Biol Fertil Soils (1989) 8:311–318

Biology and Fertility of Soils

Competition among strains of *Bradyrhizobium* and vesicular-arbuscular mycorrhizae for groundnut (*Arachis hypogaea* L.) root infection and their effect on plant growth and yield*

P. T. C. Nambiar and V. Anjaiah

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh 502 324, India

Summary. Strains of *Bradyrhizobium* influenced root colonization by a species of vesicular-arbuscular mycorrhizae (VAM), and species of VAM influenced root nodulation by strains of *Bradyrhizobium* in pot experiments. In a field experiment, the effects of VAM on competition amongst inoculated bradyrhizobia were less evident, but inoculation with *Bradyrhizobium* strains increased root colonization by VAM. Certain VAM/*Bradyrhizobium* inoculum strain combinations produced higher nodule numbers. Plants grown without *Bradyrhizobium* and VAM, but supplied with ammonium nitrate (300 $\mu g\ ml^{-1}$) and potassium phosphate (16 $\mu g\ ml^{-1}$), produced higher dry-matter yields than those inoculated with both symbionts in the pot experiment. Inoculation with either symbiont in the field did not result in higher pod and haulm yields at harvest.

Key words: N_2 fixation – *Arachis hypogaea* – Vesicular-arbuscular mycorrhizae (VAM) – *Bradyrhizobium* – Groundnut

Many legumes benefit from the symbiotic association with bradyrhizobia and with VAM. In this tripartite association, the symbiotic partners interact with each other. Nodules formed by bradyrhizobia on legumes fix atmospheric N_2, while the major role of VAM is aiding plant nutrition, mainly by improving phosphate uptake (review by Hayman 1986). The extraradical VAM fungal hyphae are broadly analogous to extra root hairs that increase the volume of soil exploited for nutrients. By improving the P status, the VAM indirectly enhance the N status of the legumes (Hayman 1986). The benefits of VAM are mainly observed on plants grown with insoluble P sources, such as rock phosphate. Benefits from inoculation with VAM and bradyrhizobia have been reported in many legumes (Daft and El-Giahmi 1976; Smith and Daft 1977; Bagyaraj et al. 1979; Azcon-Aguilar et al. 1982; Parvathi et al. 1985; Hayman 1986; Karunaratne et al. 1987; Subba Rao and Krishna 1988). Inoculation with strains of bradyrhizobia have increased groundnut yields under certain circumstances. Inoculation with one strain, NC 92, has increased groundnut pod yields in many trials. However, inoculation with another strain, NC 43.3, did not, although strain NC 43.3 showed a greater N_2-fixing efficiency in glasshouse experiments and formed a similar percentage of nodules to that formed by NC 92 in the field in competition with native bradyrhizobia (Nambiar 1985). Inoculation with VAM species has also increased pod yields in fields (ICRISAT 1987). We examined the effect of these symbionts on each other in a pot experiment and in field experiments, in order to study competition among strains of *Bradyrhizobium* and VAM for groundnut (*Arachis hypogaea* L.) root infection, to determine the effects of both symbionts on plant growth, and to assess the usefulness of dual inoculation in the field is a viable strategy.

Materials and methods

Bradyrhizobium strains NC 92, NC 43.3, and TAL 176 were obtained from North Carolina State University, Raleigh, and NifTAL, Hawaii, USA). These three strains ranked high in N_2-fixing efficiency in pot experiments with over 50 strains. Strains FB 103, FB 105, and IC 6012 were isolated from nodules collected from groundnut plants grown in ICRISAT (Patancheru) fields, and were included in the pot experiment as representatives of the most efficient local field bradyrhizobia. The *Bradyrhizobium* inoculation was carried out as described by Nambiar et al. (1983, 1987). Species of my-

* ICRISAT Journal Article No. 886

Offprint requests to: P. T. C. Nambiar

corrhiza (Table 1) were obtained from D. J. Bagyaraj (University of Agricultural Sciences, Bangalore, India) and from A. S. Rao (Nagarjuna University, Guntur, India). The VAM species were multiplied on *Chloris gayana*, grown in pots containing a mixture of soilrite and perlite (1 : 1), and inoculated as described by Sreenivasa and Bagyaraj (1988a, b). The bradyrhizobia were inoculated at the rate of 10^8 cells seed^{-1}, and the VAM at 50 spores pot^{-1}.

