RESPONSE OF SEEDLINGS OF *GREVILLEA ROBUSTA* A. CUNN TO PHOSPHORUS FERTILIZATION IN ACID SOILS FROM KENYA[1]

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Abstract. Three experiments were conducted to assess the response of *G. robusta* to phosphorus fertilization using acid low-P soils from Eastern (Andosols, Gituamba) and Western (Acrisols, Kakamega) Kenya. In the first experiment, P was applied as Minjingu Phosphate rock (MPR, 12.9% total P) at 0, 25.8 and 38.7 kg P/kg soil into pots containing five kg soil. In the second experiment, 2g VAM soil + roots inoculum/5 kg soil was included in addition to the same MPR rates but only to Acrisol, Kakamega. In the third experiment, MPR and TSP were added to 2 kg soil (Acrisols, Kakamega) at a rate of 25.8 mg P kg^{-1} soil and ^{32}P isotope dilution techniques were used to assess P uptake and divided into two destructive shoot harvests at 3 and 6 MAT (months after transplanting). Application of MPR in Andosols significantly ($P < 0.05$) reduced height and root collar diameter of *G. robusta* as compared to the control whereas significant increases ($P<0.05$) in height and root collar diameter were recorded in the Acrisol in the P-fertilized treatments compared to control. Interaction soil with P fertilizer rates was highly significant ($p<0.001$) for both height and root collar diameter growth. The roots were not infected with VA-mycorrhizae after 12 months. At 3 MAT the percentage P derived from the MPR and TSP (%Pdff) was 3% and 6% respectively. P uptake decreased significantly ($p<0.05$) between 3 and 6 months. The results indicate that addition of P fertilizer and inoculation with VA-mycorrhizae to *G. robusta* in the two soils was probably required at the early stages of growth. Further research, especially extensive root studies (nursery and field) are required to explain the above observations.

1. INTRODUCTION

Grevillea robusta A. cunn was introduced in Kenya mainly as a shade tree for coffee and tea and from 1910 as a mixture with *Cupressus lusitanica*. Streets, 1962; Milimo, 1988 [1, 2]. The species is currently well accepted in Western [3] and Central [4] Kenya. *Grevillea robusta* is well established in subtropical and tropical highland environments [6] and in densely populated zones it is an important source of fuelwood and income from sale of construction timber [4]. Harwood and Booth [7] reported that the species is popular with African farmers because it provides viable products, it is easy to propagate and its proteoid roots help it grow in low fertility soils. Further, *G. robusta* does not compete with adjacent crops. Annual growth rates of 2 m in height and 2 cm in diameter over the first 5 years are commonly achieved in a number of countries where climate and soils are suitable [7, 4]. Therefore, this tree is a good candidate in Agroforestry systems. The majority of the areas in Western Kenya and parts of Central Kenya highlands are dominated by acid soils with low levels in phosphorus (P) and nitrogen (N) [8].

P addition up to 500 kg P_2O_5 ha^{-1} as triple super phosphate (TSP) has produced a response in above ground growth of *G. robusta* in experimental farms in Maseno, W. Kenya (Bashir, pers. comm.).

[1] Original published in Biotechnol. Agron. Soc. Environ. 3 (1), 57-64, 1999.
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However TSP and other commercial fertilizers are expensive and unaffordable by the majority of the smallholder farmers who would like to increase their yields on these inherently infertile soils [9]. Use of phosphate rock has been suggested as an alternative [10, 9] in replenishing their low P status.

