MANAGEMENT AND REHABILITATION OF DEGRADED LANDS AND SECONDARY FORESTS IN AMAZONIA:

An International Symposium/Workshop

April 18-22, 1993
Hotel Tropical
Santarém, Pará - Brazil

Organized by:

International Institute of Tropical Forestry
USDA - Forest Service
Southern Forest Experiment Station
Rio Piedras, Puerto Rico, USA

EMBRAPA/CPATU
Centro de Pesquisa Agroflorestal da Amazônia Oriental
Belém, Pará, Brasil

In Collaboration with:

Instituto Brasileiro do Meio Ambiente e
dos Recursos Naturais Renováveis - IBAMA
Santarém, Pará, Brasil

With Support from:

Tropical Forestry Program
International Forestry
U.S. Department of Agriculture - Forest Service
Washington, DC, USA

and

Man and the Biosphere Programme (MAB)
UNESCO
Paris, France

ISOLATION, SELECTION AND PRODUCTION OF VESICULAR-ARBUSCULAR MYCORRHIZAL FUNGI (VAMF) AND THEIR APPLICATION IN MIXED CROPPING SYSTEMS

F. Feldmann[1], I. Müller[1], J. Weritz[2], J.L.V. Macedo[3], E. Idczak[1]

[1]*Universitat Hamburg, Institut für Angewandte Botanik, Hamburg, Germany*
[2]*Biologische Bundesanstalt, Institut für Pflanzenschutz im Gartenbau, Braunschweig, Germany*
[3]*Centro de Pesquisa Agroflorestal da Amazônia Ocidental, CPAA/EMBRAPA, AM, Brazil*

SUMMARY

The introduction of vesicular-arbuscular mycorrhizal fungi (VAMF) into agricultural systems benefits the growth and the health of the crops, especially in abandoned areas with poor soils. As a prerequisite for the application of the symbionts, the fungi must be isolated and selected with respect to their efficiency for the plants and their adaptability to the environmental conditions in the field. Different methods of isolation and the selection procedure are described. The mass production of the selected fungi can be carried out in the greenhouse or in the field. The inoculation of the plants with the VAMF is possible in the nurseries as well as in the field. Two methods of application - inoculation of the plants with a homogeneous mixture of the inoculum in the substrate and with a concentrated inoculum layer respectively - are presented.

INTRODUCTION

In tropical habitats the benefits of vesicular-arbuscular mycorrhizal fungi (VAMF) to their host plants are of special importance. By means of improved nutrient supply the fungi cause significant growth enhancement of the plants in the poor soils of the tropics (Sieverding 1987; Lin 1986). Furthermore, the colonization of roots by mycorrhizal fungi increases the resistance of the host plants against root pathogens (Zambolim 1986) and reduces the severity of foliar disease (Feldmann 1991). The tolerance of mycorrhizal plants against stress (*e.g.* extreme temperatures, high humidity or drought and transplanting) is generally higher compared to non-mycorrhizal plants (Schonbeck 1987; Müller 1991). In agricultural systems a high deficiency of the symbiotical fungi has been observed (Feldmann 1991). To regain the "vitality of the soil" with respect to VAMF, the application of the fungi at the time of sowing or planting or even earlier in the nurseries is necessary. In Manaus, Amazonas State, a mixed cropping system of perennial plants has been installed in which the management takes account of the requirements of the fungi (less fertilization, less pesticides, more secondary vegetation). The plantation has been established in an abandoned area (former rubber plantation). To improve the "ecological fitness" of the plants, they have been inoculated with VAMF in the nursery or at the time of planting into the field.

So far the use of VAMF in plant production systems and agriculture is not a common practice. This is mainly due to the difficulties involved in the isolation, selection, multiplication and application of the VAMF. The purpose of this paper is to demonstrate the methods from the first step of isolation of the mycorrhizal fungi to the final step of application in the nurseries and in the field.

