

8. Encouraging and using natural symbionts

8.1 General remarks

If the agricultural sector aims (or is forced) to use non-renewable resources sparingly, it must try to make the best possible use of those resources that nature has provided.

In addition to chemical and physical factors, such as light, of which optimal use should be made, biological factors are of vital importance. Although scientists have recognized the potential of genetic manipulation, other biological factors have received little attention until recently. When optimizing plant or production systems, priority is usually given to inputs brought in from the outside (fertilizer, machines, pesticides). These priorities do not take into account the needs of smallholder farmers with limited cash available to purchase inputs. They often lead to a weakening rather than a promotion of natural symbioses.

The promotion of an ecologically sound approach to agriculture would reduce the need for smallholders to purchase external inputs. Such an approach would focus primarily on mobilizing the resources available within the system. External inputs would then play only a supplementary role.

The goal of reduced dependence on external inputs can be achieved by increasing the use of natural symbioses. This also implies avoiding measures that may negatively affect biological relationships.

The following section examines more closely the character and potential importance of natural symbioses, and possible ways of influencing them.

8.2 The vesicular-arbuscular mycorrhiza (VA mycorrhiza)

8.2.1 Characteristics and importance

Mycorrhizae are symbiotic associations between the roots of higher plants and soil fungi. The most commonly occurring forms are the vesicular-arbuscular mycorrhizae (VAM). The fungi species belong to the family of Endogonaceae (Endogonales). They are distributed worldwide. For example, the species *Alomus fasciculatum* and *Glomus mosseae* have been identified in all parts of the world (RHODES 1980).

These are obligatory symbionts, meaning that they require the presence of host plants in order to reproduce. They can, however, survive for several years in the soil in the form of resting spores.

In nature plants are usually infected with more than one species of mycorrhizal fungus. This means that fungi do not specialize in the way that certain rhizobia (nodule bacteria) do, being able to join in symbiosis only with a specific legume. A mycorrhizal fungus does not need a specific host plant. Nor do host plants require a particular mycorrhizal species, being able to enter symbiosis with any species of fungus.¹³⁰

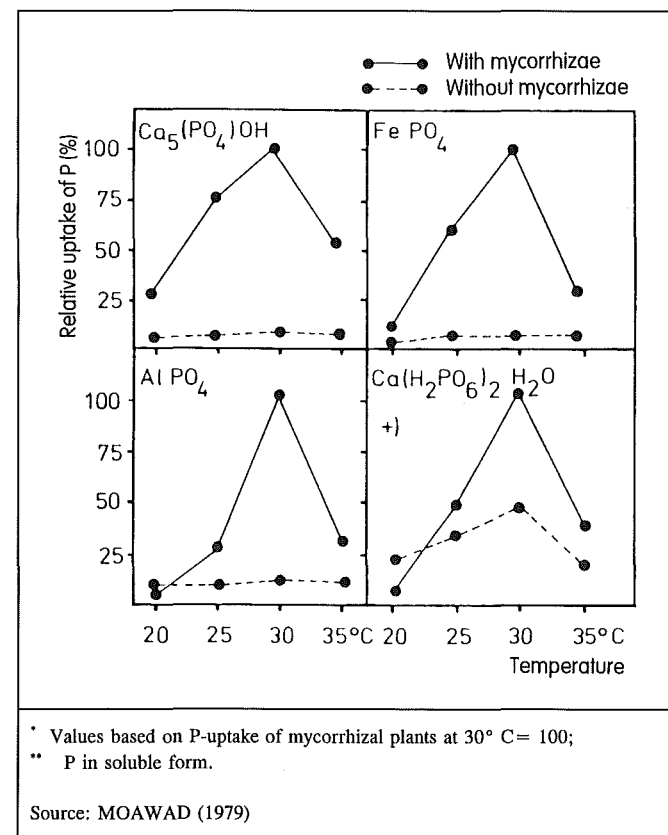
The symbiosis develops in the following way: first the mycorrhizal fungus infects the cortical layer of the roots. Fungal hyphae (filaments) then begin to grow out of the fine roots of the host plant and penetrate through the soil. This vastly improves the ability of the host plant to assimilate nutrients, especially less mobile nutrients such as phosphorus (Figure 8.1). By enlarging the absorption area of its host's roots the fungus supplies the plant with mineral nutrients, while the plant in turn supplies the fungus with the products of photosynthesis (energy).

The extent to which plants depend on mycorrhizae varies from species to species. There are groups of plants that have virtually no mycorrhizae. According to SIEVERDING and JOHN (1982), these include most, though not all, species

¹³⁰ Certain preferences have nevertheless been observed in trials (GRAW 1978; GTZ 1984).

belonging to the families Chenopodiaceae, Cruziferae and Cyperaceae. JANOS (1980) also includes the Commelinaceae, Juncaceae, Amaranthaceae, Caryophyllaceae, Fumariaceae, Phytolaccaceae, Nyctaginaceae, Polygonaceae and Urticaceae.¹³¹

Figure 8.1. P-uptake of *Eupatorium odoratum* with (—) and without (---) mycorrhizae at various soil temperatures and with different forms of P fertilizers under greenhouse conditions.*



¹³¹ It is striking that many indicator plants, which supply a large amount of nutrients (e.g. nettle, goosefoot, hollowroot), are members of these families.

However, the vast majority of plants enter a loose symbiosis with mycorrhizal fungi. The extent to which the roots are infected depends partly on the amount of infectious fungal material in the soil and partly on soil conditions. Here the P content plays an important role. A high content can (but need not necessarily) reduce root infection, thereby influencing the dependence of the plant species on a symbiosis.

A plant's dependence on mycorrhizae is determined in large part by the extent of its root system. Plants with a system of well developed, fine, dense roots, such as many grasses, are dependent on mycorrhizae only in nutrient-poor soils. These are known as optimal mycotrophic plants.

Plants with a weakly developed, coarse root system and few root hairs are dependent on mycorrhizae under all conditions. These are obligatory mycotrophic plants. According to SIEVERDING and JOHN (1982), this applies to cassava, onions and young citrus plants.

The importance of mycorrhizal symbiosis for the uptake of phosphorus by various species of plant has been demonstrated many times in experiments. Recent works on this topic include BAREA et al. (1988), RAJU et al. (1990); ARIAS et al. (1991) and VIVEKANANDAN and FIXEN (1991). SIEVERDING and JOHN (1982) cite results in which the inoculation of the host plant with a well chosen strain of mycorrhiza produced increased yields, even under natural conditions. These yield increases were equivalent to those obtained by fertilizing perennial ryegrass with 30 kg P/ha, maize with 56 kg P/ha, cassava with 160 kg P/ha, soybean with 176 kg P/ha, and bitter orange with 556 kg of P/ha. Table 8.1 lists tropical and subtropical crops known to benefit from mycorrhizal associations.

Table 8.1. Some important cultivated plants of the tropics and subtropics on which mycorrhizal infection has been shown to have a positive effect

Common name	Latin name	Family
a) Annual crop plants		
Cotton	<i>Gossypium hirsutum</i>	Malvaceae
Bean	<i>Phaseolus vulgaris</i>	Leguminosae
Groundnut	<i>Arachis hypogaea</i>	Leguminosae
Winged bean	<i>Psophocarpus tetragonolobus</i>	Leguminosae
Barley	<i>Hordium vulgare</i>	Gramineae
Summer squash	<i>Cucurbita pepo</i>	Cucurbitaceae
Millet	<i>Sorghum bicolor</i>	Gramineae
Potato	<i>Solanum tuberosum</i>	Solanaceae
Cowpea	<i>Vigna unguiculata</i>	Leguminosae
Maize	<i>Zea mays</i>	Gramineae
Pumpkin	<i>Cucurbita moschata</i>	Cucurbitaceae
Cassava	<i>Manihot esculentum</i>	Euphorbiaceae
Paprika	<i>Capsicum annum</i>	Solanaceae
Rice	<i>Oryza sativa</i>	Gramineae
Lettuce	<i>Lactuca sativa</i>	Compositae
Soybean	<i>Glycine max</i>	Leguminosae
Tobacco	<i>Nicotiana tabacum</i>	Solanaceae
Tomato	<i>Lycopersicum esculentum</i>	Solanaceae
Yam bean	<i>Pachyrhizus tuberosus</i>	Leguminosae
Onion	<i>Allium cepa</i>	Alliaceae
b) Grasses		
Brachiaria	<i>Brachiaria</i> sp.	
Bahia grass	<i>Paspalum notatum</i>	
Ryegrass	<i>Lolium perenne</i>	
Zebra grass	<i>Hyparrhenia rufa</i>	
Lemon grass	<i>Cymbopogon citratus</i>	

Table 8.1 Continued. Some important cultivated plants of the tropics and subtropics on which mycorrhizal infection has been shown to have a positive effect

