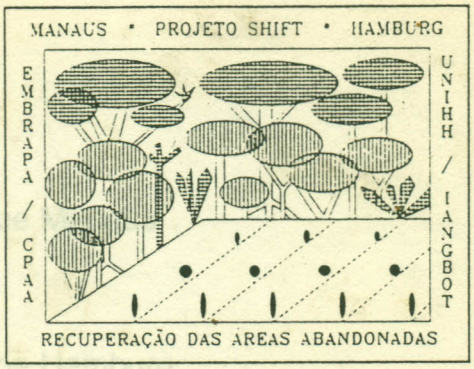

SHIFT-Projekt ENV-23

Rekultivierung degradierter, brachliegender Monokulturflächen in ausgewogene Mischkulturflächen unter besonderer Berücksichtigung bodenbiologischer Faktoren

Förderkennzeichen 0339457A

Jahresbericht 1993

EMBRAPA/CPAA - Universität Hamburg

Proceed. "Manejo ..." Santarem 1993, in Druck

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

by

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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 pre-requisite 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 (Schönbeck 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, AM, 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 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

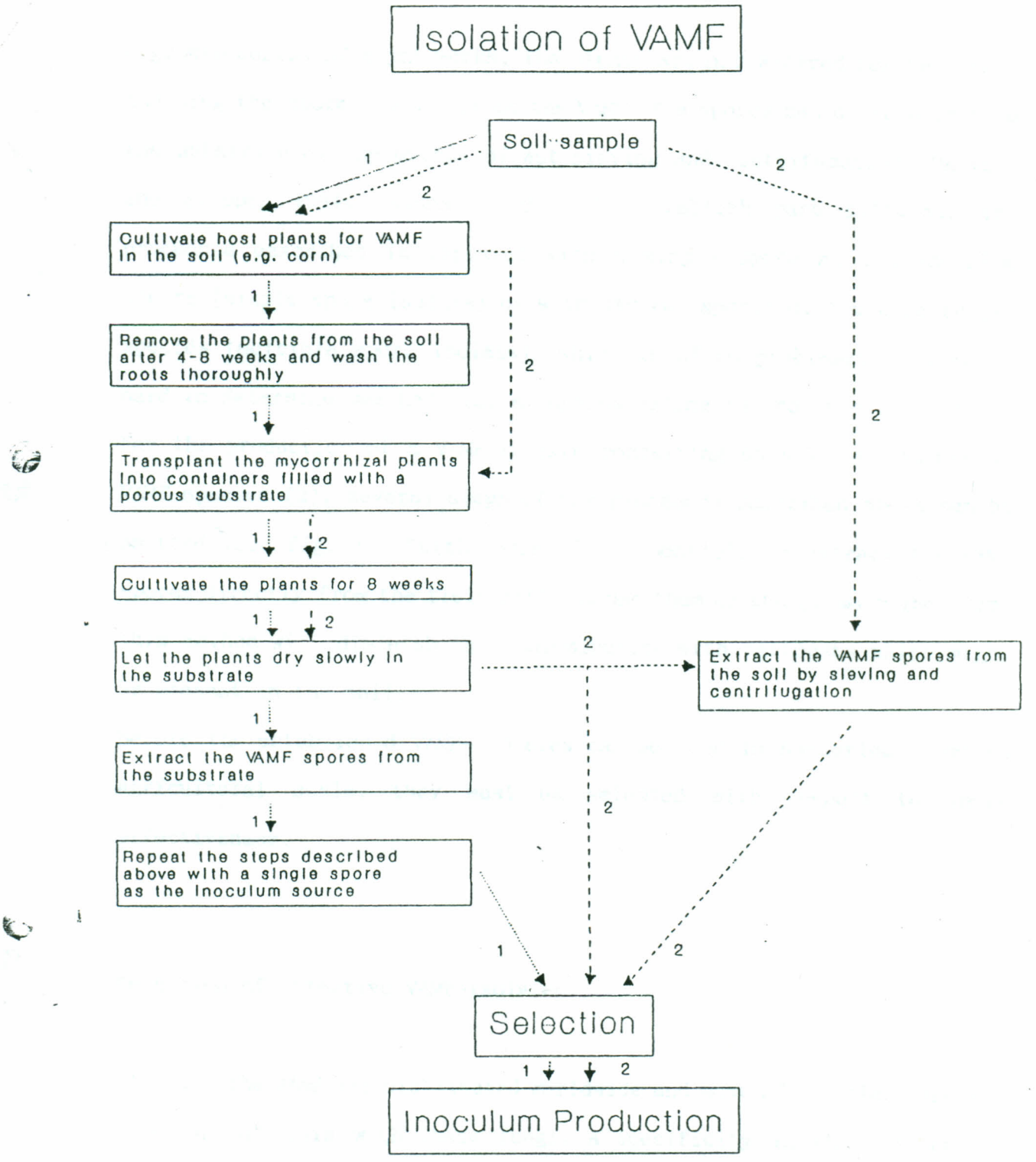

Fig. 1: Procedure of isolation of VAMF

(1) pure isolate, (2) isolate of an unknown mixture of VAMF

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 fig. 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 (Estaún 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 (fig. 2) and mass production (fig. 3).

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.

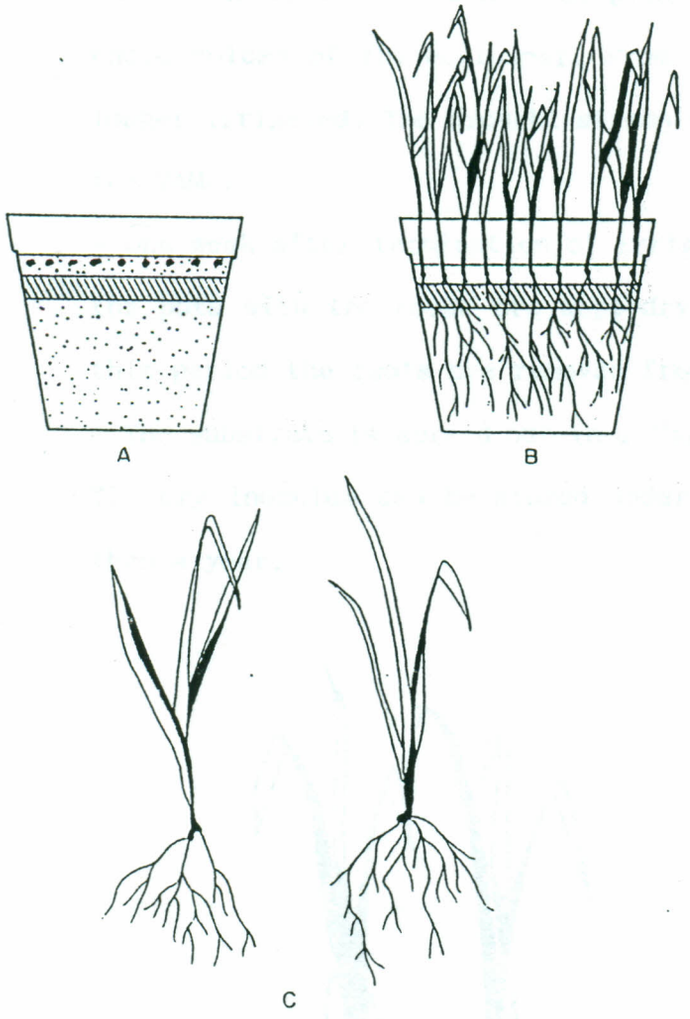

Fig. 2: Initial phase of inoculum production (Feldmann & Idczak 1992). See text for details.

Mass production:

- The mycorrhizal plants are transplanted into larger pots (volume about 5l) 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.