ISOLATION OF VESICULAR-ARBUSCULAR MYCORRHIZAL FUNGI (VAMF)

VAMF can be used in the form of:

- pure isolates derived from single or multiple spore cultures of one fungal species
- a mixture of defined pure isolates
- a mixture of unknown VAMF species with proven stability of the population and its effects.

The establishment of pure isolates (1) and isolates of an unknown mixture of VAMF (2) is shown in Figure 1. In both cases the origin of the fungi is field soil. Besides the living VAMF spores, field soil often contains large numbers of dead spores of

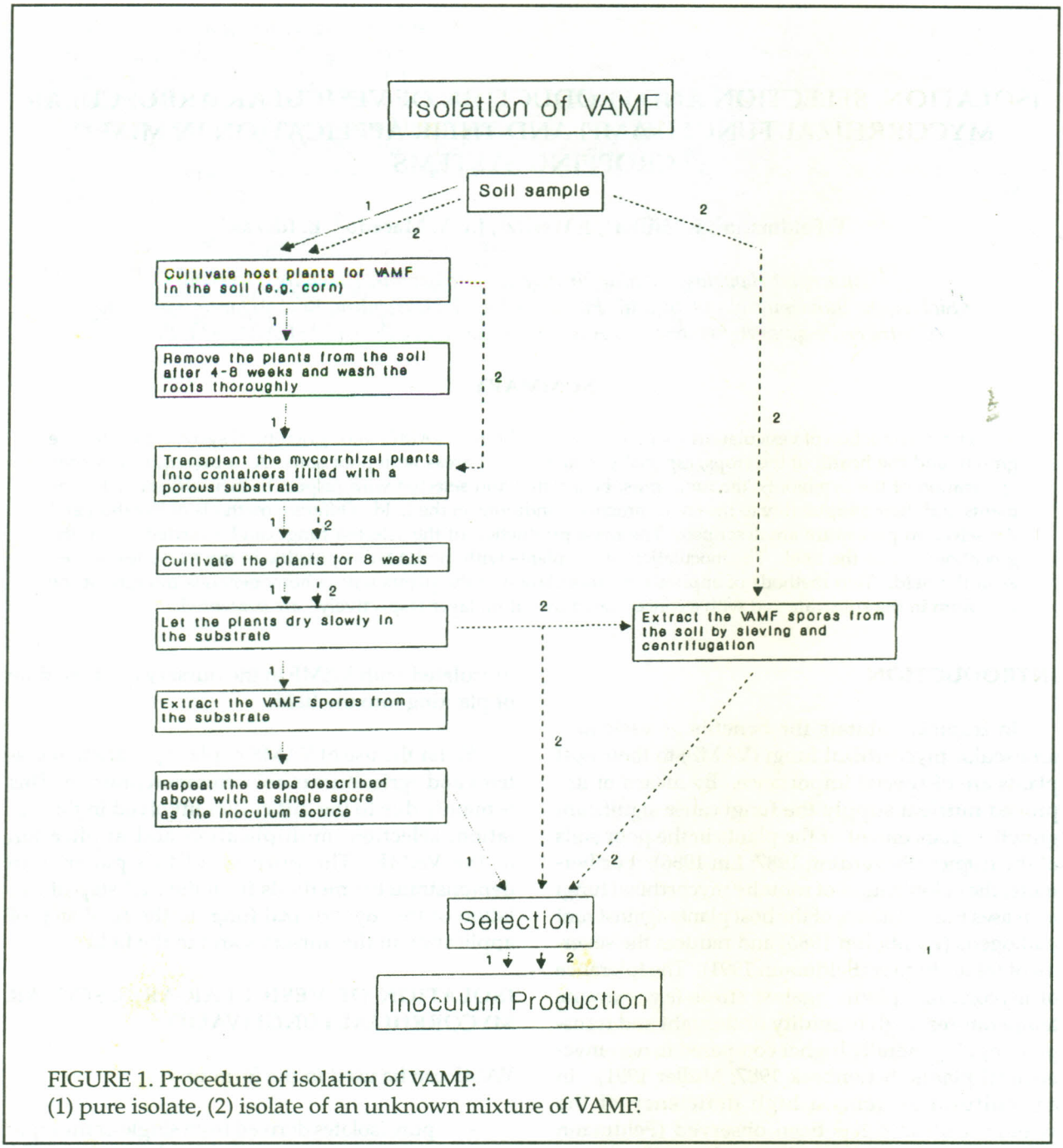

FIGURE 1. Procedure of isolation of VAMP.
(1) pure isolate, (2) isolate of an unknown mixture of VAMF.

mycorrhizal fungi. As it would be time consuming to establish single spore isolates directly from the original soil sample, we prefer to enhance the number of living spores by cultivation of a host plant (*e.g.* corn, onion, bell pepper) in the soil. This will cause the propagation of the fungi in the substrate. A disadvantage of this method at increasing the number of living VAMF spores is the possibility of pre-selection of those fungi which are more adapted to the given growth conditions. To minimize this pre-selection, the use of different plant species and growth conditions is recommended. Four to eight weeks after sowing or planting, the plant roots will be colonized by the VAMF. For the establishment of pure isolates, the plants are taken from the substrate and the roots are rinsed thoroughly. To avoid contamination with root pathogens a surface sterilization (*e.g.* with ethanol) of the roots can be an

advantage. The plants are then transplanted into plastic sacks or other containers such as pots or boxes containing a porous substrate. They are irrigated moderately and fertilized once a week with a low phosphate fertilizer (< 60ppm P). After a growth period of eight weeks, the plants are not watered further, thus inducing the spore production of the VAMF. The spores can be removed from the substrate by the method of wet sieving and centrifugation (Daniels and Skipper 1984; Feldmann 1991). To establish pure cultures, the procedure described is repeated with a single spore as the inoculum source (single spore isolate) or with several spores of the same fungal species (multiple spore isolate), which is often problematic as it is hard to determine whether similar spores belong to the same species.