Common name	Latin name	Family
c) Fodder legumes		
Centro	<i>Centrosema pubescens</i>	
Centro	<i>Centrosema macrocarpa</i>	
Subclover	<i>Trifolium subterraneum</i>	
Kudzu	<i>Pueraria phaseoloides</i>	
Lucerne	<i>Medicago sativa</i>	
Red clover	<i>Trifolium pratense</i>	
Stylo	<i>Stylosanthes viscosa</i>	
Stylo	<i>Stylosanthes capitata</i>	
Stylo	<i>Stylosanthes guianensis</i>	
d) Permanent crops		
Bitter orange	<i>Citrus aurantium</i>	Rutaceae
Cashew nut	<i>Anacardium occidentale</i>	Anacardiaceae
Bucare	<i>Erythrina glauca</i>	Leguminosae
Gmelina	<i>Gmelium arborea</i>	Verbenaceae
Guava	<i>Psidium guajava</i>	Myrtaceae
Rubber tree	<i>Hevea brasiliensis</i>	Euphorbiaceae
Inga	<i>Inga edulis</i>	Leguminosae
Coffee	<i>Coffea</i> ssp.	Rubiaceae
Cocoa	<i>Theobroma cacao</i>	Sterculiaceae
Plantain	<i>Musa x paradisiaca</i>	Musaceae
Mango	<i>Mangifera indica</i>	Anacardiaceae
Orange	<i>Citrus sinensis</i>	Rutaceae
Oil palm	<i>Elaeis guinensis</i>	Palmae
Pepper	<i>Piper nigrum</i>	Piperaceae
Castor	<i>Ricinus communis</i>	Euphorbiaceae
Lemon	<i>Citrus limon</i>	Rutaceae
Source: SIEVERDING and JOHN (1982), ST. JOHN (1980)		

Plants appear to take up only P that is dissolved and easily fixed via the mycorrhizal symbiosis (MOSSE 1981). The fungal hyphae thoroughly permeate the soil, even growing into the surface litter. This means that the danger of soluble or already dissolved P (e.g. from Fe-Al oxides) becoming fixed again is reduced considerably

because the route taken by the nutrients up through the plant is shortened and the circuit tightly closed. From an ecological standpoint, mycorrhizae are therefore an important component in the cycling of nutrients (especially in humid ecosystems). Mycorrhizae can also cause plants to reduce the P content of soil, as insoluble forms are slowly but steadily mobilized. This may amount to a significant quantity, at least with regard to perennial plants (MOSSE 1981, MAJUNATH et al. 1989).

In addition to improving the absorption of P, the capacity of plants to assimilate sulphur, zinc and copper is also improved (MAJUNATH et al. 1989), whereas the uptake of calcium is not affected (VAN DER ZAAG et al. 1979).

In legumes, mycorrhizae promote N fixation through the improved supply of P (YOUNG et al. 1988). In Malaysia, SHAMSUDDIN and MANAP (cited in GAILLARD 1981) observed a close synergistic effect of mycorrhizae and rhizobia in *Leucaena leucocephala*, especially on poor sites.

Experiments by SIEVERDING (1979) showed that, because they were better supplied with nutrients, plants infected with mycorrhizae had better transpiration coefficients. In other words, they produced more dry matter per unit of water than non-infected plants.

The supply of water to plants is usually improved through mycorrhizal infection. The water in a larger volume of soil becomes available, so the plants are more able to tolerate water stress. This was clearly shown in trials by MENGE et al. (1978), in which avocado seedlings associated with mycorrhizae withstood transplantation to the field better than mycorrhizae-free plants (Table 8.2)(see also BETHLENFALVAY et al. 1987; BETHLENFALVAY et al. 1988; WATERER and COLTMAN 1989).

Table 8.2. Effect of the mycorrhiza fungus *Glomus fasciculatum* on reducing transplantation losses in avocado seedlings

	% injured plants	Wilting index*
Without mycorrhiza	80	2.6
With mycorrhiza, inoculated	20	0.4
* Wilting index: 0 no symptoms; 1-2 necrotic and wilted leaves; 2-3 severe necrosis, wilted; 4 destruction of meristem		
Source: MENGE et al. (1978)		

Sometimes the infection of a plant with VA mycorrhizae increases its resistance to pests. SIEVERDING and JOHN (1982) report, for example, a lower incidence of nematode parasitism in cotton and soybean. However, RHODES (1980) regards a direct endomycorrhizal effect as unlikely and believes that the higher resistance observed is due to the improved nutritional status of the plants (see also DIEDERICHS 1986 and CARON 1989).

Mycorrhizae have a yield-increasing effect on saline soils, and the plant's uptake of sodium is reduced (ROZEMA et al. 1986; PFEIFFER and BLOSS 1987).

MOSSE (1981) points out another significant characteristic of mycorrhizae, namely their importance in building soil aggregates. Mycorrhizae help to improve the structure of soils and hence their ability to resist erosion.

Mycorrhizae are present nearly everywhere, with the result that at most sites inoculation is superfluous. Nevertheless, the effectiveness of symbiosis can be influenced by human beings, chiefly because:

- * Agricultural and forestry activities strongly influence the amount of mycorrhizal fungi present, and their effectiveness. The relationship of different species to each other may also be affected (JANOS 1980; GTZ 1984).

- * The species and strains of mycorrhizal fungi that occur in nature have different characteristics. Some are particularly effective in the assimilation of P, while others are relatively ineffective. It is possible to make practical use of this by inoculating the plants or soils with selections that are better adapted to the specific site.

The fact that these symbionts have no specificity is an advantage with regard to making use of both the points mentioned above.

8.2.2 Influencing the fungus population of the soil

Almost all cultivation measures affect the occurrence and activity of vesicular-arbuscular mycorrhizae. Many of the effects on plant growth achieved through tillage, crop rotation, etc are partially due to changes in mycorrhizal symbiosis.

Theoretically, **cultivation techniques** can be used to promote mycorrhizal fungi, but SIEVERDING and SAIF (1984) concede that the options for doing so are limited in practice.

As mentioned above, high levels of soil phosphorus can impair mycorrhizal activity. In general, high doses of **phosphate or nitrogen fertilizer** can render plants immune to mycorrhizal infection (ROSS 1971, MOSSE 1981). Applying more than 50 kg/ha of P fertilizer to mycotrophic plants will often produce increased yields only if the species of fungus in the soil is adapted to such high levels of P (SIEVERDING and JOHN 1982).

Although mycorrhizal activity is reduced by these high doses, the crop plants' uptake of P is usually not less in the end, because the decreased capacity to assimilate P is compensated by the higher level of P in the soil solution (as long as this is present).

On the other hand, plants can develop deficiencies of other, less mobile nutrients such as zinc or copper, because the roots, being only slightly infected, are unable to absorb more than small amounts. This is an indirect P-induced micronutrient deficiency

(RHODES 1980). Small amounts of P fertilizer usually have a stimulating effect on mycorrhizae. In particular, limited P fertilization with rock phosphate has a beneficial effect on symbiosis (ROSS 1971; GTZ 1984).

Clearing and burning land for crops generally results in a severe reduction of the natural mycorrhizae population. The "pioneer plants" that subsequently colonize such sites are usually not mycotrophic, at least where there are relatively high nutrient levels. If crops that do well without mycorrhizae are then planted, the stock of mycorrhizal fungi in the soil will fall sharply, as REEVES et al. (cited in RHODES 1980) showed with *Chenopodiaceae* and *Cruziferae*.

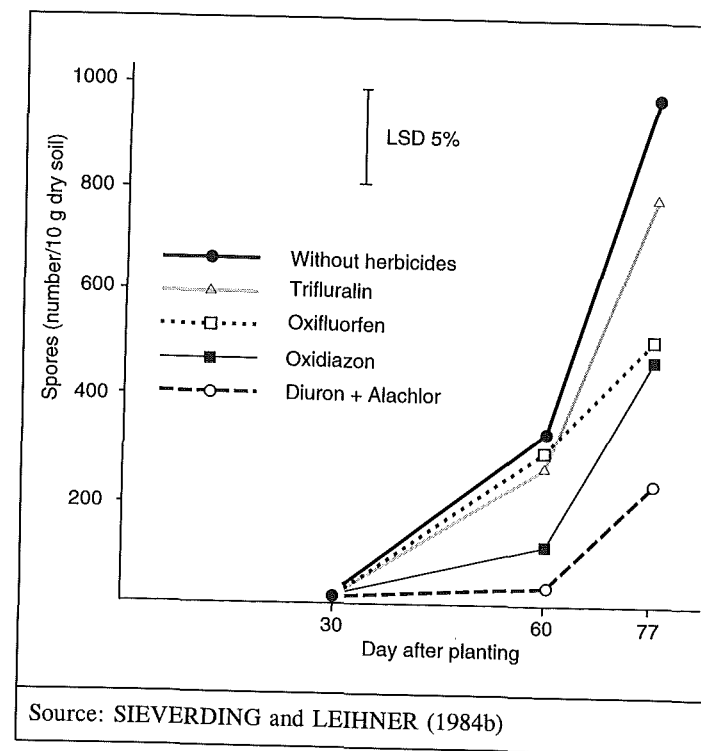
Crop rotation also has a strong impact on mycorrhizae. In field trials carried out at CIAT in Colombia, the mycorrhizal activity of cassava planted in rotation with legumes (*Vigna unguiculata*, *Vigna radiata* and groundnut) was markedly higher than in cassava planted in monoculture. The stimulating influence of the legumes, found with mixed cropping as well as in rotations, was pronounced on sites having a low original fungus population (SIEVERDING and LEIHNER 1984a).

KRUCKELMANN (cited in RHODES 1980) found that, after 16 years of a rotation involving oats and a non-mycorrhizal plant (turnip), the natural mycorrhizal population in the crop soil was far lower than in soil under mycorrhizal host plants.

In theory it is not difficult to grow crops that help maintain a high mycorrhizal population and activity, but in practice socio-economic pressures often dictate the growing of non-mycotrophic plants as cash crops. The solution is to integrate the production of mycotrophic and non-mycotrophic crops through mixed cropping, relay cropping or alley cropping, for example.

Weeds play an important but frequently neglected role in helping to maintain or stimulate mycorrhizae. Symbiosis can be fostered by tolerating a few "noble" weeds, or by practising only mechanical or crop-specific weed control.

Figure 8.2. Effect of herbicides on the spore production of *Glomus manihotis* in cassava



Herbicides can have a negative influence on mycorrhizae. They decimate the number of host plants, and often cause direct injury to fungi, inhibiting root infection and spore production (see Figure 8.2).