Pot experiment. The plants were grown in a glasshouse at temperatures of 28° ± 4 °C and 23 ± 4 °C day and night, respectively. The medium used was a sterilized mixture of soilrite and perlite (Sreenivasa and Bagyaraj 1988a), at the rate of 500 g in a 15-cm diameter pot, and incorporating rock phosphate (5 g kg^{-1} of soilrite/perlite). The plants were supplied with a nutrient solution as described by Nambiar et al. (1983), except that no P was included. Other growth conditions were similar to those described by Nambiar et al. (1987). Eight seeds were sown per pot, which were thinned to three plants soon after emergence. Nine strains of VAM and one control P treatment were tested with two N-source treatments (Tables 1 and 2). Under the first treatment, seeds were inoculated with the following *Bradyrhizobium* strains: (1) A mixture of NC 92 and NC 43.3; (2) a mixture of NC 92 and three native strains (FB 103, FB 105, and IC 6012); and (3) a mixture of NC 43.3 and three native strains (FB 103, FB 105, and IC 6012; all mixtures contained 10^6 cells per seed of each strain). The second N-source treatment comprised plants grown without a source of symbiotic N but with mineral N only, supplied as ammonium nitrate (300 µg ml^{-1}). This was the control treatment. The treatments were arranged in a factorial design, with four replications. The volumes of nutrient solution added to each pot were: 100 ml until 10 days after sowing; 200 ml until 20 days after sowing; 300 ml until 30 days after sowing; 400 ml until 40 days after sowing; and 500 ml until 50 days after sowing. The plants were harvested 50 days after sowing. The cultivar used was Robut 33-1.

Field experiment. Two field experiments were conducted in Alfisol fields. Chemical properties of Alfisols at the ICRISAT Center, in general, are pH 6.3, organic C 0.62%, total N 0.06%, total P 140 mg kg^{-1} soil, and NO_3^- 1.7 mg kg^{-1} soil. All plots were fertilized with 40 kg ha^{-1} P_2O_5 as single superphosphate. The indigenous rhizobial population in these soils was 10^4 to 10^5 cells g^{-1} dry soil (by most probable number counts). Each plot comprised a 1.5-m bed with four rows 30 cm apart and plants spaced at 10 cm within the rows. Other growth conditions were as described earlier (Nambiar et al. 1984a). Inoculants, using γ-irradiated peat as a carrier, were used in these experiments. The inoculum was prepared by thoroughly mixing the peat in water (0.7 g litre^{-1}) and pouring the mixture below the seed (4–5 ml seed^{-1}) into the furrow, to give a minimum population of 10^6 cells seed^{-1}. Uninoculated plots, nodulated by the native *Bradyrhizobium* population, served as a control. In the first experiment, which was conducted during the 1986 rainy season, five genotypes (Robut 33-1, JL 24, J 11, PI 259747, and NC Ac 2821) inoculated with three strains of bradyrhizobia (NC 92, NC 43.3, TAL 176, and a non-inoculated control) were tested in a factorial design. The seeds were sown on 23 June 1986, and the plants were sampled 60 days after sowing and harvested on 20 October 1986. In the second experiment, tests were conducted during the 1986–1987 postrainy season on the nine strains of VAM and two strains of bradyrhizobia, with non-inoculated control treatments for both symbionts. The treatments were arranged in a split-plot design with strains of mycorrhiza as the main plot and strains of *Bradyrhizobium* as the subplot; there were four replications. The genotype used was Robut 33-1. The plants were irrigated during the postrainy season at intervals of 7–10 days, and were regularly sprayed for protection against insect pests. The crop was sown on 2 December 1986; the plants were sampled 70 days after sowing and harvested on 6 April 1987.

Observations. The nodules were separated and 50 nodules per treatment were typed for the idenity of the nodulated strain by enzyme-linked immunosorbent assay as described by Nambiar et al. (1984b). The roots were stained with 0.1% trypan blue, and the percentage of roots infected with mycorrhizae were recorded as described by Phillips and Hayman (1970). The N and P contents in plant tissues were determined by Technicon (Autoanalyser II, industrial method 218-72 A, 11, Technicon Industrial Systems, Tarrytown, NY 10591, USA).

Statistical analysis. The results were analyzed using conventional techniques. Wherever necessary, the data were transformed to the arcsine variable for the analysis of variance. All results were tested for significance at the 5% level of probability.