For the production of a VAMF-isolate containing an unknown mixture of VAMF species (2), several steps of the procedure described above can be omitted (see Figure 1). Furthermore, it is possible to extract the VAMF spores directly from the field soil and use them as the primary inoculum. This method will diminish the contamination with pathogens which might be present in the soil.

Before the established VAMF-isolates can be used in nurseries or on an agricultural scale, they must be selected with respect to their effectiveness.

SELECTION OF EFFECTIVE VAMF-ISOLATES

Although the VAMF are distributed worldwide and most of the plant species live in symbiosis with these fungi, a specificity in the interaction between fungus and host plant exists (Estaun *et al.* 1987, Graw *et al.* 1979, Idczak 1992). Specificity is revealed with respect to

- the host plant (plant species / plant cultivar)
- the nature of the effect (which also depends on the environmental conditions).

Therefore the selection of effective VAMF isolates is necessary.

THE SELECTION PROCEDURE

List:

* the nature of the effect(s) desired (*e.g.* plant growth enhancement, increase of resistance against pathogens...),
* the given abiotic factors during the selection which should be similar to the final conditions under which the fungi will be used (substrate, temperature, pH...).

- Ensure the infectivity of the fungal inoculum which will be tested (check whether a potential host plant, *e.g.* corn, is colonized by the fungi).

- Determine whether the plant for which the fungal isolate is to be selected is mycotroph (Janos 1987; Feldmann 1991), *i.e.* test whether the VAMF are able to colonize the roots and whether the mycorrhizal plants benefit from the symbiosis.

- If the plant is mycotroph, analyse the degree of mycorrhizal dependency (obligate or facultative dependency; Feldmann 1991).

- Determine again the abiotic selection factors, considering the degree of dependency, the conditions in the area where the fungi will be used and the kind of management.

- Compare the effectiveness of the isolates with respect to the desired effect.

