Biol Fert Soils (1985) 1: 15-23

Biology and Fertility of Soils

Inoculation with *Glomus mosseae* improves N_2 fixation by field-grown soybeans

F. **Ganry**[1], H.G. **Diem**[2], J. Wey', and Y.R. **Dommergues**[2]

1 IRAT/ISRA Centre national de recherches agricoles. Bambey. Sénégal
2 CNRS/ORSTOM; B.P. 1386. Dakar. Sénégal

Summary. A field study carried out in a sandy, relatively acid Senegalese soil with a low soluble P content (7 ppm) and low vesicular-arbuscular mycorrhizal (VAM) populations showed that soybean responded to *Glomus mosseae* inoculation when the soluble P level in the soil had been raised by the addition of 22 kg P ha^{-1}. In P-fertilized plots, N_2 -fixation of soybean, assessed by the A value method. was 109 kg N_2 fixed ha^{-1} when plants were inoculated with *Rhizobium* alone and it reached 139 kg N_2 fixed ha^{-1} when plants were dually inoculated *wi* th *Rhizobium* and *Glomus mosseae* using an alginate bead inoculum. In addition to this N_2 fixation increase (+28%), *Glomus mosseae* inoculation significantly improved grain yield (+ 13%) and total N content of grains (+16%). This success was attributed mainly to the low infection potential of the native VAM populations in the experimental site. In treatments without soluble P or with rock phosphate, no effect of VAM inoculation was observed.

Key words: *Glomus mosseae* – Soybean – Inoculation – A value method – Senegal

Laboratory and greenhouse experiments have clearly demonstrated the beneficial effect of vesicular-arbuscular mycorrhizae (VAM) on N_2 fixation by legumes especially in P-deficient soils. Because of the generally low availability of P in tropical soils, the potential for the exploitation of VAM in the culture of legumes seems to be greater than in temperate soils. However, this view should be tempered by the fact that limitations exist in the field that often obliterate the stimulation of legume N_2-fixing activity by VAM. Thus field experiments are needed to find out whether inoculation with VAM can improve N_2 fixation by legumes. Up to now only a few field experiments dealing with legumes have been set up in the tropics (e.g., Islam et al. 1980; Islam and Ayanaba 1981; Bagyaraj et al. 1979). In some cases the results were inconclusive and no reliable method was used to assess the effect of VAM inoculation on N_2 fixation.

A previous field study in Senegal, Ganry et al. (1982), indicated that inoculation of soybean with *Glomus mosseae* increased the harvest index and N_2 fixation assessed using the A value method (Fried and Broeshart 1975). The rainfall was irregular during the growth cycle and drought spell occurred during pod filling. These unfavorable climatic conditions probably affected the N_2-fixing activity of the plants since even under the best conditions (i.e., in P-fertilized plots inoculated with *Glomus mosseae)* the total amount of fixed N_2 was relatively low (60 kg ha^{-1}) and only 41% of crop N was derived from N_2 fixation. So it appeared necessary to repeat this field experiment, hopefully under more favorable

Offprint requests to: Y. R.Dommergues

climatic conditions. In addition, we carefully chose an experimental site with a low VAM inoculum potential, so that we could expect a satisfactory response of the crop to VAM inoculation.

Material and methods

The field experiment was carried out at the ISRA (Institut Sénégalais de Recherches Agricoles) research station of Sefa, South Senegal, in 1982. The soil was a leached ferruginous tropical soil (alfic entrustox) in which soybeans had never been grown and which had lain fallow for 5 years before the experiment.

Materials

The soybean cultivar used was cv. ISRA-IRAT 26/72 obtained from the CNRA (Centre National de Recherches Agronomiques) station, Bambey, Senegal. The *Rhizobium* peat base inoculum, which contained 3×10^8 living cells g^{-1} (fresh weight), was applied by hand to the seedling bed at the rate of 25 kg ha^{-1}. Two strains of *Rhizobiurn* were used: an effective strain, USDA 138, and an ineffective one, strain G1 (Lagacherie et al. 1977). G1 was used in the standard crop. The VAM inoculant was prepared as wet beads of *Glomus mosseae* entrapped in alginate according to the method proposed by Diem et al. (1981). Each bead contained ca. 12 mg (fresh weight) of infected roots, spores, and hyphae. Inoculation was performed by introducing 10–15 beads beneath the seed 3-4 cm deep into the soil. It is interesting to note that to obtain 1 l of this *Glomus mosseae* inoculant it is necessary to grow an area of *0.3* m^2 of *Vigna unguiculata* (uninoculated with *Rhizobium),* the plant we routinely use to multiply *Glomus mosseae* in the greenhouse.

