Effect of Phosphate Rock Fertilization and Arbuscular Mycorrhizae (AM) Inoculation on Growth and Nodulation of Agroforestry Tree Seedlings

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Abstract

Phosphate rocks (PR) have been identified as cheap complements of easily soluble phosphate fertilizers for low pH soil. A-mycorrhizae improve plant uptake of P and other nutrients in acidic, low-P soils. Using two acid soils, Acrisols and Andosols, two greenhouse studies were carried out to evaluate effect of Minjingu Phosphate Rock (Minjingu PR) on growth of four agroforestry multipurpose trees: *Leucaena leucocephala, Senna siamea, Grevillea robusta,* and *Eucalyptus grandis.* In the first experiment, one-month-old seedlings received Minjingu PR at 0 (PR$_0$), 52 (PR$_1$) and 77 (PR$_2$) kg P ha^{-1} in 2 kg soil. In a second experiment, the Minjingu PR rates of the first experiment were maintained while *G. robusta* and *L. leucocephala* were used as the test trees on the Acrisol only. A-mycorrhizae inoculum in the form of *A. tortilis* roots mixed soil was included in this study. There was a slower response to Minjingu PR fertilizer application in Andosols than in Acrisols. At 19 weeks after transplanting, PR$_2$ had caused a significant ($P \leq 0.05$) height increase over PR$_0$ in *L. leucocephala.* Addition of PR$_2$ had a negative effect on the height of *C. siamea* whereas *E. grandis* did not respond to PR additions. Application of PR in Andosol, significantly reduced ($P \leq 0.05$) height and root collar diameter of *G. robusta* and *S. siamea* as compared to the control. In the second study, there were significant increases of up to 121% in height ($P \leq 0.001$) and root collar diameter ($P \leq 0.05$) and 4.5 times biomass over the controls when *L. leucocephala* seedlings received PR alone and PR + mycorrhizae at 12 months after planting. Nodulation of *L. leucocephala* was significantly affected by P application and/or A-mycorrhizae inoculation but was variable within any similar treatments except for the controls, where no nodulation was observed. Species × treatments interactions were significant, $P \leq 0.05$ for shoot dry weight and $P \leq 0.001$ for root dry weight. It is probably not necessary to add Minjingu PR fertilizer to *G. robusta* in either soil and to *S. siamea* in the Andosols soils. PR and mycorrhizae inoculation have the potential to improve legume performance in these acidic soils.

Introduction

Increasing over-exploitation of woody vegetation for timber and/or fuelwood, and declining soil fertility of cultivated land now characterize over 40 of the 50 tropical African countries (Sanginga 1989). The establishment of low-input sustainable land-use systems especially for resource-poor-small scale farmers has therefore become a priority to meet these demands in the developing countries. The high cost of soluble phosphate fertilizers such as triple superphosphate (TSP) has generated considerable interest in the use of cheap sources of phosphorus, in particular, Rock Phosphates (Nnandi and Haque, 1988; Mathur and Suresh, 1989; Minjingu Co., 1991) for forestry and agricultural purposes. The positive effects on growth by applying nitrogenous and phosphorus fertilizer to potted tree seedlings are well studied (Osunde *et al.*, 1989) as well as inoculations with mycorrhizae and *Rhizobium* in soils of low fertility (Howeler *et al.*, 1987). Although mainly with agricultural crops, inoculations are a recommended practice where P and N are limiting, especially in the tropical acid soils (Danso, 1992).

In Kenya, there has been emphasis on short rotation multipurpose trees such as *Eucalytus grandis*, *G. robusta*, *C. siamea* and nitrogen fixing legumes *L. leucocephala* and *G. sepium*. Their establishment in acid soils in Western and Eastern Kenya is nonetheless poor. Aluminium toxicity and P deficiency have been identified as probable factors inhibiting the growth of these trees in acid soils (FAO, 1986). A major problem also of *L. leucocephala* seedlings is their slow initial growth which is attributed to several factors including lack of nodulation (Ahmad and Ng, 1981). In legumes, an adequate supply of phosphorus is necessary for their optimum growth, survival of rhizobia in the soil, nodule formation and N_2-fixation (Azcon-Aguilar *et al.*, 1979; Mosse *et al.*, 1976; Smith *et al.*, 1979; Daft and El-Ghiami, 1975). Efficient mycorrhizal association can also increase P-uptake and hence yields (Howeler *et al.*, 1987; LeTacon *et al.*, 1987) and also plant resistance to water stress (Fortin, 1982). In Kenya, introduction of VAM inoculum in some semi-arid areas was shown to enhance the growth and survival of *Acacia tortilis* and *Prosopis juliflora* (Wilson *et al.*, 1991) and *Melia volkensii* (Mwangi *et al.*, 1989) in the nursery and field.