Other **pesticides** and especially fungicides often have a devastating effect on mycorrhizae.¹³² RHODES (1980) singles out methylbromide, benomyl, pentachloronitrobenzol (PCNB) and chlorthalonyl for special mention. GRAHAM et al. (1980) were able to substantiate the destructive effect of PCNB.

¹³² In exceptional cases a "positive" impact may result from eliminating antagonists and selectively suppressing less efficient strains of mycorrhizae.

Soil cultivation, i.e. creating or maintaining a loose, well aerated soil structure with good water-holding capacity, has a favorable effect on mycorrhizae. According to JANOS (1980), however, the soil should not be turned to too great a depth as this buries the active topsoil layer and possibly reduces its activity.

Stabilizing the soil temperature at 25-30°C, either with mulch or through some other form of management, also stimulates mycorrhizal activity. Thus CHALKER (1966) attributes the success of mulching on avocado plantations partly to the favorable effect this has on the mycorrhizae. Temperatures under 20-25°C and over 30°C (see Figure 8.1) inhibit mycorrhizae (SIEVERDING 1979, MOAWAD 1979).

Mycorrhizae that naturally occur on a site are closely related to its climate and soil properties. This is why drastic, short-term changes in the soil environment, such as heavy liming, adversely affect mycorrhizal activity. The adaptation of fungi to the new conditions can be extremely slow, especially if artificial inoculation with suitable species is not carried out (MOSSE 1981).

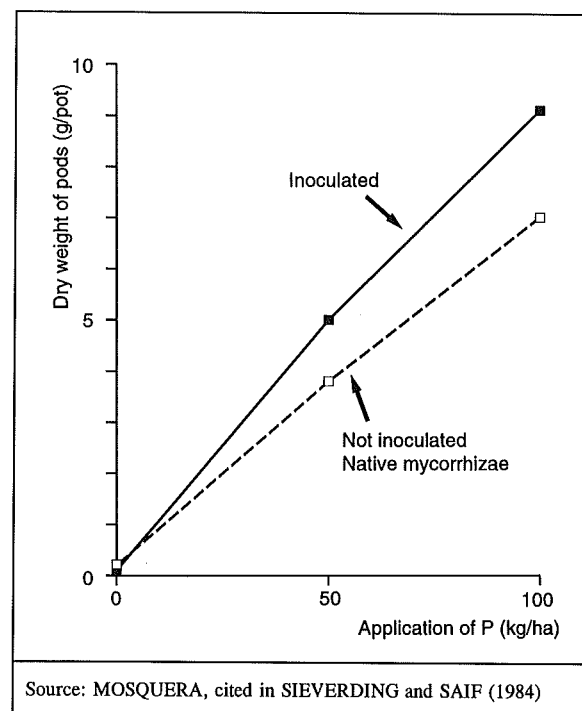
Crops vary genetically in their capacity to become infected with mycorrhizae, and many facultative mycotrophic plants enter a symbiosis only at relatively low P levels. It is highly likely that modern cultivars selected for high nutrient levels are less able to enter symbioses with local strains than are traditional varieties or hybrids: the latter are better adapted to low P levels and to utilizing mycorrhizae, and they have a higher affinity to mycorrhizae (JANOS 1980).

Another way of managing natural mycorrhizae is to inoculate crop plants with mycorrhizae-rich earth. According to YANTASATH (1975), inoculating plants with earth from the root area of known mycotrophic plants (such as *Tagetes*) can prove very effective. When such earth (together with spores and infected pieces of roots) is placed in the planting holes, nursery pots or seedbeds of other mycotrophic plants, these often develop better and more robustly, especially if previously sterilized potting earth is added. The inoculation material should, however, always be from a different plant family, so that injurious agents are not carried over at the same time.

8.2.3 Inoculation with selected, efficient strains of mycorrhizae

As mentioned above, inoculation is unnecessary in many locations. However, experiments in recent years have shown that better yields can often be achieved by inoculating soils with species and strains adapted to the site (GTZ 1984)(see Figure 8.3).

Figure 8.3. Effect of inoculation with *Glomus manihotis* and of different applications of P on the yield of beans grown in a greenhouse on non-sterilized soil



In experiments by JACKSON et al. (1972), maize inoculated with an efficient strain of mycorrhiza yielded 50% more than infected plants with native mycorrhizae.

In field trials carried out by CIAT on Inceptisols and Oxisols, each of which was fertilized with 50 kg/ha of rock phosphate, cassava yields were on average 29% higher with inoculation, increasing from 15.6 t/ha to 21.1 t/ha. After fertilization with superphosphate, the increased yield was 26%. Yield increases of 65% in fodder legumes fertilized with 20 kg/ha of rock phosphate were achieved following inoculation with efficient strains under natural field conditions (SIEVERDING and SAIF 1984).

Trials by CIAT have also revealed that small applications of phosphate improve the efficiency of field inoculation, and that the requirement for phosphate fertilizer is considerably lower with mycorrhizal inoculation than without. On acid tropical sites the use of industrially produced, soluble P fertilizers could be replaced in almost all cases with a combination of rock phosphate and mycorrhizae (see Table 8.3).

A prerequisite for successful inoculation is the selection of strains suited to local conditions that are able to assert themselves against the naturally occurring species. RHODES (1980) affirms that success also depends more on the soil and on climatic factors than on the presence of a particular host.

Table 8.3. Effect of field inoculation (inoc.) and fertilization with soluble triple-superphosphate (TSP) or with Huila-rock phosphate (HRP) on the yield of cassava tubers grown on three different crop soils in Colombia

Treatment	Yield of tubers (t/ha)			Mean
	Site a	Site b	Site c	
50 kg/ha TSP	13.1	18.5	7.0	12.9
50 kg/ha TSP and inoculum	18.1	22.9	8.2	16.9
50 kg/ha HRP	12.7	11.3	6.2	10.1
50 kg/ha HRP and inoculum	15.5	20.4	9.5	15.1

Source: SIEVERDING and SAIF (1984)

Methods of selecting and using VA mycorrhizae have now been developed to the point at which they can be applied in practice. The procedure for mycorrhizal inoculation is relatively simple and inexpensive. According to MOSSE (1981), not much more is required than a good microscope and a sieve.

In many areas, introducing this technique would make the use of rock phosphate more profitable. It thus represents an important opportunity to become less dependent on expensive soluble phosphates and to improve the supply of P in soils where its lack is often the yield-limiting factor.

Unfortunately, there are still too few institutions which produce and supply the basic inoculation material.¹³³ According to SIEVERDING and SAIF (1984) and SIEVERDING (1991), if such basic inoculation material were available, all the other necessary preparations could be made by farmers. For example, to produce enough inoculation material for 1 ha (about 5 t), a farmer needs 3-6 kg of infected soil as basic inoculation material. This is spread over and then worked into a 25 m² area (20 cm deep) of sterilized soil, which is then sown with a mycorrhizae-increasing plant such as *Pueraria phaseoloides*. The inoculation material thus produced would be ready in 4-6 months (preferably when the plants are mature). Preliminary calculations by CIAT suggested that this procedure would be economically viable for smallholdings in Colombia.¹³⁴

It is conceivable that such operations will be possible in the near future. If so, they should lead to a marked improvement in productivity, especially on nutrient-poor sites with scanty or ineffective natural mycorrhizae populations (Table 8.4). Such sites include many savanna locations, sites with degraded soils in all zones, and sites where cultivation has drastically altered the soil.

¹³³ There is still no method of propagating VA mycorrhizae in an artificial medium.

¹³⁴ The cost of producing 5000 liters of soil inoculum "on farm" was calculated at US\$ 58 (1986 prices) for Colombia (SIEVERDING and SAIF 1984; SIEVERDING 1991).

Table 8.4. Correlation between soil chemical and biological properties and the increase in yields resulting from field inoculation for cassava

Use of field before trial	Soil organic matter (%)	Native mycorrhiza propagulum in soil	P (ppm)	Tuber yields (t/ha)		Yield increase due to inoculum (t/ha)
				No inoculum	Inoculum	
3 years cassava	18.9	1717	1.4	17.7	15.7	-2.0
1 year cassava	8.5	823	2.9	24.7	27.8	+3.1
Pasture	3.2	103	1.2	21.2	27.2	+5.9

* Inoculation with 400 g of inoculation material in the cuttings to be planted; the inoculum consists of soil infected with a mixture of *Glomus manihotis* and *Entrophospora colombiana*

Source: GTZ (1984)

