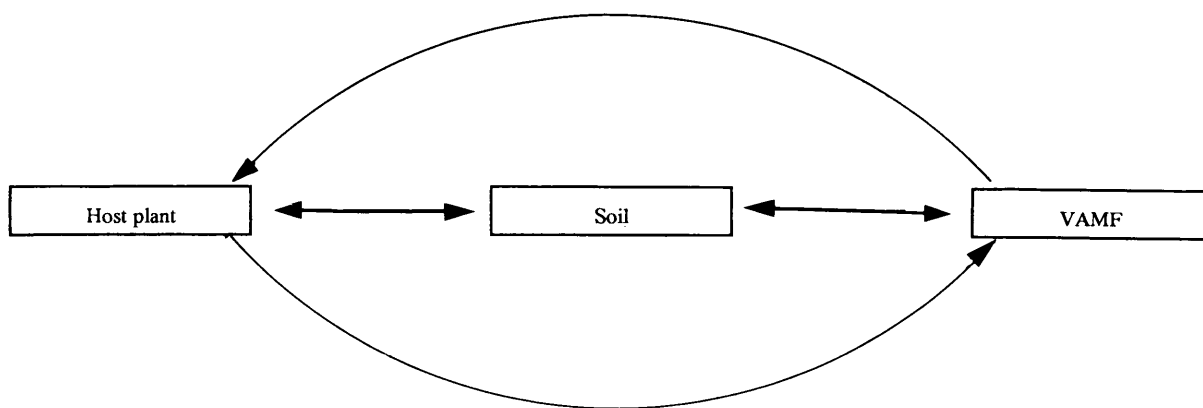
Fig. 6.1  Interaction between the host plant, the soil and the fungus during VAM symbiosis

of the soil during agricultural practices, such as the tilling of the soil, can decrease this activity of VAMF hyphae.

Johansen & Pfleger (1992) state that practices such as tillage and fallowing of agricultural soil can reduce the inoculum potential of VAMF thus affecting crop yield. They pointed out that by rotating the type of crop planted in a specific area might change the composition of the species of VAMF due to the preference by certain VAMF to certain crops. This could later decrease the effectivity of certain VAMF in a spesific area because of a decrease in VAMF propagule formation especially when different types of crops are rotated fast.

The continuous planting of monocultures will only stimulate certain VAMF to survive and subsequently decrease the inoculum potential of other VAMF species. This will result in insufficient nutrient supply to other host plants dependent of VAMF (Baltruschat & Dehne 1988).

Khadge *et al.* (1992) investigated the transfer of VAMF inoculum from one crop to another, for example from maize to mungbean. They found that this carry-over effect is not successful between crops of short duration. This might be because VAM infection is not fast enough to establish at maximum capacity before crop maturity and therefore a very few VAMF propagules will be available to the next crop. The disturbance of the soil by preparation for the next crop will also reduce the inoculum potential of the VAMF propagules in the siol. Therefore, the carry-over effect will not be successful in the tropics where crop rotation is rapid. Reinoculation of following crops may be a better proposition.

In conclusion, soil conditions have defenite effects on VAM fungal infection, thus influencing different crop plants. On the other hand, the soil will influence the physiology of the host plant, thus affecting VAM infection. Both the VAMF and crop plants will affect ecological conditions of the soil. These three factors interact with each other. Should one of these components change drastically the unique balance between the three components will also be disturbed. This disturbance can lead to crop failure (Fig. 6.1).

## 6.3.2.2 Growth stimulation

VAM can cause enhanced growth effects in host plants other than by enhanced nutrient absorption. Abbott & Robson (1986) indicated that the enhanced hormonal production and water absorption due to VAMF infection can cause a considerable increase in host plant growth. The physiological aspects of this phenomenon was already discussed in chapter 4.

Menge *et al.* (1978) and Khaliel & Elkhider (1987) found that the inoculation of avocado seedlings prior to transplanting can prevent stunting of the seedlings caused by soil fumigation and sterilization. VAM will in addition help the seedlings to overcome transplant injuries.

## 6.3.2.3 Growth depression

There is evidence that VAM may sometimes suppress plant growth due to several factors. This effect may be transitory or persistent. At the early stages of VAM infection there may be competition between the fungus and the host plant for photosynthate produced by the host. At later stages the host will compensate for the demand in photosynthate (Abbott & Robson 1986). Abbott & Robson (1986) report that pathogen invasion of the host plants after VAMF infection can also cause growth suppression due to the carbon demand of both the fungus and pathogen.

Low light intensity and low temperatures during winter can also influence VAM fungal infection negatively due to the effect of these two factors which lower the photosynthesis rates, thus inhibiting hyphal growth. This will subsequently lead to a growth depression of host plants in low fertile soils (Abbott & Robson 1986).

Growth depression of a host plant will also occur when a plant with a certain level of VAM fungal infection is transplanted to more fertile soil where the host plant will be less dependant on VAM. This will be caused by the still high carbohydrate demand from the fungus which could lead to less carbohydrate available to the host plant. This will, however, be temporarily untill the fungus adapt to the lower level of carbohydrate available (Abbott & Robson 1986).

Studies of Koide (1985) showed that growth depressions in the leaves of host plants infected by VAMF can occur due to stresses other than lowered photosynthesis rates.

## 6.4 Biological interactions between VAM and other soil organisms

### 6.4.1 The effect of VAM on plant disease

One of the most important aspects in agriculture, horticulture and afforestation is the protection of plants against disease, because disease can reduce crop production and have extensive economical consequences.

According to Kendrick (1992) several reports were written on the effect of VAM on plant disease and most of them showed that VAM reduces the incidence and severity of plant disease. There are some reports, however, which show that VAM has no effect on the disease or even may enhance the severity of the disease (Dehne 1982; Bagyaraj 1986; Schenck 1981; Quispel *et al.* 1993; Reddy *et al.* 1989; Chaturvedi *et al.* 1987; Bååth & Hayman 1983; Grey *et al.* 1989; Smith 1988). Kendrick (1992) gives three reasons why VAMF can in most cases reduce the severity of disease in the host plant:

  i) the host plants are healthier and therefore more resistant to pathogens;

 ii) the host plant may digest the arbuscules to use the chitinolytic enzymes to protect it against penetrating pathogens, and

iii) VAMF will occupy possible infection sites on the host root before other pathogens.

Schenck (1981) and Bagyaraj (1986) report that the damage of large vesicles and chlamydospores of some VAMF in the host tissue, provides a passage for pathogens to penetrate the host. Schenck (1981) reports that VAMF may even attract the zoospores of some pathogens such as *Phytophthora parasitica* towards the host plant. However, there are many reports which state that the inoculation of host plants prior to transplanting make the plant more resistant to the attacks of pathogens (Bagyaraj 1986; Ingham 1988; Smith 1988).

The effects of VAM on plant disease depends on the species of VAMF, for different species will act differently to the severity of the disease (Bagyaraj 1986). This information should

be very carefully evaluated when selecting VAMF species for agricultural, horticultural and forestry uses.

Sharma *et al.* (1992) and Bagyaraj (1986) report on the effects of VAMF on host root pathogens and give categories of mechanisms by which VAM can make the host plants more tolerant to these pathogens:

**i) Physical mechanisms.** There is evidence that VAMF cause the host cell walls to thicken through lignification and other polisaccharides to prevent pathogens from intruding into the host root. The vascular system of VAM infected plants are also stronger compared to non-mycorrhizal plant and this increased strength of the vascular system prevent the intrusion of vascular pathogens.

**ii) Physiological mechanisms.**

   a. Nutritional changes. VAMF increase the uptake and flow of nutrients in the host. This results in enhanced root growth, a larger absorbing surface and thus a greater absorbing capacity of the roots. This in turn will increase the tolerance of the host to pathogens because high phosphorus levels in the host plant which is caused by VAM infection suppresses nematode infection and the development of the juviniles into adults. This is due to less leakage of root exudation from the host roots due to the higher phosphorus levels in the roots.

   b. Biochemical changes. A change in the composition of root exudates occurs because of VAMF in the host root. This change influences the pathogens negatively because VAMF causes higher amounts of amino acids such as arganine and reducing sugars in the root exudates. These compounds can reduce the spore production of some pathogens such as *Thielaviopsis basicola*. VAMF in the roots are also responsible for the formation of phenylalanine and serine which inhibit root knot nematode development. VAMF are furthermore responsible for an increase of phytoalexins and peroxidase within the host root to make the host more resistant to pathogens.

**iii) Biological mechanisms**

   VAMF are important soil microorganisms which indeed influence other soil organisms by controlling their numbers in the population. This control effect of VAMF can affect host root pathogens in the soil such as *Phytophthora cinnamoni, Fusarium solani* and *Pseudomonas solanacearum* too.

Smith (1988) concluded that the resistance of VAMF is due to the better phosphorus nutrition and the change in the composition of host root exudates.

Ingham (1988) and Grandison & Cooper (1986) pointed out that the physiological changes in the host roots such as enhanced nutrient absorption due to VAMF activities, can reduce the invasion of root pathogens. In contradiction to this beneficial effect of VAM there is evidence that the more vigorous growth of VAMF infected plants, makes the host plant more susceptible to foliar pathogens such as *Helminthosporium sativum* and *Erysiphe graminis* (Bagyaraj (1986; Dehne 1982). This, however, must not be overlooked when VAMF are considered for farming purposes.

Many plant diseases are caused by viral infections such as mosaic viral disease on tobacco and bushy stunt virus on tomato (Lehninger 1973). Until today only a few studies were done on the effect of VAM on viruses which cause disease to the plants. These reports showed that VAM cannot reduce the severity of these viral diseases but rather make the plant less resistant to viral diseases due to more nutrients available to the virusses in healthier plants (Dehne 1982; Bagyaraj 1986).

Diseases caused by nematodes can be very harmful to crops, because these parasites can severely reduce plant growth and crop production. Today there are many nematicides that can effectively prevent or reduce nematode infection such as paclobutrazol and DBCP which can be applied to the soil.