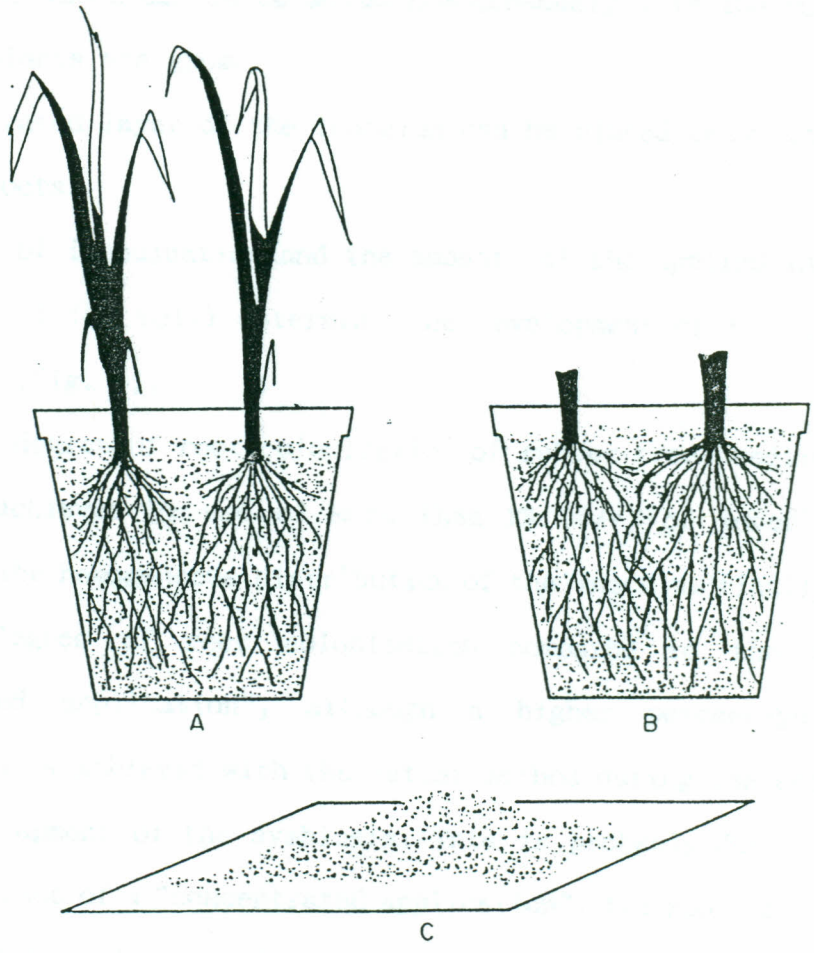

Fig. 3: Mass production of inoculum (Feldmann & Idczak 1992). See text for details.

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 inoculuation and the amount of the applied inoculum (0; 0.2; 1; 5 and 10% Vol.) determine the development of the mycorrhizal association (fig. 4).

The highest degree of root colonization of rubber tree seedlings (Hevea spec.) 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.