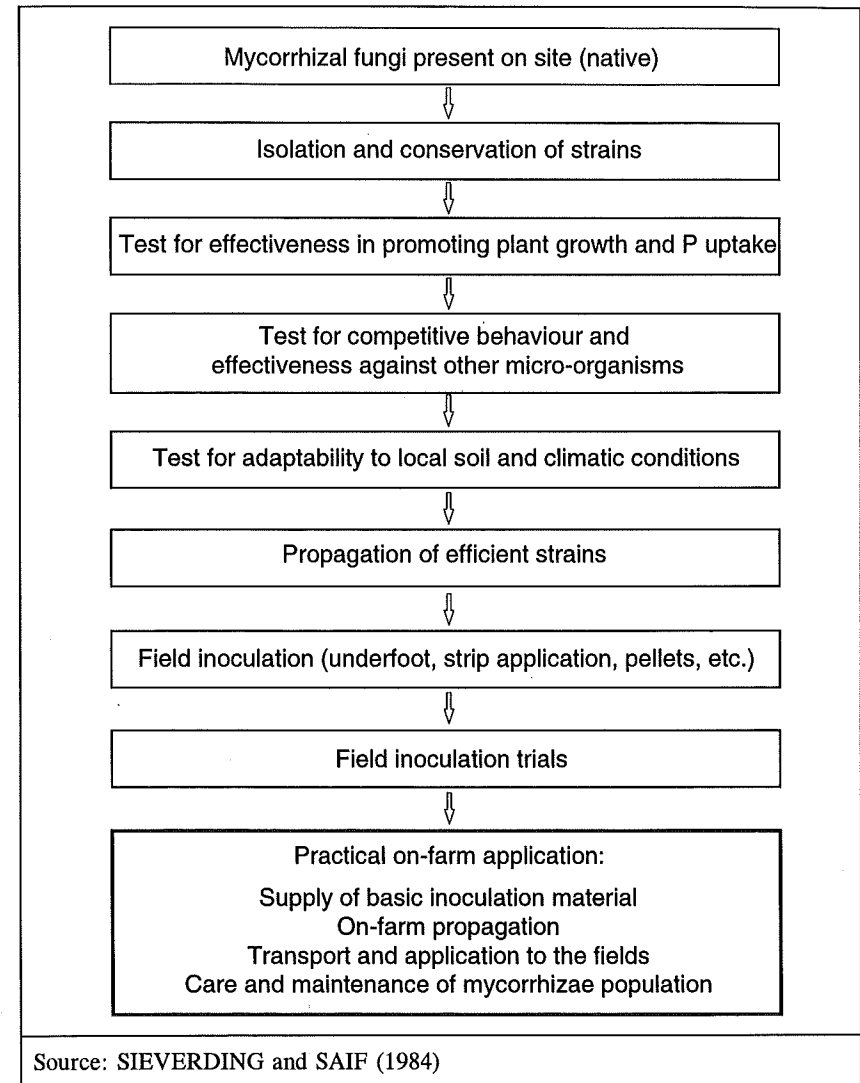
Table 8.5. Effect of inoculating with *Glomus fasciculatum* on the growth of Topa Topa avocado seedlings*

	Growth height (cm)	Dry weight of shoots (g)	Dry weight of roots (g)
Not inoculated with mycorrhiza	5.7	17.2	15.6
Inoculated	20.4	31	34.8

* Measured after 6 months

Source: MENGE et al. (1978)

Figure 8.4. Flow chart showing how efficient mycorrhizae strains are selected for field inoculation



Finally, an important field of application for inoculation with mycorrhizae has opened up in connection with the increased propagation and raising of forest and permanent crops. This is strikingly demonstrated through the results by MENGE et al. (1978) shown in Table 8.5. Figure 8.4 outlines the steps involved in inoculation, from isolating efficient strains through to practical application.

8.3 Biological nitrogen fixation

8.3.1 General remarks

Although the earth's atmosphere contains inexhaustible amounts of nitrogen (around 4×10^5 t, according to OKON 1982), this fundamental nutrient is a yield-limiting factor for most plants. This is because plants cannot utilize molecular nitrogen (N_2) but depend for nutrition on bonded nitrogen (NH_4^+ , NO_3^-).

Conversion to bonded nitrogen has been taking place since primeval times through the activity of nitrogen-fixing bacteria and algae. Aside from organic fertilizers, this was the essential basis of nitrogen nutrition for plants until the invention of the Haber-Bosch process of ammonium production. With the widespread production and use of mineral fertilizers, interest in legumes and their capacity to fix nitrogen diminished. Research and technology all over the world focused primarily on the distribution and application of mineral nitrogenous fertilizers. Wherever nitrogen fertilizers were available the yields of useful plants, especially non-legumes such as annual grain crops, increased sharply. The field area for these crops was greatly extended, while that under legumes shrank.

Subsistence-oriented smallholdings, however, were for the most part unable to afford mineral fertilizers; hence they did not benefit from this "positive" development. Only with the energy crisis of the 1970s and the failure of the Green Revolution in less favored agricultural areas did scientific attention return to legumes and to biological

nitrogen fixation - that is, to a technology that, unlike the chemical production of N, does not use up large amounts of non-renewable energy and raw materials, but instead taps the energy from sunshine to supply the nitrogen required for plant growth "on the spot".

N-fixation. Of the many micro-organisms, only a small number are capable of biologically fixing atmospheric nitrogen. They do this with the help of a special enzyme, the nitrogenase, which functions in roughly the same way in all N-fixing bacteria (RICHTER 1982). Molecular nitrogen is thus changed to NH_3 (NH_4^+) and then metabolized into amino acids and protein compounds in the host plant or within the organism itself.

As in artificial N production, micro-organisms expend considerable energy in this process (12-15 mol ATP and more for 1 mol N_2). The bacteria acquire this energy either through the intake of carbohydrate compounds (products of photosynthesis) or, like the blue-green algae, through their own photosynthetic production (DAY and WITTY 1977; NEVES 1982).

Roughly three kinds of nitrogen-fixing bacteria can be distinguished, based on their mode of living:

- * **Symbiotic group.** These bacteria invade the root tissues of higher plants. They are fairly strictly bound to a host, are protected from microbial antagonists, and are highly efficient (e.g. rhizobia and Frankia).
- * **Associative group.** These are free-living or found in the immediate vicinity of grass roots (rhizosphere). The plant micro-organism relationship is only indirect and the bacteria are subject to competition from other soil organisms (e.g. spirillum).
- * **Free group.** The activity of this group is not necessarily reciprocal with the higher plants. To be present and active they need easily available organic matter in the soil. They are exposed to strong competition from other micro-organisms (e.g. Azotobacter).

A special position is occupied by the chlorophyllose, nitrogen-fixing algae. These supply energy rich, organic compounds and play an especially important role in aquatic agro-ecosystems such as wetland rice.

The N-fixers important for agriculture are those that can obtain sufficient quantities of readily available carbohydrates (sugar or similar). This was illustrated in trials in Egypt (ODU 1977), in which nitrogen fixation by free-living *Azotobacter* bacteria was markedly improved by adding sugar-rich molasses to the soil.

The closest approximation of this under natural conditions is found in the case of free-living nitrifying bacteria, which feed off the energy-rich root exudates available in the rhizosphere of plants. Bacteria that form a symbiotic relationship with a host plant (Rhizobia, Actinomycetae) are directly supplied with energy-rich photosynthetic compounds through the flow of plant sap. Blue-green algae can photosynthesize energy themselves. These three groups are of the most profound importance to biological nitrogen fixation.

Because the nitrogenase enzyme (responsible for fixing nitrogen) is highly sensitive to high concentrations of oxygen, (aerobic) bacteria have had to develop special mechanisms to protect themselves from high oxygen concentrations and to ensure efficient fixation of nitrogen. Here too, nitrifying bacteria that live free in the soil are at a disadvantage, because they must transpire a high proportion of their carbohydrate compounds to protect their nitrogenase system from excessive concentrations of oxygen by forming CO₂. According to NEVES (1982), this can take up 80-90% of their energy. Bacteria like *Azospirillum*, which can invade the inner parts of the rhizosphere or even the roots themselves (DÖBEREINER 1982), or rhizobia that live in special nodules in the plant roots, can almost always function at low oxygen concentrations, whereas this is seldom possible for free-living organisms.

A third factor that considerably influences nitrogen fixation or nitrogenase activity is the concentration of ammonium and nitrate in the soil. If the concentration of ions containing N increases (e.g. through mineral N fertilizers), nitrogen fixation is inhibited and nitrogenase activity reduced (RICHTER 1982). This weakens the competitive power of legumes and other N-fixing plants and may cause them, for

example, to be easily overgrown by weeds on cropland or completely suppressed on pastureland. For more on the problems of legumes on pastureland, see SKERMAN (1977) and WHITNEY (1982).

Of all the natural N-fixation processes, the symbiosis between legumes and rhizobia is by far the most important in terms of quantity and (with the Actinomycetae) also the most energy-efficient. In the following sections, the conditions for optimum N-fixation will be described in more detail, as well as ways of influencing this for agricultural purposes.¹³⁵

8.3.2 Using the legume-rhizobia symbiosis

The rhizobia occurring in nature are highly specialized as to their host plant or group of host plants. This means that a species of rhizobium that enters a symbiosis with soybean cannot do so with clover (thus differing significantly from mycorrhizae). The most important rhizobium groups are:

- * Cowpea rhizobia (*Vigna*, *Cajanus*, *Pueraria*, *Arachis*)
- * *Rhizobium japonicum* (soybean group)
- * *Rhizobium lupinii* (lupin group)
- * *Rhizobium meliloti* (medicago group)
- * *Rhizobium phaseoli* (bean group)
- * *Rhizobium trifolii* (clover group)

These groups can be further subdivided into races and strains specific to plant groups or even to species and varieties. Because of the high degree of specificity between host and rhizobium it is vital, when introducing a new crop to an area, to determine whether it will be able to fix nitrogen or if its specific rhizobium species must be added (inoculation).

¹³⁵ Despite the recent spate of research on tropical legumes, including highly specialized work on topics such as the behavior of rhizobia in nodules, the course of infection, etc, relatively little general information is available on the prospects for promoting this symbiosis in agriculture.

In areas where a particular legume has been grown for a long time, the appropriate rhizobium is almost always present in sufficient quantities. For instance *Rhizobium leguminosarum*, specific to field beans, is found everywhere in Egyptian soil, whereas the newly introduced soybean was able to flourish only after strains of *R. japonicum* suited to the soils and climate of Egypt had been inoculated into the fields.

