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EFFECT OF VESICULAR - ARBUSCULAR MYCORRHIZAE ON SEEDLINGS OF *HEVEA* AND *PUERARIA PHASEOLOIDES*

BY

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ABSTRACT

Hevea seedlings showed a slight growth response when inoculated with Gigaspora margarita out of four species, tested in sterilized soil. There were no significant differences in the uptake of nutrients by mycorrhizal Hevea plants.

Non-mycorrhizal Pueraria plants grew very poorly unless they were given liberal quantities of phosphate fertilizer, confirming previous reports. All mycorrhizal plants took up more nutrients from soil than non-mycorrhizal plants. Effective nodule formation was significantly greater in mycorrhizal plants when phosphorus was added.

INTRODUCTION

Infection by vesicular-arbuscular (VA) mycorrhizae has improved growth and nutrient uptake in numerous plant species (Covey *et al* 1981, Geddeda *et al* 1983, Mosse *et al* 1980, Crush 1972, Powell 1979, Howelar *et al* 1982, Mosse *et al* 1982.) Recent studies indicate some VA mycorrhizal species are more effective than others under given conditions (Graw 1979; Skipper *et al* 1979) and can benefit some plants but not others, (Daft *et al* 1966, Ramirez *et al* 1975).

Wastie (1965) reported that although *Hevea* plants were invariably infected by VA mycorrhizae, there was no growth response due to their presence.

It is important to find out whether VA mycorrhizae help *Hevea* and other plants in the tropics to absorb nutrients; because of the availability of apatite, an insoluble phosphate rock, used as a P source in Sri Lanka.

In this paper, the studies carried out to determine the growth responses of *Hevea* seedlings and *Pueraria* plants (which are grown as a ground cover) when inoculated with different mycorrhizae and grown in sterilized and unsterilized soil are reported.

MATERIALS AND METHODS

The lateritic clay loam soils (pH 4.5), deficient in available phosphorus (3.6 ppm NH_4 / HCl extractable P) obtained from Dartonfield Estate were used. Large cement pots (31 cm diameter and 36 cm deep) which were sterilized with 10% formaldehyde were filled with either sterilized (twice CH_3Br fumigated soil) or unsterile soil. Half (50) the pots were filled with fumigated soil and the other half with unsterilized soil. Each treatment was repeated at two levels of phosphorus. Treatments with added P received 7 g of rock phosphate (500mg/kg soil) which was mixed thoroughly with soil. Soils containing spores obtained from green house pot cultures were used as the mycorrhizal inoculum. To obtain a roughly equal level of inoculum, spore numbers were determined in each pot culture before taking the inoculum, using the wet-sieving decanting technique (Gerdemann and Nicolson 1963). Soil samples that carried about 500 spores (per/pot) were used as the inoculum.

The mycorrhizal types used were, *Glomus fasciculatus* (M_1), *Glomus mosseae* (M_2), E_8 type (M_3) and *Gigaspora margarita* (M_4), a mycorrhiza free (Mo) soil was used as control. These four mycorrhizal types were selected because these were the only types available in pure culture form in our stock cultures. All five treatments were duplicated with (+P) and without (−P) added phosphorus. All plants not receiving mycorrhizal inoculum received 100 ml of twice-filtered washings from all inoculum types used. This is to ensure that, except for VA mycorrhizal spores, all treatments were exposed to the same micro-organisms. The soils containing inoculum were placed, in the form of a thin evenly spread layer, 5-6 cm below the surface soil in pots. Six equal sized *Hevea* seedlings, which were germinated on a sterile sand bed, were transplanted into each pot. When the plants were well established, they were thinned down to four per pot. Pots were arranged according to a randomised block design in the field. After 18 weeks of growth, the height of plants, fresh and dry weights of shoots and roots were recorded separately. Dried ground leaf samples were used to analyse the nutrient (N,P,K,Ca,Mg) content of leaves using the Bray 1 method and a Technicon Auto Analyzer. Root percentege infection was determined after staining with Trypan blue (Phillips and Hayman 1970).

The same experimental procedure was carried out with *Pueraria phaseoloides*. Twelve seeds which were acid treated inoculated with *Rhizobium* (CB 756) were sown into each pot. When the plants were well established they were thinned down to six per pot. A basal fertilizer doze minus phosphorus was applied once a month. Three assessments of shoot dry matter content were taken at an interval of about 2 months between assessments. Root and effective nodule dry weights as well as root percentage infection and leaf nutrient content were determined.

