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Influence of vesicular-arbuscular mycorrhizae on phosphate fertilizer efficiency in two tropical acid soils planted with micropropagated oil palm (*Elaeis guineensis* jacq.)

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Summary. The influence of vesicular-arbuscular mycorrhizae on the efficiency of triple superphosphate and rock phosphate fertilizers was compared in two tropical, acid, P-fixing soils (Ivory Coast) in which the available P was labelled with $^{32}PO_4^{3-}$. Both soils were planted with micropropagated oil palms. The growth responses to the fertilizer applications were low unless accompanied by VAM inoculation, but both fertilizers were equally available to plants. Isotopic-dilution kinetics analyses indicated that the rock phosphate was solubilized in both soils and there was an enrichment of the labile pool of plant-available P, similar to that with superphosphate. The specific activity and the fraction of P derived from either fertilizer was similar in both mycorrhizal and non-mycorrhizal plants, showing that both absorbed P from the same labile pool of P in the fertilized soils. However, VAM inoculation increased the fertilizer utilization coefficient of plants 2.7- to 5.6-fold, depending on the soil and fertilizer. We conclude that VAM inoculation increases fertilizer efficiency, as much of rock phosphate as of superphosphate, for plants growing in acid, P-fixing soils, and the processes involved are not different for the two fertilizers.

Key words: Vesicular-arbuscular mycorrhizae – Fertilizers – Phosphorus – Acid soils – Oil palm – *Elaeis guineensis* – Glomus spp.

The symbiotic association established between plant roots and fungi belonging to the Endogonaceae, known as vesicular-arbuscular mycorrhizae (VAM), can significantly improve plant growth and development due to enhanced uptake of soil phosphate and certain other nutrients (Tinker 1978; Harley and Smith 1983; Gianinazzi-Pearson and Gianinazzi 1989). The positive role of VAM in phosphate uptake is mainly due to the capacity of mycorrhizal roots to exploit the labile pool of available soil phosphate more efficiently (Sanders and Tinker 1971; Hayman and Mosse 1972; Mosse et al. 1973; Powell 1975; Pichot and Binh 1976; Gianinazzi-Pearson et al. 1981).

The potential usefulness of VAM for agricultural production systems, and in particular for the recovery and growth of high-value micropropagated plants, has been shown for several temperate species (Morandi et al. 1979; Pons et al. 1983; Gianinazzi et al. 1986; Branzanti et al. 1990; Ravolanirina et al. 1990). In contrast, very little research has been carried out on economically important perennial tropical species, such as oil palms, that are micropropagated (Blal et al. 1987; Blal and Gianinazzi-Pearson 1990). In the tropics, crop production is often seriously limited by the deficiency of soils in P (as well as N and water), partly due to the high phosphate-fixing nature of many of them (Roche 1983). In tropical agricultural systems, soluble phosphate fertilizers are commonly used to restore the phosphate reserves in the soil, but for economic reasons insoluble phosphates, such as rock phosphates, are being increasingly considered as substitutes (Hammond and Leon 1983).

While the role of VAM in P uptake from soluble forms of phosphate is well documented (Tinker 1978; Mosse 1973; Harley and Smith 1983; Gianinazzi-Pearson 1986), results concerning their ability to mobilize P from other soil sources have been contradictory (Swaminathan and Verma 1977; Pairunan et al. 1980; Cooper 1984; Bolan et al. 1984). Furthermore, reports that VAM plants respond greatly to soil fertilization by tricalcium phosphate, iron phosphate, and rock phosphate seem to indicate an improved use of P from

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these relatively insoluble fertilizers (Murdoch et al. 1967; Jackson et al. 1972; Powell and Daniel 1978; Cabala-Rosand and Wild 1982; Bolan et al. 1987). Rock phosphate does become available to some plants in acid soils and not in neutral or alkaline soils (Cooke 1956; Sims 1959; Jackson 1966), but Mosse et al. (1976) suggested that VAM infection does not alter this relationship. In fact, studies on the role of VAM in P cycling in near-neutral soils have shown that mycorrhizae do not use ^{32}P-labelled tricalcium phosphate fertilizer to a greater extent than non-mycorrhizal roots (Raj et al. 1981; Azcon-Aguilar et al. 1986).

