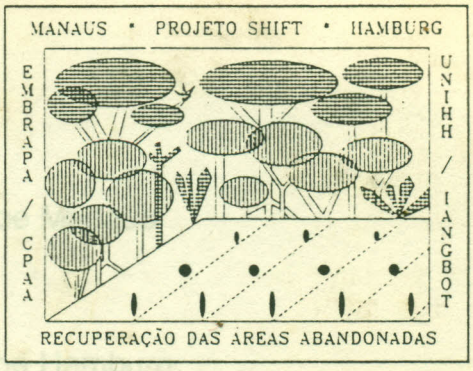


SHIFT-Projekt ENV-23

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

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# Agricultural systems in Amazonia depend on the management of mycorrhizal fungi

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## Summary

A large quantity of tropical useful plants are dependend on mycorrhizal fungi. Without the fungal symbionts they show growth depression and lower tolerance to biotic and abiotic stresses. In plant production systems normally a deficit of symbiosis is created by the common management practices. In tropical regions with the system of shifting cultivation the fallow over some years was thought to have a positively regulating effect to the mycorrhizal situation. In this contribution it is demonstrated, that over three, respectively eight years of fallow there is an increase of the inoculum potential in the soils but the effectivity of the fungal populations remains very low in comparison to the populations from natural sites. The necessity to manage the mycorrhizal fungi in situ in the field is discussed.

## Resumo

Titulo: Sistemas agricolas na Amazonia dependem do manejo de fungos micorrizicos

A maioria das plantas tropicais, de valor economico dependem de fungos micorrizicos. Na ausencia dos fungos simbiontes, as plantas apresentam baixo crescimento e baixa tolerancia aos "stresses" bioticos e abioticos. As plantas dos sistemas de producao normalmente apresentam um deficit de simbiose devido as praticas comuns de manejo. Nas regioes tropicais com os sistemas de cultivo itinerario, admitia-se que o pousio das areas teria um efeito regulado positivo na populacao de fungos micorrizicos. Neste trabalho, demenstia-se que mais os tres, ou

seja oito anos ha um aumento do potencial de inoculo no solo, mas a efetividade das populacoes fungicos permanece muito baixa em comparacao com as populacoes existentes nos sitios nativos. E discutida a necessidade do manejo dos fungos micorrizicos in situ no campo.

## 1. Introduction

The most of the tropical useful plants are facultatively or even obligatorily dependent on vesicular-arbuscular mycorrhizal fungi (VAMF) (Janos, 1987). Plants which are dependent on mycorrhizae under certain conditions show suboptimal growth and a higher susceptibility to stresses if a mycorrhization is lacking. In plant production systems normally a deficit of mycorrhiza exists: the substrate in greenhouses and in some nurseries is sterilized before use, and in the field the implantation of monocultures and the utilization of large quantities of pestizides deminishes the inoculum potential of VAMF drastically (Feldmann and Lieberei, 1992).

In several tropical regions, where shifting cultivation is still very common, the method of field preparation - normally slashing and burning - was discussed to be a very important impact for the mycorrhizal situation of the later grown useful plants on these stands (Feldmann and Lieberei, 1992).

If the preparation of an agricultural area by burning destroys the propagules of vesicular-arbuscular mycorrhizal fungi (VAMF) in the upper soil layers in the Amazon region was not yet known. Could it be that the burning of fields before installation of a plantation leads to a mycorrhizal situation that implicates an introduction of mycorrhizal fungi into the plant production system? Or could it be that a fallow of several years meliorates the situation at burned sites?

In order to step forward answering this question our group analysed the mycorrhizal situation in plantations directly after burning and after 3 years of fallow and in comparison to these stands in working plantations and natural sites.

## 2. Material and Methods

The natural vegetation of a terra firme stand in the near of Manaus (Km 28, AM 010) was cleared by fire to implant a rubber tree monoculture plantation there. After seven years this plantation was abandoned. The fallow area was located in direct contact with primary forst.