Table 1. Effect of vesicular-arbuscular mycorrhizae and bradyrhizobia on dry matter production (g plant^{-1}) in pot experiment

Strains of mycorrhiza	*Bradyrhizobium* strains			Ammonium nitrate[b]
	NC 92 + NC 43.3	NC 92 + 3 strains[a]	NC 43.3 + 3 strains[a]	
1. *Glomus fasciculatus* (D) E3	3.5	3.5	4.2	12.8
2. *Acaulospora* spp. (ICR)	3.3	3.5	4.6	12.5
3. *Glomus monosporus*	4.5	4.9	5.8	13.8
4. *Gigaspora calospora* (B) ICR	4.8	5.0	5.1	13.3
5. *Acaulospora laevis*	5.0	5.0	4.4	13.2
6. *Glomus fasciculatus*	3.5	4.8	4.5	15.8
7. *Glomus macrocarpus* (local)	4.0	5.7	4.9	13.3
8. *Gigaspora* spp.	4.0	4.8	4.4	12.8
9. *Gigaspora calospora* (Nedlands)	4.4	5.1	5.4	13.4
10. P control	4.1	7.5	7.9	15.8
LSD		1.37		

[a] FB 103, FB 105, IC 6012
[b] Values excluded for statistical analysis
SEM ±0.16 comparing *Bradyrhizobium* and mycorrhiza inoculation treatments

Results

Pot experiment

Plant growth and yield. Growth was highest in the plants supplied with ammonium nitrate, either inoculated with *Glomus fasciculatus* or grown with soluble P (Table 1). The local strains mixed with NC 92 or NC 43.3 showed a better N_2-fixing ability than the mixture of NC 92 and NC 43.3, as reflected in the dry-matter production of plants grown with soluble P. These results suggest strong interactions among *Bradyrhizobium* and VAM inoculation treatments. In some instances, there was a negative effect on plant growth from VAM colonization in the pot experiment. *Gigaspora* spp. inoculated with NC 92+NC 43.3 colonized 89% roots, but produced only 4 g dry matter plant^{-1}, whereas *Glomus monosporus* inoculated with NC 43.3 and local strains colonized only 7% roots, but produced 5.8 g dry matter plant^{-1} (Tables 1 and 2).

Effect of bradyrhizobia and VAM on competition. Strains of VAM significantly influenced nodule formation by strains of *Bradyrhizobium*. In the absence

Table 2. Effect of bradyrhizobia on root colonization by vesicular-arbuscular mycorrhizae (percentage infected roots) in pot experiment

Strains of mycorrhiza	*Bradyrhizobium* strains			Ammonium nitrate control
	NC 92+NC 43.3	NC 92+3 strains[a]	NC 43.3+3 strains[a]	
1. *Glomus fasciculatus* (D) E3	24	22	20	66
2. *Acaulospora* spp. (ICR)	44	36	46	84
3. *Glomus monosporus*	11	13	7	41
4. *Gigaspora calospora* (B) ICR	36	34	17	36
5. *Acaulospora laevis*	33	23	30	56
6. *Glomus fasciculatus*	17	24	11	68
7. *Glomus macrocarpus*	37	43	23	49
8. *Gigaspora* spp.	89	72	22	66
9. *Gigaspora calospora* (Nedlands)	27	52	24	57
10. P control[b]	ND	ND	ND	ND
LSD		45.7		

[a] FB 103, FB 105, IC 6012
[b] ND, not detected

Table 3. Effect of vesicular-arbuscular mycorrhizae on percentage of total nodules formed by strains NC 92 and NC 43.3 in pot experiment

Strains of mycorrhiza[b]	*Bradyrhizobium* strains			
	Nodules formed by NC 92		Nodules formed by NC 43.3	
	NC 92+NC 43.3	NC 92+3 strains[a]	NC 92+NC 43.3	NC 43.3+3 strains[a]
1. *Glomus fasciculatus* (D) E3	78	41	52	38
2. *Acaulospora* spp. (ICR)	63	38	55	39
3. *Glomus monosporus*	84	35	49	39
4. *Gigaspora calospora* (B) ICR	67	35	38	39
5. *Acaulospora laevis*	86	41	24	31
6. *Glomus fasciculatus*	49	38	68	31
7. *Glomus macrocarpus* (local)	68	41	51	39
8. *Gigaspora* spp.	63	31	61	31
9. *Gigaspora calospora* (Nedlands)	73	35	47	29
10. P control	89	34	34	20
LSD		12.9		

[a] FB 103, FB 105, IC 6012
[b] Strains 1, 2, 3, 4, 5, 7, and 9 were obtained from D.J. Bagyaraj and 6 and 8 from A.S. Rao

of any VAM, when mixtures of NC 92 and NC 43.3 were inoculated, strain NC 92 occupied more nodules (89%) than strain NC 43.3 (34%) (Table 3). In the presence of some strains of mycorrhiza also, NC 92 formed more nodules than strain NC 43.3; e.g., in the presence of *Acaulospora laevis*, 86% nodules in the NC 92+NC 43.3 mixture were formed by NC 92, but the presence of *Glomus fasciculatus* reduced the competitive ability of strain NC 92 (49% NC 92 nodules). Strain NC 92 was less competitive when mixed with local *Bradyrhizobium* strains; only 34% nodules were formed by NC 92 in the absence of VAM, but in this case, the addition of mycorrhizae did not significantly influence the competitive ability of NC 92 against the local strains. However, inoculation with VAM strains increased the formation of nodules by NC 43.3 when inoculated with the mixture of local strain.