Since most tropical soils are of low pH, which will affect the availability of added insoluble fertilizers, VAM could be an important factor under these conditions in facilitating plant recovery of P from these fertilizers and therefore in increasing fertilizer efficiency. The aim of the present work was to compare the efficiency with which VAM can improve the use of soluble and rock phosphate fertilizers by micropropagated oil palms growing in tropical acid soils, and to investigate the processes involved using radioisotope-labelling techniques.

Materials and methods

Soils and phosphate fertilizers. Two tropical acid soils (Dabou and Soubré), collected in oil palm plantations of the Ivory Coast, were dried, sieved (2 mm) and disinfected by γ-irradiation (10 KGy). The physical and chemical characteristics of these soils are given in Table 1. The available P in these soils was labelled by adding a carrier-free solution of $^{32}PO_4^{3-}$ ions at the rate of 3.7×10^7 Bq kg^{-1} soil. After labelling, two phosphate fertilizers were separately mixed into each soil at a rate of 66 ppm P: triple superphosphate (19.8% P) or North Carolina non-calcined rock phosphate (13.2% P : 0.1 ppm water-soluble P). Four hundred grams of $^{32}PO_4$-labelled fertilized or unfertilized soil were weighed into individual pots.

Table 1. Physical and chemical characteristics of Dabou and Soubré soils

	Dabou	Soubré
Clay (%)	9.2	15.3
Silt (%)	4.1	8.4
Sand (%)	86.7	76.3
Organic matter (%)	1.1	1.3
C (%)	0.62	0.77
N (%)	0.62	0.65
C : N (%)	10.0	12.0
Exchangeable Ca (meq 100 g^{-1})	0.66	0.75
Exchangeable Mg (meq 100 g^{-1})	0.28	0.27
Exchangeable K (meq 100 g^{-1})	0.08	0.05
Exchangeable Na (meq 100 g^{-1})	0.28	0.18
Exchangeable Al (meq 100 g^{-1})	0.19	0.16
Cation exchange capacity (meq 100 g^{-1})	1.4	1.3
Total P (mg kg^{-1})	204.0	97.0
Saunder P (mg kg^{-1})	56.0	28.0
Olsen P (mg kg^{-1})	8.3	3.9
pH (1 : 2.5) H_2O	5.1	5.2
pH (1 : 2.5) KCl	4.0	4.2

Plant, VAM inoculation, and growth conditions. Two VAM fungi (*Glomus* sp.) isolated from oil palm (*Elaeis guineensis* Jacq.) plantations at Dabou (*Glomus* isolate LPA 21) and Soubré (Glomus isolate LPA 22) in the Ivory Coast, were separately maintained as pot cultures on *Tephrosia candida*, a tropical legume, in their corresponding soils of origin. Plants of a micropropagated oil palm clone (LMC 074 from the IRHO Station La Mé, Ivory Coast) were inoculated with root fragments of *T. candida* infected by either isolate during a post in vitro acclimatization period in sterilized sand (Ravolanirina et al. 1989). Non-mycorrhizal oil palm plants were given filtered washings of infected roots to supply the same microflora as that associated with the inoculum. After 4 weeks the acclimatized plants were transplanted, one per pot, into the ^{32}P-labelled soils. Those infected with *Glomus* sp. LPA 21 and *Glomus* sp. LPA 22 were transplanted into Dabou soil and Soubré soil, respectively.