Bagyaraj (1986) and Smith (1988) divide nematodes into three different groups:
  i) **Sedentary endoparasites**. These nematodes penetrate the plant as vermiform juveniles. Once they are in the plant they start to feed and later become immobile and their body starts to swell. Their development and reproduction take place in giant host cell with syncytia cells near the head for nourishment.
  ii) **Migratory endoparasites**. These parasites stay vermiform throughout their life cycle. They penetrate the root and migrate through the host tissue to feed on different host cells.
  iii) **Ectoparasites**. These parasites remain outside the host root, but have stylets to feed on

internal cells.

Much research has already been done on the effect of VAMF on nematodes. Research reports show that in most cases VAMF are able to reduce the severity of the damage caused by nematodes as well as a reduction in the numbers of nematode galls in the host root (Kellam & Schenck 1980; Bagyaraj 1986). VAMF can also retard the development of nematode juviniles into adults and decrease their reproduction (Ingham 1988; Bagyaraj 1986; Dehne 1982). The mycelia of VAMF can even penetrate the nematode galls (Bagyaraj 1986) but if this has an effect on the nematode is not known yet. Ingham (1988), however, reports that VAMF do not invade nematode infection sites and vice versa.

Most of the work on nematodes already done, was on sedentary nematodes, especially root knot nematodes such as several *Meloidogyne* species (Bagyaraj 1986; Grandison & Cooper 1986; Strobel *et al.* 1982; Kellam & Schenck 1980)). These reports state that VAM infected host plants are more tolerant to nematode infection and that nematode infection does not significantly affect VAMF within the root. However, a few reports show that VAM can cause an increase in nematode reproduction and numbers in the plant.

Ingham (1988) and Bagyaraj (1986) conclude that the effect of VAM on nematode disease depends on:
  i) nematode inoculum level;
  ii) cultivar resistance to the nematode;
  iii) the fungal symbiont in the association, and
  iv) the timing of the inoculation of the plant with VAMF.

It is important that the seedlings in the nurseries become infected with VAMF prior to transplanting to make the plant more resistant to nematode infection (Smith 1988). In experiments done by Strobel *et al.* (1982) they found that the inoculation of citrus plants with VAMF after transplanting had no effect on the severity of the nematode disease and still caused high mortality rate of the transplanted seedlings. They discovered, however, that in soils of low fertility VAM can decrease the injury caused by nematodes and even retard nematode development.

Fewer studies were conducted on the effect of VAMF on migratory nematodes such as *Pratylenchus brachyurus* and *Radiophilus similis*. VAMF can reduce the reproduction and number of nematodes in the plant root by altering the cortex cells to unfavourable food sources for the nematode. In the case of *Radophilus similis* VAM had no effect on the nematode and there is evidence that VAM can even increase the reproduction of this nematode (Bagyaraj 1986). Jain & Hasan (1988) found that VAMF had no effect on the spiral nematode, *Helicotylenchus dihysteria*, and vice versa. There was, on the other hand, evidence that VAM still enhanced better phosphorus absorption and host plant growth.

VAM can thus be used effectively to control plant disease when it goes hand in hand with enhanced plant growth and the elimination of transplant shock when seedlings are transplanted to non-sterile field soils. Much more research on the effect of VAM as a biological control of plant disease must still be done.

### 6.4.2 VAM and beneficial soil organisms

Because VAMF are abundant in the soil they are constantly in interaction with other soil organisms. Some of the organisms such as pathogens will counteract the beneficial effects of VAMF but others will, however, interact with VAMF to enhance plant growth. This was already mentioned in chapter 5.

Also mentioned in chapter 5 is that VAMF can increase nodulation and nitrogen-fixation ability of nitrogen-fixing organisms. Dela Cruz *et al.* (1988) reports that different VAM fungal species may contribute differently to the production of nodules in legume and non-legume host plants. Many crop plants are legumes and are thus able to fix nitrogen. VAMF can contribute to enhance nitrogen-fixing by increasing rhizobial and actinomycetous nodulation but this contribution differs between different species of VAMF. This economical practice can also be applied to desert and temperate areas.

Nitrogen-fixing crops require high phosphorus levels, thus VAM infection is extremely important in soils with low levels of phosphorus as already mentioned in chapter 5 (Herrera *et al.* 1993; Bagyaraj 1986). By planting legume crops in low fertile soils, is also an

economic way to provide nitrogen to the crops especially for farming in the tropics (Herrera et al. 1993; Barea 1991). When these plants are stressed by low phosphorus levels, plants can be inoculated with VAMF to increase nodulation and nitrogen-fixation and subsequently plant growth (Manjunath & Bagyaraj 1984; Bagyaraj 1986).

As discussed in chapter 5 the dual inoculation of legume crops in soils with low levels of phosphorus with VAMF and *Azotobacter chroococcum* can enhance plant growth more than with one of the associates alone. Brown & Carr (1984) confirmed this findings.

Shivaram *et al.* (1988) state that the inoculation of nitrogen fixing plants such as legumes in a grass-legume association with both VAMF and *Rhizobium*-like bacteria, increased the dry weight and nitrogen content of both plant species.

VAM synergestically associate free-living nitrogen-fixing bacteria, phosphorus- solubilizing bacteria as well as species of *Streptomyces* in the soil (Mentioned in chapter 5). These associations are important to crop growth and production (Bagyaraj 1986; Raj *et al.* 1981).

**6.4.3 Parasites and predators of VAMF**

VAMF are also pestered by parasites and predators that feed on them and this can have a significant effect on possible crop infection. This would not only affect VAMF propagule numbers but will also be detrimental on the extramatrical mycelium which is important for nutrient absorption from the soil.

Parasites such as *Cephalosporium micromonospora* which attack VAMF spores, can penetrate the spores via the fine radial canals in the spore walls to form a fine mycelium within the spore (Bagyaraj 1986). Studies by Gerdemann & Nicolson (1963) showed spore-like structures within VAM fungal spores. There is evidence of bacteria which can erode the spore walls of VAMF (Bagyaraj 1986). This will defenitely affect the viability of the spores.

Other fungi such as *Phylyctochytrium* and *Pythium*-like fungi can reduce the sporulation of VAM fungi (Bagyaraj 1986). The fungicide, Mancozeb can be used to eliminate parasitic

fungi such as *Anguillospora pseudologissima, Humicola fuscoatra* and *Phylyctochytrium* species which parasitize VAMF without totally destroying the VAMF population. These parasitic fungi are not easily eliminated by surface sterilization with hypochlorite (Bagyaraj 1986). These parasites are a problem to using VAMF for agricultural purposes and inoculum production. They can reduce VAMF propagules to such an extent that VAMF infection of crops is not possible or very slow. These aspects will be discussed thoroughly in chapter 7.

There is evidence that *Folsomia candida*, a collumbola, and a certain nematode, *Aphelenchoides* species, feed on the external hyphae of VAMF. This could reduce VAM propagule quantities in the soil (Ingham 1988; Warnock *et al.* 1982; Bagyaraj 1986). The problem of this for the production and storage of inoculum will be discussed in chapter 7.

In the studies by Kohl & Schlosser (1989) they found that species of *Trichoderma* which are known to feed on fungi, has no effect on VAMF.

## 6.5 The effect of fumigation, application of soil chemichals and sterilization of soil on VAMF

There are many fungicides and chemicals that are used in soils to eliminate pathogenic fungi, but what will the effect of these compounds be on VAMF ? Many studies were already conducted to investigate the effect of these chemicals on VAMF. The findings of most of these studies concluded that most of them are detrimental to VAMF (Habte & Manjunath 1991).

Toth *et al.* (1990) showed that at present plants are cultivated to be more resistant to pathogens. The controversy of this is that these cultivated plants are less susceptible to VAMF.

Martin *et al.* (1973) and Berch *et al.* (1991) found that the fumigation of soil with various fumigants and soil sterilization reduce the absorption of phosphorus by plants, because of the elimination of important soil organisms such as VAMF. In greenhouses and nurseries there is evidence that fumigation of the soil against pathogens causes stunting of the plants. This is also due to the elimination of VAMF and subsequently to a nutrient deficiency in the

"Spreader" plant
inoculated with VAMF spores

Root-free zone with
pesticide to be tested

Interhyphal branches will
grow through this zone

Reciever plant

VAMF spore

Sterilized sand

A

B

C

Net with 80μm pore size
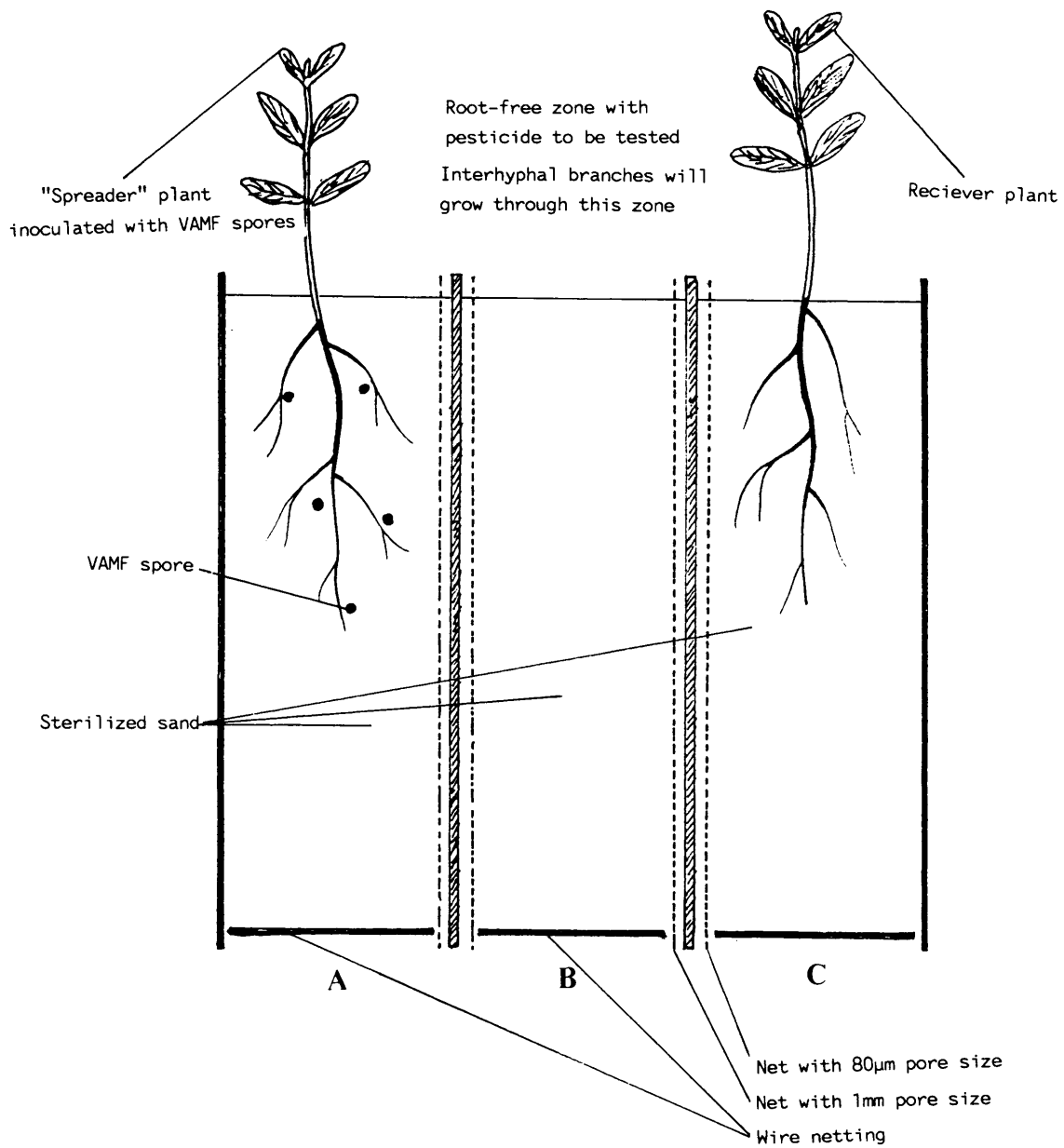
Net with 1mm pore size

