

## ORIGINAL PAPER

Benjamin D. Ahiabor · Hiroshi Hirata

## Characteristic responses of three tropical legumes to the inoculation of two species of VAM fungi in Andosol soils with different fertilities

**Abstract** The growth and mineral nutrition responses were evaluated of three tropical legumes, cowpea (*Vigna unguiculata* L. cv Kuromame), pigeonpea [*Cajanus cajan* L. (Millsp.) cv ICPL 86009] and groundnut (*Arachis hypogaea* cv Nakateyutaka) inoculated with two different species of VAM fungi, *Glomus* sp. (*Glomus etunicatum*-like species) and *Gigaspora margarita*, and grown in Andosols with different fertilities [Bray II-P: topsoil (72 ppm), subsoil (<0.1 ppm)]. Percent fungal root colonization was high in cowpea and groundnut but relatively low in pigeonpea in both soil types. Despite the low rate of root infection, significant growth responses were produced, especially in the inoculated pigeonpea plant. In all legumes, shoot dry matter production was favoured by the inoculations. Increases in shoot biomass due to mycorrhizae were greater in the subsoil than in the topsoil. Mycorrhization raised shoot concentrations of P and Ca (in cowpea and groundnut) and P and K (in pigeonpea) in the topsoil. Whereas the P concentration in shoots in the subsoil was not positively affected by VAM fungi, particularly in cowpea and pigeonpea, the concentration of K in such plants was significantly increased by VAM treatment. The results also showed that mycorrhizal enhancement of shoot micronutrient concentrations was very rare in all plants, with negative effects observed in certain cases. Cu concentration, in particular, was not affected by VAM formation in any of the plants, and Mn and Fe in pigeonpea and groundnut, respectively, remained the same whether plants were mycorrhizal or not. In both soils the three legumes responded to *Glomus* sp. better than to *Gigaspora margarita*, and the effects of the VAM fungi on each of the crops relative to the controls were greater in the subsoil than in the topsoil. However, shoot growth of groundnut was not affected as much

as cowpea and pigeonpea by the type of soil used. In spite of the relatively low infection of its root, pigeonpea was generally the most responsive of the three legume species in terms of mycorrhizal/nonmycorrhizal ratios.

**Key words** Andosol · Legumes · Mineral nutrition · Plant growth · VAM fungi

### Introduction

Vesicular-arbuscular mycorrhizal (VAM) fungi form symbiotic associations with most economically important crop plants, including the legumes. This symbiosis enhances plant growth and this is thought to be the result of improved mineral nutrition of the host plant. An increase in the concentration and/or the content of phosphorus (P) in plants is the most often described response to VAM fungi (Krishna and Bagyaraj 1984; Pacovsky and Fuller 1986). However, it has often been reported that mycorrhizal infection also increases the concentration of nutrients other than P in plant tissues but it is not clear whether this enhancement of nutrient uptake is merely a consequence of improved P supply. Whereas Murphy et al. (1981) concluded that a high P concentration in plant tissues is sometimes associated with reduced concentrations of other nutrients, particularly the micronutrients, Janos (1987) and Stribley (1987) observed a direct positive effect of VAM on Zn and Cu nutrition. Pacovsky et al. (1986a,b) also documented a reduction in Mn and Fe uptake following VAM formation.

In many countries, especially developing ones, farmers intercrop with legumes or include them in the crop rotational cycle to enrich exhausted soils; this occurs mainly through nodule dinitrogen fixation with little or no application of other fertilizer nutrients such as P. Supplementation of this agronomic practice with other soil and crop management practices geared towards yield improvement, e.g. the application of a basal P

B. D. Ahiabor (✉) · H. Hirata  
Laboratory of Plant Nutrition, Faculty of Agriculture,  
Tokyo University of Agriculture and Technology,  
Fuchu, Tokyo, 183 Japan  
Fax: 81-423-60-8830

(Manjunath and Bagyaraj 1984) and/or inoculation with selected VAM fungi, would probably increase yields further by the provision of phosphorus and micronutrients essential to the nodule dinitrogen fixation process. Therefore, the symbiotic interactions between different VAM fungal species and a variety of host legumes in different soil conditions need to be examined and understood in order to optimize the beneficial effects of VAM fungi on growth and mineral nutrition of such plants.