Three years after the abandonation the first survey of the mycorrhizal situation was made on the fallow area.

Data were collected about the degree of colonization and the Most Probable Number of VAMF Propagules in the soil.

The degree of colonization is the percentage of colonized roots which a root sample of 100 root pieces of 1cm length shows (Slide method, Giovannetti and Mosse, 1980).

The colonization of the roots was determined after they had been bleached and stained with lactophenol-cotton-blue (Philipps and Hayman, 1970).

The MPN (Most Probable Number, Porter, 1979) of propagules in a soil sample was carried out according to the description by Feldmann and Idczak (1992). Zea mays was used as the host plant. Five repetitions were carried out for each sample. The number of spores were counted after a wet sieving process (Daniels and Skipper, 1984).

Thirty samples of fine roots were taken from the surface and from the mineral soil from each surveyed stand of the rubber tree. Only samples of undoubtedly identified rubber tree roots were taken (tested by rinsing the root system). The root samples were combined and mixed. A sub-sample of 3 x 100 root pieces was taken from this collective sample and tested as described above.

The results were compared with nearby located natural stands of rubber trees in native rain forests.

After three years of fallow an effectivity test was made to compare the effect of not identified mycorrhizal fungus populations of the fallow area, monocultural areas, and natural sites. For this experiment the soil of the different sites was first tested by the MPN method. Than identical numbers of propagules were mixed with sterilized

substrate and the effect on the growth and development of the mycorrhizal plants compared with non-mycorrhizal plants. The effectivity test was made with two plant species, with an annual graminae (Zea mays) and with seedlings of a perennial tree (Hevea brasiliensis, rubber tree).

After eight years of fallow the same area was slashed and burned to prepare it for the installation of a new plantation (see Feldmann et al. elsewhere in this volume). Directly before, immediately after the burning, and six months later soil samples were taken and the survival of the spores tested by bio-essay with Zea mays and Petroselinum crispum, the MTT-method (An and Hendrix, 1987) or by MPN-estimation. The MTT-method is a vital stain with 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyl-2H-Tetrazoliumbromid.

## 3. Results

After eight years of fallow a dense secondary vegetation desenvolved in the formerly monocultural plantation. The secondary vegetation contained 178 plant species (see Feldmann et al, elsewhere in this volume) and only the occurrence of the rubber tree remembered in the former use of the area. Together with the high number of plant species a high number of spores of vesicular-arbuscular mycorrhizal fungi occurred on the area (table 1). While nearly one third of these spores were alive this meant a quiet good inoculum potential in the soil.

The slashing and burning of the secondary vegetation led to a sterilization of the soil (table 1). Still spores were found, but all of them were dead (determined with the MTT-test). A MPN-test with five repetitions showed no colonization at all. Even six months after burning a MPN-test did not show active mycorrhizal fungi.

Table 1: Influence of slashing and burning to the inoculum potential of mycorrhizal fungi in the soil

| | Spores/ 50cm3 soil | living spores | infectivity |
|---|---|---|---|
| before burning | 658±157 | 27% | yes |
| directly after burning | 539±135 | 0 | no |
| six months after burning | 412±112 | / | no |

Soil samples were taken in the upper soil layers (0-10cm depth). The spore number was counted after wet sieving, the percentage of living spores ("alive") was quantified with the MTT-test (An and Hendrix, 1987), the infectivity was tested by bioessay with Zea mays or Petroselinum crispum. (/) means "not determined".

The presented data show a dramatic impact of the burning to the VAM fungal association in the soils of implanted plantations. This impact sustains over more than six months, possibly more. Finally a regeneration of burned sites occurs: an investigation of 16 monocultural rubber tree plantations demonstrates, hat in every area one can find mycorrhizal spores. This was true in plantations of different age, in very young, 3 years old plantations, too. For example in rubber tree clone gardens (3 years old) there had been counted several living spores which were infective to testing plants. But even after 12 years the mycorrhizal situation in monocultural areas does not reach the conditions of natural sites. Only if monocultures were abandoned and lay fallow the number of spores, the root colonization and the MPN regenerated nearly to "natural" conditions. In table 2 results are shown of the analysis of undefined mixed populations of VAM fungi in still working monocultural plantations, in fallow lying plantations and - as a test standard - in near natural sites of the tropical rain forest. All sites - plantations and natural locations - were stands of the rubber tree. The spore numbers were counted and the Most Probable Number of

propagules in the soil was measured in each sample. Aditional to these measures root samples of the rubber tree from each stand were taken.

