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Response of *Leucaena leucocephala* to vesicular-arbuscular mycorrhizal colonization and rock phosphate fertilization in an Oxisol*

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Abstract

Response of *Leucaena leucocephala* (Lam) de Wit to rock phosphate application and inoculation with the vesicular-arbuscular mycorrhizal (VAM) fungus *Glomus aggregatum* (Schenck and Smith emend Koske) was evaluated in a pot experiment. VAM colonization increased as rock phosphate application increased. Using phosphorus concentration in pinnules as an indicator of VAM activity, significant VAM activity occurred at 25 days after planting at the lower levels of rock phosphate application (0, 0.34 and 0.68 g P kg^{-1}). The time required for significant VAM activity was shortened by 5 days at the higher P levels (1.36, 2.72 and 5.44 g P kg^{-1}). The highest VAM activity was associated with the highest rate of rock phosphate application.

Inoculation with *G. aggregatum* significantly increased the uptake of Cu, P and Zn and dry-matter yield at all levels of rock phosphate applied. Copper concentrations in roots of mycorrhizal Leucaena were significantly higher than that of shoots. The results indicated that Leucaena in symbiotic association with VAM fungi effectively utilized P from rock phosphate. However, high rates of rock phosphate are required to attain growth comparable to that obtained with the application of water-soluble phosphate.

Introduction

Phosphorus deficiencies in plants are common in strongly weathered soils of the tropics, in which Fe and Al oxides with high P-sorption capacities predominate (Fox, 1978). Furthermore, P applied to these soils as water-soluble fertilizers is quickly converted to forms not available to plants (Fox and Searle, 1978). Under these conditions and where rock phosphate is readily available, it is better to utilize less-soluble and less-expensive P sources, in association with microorganisms that can either solubilize P or enhance its absorption from slowly soluble forms, than expensive soluble P fertilizers.

Several investigations have shown that VAM fungi can improve plant uptake of immobile nutrients, particularly P in high P-fixing soils (Abbott and Robson, 1982; Mosse, 1981). Therefore, it is likely that plants infected with VAM fungi can significantly benefit from rock phosphate application in these soils. Indeed, VAM-dependent plants species, such as Stylosanthes and Leucaena, have been known to respond favorably to VAM inoculation at low levels of soil-solution P (Mosse, 1981; Habte and Manjunath, 1987). Furthermore, compared to soluble P fertilizers, rock phosphate application is less likely to elevate soil-solution P concentration to levels that are detrimental to mycorrhizal colonization and effectiveness.

The effect of rock phosphate application and VAM inoculation on many legumes has been the subject of numerous studies (Howler *et al.*, 1987; Mosse, 1981). However, most investigators have employed only one or two rates of rock phosphate addition; this narrow range of P makes the findings hard to interpret. It is essential to compare P uptake and growth of mycorrhizal plants over a wide range of P levels in order to get a complete picture of the relationships between P and VAM symbiotic

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activity (Harley and Smith, 1983; Linderman and Hendrix, 1982). Pairunan *et al.* (1980) evaluated the response of subterranean clover (*Trifolium subterraneum* L.) to rock phosphate fertilization in a sandy soil with and without VAM fungi. However, because of high rates of rock phosphate applied (11–1029 g pot^{-1} of which 0.05% was water-soluble P), soil-solution P concentrations must have been unusually high, thus precluding a realistic assessment of the rock phosphate–VAM interaction.

The objective of this study was to evaluate the growth response of *Leucaena leucocephala*, a tropical tree legume highly dependent on VAM fungi, to rock phosphate applications and inoculation with *Glomus aggregatum* in a highly weathered tropical soil.