The work reported here was undertaken to evaluate the effects of two VAM fungal species, *Glomus* sp. (*Glomus etunicatum*-like species) and *Gigaspora margarita* on growth and mineral nutrition of three widely cultivated tropical legumes (cowpea, pigeonpea and groundnut) grown in andosols with different fertilities.

## Materials and methods

### Growth medium

Air-dry topsoil and subsoil samples of an Andosol collected from the Tokyo University of Agriculture and Technology farm field were used as the culture media. The topsoil of this field continuously received farmyard manure at the rate of 15–40 t/ha and compound chemical fertilizer [14(N) – 14(P<sub>2</sub>O<sub>5</sub>) – 14(K<sub>2</sub>O)] at 600 kg/ha for more than 10 years at each cropping time. The soils were sieved through a 4-mm mesh and then sterilized by  $\gamma$ -ray irradiation (1.5 Mrad). The available P contents were 72 ppm (topsoil) and <0.1 ppm (subsoil) (Bray II-P; 1N NH<sub>4</sub>F, 0.5N HCl). Before being loaded into 16.4-cm-diameter wagner pots at the rate of 2.4 kg soil/pot, the pH of the soils were adjusted to 6.0 (in water) using lime (ground dolomitic limestone). The soils were amended with the following essential nutrients (mg/pot): Topsoil: N (urea) 100, K (K<sub>2</sub>SO<sub>4</sub>) 500, (KCl) 500. Subsoil: P [Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub>] 500, N (urea) 500, K (K<sub>2</sub>SO<sub>4</sub>; KCl) each 500, Fe (FeCl<sub>3</sub>·6H<sub>2</sub>O) 50, Cu (CuCl<sub>2</sub>·2H<sub>2</sub>O) 10, Mn (MnCl<sub>2</sub>·2H<sub>2</sub>O) 30, B (H<sub>3</sub>BO<sub>3</sub>) 25, Zn (ZnSO<sub>4</sub>) 100, Mo [(NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>·4H<sub>2</sub>O] 10, Co (CoCl<sub>2</sub>·6H<sub>2</sub>O) 5. Solutions of these salts were mixed thoroughly with the soil samples packed in polythene bags and then placed into the pots.

### Plant materials

Cowpea (*Vigna unguiculata* L. cv Kuromame), pigeonpea [*Cajanus cajan* L. (Millsp.) cv ICPL 86009] and groundnut (*Arachis hypogaea* cv Nakateyutaka) were grown as the test crops. Uniform seeds, sterilized with 1% NaOCl for 4 min and pregerminated on moist filter paper at 25°C, were sown at 4 seedlings/pot and inoculated with suitable *Rhizobium* strains. Plants were later thinned to 2 seedlings/pot and cultivated to reach their respective maturity stages (cowpea: 73 days, pigeonpea: 84 days, groundnut: 91 days), with soil moisture maintained at 60% of the maximum water holding capacity with deionized water.

### Microbial treatments

One week before planting, each pot soil was preinoculated with 100 spores of either *Glomus* sp. (*Glomus etunicatum*-like yellow spore type) or *Gigaspora margarita* at a depth of 5 cm, or not preinoculated. Spores of both *Glomus* sp. and *Gigaspora margarita* were collected from the University farm field (humus-rich andosol) and proliferated on soybean and wheat, respectively. Spores of these mycosymbionts were surface-sterilized by expo-

sure to 5% (w/v) Chloramine-T (sodium p-toluenesulfon chloramide trihydrate) for 15 min followed by 0.025% (w/v) streptomycin sulfate for 20 min and then rinsed with several changes of sterilized distilled water. The germination rates (25°C; 14 days) for spores of *Glomus* and *Gigaspora margarita*, determined prior to inoculation, were 98% and 60%, respectively. During mycorrhizal inoculum application, all treatments received a wash of the VAM inocula, sieved free of VAM propagules using Whatman no. 2 filter paper, to minimize differences in the soil microbiota.

### Shoot and root sampling

At maturity, the tops of all plants were harvested and weighed (leaves and stems were pooled) after drying in a forced-air oven at 80°C for 48 h. The roots in each pot were washed free of soil particles and organic debris on a 2-mm mesh sieve under a jet of ordinary tap water. After nodules were removed with a pair of forceps and counted, their dry weights together with that of the roots were determined as for shoots. Prior to drying, 2 g of fresh root samples with segments less than 2 mm in diameter were cut into approximately 2-cm pieces and stored in formalin acetic acid solution (Kormanik and McGraw 1982). The root lengths of these samples were determined with a Comair Root Length Scanner using the equation:  $A = 0.2246 + 0.9655E + 0.00123E^2$ , where A represents the actual root length and E the means of two readings. Lengths of roots with diameters of more than 2 mm were measured separately with a ruler. Values were pooled to obtain the total length.