Table 2: Mycorrhizal situation of rubber tree stands

| | Monocultural plantations n = 16 | fallow areas n = 5 | natural sites n = 11 |
|---|---|---|---|
| Root colonization[%] | 30,4 ± 6,3 | 63,4 ± 9,5 | 72,2 ±10,8 |
| Spore number/ $cm^3$ [n] | 4,3 ± 2,1 | 15,6 ± 2,3 | 14,4 ± 5,9 |
| Most Probable Number [n] | 3,5 ± 1,4 | 12,5 ± 2,1 | 14,1 ± 3,2 |

The 16 monocultures of the rubber tree were of different age (3-12 years), the fallow areas were formerly used as monocultures and remained fallow lieing for three years.

In areas which were used as monocultures of the rubber tree the value for spore number and root colonization remains very low. Respecting the manyfold positiv effects of mycorrhizal fungi for the plant these circumstances can indicate a severe deficacy of symbiosis (compare Feldmann and Lieberei, 1992). In monocultural areas, which were let to lie fallow the number of infective propagules or spores were found to be as high as at natural stands of the rubber tree already three years after abandonation. But does this quantitative regeneration mean a qualitative melioration of the mycorrhizal situation, too?

To estimate the importance of mycorrhizal fungi for useful plants we selected two of them, the Graminae Zea mays and the Euphorbiacea Hevea brasiliensis for an effectivety test with VAM fungal populations.

Substrate from the testing areas of known infectivity was mixed with sterilized soil to reach an equal MPN in the planting substrate. Every treatment had its own control

with sterilized substrate from the proprior stand. In table 3 the results of the effectivity test are shown.

The surprising result was, that in spite of a quantitative regeneration the populations in monocultures of the rubber tree as well as those from fallow areas were of low effectivity for the two crops in relation to mycorrhizal populations from native rainforest stands. The low effectivity was true especially for the rubber tree.

These data demonstrate that with the indigenous VAMF of rubber tree monocultures probably only suboptimal growth and a higher susceptibility to stresses of the crops can be expected in a field without a management of the symbiotic fungi. The data are urging upon the necessity to introduce management practices which allow the establishment of effective VAM fungi in this plant production system.

Table 3: Effectivity of undefined VAM fungal populations on the growth of Hevea brasiliensis and Zea mays

| | VAMF of monocultural plantations | | VAMF of fallow areas | | VAMF of natural sites | |
|---|---|---|---|---|---|---|
| Testing plants | Zea | Hevea | Zea | Hevea | Zea | Hevea |
| Initial MPN [$n/cm^3$] | 3 | 3 | 3 | 3 | 3 | 3 |
| Root colonization [%] | 81 | 68 | 75 | 68 | 78 | 67 |
| VAM growth response | 1,22 | 1,03 | 1,35 | 1,05 | 1,72 | 1,41 |

The growth response is calculated by dividing the value for dry weight of mycorrhizal plants by the value of non-mycorrhizal plants. That means a positiv growth response due to mycorrhizal fungi if the quotient is above 1 and a growth depression if it is below 1. The plants were harvested three month (Zea) respectively six month (Hevea) after inoculation. n=50 (Zea) respectively 30 (Hevea) plants per treatment.

The most obvious method to meliorate the VAM fungal situation is to inoculate the crops with selected VAM fungi. Normally the VAM fungi are introduced into production systems whithout indigenous mycorrhizal fungi, e.g. into sterilized substrate. A more problematical approach it is, to add multiplied VAM fungi to non-sterilized soil. Our results (table 4) show that selected introduced mycorrhizal fungi are able to compete successfully with indigenous VAM fungi even under nursery (rubber tree) or field conditions (corn) in Amazonia.