Rainfall

The total rainfall before sowing (May 1 to July 17) was 170 mm and during the growth cycle (July 17 to October 10, harvest time) it was 692 mm. The rainfall distribution was fairly even, without any marked dry spell.

Preliminary pot experiment for choosing the experimental size

The experimental site, which was selected from three sites (A, B, C) for its lower VAM infection potential, necessitated a preliminary pot experiment. We compared the endomycorrhizal infection percentage of soybean (cv. 44/A/73) grown in pots filled with 3 kg of soils A, B, C (nonsterile soils). For each soil there were two treatments: treatment 0: no inoculation: treatment G: inoculation with *Glomus mosseae. Inoculation was achieved by introducing 20 beads of alginate-entrapped Glomus mosseae* in the rhizosphere of the soybean seedlings when they were at their first-leaf stage. Pots were placed in a greenhouse under the climatic conditions prevailing at Dakar in February and March.

Preliminar experiment for choosing the standard crop

To choose the standard crop (i.e., the non-N_2-fixing crop to be compared with the N_2-fixing one) we planted soybean cv. ISRA-IRAT 26172 (the same cv. as the one used in the field experiment) with two treatments: (1) no inoculation (to avoid contamination, plots were separated by sheets of corrugated iron) and (2) inoculation with the ineffective strain G 1. Uninoculated soybeans were found to be slightly nodulated either by native strains compatible with soybean as already observed by P. Jara (personal communication) in many Senegalese soils. The atom percentage ^{15}N excess in soybeans inoculated with the ineffective strain was higher than that of inoculated soybeans, indicating that even if the former soybeans fixed a small amount of N_2, their fixation was lower than that of uninoculated soybeans. Thus soybeans inoculated with the ineffective strain were chosen as the standard crop.

Experimental design

A split-plot experimental design was used with eight replicates. The main treatments were:

1. Inoculation with the ineffective *Rhizobium* strain; N fertilizer: 90 kg N ha^{-1} (I-90 N)
2. Inoculation with the effective *Rhizohium* strain; starter N fertilizer: 17 kg N ha^{-1} (R-17 N)

3. Inoculation with the effective *Rhizobium* strain and *Glomus mosseae;* starter N fertilizer: 17 kg N ha^{-1} (RG-17 N)

The subreatments were:

1. No P addition (OP)
2. P added as supertriple, 22 kg P ha^{-1} (super)
3. P added as Taiba rock phosphate, 22 kg P ha^{-1} (rock P)

Each main plot (40.25 m^2) was divided into three subplots (12.25 m^2). In each subplot an area of 6.10 m^2 was used for yield estimation and an area of 1.65 m^2 was used for the ^{15}N labeling. All the experimental plots were fertilized with KCl at the rate of 90 kg h^{-1}. Labeled N fertilizer was applied as $(^{15}NH_4)_2SO_4$ with 1.01 atom percent ^{15}N excess for the 90 kg N ha^{-1} application and 4.73 atom percent ^{15}N excess for the 17 kg N ha^{-1} application.

Analysis of the plants

Plants were carefully harvested avoiding contamination with soil N; leaves, stems, husks, and grains were sampled and analyzed separately. The samples were dried at 65°-70°C for 24 h, weighed, ground into a 40-mesh powder, and anaiyzed for total N content according to the Kjeldahl method. ^{15}N analyses were carried out at the Seibersdorf Laboratory (IAEA) using Dumas' method (the combustion performed in this technique converts total N directly to N_2) and emission spectrometry. For the sake of simplification figures related to leaves. stems, and husks were pooled under the term shoot, but data related to grains were presented separately.

The amount of N_2 fixed was evaluated according to the A value method (Fried and Broeshart 1975).

Root samples from each treatment were stained with trypan blue in lactophenol using the method of Phillips and Hayman (1970).

Frequency and intensity of VAM infection were then assessed according to Ollivier et al. (1983). The interpretation of the whole set of data was performed according to the test proposed by Quidet and Masmejean (1982), which indicated the level of significant (P = 0.05) of the main treatments and their possible interaction. Within each treatment, subtreatments were compared and within each subtreatment the main treatments were also compared. Related LSDs are indicated at the bottom of each table of data.

