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EFFECTS OF MYCORRHIZAL INOCULATION ON GROWTH, NITROGEN AND PHOSPHORUS CONTENTS OF TWO TROPICAL FORAGE LEGUMES

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INTRODUCTION

The use of forage legumes associated with tropical grasses to increase forage production is a common pratice to reduce the requirement of N fertilization, and also because the forage legumes have better nutritious value. Phosphorus is often a growth limiting factor to many tropical forage legumes (FRANÇA & CARVALHO, 1970; JONES & FREITAS, 1970; WERNER & MONTEIRO, 1974; PAULINO *et alii*, 1985). For legume establishment and optimum growth large applications of P fertilizer are required.

A possible alternative mechanism for maximizing fertilizer efficiency is via inoculation with vesicular-arbuscular mycorrhizal (VAM) fungi. Such a mycorrhizal effect is mainly due to enhance nutrient uptake (translocation and transfer of phosphate ions from soil solution to the root cells), and consequently plant growth, through an extensive network of external mycelium (HAYMAN, 1975; MOSSE, 1981).

However, most studies with the VAM fungi include temperate species, e.g., lucerne *Medicago sativa* (AZCÓN-AGUILAR & BAREA, 1978), white clover *Trifolium repeos* (NEWBOULD & RANGELEY, 1984). There is little information on tropical forage legumes (CRUSH, 1974; PAULINO *et alii*, 1986). Other studies are needed on the effects of VA mycorrhizae on some promising forage legumes.

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The aim of this paper was to evaluate the effects of three VAM fungi and the interactions with three levels of phosphate fertilizer on growth and N and P uptake in two tropical forage legumes.

MATERIAL AND METHODS

Site and Soil

The trial was carried out in 1986 in Porto Velho, State of Rondonia, Brazil.

The top 20 cm of Yellow-Latosols clay (Oxissol) was collected from the Experimental Station of UEPAE--EMBRAPA, Porto Velho city, the low-P soil was air-dried and sieved through a 4 mm mesh screen.

The soil had an initial pH of 4.2 (1:2 soil:water suspension) and P, Ca, Mg, Al and K concentrations of 2, 200, 84, 297 and 75 ppm, respectively.

The soil was steam sterilized at 100°C for one hour during three consecutive days to destroy its indigenous endophites and then it was distributed in pots (2,5 kg/pot).

Treatments

There were three phosphate treatments: P_0 (without P addition), P_1 (25 kg of P_2O_5/hectare), P_2 (50 kg of P_2O_5/hectare). The fertilizer employed was soluble calcium phosphate containing 20% of P_2O_5. It was used alone or combined with addition of three VA mycorrizal endophytes: *Glomus macrocarpum*, *Glomus etunicatum* and *Gigaspora margarita*. The VA mycorrhizal inoculum consisted of spores, hyphae and infected root fragments thoroughly homogenized and divided into similar aliquots.

Experimental design

The pots were placed on greenhouse benches in a randomized blocks design with a 3 (phosphate levels) x 3 (mycorrhizae species) factorial arrangement with three replications per treatment.

Growth of plants

The forage legumes used in the experiment were: *Centrosema macrocarpum* cv. Ciat 5065 and *Pueraria phaseoloides* cv. Ciat 9900. Seeds were scarified with sandpaper. Ten seeds of the corresponding legumes were sown per pot, and the plants were thinned to five per pot 10 days after emergence, and were harvested after 12 weeks.

Plants were watered daily with distilled water to maintain soil moisture near field capacity. The average maximum and minimum greenhouse temperatures were 35°C and 23°C, respectively.

Measurements

Shoots and roots were dried at 70°C during 48 h and weighted. The shoots were analysed for P and N. The percentage of mycorrhizal roots length was determined by the gridline intersect method (GIOVANNETTI & MOSSE, 1980) after roots segment were stained (PHILLIPS & HAYMAN, 1970; HAYMAN *et alii*, 1976).

The statistical evaluation was made by analysis of variance and the means compared by Tuckey's test.