RESULTS

Hevea plants

All four endophytes readily infected both *Hevea* and *Pueraria* plants. There was on significant difference in height between *Hevea* plants under different treatments. The dry weights of stem, leaves plus petioles, roots and the leaf nutrient contents are shown in

Table 1. There were no significant growth differences between the mycorrhizal and non-mycorrhizal *Hevea* seedlings, except in the case of dry weights of leaves plus petioles of mycorrhizal plants (*Gigaspora margarita*) in sterilized soil. The dry weights of leaves+ petioles of these mycorrhizal plants were significantly greater than non-mycorrhizal plants in sterilized soil.

Root percentage infection at the end of the experiment was significantly lower in sterilized soil than in unsterilized soil. Added P tends to lower the level of infection in both soils, but the differences were significant at 5% level only when roots were infected with *Glomus fasciculatus* and *Gigaspora margarita*, in sterilized soil.

The percentage P levels of plants grown in sterilized soil were always significantly lower than plants grown in unsterilized soil. Plants with added rock phosphate had higher levels of P than those without. The N and K content of plants grown in unsterile soil were significantly higher than plants grown in sterile soil. Further the K percentage in leaves of the plants inoculated with *Gigaspora margarita* with added P were significantly higher than non-mycorrhizal plants with and without added phosphorus as well as *Gigaspora margarita* inoculated mycorrhizal plants without added P. There were no significant differences seen in the case of leaf percentage nitrogen content. The differences in leaf calcium percentage between plants inoculated with *Glomus fasciculatus* with added P and non-mycorrhizal plants with and without added phosphorus were significant.

Pueraria plants

Table 2 shows that shoot dry matter content in all three assessments were significantly greater in sterilized soil than in unsterile soil, except in the case of M_0 treatment. It is also observed that the shoot dry matter content of mycorrhizal plants were significantly higher than non-mycorrhizal plants, in sterilized soil. All mycorrhizal plants in sterilized soil with added rock phosphate grew significantly better than plants in unsterilized soil with added P. The differences in shoot and root dry matter content with and without P were significantly different in both soil treatments. Effective root nodule dry weights were significantly greater in sterile than in non-sterile soil. Further, the mycorrhizal plants had a significantly greater nodule dry weight than non-mycorrhizal plants in sterile soil. All mycorrhizal *Pueraria* plants in sterilized soil, except *Glomus mosseae* inoculated plants, contained a higher percentage of phosphorus than non-mycorrhizal plants. There were also a higher level of phosphorus in plants grown in unsterile soil than plants in sterilized soil. In sterilized soil, the highest amount of P were taken up by the plants inoculated with the E_s type, followed by *Glomus fasciculatus* and *Gigaspora margarita.*

As in the case of phosphorus, the percentage potassium content of plants grown in unsterile soil were significantly higher than plants grown in sterilized soil. The differences in percentage K content between plants with and without added phosphate were highly significant. Plants with added P always had a high level of K. Further the plants grown in sterilized soil had a significantly lower percentage of N than plants grown in unsterile soil. The mycorrhizal plants with added rock phosphate had a higher percentage of N than non-mycorrhizal plants.

Table 1. Mean stem, leaves + petioles and root dry weights (g), height (cm) and percentage root infection of Hevea *seedlings (per pot) inoculated with four types of va mycorrhizae in sterilized and in unsterilized soil at two levels of P.*

Treatment	Dry weights (g) Stem	Leaves Petioles	Roots	Height (cm)	Leaves nutrient Percentages (%) N	P	K	Ca	Mg	Root percentage infection
Sterile soil										
M_1 + P	36.3	18.0	31.1	65.1	2.54	0.091	0.973	0.481	0.170	11
M_1 — P	36.3	17.8	33.4	63.8	2.49	0.066	0.934	0.620	0.174	26
M_2 + P	28.3	19.9	30.5	58.7	2.70	0.110	1.094	0.573	0.160	30
M_2 — P	34.8	22.1	32.4	63.6	2.58	0.081	0.989	0.618	0.127	33
M_8 + P	36.3	20.1	34.7	61.6	2.39	0.085	1.001	0.513	0.144	5
M_8 — P	30.4	13.9	31.2	47.8	2.36	0.080	0.880	0.529	0.163	10
M_4 + P	33.2	26.0	34.4	62.6	2.80	0.110	1.113	0.551	0.154	36
M_4 — P	37.4	20.8	32.2	63.2	2.61	0.095	0.975	0.561	0.143	53
M_O + P	27.2	15.3	30.9	56.0	2.77	0.100	1.122	0.625	0.145	Nil
M_O — P	28.6	17.3	27.2	58.8	2.36	0.076	0.973	0.627	0.190	Nil