Results

Pot experiment

Table 1 shows that soil C had a lower VAM infection potential than soils A and B; thus soil C was chosen for the field experiment. The main characteristics of the soil C (O-20 cm horizon) were as follows: sand (20–2000 μm), 83% pH H_2O (1:2.5), 6.2; organic C, 0.40%; organic N, 0.038%; total exchangeable cations, 1.29 mEq 10^{-2}g; total exchange capacity, 1.66 mEq 10^{-2}g; total P. 197 ppm; available P (Truog), 7 ppm. One should note that soil C differed only from soils A and B in the fact that the root infection by native VAR4 fungi was significantly lower at the 20th and 38th days, but was the same when the plants wer 60 days old. In all soils, plants responded similarly to the inoculation with *Glomus mosseae,* indicating that no limiting factor occurred in preventing the establishment of G. *mosseae.*

Table 1. VAM infection frequency of soybeans grown in nonsterile soils A, B, and C from the Sefa experimental station (preliminary pot experiment)

Soils	Treatments	VAM infection frequency (%) at 20th day	38th day	40th day
A	0	9	17	55
	G	14	19	57
B	0	19	20	55
	G	18	25	54
C	0	4	12	56
	G	18	24	61

0, no inocultion; G, inoculation with G. mosseae
Each value is the mean of the results from five replicates

Field experiment

Infection by VAM. Table 2 shows that there was no significant interaction between the main treatments and the subtreatments. The only significant main effect on infection frequency was that of inoculation with *Glomus mosseae* within subtreatment Super (application of superphosphate): without G. *mosseae* inoculation, the infection frequency was 65% and with inoculation it was 87% at the 26th day. This effect disappeared when plants were older (40th day). There were significant effects of G. *mosseae*

Table 2. Nodule weight, frequency, and intensity of VAM infection of soybean roots 26 and 40 days after inoculation with *Rhizobium japonicum* (USDA 138) alone (R) or with *Rhizobium japonicum* (USDA 138) plus Glomus mosseae (RG)

Treatments			26 days			40 days		
Main treatments[a]		Subtreatments	Nodule	VAM infection		Nodule	VAM infection	
Inoculation	N fertilizer	(P fertilizer)	dry wt[b]	Frequency (%)	Intensity (%)	dry wt[b]	Frequency (%)	Intensity (%)
R	17N	OP	14	70	25	74	98	42
R	17N	Super	27	65	25	103	96	42
R	17N	Rock P	21	68	27	98	96	45
RG	17N	OP	22	73	34	76	91	39
RG	17N	Super	61	87	35	98	92	40
RG	17N	Rock P	45	75	28	87	93	42
LSD between the main treatments within the same subtreatment				10	6		5	NS
LSD between the subtreatments within the same main treatment				12	5		4	NS

a 17N: N fertilizer added at the rate of 17 kg N ha^{-1}
b Geometrical mean for dry wt. at the 26th day

inoculation on infection intensity: one within the subtreatment Super (25% versus 35%) and the other within the subtreatment OP (25% versus 34%).

The coefficient of variation of infection frequency was 4.5% for the whole set of plots inoculated with G. *mosseae*, which is a much lower figure than that for plots not inoculated with *G. mosseae* (10.9%).

N_2 Fixation. Nodulation (Table 2): There was no significant interaction between the main treatments and the subtreatments. There were two significant main effects:

1. Inoculation with G. mosseae increased dry weight of nodules per plant in the earlier growth stage (from 21 to 45 mg). This effect disappeared when plants were older (40th day)
2. Effect of P addition, whatever form was applied

Percentage of N derived from N_2 fixation (Table 3): There was no significant interaction between the main treatments and the subtreatments. There were two significant main effects:

1. Effect of *G. mosseae* inoculation on percentage N derived from N_2 fixation within the subtreatment Super (R, 69.8%; RG, 75.9%)
2. Effect of P addition, whatever form was applied

Grain yield (Table 4): Significant interactions between inoculation with G. *mosseae* (R-17N versus RG-17N) and form of P fertilizer (Super versus rock phosphate) were observed for (1) grain yield expressed as kilograms dry weight per hectare, (2) grain yield expressed as kilograms N per hectare, (3) grain N concentration, and (4) grain and shoot total N expressed as kilograms N per hectare.