### Analysis of VAM

After root length measurement, retrieved samples were cleared in 10% (w/v) KOH solution at 90°C on a hot plate for 1 h for cowpea and pigeonpea and 3 h for groundnut, since the latter's cytoplasm was difficult to clear. Staining was with 0.05% (w/v) trypan blue in lactoglycerol (modified from Kormanik and McGraw 1982) at 90°C for 25 min for cowpea and pigeonpea and 40 min for groundnut. Percentage colonization of host roots was estimated by visual observation of stained root segments mounted in lactoglycerol by the grid-line intercept method (Giovanetti and Mosse 1980).

### Tissue nutrient analysis

Concentrations of N, P, K, Ca and Mg as well as Cu, Fe, Mn and Zn were determined for shoots (leaves and stems combined). Dried plant tissues were homogenized in a motor grinder into very fine particles and digested in concentrated sulfuric acid (36 N H<sub>2</sub>SO<sub>4</sub>) using hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) as an oxidant. N was determined by micro-Kjeldahl distillation, collecting the distillate in 2% (w/v) H<sub>3</sub>BO<sub>3</sub> and P was measured by the ammonium paramolybdate-vanadate method (Murphy and Riley 1962, modified by Watanabe and Olsen 1965). K was measured by flame photometry and Ca, Mg, Cu, Fe, Mn and Zn by atomic absorption spectrophotometry.

Analysis of variance (ANOVA) was conducted on the data, and treatment means in all tests were compared using Duncan's Multiple Range test.

## Results

### VAM formation and plant growth

Colonization by the two fungi species was higher in cowpea roots than in pigeonpea and groundnut in both soils (Tables 1–3). In general, there were no significant

**Table 1** Responses of cowpea to inoculation with *Glomus* sp. and *Gigaspora margarita* in an Andosol (after 73 days). Values (means of three replicates) followed by different letters within a

column are significantly different at  $P < 0.05$ . Shoot weight includes both stem and leaves

Soil type and inoculum	Root colonization (%)	Pod dry wt. (g/pot)	Shoot dry wt. (g/pot)	Root dry wt. (g/pot)	Root length (m/pot)	Nodule number (/pot)	Nodule dry wt. (g/pot)	Harvest index (%)
Topsoil								
Control	0 c	3.46 b	8.82 cd	1.50 b	75.7 ab	61 c	0.02 c	39.3 b
<i>Glomus</i> sp.	62 b	11.7 a	24.76 a	2.00 a	108.7 a	169 a	0.63 a	46.7 ab
<i>Gigaspora margarita</i>	82 b	9.46 a	16.50 b	1.42 b	112.3 a	120 b	0.29 b	57.3 a
Subsoil								
Control	0 c	0 b	0.40 e	0.40 c	32.56 b	0 d	0 c	—
<i>Glomus</i> sp.	67 b	1.42 b	10.39 c	1.85 ab	93.13 a	25 d	0.01 c	13.8 c
<i>Gigaspora margarita</i>	63 b	1.55 b	7.52 d	1.83 ab	102.7 a	9 d	0.002 c	20.9 c

**Table 2** Responses of pigeonpea to inoculation with *Glomus* sp. and *Gigaspora margarita* in an Andosol (after 84 days). Abbreviations and symbols are as in Table 1

Soil type and inoculum	Root colonization (%)	Pod dry wt. (g/pot)	Shoot dry wt. (g/pot)	Root dry wt. (g/pot)	Root length (m/pot)	Nodule number (/pot)	Nodule dry wt. (g/pot)	Harvest index (%)
Topsoil								
Control	0 c	0.14 b	3.37 d	1.56 c	167 c	20 c	0.004 b	4.4 b
<i>Glomus</i> sp.	45 a	5.34 a	26.09 a	5.78 a	394 a	602 a	1.61 a	20.3 a
<i>Gigaspora margarita</i>	44 a	3.12 a	23.97 a	5.44 a	370 ab	579 a	1.36 a	13.1 ab
Subsoil								
Control	0 c	0 b	0.427 e	0.37 d	15 d	0 c	0 b	—
<i>Glomus</i> sp.	43 ab	0.25 b	14.71 b	5.76 a	330 ab	192 b	0.18 b	1.7 b
<i>Gigaspora margarita</i>	34 b	0.25 b	7.66 c	3.98 b	313 b	66 c	0.01 b	3.6 b

