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ORIGINAL PAPER

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Effect of phosphorus source and rate of application on VAM fungal infection and growth of maize (*Zea mays* L.)

Abstract The effects of two phosphorus (P) sources (triple superphosphate and Ghafsa phosphate rock), applied at rates equivalent to 44 kg ha^{-1} and 22 kg ha^{-1}, on vesicular-arbuscular mycorrhizal (VAM) fungal infection in roots, dry matter yield and nutrient content of maize grown in an oxisol and an alfisol, were investigated in a growth cabinet. The application of 44 kg P ha^{-1} resulted in root infection by VAM fungi not was significantly different ($P \leq 0.01$) from when no P was applied. Root infection was significantly greater when P was applied as triple superphosphate at the rate of 22 kg ha^{-1} the higher rate. Phosphate rock treatments at both rates of application resulted in significantly greater root infection than in controls with no P or when triple superphosphate was applied at 44 kg ha^{-1}. Plant P uptake increased in all soils with the different P treatments compared with the control. No direct effects of the treatments on the aluminium and zinc contents of maize plants were observed. In the gleyic alfisol, reduced Mn uptake as a result of increased infection of plants with the superphosphate treatments was observed. Higher Mn was also found in plants with the higher rate of superphosphate treatment than with the phosphate rock treatments in the haplustox, although infection rates in plants with the latter treatments were higher. With the exception of plants with the phosphate rock treatment applied at 22 kg ha^{-1}, dry matter yields of plants with all P sources were significantly greater than the controls.

Key words Vesicular-arbuscular mycorrhiza
Phosphate rock · Triple superphosphate · Infection

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Introduction

For many decades, the dominant P sources used by farmers in tropical sub-Saharan Africa have been the manufactured single/triple superphosphates and other high-analysis compound fertilisers. With the ever-increasing need to reduce farm inputs, phosphate rock, which is about one-third of the price of single superphosphate (Nye and Kirk 1986), is being adopted for application to acid soils with inherently low P levels. It has been recognised that such alternative sources of P may be appropriate to replace or complement the conventional sources to fill the needs of specific user-groups such as peasant farmers with limited capital (Hammond et al. 1986).

Although some information is available on the influence of phosphate applied as single/triple superphosphate on the growth of some crops in sub-Saharan Africa, there is a dearth of information on the effect of these sources on vesicular-arbuscular mycorrhizal (VAM) fungi and their role in the nutrition of crop plants.

Investigations into rock phosphates in the sub-region have mainly centred on their agronomic potential (Juo and Kang 1978, 1979). Little or nothing is known about the effects of phosphate rock or superphosphate fertilisers on VAM associations in plants. Additionally, the enormous variability in agronomic effectiveness of different types of phosphate rock (Khasawneh and Doll 1978), due to variations in physico-chemical and edaphic factors, has made it increasingly difficult to extrapolate results from one location to another.

Recent evidence from other regions suggests differences between VAM fungi in their sensitivity to phosphate (Plenchette et al. 1983; Salinas et al. 1985; Schubert and Hayman 1986). Instances have been reported where VAM fungi have been effective with the application of phosphate from one source and ineffective with other sources (Jehne 1980; Powell 1980a). This also suggests sensitivity of the mycobionts to phosphate sources.

The application of high rates of expensive phosphate fertiliser on high phosphate-fixing, acid tropical soils could be suppressing or reducing the beneficial effects of VAM associations, if reports (Powell 1980b; Saif 1986) on the negative influence of high phosphate applications on the mutualistic association in temperate soils can be extrapolated to tropical regions.

As national agricultural policies change and peasant agricultural systems begin to adopt fertiliser management practices which involve the use of phosphate rocks, either singly or in combination with high-analysis manufactured phosphate fertilisers, there is a need to determine the effect of these nutrient sources on the mutualistic endomycorrhizal association. This must be done in different agroecological zones if the potential of the mutualistic association for improved growth and restoration of soil fertility is to be fully exploited.

This present investigation was conducted to determine the effect of high-analysis triple superphosphate and unacidulated rock phosphate on VAM fungal infection in maize roots and its related effects on overall plant growth.