Harvest index (grain/shoot ratio): Comparing soybean inoculated with *Rhizohium* USDA 138 and G. *mosseae* (treatment RG), we note (Table 5) that in the absence of P fertilizer, dual inoculation (treatment RG) increased only slightly the harvest index expressed as dry weight (+2%), total N (6%). and total P (+9%); in the presence of P fertilizer (Super) the effect of dual inoculation (treatment RG) on harvest index was much more marked: the increase of harvest index expressed as dry weight, total N, and total P was 6%, 13%, and 22% respectively.

Table 5 shows that inoculation of soybean with *Rhizobium* USDA 138 (R) instead of the ineffective strain of *Rhizobium* (1) improved the harvest index when expressed on dry weight and total N basis.

Concluding remarks: When the level of soluble P in the soil was raised by adding 22 kg P ha^{-1}, inoculation of soybean with *Glomus mosseae* increased N_2 fixation (+28%), the grain yield (+ 13%). and the harvest index based on dry weight (+12%). The increase in grain protein resulting from G. *mosseae* inoculation was relatively modest (+16%) but the

Table 3. Sources of N (expressed as a percentage of total plant N or in kg N ha^{-1}) in soybean inoculated with ***Rhizobium japonicum*** (USDA 138) alone (R), or with ***Rhizobium japonicum*** (USDA 138) plus *Glomus* mosseae (KG)

Treatments			Fertilizer N		Soil N[b]		Fixed N_2	
Main treatments		Subtreatments						
Inoculation	N fertilizer[a]	(P fertilizer)	%	kg N ha^{-1}	%	kg N ha^{-1}	%	kg N ha^{-1}
R	17N	OP	2.6	2.8	30.1	32.8	67.3	73.1
R	17N	Super	2.0	3.1	28.2	44.0	69.8	109.0
R	17N	Rock P	2.0	2.9	26.2	39.2	73.8	110.3
RG	17N	OP	2.6	3.1	30.6	36.7	66.9	80.2
RG	17N	Super	1.6	3.0	22.5	41.3	75.9	139.3
RG	17N	Rock P	2.1	3.2	26.3	40.0	71.6	108.6
LSD between the main treatments within the same subtreatment			0.40	NS			5.3	14.5
LSD between the subtreatments within the same main treatment			0.44	NS			5.7	13.4

a Applied at the rate of 17 kg N ha^{-1}
b Calculated from fixation and labeled fertilizer data

Table 4. Grain yield (expressed as kg dry wt ha^{-1} or kg total N ha^{-1}), grain N content (%), grain and shoot total N and total P (expressed as kg ha^{-1}) of soybean inoculated with an ineffective strain of ***Rbizobium japonicum*** (1), with ***Rhizobium japonicum*** (USDA 138) alone (R), or with ***Rhizobium*** *japonicum* (USDA 138) plus *Glomus* mosseae (KG)

Main treatments		Subments	Grain yield		Grain N content	Grain and shoot total N	Grain and shoot total P
Inoculation	N fertilizer	(P fertilizer)	kg dry wt ha^{-1}[a]	kg N ha^{-1}	(%)	kg N ha^{-1}	kg P ha^{-1}
I	90N	OP	1093 (16)	65.7	6.01	84.2	5.6
I	90N	Super	1725 (15)	101.6	5.89	127.2	11.1
I	90N	Rock P	1482 (11)	86.2	5.81	109.1	8.2
R	17N	OP	1423 (21)	90.3	6.47	112.0	6.0
R	17N	Super	2017 (16)	133.8	6.58	161.0	10.0
R	17N	Rock P	1888 (9)	124.6	6.62	150.2	8.2
RG	17N	OP	1431 (21)	98.2	6.60	120.0	6.9
RG	17N	Super	2290 (11)	154.7	6.76	183.7	11.8
RG	17N	Rock P	1892 (7)	126.0	6.69	152.0	8.3
LSD between the main treatments within the same subtreatment			192	12.8	0.14	16.2	0.79
LSD between the subtreatments within the same main treatment			197	13.5	0.14	17.0	0.81

a In parentheses, coefficient of variation (%)