When the crops being grown are not newly introduced, costly inoculation efforts are justifiable only for certain very specifically symbiotic legumes (e.g. *Stylosanthes guyanensis*, soybean, *Centrosema pubescens* and *Leucaena* (DÖBEREINER 1977)). In these cases the species of rhizobia naturally present in the soil may be well adapted to local conditions but ineffective with regard to nitrogen fixation. They may well be completely suppressing far more effective rhizobia. Inoculating the soil is not worthwhile for less specifically symbiotic legumes, such as cowpea, siratro (*Macroptilium atropurpureum*), kudzu (*Pueraria phaseoloides*) or perennial soybean (*Glycine wightii*), since the resulting yield increases are usually insufficient to justify the effort. This is especially true in tropical regions, where a mixed assortment of rhizobia and legume species occur (more so than in temperate regions).

Much more needs to be known about the promotion of symbiosis in tropical legumes through cultivation methods (BERGERSEN 1977, JOHN 1982). Because experimental data have been isolated and are sometimes conflicting, it is difficult to generalize (ALEXANDER 1977). Nevertheless, we will attempt to provide an overview, since space precludes a detailed discussion of the varied characteristics of specific legumes and rhizobia.

The first and most important determinant of good N fixation by the legume-rhizobia symbiosis is the healthy growth of the host plant. The natural factors that hinder plant growth also hinder symbiosis (e.g. shallow soil, water scarcity, salinity, toxicity, etc).¹³⁶

¹³⁶ The more the nutritional, climatic, soil, temperature and light demands of the crop plant are met, the better the plant will be able to supply the rhizobia with energy and hence itself with the nitrogen for growth (see also Table 4.19 in Chapter 4).

Lack of light or heavy shade, for example, lead to a reduction in nitrogen fixation if the plant is no longer able to photosynthesize enough for its own growth and to supply the nodules with sufficient energy (DART 1977). MINCHIN and PATE (cited in BERGERSEN 1977) found that up to 15% of the products of photosynthesis in peas are stored in the nodules (32%, if the carbon structure for the formation of amino acids is included).

Many examples can be found of legumes receiving too little light and being suppressed, for example on pastures where the grass is tall, or under trees. In crop mixtures such as maize and beans, planting beans too late produces adverse results because N fixation suffers when the beans are shaded too early. This is especially the case when high levels of mineral N fertilizer are used.

When applying fertilizers it is important to meet not only the nutrient demands of the host plant but also the special needs of the rhizobia. It should be noted here that legumes have a relatively high demand for P compared with other crop plants. MUNNS and FRANCO (1982), as well as others, regard P deficiency as the most significant factor affecting growth and N fixation in tropical legumes. GRAHAM (1981) found that in Phaseolus beans an improved supply of P not only encouraged nodule formation but caused it to begin earlier; hence the length of time for binding N was extended. The nitrogen fixation activity of the nodules was thus markedly higher. Studies by EDWARDS (1977) confirm the special importance of the P supply.

There are several ways of improving the supply of P:

- * by applying fertilizers (rock phosphate or a combination of rock phosphate sulfur and *Thiobacillus* (DOMMERGUES 1982, SWABY 1982)).
- * through cultivation practices that promote root extension and development, leading to improved P absorption.
- * by promoting (or inoculating with) mycorrhizae.
- * by choosing appropriate legumes. According to ANDREW and ROBINS (cited in MUNNS and FRANCO 1982) *Stylosanthes humilis* and *Centrosema pubescens* tolerate low P availability considerably better than *Macroptilium atropurpureum* (siratro), *Desmodium* and *Glycine*.

Nitrogen fertilizers (NH_4^+ and NO_3^-) usually have a negative effect on nitrogen fixation. As already mentioned, they inhibit nitrogenase formation and cause enzyme activity to decline appreciably (RICHTER 1982). This eventually leads to disintegration of the root nodules, and biological nitrogen fixation comes to a halt (BERGERSEN 1977). Plants and various rhizobia strains respond with varying sensitivity to N fertilization (DART 1977). GRAHAM (1981) measured a decline of 39% in nitrogen fixation after an application of just 15 kg/ha of N fertilizer. HAMDI and ALAA EL-DIN (1982) also detected a drop in nitrogen fixation by soybeans at only 15 kg N/ha.

On the other hand, light dressings of N (5-15 kg/ha, depending on plant species and nitrogen status of the soil) can have a positive effect, as legumes often suffer from a temporary shortage of N in the juvenile phase, before nodules are formed. A small application of N ("starter dressing") can bridge this deficit phase, promoting the early growth of the legume and thus its overall development.

According to GRAHAM (1981), N deficits of this kind are especially common in the cooler seasons or at higher tropical elevations. They are less likely to occur in the subhumid tropics because here a natural surge in mineralization occurs at the beginning of the rainy season. This can make a starter dressing superfluous.

YOSHIDA (cited in GRAHAM 1981) found that chicken manure, a fertilizer often used by smallholders, had a less negative effect on N fixation than the equivalent amount of urea. Very probably this is also the case with other organic fertilizers.

DOMMERGUES (1982) notes that the N-binding activity of leguminous trees often declines with increasing age. The author suggests that this is due to the increasing concentration of nitrogen around the base of the tree. It is therefore not advisable to leave the leaf litter of leguminous trees where it falls, as the N contribution of the trees is better utilized if the leaves are spread on the field crops.

A good supply of **potassium** usually strengthens the competitive power of legumes, thus also indirectly benefitting symbiosis.

Calcium encourages infection in the roots of legumes (EDWARDS 1977). The optimal Ca content for rhizobial infection is usually substantially higher than the requirement for Ca of the legume itself. The demand for Ca of rhizobia or of the nodules is relatively low once infection has taken place (Table 8.6).

Table 8.6. Effect of calcium concentration in a bacterial culture on nodule formation in subterranean clover (*Trifolium subterraneum*)

	Calcium concentration of bacterial culture (μM)					
	4	9	30	81	246	720
Plants with nodules (%)	0	5	12	27	98	100
Average size of nodules	-	2.5	2.3	2.3	2.2	2.4
Source: LOWTHER and LONERAGAN (1968), cited in EDWARDS (1977)						

Considerable yield increases are often achieved by liming (see Section 2.3.2). With or without adding lime, it is important to enhance and conserve the calcium level in the soil through high biomass turnover in order to counteract acidification. This can be achieved by periodic applications of stable manure, by mulching or by planting green manure/intensive fallows. The latter have deep-rooting plants that bring the leached calcium back up to the upper horizon, where it is accessible to crops (see Chapter 4).

Of the trace elements, the most important are molybdenum (Mo) and sulfur (S). According to BERGERSEN (1977), it is deficiencies in these elements that are most responsible for inadequate N-fixation in the tropics. **Molybdenum** is an important element of the nitrogenase enzyme, which is why demand for it is higher in the root nodules than in the plant as a whole. Because the availability of molybdenum decreases with the pH level, deficiencies are especially likely to occur on acid soils (DÖBEREINER 1977). They may be alleviated by fertilizing with lime.

Molybdenum may be added when leguminous seed is pelletized or inoculated before planting. If this is not possible, or where little or no molybdenum fertilizer is available, the supply of molybdenum can be improved by gathering the seed to be planted from fields that have been fertilized with molybdenum or in which molybdenum availability has been improved (SANCHEZ and SALINAS 1981).

Sulfur is one of the most important nutrients for legumes, since it promotes the quantity and quality of protein. It is also an important component in the N-binding nitrogenase enzyme of nodule bacteria (RICHTER 1982). One important way of conserving sulfur is to avoid burning crop residues. The sulfur contained in the residues becomes gaseous when burned and is lost. Moreover, burning destroys organic matter, which is vital for the storage of sulfur in tropical soils.

Considerable quantities of sulfur can enter the soil via fertilizers containing sulfates. Deficiencies are unlikely to arise on land fertilized in this way. Sulfur deficiency is also unlikely to occur in industrial regions, where large amounts of sulfur reach fields in the rain. Fertilizing with rock phosphate contributes almost no sulfur to the soil.

Another very effective measure for promoting N fixation and the growth of legumes is to alleviate **acidity problems** (low pH, aluminum toxicity, manganese toxicity, and deficiencies in calcium, phosphorus and molybdenum). By adding lime to an acidic, sandy-loamy soil in Senegal, CHARREAU (1975) solved the problem of aluminum toxicity, significantly improving groundnut yields.

Acidity is not only a problem for plant growth. Acid soil conditions also have a negative impact on the survival and activity of rhizobia. According to DANSO (1977) the survival rate of *Rhizobium meliloti* in limed soil was considerably better with than without liming (Table 8.7)

Table 8.7. Effect of liming on the survival of *Rhizobium meliloti* in an acidic loamy-sandy soil

Observation period	<i>R. meliloti</i> (millions per g of dry soil)	
	Without CaCO ₃	With CaCO ₃
Beginning of trial	30	30
1 week	14	30
4 weeks	6.8	34
8 weeks	1.0	14
Source: DANSO (1977)		

According to EDWARDS (1977), nitrogen fixation in acid soils may be limited even when a large stock of rhizobia is present. His explanation is that infection of the roots with rhizobia is often disrupted at pH levels at which the crop plant is still able to flourish. The critical pH value for *Rhizobium meliloti* is around pH 5. In trials by GRAHAM (1981), Phaseolus beans almost always exhibited good nodule formation beginning at pH 5.5. VIDOR et al. (1982) found that liming *Phaseolus* had a positive effect on symbiosis only when the pH value of the soil was less than 5.5. Adding lime brought no further improvement at higher pH levels.