Each treatment comprised eight replicates and all pots were completely randomized in a growth chamber (27 °C/25 °C, 80%–90% relative humidity, 250 μmol^{-2} m^{-2} s^{-1}, 16-h day). Each pot was watered daily with distilled water and weekly with 50 ml of a nutrient solution containing KCl (3 g l^{-1}), NH_4NO_3 (2.5 g l^{-1}) and $MgSO_4\cdot7H_2O$ (3 g l^{-1}).

Measurements. The plants were harvested after 12 weeks' growth, and the dry weights of roots and shoots were determined. Mycorrhizal infection was estimated microscopically on a sample of fresh root as described by Trouvelot et al. (1986), after clearing and staining (Phillips and Hayman 1970). Mineralization of shoots and roots was carried out separately at 580 °C over 5 h. The ashes of each sample were dissolved in 20 ml 1.2 *N* HNO_3 and made up to 50 ml with H_2O. The P content was determined by an L-ascorbic acid method, as described by John (1970); ^{32}P radioactivity was measured in a liquid scintillation counter by the Cerenkov effect. The P taken up by plants from both the soil and the phosphate fertilizers during the growth period was calculated by subtracting the quantity of P present in the plant just before planting.

The fertilizer effect on the P nutrition of the plants was determined by measuring the specific activity (^{32}P/total P) in the soils and in the plants after their growth on the labelled soil with or without P fertilizer. Two parameters were considered: the utilization coefficient (UC%) of the phosphate fertilizer (percentage quantity of P fertilizer in the plant sample/quantity of P fertilizer applied) and the amount of P in the plant derived from fertilizer (PdfF%; percentage quantity of P fertilizer in the plant sample/quantity of total P in the plant). These parameters were calculated according to Fardeau et al. (1984) by the following equations:

$$UC\% = \frac{^{32}\text{P activity of plant sample}}{\text{quantity of P fertilizer applied}} \times \left(\frac{1}{\text{plant specific activity}} - \frac{1}{\text{soil specific activity}}\right) \times 100$$

$$PdfF\% = \left(1 - \frac{\text{plant specific activity}}{\text{soil specific activity}}\right) \times 100$$

The available-P status of the soils, with triple superphosphate, or non-calcined rock phosphate, or without fertilizer, was analyzed before and after incubation under plant growth conditions without cropping, and after cropping with mycorrhizal or non-mycorrhizal plants by the isotopic-dilution kinetics method. The isotopic-dilu-

tion kinetics were determined using a mixture of 10 g soil in 100 ml water as described by Fardeau and Guiraud (1974). Larsen (1967) and Fardeau and Jappé (1976) have previously shown that for times of isotopic exchange between 0.5 min and 2.5 months, the relation between r (radioactivity present in solution at time t) and R (radioactivity injected in the soil system at time $t = 0$) can be written:

$$\frac{r}{R} = \frac{r_1}{R} t^{-b} \left(\text{or } \log \frac{r}{R} = \log \frac{r_1}{R} - b \log t \right)$$

where r^1 is the radioactivity present in solution at time $t = 1$ min and b an exponential characteristic of the soil sample. Values for r_1/R and b were obtained for each sample by analyzing aliquots of solution after 1, 10, 40, and 100 min of contact between the soil solution and the isotope. Two other parameters were also estimated to describe the labile pool of soil PO_4, M_1, the quantity of PO_4 ions in solution measured using the method described by John (1970), and E_1, the pool of directly available P calculated by $E_1 = M_1 \times R/r_1$.

All data were analyzed by analysis of variance and Duncon tests.

Results

Crop analyses

Mycorrhizal infection. The VAM-inoculated plants developed a high level of infection in both acid soils (Table 2), which indicated good ecological adaptation in each mycorrhizal fungus to the soil of origin. No infection was observed in the uninoculated control plants. In these soils phosphate-fertilizer applications of 66 ppm P caused only a slight and insignificant decrease in the frequency and intensity of mycorrhiza development.