Table 5. Harvest index of soybean inoculated with an ineffective strain of ***Rhizobium japonicum*** (I), with ***Rhizobium japonicum*** (USDA 138) alone (R), or with *Rhizobium japonicum* (USDA 138) plus *Glomus mosseae* (RG)

Main treatments		Subtreatments	Harvest index expressed on the bases of		
Inoculation	N fertilizer[a]	(P fertilizer)	Dry weight	Total N	Total P
I	90N	0 P	0.33	3.55	3.8
R	17N	0 P	0.43	4.18	4.3
RG	17N	0P	0.44	4.42	4.7
I	90N	Super	0.36	3.98	4.4
R	17N	Super	0.48	4.71	4.5
RG	17N	Super	0.51	5.33	5.5

Harvest index: grain/shoot ratio
a 90N, 17N: N fertilizer applied at the rate of 90 or 1' 7 kg N ha^{-1}

absolute value was + 132 kg ha^{-1}, which is substantial gain if we consider that this figure is equivalent to 1300 kg pearl millet grain (assuming that the protein content of this cereal is 10%). Interestingly, *G. mosseae* inoculation reduced the coefficient of variation of the grain yield expressed in weight, which confirms previous observations (Ganry et al. 1982). In the 1980 experiment (Ganry et al. 1982) when no Super was added, total P content of grain and shoots expressed as kg P ha^{-1} was not affected by *G. mosseae* inoculation since it was 6.9 in the uninoculated plots and 6.5 in the inoculated ones, but when Super was added total P content was raised from 10.6 in the uninoculated plots to 11.2 in the inoculated ones. Similar effects were observed in the 1982 experiment (present report), the related figures being respectively 6.9 (uninoculated plots) and 6.0 (inoculated plots) in the absence of Super, and 10.0 (uninoculated plots) and 11.8 kg P ha^{-1} (inoculated plots) in the presence of Super.

Discussion

Field inoculum preparation

Although procedures usually proposed for mycorrhizal inoculum preparation and field inoculation have been reviewed by Hayman et al. (1981) and Menge and Timmer (1982), only a few reports have dealt with field trials performed with the new forms of inoculum that have been proposed in the past years. Soil pellets that were devised by Hall (1979) have been used successfully on a small scale, but the method would require nearly 2 t ha^{-1} of soil inoculum and the technology of pellets production needs to be improved (Hayman et al. 1981). Witty and Haymnan (1978) used the fluid drilling of mycorrhizal soil wet-sicvings suspended in methylcellulose. Unfortunately such a technique is not suitable when drought conditions coincide with sowing (Hayman et al. 1981).

In the field experiment reported in this paper we used the same type of alginate bead inoculum as the one used earlier (Diem et al. 1978; Ganry et al. 1982). The results presented here clearly confirm the conclusion of this previous trial that VAM inoculum made of spores, mycelium. and homogenized mycorrhizal roots entrapped in alginate beads can successfully be used for large-scale field inoculation. The alginate bead inoculum presents the following advantages:

1. Simple preparation of a large amount of inoculum
2. Easy storage and transportation of the inoculum to the field
3. Easy incorporation into the soil at planting time

Forms of phosphate to be used in combination with VA M inoculation

A number of greenhouse experiments have shown that in sterile P-deficient soils addition of soluble P up to an appropriate level is required to obtain a significant response to VAM inoculation (Gianinazzi-Pearson and Diem 1982). In other words, in these soils we can expect a positive interaction between VAM inoculation and fertilization with soluble P. but such an interaction does not occur if soluble P is replaced by insoluble P except when insoluble P (rock phosphate) is solubilized subsequently to its application. These conclusions were confirmed in the field by a previous study (Ganry et al. 1982) and reconfirmed here. thus it can be claimed that, in some tropical P-deficient soils. an appropriate addition of soluble P may be required to optimize the response to VAM inoculation. Besides this concept our experiment indicated that the form of added rock phosphate should be carfullyconsidered. Taiba rock phosphate that was used in our experiment was not taken up by mycorrhizal plants growing in the field although the soil was slightly acidic (pH 6.2). This result is apparently not consistent with those obtained by

Mosse et al. (1976), who found that inoculation with VAM fungi greatly improved the utilization of Gafsa rock phosphate in some acidic soils (pH, 5-6.4). The reason for the discrepancy is probably that the rock phosphate we used was very resistant to solubilization or that some limiting factors occurred hindering activity of rhizospheric phosphate-dissolving bacteria (e.g., unfavorable water regime; lack of energetic substrates).