While very few propagules of indigenous fungi (from fallow stands) can lead to a colonization of the root system of the two testing plant species even a better mycorrhization occurs when VAM fungi are introduced. Together with a better mycorrhization a better growth response in the mycorrhizal plants exists due to the changed degree of colonization or to qualitatively changed specific interactions between the symbionts.

Table 4: Effect of introduced VAM fungi competing with indigenous VAM fungi on the growth of rubber tree and corn

| | Indigenous VAM fungi | | Introduced Gl. etunicatum | | Introduced Gl. manihotis | |
|---|---|---|---|---|---|---|
| Testing plants | Zea | Hevea | Zea | Hevea | Zea | Hevea |
| Initial/Inoculated MPN [n/plant] | 4 | 4 | 30 | 30 | 30 | / |
| Root colonization [%] | 63 | 24 | 76 | 45 | 91 | / |
| VAM growth response | 1,00 | 1,00 | 1,61 | 1,39 | 1,71 | / |

The growth response is calculated by dividing the value for dry weight of mycorrhizal plants by the value of non-mycorrhizal plants. That means a positiv growth response due to mycorrhizal fungi if the quotient is above 1 and a growth depression if it is below 1. The plants were harvested three month (Zea) respectively six month (Hevea) after inoculation. n=50 (Zea) respectively 30 (Hevea) plants per treatment.

For the practical use of mycorrhizae in plant production systems there are two main possibilities for the introduction of mycorrhizal fungi: mycorrhizal inoculum can be mixed with the substrate of useful plants in nurseries of perennial plants or inoculum can be brought out together with the seeds of annual plants directly in the field. The latter method gives a third possibility; the annual plants, which are inoculated in this way, can function as "nurse plants", i.e. multiplicate the inoculum in situ in the field and transfer the propagules with their own root system to the roots of already planted perennial plants in the field (see also Feldmann et al., elsewhere in this volume).

An utilization of VAM fungi will only be accepted by producers, if the effect is decisive, long lasting, and profitable. Because of the deficacy of symbiosis in plant production systems normally the introduction of VAMF leads to economically interesting changes. But how long does the effect occur? This will be shown here in an example of the plant production system of the rubber tree (compare Lieberei et al., 1989).

Since nearly 100 years the rubber tree plantations in Amazonia are destroyed by a severe foliar disease, the Rubber Tree Leaf Blight, caused by the Ascomycete Microcyclus ulei. The introduction of mycorrhizal fungi into the plant production system of rubber tree plants (nursery use) can lead to a biological control of the foliar disease by the root symbionts (Feldmann et al., 1989). Mycorrhizal plants which are infected with the leaf pathogen show smaller lesions with drastically reduced sporulation of the pathogen. This effect was measured at four times after inoculation with the mycorrhizal fungus Glomus etunicatum. Everytime the same plants of three different rubber tree clones were used for the test. All plants which were inoculated with Gl. etunicatum were controlled to be mycorrhizal during the whole test. The results are demonstrated in table 5.

The interrelationship between the rubber tree, the leaf pathogen and the root symbionts is very clone specific. In the highly susceptible clone RRIM 600 the dramatic increase of the leaf resistance lasts more than 16 months. This effect decides on the survival of the plants with higher resistance. The more resistent clone Fx 4098

showed a resistance reaction which was strongly influenced by mycorrhization, too. But the effect disappeared 12 months after inoculation with mycorrhizal fungi.
The clone specifity of the mycorrhizal effect can also lead to the contrary effect in the early colonization phase: on leaves of plants of the clone Fx 3925, the most resistant of the tested three clones, primarily a slightly higher susceptibility to Microcyclus ulei occurred, but finally turned to a long lasting slight increase of resistance.