RESULTS

Centrosema growth mycorrhizal root colonization

Mycorrhizal inoculation improved ($P < 0.05$) shoots yields of centro. In the absence of phosphate additions the inoculation VA fungi increased the yield of this forage legume about 3 to 4 fold (table 1), and there was no difference among micorrhizae fungi. However in presence of P_1 rate only *Gigaspora margarita* inoculation increased the yield by about a further 60% ($P < 0.05$). In the highest dose of P_2 both *Glomus etunicatum* and *Gigaspora margarita* increased the growth a further 17 and 46%, respectively.

Control centrosema plants and those inoculated with *Glomus etunicatum* and *Gigaspora margarita* showed an increasing response to P fertilizer but none response to P was obtained in *Glomus macrocarpum* inoculated plants (table 1).

The VA mycorrhizal fungi differed in levels of root mycorrhizal colonization of centrosema (table 1). *Gigaspora margarita* - inoculated plants had more colonization (42.3 - 57.0%) than *G. macrocarpum* - inoculated plants (37.6 - 39.4%) and *G. etunicatum* - inoculated plants (35.2 - 41.7%). The levels of soluble phosphate added significantly (P < 0.05) depressed mycorrhizal colonization by *Gigaspora margarita* elsewhere P fertilizer slightly depressed the roots colonization of *G. macrocarpum* and *G. etunicatum*.

Pueraria growth and mycorrhizal root colonization

The growth of *Pueraria* was significantly (P < 0.05) increased by mycorrhizal inoculation (table 1). Figure 1 shows the development of *Pueraria* plants under the influence of the different fungi inoculation treatments. Results are given as percentage of increase due to VA fungi over controls without VA inoculum by comparing the yields of mycorrhizal plants vs. non mycorrhizal plants (MENGE *et alii*, 1982; PLENCHETTE *et alii*, 1983).

For *Pueraria* legume, in the absence of phosphate (fig. 1), *G. macrocarpum* and *G. margarita* were more effective than the *G. etunicatum* in terms of mycorrhizal efficiency, although in the presence of phosphate the three VA fungi had positive effects, there were no significant differences among the VA fungi.

The percentage of root colonization ranged from 53.2 to 79.8 *G. margarita* inoculated plants presented the highest level of root colonization. For *G. macrocarpum* and *Gigaspora margarita* fungi P fetilizer significantly (P < 0.05) reduced the root colonization while for *G. etunicatum* the levels of phosphate added slightly decreased colonization rates.

Table 1 - Effects of mycorrhizae fungi (M) and soluble calcium phosphate (P) on *Centrosema macrocarpum* and Pueraria phaseoloides growth and mycorrhizal root colonization.

TREATMENTS		*Centrosema*		*Pueraria*	
P	M	Shoots dry weight (g/plant)	Root colonization (%)	Shoots dry weight (g/plant)	Root colonization (%)
P	C	0.64	-	1.13	-
	Gm	2.37	39.4	3.12	65.1
	Ge	2.23	41.7	1.44	63.2
	Gim	1.19	57.0	3.26	79.8
P	C	2.41	-	1.92	-
	Gm	2.34	38.5	2.98	59.3
	Ge	2.40	38.3	4.07	60.4
	Gim	3.83	47.2	3.30	73.6
P	C	3.57	-	2.90	-
	Gm	2.39	37.6	3.11	53.2
	Ge	4.16	35.2	2.93	58.5
	Gim	5.22	42.3	3.38	71.8
Tukey (M/P) 0.05		0.76	6.2	1.30	9.2

P_0 = no phosphate addition; P_1 and P_2 = addition of 25 and 50 kg P_2O_5/hectare respectively; C = non mycorrhizal plants; Gm = inoculated with Glomus macrocarpum; Ge = inoculated with Glomus etunicatum; and Gim = inoculated with Gigaspora margarita.