Response of field-grown plants to VA M inoculation

In contrast with pot experiments, large-scale field experiments on VAM inoculation have seldom been successful. Thus the satisfactory response to VAM inoculation we obtained here for the second time in Senegalese soils should be discussed. Probably the main cause of this response is that the experimental design was set up in a soil chosen for its low VAM infection potential, but one cannot totally exclude the hypothesis that the introduced strain of *Glomus mosseae* was more effective than the native VAM strains and that it was competitive enough to outclass them.

Low infection potential of the native VAM population. Actually this expression refers to the inability of the native VAM population to infect the plant in its early growth stage. the late infection being possibly attributed either to the low number of native VAM propagules or to some intrinsic characteristics (such as low infectivity ability) of these propagules.

Late infection by native VAM populations probably explains why Howeler et al. (1982) found that cassava -- a mycorrhiza-dependent plant which is most easily infected by VAM fungi - responded positively to inoculation only when VAM populations were low. Similarly we obtained a positive response of soybean to VAM inoculation in soil C chosen for its low VAM infection potential (Table 1). It is also necessary to indicate that in our experiment inoculation was performed by placing the *Glomus mosseae* propagules (entrapped in the alginate beads) in the seedbed (that is close to the germinating soybeans), which is a strategic position conferring a marked advantage to *Glomus mosseae* upon the native VAM fungi (probably irregularly and widely scattered throughout the soil) and also making possible an early infection of the soybean roots.

Based on several papers on the effect of mycorrhizal inoculation (Powell et al. 1980: Abbott and Robson 1978; Owusu-Bennoah and Mosse 1979), it is suggested that inoculated plants benefit especially from an early mycorrhizal infection compared with uninoculated ones. Early mycorrhizal infection allows the host plants, especially legumes, to increase P uptake in the first growth stage when P requirements are high. Carling et al. (1979) wrote that soybean derives maximum benefit from a VAM fungus only if it receives maximum exposure at the early seedling stage.

The effect of earlier VAM infection resulting from inoculation upon nodule weight of soybean is obvious when comparing the data of Table 2, subtreatment Super (the most favorable to the expression of the beneficial effect of VAM). In the early growth stage (26th day) *Glomus* infection (frequency and intensity) and nodule weight in plants inoculated with *Glomus mosseae* (treatment RG–17N, Super) were 87%, 35%, and 61 mg respectively. whereas the corresponding figures for plants uninoculated with *Rhizobium* alone (treatment R–17N, Super) were only 65%, 25%, and 27 mg respectively. Later (40th day) there were no differences between the treatments. suggesting that assessment of infection at a late stage in plant growth is inadequate to ascertain the effect of inoculation. This is in agreement with the suggestion of Abbott and Robson (1981) that only an early assessment of infection is valuable in studies of plant growth response to mycorrhizal inoculation.

Whereas the "rhizobiologist" is familiar with the idea of evaluating the best locations for successful inoculation trials, the "mycorrhizologist" very seldom cares about that problem. which explains most failures of VAM inoculation that are all the more frequent as VAM fungi are ubiquitous organisms. From this point of view preliminary inoculation experiments in the laboratory of greenhouse for selecting sites of inoculation in the field are recommended and methods for assessing indigenous VAM populations in soil (Porter 1979: Wilson and Trinick 1983) should be developed.

Effectivity and competitive ability of the introduced strain of Glomus mosseae. The double concept of VAM effectivity (or capability to enhance absorption of nutrient by the host plant) and competitiveness is still not very well established. However, a number of experiments support this concept. Thus Barea et al.

(1980) and Kucey and Paul (1982) have shown that Glomus mosseae improved P uptake more than indigenous VAM fungi.

Similary Powell et al. (1980) found that indigenous VAM fungi were ineffective in many soils and that inoculation by more effective VAM fungi would result in positive responses even in nonsterile soils containing a high indigenous VAM population,

Kucey and Paul (1982) related the effectiveness of the introduced VAM fungus in relation to its compatibility with the host. They have shown that *Faba* beans growing in a field previously planted with wheat would benefit from inoculation with *G. mosseae* because indigenous VAM species spontaneously selected by wheat may not be efficient for promoting growth of *Faba* beans. Furthermore, Carling and Brown (1980) and Schenck and Smith (1982) have found that *G. mosseae* was more stimulatory to soybean than many other species of VAM fungi and response of soybean to *G. mosseae* inoculation was particularly marked at a high temperature (36°C).