Table 5: Stability of the resistance enhancement in mycorrhizal rubber tree plants: reduction of the pathogen's sporulation (difference to control plants [%])

| | 4 months after VAM-inoculation | 8 months after VAM-inoculation | 12 months after VAM-inoculation | 16 months after VAM-inoculation |
|---|---|---|---|---|
| clone FX 3925 | -20 | 18 | 22 | 27 |
| root colonization | 27 | 27 | 30 | 32 |
| clone FX 4098 | 76 | 60 | 76 | 0 |
| root colonization | 25 | 42 | 37 | 30 |
| clone RRIM 600 | 78 | 80 | 55 | 75 |
| root colonization | 41 | 47 | 39 | 41 |

The example of the rubber tree demonstrates that the introduction of mycorrhizal fungi into the plant production system is very senseful and gives long lasting effects which can be of economical interest. This is true especially if the costs of the inoculum production are compared with the benefits of the VAM fungi. A detailed study on this subject will be publicated soon.

## 4. Discussion

In Amazonas the natural vegetation is normally cleared by fire to make place for plantations. The fire kills most of the host plants of mycorrhizal fungi in the burned areas and destroys the root layer growing on the soil surface. Therefore, a direct effect on the fungal populations is caused (Dhillion et al., 1987). Only after a rather long period of time, the burned areas re-attain infection potentials which they had before the fire. Wicklow-Howard (1989) noticed a period of 3-5 years necessary to re-establish the original infection rate caused by mycorrhizal fungi to host plants tested from the burned stands in Idaho (USA). The results presented in this research show that the infectivity of the soils due to mycorrhizal fungi reaches after 3 years of fallow the same value which natural stands of rubber trees have.

The re-colonization of destroyed soil layers by mycorrhizal fungi may be effected by inoculum spread caused by wind, water, and animals (Rabatin et al., 1987). Which species of mycorrhizal fungi colonize depends on the environmental conditions as well as on the plant species which are planted into the soil (Bevege and Bowen, 1975). In addition to negative influences of the fire clearance and the monoculture of a useful plant, several management practices utilized in the rubber tree cultivation affected the mycorrhizal potential of plantation soils (Feldmann and Lieberei, 1992).

If the plantation is a monoculture, the mycorrhizal community will generally be species-poor and the mycorrhizal populations are not much differentiated (Toro and Herrera, 1987). The diversification of host plants in a monoculture by means of growing some soil-covering plants improves the situation of mycorrhizae in plantations, however, this management practice alone is not sufficient to create natural growing conditions for rubber trees as shown in the results of our research on the occurrence of different soil-covering plants in rubber trees.

The comparison between the occurrence of mycorrhiza in rubber tree plantations and in their natural stands as well as the study of the effects which different management practices have on mycorrhizal colonization show a deficiency of mycorrhizal symbiosis in plantations. Taking the potential influences of mycorrhiza into

consideration, this deficiency of symbiosis may probably lead to malnutrition, insufficient growth, and high susceptibility of rubber trees in plantations. A comparison between the mycorrhizal characteristics in rubber trees cultivated in plantations and those growing in their natural stands as well as their probable influences confirms this hypothesis. The data demonstrated here indicate that a management of the VAM fungal populations in the production systems of Amazonia is needed to optimize the possibility to include the benefits of the symbiosis between the crop and the mycorrhizal fungi.

While the re-establishment of growth stimulating mycorrhizal fungi takes place too slowly it seems to be necessary to inoculate the degenerated areas with selected, effective mycorrhizal fungi.

The use of mycorrhizal fungi in nurseries is unproblematic as shown in the cultivation of rubber trees. The inoculum of mycorrhiza can be mixed with the substrate at the time the rubber trees are planted or re-planted (Feldmann et al., elsewhere in this volume).

The introduction of mycorrhizae into old plantations, where the existence of mycorrhizae is insufficient in number or lacking, is, however, quite different. Inoculations can be carried out there - after introducing the management practices favourable to mycorrhiza - by means of the so-called "nurse plant", e.g. annual, useful plants which assist in an in situ-multiplication of mycorrhizal inoculum.