Effect of Bradyrhizobium strains on VAM infection. In some instances, a higher root colonization by VAM was observed in the plants supplied with ammonium nitrate supplied than in the nodulated plants. Root colonization by *Gigaspora* spp. was significantly higher (72%) in the presence of NC 92 mixed with local

Table 4. Effect of vesicular-arbuscular mycorrhizae and bradyrhizobia on number of nodules plant^{-1} in pot experiment

Strains of mycorrhiza	*Bradyrhizobium* strains			Ammonium nitrate[b]
	NC 92+NC 43.3	NC 92+3 strains[a]	NC 43.3+3 strains[a]	
1. *Glomus fasciculatus* (D) E3	55	34	57	3
2. *Acaulospora* spp. (ICR)	52	35	57	9
3. *Glomus monosporus*	90	56	75	8
4. *Gigaspora calospora* (B) ICR	117	55	74	20
5. *Acaulospora laevis*	82	70	53	20
6. *Glomus fasciculatus*	81	51	62	5
7. *Glomus macrocarpus* (local)	80	65	79	15
8. *Gigaspora* spp.	102	47	61	3
9. *Gigaspora calospora* (Nedlands)	84	58	73	4
10. P control	143	112	136	15
LSD		30.6		

[a] FB 103, FB 105, IC 6012
[b] Values excluded from statistical analysis

Table 5. Effect of vesicular-arbuscular mycorrhizae and bradyrhizobia on N assimilation (mg plant^{-1}; dry matter percentage N in parentheses) in pot experiment

Strains of mycorrhiza	*Bradyrhizobium* strains			Ammonium nitrate
	NC 92+NC 43.3	NC 92+3 strains[a]	NC 43.3+3 strains[a]	
1. *Glomus fasciculatus* (D) E3	95 (2.7)	98 (2.7)	121 (2.9)	375 (2.9)
2. *Acaulospora* spp. (ICR)	91 (2.6)	104 (3.0)	136 (3.0)	351 (2.8)
3. *Glomus monosporus*	133 (3.0)	144 (2.9)	209 (3.6)	354 (2.6)
4. *Gigaspora calospora* (B) ICR	143 (3.0)	151 (3.0)	151 (3.0)	368 (2.8)
5. *Acaulospora laevis*	152 (3.1)	156 (3.1)	133 (3.0)	368 (3.5)
6. *Glomus fasciculatus*	100 (2.8)	141 (2.9)	131 (2.9)	363 (2.3)
7. *Glomus macrocarpus* (local)	115 (2.9)	166 (2.9)	146 (3.0)	365 (2.8)
8. *Gigaspora* spp.	109 (2.7)	150 (3.1)	123 (2.8)	347 (2.7)
9. *Gigaspora calospora* (Nedlands)	129 (2.9)	155 (3.0)	155 (2.9)	387 (2.9)
10. P control	118 (2.9)	228 (3.0)	238 (3.0)	420 (2.7)
LSD		64.4 (0.56)		

[a] FB 103, FB 105, IC 6012

Table 6. Effect of vesicular-arbuscular mycorrhizae and bradyrhizobia on P uptake (mg plant^{-1}; dry matter percentage N in parentheses) in pot experiment

Strains of mycorrhiza	*Bradyrhizobium* strains			Ammonium nitrate
	NC 92+NC 43.3	NC 92+3 strains[a]	NC 43.3+3 strains[a]	
1. *Glomus fasciculatus* (D) E3	12 (0.34)	11 (0.33)	13 (0.32)	36 (0.28)
2. *Acaulospora* spp. (ICR)	11 (0.34)	12 (0.36)	15 (0.33)	39 (0.31)
3. *Glomus monosporus*	17 (0.37)	16 (0.34)	18 (0.32)	40 (0.29)
4. *Gigaspora calospora* (B) ICR	16 (0.33)	15 (0.31)	15 (0.30)	39 (0.30)
5. *Acaulospora laevis*	16 (0.32)	16 (0.33)	15 (0.34)	38 (0.35)
6. *Glomus fasciculatus*	12 (0.34)	15 (0.32)	14 (0.32)	43 (0.27)
7. *Glomus macrocarpus* (local)	13 (0.34)	17 (0.31)	16 (0.33)	37 (0.28)
8. *Gigaspora* spp.	14 (0.35)	16 (0.33)	14 (0.31)	34 (0.26)
9. *Gigaspora calospora* (Nedlands)	14 (0.32)	16 (0.31)	18 (0.34)	43 (0.32)
10. P control	21 (0.50)	34 (0.46)	36 (0.46)	75 (0.47)
LSD		6.2 (0.056)		