Materials and methods

Soils

Two soils (Basachia series, a typic haplustox, and Udu series, an aquic paleustalf) from the rainforest and coastal savanna zones of Ghana were analysed for some physical and chemical properties.

P sources

Two P sources were used in the investigation: triple superphosphate (TSP, manufactured by BASF) and a carbonate-substituted, phosphate rock from Tunisia (Ghafsa). The phosphate-containing materials were ground to allow 70% of the material to pass through an 80-mesh sieve and the phosphate rock source was analysed for P, Ca, aluminium oxide (Al_2O_3), and iron oxide (Fe_2O_3).

Treatments

Two rates of application were used: 44 kg P ha^{-1}, the full rate recommended for maize, and 22 kg P ha^{-1}. For the full application rate, phosphate carriers containing 19.5 mg P were added to 1.0 kg of soil per pot and thoroughly mixed. All replicates of the same treatment were bulked together and mixed before potting. For half the full rate, the P sources contained 9.8 mg P. All rates used for P sources were calculated on the basis of ammonium citrate and citric acid solubilities for triple superphosphate and rock phosphate, respectively. Controls without phosphate were taken through the same routine. Nitrogen was applied at a rate equivalent to 30 kg N ha^{-1}, and 25 g of mixed inoculum consisting of root, spores and mycelia of mycorrhizal fungi were added to each pot and mixed into the soil by stirring. The pots were brought to a matric potential of −30 kPa by the addition of deionised water and placed in a growth cabinet (Fitotron 600) with environmental settings as follows: temperature 27/24° C (day/night), relative humidity 70%, photoperiod 12 h, light intensity 20000 lux.

Seeds of maize (variety Dobidi) were surface sterilised and germinated in Petri dishes. Seedlings were transplanted into the pots (1/pot) after 3 days, when the radicles had appeared. The plants were allowed to grow for 6 weeks and then harvested.

Plant analysis

Plant shoots and roots were rinsed in deionised water and samples were dried at 60° C. Dried plant material was digested using a sulphuric/perchloric acid digestion procedure (Cresser and Parsons 1979). Phosphorus in the digests was determined by a colorimetric method (Murphy and Riley 1962). Manganese and zinc in digests were determined by atomic absorption spectrometry.

Aluminium in plant material was analysed using a modification of the the digestion procedure of Wilson (1984) as follows: duplicate 2-g samples of oven-dried plant material were weighed into digestion tubes and 8 ml of 15 M HNO_3 and 2 ml of $HClO_4$ were added. The tubes were allowed to stand overnight and then placed in a digestion heating block at 105° C for 1 h and then 200° C for a further 1 h. The samples were allowed to cool and the digests were quantitatively transferred into 50-ml volumetric flasks and made up to volume with deionised water. The aluminium concentration in the digests was determined colorimetrically by the method of Pritchard (1967).

Estimation of VAM fungal infection

Root subsamples were processed and the percentage root length infected was estimated by the method of Koske and Gemma (1989).

Statistical design and analysis

The pots were arranged to conform to a randomised complete block design consisting of 10 treatments, with four replications. Analysis of variance procedures were used to analyse the effect of treatments on plant growth, mycorrhizal fungal infection and nutrient uptake. Mean comparisons were made using the least significant difference (LSD) test.

Results

Soils

Some physical and chemical properties of the soils are presented in Table 1.

P sources

The chemical compositions of the Ghafsa phosphate rock and triple superphosphate are presented in Table 2. Applications of triple superphosphate and phosphate rock at the two different rates are designated as TSP1, TSP2, PR1 and PR2, respectively.

Root infection

There were no significant differences in percentage root length infection between control plants and plants

Table 1 Some physical and chemical properties of the soils used

Soil series	pH (1:2 $CaCl_2$)	Ca	Mg	K	Na	Al	H	ECEC	Al sat %	Zn $\mu g\ g^{-1}$	P $mg\ kg^{-1}$	Available N ($mg\ kg^{-1}$)		Mn $\mu g\ g^{-1}$	Org C %	Clay %	Silt %
		(cmol kg^{-1})										NH_4–N	NO_3–N				
Udu (aquic paleustalf)	5.31	2.32	0.39	0.20	0.32	0.11	1.02	4.36	2.52	4.35	8.78	37.84	23.52	44.47	2.22	30.14	20.22
Basachia (typic haplustox)	4.65	1.14	0.33	0.23	0.17	0.32	1.08	3.27	9.79	3.2	4.85	31.33	21.22	22.57	2.31	18.56	17.23