Associated problems such as Al toxicity appear to cause problems in Phaseolus beans even at 3-5 ppm, depending on the variety (GRAHAM 1981). Free Al has a negative effect on both the plants and the rhizobia (EDWARDS 1977). The extent to which nodule formation and symbiotic activity are influenced by lower pH values depends greatly on the species of legume, the particular rhizobia and the soil. For example, NORRIS (1969) observed in the rainforest of Brazil that plants that were still able to form nodules at pH 4.5 on a sandy grey soil showed no signs of symbiosis on a neighboring yellow loam (see also SYLVESTER-BRADLEY et al. 1980).

It is known that cowpea rhizobia and cowpea varieties are relatively resistant to Al toxicity. Selecting acid-tolerant varieties and rhizobia has proved successful with other legumes (e.g. Phaseolus). However, it will certainly be some time before the results

of strategic research on this topic find their way into practice, especially on smallholdings. More promising in the immediate future is the selection and breeding of tolerant host plants (MUNNS and FRANCO 1980). Here it can be assumed that traditional varieties will usually be better adapted to unfavorable local conditions than most modern cultivars (SANCHEZ and SALINAS 1981).

Soil moisture and soil temperature also influence symbiosis. Waterlogging inhibits gas exchange in the root and nodule area. When the oxygen concentration in the soil falls below 0.2 atm, as is not unusual in field soils, the result is a decline in the formation of nodules and the amount of nitrogen they fix (MINCHIN and PATE, cited in EDWARDS 1977). In Phaseolus beans it was found that waterlogging caused nodule activity to fall sharply by 40-50% (SPRENT, cited in GRAHAM 1981). Measures to prevent waterlogging (biological activity, organic matter, loose tilth, proper drainage, etc) are useful in such situations.

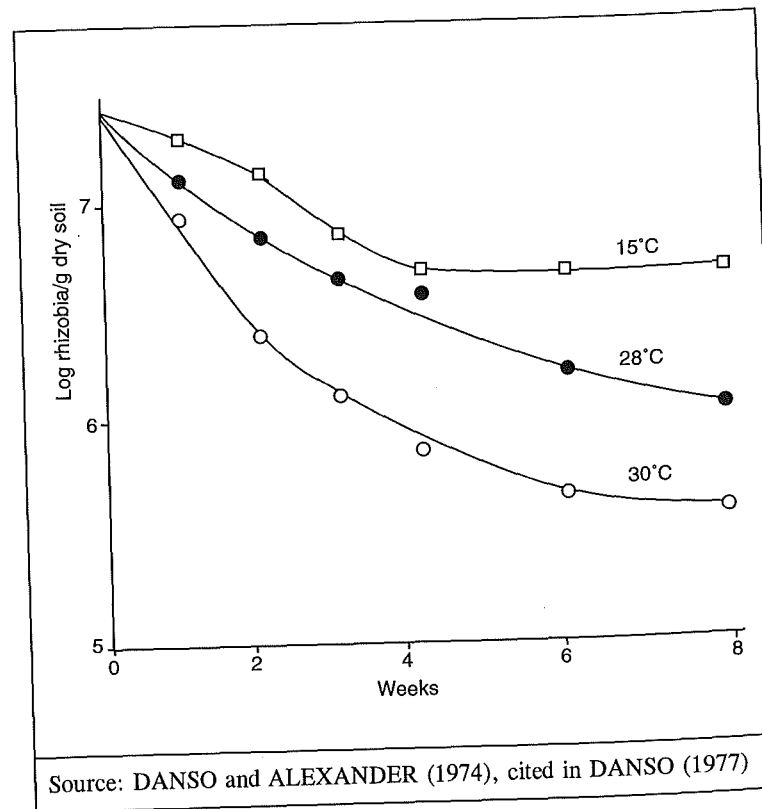
Drought stress is no less harmful in its effects (ALEXANDER 1977). It was observed that drought stress caused a marked decrease in N fixation in clover, Phaseolus and soybean. GRAHAM (1981) cites studies by SPRENT which showed that both the number and size of nodules decreased in stressed Phaseolus beans, causing a 90% reduction in N fixation. The decline in N-fixation activity at the end of flowering, which is typical for most annual legumes, took place earlier and more rapidly under drought stress. In this way the time available for nitrogen fixation was shortened (DUQUE et al. 1982).

For higher nitrogen production, an adequate water supply should be ensured at the time of maximum N fixation (flowering), especially in annual legumes. Leguminous shrubs and trees are less sensitive to dry topsoil conditions. The nodules in the dry upper soil can continue their activity if the taproots of the plant still have access to water (DART 1977). Drought is particularly harmful when accompanied by heat. Not only is the host plant exposed to stress, but the survival rate of the rhizobia in the soil also declines.

High temperatures have an especially negative impact on the survival of rhizobia in sandy soils, while the influence of heat and drought is not so extreme on clayey soils.

It appears that the clay colloid acts to protect rhizobia (DANSO 1977, Figure 8.5). *Rhizobium trifolii* was considerably more sensitive than *R. japonicum* and *R. lupinii*; apparently differences exist between species and even between strains. BOONKERD et al. (1982) found that high temperatures accompanied by either drought or waterlogging had a decimating effect on cowpea rhizobia.

Figure 8.5. Effects of storage temperature on the survival of *Rhizobium meliloti* M₃V₂-S in a sandy soil



Low temperatures can be just as detrimental as high ones to the performance of rhizobia, although in the tropics it is usually high temperatures that inhibit N fixation.

Low temperatures in the rhizosphere hinder the formation of root hairs in the host plant, infection and the development of nodules. Inhibition of nitrogenase activity has also been shown for many plants (MUNEVAR and WOLLUM 1982).

Phaseolus, for example, exhibits optimum nitrogen fixation in the temperature range 20-25°C, whereas fixation falls off sharply at temperatures over 33° and under 15°C. At a day-night fluctuation of 25/15°C, beans fixed twice as much nitrogen as when temperatures fluctuated between 25° and 35°C (GRAHAM 1979, 1981). In soybean, many varieties and rhizobia strains reacted to temperatures exceeding 28°C with a drop in performance (MUNEVAR and WOLLUM 1982). According to DOMMERGUES (1982), most legumes cease to form nodules at temperatures over 30°C in the root area, with the exception of a few specialists (e.g. *Acacia mellifica*, only at 30-35°C).

The influence of temporarily higher temperatures has received little attention so far. The rhizobia in *Vigna unguiculata* were able to recover within 5-10 days from a temperature cycle that rose to a maximum of 40°C every day. This means that high temperatures over just a short period of time need not have a negative effect on the final yield (DART 1977).

Mulching trials on Phaseolus indicate that the symbiosis clearly benefits from even temperatures. Beans were mulched with 4 cm of rice husks, lowering temperatures at a soil depth of 10 cm by an average of only 1.3°C. But by keeping the temperature from rising over the critical level of around 28°C, the yield of beans was increased by 38% (fresh matter) and nitrogen fixation from increased nodule formation was three times as high at flowering and twice as high at harvesting (WATERS et al. 1980).

Another positive effect of mulch is that it helps prevent the upper soil from too drastic an alternation between drying and rewetting. Repeated wetting and drying can lead to the bursting of previously dehydrated rhizobia bacteria (SALEMA et al. 1982). Because exposed topsoil heats up quickly, the protection of rhizobial activity is another reason for not leaving a soil exposed.

It is possible to promote nitrogen fixation through the choice of appropriate varieties. It has been shown that great differences exist in the capacity of legumes to fix nitrogen, not only between species but also between varieties (e.g. in clover, soybean, *Desmodium*, lucerne, vetch, peas and Phaseolus beans). Several factors are responsible for this. In addition to the resistance and tolerance characteristics already mentioned, varieties also differ in their ability to enter symbiosis with naturally occurring rhizobia.

Research indicates that the main reason for this difference lies in the varying capacities of different species and varieties to supply their root nodules with sufficient carbohydrates (GRAHAM 1982). Numerous studies in the past few years have shown that, even when a plant is otherwise well supplied, its internal supply of photosynthesis products to the nodule bacteria is the limiting factor for nitrogen fixation. Some varieties store a lot of carbohydrates in their roots (more from the lower than from the upper leaves), but deliver little to the nodules.

Other legumes assimilate large amounts of nitrogen in the juvenile stage and use the energy-rich products of photosynthesis to convert this immediately into above-ground biomass. With this type of legume there is little left over from the products of photosynthesis to develop nodules, with the result that the plants form fewer of them or that the nodules they have do not fix as much nitrogen. This is the case with many Phaseolus bean types that exhibit early flowering, early maturity and determinate growth.¹³⁷

Annual legumes have a longer growth phase, lasting until the end of flowering, and they tend to fix more nitrogen. This is because a physiological change in the plant begins to take place with the formation of the pods: there is an increase in the delivery of carbohydrates to the new center of attraction (physiological "sink"), and this causes a decrease in supply to the nodule bacteria. According to DART (1977), the

¹³⁷ Determinate growth means that on almost all shoots flowering and maturity occur almost simultaneously (short flowering phase). The behavior described here and the rapid drop in N fixation after flowering are regarded as the main reason why Phaseolus beans bind relatively little nitrogen compared with other grain legumes; according to GRAHAM (1981) they fix 25-70 kg/ha, depending on variety.

relationship between pods and roots as competing centers of attraction for the supply of carbohydrates varies with the variety of soybean, i.e. the ability to fix nitrogen still differs, even in varieties with synchronous flowering.

As a rule, late flowering over a long period promotes nitrogen fixation. It extends the time in which root nodules are supplied with carbohydrates, thus increasing their growth and fixation capacity. HARDY et al. (cited in GRAHAM 1982) doubled nitrogen fixation in soybeans by extending the pre-flowering phase by 9 days through periodic illumination.