[a] FB 103, FB 105, IC 6012

Table 7. Effect of *Bradyrhizobium* inoculation on root colonization by vesicular-arbuscular mycorrhizae (percentage infected roots) in the field during the 1986–1987 postrainy season

Strains of mycorrhiza	*Bradyrhizobium* strains		Non-inoculated (without bradyrhizobia)
	NC 92	NC 43.3	
1. *Glomus fasciculatus* (D) E3	27	33	31
2. *Acaulospora* spp. (ICR)	32	27	32
3. *Glomus monosporus*	29	51	24
4. *Gigaspora calospora* (B) ICR	40	31	42
5. *Acaulospora laevis*	49	50	49
6. *Glomus fasciculatus*	44	46	38
7. *Glomus macrocarpus* (local)	50	34	50
8. *Gigaspora* spp.	47	51	39
9. *Gigaspora calospora* (Nedlands)	37	24	37
10. Non-inoculated control (without mycorrhiza)	45	25	31
LSD		15.4	

strains, but only 22% in the presence of strain NC 43.3 (Table 2). The presence of NC 92 increased root colonization by *Gigaspora* spp. (72%–89%, compared to 23% in the absence of strain NC 92).

Effect of VAM on nodulation. Apart from their effect on the competitiveness of individual *Bradyrhizobium* strains, the VAM strains also influenced the total number of nodules formed on each plant (Table 4). For example, when mixtures of NC 92 and NC 43.3 were inoculated, the maximum nodulation among the VAM-inoculated plants occurred with *Gigaspora calospora* (117 nodules plant^{-1}), and the minimum with *Acaulospora* spp. (52 nodules plant^{-1}). However, the overall maximum nodulation was observed in the plants grown with soluble P alone.

Accumulation of N and P. The N and P accumulated in plants and the percentages of N and P at harvest also varied considerably with the bradyrhizobia-VAM combinations used (Tables 5 and 6). The application of ammonium nitrate did not significantly influence the percentage of N, but did promote a higher N uptake. Among the plants that were dependent on symbiotic N, those supplied with soluble P accumulated the highest N levels, followed by those inoculated with *Glomus monosporus* and NC 43.3 with the three local isolates (Table 5). The plants inoculated with local strains mixed with NC 92 and NC 43.3 and supplied with soluble P absorbed more P and N than those inoculated with mixtures of NC 92+NC 43.3. The highest level of P was accumulated in the plants supplied with soluble P and ammonium nitrate (Table 6). Among the VAM strains, *Gigaspora calospora* (Nedlands) in combination with NC 43.3 and local bradyrhizobia strains accumulated 18 mg P plant^{-1}, while NC 92+NC 43.3 with *Acaulospora* spp. accumulated 11 mg P plant^{-1}. The differences in the percentage of dry-matter P were greater than the differences in the percentage of N. Certain *Bradyrhizobium*-VAM combinations gave N levels comparable to those in the plants supplied with ammonium nitrate, but the P level was high in all soluble-P supplied plants, the highest being in those inoculated with mixtures of strains NC 92 and NC 43.3 (Tables 5 and 6).

Field experiments

Pod and dry-matter yields. In both field experiments, inoculation with VAM, alone or in combination with *Bradyrhizobium*, did not significantly influence the pod yields. No response to *Bradyrhizobium* or VAM inoculation was observed in these experiments (mean haulm yield 4200 kg ha^{-1}, and pod yield 4000 kg ha^{-1}).

Table 8. Effect of *Bradyrhizobium* inoculation on root colonization by vesicular-arbuscular mycorrhizae (percentage infected roots) in the field during the 1986 rainy season

Bradyrhizobium strain	Root colonization by VAM (%)[a]
Non-inoculated control	48[b]
NC 92	72
NC 43.3	66
TAL 176	63
LSD	11.5

[a] Means of five genotypes: Robut 33-1, JL 24, J 11, PI 259747, and NC Ac 2821; no VAM was inoculated
[b] Non-inoculated plants were nodulated by native bradyrhizobia

Table 9. Effects of vesicular-arbuscular mycorrhizae on the number of nodules[a] $plant^{-1}$ and the competitiveness of inoculated bradyrhizobia in the field during the 1986–1987 postrainy season