Table 2 Chemical characteristics of the phosphorus sources. The data for TSP are taken from the manufacturers analysis. Citric acid-soluble P is expressed as percent total P. *PR* Phosphate rock, *TSP* triple superphosphate

	TSP	Ghafsa PR
Total P (%)	—	12.1
Citric acid-soluble P (%)	19.6	33.9
SiO (%)	—	—
Ca (%)	13.6	31.0
Al_2O_3 (%)	—	4.0
Fe_2O_3 (%)	—	4.0
Other constituents (%)	—	8.0

grown under treatment TSP2 in the Udu soil. The percentage root length infection in plants grown under all other treatments was significantly ($P \leq 0.01$) higher than that of control plants (Table 3). In the typic haplustox soil, the percentage root length infections of maize plants in treatments TSP1 and TSP2 and the controls were not significantly different. However, the infection of control plants was significantly higher than the plants in the PR treatments.

In both soils, the percentage root length infection of plants grown under treatment TSP2 was significantly ($P \leq 0.01$) lower than that of plants grown under treatments PR1 and PR2. There were no significant differences in percentage root length infection between plants grown in the haplustox under all treatments and those in the paleustalf.

Plant P concentration and uptake

The concentration of P in control plants was significantly ($P \leq 0.01$) lower than that in plants grown under treatments TSP2 and PR2 in the aquic paleustalf. There were no significant differences in P concentration between control plants and plants grown under treatments TSP1 and PR1 (Table 3).

The P concentration in control plants was significantly ($P \leq 0.01$) lower than that in plants grown under treatment TSP1 in the haplustox. In the same soil, there were no significant differences in P concentration between control plants and plants grown under treatments TSP2, PR1 and PR2. Phosphorus uptake per pot by control plants grown in both soils was significantly ($P \leq 0.01$) lower than that found in all other treatments. No significant differences were found between treatments PR1 and PR2 and control plants grown in all soils.

The highest P uptake was associated with plants grown under treatment TSP2, this being significantly ($P \leq 0.01$) higher than that of plants under all other treatments in the paleustalf. Phosphorus uptake by plants in the haplustox treatments TSP1 and TSP2 was not significantly different, and P uptake by plants in treatment TSP1 was not significantly different from that of plants in treatments PR1 and PR2.

Table 3 Relationships between phosphorus sources applied and root infection, nutrient concentration and uptake, and dry weight of maize. *TSP1* Triple superphosphate 44 kg P ha^{-1}, *TSP2* triple superphosphate 22 kg P ha^{-1}, *PR1* rock phosphate 44 kg P ha^{-1}, *PR2* rock phosphate 22 kg P ha^{-1}

Soil series	P source	Root infection (%)	Nutrient concentration				Nutrient uptake				Dry wt. (g/pot)
			P	Al	Mn	Zn	P (mg/pot)	Al (μg/pot)	Mn (μg/pot)	Zn (μg/pot)	
				(μg g^{-1})							
Udu (paleustalf)	Control	47.50	1.26	289.31	77.19	44.98	1.65	379.00	101.12	58.92	1.31
	TSP1	78.30	1.27	286.52	68.18	38.12	2.58	581.64	138.41	77.38	2.03
	TSP2	52.30	2.18	248.97	76.16	36.77	4.38	500.43	153.08	73.91	2.01
	PR1	68.90	1.61	245.53	74.80	41.72	2.71	412.49	125.66	70.09	1.68
	PR2	75.00	1.61	237.45	72.23	39.00	3.73	489.15	148.79	80.34	2.06
Basachia (haplustox)	Control	58.00	0.99	486.70	79.61	43.65	1.12	549.97	89.96	49.32	1.13
	TSP1	63.80	1.40	484.55	71.00	38.62	2.81	973.95	142.71	77.63	2.01
	TSP2	50.00	1.37	455.08	82.34	36.00	3.21	1064.89	192.68	79.64	2.24
	PR1	77.60	1.12	423.65	65.11	35.63	2.18	826.12	126.97	69.48	1.95
	PR2	80.25	1.16	416.96	72.31	36.57	2.33	838.09	145.34	73.51	2.01
LSD	($P<0.01$)	16.48	0.39	97.84	9.64	8.54	0.68	284.31	44.13	31.76	0.59

Plants grown under treatment TSP2 in the paleustalf had a significantly ($P \leq 0.01$) higher P uptake than plants grown under the corresponding treatment in the haplustox.