In addition to the described steps in the selection of effective VAMF isolates, there are other factors which should be taken into account during the selection process, such as the competitive ability and persistence of the fungi under natural conditions (Abbot *et al.* 1992). Once a VAMF isolate has been chosen, inoculum production on a large scale is a pre-requisite for the application in nurseries or in the field.

INOCULUM PRODUCTION

A detailed description of the production of VAMF inoculum for use in the tropics has been presented by Feldmann and Idczak (1992). Therefore only the main aspects of inoculum production are described here.

The inoculum production can be differentiated into an initial phase (Figure 2) and mass production (Figure 3).

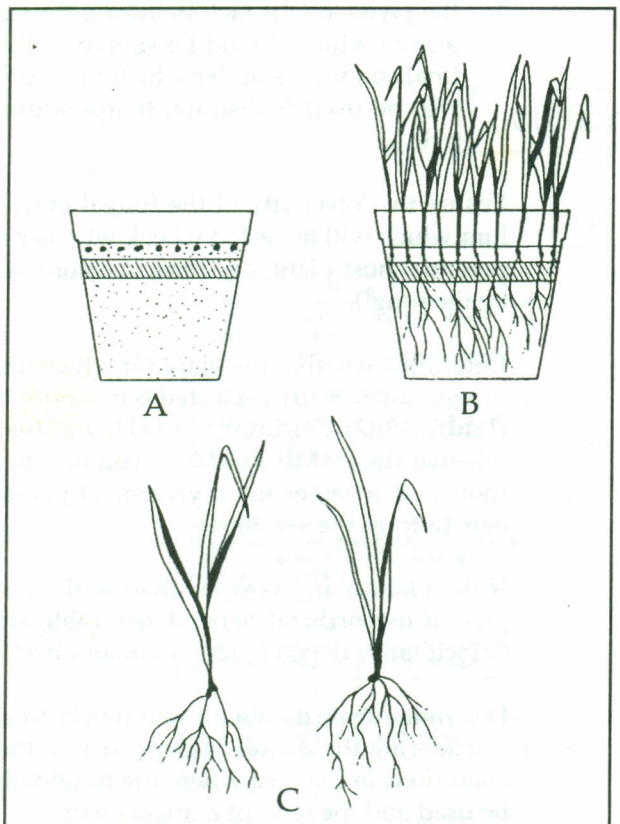

FIGURE 2. Initial phase of inoculum production (Feldmann & Idczak 1992). See text for details.

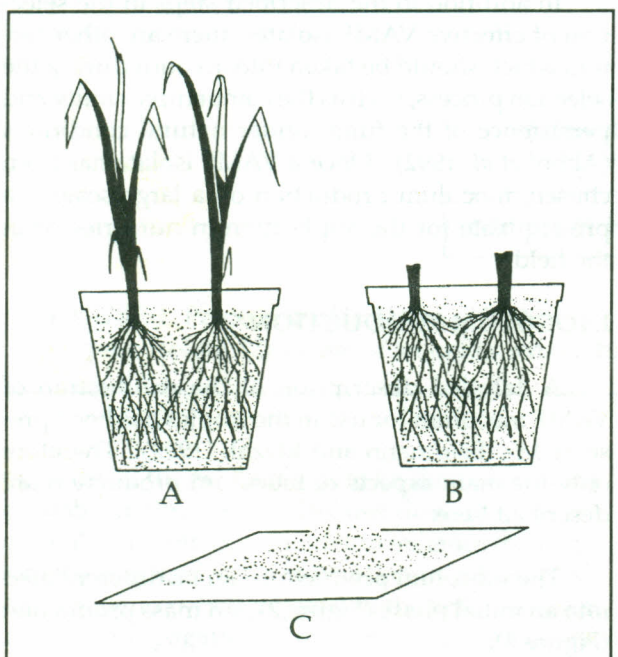

FIGURE 3. Mass production of inoculum (Feldmann & Idczak 1992). See text for details.