Unsterile soil

M_1 + P	30.6	20.0	28.8	61.8	3.01	0.120	1.156	0.406	0.156	36
M_1 — P	32.8	20.3	30.7	64.4	2.80	0.100	1.362	0.883	0.157	42
M_2 + P	29.7	21.9	31.8	61.6	2.74	0.117	1.383	0.561	0.147	31
M_2 — P	28.9	18.1	26.3	58.1	2.69	0.103	1.329	0.611	0.172	38
M_3 + P	32.7	22.7	35.3	64.4	2.92	0.123	1.393	0.651	0.169	41
M_3 — P	26.1	19.6	29.6	60.6	2.62	0.110	1.205	0.518	0.150	48
M_4 + P	29.3	18.6	34.3	63.4	3.10	0.133	1.520	0.771	0.163	61
M_4 — P	30.1	19.2	26.7	61.4	2.72	0.100	1.161	0.595	0.132	63
M_0 + P	32.3	21.4	40.9	63.1	2.78	0.111	1.150	0.631	0.147	44
M_0 — P	28.7	17.4	28.1	59.7	2.91	0.119	1.369	0.586	0.136	64
*LSD I	+NS	5.95	NS	NS	NS	NS	0.170	0.1502	NS	9
**LSD II	NS	NS	NS	NS	NS	NS	NS	0.1502	NS	9

*LSD I — Within a main treatments

**LSD II — Between main treatments

+ NS — not significant.

Table 2. Mean shoot, root dry weights (g) (per pot) and shoot nutrient content of pueraria *plants inoculated with four types of va mycorrhizae in sterilized and in unsterilized soil*

Treatment	Dry weight (g) shoot					Leaves nutrient content (%)			Effective root nodule dry wt. (g)	Root % infection
	(1st)	(2nd)	(3rd)	Total	Root	N	P	K		
Sterilized soil										
M_1 + P	31.8	34.9	57.9	124.6	62.6	3.80	0.117	1.278	0.853	38.6
M_1 — P	17.1	22.1	31.2	70.4	43.9	3.42	0.089	1.857	0.330	46.8
M_2 + P	40.6	49.3	62.2	156.1	79.3	3.35	0.089	1.345	1.003	48.6
M_2 — P	24.8	32.1	29.3	86.2	51.4	3.74	0.099	1.825	0.305	56.0
M_3 + P	33.5	44.4	62.2	140.1	77.3	3.90	0.140	1.502	0.649	41.6
M_3 — P	22.8	28.4	24.9	76.1	49.2	3.42	0.085	1.738	0.670	67.2
M_4 + P	41.6	51.3	67.2	160.1	75.8	3.87	0.118	1.310	1.027	52.0
M_4 — P	21.9	26.5	28.3	76.7	47.1	3.42	0.090	1.690	0.523	61.2
M_0 + P	17.1	27.3	57.1	101.5	59.2	3.14	0.088	1.467	0.856	Nil
M_0 — P	9.7	17.9	23.0	50.6	41.2	3.52	0.096	1.527	0.311	Nil

Unsterilized soil

M_1 + P	25.4	43.1	50.5	119.0	60.4	4.01	0.140	1.443	0.411	65.6
M_1 — P	12.5	11.7	26.2	50.4	26.0	3.80	0.107	2.125	0.222	74.8
M_2 + P	27.2	41.7	53.3	122.2	64.5	4.15	0.127	1.895	0.599	58.8
M_2 — P	10.9	15.3	19.8	46.0	31.4	3.84	0.134	2.019	0.141	66.0
M_3 + P	24.5	51.2	55.4	131.1	51.9	3.90	0.151	1.833	0.659	36.4
M_3 — P	10.3	15.8	33.3	59.4	32.5	3.58	0.107	2.204	0.164	45.0
M_4 + P	25.6	40.6	58.4	124.6	63.7	3.80	0.135	1.848	0.899	82.4
M_4 — P	11.3	13.7	19.6	44.6	35.1	3.96	0.109	2.288	0.118	83.8
M_0 + P	24.0	43.3	52.0	119.3	60.2	4.20	0.122	1.917	0.545	59.8
M_0 — P	8.6	15.1	21.6	45.3	29.2	4.15	0.140	2.320	0.141	70.6
*LSD I	4.42	5.40	+NS	12.25	NS	NS	NS	0.060	0.232	8.51
**LSD II	6.75	8.97	NS	16.32	NS	NS	NS	NS	0.310	8.67