Material and methods

The soil used in this study was subsurface (38–50 cm) material of an Oxisol (Tropeptic Eutrustox, Wahiawa series, with 42% clay, 38% silt, and 20% sand; and 0.22% organic C). Subsoil with low initial P and organic matter content was selected to minimize the influence of organic P on P availability. The soil was passed through a 4-mm sieve and mixed with an equal quantity of sand (by weight). Pots of 15 cm diameter were filled with 2 kg of the sand:soil mixture. The pH of the mixture was raised to 6.2 with $Ca(OH)_2$ (1.14 g kg^{-1} soil) to eliminate the potential adverse effects of Al and Mn. A nutrient solution containing K, N, Mg, Cu, B, Mo and Zn was added to the sand:soil mixture at rates of 100, 34.6, 50, 5, 10, 0.5 and 10 mg kg^{-1} soil, respectively. Rock phosphate (16% P) was added at six rates: 0, 0.34, 0.68, 1.36, 2.72 and 5.44 g P kg^{-1} soil. All nutrient additions were made 3 weeks before planting. There was no leaching from the pots. Rock phosphate fertilizations did not measurably alter soil-solution P concentration in 0.01 *M* $CaCl_2$ extract as determined by the method of Fox and Kamprath (1970). All pots were fumigated with 48 g of methyl bromide and 1 g chloropicrin in a gastight chamber for 4 days. After fumigation, the pots were placed on a greenhouse bench for 10 days for the fumigants to dissipate.

A crude inoculum (50 g) of *G. aggregatum* that consisted of sand, spores (110 g^{-1}), bits of hyphae and infected corn root segments was mixed with 2 kg of the sand:soil mixture. Uninoculated controls received 50 g of sterilized sand along with washings of crude inoculum after removal of *G. aggregatum* propagules by filtration (Whatman no. 1 filter paper).

Seeds of *L. leucocephala* (CV. K-8) were pregerminated on water agar (0.9% agar) after scarification in concentrated H_2SO_4 for 30 min. Three pregerminated seeds were planted into each pot on March 25, 1986 and later thinned to two seedlings per pot. The experiment consisted of six application rates of rock phosphate and one of water soluble KH_2PO_4, all with and without inoculation, making 14 treatments in total. There were four replications per treatment. The soluble P treatment (KH_2PO_4, 0.26 g P kg^{-1} soil, added along with nutrient solution) yielded 0.021 mg P l^{-1} in the soil solution, a P level considered optimum for the VAM symbiosis in Leucaena (Habte and Manjunath, 1987). Pots were arranged on greenhouse benches in a randomized complete block design. Plants were grown under greenhouse conditions (temperature 25–30 °C, relative humidity 75–95% and quantum flux density 500–1200 μmol sec^{-1} m^{-2}). Moisture was maintained at near field-water holding capacity.

The development of VAM activity was monitored by measuring P status of the second pinnule of the youngest fully expanded leaf at 5-day intervals, starting on the 10th day after planting (Habte et al., 1987). Plants were harvested after 45 days of growth on May 8, 1986. Dry weights of shoots and roots were measured. The proportion of root length colonized by VAM fungi was determined by the grid-line intersection method (Giovannetti and Mosse, 1980). Phosphorus in plant tissues was determined by the molybdate-blue method (Murphy and Riley, 1962) after dry ashing. Copper and Zn were determined by atomic absorption spectroscopy (Hue and Evans, 1986).

Data were statistically analyzed by the procedure of Analysis of Variance to test the effect of VAM inoculation and P application on dry weight and nutrient concentration (SAS Institute, Inc., 1982). Least significant difference (LSD) was used to separate treatment means when the F-test was significant.