Initial phase:

- Fill two thirds of a pot (volume about 500 ml) with quartz sand and layer the start inoculum (soil containing the VAMF, infected root pieces or fungal spores) on top.
- Surface sterilize the seeds of a host plant (*e.g.* corn or onion), rinse them with water afterwards to remove all pesticides, sow them into the pots and cover them with sand.
- Water the pots moderately (the humidity of the substrate should be a little below field capacity) and fertilize the plants with a low phosphate fertilizer (P < 60ppm, details see Feldmann and Idczak 1992). Avoid the use of pesticides.
- After a growth period of three to four weeks, the roots of the host plants should be colonized by the VAMF. Control the root colonization with a microscope. In the event of low infectivity of the start inoculum the culture period can be extended by one to two weeks.
- When the roots of the host plant are well colonized, separate the plants from each other and wash the roots. Sometimes it might be necessary to surface sterilize the roots (70% ethanol, two minutes) to eliminate pathogenic microorganisms.
- The plants treated in this way are used for the mass production of VAMF inoculum.

Mass production:

- The mycorrhizal plants are transplanted into larger pots (volume about 51) or comparable containers, which might be even larger, but still allow the roots to explore the whole volume (A). The use of expanded clay as the substrate (method described by Dehne and Backhaus 1986) has various advantages such as its low weight, easy application and the fact that contamination by pathogens can be largely avoided and subsequent decontamination is also possible. But other substrates like sterilized sandy soils can be used as well.

- Irrigation and fertilization should be carried out as described above (initial phase).

- After three to four months of plant growth, the roots should fill the whole volume of the container. From this moment on, the plants are no longer irrigated. The drought stress stimulates the spore production of the VAMF.

- One week after termination of irrigation, the plants are cut off and the pots with the roots are kept dry for another two weeks (B). After this period the roots are removed from the substrate.

- The substrate is spread out in a thin layer and air-dried rapidly (C). The dry inoculum can be stored under cool and dry conditions for more than a year.

Alternatively to the procedure of inoculum production described above, the multiplication of the VAMF can be carried out directly in the field as well (in-situ production). Feldmann (1991) showed that the inoculation of host plants (*e.g.* corn) with VAMF in the field at the time of sowing or planting results in an increase of the number of VAMF spores from 4 to 57 spores per gram of soil after two months.

APPLICATION OF VAMF INOCULUM

The inoculation of plants with mycorrhizal fungi can be performed in different ways:

- The fungal inoculum can be mixed homogeneously with the substrate in which the plants are grown.

- A concentrated layer of the inoculum can be placed below the seeds or the plant roots.

The method of inoculation and the amount of the applied inoculum (0; 0.2; 1; 5 and 10% Vol.) determine the development of the mycorrhizal association (Figure 4).