Wire netting

Fig. 6.2 The cuvette system used by Schuepp & Bodmer (1988) to test the effects of different pesticides on VAMF

plants. However, Mosse (1973) reports that VAMF can prevent stunting of plants grown in fumigated soils.

Krikun *et al.* (1990) examined the effect of methyl bromide and found that it suppresses the growth of VAMF in the host plant. Michelini *et al.* (1989) investigated the effect of Paclobutrazol on VAMF. This fungicide is widely used in citrus nurseries. It does not radically affect VAMF and it can thus be used safely together with VAMF to control host disease and to prevent stunting of the seedlings due to fumigation.

The use of $SO_2$ to fumigate soils limit the ability of VAMF to infect a susceptible host. This is because $SO_2$ decrease photosynthesis, thus decreasing the availability of carbohydrates to the fungus (Clapperton *et al.* 1990).

Garsia-Romera *et al.* (1988) studied the effects of the herbicide, Cyanozine, which inhibit photosynthesis on the treated plant, on VAMF. Their findings showed that a small dosis of this chemical will have no significant effect on VAMF and that VAM even helped the plant to overcome this inhibition of host plant photosynthesis.

Hetrick & Wilson (1991) found that the fungicide Metalaxyl can even increase VAMF colonization in the host plant due to the decrease in competition with other pathogenic fungi which are eliminated by this fungicide. The fumigation of soil with formalin reduce VAMF but does not completely eliminate them (Khadge *et al.* 1992).

Schüepp & Bodmer (1988) described a method to test what the side-effects of certain pesticides and fumigants will be on plants. They used a system where a cuvette is devided into three compartments with fine nets which restrict root growth, but not the growth of extramatrical hyphae of VAMF. The compartments are filled with sterilized soil. A VAMF inoculated plant is planted in one of the outside compartments and another non-mycorrhizal plant in the other outside compartment. The extramatrical hyphae from the mycorrhizal plant will then grow through the nets towards the non-mycorrhizal plant via the middle root free compartment or zone (Fig. 6.2.) They applied the pesticides to the root-free zone and tested their effects on the extramatrical hyphae of VAMF. They pointed out that if the substance

is detrimental on the hyphal growth of VAMF in will consequently affect the growth of the plant negatively. They concluded that VAM fungi could be a good indicator for the monitoring of the side-effects of certain pesticides.

Menge (1982) and Johansen & Pfleger (1992) listed most of the pesticides and fumigants used in nurseries and those used during agricultural practices which have an effect on VAM. Those which inhibit or eliminate VAMF are methyl bromide, Captan, Captafol, PCNB, Benzimidasol, Benomyl, Carbendazim, Thiabendazol, Thiophanate, Chlorpropham, Phenmedipham, Chloropicrin, Formaldehyde, Mylone, Vapam, Vorlex, Thiram, Botran, Difolatan, Euparen, Lanstan, Maneb, Banrot, Calixin, Cela W524, Ethirimal, Imugan, Topsin, Triademifon and Vitavax. Others that have little or no effect on VAMF are Chlorfenvinphos, Carbaryl, Diazinon, Ethoprop, Malathion, Parathion, Carbofuran, Aldicarb, Oxamyl, copper sulphate, Terrazole, sodium azide, Demozan, Daconil, Pyroxychlor, Aliette, Prothiocarb and Ridomil. Some pesticides can even stimulate VAM infection such as Fosetyl-Al, Metalaxyl and DBCP.

## 6.7 Conclusion

A considerable amount of research data is already available on the unique symbiotic association which exists between VAMF and a large variety of plant species. Much is already known with regard to benefits which the infected plants gain via the VAMF.

The nutrient and moisture intake of infected plants, for example, improve under certain conditions via the VAMF. They are more drought resistant. They do better in impoverished soils and they can withstand certain plant diseases better than the non-mycorrhizal plants can do. The overall result is that growth is enhanced in the VAMF infected plants.

From an agricultural point of view this can be of great significance. Cultivated plants could produce better yields if they were infected with VAMF. An inoculation process by which the VAM can be manipulated is available and is a major breakthrough for improved crop production by a biological means.

Much research is done to demonstrate te beneficial effects of VAM in many types of crop plants which are also cultivated in Africa and in particular, South Africa. Most of the work, however, was done on the plants grown under greenhouse or nursery conditions but with careful manipulation of data on soil and other environmental conditions gained from the results the managing of VAM under field conditions looks promising to the farming industry. The list below contains the major crops which are susceptible to VAMF, grown in South Africa (Esterhuysen 1992) with some references of research done on these crops in other countries:

| | |
|---|---|
| MAIZE : | Evans & Miller (1990); Fairchild & Miller (1992); Khan (1972); Faber *et al.*(1990); Khadge *et al.* (1992); Toth *et al.* (1990) |
| BARLEY: | Black & Tinker (1979): Mosse *et al.* (1982); Hall (1988); Grey *et al.* (1989). |
| WHEAT: | Azcón & Ocampo (1981); Baltruschat & Dehne (1988); Kapulnik & Kushnir (1991). |
| SORGHUM: | De Miranda *et al.* (1989). |
| SOYBEAN: | Vejsadová *et al.* (1992); Heckman & Angle (1987); Kawai & Yamamoto (1986); Hall (1988); Kellam & Schenck (1980). |
| PEA TYPES: | Jakobsen (1986); Garcia-Romera *et al.* (1988); Chaturvedi *et al.* (1987); Manjunath & Bagyaraj (1984). |
| LUCERN: | Hall (1988); Grandison & Cooper (1986). |
| AVOCADO: | Menge *et al.* (1978); Martin *et al.* (1973); Vidal *et al.* (1992). |
| BANANA: | Iyer *et al. (1988) ( show presence of VAMF but unknown if VAM stimulate growth).* |
| CITRUS: | Michelini *et al.* (1989); Dixon *et al.* (1989); Hattingh & Gerdemann (1975); Dela Cruz *et al.* (1988); Davis (1980). |
| ONIONS: | Hall (1988); Manjunath *et al.* (1981); Furlan & Bernier-Cardou (1989). |
| SWEET POTATO: | Potty & Indira (1990). |
| POTATO: | Hall (1988). |
| TOMATO: | Bååth & Hayman (1983); Khaliel & Elkhider (1987); Pond *et al.* (1984). |
| APPLE: | Koch *et al.* (1982). |
| PAPAYA: | Ramirez *et al.* (1975). |

PEACH:      Strobel *et al.* (1982).

PEANUT:     Bell *et al.* (1989); Krishna & Bagyaraj (1983).

CASSAVA:   Howeler & Sieverding (1983); Sieverding & Howeler (1985).

COFFEE:     Howeler *et al.* (1987).

TEA:        Howeler *et al.* (1987).


The production of the VAM inoculum on a large scale, the storage of the inoculum for use at the right time and the inoculation of agricultural crops under extensive field conditions need to be addressed if agriculture is to gain from what is already known about VAMF. However, this important topic on VAM will be thoroughly discussed in the next chapter.

# CHAPTER 7 : VAMF inoculum

## 7.1 Introduction

Currently there is a need to inoculate plants with VAMF, not because it is only practical or useful but in some cases it is a necessity to inoculate plants to reinstate VAMF in heavily disturbed areas. When soils are fumigated, the VAMF are also eradicated and these fungi should again be inoculated into the soil to sustain crop production.

To apply VAMF to the soil to infect susceptible hosts, for example crops, VAMF propagules should first be collected from the soil or produced in one way or another. These propagules must then be applied in such a way to ensure host plant infection. Viable VAMF propagules which are used to inoculate host plants is termed VAMF inoculum.

The main aim in research to produce VAMF inoculum today is to be able to culture VAMF axenically, because the inoculum produced from these cultures will be concentrated and pathogen-free. Unfortunately up to now, VAMF could not be cultured axenically.

The lack of adequate VAMF inoculum due to the inability to grow VAMF in pure culture, makes VAM technology very difficult (Janerette 1991 and Howeler *et al.* 1987). Soil inoculum (soil obtained from around the roots of infected plants) is a very effective inoculum, but the transmittance of pathogens promote major problems. Many researchers believe that the inability to grow VAMF in pure cultures and the difficulties in producing large amounts of pathogen free inoculum, limits the use of these fungi in agricultural practices (Menge 1986). There is, however, evidence that in some countries VAMF inoculum, originating from the roots and surrounding soil of VAM fungal infected plants grown in sterilized pot cultures, is successfully produced for the use in agricultural and horticultural practices. The production of these inocula is still time consuming though, and is susceptible to pathogen contamination (Janerette 1991) as was mentioned in chapter 6.