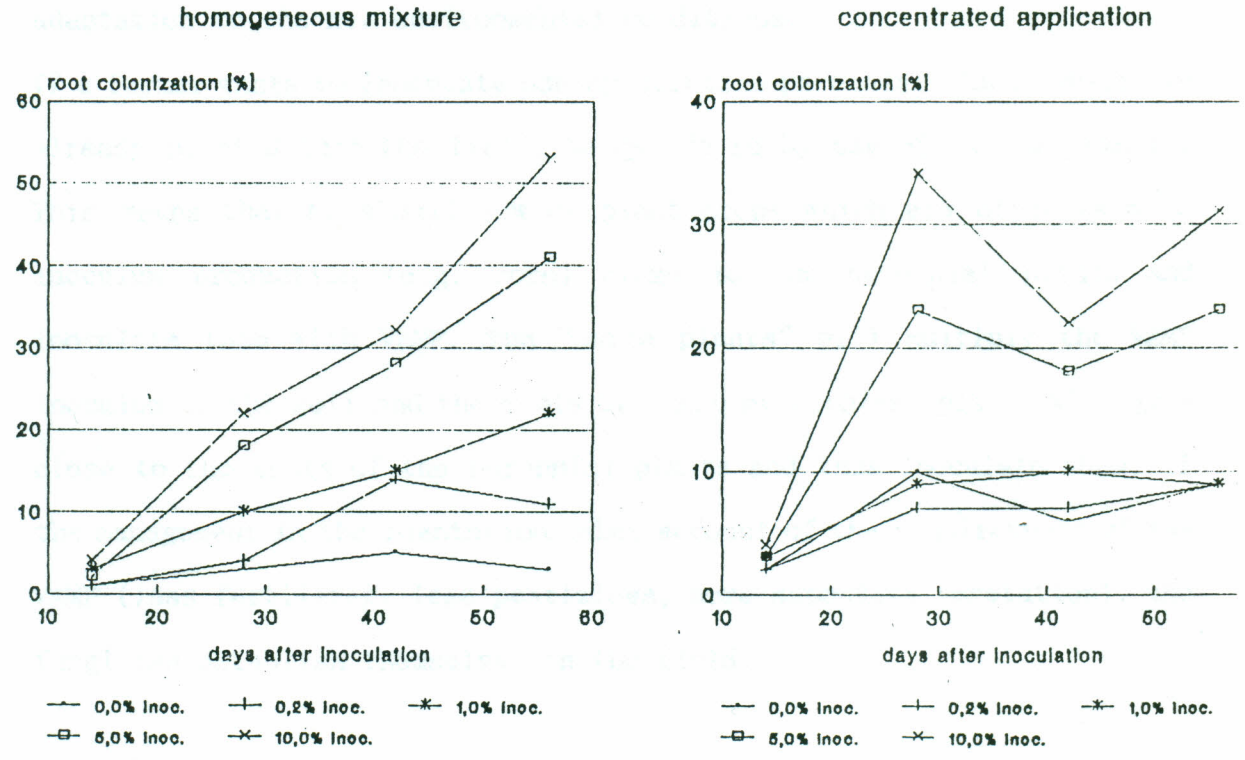

Fig. 4: Root colonization by VAMF in dependency of the method of application and the amount of the inoculum applied.

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PREPARO E APLICACAO DE FUNGOS MICORRIZICOS SELECIONADOS PARA POLYCULTURAS

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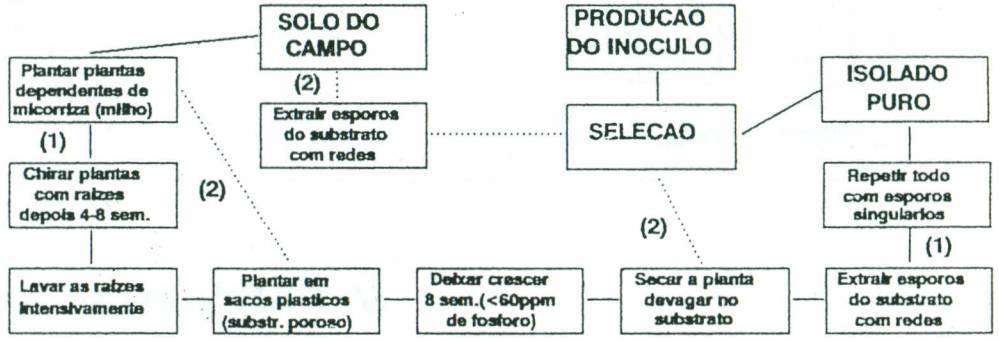
Introducao

- A maioria das plantas uteis das regioes tropicais sao altamente dependentes dos fungos micorrizicos vesiculo arbusculares (FMVA)
- Em sistemas agricultorios uma deficiencia alta dos simbiontes foi observado que implicou usar FMVA como agentes biologicos para melhorar a saude das plantas.
- Na Manaus, AM, uma polycultura das plantas uteis perennes foi installado no que o manejo respeita as necessidades dos fungos (menos adubo, menos pesticidas, mais plantas secundarias...). A polycultura foi installado numa area degradada e abandonada. Na fase da implantacao FMVA selecionadas foram usadas para aumentar o "fitness ecologico" das plantas.
- Nesta presentacao os methodos da producao e aplicacao do inoculo para este projeto sao mostrados.

PASSO I

Como ganhar isolados dos FMVA?