Vesicular arbuscular mycorrhizal (VAM) associations with plant roots plays an important role in P-nutrition of plants particularly those growing in soil having low available P [11]. Plants infected with VA-mycorrhizae on their roots have been shown to produce greater plant growth and increased P-uptake in P deficient soils by increasing the root surface area [12]. VA-mycorrhizal fungi readily absorb soluble phosphate from the labile pool of phosphorus in soils and improve the use of phosphate rock [13-15]. Tinker 1980 [12] estimated that increased P uptake from VAM-inoculation was equivalent to 100 kg ha^{-1} of fertilizer. Isotopic labeling (^{32}P) experiments have also indicated that both mycorrhizal and non-mycorrhizal plants utilize the same pool of phosphorous in soil [15, 14]. However, limited research has been conducted to investigate the role of P in the establishment of *G. robusta,* hence information is scarce or unavailable. These glasshouse studies were thus initiated to evaluate *G. robusta* growth response and P uptake after application of P fertilizers:Minjingu phosphate rock (MPR) and TSP, with and without VA-mycorrhiza inoculation.

2. MATERIALS AND METHOD

2.1. Characteristics of soil and tree seedlings

Surface soil samples (0-20 cm) were randomly collected from experimental farms. Main properties of the Andosol from Gituamba in E. Kenya were:pH (1:5 soil to H_2O ratio) 4.2, carbon = 5.9%, available P-Olsen = 2.5 mg kg^{-1}, exchangeable Al = 2.1 cmol kg^{-1}, CEC. = 22.3 cmol kg^{-1} . The Acrisol of Kakamega in W. Kenya had the following properties: pH (1:5 soil to H_2O ratio) 4.3, carbon = 1.8%, exchangeable P-Olsen = 2.1 mg kg^{-1}, exchangeable Al =1.0 cmol kg^{-1} and CEC = 3.2 cmol kg^{-1}. Seeds of *G. robusta* were pregerminated on sterile water-agar Petri dishes and after emergence transferred into sterilized sand trays for 3 weeks. Thereafter seedlings were transplanted one per pot (30 cm x 15 cm diameter) containing 5 kg of soil.

2.2. Experiment I and II: Assessing growth of the tree seedlings due to P application and VAM inoculation

In experiment I, Minjingu PR (12.9% P and 40% CaO) was applied at the rates of 0 (PRo), 25.8 (PR1) and 38. 7 (PR2) mg P kg^{-1} (equivalent to 0, 400 and 600 kg Minjingu PR ha^{-1}) and mixed thoroughly with the two soils before transplanting the one-month-old seedlings. The design of the experiment was a randomized complete block replicated three times.

In experiment II, the treatments were PR0, PR1, PR2, PRo + VAM-mycorrhizae, PR1+VA-mycorrhizae and PR2+VA-mycorrhizae using just Kakamega soil. Minjingu PR was applied and mixed thoroughly with the soil. About 200 ml of water was added (to approximately 80% field capacity) and the set-up left for a week to allow for equilibration. The VA-mycorrhizae inoculation method described by [16] was adopted at a rate of 2 g of inoculum per pot. The inoculum consisted of a mixture of roots and soil from *Acacia tortilis* and was added approximately quarter-way down the soil in the pots. A basal nutrient solution containing 200 mg N (NH_4NO_3), 400 mg K ($K_2SO_4.7H_2O$) and 400 mg Mg ($MgSO_4.7H_2O$) was applied to each pot at transplanting. The experiment was replicated four times in a completely randomized design.

In experiment I, height and root collar diameter were recorded at 21 weeks after transplanting (WAT). This age (21 WAT) is normally recommended by Forestry Department in Kenya to transplant tree seedlings to the field. In experiment II, the height and root collar diameter was recorded at 12 months after transplanting (12 MAT), after which the seedlings were harvested. Shoot dry weight was determined after drying the materials at 80° C in an oven for 24 hrs. One-centimeter root sections from experiment II were stained to assess VA-mycorrhiza infection using the methods described by [17, 18]. The grid intercept method of [19] was used to quantify the infection. The remaining portions of roots were oven-dried at 80° C for 24 hrs. for root dry weight determination.