The production of VAMF in pure culture may simplify research on these fungi and according to Hepper (1986) and Louis & Lim (1988) this will contribute to identify many more

characteristics which might be useful in the classification of VAMF. It will also contribute to the research on modifying the genetic structure of VAMF to produce more effective VAM fungal strains for agricultural use. This then will help to select the most suitable strains in agricultural practices. The most important contribution will be to produce large amounts of inoculum which are pathogen free for inoculation of plants under field conditions.

Today the production of inoculum is confined to growing VAMF in association with their hosts plant in semi-sterile soil or rooting media and then using the roots as inoculum (Hepper 1986). The growth media should contain the necessary nutrients and should be exposed to conditions necessary for optimal growth and reproduction of the fungi during the whole production process (Howeler *et al.* 1987). Howeler *et al.* (1987) also found that a wide host range can be used as suitable hosts for inoculum production, but that the VAMF in legumes tend to produce more spores. They are therefore more effective in the production of VAMF inoculum than other plants are.

Powell (1986) gives three main objectives for research in producing VAMF inoculum:
  i) to reduce fertilizer application, because VAMF can give the same growth responses to the host than the generous application of fertilizer;
 ii) to mechanize the inoculation process to effectively inoculate plants to reduce manpower and energy consumption in the farming industry, and
iii) to show that the inoculation of plants with VAMF is profitable.

Millner (1988) estimates that about 25% less fertilizer is needed to feed VAMF infected plants during horticultural practices in Florida, America but that could only be achieved when large amounts of VAMF inoculum could be applied to the soil.

Powell (1986) pointed out that the inoculation of crop plants with VAMF should have the same growth response in low phosphorus soil than with the application of high levels of phosphorus fertilizer. If this aim is not reached, VAM fungal inoculation will have no benefit to the farmer. This implicates that VAMF inoculum will only be useful in low fertile soils.

## 7.2 Progress towards growing VAMF as pure cultures

Richards (1987) points out that during the last few years the attempts of many researchers to grow and maintain VAMF in pure cultures failed. Limited growth of VAMF under axenic conditions can already be obtained (Herrera *et al.* 1993). Unfortunately all the factors necessary for this obligate symbiotic relationship of the fungi has not yet been discovered or properly defined (Hepper 1986).

In trying to culture VAMF purely, there are generally two approaches. One approach is to find out if there are any blocks in an essential metabolic pathway which prevent VAM fungi to grow non-symbiotic. If the block is identified it could probably be eliminated or altered in such a way that it won't inhibit VAMF to grow in the absence of the host plant. The other approach is to eliminate certain metabolic pathways by using certain metabolic compounds which inhibit or block the pathway. One may then determine which pathway is really necessary for the VAM fungus to grow axenically (Hepper 1986).

Hepper (1986) gives several reasons for the inability to culture VAMF purely. She states that this could be because only a small number of species has been studied as yet, and that there may be others that can be more easily grown in pure culture. Another problem may be that different VAMF have different nutrient requirements. Hepper (1986) and Millner (1988) hypothesized that VAMF, because of their obligatory biotrophic growth in a host plant, have lost some of their genetic material which stimulates certain metabolic activities, during their evolution process. It is therefore necessary to interact with the host genes to stimulate these metabolic activities. The host must thus always be present to promote fungal growth.

Up to now the simulation of host cell conditions did not promote fungal growth. VAMF feeding structures such as arbuscules, which is normally found within the host tissue where it takes up the necessary nutrients, may also be necessary to maintain hyphal growth in a pure culture.

Daniels *et al.* (1981) report that today it is generally accepted that VAMF spores can germinate in the absence of a receptive host, forming a network of hyphae with many

potential contact points. This gives a firm basis for the research to grow VAMF as pure cultures. The germination of spores and subsequent limited hyphal growth in growth mediums have already been obtained (Mosse 1973). Daniels *et al.* (1981) also found that the attraction between the fungal hyphae and the root usually occurs when the hyphae are close to the root surface.

Researchers are now already capable to cultivate VAMF by inoculating several axenic plant species with surface sterilized spores, extramatrical mycelium and infected root pieces. Millner (1988) states that these cultures are termed dual cultures and that this culturing method produces important information to the process of culturing VAMF purely. These types of cultures are already more informative about certain physiological changes in host plants, due to VAMF growing in the absence of other microorganisms which cannot be achieved with plants grown in soil (Mosse & Hepper 1975; Millner 1988; Hepper 1986).

Valuable information about stimuli from the host root which affect VAM fungal hyphae near the root, can also be obtained from axenically propagated plants infected by VAMF. VAMF can form many spores under these conditions (Hepper 1986; Hepper 1981). These spores are small and less viable than the spores normally formed during the VAM symbiosis (Louis & Lim 1988). No sporocarps could be obtained by this method yet (Hepper 1986). Most of the extramatrical mycelium and spores, as well as the infected host roots produced by this method, can be used as a possible form of inoculum. (Hepper 1986).

In experiments by Mosse (1988) on the dual culturing of VAMF and root pieces of carrots she showed that hyphae from germinated spores and infected root pieces go on growing, even forming typical arbuscules and spores outside the roots. When the host roots were taken away, further growth of the hyphae ceased within a few days. She also separated some of the hyphae from the root and these loose hyphal pieces, still in the original medium, went on growing for a while. She observed that when these pieces were transplanted to mediums wherein host roots grew previously, the hyphae still showed limited growth.

Hepper (1986) found that some types of obligatory biotrophic fungi other than VAMF, can grow axenically, sometimes loose their capability to reinfect host plants. This inability should
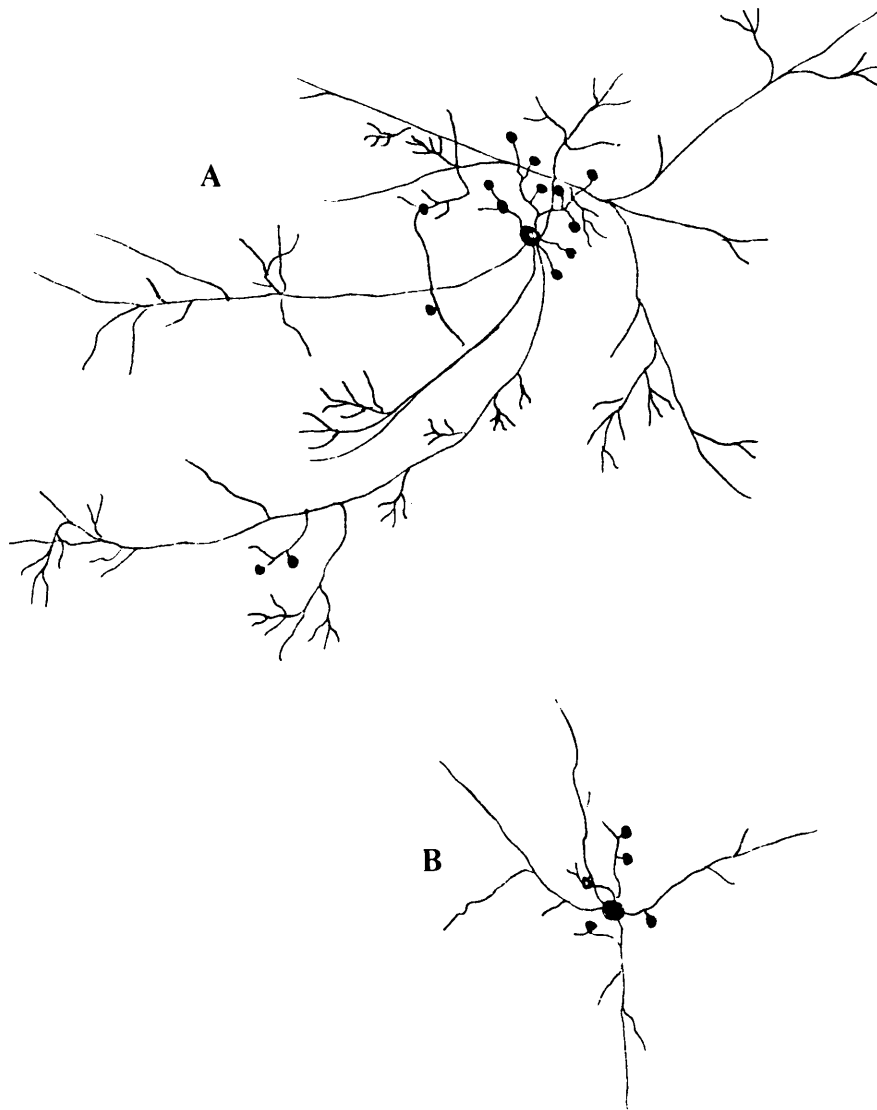
Fig. 7.1 Amount of mycelial growth in A. the presence of the host root, and B. the absence of a host root.

then first be eliminated before reinfecting the plant. This phenomenon may also be true for VAMF once they are purely grown as they would then become saprophytic and lose their ability to infect susceptible host plants again. Further studies should again be conducted to change the saprophitic state of the fungi to a symbiotic state.

According to Hepper (1986) an attempt to grow VAMF hyphae from root cutlets in water or on agar, showed that the proximal ends of intramatrical hyphae could form new hyphae and with the addition of boiled hemp seed, form the typical VAMF structures. These structures were able to infect a new host plant. Unfortunately other researchers failed to repeat these experiments so as to get the same results under the same conditions. None of the usual fungal growth media is able to sustain hyphal growth of VAMF after spore germination. (Hepper 1986).

By adding certain organic compounds to agar with germinating spores, it was found that several compounds containing organic acids are able to promote hyphal growth. Some nitrogen containing compounds such as casein, sodium nitrate and yeast extract can promote hyphal growth. The addition of bovine serum albumin promoted hyphal growth in a mixture of soil extracts. The addition of dialysate from *Chlorella* (green algae) or bean roots and inorganic sulphur-containing compounds such as potassium sulphite and potassium metabisulphate to growth mediums, can also enhance hyphal growth to some extent (Hepper 1986).