Strains of mycorrhiza[a]	*Bradyrhizobium* strains		Non-inoculated[d] (without bradyrhizobia)
	NC 92[b]	NC 43.3[c]	
1. *Glomus fasciculatus* (D) E3	135 (25)	106 (17)	132 (–)
2. *Acaulospora* spp. (ICR)	141 (30)	108 (30)	137 (–)
3. *Glomus monosporus*	128 (24)	146 (18)	143 (–)
4. *Gigaspora calospora* (B) ICR	165 (22)	121 (23)	100 (–)
5. *Acaulospora laevis*	141 (26)	122 (18)	132 (–)
6. *Glomus fasciculatus*	150 (21)	136 (22)	136 (–)
7. *Glomus macrocarpus* (local)	164 (27)	129 (25)	106 (–)
8. *Gigaspora* spp.	130 (31)	128 (19)	122 (–)
9. *Gigaspora calospora* (Nedlands)	121 (30)	175 (23)	137 (–)
10. Non-inoculated control (without mycorrhiza)	122 (26)	125 (20)	117 (–)
LSD		41.2 (8.7)	

[a] Percentage of nodules formed by inoculant strains given in parentheses; –, not detected
[b] Percentage of NC 92 nodules
[c] Percentage of NC 43.3 nodules
[d] Neither NC 92 nor NC 43.3 was detected in non-inoculated control

Root colonization by VAM. Inoculation with strains of bradyrhizobia had a marked effect on the VAM root colonization. It was not possible to differentiate colonization by native mycorrhizae and by inoculated VAM strains. *Glomus monosporus* inoculated with NC 43.3 gave 51% root colonization, but *Glomus monosporus* inoculated with NC 92, or without any added *Bradyrhizobium* inoculant, gave only 29% and 24% root colonization, respectively (Table 7). Inoculation with strain NC 92 resulted in better root colonization by native VAM (45% compared to 31% non-inoculated). This specificity was more evident in a second field experiment when four genotypes were tested. Since the genotype mean and genotype × *Bradyrhizobium* mean interactions were not significantly different, the means of four genotypes only are presented (Table 8). Inoculation with strain NC 92 increased root colonization by native VAM to 72%, while only 48% of the roots were colonized by VAM without additional *Bradyrhizobium* inoculation.

Nodules formed by bradyrhizobia. Both the *Bradyrhizobium* strains tested, NC 92 and NC 43.3, formed less nodules in the field (24%–33%) compared with pots when mixed with three isolates from similar fields (Table 9). The influence of inoculated VAM strains on the percentage of nodules formed by the inoculated *Bradyrhizobium* strain was only marginal. But inoculation with specific combinations of both symbionts under certain combinations increased the total number of nodules (Table 9; e.g., *Gigaspora calospora* (B) ICR and *Glomus macrocarpus* (local) inoculated with NC 92 and *Gigaspora calospora* (Nedlands) inoculated with NC 43.3).

Discussion

Plants grown without *Bradyrhizobium* inoculation and without mineral N showed stunted growth and started defoliating around 25 days after sowing; these were not included in the final observation. The results were similar when the plants were not supplied with any source of P.

The application of ammonium nitrate increased dry matter production threefold, but the N concentration in the plants was not affected. The *Bradyrhizobium* strains used in these experiments were among the top 10 strains for efficiency in N_2 fixation selected by screening experiments on 62 *Bradyrhizobium* strains

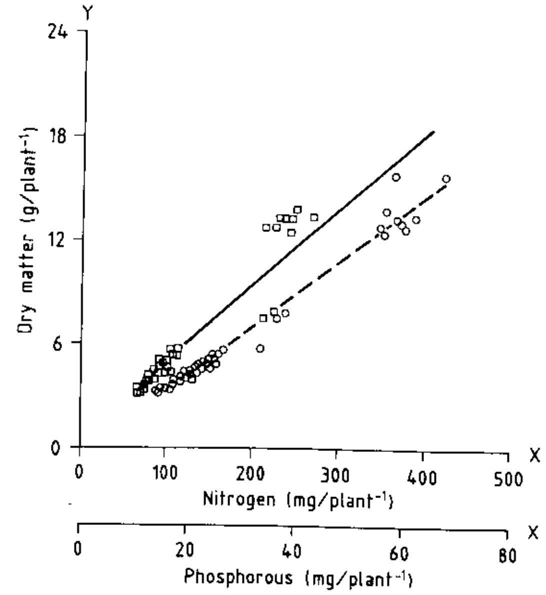

Fig. 1. Regression analysis showing relationship between plant growth and N and P uptake in pot experiment. Regression equations: ○, N uptake, $y = 0.038x - 0.057$ ($r^2 = 0.98$); □, P uptake, $y = 0.274x + 0.72$ ($r^2 = 0.85$)

from different parts of the world. In these, as well as in many other experiments conducted earlier (P. T. C. Nambiar and V. Anjaiah 1986, unpublished data) plants supplied with ammonium nitrate consistently grew better than those inoculated with the most efficient *Bradyrhizobium* strain. In contrast, in the field, the application of fertilizer N even at 200 kg N ha^{-1} did not significantly influence the growth and yield of this groundnut cultivar, Robut 33-1 (Nambiar et al. 1986). The difference between the root growth of potted plants and that of plants grown in the field, and the difference in the availability of nutrients and water could result in a variable nutrient uptake, but it is difficult to account for such a high level of variation between the pots and the field. The three most effective *Bradyrhizobium* strains from these fields were also included in the pot experiment, but the plants inoculated with these strains also grew poorly compared to those supplied with ammonium nitrate. There were no significant differences in N content.