In trials by GRAHAM (1981) with different varieties and growth types of Phaseolus beans, low-growing types of bush beans with determinate growth fixed smaller amounts of nitrogen than climbing (runner) types. Bush beans of the semi-determinate growth type and climbing-creeping bush forms occupied an intermediate position (Table 8.8 and Figure 8.6).

In these experiments only the climbing runner beans, often found with maize in traditional cropping systems, showed N fixation rates comparable to other grain legumes.

This difference in varieties with regard to their ability to fix nitrogen can be assumed for other legumes besides Phaseolus beans, though they may perhaps be less pronounced. The results for beans show that it is possible through selection and breeding to increase the competitiveness of grain legumes by improving their nitrogen fixation.

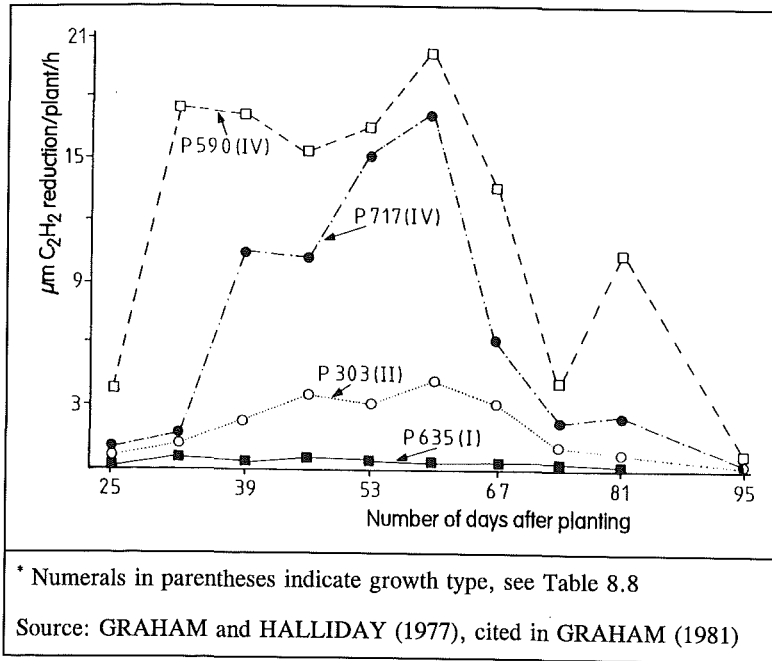
Little is known about how **plant protection products** affect the survival and effectiveness of rhizobia. According to GRAHAM et al. (1980), the damage done to rhizobia by **phytohormones, insecticides and herbicides** is usually only slight if these are applied at recommended doses. In trials by HAMDI and ALAA EL-DIN (1982), negative effects were observed from Aretit, Linuron, Treflan and Cobex. HUGE (1981) noted that several years of applying the herbicide Tribunil to grain crops reduced the number of anaerobic nitrogen-fixing bacteria in the soil.

Table 8.8. Nitrogen fixation in eight varieties of *Phaseolus vulgaris* with different growth habits

Variety	Growth habit*	Time until flowering (days)**	Seasonal nitrogen fixation***
First season, 1976			
P 635	I	42	0.40
P 417	II	55	2.72
P 589	III	60	2.52
P 717	IV	48	9.47
Second season, 1976			
P 536	I	41	6.85
P 561	II	43	7.66
P 498	III	50	13.10
P 590	IV	66	28.01
* Classification of growth habit : I determinate, bush form; II indeterminate, bush form; III indeterminate, creeping-climbing bush form; IV climbing type, string beans.			
** No. of days until 50% of the plants display at least one flower.			
*** Measured as acetylene reduction in mol C ₂ H ₂ per plant.			
Source: Adapted from GRAHAM (1981)			

Fungicides have a more toxic effect on rhizobia. In trials by GRAHAM et al. (1980), PCNB, Thiram and Captan led to higher rhizobia mortality. HAMDI and ALAA EL-DIN (1982) found that nodulation and nitrogen fixation decreased after the use of Captan, Benlate and Vitavax. Where fungicide treatments are necessary, only mildly toxic products should be used and, if inoculation is to be carried out, the inoculum should be spread on the field separately in granulated form, not combined with the dressed seed. In this way the most negative effects can be avoided.

Figure 8.6. Seasonal course of $N_2-(C_2H_2)$ reduction by four varieties of *Phaseolus vulgaris* in Popayan, Colombia



The rhizobia population is higher in fields in which legumes are frequently part of the crop rotation. This effect can last for a long or short time, depending on the persistence of the rhizobia (ALEXANDER 1977). The stimulating effect of a leguminous rotation component extends beyond the host's specific rhizobia species to other rhizobia which are unable to infect the host plant (Table 8.9).

For the farmer this affords the possibility of improving the nitrogen fixation and growth potential of a particular legume by planting any other legume he or she wishes. For example, soybean yields can be increased by first growing a fodder legume. Non-legumes may enhance or hinder the development of rhizobia; too few studies have been made of this last subject for practical recommendations to be possible (ROVIRA cited in ALEXANDER 1977).

Table 8.9. Occurrence of *Rhizobium trifolii** in the rhizosphere of different legumes

Plant	Number of rhizobia per g of soil	
	In the rhizosphere	In soil beyond the rhizosphere
Clover	1,900	900
Lucerne	700,000	4,200
Soybean	5,500	3,200
Rice	190	32

* *R. trifolii* can only infect species of clover
Source: TUZIMURA and WATANABE (1962), cited in ALEXANDER (1977)

The **inoculation** of legumes with selected strains of rhizobia can be discussed only briefly here. There is no doubt that this technology can substantially increase the effectiveness of symbiosis. When new legumes are introduced into an area, inoculation is usually a prerequisite for their successful cultivation. However, even after many years of experience this technique remains complex to carry out, and experiments with inoculation still produce highly inconsistent results. Sometimes inoculation proves completely ineffective. Site-specific soil and climate conditions often mean that a successful combination at site A is a total failure at site B (see also AYANABA and DART 1977 and GRAHAM and HARRIS 1982).

Table 8.10 shows the results of inoculation of *Phaseolus* beans. Here the influences of site, rhizobium strain and variety of crop plant - to mention just a few of the factors concerned - are evident. The "La Selva" site exhibited a higher yield potential for *Phaseolus* beans than the "Quilichao" site, at which neither fertilizer application nor inoculation with different strains of rhizobia produced an improvement in yields. At "La Selva", the bean variety P-590 benefitted from inoculation with rhizobia. In

almost all cases the inoculated rhizobium proved superior to the rhizobium species originally present in the soil (control). The yield increases with the rhizobia strains most appropriate to the crop variety and site were almost as high as those obtained by fertilizing with 50 kg of nitrogen (JOHN 1982).

Table 8.10. The influence of variety, site and rhizobium strain on the yield of Phaseolus beans (kg/ha)*

Treatment	"Quilichao" (1000 m a.s.l.) Yield of bean variety P 566 (kg/ha)	"La Selva" (2000 m a.s.l.) Yield of bean variety P 590 (kg/ha)
Rhiz. strain 45	932	2636
Rhiz. strain 57	958	3073
Rhiz. strain 140	901	2130
Rhiz. strain 147	1075	3610
Rhiz. strain 255	1095	2750
Rhiz. strain 625	1060	3346
Rhiz. strain 640	990	3590
Rhiz. strain 676	1015	3060
Rhiz. strain 839	922	3356
No Rhizobium	918	2300
100 kg urea/ha	1066	3886

Source: CIAT Annual Report: Beans (1979), JOHN, personal communication

In conclusion, accurate knowledge of the site and the identification of a suitable crop variety and rhizobium strain are prerequisites for improving nitrogen fixation by means of artificial inoculation. These factors are usually not understood for most areas of the tropics. Multilocational trial programs are one way of increasing our knowledge, but exist in a few countries only at present.¹³⁸

¹³⁸ In India, efforts in this area have been made since the mid-1970s; 29 centers have already been set up, supervising a cultivation area of about 60,000 ha (RANGASWAMI 1982).

8.3.3 Other forms of biological nitrogen fixation

Table 8.11 presents an overview of nitrogen-fixing micro-organisms other than rhizobia and mycorrhizae. Most free-living N₂-fixing bacteria are heterotrophic and either aerobic, facultatively aerobic or anaerobic. The anaerobic bacteria expend more energy on nitrogen fixation than do aerobes, because they possess a less effective energy metabolism. Their contribution to the fixation of nitrogen in natural ecosystems is therefore usually low (NEVES 1982).

Table 8.11. Orders, families and genera of free-living, nitrogen-binding bacteria

1. Eubacteriales	
Azotobacteriaceae:	<i>Azotobacter, Beijerinckia, Derxia</i>
Bacillaceae:	<i>Bacillus, Clostridium</i>
Enterobacteriaceae:	<i>Klebsiella</i>
Micrococcaceae:	<i>Micrococcus</i>
Rhizobiaceae:	<i>Chromobacterium</i>
2. Hyphomicrobiales	
Hyphomicrobiaceae:	<i>Rhodomicrobium</i>
3. Pseudomonadales	
Athiorhodaceae:	<i>Rhodopseudomonas, Rhodospirillum</i>
Chlorobacteriaceae:	<i>Chlorobium</i>
Pseudomonadaceae:	<i>Azotomonas, Pseudomonas, Xanthomonas</i>
Spirillaceae:	<i>Desulphovibrio, Methanobacterium, Spirillum</i>
Thiorhodaceae:	<i>Chromatium</i>

Source: ODU (1977)

The effectiveness of aerobic bacteria depends on the concentration of oxygen in the soil and on the availability of carbohydrate compounds. Lack of energy, typically caused by a deficit of nutritive humus or of root exudates, inhibits effectiveness just as much as a high concentration of oxygen.