Plant Al concentration and uptake

No significant differences in Al concentration were found between any of the treatments (Table 3).

Plant Mn concentration and uptake

Manganese concentrations in plants grown under all superphosphate and phosphate rock treatments in the paleustalf were not significantly different from that in control plants (Table 3). However, the Mn concentration in control plants grown in the haplustox was significantly higher than that in plants with treatment PR1 but not different from those in plants from all other treatments.

Manganese uptake by control plants grown in the aquic paleustalf was not significantly different from uptake by plants grown under treatments TSP1 and PR1. However, Mn uptake by plants under treatments PR2 and TSP2 was significantly ($P \leq 0.01$) higher than that found for control plants. There were no significant differences in Mn uptake by plants under superphosphate treatments compared with plants under rock phosphate treatments.

Manganese uptake by control plants in the typic haplustox was significantly ($P \leq 0.01$) lower than uptake by plants grown under treatments TSP1, TSP2 and PR2, but not significantly different from that of plants grown under treatment PR1. The Mn uptake by plants grown under treatment TSP2 was significantly ($P \leq 0.01$) higher than uptake by plants grown under treatments TSP1, PR1 and PR2.

Plant Zn concentration and uptake

In both soils, there were no significant differences between Zn concentration and uptake of control plants and all the superphosphate and phosphate rock treatments (Table 3).

Plant dry weight

The dry weight of maize plants grown in the aquic paleustalf without P application (controls) was significantly ($P \leq 0.01$) lower than that of plants grown under treatments TSP1, TSP2, and PR2. There was no significant difference between the dry weight of control plants and that of plants grown under treatment PR1 (Table 3).

The dry weights of plants grown in the oxisol at all superphosphate and phosphate rock rates were significantly ($P \leq 0.01$) higher than that of control plants. There were no significant differences in the dry weights of plants grown in the oxisol between treatments TSP1 and TSP2, or between treatments PR1 and PR2. In the two soil types, no significant differences were found in the dry weight of plants grown under corresponding treatments.

Discussion

The addition of phosphate rock at the rate equivalent to 19 mg nitrate-soluble P per kg soil produced a limited liming effect by raising the pH of the oxisol soil by 0.2 units. Ghafsa phosphate rock is generally regarded as being of high reactivity (Robinson and Syers 1990). The reactivity of phosphate rock in soil depends on factors such as the composition of the mineral, mineral particle size, soil pH and the P and Ca status of the soil.

Maize plants grown in the two soils under all phosphate treatments were infected by indigenous mycorrhizal fungi. The significant decrease in infection associated with plants under treatment TSP2 may have resulted from the high supply of soluble P by the triple superphosphate. Various workers have suggested that both the phosphate in rooting media (Daft and Nicholson 1972; Stribley 1987; Schubert and Hayman 1986) and the P concentration in plants (Sanders 1975; Menge et al. 1978) are responsible for reductions in VAM fungal infection of plants. It seems likely that the supply of soluble P in the soil was responsible for the reduced VAM fungal infection associated with treatment TSP2. The differences in percentage root length infection in the two soils between control plants and plants grown under the superphosphate and phosphate rock treatments could be attributed to differences in the ability of different VAM fungal species or strains to infect at increasing soil P, as suggested by Jasper et al. (1979).

Some workers (Pairunan et al. 1980; Same et al. 1983; Bolan et al. 1984) have reported increases in VAM infection of plants with increasing availability of P. For both soils, there was no effect of P source on infectivity (i.e. there was no difference in VAM infection between plants grown under the superphosphate and phosphate rock treatments at the first rate of application). The effect of P source is evident when the second rate of P application is considered. The high root length infection associated with treatments PR1 and PR2 could be attributed to low available P from these sources, since the dissolution and release of P from phosphate rock sources are slower compared to that of relatively soluble P sources such as superphosphate.