In all monocultures probably a change of the management practices and their adjustments is necessary to make it more favourable for the mycorrhizal fungi. The mycorrhiza must be included into plant protection concepts. For the rubber tree (see also Lieberei et al., 1989) that means that practices such as reducing the fungicides as well as lessening fertilization or dispensing of herbicides to destroy soil-covering plants near the trees has to be carried out. The most essential change may be the introduction of an intensive polyculture system in order to diversify the host plants for the mycorrhizal fungi. Several tropical useful plants, shown as suitable host plants for mycorrhizae, could be planted in such polycultures, e.g. Coffea arabica and

Citrus sinensis (Caldeira et al., 1983), mango (Cortes et al., 1984), Manihot esculenta (Powell, 1984), banana (Umesh et al, 1988), Bactrys gassipaes, Theobroma grandiflorum, Theobroma cacao, Carica papaya, Passiflora edulis, Bertholletia excelsa, Bixa orellana, Schizolobium amazonicum, Swietenia macrophylla, Cocos nucifera, Zea mays, Vigna uniculata and others (Feldmann et al., elsewhere in this volume).

With the cultivation of numerous different useful plants in a former monocultural plantation, the most important condition is provided keeping the survival of diverse and effective mycorrhiza populations. The adaption of management practices favourable to the root symbionts means a first step to the ecologically balanced, stabilized culture system of perennial useful plants. It is expected that this system would lead to an improvement of the environment and a more productive agriculture on nutrient deficient or otherwise problematical soils.

## 5. Acknowledgements

A part of the project was included in the bilateral SHIFT-program between Germany and Brasil. We thank the the Brasilian EMBRAPA and the German BMFT for their support.

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# SISTEMAS DE CULTIVOS NA AMAZONIA DEPENDEM DO MANEJO DOS FUNGOS ENDOMICORRIZICOS


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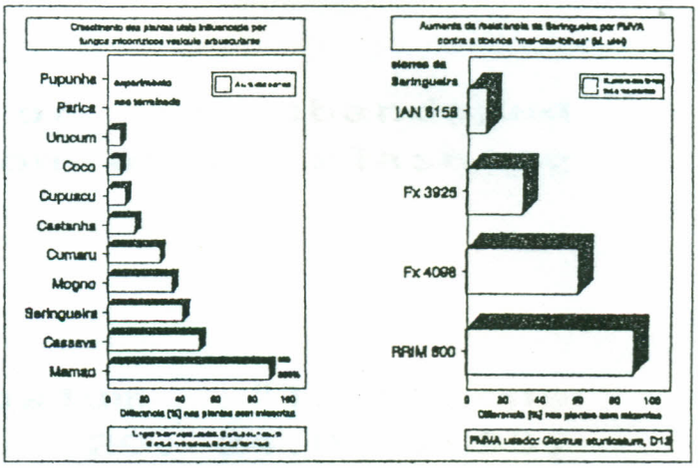
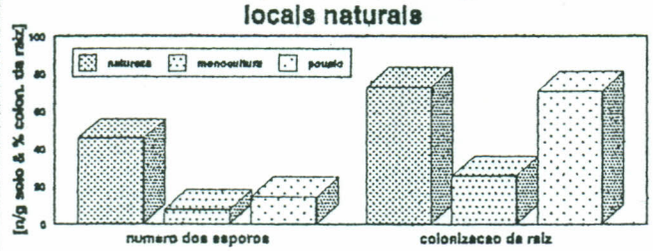
## RESUMO

**Muitas plantas uteis tropicas dependem da presencia dos fungos endomicorrizicos para o melior crescimento e saude delas. Na casa de vegetacao observamos uma aumenta da biomassa ate 200% por causa da micorizacao por exemplo no mamaoeiro. Estudos ecologicos mostraram uma deficiencia dos fungos micorrizicos em monoculturas. Correlado com isso as plantas mostraram deficiencias da nutricao e muitas doencas. Na base destes resultados concluimos que sera necessario manejar as populacoes dos fungos micorrizicos, que significa uma aplicacao dos fungos e uma introducao de um manejo que respeita a necessidades dos simbiontes.**