2.3. Experiment III: Procedures for ^{32}P labeling the P fertilized experiment

In experiment III, ^{32}P isotope dilution technique was applied to discriminate between soil and fertilizer derived phosphorus in the plant material. MPR and TSP, were applied at the rate of 25.8 mg P/kg soil equivalent to 52 kg P ha^{-1}, and the Acrisol was used as the test soil. The treatments included:

(a) Soil alone as the control
(b) Soil + Minjingu PR and
(c) Soil + TSP

To all pots, 20 ml of a 10 ppm KH_2PO_4 solution labeled at an activity of 185x104 Bq ^{32}P/kg soil (50μCi ^{32}P per kg soil) were then uniformly applied over the soil surface of all pots immediately after transplanting the 3 week-old seedlings. Small amounts of distilled water were added to each pot to wash down the labeled solution. The soils were constantly watered to approximately field capacity. The experimental design was completely randomized with 3 replications for each of the harvests done at 3 and 6 months after transplanting (3 and 6 MAT). Coinciding with the first destructive harvest of shoots at 3 MAT, a similar amount and activity of ^{32}P labeled solution was again uniformly added onto the soil surface of 3 month old seedlings which constituted the second destructive harvest of shoots that was carried out at 6 MAT. For radiation protection measures, root data were not collected.

In experiment III, P uptake and other isotopically derived parameters were determined. At each harvest the shoots of the seedlings to which ^{32}P was applied were cut into small sections and oven dried at 80°C for 24 hours to obtain dry weight. This was followed by subsampling into 2 g portions and placed into oven dried porcelain dishes. The samples were moistened by a little distilled water and dry ashed in a muffle furnace at 450°C. After cooling, the ash were dissolved using 20 ml of 1 M HCl; 10 ml of this solution was used for plant tissue P determination by colorimetric method. The rest was transferred to vials for Cerenkov counting to assess ^{32}P activity using a liquid scintillation counter Packard Model LSC 2000. The following formulae were used to derive isotopically determined parameters [20].

$$SA = \frac{\text{Disintegration per minute (dpm)}}{(\text{mg P}/2)} \quad (1)$$

where SA= specific activity of plant material and mg P/2 because only half the sample was used in the P determination;

$$\%\ Pdfs = \frac{\text{SA plant (in presence of fertilizer)} \times 100}{\text{SA plant (in absence of fertilizer)}} \quad (2)$$

where %Pdfs = The percentage of P in the plant material derived from the soil; the assumption in this method is that the higher the P availability from fertilizer (Minjingu PR or TSP) to the plant, the more it will dilute the SA of the material;

$$Pdff = \frac{(1-Pdfs)}{(100)} \times 100 \quad (3)$$

where Pdff = percentage of P in the plant material derived from the respective fertilizers; the assumption made here was that the plant absorbs P in direct proportion to its availability and that the ratio of availability of soil P and labeled carrier ^{32}P was unaffected by the addition of fertilizer.

The actual amount in the plant material derived from the fertilizers was determined as follows:

$$AAPf = \text{Total P uptake} \times \%Pdff \quad (4)$$

Where AAPf = actual amount of P in mg taken up by the seedlings from the fertilizer. The relative availability of Minjingu PR was then determined as follows:

$$RAID = \frac{AAPf\ (MPR)}{AAPf\ (TSP)} \quad (5)$$

Where RAID = relative availability of (Minjingu PR compared to TSP) as determined by isotope dilution, AAPf (MPR) = actual amount of P in plant derived from Minjingu phosphate rock as determined by isotope dilution.

2.4. Statistical analysis

The data at 21 WAT (Experiment I), 12 MAT (Experiment II), 3 and 6 MAT (Experiment III) were subjected to analysis of variance (ANOVA) using the Genstat 3.22 computer software package [21] while least significant differences (l.s.d) at ($P<0.05$) were used to compare the means.