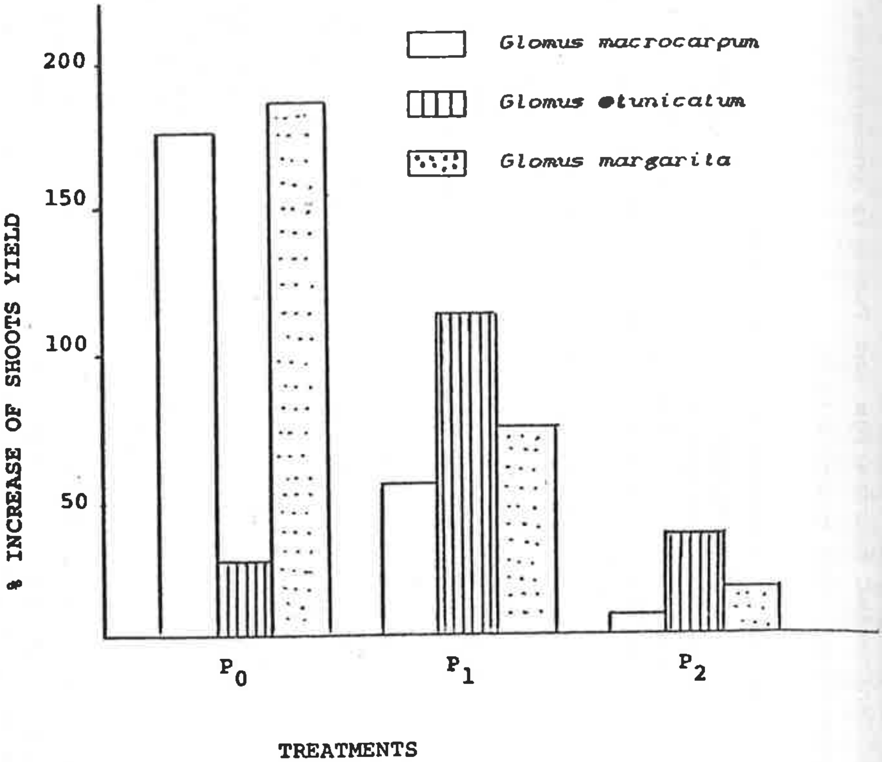

Figure 1 - Effect of different VA fungi inoculation treatments in presence of level phosphates P_0 = no phosphate addition, P_1 and P_2 = addition of 25 and 50 kg P_2O_5/hectare, respectively) on the increment (%) of shoots yield in *Pueraria*.

N and P concentrations and uptake

Centrosema

According to results (table 2) the mycorrhizal inoculation significantly increased the plant nutrition uptake (N and P). Plants inoculated with *G. margarita* and in presence of P_1 or P_2 treatments took up significantly ($P < 0.05$) more N and P than the other VA fungi.

Table 2 - Effects of different mycorrhizae fungi (M) and soluble calcium phosphate (P) on the nitrogen and phosphorus concentrations and uptake in *Centrosema*. (Shoots dry matter).

TREATMENTS		N	TOTAL N	P	TOTAL P
P	M	%	mg	%	mg
P_0	C	3.43	21.9	0.10	0.68
	Gm	3.08	73.0	0.13	3.12
	Ge	3.22	68.0	0.13	2.91
	Gim	3.21	64.0	0.14	2.84
P_1	C	2.84	68.4	0.14	5.02
	Gm	3.32	77.7	0.14	3.28
	Ge	2.84	68.2	0.14	3.36
	Gim	2.52	97.0	0.15	5.75
P_2	C	2.72	97.1	0.16	5.87
	Gm	3.04	72.6	0.17	4.06
	Ge	2.58	107.3	0.17	7.07
	Gim	2.36	123.2	0.17	8.87
Tukey (M/P) 0.05		0.16	17.50	0.008	1.26

P_0 = no phosphate addition; P_1 and P_2 = addition of 25 and 50 kg P_2O_5/hectare respectively; C = non mycorrhizal plants; Gm = inoculated with *Glomus macrocarpum*; Ge = inoculated with *Glomus etunicatum*; and Gim = inoculated with *Gigaspora margarita*.

