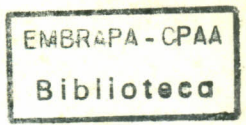


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MANAGEMENT AND REHABILITATION OF DEGRADED LANDS AND SECONDARY FORESTS IN AMAZONIA:

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AGRICULTURAL SYSTEMS IN AMAZONIA DEPEND ON THE MANAGEMENT OF MYCORRHIZAL FUNGI

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SUMMARY

A large number of useful tropical plant species are dependent on mycorrhizal fungi. Without the fungal symbionts they show growth depression and lower tolerance to biotic and abiotic stresses. In plant production systems, common management practices normally result in symbiosis deficits. In tropical regions where the system of shifting cultivation is practiced, the fallow period was thought to have a positive regulating effect on the mycorrhizal situation. In this paper, it is shown that between three and 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 need to manage the mycorrhizal fungi *in situ* in the field is discussed.

INTRODUCTION

Most useful tropical 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 mycorrhizae are lacking. In plant production systems normally a deficit of mycorrhizae exists: the substrate in greenhouses and in some nurseries is sterilized before use, and in the field the planting of monocultures and the utilization of large quantities of pesticides diminishes the inoculum potential of VAMF drastically (Feldmann and Lieberei 1992).

What effect does the preparation of an agricultural area by burning have on the propagules of vesicular-arbuscular mycorrhizal fungi (VAMF) in the upper soil layers in the Amazon region? Could it be that the burning of fields before planting leads to a mycorrhizal situation that precludes an introduction of mycorrhizal fungi into the plant production system? Or could it be that a fallow of several years ameliorates the situation at burned sites?

In order to begin to answer these questions our group analysed the mycorrhizal situation in plantations directly after burning, after 3 years of fallow,

and compared these stands to working plantations and natural sites.

MATERIALS AND METHODS

The natural vegetation of a terra firme stand near Manaus (Km 28, AM 010) was cleared by fire to establish a rubber tree monoculture plantation there. After seven years this plantation was abandoned. The fallow area was located in direct contact with primary forest. Three years after abandonment the first survey of the mycorrhizal situation was made in the fallow area.

The degree of colonization was measured as the percentage of colonized roots using a sample of 100 root pieces of 1cm length (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 (1991). *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 rubber trees. 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 those from 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 gramineae (*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 bioassay 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.

RESULTS

After eight years of fallow dense secondary vegetation developed 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 trees indicated 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 the area (Table 1). Since nearly one third of these spores were alive, this indicated 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 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.

The data presented show the dramatic impact of burning on the VAM fungal associations in the soils of plantations. This impact is sustained for at least six months. Eventually a regeneration of burned sites occurs: in a study of 16 monocultural rubber tree plantations, of different age, in very young, 3 year old plantations, too. For example in rubber tree clone gardens (3 year 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 could the number of spores, the root colonization and the MPN return

TABLE 1. Influence of slashing and burning to the inoculum potential of mycorrhizal fungi in the soil.

	Spores/ 50cm ³ 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 bioassay with *Zea mays* or *Petroselinum crispum*. (/) means "not determined".

to nearly "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 nearly all rain forest sites. All sites - plantations and natural locations - included 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. Additional to these measures, root samples of the rubber tree from each stand were taken.

In areas which were used as monocultures, spore numbers and root colonization remained very low. Considering the many positive effects of mycorrhizal fungi for the plant these circumstances can indicate a severe deficiency 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 abandonment. But does this quantitative regeneration mean a qualitative amelioration 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 Euphorbiaceae *Hevea brasiliensis* for an effective 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 same 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 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.

The most obvious method to ameliorate the VAM fungal situation is to inoculate the crops with selected VAM fungi. Normally the VAM fungi are introduced into production systems without indigenous mycorrhizal fungi, e.g. into sterilized substrate. A more problematical approach 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

TABLE 2. Mycorrhizal situation of rubber tree stands.

	Monocultural plantations n = 16	fallow areas n = 5	natural n = 11
Root coloni- zation [%]	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 for three years.

TABLE 3. Effectively of undefined VAM fungal populations on the growth of *Hevea brasiliensis* and *Zea mays*.

Testing plants	VAMF of monocultural plantations		VAMF of fallow areas		VAMF of natural sites	
	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 positive 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.

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

Testing plants	Indigenous VAM fungi		Introduced <i>G1. etunicatum</i>		Introduced <i>G1. manihotis</i>	
	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 positive 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.

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.

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.* multiply 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).

The utilization of VAM fungi will only be accepted by producers if the effect is decisive, long lasting, and profitable. Because of the deficiency 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 by example of the plant production system of the rubber tree (compare Lieberei *et al.* 1989).

For nearly 100 years the rubber tree plantations in Amazonia have been 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 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 *G. etunicatum* were controlled to be mycorrhizal during the whole test. The results are presented 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 mM 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 resistant clone Fx 4098 showed a resistance reaction which was strongly influenced by

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 published soon.

mycorrhization, too. But the effect disappeared 12 months after inoculation with mycorrhizal fungi.

The clone specificity 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.

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 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 not problematic 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 replanted (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 problem soils.

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