Dry-weight yields. The dry weights of shoots and roots after 12 weeks' growth are presented in Table 3. The oil palm plants grew very poorly in both the Dabou and the Soubré soils without mycorrhizal infection and without fertilizers, although adding either phosphate fertilizer to the non-mycorrhizal plants led to a significant increase in dry-matter production (two-to threefold). However, this fertilizer effect on plant growth was not as important as that of VAM inoculation without phosphate fertilizer (four-to fivefold). The presence of both the mycorrhiza and the phosphate fertilizer ensured the maximum growth of the oil palm plants in both soils. No difference was observed between the growth response of the inoculated plants to the rock or the soluble phosphate fertilizer. The response of the mycorrhizal plants to both types of fertilizer was significantly greater in the Soubré soil.

P uptake by plants from soil and fertilizers

Phosphate nutrition. Data concerning the P contents of oil palm plants growing in the two soils are given in Table 3. The P contents of the non-mycorrhizal plants without fertilizer were very low compared to those in the other treatments. The addition of superphosphate or rock phosphate to the non-mycorrhizal plants significantly increased the total and percentage P contents. Oil palm plants infected by a VAM fungus took up more P than fertilized non-mycorrhizal plants, even in the absence of fertilizer. The maximum phosphate uptake was obtained when either triple superphosphate or rock phosphate was supplied to the mycorrhizal plants, showing the positive interaction of VAM with the fertilizers. The greatest effect of this positive interaction was obtained in the Soubré soil, when rock phosphate was added to mycorrhizal plants.

Table 2. Effect of fertilizer applications on mycorrhizal infection in oil palm plants growing in Dabou and Soubré soils

Soil	Fertilizer	Infection parameters (%)		
		F	M	A
Dabou	0	92 a	71 a	54 a
	+TSP	75 a	54 a	49 a
	+CNC	70 a	52 a	48 a
Soubré	0	94 a	73 a	72 a
	+TSP	83 a	59 a	58 a
	+CNC	85 a	64 a	64 a

F, frequency of root infection; M, intensity of cortical infection; A, arbuscule frequency in roots (Trouvelot et al. 1986); TSP, triple superphosphate; CNC, North Carolina non-calcined rock phosphate. Values in each column followed by the same letter do not differ significantly ($P = 0.05$; Duncan's test) and each soil was statistically analyzed separately

Table 3. Dry matter and P content of mycorrhizal (M) and non-mycorrhizal (NM) oil palm plants growing in Dabou and Soubré soils

Soil		Fertilizer	Shoot dry weight (g)	Root dry weight (g)	P plant^{-1} (µg)	P (%)
Dabou	NM	0	0.54 a	0.15 a	427 d	0.064 d
		+TSP	0.93 b	0.23 a	1265 c	0.116 b
		+CNC	0.86 b	0.24 a	1232 c	0.109 bc
	M	0	2.13 c	0.56 b	2742 b	0.102 c
		+TSP	2.82 c	0.76 bc	5740 a	0.173 a
		+CNC	2.69 c	0.82 c	5495 a	0.173 a
Soubré	NM	0	0.55 a	0.09 a	433 e	0.065 e
		+TSP	1.60 b	0.47 b	2062 d	0.113 c
		+CNC	1.52 b	0.50 b	2075 d	0.118 c
	M	0	2.40 c	0.77 c	2713 c	0.086 d
		+TSP	3.14 d	0.87 c	5813 b	0.156 b
		+CNC	3.30 d	0.95 c	6854 a	0.176 a

See footnotes to Table 2

Table 4. Analysis of P use from soluble (TSP) and insoluble (CNC) fertilizer by mycorrhizal (M) and non-mycorrhizal (NM) oil palm plants growing in Dabou and Soubré soils