Pueraria

The nutrients absorbed by *Pueraria* plants under different treatments are shown in table 3. Mycorrhizal infection significantly ($P < 0.05$) increased tissue P concentration and total P uptake at almost all fertilizer rates. P contents in all plants remained low, but mycorrhizal inoculation alleviated with *Glomus etunicatum* and simultaneous P fertilizer had the highest P uptake. Conversely in non-fertilizer plants *Glomus macrocarpum* and *G. margarita* were the treatments responsible for a highest plant nutrient uptake (N and P).

Table 3 - Effects of different mycorrhizae fungi (M) and soluble calcium phosphate (P) on the nitrogen and phosphorus concentrations and uptake in *Pueraria* (Shoots dry matter).

TREATMENTS		N	TOTAL N	P	TOTAL P
P	M	%	mg	%	mg
P_0	C	3.52	39.9	0.13	1.44
	Gm	2.62	82.0	0.15	4.56
	Ge	3.07	44.0	0.15	2.19
	Gim	2.67	87.0	0.14	4.56
P_1	C	2.74	52.6	0.15	3.00
	Gm	2.90	86.4	0.15	4.47
	Ge	2.26	192.0	0.15	6.96
	Gim	2.52	83.1	0.14	4.93
P_2	C	2.46	55.0	0.17	4.94
	Gm	2.38	74.0	0.18	5.45
	Ge	2.46	72.0	0.19	7.47
	Gim	2.35	80.0	0.17	5.74
Tukey (M/P) 0.05		0.19	24.8	0.006	1.71

P_0 = no phosphate addition; P_1 and P_2 = addition of 25 and 50 kg P_2O_5/hectare respectively; C = non mycorrhizal plants; Gm = inoculated with *Glomus macrocarpum*; Ge = inoculated with *Glomus etunicatum*; and Gim = inoculated with *Gigaspora margarita*.

DISCUSSION

This investigation supports previous work (CRUSH, 1974; MOSSE, 1977; PAULINO, 1986) stating that in low-phosphate soils, plant growth and a satisfactory nutrient uptake are dependent on the mycorrhizal symbiosis.

There was a response influenced by VA fungi and the added P fertilizer. In this experiment the high rates of root colonization by VAM improved the nutrition of Centro and *Pueraria*, mainly in phosphorus. Non-mycorrhizal plants absence of P fertilizer had reduced growth, probably because in such a P-deficient test soil, P was a limiting factor for plant growth (FRANÇA & CARVALHO, 1970; JONES & FREITAS, 1970; WERNER & MONTEIRO, 1974; PAULINO *et alii*, 1985) unless the plants were mycorrhizal.

Gigaspora margarita was the most efficient microssymbiont for centrosema plant growth, however *G. etunicatum* was more effective for *Pueraria*.

Current literature commonly indicates that the most important relationship of specificity in VAM occurs between fungus and soil, for example *Gigaspora margarita* is more adapted to low-P acid soils than *G. mosseae*, which did not establish well in acid soils, sometimes even after liming (DAVIS *et alii*, 1983). The present work agrees with these observations and stressed that *G. macrocarpum* was less adapted in acid soils conditions. The original soil employed in the present work had high aluminium contents (3.3 m.eq/100 g soil). All ions were a very important component of the fungistatic property against the VAM symbiosis (SIQUEIRA *et alii*, 1984). On the other hand, soil pH did not explain the variability in root colonization in grassland (SPARLING & TINKER, 1978).

Glomus etunicatum showed positive effects in the yield of the two forage legumes tested, specially in *Pueraria*. There is little evidence of host-fungus specificity in VAM. However, the morphological features, the pattern of spread and the extent network in VAM certainly depend on the plant and fungi involved and this, which could be defined as the degree of compatibility between both partners, would affect the size of the mycorrhizal effects (HAYMAN, 1975; MOSSE, 1981; GIANINAZZI-PERSON, 1984).

The root colonization by VAM endophytes in *Pueraria* was higher than in *Centrosema*, but the results demonstrated that growth enhancement is not necessarily proportional to the amount of root colonization. It is possible that other factors, such as external hyphae distribution, were more developed in centrosema than pueraria, although this was not examined. Another possibility is that the VAM fungi induced different plant hormonal growth.