Root exudates also play a role in the hyphal growth after spore germination, although it does not promote spore germination (Millner 1988; Dixon *et al.* 1989). The hyphal growth is much more abundant in the presence of root exudate than without it in axenic systems (Fig.7.1) (Hepper 1986, Mosse 1973). When peptone, yeast extract, thiamin and lima bean agar are added into the basic growth medium, enhanced hyphal growth is observed. The addition of boiled dicotyledon seeds to the growth medium also promote growth. However, the addition of some carbohydrates such as glucose, is detrimental to hyphal growth from the germinated spore (Hepper 1986).

Mosse & Hepper (1975) pointed out that when attempting to grow VAMF axenically on White's agar or other liquid growth media, the pH of the growth media was the critical factor. They also found that White's agar inhibited spore germination and therefore they used pre-germinated spores.

After a certain extent of hyphal growth following spore germination, the whole process of hyphal growth will stop when the VAMF spore is detached from the germination tube (Hepper 1986 and Mosse 1973). The spore itself will germinate again several times thereafter and promote hyphal growth to the same extent as the previous times when it is put in a new growth medium. This ability of the spore to germinate more than once, shows that there may be some auto-inhibition factors which prevent further hyphal growth (Koske 1981; Hepper 1986).

Although VAMF spores contain enough food to sustain hyphal growth for a long period of time, studies showed that the spores do take up extra nutrients after a few days from a certain growth medium to synthesize new hyphal compounds. The effect of several nutrients on the hyphal growth under axenic conditions had already been tested by comparing the extent of hyphal growth to the addition of nutrients to the growth medium (Hepper 1986 and Louis & Lim 1988).

It is important to avoid contamination of axenically grown cultures but several antibiotics can be used together with the different growth media to eliminate bacterial contamination. Hepper (1986) found that the use of chloramphenicol in agar can inhibit bacterial growth when VAM spores are germinated.

In experiments by Persad-Chinnery *et al.* (1992) VAM fungal spores were germinated on a solution of cellulase taken from a *Trichoderma* species. They found that there was a critical value of cellulase input that enhanced spore germination and that this might be a positive contribution to culturing VAMF. They stated, however, that the cellulase extraction was not tested for its purity and that the germination of the spores could be initiated by other compounds.

Constantinescu (1988) designed an instrument that can be used to collect single VAMF spores from agar plates or placing individual spores taken from an agar plate on another. This instrument may come in handy during experiments of culturing VAMF axenically.

## 7.3 Production of VAMF inoculum

Even with all the knowledge mentioned in 6.2, VAMF could still not be grown as a pure culture, and therefore the only way to maintain and produce VAMF inoculum is in dual culture with a specific host. This method, however, is time consuming and still susceptible to possible pathogen contamination. VAMF inoculum can be infected root pieces, pieces of extramatrical hyphae, spores, or a mixture of the three together with or without soil. Most inocula used today are produced from **"pot cultures"** grown under aseptic conditions (Kendrick 1992, Menge 1983). It is important that the pot cultures must be pathogen free and the host plant must preferably be infected by only one VAM fungal species.

## 7.3.1 Isolation of VAMF from the soil

Inoculum should preferably contain the propagules of only one species of VAMF and should as far as possible be free from other unwanted organisms which could decrease the quality and quantity of the inoculum. Propagules of a specific species of VAMF should therefore be collected and used to produce inoculum.

According to Hepper (1986) and Mertz *et al.* (1979) the best way to isolate a single species of VAMF is to collect spores and sporocarps from field soils through wet sieving and decanting or other methods as already described in chapter 2. After identifying these spores or sporocarps, it can be used to inoculate host plants which grow in sterile soil. The spores of some species of VAMF are very small and not that easy to isolate and identify. It will be better to use washed, infected root pieces as inoculum. This method is not always effective, because the collected root pieces can be infected by more than one VAMF or can be contaminated with unwanted organisms such as host pathogens (Hepper 1986).

Horn *et al.* (1992) developed a method to isolate pure spore and hyphal fractions from clay expanded cultures (dual cultures of VAM fungal infected plants in porous clay). The shoots and the roots are removed from the clay before the clay is left to dry for about 8 weeks. They then removed the algae containing top layer before bigger root pieces were collected by hand. The remaining clay with VAMF propagules is then wet sieved and decanted. The wet sieved fractions are then further processed by floatation, bubble floatation and centrifugation until pure spore and hyphal material remained. These spore and hyphal fractions can be used directly as VAMF inoculum or can be used to inoculate host plants during the inoculum production process.

## 7.3.2 Surface-sterilization of VAMF spores

The spores, isolated from the soil are in most cases contaminated by unwanted microorganisms which could later contaminate the crops for which the inoculum is intended. Today there are several chemical compounds and methods to eliminate most of these unwanted microorganisms from the spores. The best way is to surface sterilize the spores by soaking them in sterilizing agents. Compounds such as 5% Chloramine T can be used for VAMF spores. This compound can be used together with Streptomycin (an antibiotic) and a surfactant such as 0.05% aqueous Tween 80. First the spores are washed in sterile water, followed by washing it in 0.05% Tween 80 and then the sterilizing agent is added to the spores in a watch-glass under vacuum conditions. For thorough surface sterilization the spores can be stored in a mixture of Streptomycin and Gentamycin (also an antibiotic) for a week at 4°C and again sterilized in chloramine T for 20 minutes (Hepper 1986, Mertz *et al.* 1979)

Another method to surface-sterilize VAMF spores, is to expose spores to a diluted solution of sodium hypochlorite but this is not as effective as the method described above. Other methods which are less successful are the treatment of spores with heat and ultrasonic irradiation. Heat and irradiation practices can be harmful to the viability of the spores, because these treatments are sometimes used to sterilize soils or to eliminate VAMF (Jakobsen & Anderson 1982; Jacobsen 1984). VAMF spores which are exposed to ultraviolet irradiation for about 7 hours, are also surface-sterilized, but this method is time-consuming
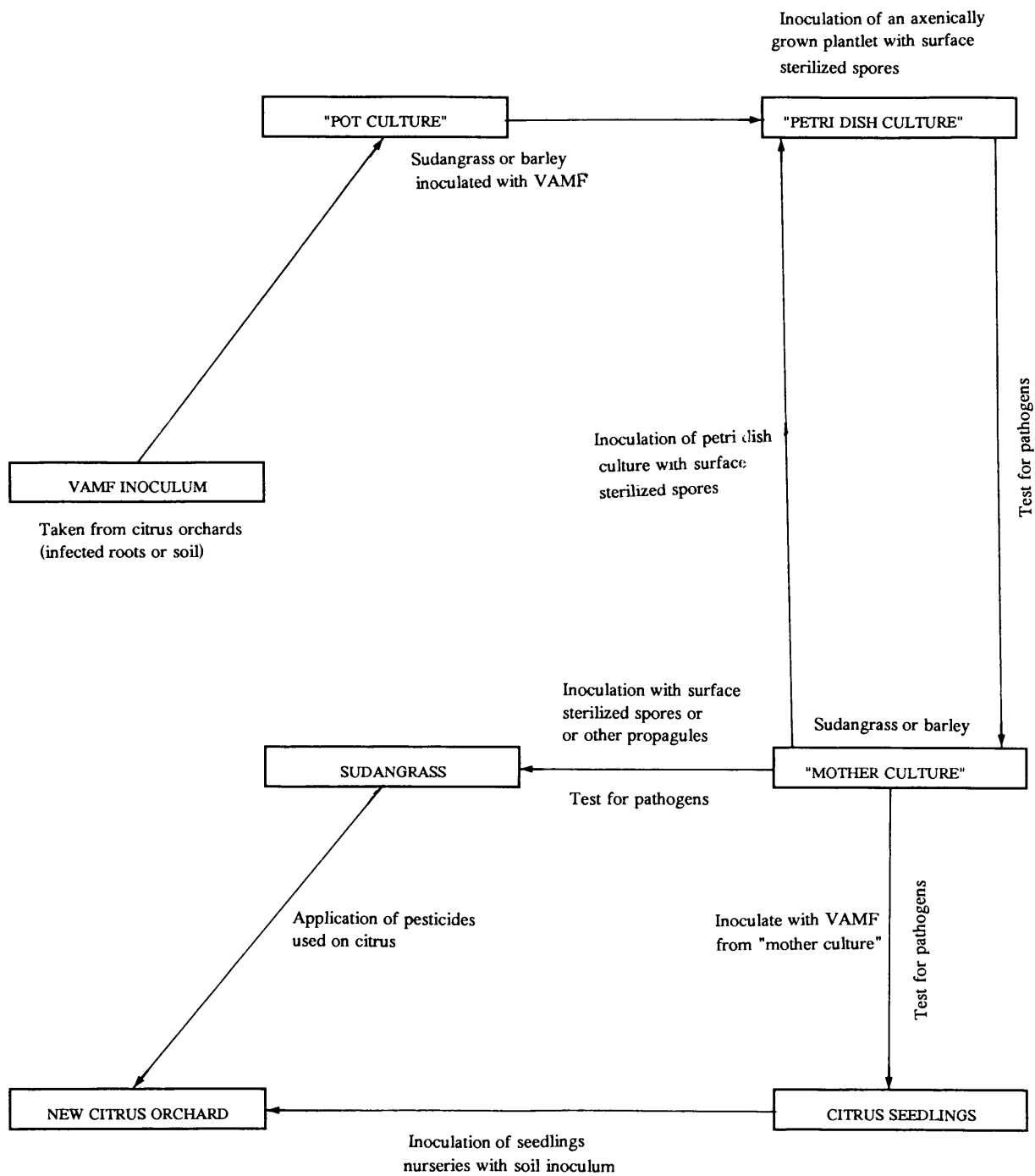
Fig. 7.2 A schematic diagram of the "pot culture" method for the commercial production of pathogen-free VAMF inoculum

and requires a lot of effort (Hepper 1986).

According to Hepper (1986) it is more difficult to surface-sterilize those spores collected from the field than spores collected from pot cultures. This may be due to the higher percentage of parasitism by fungi on VAMF spores under field conditions.