Soil		Fertilizer	% Fertilizer utilization coefficient	% Plant P derived from fertilizer	Plant-specific activity (cpm mg^{-1} P)
Dabou	NM	TSP	2.69 b	56 a	1.64 a
		CNC	2.67 b	57 a	1.78 a
	M	TSP	15.06 a	69 a	1.83 a
		CNC	14.10 a	68 a	2.03 a
Soubré	NM	TSP	6.13 b	78 a	1.93 a
		CNC	5.48 b	68 a	1.69 a
	M	TSP	16.26 a	74 a	1.98 a
		CNC	19.62 a	76 a	1.90 a

cpm, counts $minute^{-1}$. For other abbreviations see Table 2

Table 5. Isotopic-dilution kinetics of $^{32}PO_4$ in Dabou and Soubré soils analyzed in the absence of (NI, I) or after plant growth (NM, M)

Soil	Fertilizer		r_1/R	M_1 (ppm P)	E_1 (ppm P)
	0	NI	0.61	0.39	0.60
		I	0.41	0.36	0.90
		NM	0.46	0.17	0.40
		M	0.41	0.06	0.10
Dabou	+TSP	NI	0.76	9.70	12.80
		I	0.69	4.00	5.80
		NM	0.74	3.60	4.90
		M	0.66	1.90	2.90
	+CNC	NI	0.77	8.90	11.40
		I	0.79	5.30	6.70
		NM	0.75	3.90	5.20
		M	0.70	2.70	3.80
Soubré	0	NI	0.22	0.31	1.40
		I	0.15	0.30	2.00
		NM	0.12	0.10	0.83
		M	0.07	0.06	0.86
	+TSP	NI	0.42	4.20	10.20
		I	0.42	3.10	7.40
		NM	0.36	1.70	4.70
		M	0.22	0.50	2.27
	+CNC	NI	0.53	4.60	8.80
		I	0.41	2.20	5.30
		NM	0.39	1.80	4.60
		M	0.27	0.80	3.00

NI, non-incubated soil; soil in pots watered and maintained as for NM and M; NM, after growth of non-mycorrhizal plants; M, after growth of mycorrhizal plants. For other abbreviations see Table 2

P uptake from fertilizer. Values for the utilization coefficient of P-fertilizer and the amount of P in plants derived from fertilizer, which correspond to the fertilizer participation in plant P nutrition, are presented in Table 4. The VAM inoculation of oil palm plants ensured a better use of P from both types of fertilizer in the two acid soils. The utilization coefficient was 2.7- to 5.6-fold greater for mycorrhizal plants compared to non-mycorrhizal plants. There was no significant difference in the use of P from fertilizer supplied either as superphosphate or rock phosphate. The fraction of plant P derived from the fertilizer was not affected by VAM inoculation or by the type of P fertilizer added to the two soils (Table 4) and there was no significant difference in the specific activity of mycorrhizal and non-mycorrhizal plants (Table 4), indicating that all the plants had taken up P from the same pool of P in the fertilized soils, as occurs in unfertilized soils.

Soil analyses

Dissolution of P fertilizer before and after soil incubation without cropping. The amount of P in the soil solution (M_1) and the size of the labile pool of soil P (E_1) greatly increased in the two soils when measured after either superphosphate or rock phosphate had been added (Table 5, NI treatments), but both values considerably decreased with incubation of the fertilized soils during 12 weeks under plant growth conditions (Table 5, I treatments). This effect tended to be more pronounced in the Dabou soil than in the Soubré soil. The increase in available P with soil fertilization was also reflected in the ratio r_1/R. This ratio is inversely related to the P-fixing capacity of a soil ($1-r_1/R$) and it increased as the latter decreased in the fertilized soils. Overall values of r_1/R were much lower in the Soubré soil than in the Dabou soil, indicating that the P-fixing capacity of the former was higher (78%) than the latter (39%). This property was also seen in the M_1 values, which showed that proportionally less P remained in the soil solution of the Soubré than the Dabou soil after fertilization (NI treatments).