Legumes and tree species that form mycorrhizal associations have been reported to utilize the P from RP more effectively than other plants. However, few studies in this area have been conducted and lack of information on the ability of these trees to utilize P from phosphate rock is a limitation to agricultural productivity.

Two greenhouse studies were carried out. Experiment I evaluated the P uptake from locally available Minjingu phosphate rock (Minjingu PR) to four agroforestry tree species, *L. leucocephala*, *C. siamea*, *G. robusta*, and *E. grandis* growing on two acidic soils (Acrisol and Andosol). Experiment II evaluated the effects of Minjingu PR fertilization and arbuscular mycorrhizae inoculation on the growth of *L. leucocephala* and *G. robusta* growing on an acidic Acrisol.

Materials and methods

Two acids soils, an acrisol from Kakamega in Western Kenya at an altitude of 1595 metres above sea level (a.s.l.) and an Andosol from Gituamba in Central Kenya at an altitude of 2130 m a.s.l. were used(FAO/UNESCO 1974). The Andosol had a pH of 4.2, 5.9% C, 0.6% total N, and 0.5 mg kg^{-1} of P, whereas the Acrisol had a pH of 4.3, 2.4% C, 0.18% total N, and 0.21mg kg^{-1} of P. The soils were air dried and crushed to pass through a 2 mm sieve and transferred to plastic pots (2 kg pot^{-1} in experiment I and 10 kg pot^{-1} in experiment II). The phosphate source was Minjingu PR with 12.9% grade P and 40% CaO. In experiment I, Minjingu PR treatment rates of application consisted of O (PR_0), 52 (PR_1) and 77 (PR_2) kg P ha^{-1} (equivalent to 0, 400 and 600 kg Minjingu PR ha^{-1}) replicated three times. In experiment II, the treatments consisted of, PR_0, PR_1, PR_2, PR_0 + A-mycorrhizae, PR_1 + A-mycorrhizae, and PR_2+A-mycorrhizae, replicated four times in a randomized complete block design.

The Minjingu PR was applied and mixed thoroughly with the soil. About 200 ml of water was added and the set-up left for a week to allow for equilibration. A-mycorrhizae inoculation adopted the method of Wilson *et al.* (1991) where 2 g/pot of inoculum which was a mixture of roots and soil from *Acacia tortilis* was added approximately quarter-way down the soil in the pots. The seeds of *L. leucocephala*, *G. robusta*, *E. grandis* and *S. siamea* were obtained from Kenya Forestry Research Institute (KEFRI) seed centre and sown in sand trays in the glasshouse. *L. leucocephala* seeds were first pretreated overnight in hot water and sown in the trays. The seedlings were pricked out into the pots 30 days after sowing. *L. leucocephala* was not inoculated with *Rhizobium* bacteria. An amount of water not exceeding 250 ml (Exp. I), and 1000 ml (Exp. II) was added on a weekly basis to maintain the soils at field water capacity.

In experiment I, height (ht.) and root collar diameter (rcd) were recorded at 19 weeks after planting (19 WAP) whereas in experiment II the same were recorded at 12 months after planting (MAP) and destructive sampling was carried out. Parameters assayed were shoot and root dry matter for both tree species and nodule number and fresh weight for *L. leucocephala*. The shoots were cut at the base and placed in paper bags for fresh weight determination. The bags were then placed in the oven at 70°C for 48 hours then weighed. The roots were cleaned using tap water to remove all the soil after which the nodules were manually removed and placed in 50 ml beakers. Enumeration of the nodules was done in the laboratory. The roots were placed in paper bags and placed in the oven at 70°C for 48 hours for dry weight determination. The data were subjected to an analysis of variance (ANOVA) and the Duncan multiple range test ($P < 0.05$) was used to compare the means using SAS software (SAS, 1982).

Results and discussion

Soil chemical and physical characteristics

The two soils were acidic, with pH range of 4.2 to 4.3. Acidity could be due to crop removal of basic cations including calcium (Ca), magnesium (Mg), potassium (K) and sodium (Na) in exchange of H^+ or leaching of the cations to be replaced by H^+ and subsequently aluminium (Al^{+3}) (Tisdale, 1984). Decomposition of organic residues is also a factor affecting acidity with H^+ ions being left on the negatively charged organic matter (Tisdale, 1984). These soils are highly leached tropical soils (Shepherd, 1991). However, Ca content in Acrisols was still three times higher than that found in Andosols possibly due to low organic matter content in the acrisol.