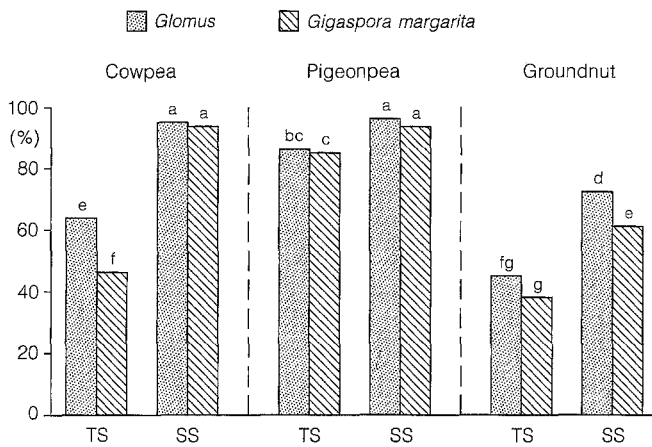
**Table 3** Responses of groundnut to inoculation with *Glomus* sp. and *Gigaspora margarita* in an Andosol (after 91 days). Abbreviations and symbols are as in Table 1

Soil type and inoculum	Root colonization (%)	Pod dry wt. (g/pot)	Shoot dry wt. (g/pot)	Root dry wt. (g/pot)	Root length (m/pot)	Nodule number (/pot)	Nodule dry wt. (g/pot)	Harvest index (%)
Topsoil								
Control	0 c	2.56 bc	12.38 b	4.31 bc	315 c	1.00 a	0.0004 b	20.7 a
<i>Glomus</i> sp.	51 ab	6.14 a	23.09 a	4.58 bc	373 a	8.00 a	0.073 a	26.7 a
<i>Gigaspora margarita</i>	61 a	5.32 a	20.27 a	4.79 ab	386 a	5.00 a	0.035 ab	26.1 a
Subsoil								
Control	0 c	1.31 c	5.68 c	3.76 c	211 d	0.00 a	0.00 b	23.3 a
<i>Glomus</i> sp.	53 ab	4.87 a	21.01 a	5.75 a	360 ab	3.00 a	0.022 ab	22.7 a
<i>Gigaspora margarita</i>	44 b	4.26 ab	15.20 b	4.94 ab	340 bc	0.00 a	0.00 b	28.2 a

differences in VAM formation by these fungi between the different legumes or soils, although *Gigaspora margarita* produced a higher infection in the topsoil with cowpea and groundnut.

Both fungal symbionts markedly enhanced pod production in cowpea and pigeonpea in the topsoil but not in the subsoil (Tables 1, 2). In contrast, shoot and root growths were much increased in both soils. Thus pod production by cowpea and pigeonpea was very much dependent on VAM formation even in the relatively highly fertile Andosol. The low pod production by cowpea and pigeonpea both in the subsoil and in the unin-

oculated topsoil control corresponds closely to low nodulation. Unlike with cowpea and pigeonpea, inoculation significantly enhanced pod yield in groundnut, even in the subsoil; as for cowpea and pigeonpea, these effects were the same with either symbiont in both soils (Table 3). Although VAM plants produced greater shoot and pod biomass than non-VAM plants, the harvest index of groundnut was the same in all treatments (Table 3). This suggests equal partitioning of plant dry matter into pod in both inoculated and noninoculated plants.



**Fig. 1** Mycorrhizal dependencies (%) of cowpea, pigeonpea and groundnut inoculated with *Glomus* sp. and *Gigaspora margarita* in an Andosol. *TS* Topsoil, *SS* subsoil. Columns with the same letters are not significantly different ( $P > 0.05$ )

In groundnut, nodulation was rarely observed after VAM fungi inoculation in both soil types, except in the case of *Glomus* inoculation in topsoil. Root production was markedly stimulated in all legumes in the subsoil, but in the topsoil mycorrhization did not stimulate root dry weight or root length in groundnut and cowpea, re-

spectively. Where positive effects were produced by mycorrhization in either soil, such benefits were more pronounced in the subsoil with inoculation than with noninoculated controls.