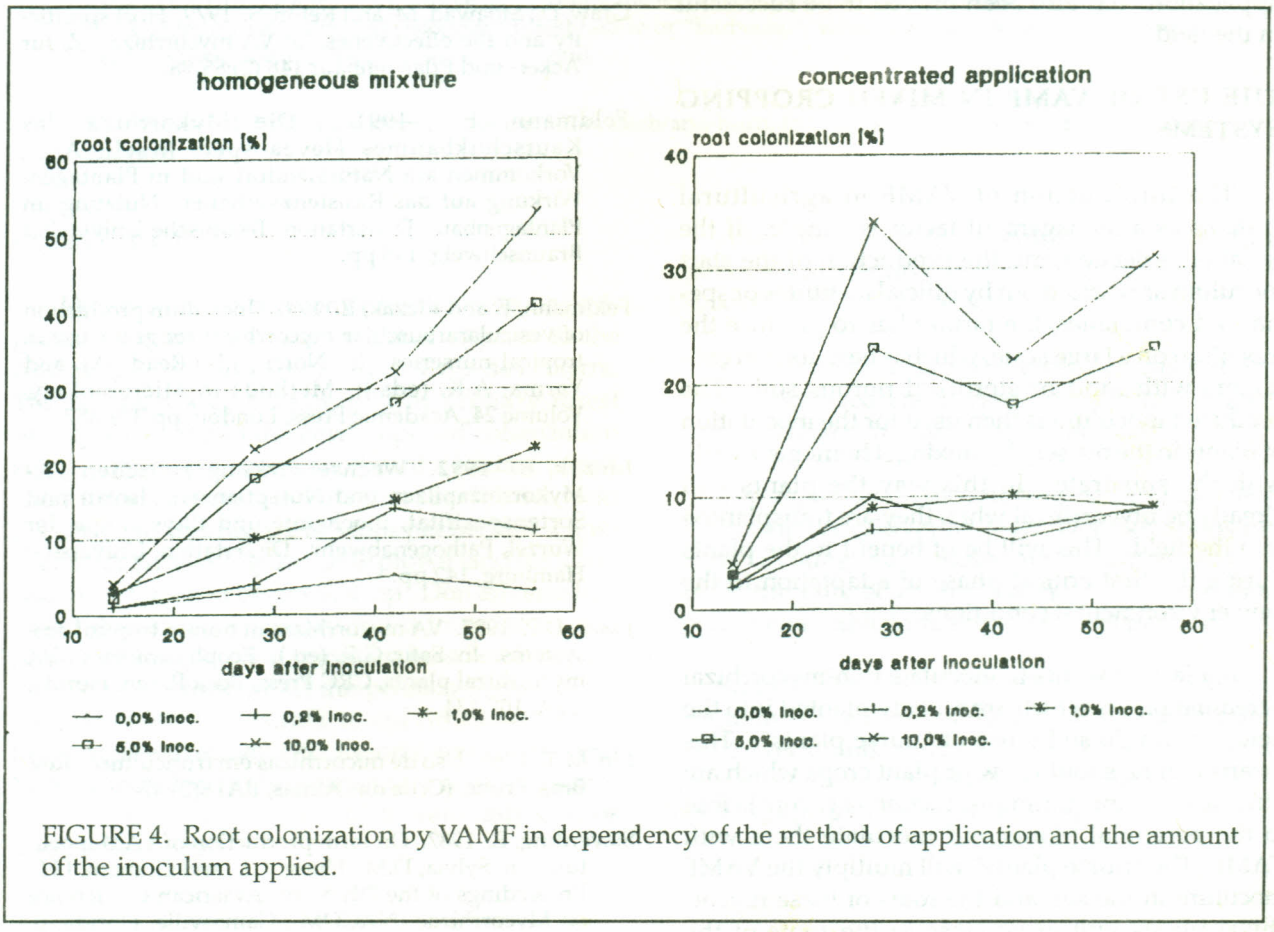

FIGURE 4. Root colonization by VAMF in dependency of the method of application and the amount of the inoculum applied.

The highest degree of root colonization of rubber tree seedlings (*Hevea* sp.) is achieved by use of more than 1% (v/v) of inoculum in the substrate. The homogeneous distribution of the inoculum finally leads to a higher degree of root colonization compared to the method of "concentrated application", although a higher percentage of root colonization is achieved with the latter method during the early stages of the development of the symbiosis. This is probably due to the fact that in the case of a "concentrated application", the roots of a seedling have contact to a large number of infection units when growing through the inoculum layer. At this moment a heavy infection of the root system occurrs. After the roots have passed the inoculum layer no further infection units of VAMF are present. The fungus has to spread over the whole root system from the site of the primary penetration points. In case of a homogeneous distribution of the fungal inoculum, new infections of the roots by the fungus are possible even after a longer time of the development of the root system. For this reason we recommend applying the inoculum in the form of a homogeneous mixture, but the "concentrated application" has also been proved to be successful in the field.