Since our experimental design was set up in a soil which had lain fallow for many years, we can assume that the introduced strain of *Glomus mosseue* was more adapted to soybean than the native VAM microflora. However, this speculation should be further explored, using reliable experimental tools such as labeled strains of VAM and ^{32}P-labeled fertilizer.

In any case, data from the literature and this paper suggest that G. *mosseae* could be one of the best VAM fungi promoting maximum growth of soybean in different soil types and climatic conditions.

inoculation versus N fertilization

Inoculation of soybean with effective strain *Rhizobium* USDA 138 increased the yield and grain/shoot ratio more than fertilizer application. The amount of N_2 fixed due to *Rhizobium* inoculation alone was estimated to be ca 109.0 kg N ha-'; when soybean was inoculated simultaneously with *Rhizobium* and *Glomus mosseae,* N_2 fixation was 139.3 kg N ha.', which is the highest figure reported in a field experimen in Senegal to date. From Tables 3 and 4 we can infer that increasing N_2 fixation made possible increase in yield without depleting soil N content.

Influence of the distribution of precipitation

When comparing the data obtained in 1980 (Ganry et al. 1982) and 1982 (present note) in P-fertilized plots inoculated with *Glomus mosseae,* we see that the amoung of N_2 fixed was more than twice as high in 1982 (139.3 kg ha^{-1}) than in 1980 (63 kg ha"). Similarly the percentage of plant N derived from N_2 fixation was much higher in 1982 (75.9%) than in 1980 (41.4%). These differences can be attributed to the harmful influence of drought periods on N_2 fixation that occurred in 1980, a year when the distribution of precipitation was very irregular.

By contrast, in the P-fertilized plots the increase in N_2 fixation resulting from *Glomus mosseae* inoculation was identical in the 1980 (+29 kg ha") and in the 1982 (+30 kg ha-') experiments. Similarly the yield increase due to *Glomus mosseae* inoculation was roughly the same in 1980 (+302 kg dry weight ha") and 1982 (t273 kg dry weight ha-').

In 1982, the beneficial effect of VAM inoculation was attrihuted to the fact that it had induced early infection in the roots of soybean grown in a soil with a low VAM infection potential whereas in 1980 the inoculation effect was mostly attributed to the fact that during drought periods, which had occurred throughout the growth cycle of soybean, VAM protected the host plants against the harmful effects of water stress (Sprent 1979; Gianinazzi-Pearson and Diem 1982).

Acknowledgments. We thank Youssouph Ndiaye and Moussa Niang for technical assistance. The investigation was funded in part by IAEA (joint FAO/IAEA coordinated Research Programme. Contrat n°D1–SEN 2375).

References

Abbott LK, Robson AD (1978) Growth of subterranean clover in relation to the formation of endomycorrhizas by introduced and indigenous fungi in a field soil New Phytol 81:575–585

Abbott LK. Robson AD (1981) Infectivity and effectivcness of vesicular-arhuscular mycorrhizal fungi: effect of inoculum type. Aust J Agric Res 32:631–639

Bagyaraj DJ, Manjunath A, Patil RB (1979) Interaction hetween a vesicular-arbuscular mycorrhiza and *Rhizobium* and their effects on soybean in the field. New Phytol 82:141–146

Barea JM. Escudero JL, Azcon-G de Aguilar C (1980) Effects of introduced and indigenous VA mycorrhiza fungi on nodulation. growth and nutrition of *Medicago sativa* in phosphate-fixing soils as affected by P fertilizers. Plant Soil 5432X3-296

Carling DE, Brown MF (1980) Relative effect of vesicular-arbuscular mycorrhizal fungi on the growth and yield of soybeans (*Glycine max*). Soil Sci Soc Amer J 44:528–532

Carling DE, Brown MF. Brown FA (1979) Colonization rates and growth responses of soybean plants infected by vesicular-arbuscular mycorrhizal fungi. Can J Bot 57: 1769–1772

Diem HG, Jung G, Mugnier J, Ganry F. Dommergues Y.R. (1981) Alginate-entrapped *Glomus mosseae* for crop inoculation. In: Proc 5th Nacom. University Laval, Quebec, p 23