#### VAM dependencies

Mycorrhizal dependencies of the three legume crops, calculated as the difference in total shoot dry weight of VAM and non-VAM plants as a percentage of VAM plants, are shown in Fig. 1. In both soil types, groundnut showed the lowest dependency irrespective of inoculant type. Furthermore, for each crop VAM dependency was greater in the subsoil regardless of VAM species, suggesting that both fungi species were more effective in promoting shoot growth of these legumes in the comparatively less fertile subsoil.

#### Nutrient concentrations in shoots at maturity

The concentrations of both macro- and micronutrients in shoots (leaves plus stem) of the three legumes at harvest are shown in Tables 4–6. In general, the concentration of P in these legumes in the topsoil was maintained at a high level by both inoculants, whereas in the sub-

**Table 4** Concentrations of mineral nutrients in shoots (stem plus leaves) of cowpea inoculated with VAM fungi (after 73 days). Symbols are as in Table 1

Soil type and inoculum	Nutrient								
	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Cu (ppm)	Fe (ppm)	Mn (ppm)	Zn (ppm)
<b>Topsoil</b>									
Control	2.24 c	0.063 b	0.975 a	1.82 b	0.459 a	8.07 a	683 a	517 b	53.3 c
<i>Glomus</i> sp.	2.23 c	0.166 a	1.13 a	2.32 a	0.324 b	10.0 a	727 a	217 c	83.3 bc
<i>Gigaspora margarita</i>	2.03 c	0.145 a	1.24 a	2.64 a	0.330 b	13.3 a	767 a	543 b	103.3 b
<b>Subsoil</b>									
Control	7.26 a	0.145 a	0.469 b	0.746 d	0.433 a	10.0 a	693 a	1270 a	193.3 a
<i>Glomus</i> sp.	2.78 b	0.186 a	0.947 a	1.206 c	0.341 b	13.3 a	473 b	287 bc	66.7 c
<i>Gigaspora margarita</i>	2.91 b	0.096 b	0.996 a	1.041 cd	0.312 b	10.0 a	577 ab	520 b	53.3 c

**Table 5** Concentrations of mineral nutrients in shoots (stem plus leaves) of pigeonpea inoculated with VAM fungi (after 84 days). Symbols are as in Table 1

Soil type and inoculum	Nutrient								
	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Cu (ppm)	Fe (ppm)	Mn (ppm)	Zn (ppm)
<b>Topsoil</b>									
Control	4.39 b	0.108 bc	0.617 c	0.802 a	0.164 a	10 a	430 b	450 b	46.7 b
<i>Glomus</i> sp.	2.44 d	0.174 a	1.270 a	0.801 a	0.123 b	17 a	723 a	503 ab	43.3 b
<i>Gigaspora margarita</i>	2.46 d	0.172 a	1.250 ab	0.776 a	0.128 b	10 a	533 ab	337 b	26.7 c
<b>Subsoil</b>									
Control	6.19 a	0.115 bc	0.473 d	0.472 b	0.161 a	13 a	363 b	677 ab	66.7 a
<i>Glomus</i> sp.	2.65 d	0.130 b	1.290 a	0.495 b	0.133 b	10 a	623 ab	463 ab	40.0 bc
<i>Gigaspora margarita</i>	3.30 c	0.104 c	1.160 b	0.704 a	0.139 ab	10 a	473 ab	797 a	36.7 bc

**Table 6** Concentrations of mineral nutrients in shoots (stem plus leaves) of groundnut inoculated with VAM fungi (after 91 days). Symbols are as in Table 1

Soil type and inoculum	Nutrient								
	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Cu (ppm)	Fe (ppm)	Mn (ppm)	Zn (ppm)
Topsoil									
Control	1.99 b	0.091 cd	1.72 b	1.95 b	0.424 a	6.33 a	720 a	220 d	30.0 c
<i>Glomus</i> sp.	1.09 c	0.293 a	1.45 c	2.35 a	0.468 a	10.0 a	903 a	567 c	80.0 a
<i>Gigaspora margarita</i>	1.09 c	0.202 b	1.32 d	2.53 a	0.449 a	8.0 a	737 a	897 a	53.3 bc
Subsoil									
Control	2.90 a	0.075 d	1.86 a	1.52 cd	0.409 a	4.0 a	613 a	667 bc	33.3 c
<i>Glomus</i> sp.	2.05 b	0.135 c	1.60 c	1.43 d	0.445 a	10.0 a	707 a	577 c	63.3 ab
<i>Gigaspora margarita</i>	1.94 b	0.082 d	1.80 ab	1.82 bc	0.450 a	7.33 a	650 a	760 ab	50.0 bc