### 7.3.3 Methods to produce VAMF inoculum

The most popular method of producing VAMF inoculum for commercial use, is the pot culture method (Fig. 7.2). After the isolation of propagules from the field, hosts grown in sterilized soil are inoculated with the spores (Menge 1983, Menge 1986). According to Menge (1983) Sudan grass is most commonly used, but plants such as peanut, corn, soybean and safflower are also successfully used. These are known as **"field pot cultures"**. These cultures can still be invaded by many pathogens as well as more than one VAM fungal species if VAMF propagules are used directly from the field without sterilization. The following step is to isolate the spores of one single VAM fungal species from the "field pot culture" and then surface-sterilize them. About 20 healthy spores are then brought into contact with the roots of host seedlings grown in aseptic greenhouse conditions. A single spore from these **"pot cultures"** is then used to inoculate an axenically grown host plant known as a **"petri-dish culture"**. A pathogen free culture of a VAM infected plant is now established and spores from this culture can be used to infect a **"mother plant"** to form a **"mother culture"**. The host plants used during the process is usually another plant than the crop for which the inoculum is intended. For example, if the inoculum is intended for tobacco, a host plant such as barley or sudan grass which is not usually pathogenized by the same organisms as tobacco, can be used. This will insure that no pathogens of the intended crops will contaminate the inoculum. This culture can then be maintained as a source to inoculate the intended crop or other "petri-dish cultures" or other "pot cultures". These "mother cultures" must be checked regularly for the presence of pathogens. Pathogens can be further avoided by constantly drenching inoculum in pesticides used on the intended crop which has no effect on VAMF (Menge 1986, Menge 1983).

The most popular growth medium in which to enable the host plant to produce the inoculum is sterilized soil from which all VAMF are eliminated. Plants that are grown in this soil can then be inoculated with single strains of VAMF. The best way to eliminate native VAMF from the soil together with other unwanted organisms, is to pasteurize the soil for 8 hours, or by autoclaving the soil. Using substances such as methyl-bromide and a fumigant such as Basamid can also be successfully used for this purpose (Howeler *et al.* 1987).

VAMF inoculum can also be easily produced in vermiculite, peat, sawdust, bark, perlite, pumice or mixtures of these media (Menge 1986).

Vestberg & Uosukainen (1992) used a polymeric hydrogel product, **"Water-Works"**, to produce VAMF inoculum from micropropagated strawberry plantlets. This "Water-Works" can be mixed with vermiculite and then inoculated with a mixture of VAM fungal infected strawberry roots and sand. After 7 weeks they found that VAMF produced an abundance of spores into the hydrogel. The spore containing hydrogel can then be used as pathogen-free inoculum if the strawberry plants are not contaminated by unwanted organisms.

The media wherein all the different cultures are maintained should be regularly supplemented with sufficient nutrients for the host plant to grow healthy and not inhibit VAM fungal colonization. A half standard of Haoglands solution without phosphorus proves to be efficient to fertilize these cultures (Menge 1983).

One must always remember that the same principles that sustain the symbiotic relationship should be incorporated into the production of VAMF inoculum. Anything that will affect the growth of the plant will subsequently affect the production of inoculum. The host plant which is to produce VAMF inoculum must:

i) be able to adapt to the growth conditions during the production process as well as the conditions under which the intended crop grows (Howeler *et al.* 1987, Menge 1986);

ii) be a suitable host to the specific VAMF species (Menge 1986);

iii) produce a dense and fast growing root system while growing fast (Menge 1986), and

iv) not have pathogens common to the plants for which the inoculum is intended. These precautions will eliminate contamination with pathogens of the intended crop

(Howeler et al. 1987, Menge 1986).

Other factors such as fertilization, water-aeration, pH, light intensity and photoperiod, temperature, pruning, pot size and chemical applications has an important impact on the quantity and quality of VAMF inoculum. These factors should be carefully manipulated for the maximum inoculum production and can differ with each species or strain of VAMF (Menge 1986).

Once VAMF are established in plants which are going to be used as inoculum, the plant tops are removed and the roots together with the spore filled soil are ground into inoculum. The inoculum can then be stored under suitable conditions for further use in farming practices and afforestation (Menge 1986 & Menge 1983).

Menge (1986) concludes that the ideal conditions to produce VAMF inoculum is to use healthy plants which grow under long photoperiods and high light intensities. The phosphorus levels should also be slightly lower than the levels necessary for optimal plant growth without VAMF.

The growth pattern or dynamics of VAMF after the infection of the host and the subsequent colonization should also be taken into account during the VAMF inoculum production process. As mentioned in Chapter 4, the growth of VAM fungi in the host show a sigmoid pattern with an initial lag phase at the start of infection, a period of rapid exponential growth and a plateau phase with maximum growth of the fungus. Therefore, maximum inoculum will be harvested when the plateau phase is reached. The growth dynamics vary in different species of VAMF and host plants being used. (Menge 1986).

Other successful methods for producing VAMF inoculum are the nutrient film technique (NFT) and the hydroponic or aeroponic culture method (Herrera et al. 1993; Millner 1988; McDonald 1981). These two methods are based on the recycling of a nutrient solution around the VAM fungal infected roots (Menge 1986). According to Elmes & Mosse (1984) the levels of nutrients for this technique should be constantly kept low. Critical conditions to these methods of inoculum production is aeration, phosphorus and nitrogen supply (Menge

1986, Elmes and Mosse 1984). Mosse and Hepper (1975) emphasize the importance of pH in the initiation and spread of VAM fungal infection because of its role on the release of nutrients such as phosphorus from rock phosphate often used during these procedures. The production of VAMF inoculum for future use looks promising with both methods. The NFT and hydroponic cultures can also contribute to metabolic studies of VAMF (Menge 1986).

Mosse & Thompson (1984) experimented with beans in NFT cultures to produce VAMF inoculum. They found that the roots of the bean plants showed normal VAM fungal infection structures and confirmed the use of this method for future inoculum production.

Another method that can be developed for the production of VAMF inoculum is the expanded clay culture method. Expanded clay with large pores is used as the VAMF propagule carrier. As colonization of the host plant takes place, the extramatrical hyphae colonize the pores with subsequent sporulation within the pores. The VAMF propagule filled clay are then used as VAMF inoculum (Horn *et al.* 1992).

Another important factor to be remembered when selecting a species of VAMF for the production of VAMF inoculum is the inoculum potential of the selected fungus (Daniels *et al.* 1981; Menge 1986; Millner 1988). The infectiveness or inoculum potential of VAMF depends on the quality of the inoculum and not always on the quantity of the inoculum. There is also evidence that some VAMF which produce large spores such as *Glomus mosseae*, have higher inoculum potentials, but usually there is no correlation between spore size and inoculum potential (Daniels *et al.* 1981). One must also remember that the infectiveness of different VAMF can vary depending on the type of host plant and the soil conditions such as phosphorus levels as mentioned in chapter 6.

Daniels & Menge (1981) report that VAMF which produce an abundance of spores within big epigeous sporocarps can have great commercial potential as a type of inoculum if the specific fungus is a host growth stimulator. The sporocarps can easily be collected from the surface of the soil and can be stored fresh for up to 3 months. *Glomus epigeous* shows all the above mentioned characteristics. Another attribute that makes *G. epigeous* sporocarps excellent inoculum is that they are naturally resistant to VAM fungal parasites.

## 7.3.4 The Control of contamination of VAMF inoculum

VAMF inoculum can spread disease to host plants when the inoculum used is not pathogen free. Therefore it is important to take the necessary precautions against pathogen invasion when inoculum is produced.

Apart from the usual procedures to eliminate pathogens such as sterilization, heat treatment and fumigation, it is also essential to alter host species used during the inoculum production process frequently (Menge 1986). However, as already mentioned earlier in this chapter, it is very important to select hosts which are free of pathogens common to the hosts for which the inoculum is produced (Menge 1983, Menge 1986).

Menge (1986) give five rules that can be followed to keep sanitary conditions in greenhouses to produce pathogen-free VAMF inoculum:

i) the pots and growth media should always be sterilized before use;

ii) the propagules isolated from the field should be free from pathogens and other unwanted microorganisms by thoroughly checking the roots of the host from which the propagules are collected and to surface sterilize spores;

iii) the inoculum used during the production process should regularly be tested for unwanted pathogens and must immediately be eliminated from the greenhouse if contaminated;

iv) host plants should be changed regularly, and

v) selected pesticides which do not eradicate VAMF, should be applied generously especially those pesticides used to control pests on the intended crops.

Parasites of VAMF can also be a major problem to the VAMFinoculum production process, for these parasites can decrease the inoculum potential of VAMF. To prevent this Menge (1986) concluded that one must start with uncontaminated spores at the beginning of the process and use pesticides which kill VAMF parasites.

Ames *et al.* (1989) discovered that there are several Actinomycete fungi which decompose the chitin in the walls of VAMF spores. These fungi can be a serious threat to VAMF inoculum because it can reduce large quantities of the spore propagules of VAMF inoculum.

## 7.3.5 Storage of VAMF inoculum

Some of the main objectives in producing any inoculum for agricultural use should be that the inoculum must be produced in large quantities and should be pathogen free. These inocula should also be able to stay viable over long periods of time and must also be light and easy to transport.

The storage of VAMF inoculum depends on the type of inoculum. Soil inoculum which contains a mixture of soil, infected root pieces, pieces of extramatrical hyphae and spores, is usually air dried to a 10 % or less moisture content, packed in plastic bags and stored at 5°C. Under these conditions the inoculum can stay viable for up to 4 years (Menge 1986, Tommerup & Abbott 1981). L-drying is a another process where inoculúm is dried at 22°C over $P_2O_5$ or silica gel. This method can be successfully used to dry soil inoculum of VAMF and especially spore inoculum before long term storage (Tommerup & Kidby 1979).

Spores of some VAMF can be cryopreserved and still be viable as shown in experiments by Douds & Schenck (1990). The best way is to dry the soil with VAM fungal spores of pot cultures slowly and then freeze it up to -60 - -70°C. This method is more effective than storing VAM fungal spores within soil at 5°C.

If peat blocks are used as a growth medium for the commercial production of VAMF inoculum with NFT cultures, the whole block with the VAM fungal infected roots and spores can be ground up and can then be used as VAMF inoculum which are light and easy to ship to other countries (Menge 1983).