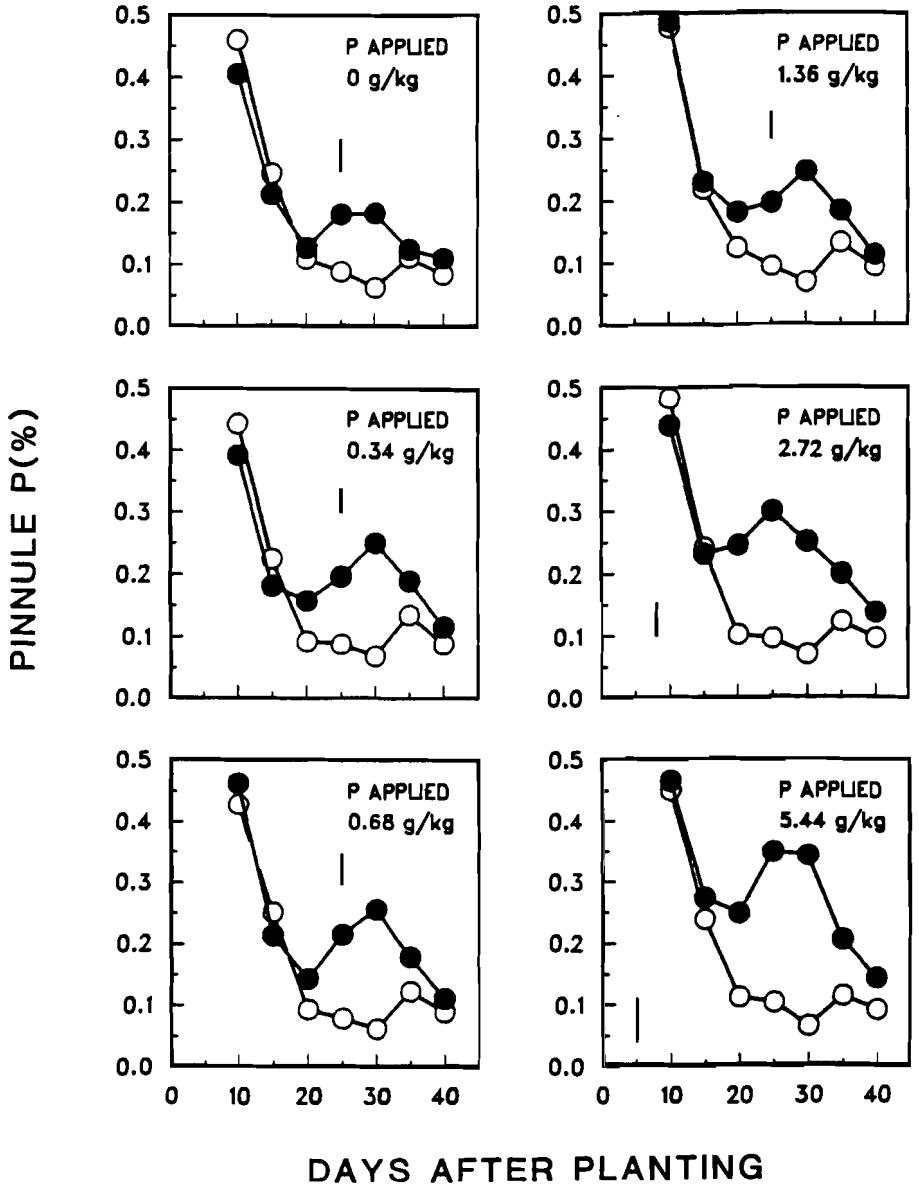

Fig. 1. Influence of rock phosphate application on the concentration of phosphorus in pinnules of mycorrhizal (●) and nonmycorrhizal (○) Leucaena. *Vertical bars* represent least significant difference (LSD) at 5% level.

Results and discussion

Plant response to P application rates and fungal inoculation

There were no significant differences in the P concentrations of pinnules between inoculated and uninoculated Leucaena, regardless of P treatments at the first sampling date (10 days after planting, Fig. 1). However, significant reduction in P concentration of pinnules was observed at 15 days after planting in all treatments (Fig. 1). This was probably due to the dilution effect of growth because dry matter usually accumulates faster than P uptake (Jarrel and Beverly, 1981).

The effect of VAM inoculation on P concentra-

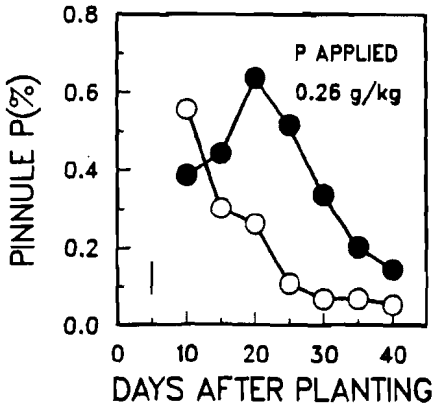

Fig. 2. Influence of soluble phosphate application on the phosphorus concentration of pinnules of mycorrhizal (●) and nonmycorrhizal (○) Leucaena. *Vertical bar* represents LSD at 5% level.

tion of pinnules was apparent after 20 days in the rock phosphate-applied treatments and after 25 days in the untreated soil (Fig. 1). However, differences in P concentration of pinnules between VAM and non-VAM Leucaena became insignificant after 40 days. This is not because non-VAM plants grew better with time, but because VAM activity declined as a result of limited supply of substrates (Pacovsky and Fuller, 1986).