3. RESULTS AND DISCUSSION

3.1. Experiment I: Height and root collar diameter of 21 week-old seedlings

Results for height and root collar diameter for experiment I are shown in Table I. When the soil types and P rates were separated during the data analysis, a significant reduction of up to 1.5 times for both tree height and root collar parameters was observed upon addition of P fertilizer to the Andosol, Gituamba compared to the control. With the Acrisol, Kakamega there was a significant increase in height and root collar diameter of up to 1.5 times when P was applied compared to the control. Soil and P rates interaction were significant ($P<0.001$) confirming that the two soils behaved differently to P application. The mineralization of high organic matter in the Andosol,Gituamba under greenhouse conditions could have contributed to the negative response to P application. Furthermore growth response to P application in the Acrisol, Kakamega still remained lower than that recorded in the Andosol, Gituamba control.

The Andosols, Gituamba are volcanic soils, which typically have amorphous alumino-silicates and humus-Al complexes that have a very high capacity to sorb P [22, 23]. This sorption tends to be essentially irreversible, and even high rates of P application do not satisfy the P sorption capacity [24].

TABLE I. EFFECT OF P FERTILIZATION ON THE HEIGHT AND ROOT COLLAR DIAMETER INCREASE OF *G. robusta* AFTER 21 WEEKS WHILE GROWING IN TWO ACIDIC SOILS FROM KENYA

	P rate (kg P_2O_5/ha)			
	0	52	77	LSD ($p<0.05$) soil x P rates
Soil		Height (cm)		
Andosol, Gituamba	31.8	15.4	18.3	9.8
Acrisol, Kakamega	15.9	27.6	28.7	
		Root collar diameter (cm)		
Andosol, Gituamba	0.6	0.4	0.4	0.1
Acrisol, Kakamega	0.3	0.6	0.5	

However P fixed by amorphous materials in Gituamba Andosols was probably easily extracted by *G. robusta* through its special root structure [25]. This may have accounted for the poor response to P application. Moreover, the reduction in growth observed with larger P applications might be due to toxic amounts of exchangeable Al^{3+} released from the mineralizing organic matter-Al complexes influenced by the Ca^{2+} ions form Minjingu PR that contains 40% CaO as it solubilized to release nutrients into the soil solution. Such an effect of Ca^{2+} on organic matter-Al complexes in Andosols has also been reported by [26].

The seedlings in the control of Acrisol, Kakamega had poor growth compared to those of Andosol, Gituamba control because of inherently low P level in Acrisols [27]. The positive response to P application was probably due to the low organic matter levels, which were unable to supply sufficient P through mineralization [28]. Buresh et al., 1997 [22] reported that a decrease in soil organic matter is likely to lead to a reduction in supply of plant-available P. However, the low response to P fertilization in the Acrisols, Kakamega could be explained by their high Al content (approx. 37%) when compared to the Andosol, Gituamba control leading to an increase in the sorption of the P released from the applied Minjingu PR or directly affecting the growth of *G. robusta* seedlings.

3.2. Experiment II: Dry matter production, height and root collar diameter of 12 month-old seedlings

Results of experiment II on seedlings growing in the Acrisol, Kakamega are shown in Table II. There were no significant differences in response in terms of height; root collar diameter and root dry weights to P application or VA-mycorrhizae inoculation by *G. robusta*. No VAM infection was detected on the root samples and the seedlings looked healthy without symptoms of nutrient deficiencies. The results though inconclusive, imply that mycorrhizal inoculation may not be necessary when *G. robusta* is grown in this Acrisol. There was however, no trend in Minjingu PR addition and VAM inoculation at 12 MAT.