An & Hendrix (1988) proposed a vital stain to determine the viability of VAMF spores. Stock solution (a mixture of a mutagen, MTT, with deionized water) of 0,5 mg MTT/ml can be used to stain the spores by incubating equal amounts of Stock solution and spore suspension at 25°C for 40 hours. Viable spores usually stain bright red with MTT and dead spores will, however, not stain or will stain blue when incubated longer than 40 hours. With this method one can determine the viability of VAMF spore inoculum after a certain period of time by staining a few of the spores.

## 7.4 Methods to inoculate different host plants with VAMF

For the inoculation of crops in the field, the most suitable VAMF inoculum should be selected. This selected inoculum should be adaptable to the host plant and to the soil and environmental conditions of the particular area where the inoculum is going to be used. The most suitable and most successful inoculation method to use should be determined prior to the inoculation of a certain crop or host plant.

Howeler *et al.* (1987) indicate that the method of inoculation depends on the type of inoculum to be used, the planting system and the growth cycle of the specific crop. Soil inoculum which is derived from chopped infected root pieces, spores and the soil that surrounds the roots, after removing the parts of the plants above the ground, is most commonly used. Mosse & Thompson (1984), on the other hand, indicate that soil inoculum is too bulky to use and not easily sterilized. It is also difficult to keep this inoculum from contamination of unwanted microorganisms (mentioned earlier in this chapter). They suggest that growing VAMF in roots in water cultures such as the nutrient film technique will eliminate much of the above mentioned problems.

For some crops spore inoculum is sufficient to infect the host plant successfully. Some researchers found that concentrated inoculum from which most of the soil is removed, can also be successful. The type of inoculum to be used depends on the crop and the area (field or container) where the crop grows. The method of the application of inoculum also depends on the place where the host grows (Powell 1986).

Michelsen (1993) found that inoculum such as chopped, infected roots of plants collected from the field can sometimes be used as inoculum to other plants of the same species or other plant species susceptible to the same VAMF in the same field area. This inoculum can be as effective as selected inoculum to enhance plant growth in drought stressed conditions.

How VAMF inoculum is applied to the host, can also play a role in the successful inoculation of crops. If host plants are raised in nurseries or greenhouses, it is preferable to inoculate the seedlings prior to transplanting (Powell 1986; Hatting & Gerdemann 1975).

Some researchers found that seeds can be pelleted with inoculum prior to sowing, thus the seedlings become infected shortly after the germination of the seeds. This is very successful in the establishment of pasture (Hattingh & Gerdemann 1975; Howeler *et al*. 1987). In most cases of short season crops the inoculum is put as a continuous layer into the furrow prior to sowing, thus the seeds are placed on top of the inoculum (Powell 1986; Howeler *et al*. 1987). Another method is to mix the seeds and the inoculum before sowing into seed beds (Powell 1986).

The mixing of VAMF inoculum with topsoil, through the rototilling or ripping of the field soils when preparing for planting crops, can also be a very effective inoculation method (Howeler *et al*. 1987). In nurseries the inoculum can be placed under the roots of seedlings before the seedlings are planted or seedlings can be planted in soil which is already mixed with VAMF inoculum (Howeler *et al*. 1987; Powell 1986). Reinoculation of trees after prolonged drought conditions can be accomplished by placing inoculum near the host roots.

Sterilized sand can be used to distribute the inoculum more sparsely, thus be more economical (Howeler et al. 1987). The time of inoculation can also determine the success of VAM fungal infection. In agricultural, horticultural and forestry practices the main objective is that the inoculated VAMF should infect the host before indigenous fungi do to obtain the best results (Powell 1986; Millner 1988).

The inoculation of horticultural plants with VAMF can prevent stunting of these plants due to soil sterilization and fumigation as previously reported in chapter 6. VAMF can even promote host growth under these conditions. This beneficial effect of VAMF can also be implemented in nurseries during the production of enough seedlings for afforestation purposes. This can then lead to a decrease in seedling mortality after transplanting them to field soils. This implies that VAMF inoculum should be applied on the seeds before germination or the inoculum should be placed under the seeds in sterile soil. The seedlings will thus be infected at a very early stage of plant development. More of these pre-inoculated seedlings will thus be suitable for transplanting into the unsterile field soils and they will survive transplant injury better. Pre-inoculated timber plants will also survive better after transplanting into field conditions (Powell 1986).

Vidal *et al.* (1992) found that micropropagated tissue cultures of avocado can be inoculated by VAMF and that these inoculated plantlets will survive better after transplanting. This phenomenon indicates that this is a successful method for the inoculation of horticultural plants.

Boudarga *et al.* (1990) found that *Eucalyptus* plants can be dually inoculated by VAMF and ectomycorrhiza fungi in vitro. Pieces of the dual infected root tips can then be cultured on a growth medium and also be used as inoculum to establish *Eucalyptus* plants in the field.

VAMF inoculum can promote the growth and establishment of pasture plants which are used to restore disturbed soils where the inoculum potential of indigenous VAMF is usually low due to disturbing activities such as mining and deforestation (Powell 1986).

The amount of inoculum necessary to stimulate sufficient plant growth is very important. Daniels & Menge (1981) indicate that when using spore inoculum, the amount (density) of spores used must not be low. They suggest that the smaller the spores, the higher the amount of spores are needed to effectively colonize the host. Millner (1988) confirms that the propagule density play a major role in the establishing of VAM fungal infection in host plants. The amount or density needed for inoculation depends on the type and age of the crop or host plant, the type of soil and the agricultural practices in a certain area.

## 7.5 The development of appropriate technology for inoculation with VAMF

Other objectives in the production and use of VAMF inoculum is that it must be economically viable and that machinery and labour resources must easily be adapted or modified to inoculate crops effectively.

In some U.S. nurseries it is standard procedure to use VAMF inoculum to produce healthier host plants. Soil inocula and "starter VAM cultures" are also sold to farmers and other plant growers. These cultures are even exported to other countries (Powell 1986). In Colombia VAM fungal inoculum is already being commercialized by producing bags of inoculum of a certain VAM fungus and then sold to the public (Dodd & Sanchez 1989).

VAMF inoculum can be of extreme importance in third world countries where most of the crops are planted by hand. The inoculum can thus be added with the seedlings or seeds by hand. In these countries where fertilizer is very expensive VAM fungal inoculation will reduce those high costs of fertilizer. In industrialised countries where the planting of crops is highly mechanized, the machines should be modified to place VAMF inoculum in the proper position in the seed beds. These machines will then reduce labour, and the inoculum will be effectively placed to infect the crops and thus reduce fertilizer requirements (Powell 1986).

Howeler *et al*. (1987) proposed that the production of VAMF inoculum on the farm itself should reduce the high costs of transportation of VAMF inoculum. These inocula can be infected root pieces and spores in soil or the soil can be removed from the inoculum to produce a concentrated inoculum. These inocula can then be used freshly or can be stored in cold, dry conditions for up to 3 years.

## 7.6 Conclusion

The production of VAMF inoculum is not an easy task. This can only succeed if the objectives with and qualities of VAMF inoculum previously mentioned are achieved. These are economical viability, manageability and the production of light-weighted inoculum which is pathogen free. The inoculum should promote the same quality of plant growth as large doses of fertilizer do, thus reducing the need for fertilizer and pesticides. Much more research is required to produce these inocula successfully.

The main aim in VAMF inoculum research, however, should still be to culture VAMF axenically. When this is achieved the inoculum produced from these cultures will succeed in all the objectives to utilize VAMF for farming and afforestation purposes. Many results show that the inoculation of plants under nursery and greenhouse conditions is feasable and that it may be possible to inoculate plants under field conditions to achieve the same results.

# CHAPTER 8 : Conclusion

## 8.1 Summary of VAM aspects

VAM are the most common type of mycorrhiza in natural ecosystems. They are necessary for the survival of many plants. Between the two mycorrhizal associates, the host plant and the fungus, there is an interdependency which manifest in a continuous transfer of important metabolites. Man, especially those who cultivate plants, should therefore be aware of the fact that the interruption of this continuous transfer of metabolites will lead to an imbalance of the VAM relationship. This will result in endangering the survival of the fungus and consequently decrease host plant vigour and crop yields in farming industries.

Many reports by researchers emphasized that the beneficial effects of VAM can be applied to plants grown in nurseries and greenhouses but that with much research it may also be effectively applied in agricultural, horticultural and afforestation practises under field conditions. This can happen when enough VAM inoculum could be produced to infect these cultivated plants and be applied to crops cost effectively in the field. The beneficial effects of VAMF, as already mentioned, are:

  i) enhancing host plant growth;

  ii) enhancing the nutrient absorption of the host plant, especially in nutrient deficient soils;

  iii) making the host plant more tolerant to some plant diseases, and

  iv) making the plant more tolerant to drought stress.

## 8.2 Economical viability of VAM in agriculture, horticulture and afforestation

Man's ability to manipulate some of the soil ecosystems can lead to economizing on fertilizer and pesticide expenses and can even be less energy consuming. With enough research man might be able to manipulate the VAM symbiosis to have this economizing effect.

Today there are many countries where agricultural and industrial malpractice have already lead to the impoverishment of soil. This will consequently result in spending huge sums of money on fertilizers to recuperate the fertility of the soil for further maximum crop yield and

efficient food production. Huge sums of money will also be needed to successfully revegetate disturbed areas successfully.

The inoculation of crops with carefully selected VAMF, can positively influence the growth and the health of crops in soils with a low phosphorus content. So far this phenomenon has mostly been investigated under green house conditions. The researchers, however, predicted that this could also be applicable under field conditions. If this is the case, the use of VAMF in agriculture, horticulture and forestry will possitively affect the economy, especially of those countries such as South Africa and most tropical and sub-tropical countries with natural phosphorus poor soil. Research fortold the beneficial effects of VAM on combatting plant diseases and reducing drought stress. VAM can also become very important to more profitable agricultural and horticultural practices in the new South Africa.