In the absense of P fertilizer inoculated treatment did not have such a high N concentration because of a dilution effect associated with growth (table 2 and 3). The VA mycorrhizae inoculation highly increased plant nutrient uptake.

CONCLUSIONS

Mycorrhizal inoculation of legumes such as *Centrosema* and *Pueraria* when combined with a sufficiently low application of soluble phosphate had potential as an economic way to increase the productivity of pasture on poor soil.

Species of VAM fungi differ in their adaptation to soil acidity. Acid-soil tolerant species of VAM fungi improve the forage legume nutrition.

RESUMO

Estudaram-se as respostas em termos de produção de matéria seca e absorção de nitrogênio e de fósforo das leguminosas forrageiras tropicais, *Centrosema macrocarpum* e *Pueraria phaseoloides* inoculadas ou não com os fungos micorrízicos (MVA) *Glomus macrocarpum*, *Glomus etunicatum* e *Gigaspora margarita* na ausência ou na presença de 25 ou 50 kg de P_2O_5/ha. O solo estudado foi um Latossolo Vermelho Amarelo (Oxissol) pobre em fósforo. A inoculação micorrízica das leguminosas *Centrosema* e *Pueraria*, quando combinada com uma aplicação de fosfato solúvel (superfosfato triplo) suficientemente baixa, mostrou-se como um caminho potencial e econômico para aumentar a produtividade dessas forrageiras em solo pobre em fósforo. As diversas espécies de fungo MVA diferiram na sua adaptação nas condições de acidez do solo, *G. macrocarpum* foi mais sensível que os demais fungos. Na ausência de aplicação de fosfato, para a *Centrosema*, *Gigaspora margarita* e *G. etunicatum* foram mais eficientes, porém, na presença de 25 kg de P_2O_5/ha, *G. margarita* foi mais eficiente, entretanto com 50 kg de P_2O_5/ha destacaram-se *G. etunicatum* e *G. margarita*. Para *Pueraria* na ausência de adição de fosfato, *G. macrocarpum* e *G. margarita* foram mais efetivos, por outro lado na presença de fosfato os três fungos MVA tiveram efeitos positivos, embora não houvessem diferenças entre os fungos em termos de produção de matéria seca e conteúdos de N e P nas plantas. As plantas micorrizadas sempre contiveram quantidades superiores de N e P em relação às plantas não micorrizadas.

SUMMARY

EFFECTS OF MYCORRHIZAL INOCULATION ON GROWTH, NITROGEN AND PHOSPHORUS CONTENTS OF TWO TROPICAL FORAGE LEGUMES

Response of two tropical forage legumes: *Centrosema macrocarpum* and *Pueraria phaseoloides* to inoculation with the mycorrhizae fungi *Glomus macrocarpum*, *Glomus etunicatum* and *Gigaspora margarita* without and with added P (25 or 50 kg P_2O_5 ha^{-1}) were studied in a P-deficient sterile soil. Mycorrhizal inoculation of legumes such as *Centrosema* and *Pueraria* when combined with a sufficiently low P application had potential as an economic way to increase the productivity of pasture on poor soil Species of VAM fungi differ in their adapta-

tion to soil acidity. Acid-soil tolerant species of VAM fungi improve the forage legume nutrition. Non-mycorrhizal plants contained always lower quantities of phosphorus and nitrogen than mycorrhizal plants. For *Centrosema*, in the absence of added phosphate, *G. margarita* and *G. etunicatum* increased significantly the growth (shoots dry weight) and N and P contents and uptake, but in presence of 25 kg P_2O_5 ha^{-1} *G. margarita* was more efficient, while with 50 kg P_2O_5 ha^{-1} the greater response was exhibited by plants inoculated with *G. etunicatum* and *G. margarita*. For *Pueraria* in absence of phosphate, *G. macrocarpum* and *G. margarita* were more effective, but in the presence of phosphate the three VA fungi had positive effects and there were no significant differences among the VA fungi.

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