At a given sampling date, P concentration in pinnules of VAM plants increased with increasing rock phosphate application rate (Fig. 1). For example, at day 25 pinnule P concentrations were 0.18, 0.21, 0.30 and 0.34% corresponding to the application rates of 0, 0.68, 2.72 and 5.44 g of rock phosphate per kg medium. However, these values were much lower than those of VAM plants grown in the KH_2PO_4 treatment (P concentration was 0.50% at day 25, Fig. 2). This further confirmed that P concentration in the sand:soil mixture amended with 5.44 g P/kg as rock phosphate was lower than 0.021 mg l^{-1} (< 0.01 mg P l^{-1} by direct measurement).

Generally, maximum P concentration in pinnules of mycorrhizal Leucaena occurred at about 30 days after planting, but the time required to attain maximum concentration was shortened by 5 days in plants grown in the sand:soil mixture fertilized with higher rates of rock phosphate. Rock phosphate applications did not increase P concentration in pinnules of non-VAM Leucaena. This demonstrates the inability of unaided Leucaena roots to take up P at low solution P concentrations, in agreement with an earlier study (Habte and Manjunath, 1987). The results also support the observation of Habte *et al.* (1987) that VAM fungal activities can be monitored by measuring P in pinnules.

Dry weights of shoots and roots of non-VAM Leucaena were not significantly influenced by rock phosphate application. In contrast, inoculation with *G. aggregatum* significantly increased dry weights of shoots and roots, regardless of P addition (Table 1). Root dry weights of non-VAM Leucaena were higher than shoot dry weights, but the opposite was true with VAM Leucaena. Plants probably allocate a greater proportion of assimilates to roots when nutrients such as nitrogen and phosphorus are limiting (Clarkson, 1985).

At harvest, P concentration of non-VAM Leucaena remained constant, irrespective of rock phosphate addition. Lower rates of rock phosphate addition ($\leqslant 0.68$ g P kg^{-1}) did not significantly increase P concentration of VAM Leucaena, while higher rates did (Table 1). Our results support the findings of Murdoch *et al.* (1967), Powell and Daniel (1978), and Mosse *et al.* (1976) that indicate that VAM plant were more efficient in utilizing P from rock phosphate than non-VAM plants. The increased P concentration in inoculated plants suggests more efficient absorption of P from the soil. This was probably due to the extensive development of VAM extramatrical mycelium, that reduced the mean distance between VAM hyphae and either rock phosphate particles or soil P-adsorbing sites as suggested by Tinker (1980) and Howeler *et al.* (1987).

In non-VAM Leucaena, Cu and Zn concentrations were not affected by the level of rock phosphate applied; whereas in VAM Leucaena, concentrations of Cu and Zn in roots were significantly reduced by high levels (> 1.36 g P pot^{-1}) of rock phosphate application (Table 2). Increases in Cu and Zn uptake can only be attributed to inoculation with VAM fungi (Cooper and Tinker, 1978; Pacovsky, 1986).

Mycorrhizal colonization as affected by P application rates

Mycorrhizal colonization of Leucaena roots was significantly increased by the application of rock

Table 1. Influence of VAM inoculation and P applications on dry-matter yield, P concentration in shoots and roots, and VAM colonization of Leucaena

P applied (g P pot^{-1})	Dry weight (g plant^{-1})						VAM colonization (%)
	Shoot			Root			
	VAM	non-VAM	F-test	VAM	non-VAM	F-test	VAM
Rock phosphate							
0.00	0.52	0.14	**	0.41	0.17	**	41.5
0.34	0.61	0.12	**	0.42	0.16	**	63.9
0.68	0.62	0.15	**	0.44	0.17	**	66.1
1.36	0.65	0.14	**	0.52	0.17	**	64.1
2.72	0.73	0.15	**	0.53	0.16	**	62.4
5.44	0.75	0.14	**	0.59	0.16	**	75.9
KH_2PO_4							
0.26	0.82	0.28	**	0.62	0.16	**	57.5
LSD							
1%	0.09	0.03		0.09	NS		17.28
5%	0.06	0.02		0.07	NS		12.61