**Table 7** Mycorrhizal/nonmycorrhizal ratios for dry matter yield and mineral nutrient contents of shoots (stem plus leaves) of cowpea after 73 days

Soil type	Inoculum	Dry matter	N	P	K	Ca	Mg	Mn	Fe	Zn	Cu
Topsoil	<i>Glomus</i> sp.	2.81	2.8	7.4	3.3	3.6	2.0	1.2	3.0	4.4	3.4
	<i>Gigaspora margarita</i>	1.9	1.7	4.3	2.4	2.7	1.3	2.0	2.1	3.6	3.0
Subsoil	<i>Glomus</i> sp.	26.0	9.9	33.3	52.3	42.0	20.5	5.9	17.7	9.0	34.5
	<i>Gigaspora margarita</i>	18.8	7.5	12.4	39.8	26.3	13.6	7.7	15.7	5.2	18.8

**Table 8** Mycorrhizal/nonmycorrhizal ratios for dry matter yield and mineral nutrient contents of shoots (stem plus leaves) of pigeonpea after 84 days

Soil type	Inoculum	Dry matter	N	P	K	Ca	Mg	Mn	Fe	Zn	Cu
Topsoil	<i>Glomus</i> sp.	7.7	4.3	12.5	15.9	7.7	5.8	8.7	13.0	7.2	13.2
	<i>Gigaspora margarita</i>	7.1	4.0	11.3	14.4	6.9	5.5	5.3	8.8	4.1	7.1
Subsoil	<i>Glomus</i> sp.	34.4	14.8	38.9	94.0	36.0	28.5	23.6	59.1	21.0	24.5
	<i>Gigaspora margarita</i>	17.9	9.6	16.2	44.0	26.7	15.5	21.1	23.4	10.0	12.8

soil this was only the case for *Glomus* with groundnut. In contrast, the N concentration in all three legumes in both soils was appreciably reduced by both inoculants, probably due to the dilution effect of increased shoot growth and/or N transfer to pods from leaves and stems during plant ageing.

There were marked responses to both fungi in the concentrations of other nutrients. The K concentrations in the shoots of pigeonpea in both soils and of cowpea in the subsoil was nearly doubled by the VAM fungi, whereas groundnut maintained a high level without VAM formation. The mean Ca concentration in shoots of pigeonpea at maturity was less than half that of cowpea or groundnut, which were also increased by VAM fungi only in the topsoil. The mean Mg concentration of pigeonpea was less than half that of cowpea and groundnut; unlike in groundnut, the increased size of VAM plants diluted this element in cowpea.

With regard to micronutrient concentrations, a slight stimulation of Fe by both fungi was observed only in

pigeonpea in the topsoil (Table 5). The Mn concentration in groundnut was also increased by both inoculants in the topsoil (Table 6). Zn was consistently increased by *Glomus* in both soils, and also with cowpea by *Gigaspora margarita* in the topsoil. The concentration of Zn in pigeonpea, however, was reduced by VAM formation, especially in the subsoil.

#### VAM effects on mineral nutrient accumulation and dry matter increase

In terms of VAM/non-VAM ratios for mineral nutrient content and dry matter yield of shoots (leaves plus stem), the results in Table 7 reveal that mycorrhiza-mediated absorption of P, K, Ca, Fe, Zn and Cu made the most consistent contributions to shoot biomass production in topsoil cowpea. In pigeonpea, however, only P, K and Fe contributed (Table 8), and in groundnut only P, Ca, Mn, Zn and Cu (Table 9). In addition, absorbed

**Table 9** Mycorrhizal/nonmycorrhizal ratios for dry matter yield and mineral nutrient contents of shoots (stem plus leaves) of groundnut after 91 days

Soil type	Inoculum	Dry matter	N	P	K	Ca	Mg	Mn	Fe	Zn	Cu
Topsoil	<i>Glomus</i> sp.	1.9	1.0	6.0	1.6	2.2	2.1	4.8	2.3	5.0	2.9
	<i>Gigaspora margarita</i>	1.6	0.9	3.6	1.3	2.1	1.7	6.6	1.7	2.9	2.1
Subsoil	<i>Glomus</i> sp.	3.7	2.6	6.7	3.2	3.5	4.0	3.2	4.3	7.0	9.3
	<i>Gigaspora margarita</i>	2.7	1.8	2.9	2.6	3.2	2.9	3.0	2.8	4.0	4.9

Mn and Cu (in pigeonpea) and Mg and Fe (in groundnut) mediated by *Glomus* also made up significant portions of the respective shoots.