The nitrogen (N) and phosphorus (P) levels in both soils were low. N and P levels are generally limiting in tropical soils and this has been attributed to low organic-matter levels and fixation of P by Al in acid soils (FAO, 1986).

Experiment I

Effect of Minjingu PR on growth of the seedlings

The effect of Minjingu PR application on the height and root collar diameter of the four species used in experiment I is shown in Table 1. At 19 WAP, *L. leucocephala* growing on Acrisols showed significant increases in height ($P \leq 0.05$) and root collar diameter ($P \leq 0.01$) in the presence of Minjingu PR compared to the control but the two PR rates were not significantly different. In the case of *G. robusta* and *S. siamea*, positive increases in height and root collar diameter were observed up to 52 kg P ha^{-1}, above which a negative growth response was observed probably due to nutrient imbalance phenomenon. The

TABLE 1

Effect of P rates as Minjingu PR on height (cm) of four tree species growing on two acid soils at 19 weeks after transplanting.

	P application rate					
	Control PR_0(0 kg P/ha)		PR_1 (52 kg P/ha)		PR_2 (77 kg P/ha)	
	Andosol[1]	Acrisol[2]	Andosol	Acrisols	Andosol	Acrisol
Species						
S. siamea	0.8	0.7	0.7	0.7	0.6	0.5
E. grandis	0.4	0.3	0.6	0.4	0.3	0.5
G. robusta	0.9	0.6	0.6	0.9	0.7	0.9
L. leucocephala	0.4	0.4	0.7	0.7	0.6	0.7
[3]$Lsd_{0.05}$						
Species				0.09***		
P rate				0.07**		
Soil				0.06ns		
Species x P rate				0.16**		
Species x soil				0.13ns		
P rate x soil				0.11**		
Species x P rate x soil				0.22**		

[1]Soils from Gituamba; [2] Soils from Kakamega; [3] *, **, *** significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$ respectively; ns= not significant.

highest height was recorded with *E. grandis* when 77 kg P ha^{-1} (PR_2) was added in both soils.

Addition of 77 kg P ha^{-1} had a negative effect on *L. leucocephala* growth whereas *G. robusta* and *S. siamea* had increased height and root collar diameter where no fertilizer was added in Andosols. Generally plants growing on Acrisols were 2.05 times taller and had 1.55 times broader root collar diameter where Minjingu PR was added as compared to the controls at 19 WAP (Tabbe 1). The responses observed in *L. leucocephala* when PR was applied are similar to the findings by Osunde *et al.* (1992) in Nigerian acidic Ultisols. The positive growth by *L. leucocephala* to PR application could be attributed to the enhanced solubility of Minjingu PR on the acidic soils. High effectiveness of finely ground rockphosphates has also been found to be greatest on acid Oxisols and Ultisols in S. American soils (Hammond and Leon, 1983).

Responses to Minjingu PR fertilization by the four tree species were varied in Andosols, whereby *L. leucocephala* and *E. grandis* had increases in height and root collar diameter of 1.80 times and 1.31 times over the control when 52 kg ha^{-1} of Minjingu PR was applied. Andosols with 5.9% C may have been supplying enough P after mineralization (Zapata, pers. comm.) There was a reduction in height of *S. siamea* at both levels of Minjingu PR but increased in root collar diameter at 77 kg P ha^{-1}. *G. robusta* displayed the opposite trend. The positive responses to P by *L. leucocephala* and *E. grandis* in both soils probably indicates that the plants are more efficient at utilizing P from Minjingu PR as compared to the other tree species in these acidic soils (Table 2). Osunde *et al.* (1992) calculated amounts of available soil P to plants in P-source equivalent and showed that *L. leucocephala* was more efficient in utilizing P from Gafsa PR even when compared to TSP in acidic soils found in South-

TABLE 2
Effect of two P rates as Minjingu PR on height (cm) of four tree species growing on two acid soils at 21 weeks after transplanting.