THE USE OF VAMF IN MIXED CROPPING SYSTEMS

The introduction of VAMF in agricultural systems as a management factor is simple. If the isolation, selection and the production of the start inoculum are carried out by official institutes or specialized companies, the farmer has to produce the inoculum on a large scale, which is best done in containers with sand or sterilized porous soil. The produced inoculum is then used for the inoculation of plants in the nursery by mixing it homogeneously with the substrate. In this way the plants will already be mycorrhizal when they are transplanted into the field. This will be of benefit to the plants during the first critical phase of adaptation to the new environmental conditions.

If a farmer wants to inoculate non-mycorrhizal perennial plants which are already planted into the field, he can do so by use of "nurse plants". This means that he should sow or plant crops which are often used in inoculum production (*e.g.* corn) close to the perennial plants and inoculate them with VAMF. The "nurse plants" will multiply the VAMF inoculum in the soil and the roots of these mycorrhizal plants will grow close to the roots of the perennial plants and thus inoculate them. If the management in the plantation takes into account the requirements of the VAMF (less fertilizer, less pesticides, more secondary vegetation), the fungi can establish themselves in the field.

REFERENCES

Abbot, L.K.; Robson, A.D. and Gazey, C. 1992. Selection of inoculant vesicular-arbuscular mycorrhizal fungi. In: Norris, J.R.; Read, D.J. and Varma, A.K. (eds.): Methods in Microbiology, Volume 24, Academic Press, London, pp. 1-21.

Daniels, B.A. and Skipper, H.D. 1984. Methods for the recovery and quantitative estimation of propagules from soil. In: Schenck, N.C. (ed.): Methods and principles of mycorrhizal research. Am. Phytopath. Soc., St. Paul, MI, USA, pp. 29-35.

Dehne, H.W. and Backhaus, G.S. 1986. The use of VAM in plant production 1. Inoculum production. Z. Pflanzenkrankheiten und Pflanzenschutz 93:415-424.

Estaun, V.; Calvet, C. and Hayman, D.S. 1987. Influence of plant genotype on mycorrhizal infection: response of three pea clutivars. Plant and Soil 103:295-298.

Graw, D.; Moawad, M. and Rehm, S. 1979. Host specificity and the effectiveness of VA-mycorrhiza. Z. fur Acker- und Pflanzenbaur 148 (2):85-98.

Feldmann, F. 1991. Die Mykorrhiza des Kautschukbaumes Hevea spec. Muell. Arg.: Vorkommen am Naturstandort und in Plantagen, Wirkung auf das Resistenzverhalten, Nutzung im Plantagenbau. Dissertation, Technische Universitat Braunschweig, 148 pp.

Feldmann, F. and Idczak, E. 1992. Inoculum production of vesiculararbuscular mycorrhizal fungi for use in tropical nurseries. In: Norris, J.R.; Read, D.J. and Varma, A.K. (eds.): Methods in Microbiology, Volume 24, Academic Press, London, pp 339-357.

Idczak, E. 1992. Wechselwirkung zwischen VA-Mykorrhizapilzen und Nutzpflanzen: Isolat- und Sortenspezifitat, Biochemie und Physiologie der Wurzel, Pathogenabwehr. Dissertation, Universitat Hamburg, 142 pp.

Janos, D.P. 1987. VA mycorrhizas in humid tropical ecosystems. In: Safir, G.R. (ed.): Ecophysiology of VA mycorrhizal plants, CRC Press, Boca Raton, Florida, USA, 107-134.

Lin, M.T. 1986. Uso de micorrhizas em fruticultura. Rev. Bras. Frutic. (Cruz das Almas, BA) 8(3):47-55.

Sieverding, E. 1987. On farm production of VAM inoculum. In: Sylvia, D.M.; Hung, L.L. and Graham, J.H.: Proceedings of the 7th North American Conference on Mycorrhizae (NACOM), Gainesville, Florida, p. 284.