In the subsoil, both fungi caused greater increases in the contents of K and Ca than the shoot dry matter yield in cowpea (Table 7), and in pigeonpea Fe also appears to be involved (Table 8). In mycorrhizal groundnut, however, increases in shoot dry matter production could be accounted for mostly by increases in the contents of P, Mg, Zn and Cu (Table 9). *Glomus*-induced P and Cu absorption in cowpea, P in pigeonpea and Fe in groundnut contributed markedly to shoot dry matter production to a similar extent as Ca and Mn or Mn in *Gigaspora margarita*-inoculated groundnut and pigeonpea, respectively.

Where the same nutrient contributed to increases in shoot biomass production in both soils, the contribution was always greater in the subsoil. Differences in topsoil/subsoil ratios were very low for groundnut when compared to cowpea and pigeonpea, i.e. under the conditions of this experiment, the type of soil medium used was not as important for the growth of groundnut as it was for cowpea and pigeonpea.

## Discussion

This investigation shows the importance of mycorrhizal infection for the growth of legume plants, confirming other reports of favourable legume response to VAM fungi inoculation, whether in sterilized or unsterilized soils (Daft and El-Giahmi 1976; Manjunath and Bagyaraj 1984). Unlike cowpea and groundnut, colonization of pigeonpea roots was relatively low. This differs from the results of Manjunath and Bagyaraj (1984), who obtained relatively high levels (greater than 60%) of root infection in pigeonpea. Despite the low infection of its roots, significant growth responses were induced in pigeonpea as well as in cowpea and groundnut, by inoculation, even under the relatively high soil fertility. The effectivity rather than infectivity of these fungi species appears to have been highest in pigeonpea.

VAM inoculation stimulated increases in shoot and root dry weights of cowpea and pigeonpea in both soils. Even though the yield was increased in the topsoil, no yield benefit was obtained through mycorrhization in the subsoil. This indicates a strong mycorrhizal effect in increasing yield in cowpea and pigeonpea. In ground-

nut, however, increased pod production in both soils may be attributed to the interaction of mycorrhization and improved shoot growth. Enhanced shoot and root growth of mycorrhizal cowpea and pigeonpea was also obtained by Manjunath and Bagyaraj (1984). However, Van Nuffelen and Schenck (1984) observed that inoculation with *Gigaspora margarita* and *Glomus etunicatum* produced no significant increases in shoot dry weight of soybean. Mycorrhizal stimulation of growth in the present work agrees with the results of Krishna and Bagyaraj (1984), who showed that inoculation of groundnut with *Glomus fasciculatum* enhanced growth in both sterilized and unsterilized soils and resulted in a significant increase in dry matter accumulation. The significant stimulation of root dry weight by either fungi reported here, especially in the topsoil with cowpea and pigeonpea, may be the result of altered source-sink relationships as well as effects of nodule weight. It has been reported that nodulated VAM root systems respire CO<sub>2</sub> at a greater rate than nodulated, non-VAM roots and, therefore, consume more carbon (Pang and Paul 1980). Snellgrove et al. (1982) reported that approximately 7% more carbon was translocated from shoots to mycorrhizal leek roots than from shoots to roots of nonmycorrhizal plants. This observation was confirmed by Koch and Johnson (1984) in a citrus split-root system. This differential demand for carbon may have supported the greater growth and nodule activity of VAM cowpea and pigeonpea root systems, as well as the nodule dry biomass yield of VAM groundnut in the topsoil.