	Phosphorus (%)					
	Shoot			Root		
	VAM	non-VAM	F-test	VAM	non-VAM	F-test
Rock phosphate						
0.00	0.059	0.036	*	0.060	0.030	*
0.34	0.057	0.040	NS	0.069	0.029	**
0.68	0.052	0.038	NS	0.071	0.033	**
1.36	0.081	0.040	**	0.094	0.032	**
2.72	0.102	0.035	**	0.109	0.033	**
5.44	0.104	0.033	**	0.109	0.037	**
KH_2PO_4						
0.26	0.204	0.048	**	0.286	0.060	**
LSD						
1%	0.027	0.009		0.020	0.007	
5%	0.020	0.007		0.014	0.005	

LSD, least significant difference; NS, not signficant; * and **, significant at 5% and 1% level, respectively.

phosphate (Table 1). Lower rates of P application have shown to increase VAM colonization in Leucaena (Habte and Manjunath, 1987) and in other legumes (Asimi *et al.*, 1980; Manjunath and Bagyaraj, 1984). VAM colonization can be depressed at higher P levels (Mosse, 1981; Stribley *et al.*, 1981), possibly by increasing P concentration in roots (Menge *et al.*, 1978); however, depression of VAM colonization was not observed in this study. This is because P concentrations in the soil solutions and in Leucaena roots were still lower than those inhibitory to VAM colonization.

Significant increases in Cu concentration in roots of VAM plants were worth noting (Table 2). In non-VAM plants, root Cu was essentially equal to shoot Cu, whereas Cu concentration was about 10 times greater in VAM Leucaena roots (74.26 vs. 7.23 $\mu g\,g^{-1}$). The possibility of contamination with soil particles was ruled out because Mn concentration in roots of VAM Leucaena (61.6 $\mu g\,g^{-1}$) was lower than that of non-VAM Leucaena (106.0 $\mu g\,g^{-1}$). Therefore, it is likely that the accumulation of Cu must have come from the fungi themselves, suggesting that Cu requirement of the

Table 2. Influence of VAM inoculation and phosphorus fertilization on copper and zinc concentration in shoots and roots of Leucaena

P applied (g P pot^{-1})	Copper (μg g^{-1})					
	Shoot			Root		
	VAM	non-VAM	F-test	VAM	non-VAM	F-test
Rock phosphate						
0.00	6.25	7.20	NS	101.73	7.90	**
0.34	6.06	8.76	*	99.36	8.81	**
0.68	7.05	9.49	NS	94.47	8.34	**
1.36	5.91	9.02	NS	81.31	7.45	**
2.72	6.90	8.30	NS	77.44	7.36	**
5.44	7.23	7.98	NS	74.26	6.62	**
KH_2PO_4						
0.26	12.75	8.90	*	68.00	21.52	**
LSD						
1%	2.55	NS		34.90	2.41	
5%	1.86	NS		25.48	1.76	
	Zinc (μg g^{-1})					
	Shoot			Root		
	VAM	non-VAM	F-test	VAM	non-VAM	F-test
Rock phosphate						
0.00	66.14	43.26	NS	31.11	12.73	**
0.34	63.51	49.07	*	29.56	11.88	*
0.68	58.55	49.26	NS	28.76	11.42	**
1.36	56.38	42.73	NS	24.37	9.26	**
2.72	53,90	37.24	*	23.31	8.24	**
5.44	57.48	44.03	**	21.44	8.20	**
KH_2PO_4						
0.26	38.90	18.63	**	25.09	24.70	NS
LSD						
1%	16.08	14.54		8.09	5.11	
5%	11.74	10.61		5.90	3.73	

Notations same as in Table 1.

fungi may be very high relative to that of the host. However, other possibilities such as (1) increased Cu requirement of VAM Leucaena or (2) increased Cu absorbing capacities of the symbiotic system cannot be eliminated.

In summary, our results demonstrate that mycotropic plant species like Leucaena can utilize P from rock phosphate in association with VAM fungi. However, large quantities of rock phosphate are required to attain growth comparable to that attained with a soluble phosphate source.

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