	P application rate					
	Control PR_0 (0 kg P/ha)		PR_1 (52 kg P/ha)		PR_2 (77kg P/ha)	
	Andosol[1]	Acrisol[2]	Andosol	Acrisol	Andosol	Acrisol
Species						
S. siamea	32.0	27.3	25.5	29.6	19.3	17.4
E. grandis	39.4	33.0	50.2	44.7	35.0	50.0
G. robusta	48.3	29.0	28.8	47.8	3.8	48.5
L. leucocephala	28.1	7.8	14.7	13.8	15.6	15.3
[3]Lsd $_{0.05}$						
Species				6.2***		
P rate				5.4ns		
Soil				4.4ns		
Species X P rate				10.8ns		
Species X soil				8.8ns		
P rate X soil				7.6ns		
Species X P rate X soil				15.2**		

[1]Soils from Gituamba;[2] Soils from Kakamega;[3] *, **, *** significant at $p < 0.05$, $p<0.01$ and $p< 0.001$ respectively; ns= not significant.

eastern Nigeria. *E. grandis* and *L. leucocephala* have been reported to have acid excretions from their roots which assist in solubilization of PR (Mada and Bagyaraj, 1993). These exudates are capable of increasing the dissolution of P from PR (Nye and Kirk, 1978).

Experiment II

Growth response of tree seedlings to Minjingu PR application

Results of height, root collar diameter, shoot and root dry weight of *L. leucocephala* and *G. robusta* responding to Minjingu PR application and A-mycorrhizae inoculation at 12 MAP are shown in Tables 3 and 4. There was a significant difference in height ($P < 0.001$), root collar diameter, shoot and root dry weight ($P < 0.05$) between *L. leucocephala* seedlings receiving Minjingu PR and Minjingu PR + A-mycorrhizae combinations and those inoculated with A-mycorrhizae alone and the control. PR_1+A-mycorrhizae resulted in about 121% increases in both height and root collar diameter above the control.

A-mycorrhizae inoculation alone did not enhance the growth of *L. leucocephala* as expected. The initial low levels of P in the soil probably caused by high fixation as Al-PO_4 (Zapata, 1994, pers. comm.) could have hindered effective A-mycorrhizae colonization on the plant roots. Minimum level of P in the soil is required to have any significant mycorrhizal infection on the roots (Baylis, 1967). Growth depressions due to A-mycorrhizae inoculant have been observed by Simpson and Daft (1990) in maize and in some Kenyan soils (Musandu and Giller, 1994). These depressions were attributed to limited carbohydrate availability in the host plant (Bethlenfalvy *et al.*, 1982; Buwalda and Goh, 1982), or competition for photosynthates between roots and A-mycorrhizae (Musandu and Giller, 1994) suggesting that the A-mycorrhizae inoculant could be partially parasitic (Awotoye *et al.*, 1992).

No significant differences were observed in the root and shoot biomasses of the seedlings that received treatment PR_1+Mycorrhizae, PR_2 alone and the ones that received PR_2+Mycorrhizae, thus with inoculation

TABLE 3

Effect of different rates of Minjingu PR application and A-mycorrhizae inoculation on height (cm) and root collar diameter growth of 2 MPTs at 12 month after transplanting.

	Height		Root collar diameter	
	L. leucocephala	*G. robusta*	*L. leucocephala*	*G. robusta*
Control	11.1	66.3	0.7	1.8
Arbuscular mycorrhiza	22.4	75.0	1.2	1.9
RP_1	21.9	77.6	1.1	1.8
RP_2	24.8	70.8	1.3	1.8
RP_1 + A-mycorrhiza	26.1	76.2	1.6	1.8
RP_2 + A-mycorrhiza	24.9	72.7	1.3	1.7
$Lsd_{0.05}$				
Treatment	7.3[1]		0.2**	
Species	4.2***		0.1***	
Treatment x Species	10.3ns		0.3**	

[1] *, **, *** significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively ns= not significant.

less P fertilization could be required. These results agree with the observation of Reddy *et al.* (1989) who found that the application of phosphatic fertilizers and inoculation with A-mycorrhizae fungus increased the productivity of *Leucaena* species. Dodd *et al.* (1990) also reported that the combination of inoculation with less soluble P source (phosphate rock) significantly increased plant yields and was as effective as the more expensive, more soluble, superphosphate alone. Results from statistical analysis indicated that treatments, species and treatment x species interactions were significant for both shoot and root dry weights. This suggested that the species responded differently to all the treatments but this was more pronounced with root dry weight. Phosphorus is known to play a significant role in root development and growth in most plant species. These differences in response to A-mycorrhizae inoculum between *G. robusta* and *L. leucocephala* may be related to the effectiveness of the symbionts (Awotoye *et al.*,1992).