Total N and P uptake is well correlated with plant growth in pot experiments ($r^2 = 0.98$ for N and $r^2 = 0.85$ for P; Fig. 1). The total nodule number and plant growth (excluding values for the ammonium nitrate treatment) were poorly correlated ($r^2 = 0.27$), mainly because of the interference from P sources. In earlier pot experiments when only N was the limiting factor, we observed a high correlation between nodule numbers and plant growth (Nambiar et al. 1983). This indicates that even if the number of nodules is increased as a result of *Bradyrhizobium* inoculation, under P-limiting conditions this may not result in better plant growth and yield.

Concentrations of N and P in the plants were influenced by VAM and the P status, and by the *Bradyrhizobium* and ammonium nitrate treatments. In the *Bradyrhizobium* treatment with mixtures of NC 92 and NC 43.3, there was a higher P uptake by the P-supplied plants, but this was not reflected in the dry matter; this high P uptake represents either luxury consumption or differences in metabolism under different nutritional conditions.

Species of VAM differ in their root colonization abilities and this is well documented (Hayman 1983). Strains of bradyrhizobia influence this process. Hicks and Loynachan (1987) observed that while the inoculation of soybeans with *Glomus mosseae* did not alter the competitiveness of *Bradyrhizobium* strains USDA 123 and USDA 110, P fertilization influenced the nodule occupancy of the native *Bradyrhizobium* strains in the field. They also observed that the relative competitive abilities of the two *Bradyrhizobium* strains were unrelated to the level of infection by *Glomus mosseae* in a glasshouse experiment. Ames and Bethlenfalvay (1987) observed that neither VAM nor P application affected the competitive interaction by bradyrhizobia for nodule formation on cowpea roots. Contrary to these reports, we observed that VAM strains influenced the competitiveness of *Bradyrhizobium* strains, and that *Bradyrhizobium* strains also influenced the root colonization by VAM.

Several *Bradyrhizobium* inoculation trials have been conducted in fields at the ICRISAT center (Nambiar 1985), and in none of these experiments have we observed an increase in the number of nodules as a result of *Bradyrhizobium* inoculation. However, the data presented here indicate that under certain combination of VAM and *Bradyrhizobium* inoculation, the total number of nodules was higher on plants grown in the inoculated plots. The metabolic processes in the rhizosphere are complex, and interactions between the symbiont and the host plant could influence the recognition, infection, and establishment of effective symbioses. The magnitude of these interactions could be large when the total microflora in the rhizosphere is considered. Ames et al. (1984) studied changes in rhizospheric bacterial populations in mycorrhizal and non-mycorrhizal roots, and observed that populations of only one bacterial isolate were significantly increased by the presence of *Glomus mosseae*. Krishna et al. (1984) reported an antagonistic interaction between a VAM fungus and an actinomycete. There are several other reports on similar interactions (Barea et al. 1975; Azcon et al. 1976; Bagyaraj and Menge 1978). These factors will influence experiments designed to test responses to inoculation in different locations and will make it difficult to explain any inconsistencies in yield responses. However, we have observed no significant responses to inoculation in the yields of field experiments.

Acknowledgment. We thank Drs. D.J. Bagyaraj and M.N. Sreenivasa (University of Agricultural sciences, GKVK Campus, Bangalore, India) and A.S. Rao (Nagarjuna University, Guntur, India) for the supply of strains of VAM and their help and advice on techniques, and Dr. K.L. Sahrawat for chemical analyses.