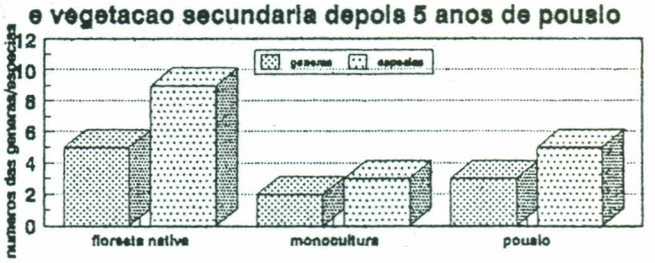




### No caso da Seringueira existe uma deficiencia da micorrizacao nos plantios em comparacao com locais naturais



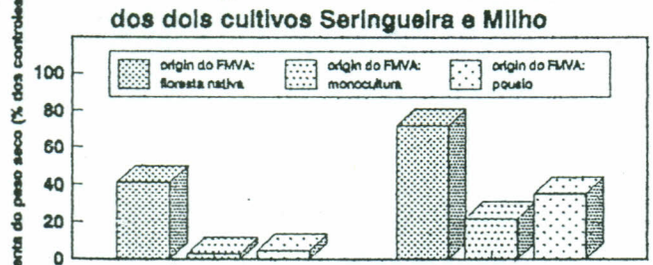

**Numa fase de pousio (5 anos) a situacao da micorriza pareceou melhorar: a colonizacao dos raizes aumentou-se**

### Diversidade das populacoes dos FMVA em tres tipos de vegetacao: floresta nativa, monocultura e vegetacao secundaria depois 5 anos de pousio




**Resultados preliminarios sugerem: o numero das especias dos FMVA aumenta com o numero dos hospites**

### Efeito dos fungos micorrizicos no crescimento dos dois cultivos Seringueira e Milho

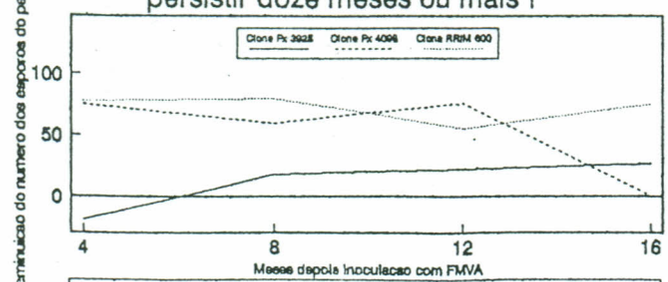



**Alem 5 anos do pousio a efectividade dos FMVA dos plantios fica muito baixa**

### Efeito dos FMVA introduzidos em competicao com fungos micorrizicos indigenos


**Fungos micorrizicos introduzidos competem com succeso com FMVA indigenos e sao mais efectivos**

### No practico o efeito da micorrizacao pode persistir doze meses ou mais !




A intensidade do efeito depende do clone !

## CONCLUSAO:

- A importancia dos fungos micorrizicos para o crescimento e a saude dos hospites cria a necessidade da presentia dos fungos numa quantidade e qualidade sufficiente num sistema agricultorio.
- Porquanto uma deficiencia alta dos simbiontes existe em areas agricultorias (tambem em areas abandonadas com vegetacao secundaria), fungos efectivos tem que ser introduzidos quando uma producao dos cultivos e planejado.
- Fungos micorrizicos podem competir com fungos indigenos com bom succeso, mas o produtor que usa FMVA tem que selecionar fungos efectivos.
- O efeito tem uma duracao que seria economicamente lucrativo.

Idczak, E., Feldmann, F. (1992):

# Mycorrhizal status of an abandoned rubber tree plantation after slashing and burning

International Symposium "Tropische Nutzpflanzen", 20.-24.09.1993, Hamburg, FRG; Tagungsband S. 88