The results of many studies done on the implementation of VAM in heavily disturbed areas by malpractices such as deforestation and mining activities, showed that the inoculation of plants with VAMF could revegetate these areas and could also render the economy more viable. The reports showed that VAM fungal infected seedlings survive better after transplanting them from nurseries into these disturbed areas which will also be more economically.

## 8.2.1 VAM versus fertilizer application

Many researchers found that VAM can not be a substitute for fertilizer but it can step up the utilization of fertilizer which is applied. This activity of VAM can lead to less fertilizer less often and will consequently save money for the farmer and horticulturalist.

In some countries the introduction of VAM in horticultural practices, already showed an estimated decrease in fertilizer application. As already mentioned Millner (1988) implied that VAM implementation in horticulture could result in a 25% cut in fertilizer costs. This economizing practices can also be applied in South Africa and with further research can even be implemented in the agriculture and afforestation industries of this country.

## 8.2.2 VAM versus pesticidal application

During the study of many reports on the effect of VAM on plant disease and the tolerance of the plant against pathogens the writer of this thesis has found that VAM infected plants can cope better with plant disease. There is evidence that VAM can reduce the severity of most plant diseases, for example, nematode infection and common root rot disease. Even when VAMF cannot reduce the severity of disease, it will still enhance plant growth and crop yield despite the presence of the disease. Some of these reports also showed that many pesticides used today to prevent plant disease, have little or no effect on the VAM symbiosis when applied in low concentrations. This shows that future use of VAM in agriculture, horticulture and forestry can lead to the biological control of some plant diseases. Even if VAM cannot be a substitute for pesticides, it can, with careful manipulation and together with less pesticidal application, be a more economical proposition.

Vast sums of money is spent yearly to combat plant diseases in South Africa, because the climate and environment favour pests and plant pathogens. The implementation of VAM as a biological control together with a reduced pesticidal application could lead to higher production of food, more profitable farming and afforestation industries and very important, to reduce the risks of high pesticidal applications for man, animals and the ecosystem.

## 8.3 The need for further research on the VAM symbiosis

Researchers as already mentioned, emphasize the urgent need for futher research regarding the effects of VAM on plant growth and health, agricultural, horticultural and afforestation practices. The main obstacle is a lack of sufficient funds.

As Hepper (1986), for example mentioned that the effects of most species of VAMF on host plants have not yet been investigated. This lack of information jeopardises the selection of the most efficient VAM fungus to maximize the beneficial effects of VAM in plants which are inoculated. Only when this objective is achieved, can VAM be utilized effectvely.

To compliment the research of VAM in relation to farming and afforestation, it is necessary to do more research in the ecology and incidence of VAM. Some researchers believe that VAM may play a significant role in natural nutrient cycling systems and this phenomenon should also be thoroughly investigated.

The importance of VAM fungal interconnections between plants is another aspect to be investigated more extensively so that this aspect of VAM could also be utilized to, may be, reduce fertilizer and pesticidal requirements.

One of the greatest needs in VAM research today is to improve techniques and information to culture VAMF axenically for production of pathogen-free inocula on a large scale for use in agriculture. There should further more be improvement in techniques to introduce selected VAMF effectively to the crop production systems.

Most of the people in rural South-Africa are poor and cannot afford fertilizer to restore the fertility of soils. Instead they will deforest another piece of natural vegetation. One of the most important aims for research in South Africa should therefore, apart from the use of VAM in agriculture, be concentrated on the restoration and rehabilitation of the vast areas where natural vegetation has already been destroyed by people in need for more fertile agricultural soils.

Other activities such as mining industries are also responsible for the deforestation of natural vegetation sites. Today companies are obligated to invest huge sums of money to restore the natural vegetation disturbed by these practices. As research in other countries has already proved, VAMF can be used effetively to speed up the process of restoring natural ecosystems. Susceptible host plants can be infected with VAMF prior to transplanting them in disturbed areas. The survival of VAMF infected plants in such areas proved to be good. Economically this will be less money consuming because the VAM fungal infected plants have a much greater chance to survive the adverse soil conditions than uninfected plants.

To cunclude the necessity for more research on VAM, it remains to encourage large industrial companies to invest money in VAM research. This will not only help to save the

deteriorating ecological environment but will also have a possitive effect on the viability of the economy of the country. This could lead to an increase in the food production to feed the ever growing populations of third world countries.

# SUMMARY

**A survey of vesicular arbuscular mycorrhizae (VAM), with special reference to taxonomy, physiology and economical importance**

Vesicular-arbuscular mycorrhizae (VAM) are the most common type of mycorrhiza found in nature. They are ubiquitously found in soils throughout the world where they form symbiotic associations with most plants as well as crops. VAMF are classified as zygomycetes fungi and can be divided into six genera: *Glomus, Gigaspora, Sclerocystis, Acaulospora, Scutellospora* and *Entrophospora*.

Vesicular-arbuscular mycorrhizal fungi (VAMF) form complex extramatrical and intramatrical mycelia within the roots of host plants. When hyphae of VAMF get into contact with host roots, appressoria are formed on the surface of roots. The penetrating hyphae branch to form coils, intercellular and intracellular hyphae throughout the cortex of host roots. The intramatrical hyphae form typical arbuscules within the cortex cells as well as vesicles which can be formed inter- or intracellular. Arbuscules are considered as the main centre of nutrient exchange between the host plant and the fungus.

VAMF also form extensive mycelia outside host roots where they are able to explore large volumes of soil for nutrients, beyond the depletion zone of plant roots.

During the symbiotic association of VAMF and host plants the fungi benefit the host plants mainly by absorbing nutrients from nutrient deficient soils and transferring it to the host. The fungi benefit from hosts by getting carbon compounds and protection.

This association is influenced by environmental and climatic factors such as soil fertility, Ph and moisture content of the soil, temperature and light as well as the presence of other soil organisms.

VAMF usually have several beneficial effects to host plants. The host plant benefit by enhanced growth and crop yield, better absorption of nutrients, especially phosphorus, from low fertility soils and increased resistance against disease, increased tolerance to water stress.

These beneficial effects of VAMF can, with careful investigation and selection of most suitable VAMF, be manipulated for agriculture and horticulture uses to enhance crop production. VAMF can not be considered as substitutes for fertilizer application but can be implemented to utilize applied fertilizer more efficiently, thus reducing the rate and amount of fertilizer application. The application of pesticides can also be reduced on VAMF infected plants. This can lead to more economical agriculture, horticulture and afforestation practices.

To enhance crop yield, it is necessary to produce VAMF inoculum a large scale basis. Unfortunately the production of pure pathogen-free inocula has not been achieved as the growing of VAMF axenically is not yet possible. However, there are several other methods to produce viable inocula but the contamination with unwanted organisms is still at risk.

There is still a need for further research to test the viability of inoculum production to the economy and only then will VAMF be successfully utilized for horticultural, agricultural and afforestation enterprises.

# OPSOMMING

**'n Ondersoek van vesiculêr-arbuskulêre mikorisas (VAM) met spesiale verwysing na taksonomie, fisiologie en ekonomiese belangrikheid.**

Vesikulêr-arbusculêre mikorisas (VAM) is die mees algemene tipe mikorisa in die natuur. Hulle kom wyd versprei in grond oor die wêreld voor waar hulle in simbiose met meeste plante en landbou- en tuinbougewasse leef. VAMF word geklassifiseer as zygomycetes swamme en word in ses genera verdeel, nl: *Glomus, Gigaspora, Sclerocystis, Acaulospora, Scutellospora* en *Entrophospora*.

Vesikulêr-arbusculêre mikorisa fungusse (VAMF) vorm 'n komplekse miselium wat in en om die gasheer voorkom. Appressoriums word op die worteloppervlak gevorm voordat hifes die gasheerwortel se korteksweefsel binnedring. Die hifes sal dan 'n netwerk van inter-en intrasellulêre hifes vorm met tipiese arbuskule en vesikels. Arbuskule word intrasellulêr gevorm en vesikels kan inter- of intrasellulêr wees. Die arbuskule word beskou as die plek vir uitruiling van voedingstowwe tussen die gasheer en die fungus.

Die uitgebreide uitwendige miselium kan voedingselement bekom uit grond wat vir die gasheerwortels onbereikbaar is.

Gedurende hierdie unieke simbiose is die fungus veral voordelig vir die gasheer vir die opname van voedingselemente veral uit arm grond. Die voedingselemente word deur die uitwendige hifes geabsorbeer en na die gasheer vervoer tot by die arbuskule waar uitruiling van voedingstowwe plaasvind.

Hierdie verhouding tussen die gasheer en fungus word beïnvloed deur omgewings- en klimaatstoestande, soos grondvrugbaarheid en grondvog, suurtegraad van die grond, temperatuur en daglengte asook ander grondorganismes.

Die gasheer plant word deur die fungus bevoordeel deur 'n verbetering in groei en opbrengs van die plant, verhoging in mineraalopname uit die grond vir die gasheerplant, die plant meer patogeenbestand word en 'n beter wateropname.

Hierdie voordele van VAM-fungusse vir gasheerplante kan, met noukeurige ondersoek en seleksie, bemanipuleer word vir landbou- en tuinboukundige praktyke om die opbrengs van gewasse te verbeter. VAMF kan nie as plaasvervangers van misstowwe gebruik word nie, maar sal dit kan misstowwe beter benut en dus 'n vermindering in die toediening van sulke stowwe tot gevolg hê. Die toediening van oormatige gifstowwe teen plae kan ook verminder word deur VAMF. Dit kan dan lei tot meer ekonomiese landbou-, tuinbou- en bebossingspraktyke.

Vir die verhoging van die produksie van landbou- en tuinboukundige gewasse is dit nodig om inokulum van die fungusvennote op groot skaal te produseer. Suiwer patogeen-vrye inokulum kan nog nie geproduseer word nie omdat die VAM-fungusse tot op hede nog nie as rein kulture gekweek kan word nie. Vandag word ander metodes gebruik om inokulum te produseer, maar dis moeilik om sulke inokulums patogeen-vry te produseer en te hou.

Daar is nog baie navorsing nodig om die gebruik van VAM-fungusse en hul inokulums te toets vir ekonomiese vatbaarheid. Indien wel, kan hierdie fungusse baie suksesvol en effektief beruik word in landbou, tuinbou en bebossingsbedrywe.

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