Fried M. Broeshart H (1975) An independent measuremcnt of the amount of nitrogen fixed by a legume crop. Plant Soil 43: 707-711

Ganry F, Diem HG, Dommergues YR (1982) Effect of inoculation with *Glomus mosseae* on nitrogen fixation by field grown soybeans. Plant Soil 68:321–329

Gianinazzi-Pearson V, Diem HG (1982) Endomycorrhizae in the tropics. In: Dommergues YR. Diem HG (eds) Microbiology of tropical soils and plant productivity Nijhoff/Junk, The Hague, pp 209–251

Hall IR (1979) Soil pellets to introduce vesicular-arbuscular mycorrhizal fungi into soil. Soil Biol Biochem 1 1:85<-86

Hayman DS, Morris EJ, Page RJ (1981) Methods for inoculating field crops with mycorrhizal fungi. Ann Appl Biol 99:247–253

Howeler RH, Cadavid LF. Burckhardt E (1982) Response of Cassava to VA mycorrhizal inoculation and phosphorus application in greenhouse and field experiments. Plant Soil 69:327–339

Islam R, Ayanaba A (1981) Growth and yield responses of cowpea and maize to inoculation with *Glomus mosseae* in sterilized soil under field conditions. Plant Soil 63:505–509

Islam R, Ayanaba A. Sanders FE (1980) Response of cowpea (*Vigna unguiculata*) to inoculation with VA-mycorrhizal fungi and to rock phosphate fertilization in some unsterilized Nigerian soils. Plant Soil 54: 107-l 17

Kucey RMN, Paul EA (1982) Vcsicular-arbuscular mycorrhizal populations in various Saskatchewan soils and the effect of inoculation with *Glomus* mosseae on Faba bean growth in greenhouse and field trials. Can J Soil Sci 63:87–95

Lagacherie B, Hugot R, Amarger N (1977) Selection of *Rhizobium japonicum* strains on their competitiveness for infection. Ann Agron 28:379–390

Menge JA, Timmer LW (1980) Procedures for inoculation of plants with vesicular-arbuscular mycorrhizae in the laboratory, greenhouse, and field. In: Schenck NC (ed) Methods and principles of mycorrhizal research. The American Phytopathological Society, St. Paul, Minnesota, pp 59–68

Mosse B, Powell CL. Hayman DS (1976) Plant growth responses to vesicular-arbuscular mycorrhiza. IX. - Interactions between VA Mycorrhiza, rock phosphate and symbiotic nitrogen fixation. New Phytol 76:331–342

Ollivier B. Bertheau Y. Diem HG. Gianinazzi-Pearson V. (1983) Influence de la variété de *Vigna unguiculata* C. Walp dans l'expression de trois association endomycorrhizienne à vésicules et arbuscules. Can J Bot 61:354–358

Owusu-Bennoah E, Mosse B (1979) Plant growth responses to vesicular-arbuscular mycorrhiza. XI. Field inoculation responses in barley, lucerne and onion. New Phytol 83:671–680

Phillips JM. Hayman DS (1970) Improved procedure for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans Brit Mycol Soc 55:158–161

Porter WM (1979) The 'most probable number' method for enumerating infective propagules of vesicular-arbuscular mycorrhizal fungi in soil Aust J Soil Res 17:515–519

Powell CL, Groters M, Metcalfe D (1980) Mycorrhizal inoculation of a barley crop in the field. NZJ Agr Res 23: 107-109

Quidet P, Masmejean T (1962) Organisation. conduite et interprétation des essais fumure. Services Agronomiques de la Société Commerciale des Potasse d'alsace. SEDA, Paris, p 108

Schenck NC, Smith GS (1982) Responses of six species of vesicular-arbuscular mycorrhizal fungi and their effects on soybean at four soil temperatures. New Phytol 92:193–201

Sprent JI (1975) Nitrogen fixation by legumes subjected to water and light stresses. In: Nutman PS (ed) Symbiotic nitrogen fixation in plants. Cambridge University Press, London. New York, pp 405-420

Wilson JM. Trinick MJ (1983) Infection development and interactions between vesicular-arbuscular mycorrhizal fungi. New Phytol 93:543–553

Witty JF, Hayman DS (1978) Slurry inoculation of VA mycorrhiza. Rothamsted Experimental Station Report for 1977, Part 1:239–240

Received September 20, 1984