- FMVA sao usados como
  - Isolados puros (originados de somente um esporo singulo do FMVA) (1)
  - Misturas dos isolados puros conhecidos
  - Misturas nao conhecidas com um efeito provado e estavel (2)

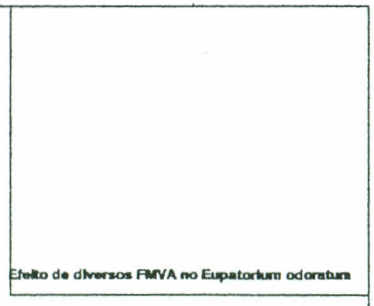


PASSO II

A procedura da selecao

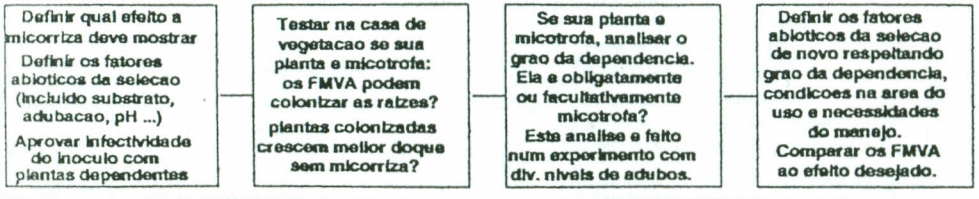
- A micorriza tem efeitos especificos:

  Existe uma especifidade em relacao
  - ao hospite
  - a natura do efeito
  - tambem o efeito depende do meio ambiente



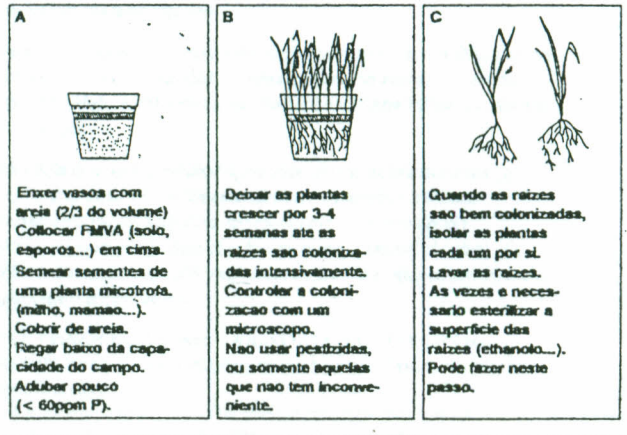
Efeito de diversos FMVA no Eupatorium odoratum

- Por isso e necessario selecionar os FMVA mais efectivos



PASSO III

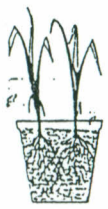
Producao do inoculo primario



Fonte:
Feldmann, F. & Idczak, E.: Inoculum production of VAM-fungi for use in tropical nurseries Methods in Microbiology (24), 339-357, 1992

PASSO IV
Producao da grande escala

A



As plantas de passo III sao transferidas para vasos grandes ou caixas de madeira. Depois 3-4 meses as raizes devem enxer todo volume do vaso ou da caixa. Neste momento nao regar mais e deixar as plantas secar.

B

Uma semana depois a irrigacao terminou, as plantas sao curtadas.
Deixar mais duas semanas secar.
Tirar as raizes secas.

C

Secar o substrato numa camada fina no ar (1 semana). Armazenar o inoculo num lugar seco e com a temperatura entre 18-25°C. A armazenagem e possivel por mais de um ano.