References

Ames RN, Bethlenfalvay GJ (1987) Localized increase in nodule activity but no competitive interaction of cowpea rhizobia due to pre-establishment of vesicular-arbuscular mycorrhiza. New Phytol 106:207–215

Ames RN, Reid CPP, Ingham ER (1984) Rhizosphere bacterial population responses to root colonization by a vesicular-arbuscular mycorrhizal fungus. New Phytol 96:555–563

Azcon R, Barea JM, Hayman DS (1976) Utilization of rock phosphate in alkaline soils by plants inoculated with mycorrhizal fungi and phosphate-solubilizing bacteria. Soil Biol Biochem 8:135–138

Azcon-Aguilar C, Barea JM, Azcon R, Olivares J (1982) Effectiveness of *Rhizobium* and vesicular-arbuscular mycorrhiza in the introduction of *Hedsarum coronarium* in a new habitat. Agric Environ 7:199–206

Bagyaraj DJ, Menge JA (1978) Interaction between a VA mycorrhiza and *Azotobacter* and their effects on rhizosphere microflora and plant growth. New Phytol 80:567–573

Bagyaraj DJ, Manjunath A, Patil RB (1979) Interaction between a vesicular-arbuscular mycorrhiza and *Rhizobium* and their effects on soybean in the field. New Phytol 82:141–145

Barea JM, Azcon R, Hayman DS (1975) Possible synergistic interaction between *Endogone* and phosphate-solubilizing bacteria in low-phosphate soils. In: Sanders FE, Mosse B, Tinker PB (eds) Endomycorrhizae. Academic Press, San Francisco, pp 409–417

Daft MJ, El-Giahmi AA (1976) Studies on nodulated and mycorrhizal peanuts. Ann Appl Biol 83:273–276

Hayman DS (1983) The physiology of vesicular-arbuscular endomycorrhizal symbiosis. Can J Bot 61:944–963

Hayman DS (1986) Mycorrhizae of nitrogen-fixing legumes. MIRCEN J 2:121–145

Hicks PM; Loynachan TE (1987) Phosphorus fertilization reduces VAM infection and changes nodules occupancy of field grown soybean. Agron J 79:841–844

ICRISAT (1987) Annual report. ICRISAT, Patancheru A.P. 502324, India

Karunaratne RS, Baker JH, Barker AV (1987) Benefits on mycorrhizae to soybeans grown on various regimes of nitrogen nutrition. J Plant Nutr 10:871–886

Krishna KR, Balakrishna AN, Bagyaraj DJ (1984) Interaction between a vesicular-arbuscular mycorrhizal fungus and *Streptomyces cinnamoneous* and their effects on finger millet. New Phytol 92:401–405

Nambiar PTC (1985) Response of groundnut (*Arachis hypogaea* L.) to *Rhizobium* inoculation in the field: Problems and prospects. MIRCEN J 1:293–309

Nambiar PTC, Ravishankar HN, Dart PJ (1983) Effect of *Rhizobium* numbers on nodulation and dinitrogen fixation in groundnut. Exp Agric 19:243–250

Nambiar PTC, Dart PJ, Srinivasa Rao B, Ravishankar HN (1984a) Response of groundnut (*Arachis hypogaea* L.) to *Rhizobium* inoculation. Oleagineix 39:149–155

Nambiar PTC, Srinivasa Rao B, Anjaiah V (1984b) Studies on competition, persistence and methods of application of a peanut *Rhizobium* strain NC 92. Peanut Sci 11:83–87

Nambiar PTC, Rego TJ, Srinivasa Rao B (1986) Comparison of the requirements and utilization of nitrogen by genotypes of sorghum (*Sorghum bicolor* (L.) Moench), and nodulating and non-nodulating groundnut (*Arachis hypogaea* L.). Field Crops Res 15:165–179

Nambiar PTC, Anjaiah V, Srinivasa Rao B (1987) Factors affecting competition of three strains of rhizobia nodulating groundnut, *Arachis hypogaea*. Ann Appl Biol 110:527–533

Parvathi K, Venkateswarlu K, Rao AS (1985) Response of groundnut (*Arachis hypogaea* L.) to combined inoculation with *Glomus mosseae* and *Rhizobium* sp. Proc Indian Acad Sci (Plant Sci) 95:35–40

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

Smith SE, Daft MJ (1977) Interactions between growth, phosphate content and nitrogen fixation in mycorrhizal and non-mycorrhizal *Medicago sativa*. Aus J Plant Physiol 4:403–413

Sreenivasa MN, Bagyaraj DJ (1988a) Selection of a suitable substrate for mass multiplication of *Glomus fascisulatum*. Plant and Soil 109:125–127

Sreenivasa MN, Bagyaraj DJ (1988b) *Chloris gayana* (Rhodes grass) a better host for the mass production of *Glomus fasciculatus* inoculum. Plant and Soil 106:289–290

Subba Rao NS, Krishna KR (1988) Interaction between vesicular-arbuscular mycorrhiza and nitrogen fixing microorganism and their influence on plant growth and nutrition. In: Subba Rao NS (ed) Biological nitrogen fixation: Recent developments. Oxford and IBH Publication, New Delhi, India, pp 53–71

Received March 12, 1989