VAM formation induced a higher concentration of P in the shoot dry matter of all three legumes in the topsoil than the 0.1% P or less generally observed in P-deficient plants (Mengel and Kirkby 1987a). The P concentration in groundnut was more than double this value. This enhanced P nutrition is most likely the cause of both the improved shoot growth and yield in these crops. Our finding agrees with the proposal for cowpea (Manjunath and Bagyaraj 1984) and other legumes (Carling et al. 1978) that the effects of VAM in increasing plant growth and nodulation are explained by enhanced P nutrition. In cowpea and groundnut, these increases could also be attributed to the high concentration of Ca in the shoots, and with pigeonpea K could also be implicated. The level of Ca observed in such plants falls within the upper limits of the concentration range (0.5–3%) generally found in the dry matter of

higher plants (Mengel and Kirkby 1987b). According to Jarrell and Beverly (1981) and Cooper (1984), an increase in both shoot weight and shoot nutrient concentration is evidence of a treatment effect. Increases in P concentration following VAM inoculation were also reported by other authors (Nielsen and Jensen 1983; Raju et al. 1990). However, Nielsen and Jensen (1983) observed a decrease in Ca concentration in the dry matter of lucerne (*Medicago sativa*) when the plants were inoculated with *Glomus caledonius*. Comparing mycorrhizal and nonmycorrhizal plants, the results for Ca have been inconsistent (Mosse 1957; Gerdemann 1964); the concentrations of Ca in VAM plants were higher, lower, or not significantly different from those in corresponding plant parts of nonmycorrhizal controls. One explanation for the increased concentration of these elements in this present work following VAM inoculation, especially of P, is that they are absorbed by VAM fungal hyphae and transported across the roots to the shoots. On the other hand, the high concentrations of K and Ca might be a consequence of mycorrhizal improvement in P supply. VAM inoculation either had no effect on or decreased Mg and N concentrations in shoots of all three legumes in both soils. This result contradicts that of Raju et al. (1990), who observed higher concentrations of Mg in shoots of mycorrhizal than nonmycorrhizal maize plants. N concentrations, however, were similar in the two treatments. The decreases in shoot concentrations of N and Mg are probably due to the dilution effect of the increased size of VAM plants.

Mycorrhizal enhancement of shoot micronutrient concentrations was rare in all plants. Negative responses (signifying a dilution effect) were observed in certain cases, especially with Zn in subsoil cowpea and pigeonpea and also with Mn in the former. High concentrations of P in plant shoots (as commonly observed in this study) are sometimes associated with reduced concentrations of micronutrients (Murphy et al. 1981). In agreement with the findings of Raju et al. (1990), Cu concentrations did not change with mycorrhiza treatment in any of the plants.

Thus VAM plants generally produced higher yields, had greater shoot biomasses and higher shoot mineral contents (i.e. the product of dry weight and mineral nutrient concentrations of shoots) than non-VAM plants. The VAM plants, with their more extensive root systems, probably scavenged a greater soil volume for nutrients than the noninoculated controls. Nevertheless, the enhanced shoot contents of all the nutrients cannot be explained by increased plant dry matter yield alone, because the increases in the contents of some of the nutrients were greater than can be accounted for by the increases in biomass production. The VAM fungi promoted the uptake of such nutrients above normal levels by some undefined mechanism.

Although both fungal species caused increased dry matter yield, inoculation with *Glomus* sp. induced greater increases than with *Gigaspora margarita* in both

soils compared to the controls. Mycorrhizal plants are known to grow more actively and, as a result, have a higher ash content than those not infected. This is because in VAM plants the fungus stimulates respiration and additional release of H<sup>+</sup> ions from the host root for use in exchange reactions with the soil, leading to increased absorption of salts (Routien and Dawson 1943).

In either soil, all three legumes responded to *Glomus* sp. better than to *Gigaspora margarita*. Apart from the possibility that the two fungi have inherently different characteristics, one reason for their contrasting performances may be the different preculture host plants used (Simpson and Daft 1990). *Glomus* was cultured on soybean (a legume) whilst *Gigaspora margarita* spores were raised on wheat (a cereal). Furthermore, the effects of the VAM on each of the test crops relative to the controls were greater in the subsoil than in the topsoil. This might be due to an inhibition of VAM performance by the higher native fertility of the topsoil.

Finally, the differences between the topsoil and subsoil VAM/non-VAM ratios for groundnut compared to cowpea and pigeonpea were very small, i.e. under the conditions of this experiment the type of soil medium used was not as important for the growth of groundnut as it was for cowpea and pigeonpea. Further work on the ecophysiological factors operating in the soil-plant continuum in relation to VAM formation is necessary in order to understand the different responses of the legumes studied.

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