3.3. Experiment III: Height, root collar diameter and P uptake as estimated through isotope dilution method of 3 month-old and 6 month-old seedlings

Minjingu PR and TSP gave significant improvement on seedling height (for Minjingu PR only), root collar diameter, P uptake above the control (Table III). Addition of phosphate fertilizers to the Acrisol of low fertility probably enhanced root proliferation hence increased P uptake by the fertilized tree seedlings. Although height, root collar diameter and shoot dry weight increased between 3 and 6 MAT, P uptake declined significantly with increase in age of the tree seedlings (Table IV). The reduction of P uptake at 6 MAT was likely due to a dilution effect of P by the increase in tissue biomass. I is reported that there is a dilution effect of P due to additional growth because dry matter usually accumulates faster than P uptake[29]. However, at both 3 and 6 MAT P uptake by *G. robusta* from the two sources were not significantly different (Table V).

At 3 MAT of the total P taken up by the seedlings only 3% (0.09 mg P) was derived from Rock-P and 6% (0.18 mg P) from TSP. This supports observations made in experiments I and II to the effect that *G. robusta* showed a preference for inherent soil derived P. The relative availability of Minjingu PR, as determined by isotope dilution (RAID) value indicated that Minjingu PR was 50% as effective as TSP in supplying P to *G. robusta* seedlings. At 6 MAT the negative results for % Pdff and AAPf could mean that little or almost no P was taken up from either Minjingu PR or TSP between 3 and 6 MAT and thus, it was assumed that all the P in the plant was derived from the inherent soil P. Kato *et al.*, 1994 [30] reported that % Pdff in the plant seems to be affected by various soil and/or plant borne factors. However, similar studies conducted with other five fast growing tree species gave consistent % Pdff results [31].

These soils are high P-fixing and it is possible that the P applied as Minjingu PR or TSP was fixed immediately after dissolution in the soil. As the soil retained more strongly newly added cation (anion) than the one it had before [32], the plant had to exploit the P retained in the soil using other mechanisms, such as cluster roots [25]. These structures (roots) cling onto soil clods with nutrient

TABLE II. EFFECT OF MINJINGU PR APPLICATION AND VA-MYCORRHIZAE INOCULATION ON HEIGHT, ROOT COLLAR DIAMETER, STEM AND ROOT DRY WEIGHT OF *G. robusta* AFTER 12 MONTHS GROWING IN THE ACRISOL, KAKAMEGA

Treatments	Height (cm)	Root collar diameter (cm)	Shoot dry weight (g/plant)	Root dry weight (g/plant)
PR_0	66.3	1.8	94.7	34.1
PR_0 + M[1]	75.0	1.9	102.4	36.6
PR_1[2]	77.5	1.8	93.5	44.0
PR_1 + M	76.2	1.8	66.5	38.5
PR_2	70.8	1.8	79.4	35.9
PR_2 + M	72.7	1.7	103.2	36.4
LSD ($p<0.05$)	12.3 ns[3]	0.2 ns	29.9 ns	13.4 ns

[1] VA-mycorrhizae inoculum. [2] Minjingu rock phosphate. [3] ns= not significant.

TABLE III. EFFECT OF TWO P SOURCES ON HEIGHT, ROOT COLLAR DIAMETER, SHOOT DRY WEIGHT AND P UPTAKE OF *G. robusta* AT 6 MONTHS AFTER TRANSPLANTING INTO KAKAMEGA SOIL

P sources	Height (cm)	Root collar diameter (cm)	Shoot dry weight (g/plant)	P uptake (mg P/plant)
Minjingu PR[1]	38.1	0.5	8.0	2.0
TSP[2]	31.2	0.6	8.5	2.0
Control	30.0	0.5	6.8	1.2
LSD ($p<0.05$)	6.1*[3]	0.1*	1.8 ns[4]	0.7*

[1]Minjingu phosphate rock. [2]Triple superphosphate. [3] *= $p<0.05$. [4]ns=not significant.