Phosphate in the soil solution derived from the Ghafsa phosphate rock source could not be expected to be different to the superphosphate source, and so the difference in infectivity associated with the higher rate of superphosphate could also have been due to the differences in the rates of dissolution of superphosphate and phosphate rock to provide P in the soil solution over a given period.

Plant P uptake increased in all soils with the different P treatments compared with the control. The higher P uptake associated in treatment TSP2 with low VAM infection suggests that root uptake of readily available P from the relatively soluble superphosphate source was the dominant mechanism compared with other treatments where root infection was significantly higher. Reduction in VAM infection with increased uptake has also been reported by Arines et al. (1989).

Aluminium concentration in maize plants was not influenced by the different treatments. It has been suggested that Ca liberated from phosphate rock sources promotes substitution of Ca for Al on the soil exchange complex (Hammond 1978), with the result that there is hydration or precipitation of released Al with $H_2PO_4^-$ in the soil solution (Easterwood et al. 1989).

In the aquic paleustalf, it seemed that higher root infection by VAM fungi was associated with lower Mn uptake by plants with the superphospate treatments. Mn uptake by plants grown in the oxisol soil under treatment TSP2 was significantly higher than that by plants grown under phosphate rock treatments, while the percentage root length infection was significantly higher in plants under treatments PR1 and PR2. Reduced Mn uptake in plants colonised by various VAM endophytes has also been reported by Pacovsky (1986) and Arines et al. (1989).

With regard to Zn concentration and uptake, no direct effects of the different phosphate treatments were observed.

Plants grown in the paleustalf under treatment PR1 did not differ in dry weight from control plants without phosphate. The plant P concentration associated with treatment PR1 was not below critical levels and plants did not show deficiency symptoms. A possible explanation for the absence of a significant increase in dry weight in this treatment may be found in the suggestion by Bolan (1991) that mycorrhizal plants use more carbon for purposes other than growth of photosynthetic tissue. Dry weight loss in mycorrhizal plants has also been associated with a drain in energy by infecting fungi (Stribley et al. 1980).

In the typic haplustox, no influence of P source was evident and dry weight increases over the control did not differ between the different P sources. In other field investigations, the dry weight of above-ground parts of maize after 30 days of growth was found to be lower when P was applied as carbonate-substituted phosphate rock (Kodjari phosphate rock) than as superphosphate; however, no differences in dry weights were found when the soils were cropped again (Easterwood et al. 1989). Such observations have been attributed to the slow P release characteristics of phosphate rock (Hammond 1978).

The effect of triple superphosphate applied at recommended rates was to reduce VAM fungal infection of the roots of maize plants. The direct application of phosphate rock in other locations has not always given satisfactory results in terms of yield when low-reactive rock or inappropriate soil/crop combinations have been used (Chien and Hammond 1991).

The long-term effectiveness of phosphate rock with various compositions and reactivity has been recognised and the effectiveness of unacidulated phosphate rocks such as Ghafsa phosphate rock may well vary from location to location, depending on soil properties and the mineralogy of the rock.

Infection by indigenous VAM fungi, however, was reduced with the application of the full recommended rate of the relatively soluble triple superphosphate. This reduction in infection could reduce or offset the many other benefits that VAM impart to plants in addition to improved P nutrition. The absence of a difference in dry matter yields between maize plants in treatments TSP1 and TSP2 suggests that the lower rate of application may be appropriate for the first 6 weeks of growth and that this ensured increased formation of

mycorrhizas. The relatively high levels of infection achieved with treatment TSP1 during the period before the reproductive stages of growth would be beneficial in the subsequent grain-filling stages since the amount and quality of maize grain has been found by Hanway (1989) to be influenced by the nutrients available to the maize plant during this period.

In developing countries, alternative soil management strategies are needed for resource-poor farmers to improve yields of crops, especially on acid soils, without additional inputs such as expensive manufactured phosphate fertilisers. Results obtained in this investigation show that the application of Ghafsa phosphate rock to acid, marginal soils offers a useful prospect for enhanced crop growth and mycorrhiza formation in cropping systems, with the added benefits of soil fertility restoration.

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