*LSD I — Within a main treatment

**LSD II — Between main treatments

+ NS — not significant

Root percentage infection of plants grown in non-sterile soil were significantly higher than plants grown in sterile soil. Addition of rock phosphate lowered the percentage root infection.

DISCUSSION

In these studies it is been reported for the first time that the *Hevea* plants show a growth response to the inoculation with VA mycorrhizae (*Gigaspora margarita*). Therefore it is evident that certain VA mycorrhizal species can have a beneficial effect on *Hevea* plants. However, before coming to any conclusion, further experimental details under different environmental conditions are necessary.

The structure and physio-chemical properties of soil may be altered during the process of sterilization, which usually releases soil nutrients such as N, P, K into the soil. Therefore it is to be expected that plants grown in sterilized soil will have high levels of nutrients. But sterilization with CH_3Br has increased the soil pH from 3.4 to 4.1. Therefore, more essential nutrients should be released into the soil solution of unsterile soil which is more acidic. Significantly higher levels of nutrients in unsterile soil could be attributed to this and also to the higher root percentage infection in these plants.

There were interactions between added phosphorus and Ca^{2+} ion content in the shoots of *Hevea* plants. In sterilized soil Ca^{2+} ion uptake showed an inverse relationship to added phosphorus while K^+ and N^+ ion showed a direct relationship. In contrast to this in unsterilized soil there was an improved uptake of Ca^{2+} ions with the addition of phosphorus. These differences in Ca^{2+} ion uptake in sterilized and unsterilized soil may be caused by differences in soil pH. The release of Ca^{2+} ions from rock phosphate were much greater in the more acidic soils.

These results confirmed the findings of Waidyanatha *et al* (1979), that plant growth and symbiotic nitrogen fixation of *Pueraria* plants was inhibited in sterilized soil, unless the plants were infected with VA mycorrhizae or supplied with liberal quantities of phosphate fertilizer.

As in the case of *Hevea* plants the higher nutrient levels of *Pueraria* plants in unsterile soil could be attributed to the pH differences in soil and higher percentages of root infection. All mycorrhizal *Pueraria* plants had a higher level of N, P, K than non-mycorrhizal plants. This confirmed the earlier findings of many workers for other plants (Mosse 1973, 1977; Gerdemann 1975). In rubber plantations, *Pueraria* covers are rarely fertilized although the levels of available phosphorus in the soil in most situations are sub-optimal for plant growth. Therefore it appears that efficient types of mycorrhizal inoculation will be a very important requirement for satisfactory growth of these plants in such soils.

As in *Hevea* plants, *Gigaspora margarita* was the most efficient variety among the VA mycorrhizas tested for *Pueraria* plants. Inoculation with it has stimulated growth of *Pueraria* plants significantly in unsterile soil too, showing that there is a strain superiority over the natural population of endophytes. Therefore before coming to any conclusion, *Gigaspora margarita*, an exotic species for our soils should be tried out

in more detail on both *Hevea* and *Pueraria* plants under our soil conditions. Strain superiority over the natural populations is very important. Thus if it can be grown in competition with indegenous species it will be possible to inoculate with these highly efficient species of VA endophytes in the field to obtain growth improvements. But these growth responses were more pronounced only at low levels of soil phosphorus.

These experiments also confirm the findings of Crush (1974) and Daft and El-Giahmi (1975), that VA mycorrhizae can have an important effect on nodulation and nitrogen fixation. There was no comparable effect of endophyte inoculation on nodulation in unsterile soil. Effective nodule formation with added rock phosphate was much greater with added rock phosphate than without. The role of phosphorus in symbiotic nitrogen fixation is not completely understood. As in many other plants (Mosse 1973, 1977 and Gerdemann 1975) this improved uptake of phosphorus from soil should be the major factor contributing to the stimulated growth of *Pueraria* plants.

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