TABLE IV.EFFECT OF P APPLICATION ON HEIGHT, ROOT COLLAR DIAMETER, SHOOT DRY WEIGHT AND P UPTAKE OF *G. robusta* AT 3 AND 6 MONTHS AFTER TRANSPLANTING INTO THE ACRISOL, KAKAMEGA

	Harvest period		
	3 MAT[1]	6 MAT	LSD ($p<0.05$)
Height (cm)	16.5	49.7	5.0***[2]
Root c.d (cm)[3]	0.3	0.8	0.1***
Shoot dry weight[4]	1.0	14.6	1.5***
mg P/plant	2.6	0.9	0.6***

[1] Months after transplanting. [2] ***= $p<0.001$. [3] Root collar diameter(cm). [4] Shoot dry weight (gm/plant)

patches, and probably exudate citrates, which move in rhizosphere for the mobilization of iron phosphates and acquisition of P [33]. It was observed during this study through low power microscopy that *G. robusta* root had such cluster roots. Skene *et.al.* [25] suggested that these roots occur at fixed distances and provide *G. robusta* with the opportunity of growing in soils of low available phosphate. These roots are specifically designed for nutrient patches exploration, exploitation and exportation. Moreover, Grierson and Attiwell, 1989 [34] showed that cluster roots produce hydrogen ions, reductants and possible chelating agents. These cluster roots in *G. robusta* exude citrate[25]. Some species with cluster roots exude as much as 23% of the total plant dry weight as citrate [35].

TABLE V. EFFECT OF TWO P SOURCES ON THE P UPTAKE, %Pdfs, %Pdff, AAPf AND RAID OF *G. robusta* AT 3 AND 6 MAT

P sources	Total P uptake (mg plant^{-1})	%Pdfs[1]	%Pdff[2]	AAPf (mg)[3]	RAID[4]
		3 MAT			
Minjingu PR	3.1	87	3	0.09	50
TSP[5]	3.0	84	6	0.18	n/a[6]
Control	1.7	n/a	n/a	n/a	n/a
	LSD($p<0.05$-P sources) 1.5				
		6 MAT			
Minjingu PR	1.0	124	-24	-22.8	n/a
TSP	1.0	135	-35	-35.0	n/a
Control	0.7	n/a	n/a	n/a	n/a
	LSD($p<0.05$-P sources) 0.60				

[1]Percent of phosphorus in plant material derived from soil. [2]Percent of phosphorus in plant arterial derived from fertilizer. [3]Actual amount of P taken up by the seedlings (%Pdff x Total P uptake) from fertilizer. [4]Relative availability of Minjingu PR as determined by isotope dilution. [5]Triple super phosphate. [6]n/a = value not applicable, LSD ($p<0.05$) P source * Harvest=0.99***.

4. CONCLUSIONS

The application of Minjingu PR to the Acrisol, Kakamega improved the growth of *G. robusta* seedlings at the early stage. The failure to get a response with the Andosol, Gituamba was attributed to increased availability to *G. robusta* of fixed P in Andosols and to the toxic effect of exchangeable Al^{3+} released by the Ca^{2+} input from the PR application. VAM inoculation may not be necessary with *G. robusta* and none of the seedlings were infected with VAM. Through use of ^{32}P isotope dilution techniques it was observed that availability of P from Minjingu PR was a half of that from water-soluble TSPat 3 MAT. The reduction of P uptake at 6 MAT was probably due to a P dilution effect by increased growth. *G.* robusta was shown to be able to utilize inherent soil P even in the absence of external P sources e.g. Minjingu PR and TSP. The ability of *G.* robusta to perform well in low available P and acidic Andosols is interesting. However, P fertilization might still be necessary in the Acrisols at the early stages of growth of *G. robusta.* Further investigations are needed with this tree species to establish the nature of the uptake discrimination against fertilizer P and whether the formation of cluster roots is an adaptation mechanism.

ACKNOWLEDGEMENT

The authors are indebted to the International Atomic Energy Agency (IAEA) for financial assistance and supply of ^{32}P isotope under research contract KEN-7503S. Thanks are given to S. Maingi, who helped in carrying out the plant sample analysis and V. Rutunga for helping in French translation and improvements on the manuscript.

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