Thus, for example, *Azotobacter* species fix more nitrogen on flooded rice fields than elsewhere, because the oxygen concentration is lower and there are more cellulose-decomposing, anaerobic bacteria here. The metabolic products of these bacteria (organic acids) serve as nutrition for free-living nitrogen-fixing bacteria.

Azospirillum species, which can invade the inner root sections of grasses, are able to fix substantial amounts of nitrogen. The concentration of N in the rhizosphere is very low because of the nitrogen uptake of the plants, so that nitrogen-fixing bacteria in this substratum are better able to compete for energy-rich root exudates than are other organisms.

According to ODU (1977), the amount of nitrogen fixed by free-living bacteria in the humid tropics ranges from 20 to 50 kg/ha/year. This is more than in temperate climates, because of the favorable ecological conditions such as warm temperatures and moist soils. *Azotobacter* bacteria, for example, utilize energy three times more effectively at the relatively low oxygen concentration of 0.04 atm O₂ than at 0.2 atm O₂. In other words, they are stimulated by high soil moisture and low soil aeration.

Azotobacter occur worldwide. They develop particularly well in slightly acid to alkaline soils and are strongly stimulated by readily accessible, energy-rich organic compounds such as sugar cane molasses incorporated into the soil. *Azotobacter* do not form spores, and die at temperatures over 50°C. According to AHRENS (1961), the occurrence of *Azotobacter chroococcum* can be considerably increased (+ 34%) in the long term by applying decomposed manure and (earth) composts. PK fertilizers also have a stimulating effect (+ 94%), whereas N (4 kg/ha) and fresh organic matter either inhibit or have no effect. Under waterlogged conditions stable manure and organic matter have an adverse effect (methane formation) (ISHAC et al. 1982).

Substantially higher yields can sometimes be achieved by inoculating seed and/or soil with *azotobacter*. HUSSAIN and MUHAMMAD (1973) improved maize yields in this way by 5-15%, while PILLAYARSAMY et al. (1980) obtained increased yields of 5-30% by inoculating cassava cuttings and soil. The increased cassava yield corresponded to that obtained by applying 37 kg/ha of N fertilizer. The importance of *azotobacter* bacteria, however, lies not only in their ability to fix N. The bacteria also produce

growth-promoting substances such as gibberellin and indoleacetic acids, which can improve the biomass production of a plant stand. For example, DEWAN and SUBBA RAO (1979) increased the formation of root mass in rice by inoculating the planting material with *Azotobacter chroococcum* and *Azospirillum brasilense*. DÖBEREINER et al. (cited in DÖBEREINER 1977) discovered an especially effective association in *Azotobacter paspali* and *Paspalum notatum* (a valuable meadow grass), fixing up to 90 kg of nitrogen per ha per year.

Nitrogen fixation in the root zone has been found for many other grasses, including several important grain crops. *Azospirillum species*, for example, are associated with rice, wheat, barley, maize, sorghum, *Pennisetum* and *Setaria*. Infection of the stalk (i.e. deep invasion of the plant by the bacteria) was observed in maize, sorghum, rice and *Brachiaria*. These associations can contribute considerably to the N nutrition of Gramineae, especially in the warm tropics.

In 2 years of field trials, KAPULNIK et al. (1981) and OKON (1982) achieved significantly increased yields in inoculated stands of maize, sorghum, *Pennisetum miliaceum* and *Setaria italica*. Yields of maize and sorghum were improved by 70-80 kg/ha on the loess and rendzina soils of Israel. Stands that had been both inoculated and fertilized with 40 kg N/ha produced yields as high as stands fertilized with 80 to 120 kg N/ha. Similar results were achieved by SMITH et al. (1977) with pearl millet and *Panicum maximum* after inoculation with *Spirillum lipoferum*.

According to DÖBEREINER (1982), the C₄ grasses, with their efficient use of water and light, possess a relatively high potential for associative N₂ fixation in the rhizosphere. This is because they require only about 19% of their assimilated energy for nitrogen fixation. BEUNARD and PICHOT (1981) point out that, here again, different varieties behave differently, as in the case of the association of *Azospirillum brasilense* with different varieties of rice. The authors assume that associative nitrogen fixation has been unconsciously selected for in traditional land races.

OBLISAMI and UDHAYASURIAN (1982) showed that *Azospirillum* species also possess considerable potential for association with non-grasses. They inoculated four species of *Amaranthus* with a species of *Azospirillum* which they had isolated in the

roots of *Amaranthus dubius*. In pot trials they achieved higher yields of green material (up to + 82%) and higher grain yields (+ 34 to 48%). The results of the field trials are presented in Table 8.12. Inoculation with azospirillum raised yields to levels equal to those obtained by applying 50 kg N/ha. The yields obtained with 50 kg N and inoculation equalled those achieved with 75 kg N/ha.

Like the rhizobia, *actinomycetes* (*Frankia*) invade the outer root mantle, triggering tissue excrescences that develop into nodule-like growths. Actinomycetes are found in the plant families of the Rosaceae, Betulaceae, Ramnaceae and Ericaceae, i.e. exclusively as root symbionts in woody plants.

Table 8.12. Effect of inoculation with *Azospirillum* sp. on the grain yield (g/m²) of *Amaranthus* species under different N fertilizers[†]

Treatment	Species of <i>Amaranthus</i>				Mean
	<i>A. dubius</i>	<i>A. gangeticus</i>	<i>A. leucocarpus</i>	<i>A. edulis</i>	
N ₀ I ₀	18.2	104.0	180.0	130.0	108.5
N ₀ I ₁	23.2	122.0	204.0	144.0	123.30
N ₂₅ I ₀	30.4	144.0	218.0	180.0	143.10
N ₂₅ I ₁	51.8	176.0	260.0	196.0	170.95
N ₅₀ I ₀	44.6	208.0	264.0	236.0	188.15
N ₅₀ I ₁	57.2	252.0	340.0	396.0	236.30
N ₇₅ I ₀	55.0	256.0	334.0	288.0	237.25
N ₇₅ I ₁	56.8	264.0	322.0	300.0	235.25
Mean	42.15	190.75	265.25	221.26*	---

* Differences between species and between inoculation and N fertilization, and the interaction of the varieties with the treatment, were all significant (P = 0.001).

Source: OBLISAMI and UDHAYASURIAN (1982)

The amount of N fixed through symbiosis with *Frankia* sp. is particularly high in leguminous trees. DOMMERGUES (1982) reported that *Casuarina equisetifolia*, an important multi-purpose tree in arid areas, can fix 58 kg N/ha/year, and that stands of *C. littoralis* fix up to 218 kg N/ha/year in the humid tropics. Values as high as 290 kg/ha are reported by TORREY (1982) from Australia. In New Guinea, *Casuarina* species have traditionally been used to plant fallow areas. The regeneration of land is accelerated through N fixation, and after 10 to 15 years there is the additional bonus of wood to be harvested (TORREY 1982). In South and Central America symbiosis with alders (e.g. *Alnus acuminata*), which provide good timber, is important (COMBE 1981).

The symbiotic relationships of many plants have simply not been studied. However, this field is gaining in importance, especially in agroforestry research. The inoculation of *Casuarina equisetifolia* seedlings with actinomycetes proved successful in Senegal, using a powdered suspension of nodule material spread on the nursery beds (DOMMERGUES 1982).

Blue-green algae are autotrophic organisms which are also able to fix nitrogen. Species such as *Anabaena* and *Nostoc* are greenish-brown in color, slimy, and are often found in stagnant bodies of water. They are sometimes associated with higher plants, notably with trees in tropical moist forests, and may play an important role in the nutrient status of such ecosystems (RUINEN 1956 and BERGERSEN, cited in DAY and WITTY 1977).

When the algae decompose in the soil, their nitrogen benefits other plants. The growth of blue-green algae in rice fields fixed about 20-50 kg N/ha (FAO, no date). Algae were present in only about two thirds of the fields in India, according to DAY and WITTY (1977). Inoculation with blue-green algae thus has considerable potential for saving nitrogen or increasing yields. To inoculate, farmers should obtain effective algae cultures from a research institute or a commercial dealer, since considerable differences in effectiveness exist between species and strains. In dried form algae are easy to transport and store. About 15 kg/ha of the algae material should be broadcast on the field. This is repeated for the next two rice crops (about a week after planting the rice). Subsequent crops will benefit and no further inoculation is needed.

Blue-green algae can also be used to produce bio-fertilizers. The materials required consist of a bowl, a shallow container or a 1 x 2 x 0.2 m plastic-lined frame, filled with 8 to 10 kg of soil and 200 g of superphosphate. Some 5-10 cm of water are added (if the soil is very acidic, 100 g of lime may also be needed). About 150 to 200 g of dried algae are sprinkled on top and the container is left to stand in the sun. The water may require topping up from time to time. After 1 to 2 weeks a thick, floating mat of algae forms on the surface of the water. This is removed when the water has evaporated, and stored in bags until needed.

The same method can be carried out on a large scale, for example by digging shallow pits and lining them with plastic (FAO, no date)¹³⁹.

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¹³⁹ See Chapter 4 for more information on this approach.

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This book presents a range of practices conducive to sustainable agriculture and of particular importance for the development of smallholdings in the tropics. These include agroforestry, intensive fallowing and green manuring, the use of mulch, compost, stable manure, and not least, the purposeful use of natural symbionts. The aim of all these practices is the maintenance of soil fertility using a minimum of external inputs. Much ancient knowledge related to these practices has been forgotten over the years. It is reexamined here, in the light of recent research findings.