Nodulation of the L. leucocephala seedlings

Nodulation of *L. leucocephala* was unexpected as no inoculation prior to sowing or during growth had been done. Fields with no history of *L. leucocephala* cultivation normally require that appropriate rhizobium inoculum is added at planting or during growth (Date and Halliday, 1980; Sanginga *et.al.,* 1985). Nodulation in the pots (Table 4) could however be attributed to the presence of some native legumes or rhizobia strains belonging to the same cross-inoculation group as *L. leucocephala* as it has lately been found in acidic Dakaini soils in Kenya (Anyango *et al.*, 1995) that nodulated *L. leucocephala* effectively even where there was no history of its cultivation or growth. They also showed that rhizobia that can effectively nodulate *P. vulgaris* could have been present in Africa prior to introduction of the crop. The observations by Cobbina *et al.* (1992); Mulongoy and Owoaje,(1992); Luyindula and Haque, (1992) on nodule formation by *L. leucocephala* seedling in their greenhouse studies, when soils with no history of *Leuceana* cultivation were used, corroborate our findings.

The improved nodulation where Minjingu PR and A-mycorrhizae were added could be related to improved P supply to the plant since an adequate supply of P to legumes is

TABLE 4.
Effect of different rates of Minjingu PR application and A-mycorrhizae inoculation on shoot and root dry weight of Leucaena leucocephala and Grevillea robusta 12 months after transplanting.

Treatments	Parameters					
	Stem dry weight (g/plant)		Root dry weight (g/plant)		SDW/RDW ratio	
	L. leucocephala	*G. robusta*	*L. leucocephala*	*G. robusta*	*L. leucocephala*	*G. robusta*
Control	4.0	94.7	8.0	34.1	0.6	2.8
Arbuscular mycorrhiza	2.2	102.4	18.5	36.6	0.1	2.9
RP_1	5.4	93.5	26.3	44.0	0.3	2.2
RP_2	9.7	79.4	36.5	35.9	0.3	2.2
RP_1 + A-mycorrhiza	9.7	66.5	36.8	38.5	0.3	1.8
RP_2 + A-mycorrhiza	9.7	103.2	36.1	36.4	0.3	2.9
[2]Lsd 0.05						
Treatment	14.6ns[1]		10.5**		0.5*	
Species	8.5**[2]		6.1***		0.3***	
Treatment x Species	20.7*		14.9*		0.6ns	

[1] ns= not significant; [2]*, **, *** significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively.

TABLE 5
Effect of different rates of Minjingu PR application and ~~A-mycorrh~~izae inoculation on mean nodule number on Leucaena leucocephala at 12 months after planting.

	Control	AM	PR_1	PR_2	PR_1+ AM	PR_2 + AM
Nodule number	1	6	20	45	36	35
Lsd $_{0.05}$						
Treatments	32**					

**, significant at $P < 0.01$.

necessary for both growth of host, survival of microsymbiont in the soil, nodule formation and nitrogen-fixation (Azcon-Aguilar *et al.*, 1979; Daft and El-Giahmi, 1975). This was proven by the lack of nodulation in the control treatments in this study. However nodulation of *L. leucocephala* within the same treatments was variable (see Table 5) which could be due to variability within *L. leucocephala* species.

Conclusion

Height and root collar diameter measurements are important in nurseries as they determine the vigour and health of a seedling before outplanting. These later translate to height and diameter at breast height (dbh) in the field and are crucial in evaluating tree volume in forest stands. It was apparent that either Minjingu PR or A-mycorrhizae or both had a positive effect on most tree species, especially in the Acrisols. *G. robusta* was still very healthy even with no P application or arbuscular mycorrhizae inoculation in the two soils. The lack of nodulation in the controls, however, showed that P might have been limiting the nodule formation and hence N_2-fixation, emphasizing the role of mycorrhizae and *Rhizobium* in legume–tree nutrition. Plants grown in the Andosols showed the least response for all the species to addition of Minjingu PR. The Andosols seem to supply enough P to *G. robusta, E. grandis* and *C. siamea* and it might not be necessary to add phosphates to these nursery soils. Plants grown in Acrisols, however, would require an addition of phosphates (50 kg P ha^{-1}) to enhance the growth of all the species except *E. grandis*. This requirement can probably reduce the time seedlings stay in the nursery before they are outplanted and so enhance their survival and growth.

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