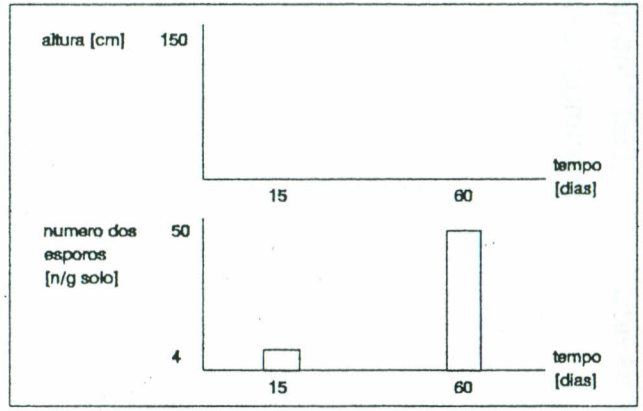
Inoculo de argila expandida

D

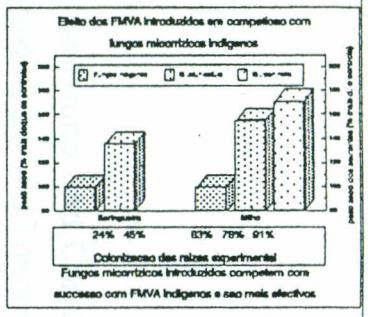
Em nossos experimentos com plantas perenes usamos um inoculo da argila expandida (metodo de Dehne e Backhaus, 1986). Argila expandida e misturavel muito simples com outros substratos, nao e muito pesado e nao favorece patogenos. Tambem usamos areia e terico para a multipilcacao dos fungos.

VARIANTE DE PASSO IV
In-situ producao do inoculo

Uma inoculacao das plantas dependentes no campo resulta numa aumenta do numero dos esporos dos FMVA: por exemplo MILHO

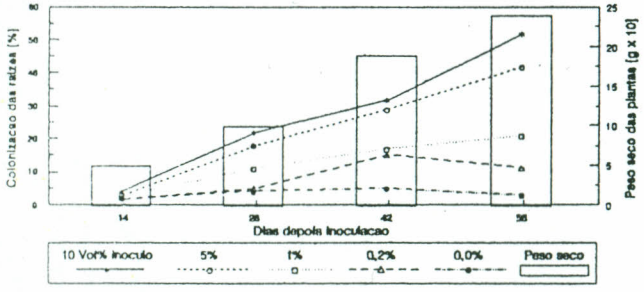


Producao no campo com Milho

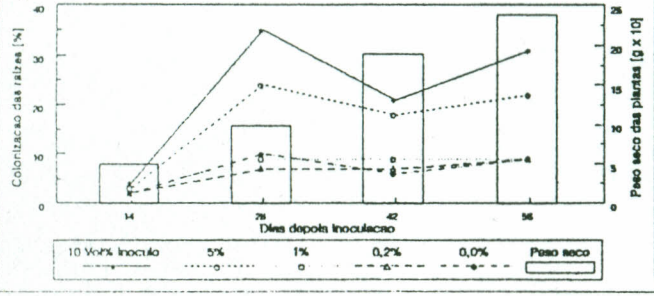


METODOS DA APLICACAO

Mistura homogenica



Aplicacao concentrada



Aplicacao concentrada

O successo da inoculacao depende
- do metodo da aplicacao
- da quantidade do inoculo

Observamos o melior colonizacao com mais de 1%Vol inoculo e a forma da aplicacao "mistura homogenica" (feito em vasos).

No campo uma "aplicacao concentrada" debaixo de semente e bem sucedido (veja foto.).

Uso na polycultura

Incluir o uso da micorriza nas sistemas da producao agricultoria e simple:

- **Se os primeiros 3 passos sao feitos por institutos oficiais ou empresas especiais o produtor tem que produzir inoculo na grande escala, melior em caixas de madeira com areia ou solo (terico) esterilizado. A esterilizacao pode ser feito com brometo de metilo ou solarizacao.**
- **Depois o inoculo e misturado homogenico com o substrato no viveiro. Entao as plantas plantadas fora no campo ja estao micorrizadas. A micorriza vai favorecer a fase initial da planta no campo.**
- **No caso que um produtor quer inocular plantas perenes ja plantadas sem micorrizacao ele pode inocular plantas dependentes como milho ou mandioca semeando ou plantando-as perto das plantas perenes ("nurse plants"). As raizes das nurse plants vao crescer para as raizes das plantas perenes e inocular-as.**
- **Se o manejo na plantacao e adaptada, as FMVA podem estabilizar-se (menos adubo, menos pesticidas, mais plantas secundarias).**

plantas secund. substituem a estabilizacao dos simbiontes

Appendix 4

Kubrom, I. (1993): Isolierung und Charakterisierung der Rhizosphärenbakterien von Hevea brasiliensis und Theobroma grandiflorum;
Diplomarbeit, Universität Hamburg, April, 1993
here: Summary