Influence of soil and cropping on the labile pool of P. The growth of oil palm plants induced a decrease in M_1 and E_1 values in the two acid soils (Table 5, NM and M treatments). M_1 values were lower in the Soubré soil than the Dabou soil while E_1 values in the two soils were comparable after plant growth. This effect was more important with VAM inoculation, reflecting the higher P uptake by the mycorrhizal plants.

Discussion

The present study confirms previous work on the interest and importance of mycorrhiza for normal growth of micropropagated oil palm clones (Blal et al. 1987; Blal and Gianinazzi-Pearson 1989) or for oil palm seedlings (Sheriff 1981). The increase in oil palm growth obtained either by the addition of P fertilizer

to non-mycorrhizal plants or by VAM inoculation reflected the low P-uptake capacity of oil palm roots and the high mycorrhiza dependency of oil palm plants. The growth responses to P fertilizer applications were low unless accompanied by VAM inoculation, and depended on the "soil-fertilizer-plant" combination. The fact that maximum growth was only obtained when fertilizer was added to mycorrhizal plants reflected the poor P status of the two acid soils Dabou and Soubré, also indicated by their low M_1, E_1, and Olsen-P values.

Many experiments comparing P uptake by mycorrhizal and non-mycorrhizal plants from $^{32}PO_4$-labelled unfertilized soils over a range of pH values have concluded that mycorrhizal plants do not use sources of phosphate that are unavailable to non-mycorrhizal plants (Sanders and Tinker 1971; Hayman and Mosse 1972; Mosse et al. 1973; Powell 1975; Pichot and Binh 1976; Gianinazzi-Pearson et al. 1981). A similar conclusion can be drawn from the present study on oil palms planted in two fertilized acid soils from the Ivory Coast. No significant differences in specific activity values were found between mycorrhizal and non-mycorrhizal plants growing in the presence of either triple superphosphate or rock phosphate, indicating that the extra P taken up by all plants came from the same pool of P in the fertilized soils.

There were no differences in the growth response of mycorrhizal or non-mycorrhizal oil palms to either fertilizer, suggesting that both were equally available to the plants in these two acid soils. Isotopic-dilution kinetics analyses showed that, in fact, the low pH of these two soils caused a solubilization of the rock phosphate, so that this fertilizer led to a similar enrichment of the labile pool of plant-available P as did superphosphate. Since the fraction of plant P derived from either fertilizer was unaffected by VAM inoculation, it can be concluded that in these soils both mycorrhizal and non-mycorrhizal oil palm plants obtained extra P from this enriched labile pool. The enhanced growth and P nutrition of the mycorrhizal oil palms in the two fertilized acid soils was therefore the result of a better exploitation of the enriched labile pool of soil P, and consequently a more efficient use of the applied fertilizers, as shown by the higher values of their fertilizer utilization coefficients. This explains previous observations of an apparent use of tricalcium or rock phosphates by VAM plants growing in either temperate or tropical acid soils (Murdoch et al. 1967; Jackson et al. 1972; Powell and Daniel 1978; Cabala-Rosand and Wild 1982) but not in neutral or near-neutral soils (Mosse et al. 1976; Gianinazzi-Pearson et al. 1981; Azcon-Aguilar et al. 1986), and also results showing similar responses by mycorrhizal plants to additions of KH_2PO_4, colloidal or crystalline iron phosphate (strengite) in an acid soil, as long as these different forms of phosphate supplied the same amount of water-soluble phosphate (Bolan et al. 1987).

Since the Soubré and the Dabou soils had high and moderate P-fixing capacities, respectively, it can be concluded that efficient VAM fungi will play a vital role in the recovery of P fertilizers, P uptake and fertilizer efficiency, not only in highly P-fixing soils, as Powell and Daniel (1978) suggested, but also in those with a moderate P-fixing capacity. In conclusion, our results demonstrate that the presence of VAM ensures an efficient use, as much of rock phosphate as superphosphate, by plants growing in acid P-fixing soils and that the processes involved are not different for the two fertilizers. Our results also underline the importance of considering VAM as an essential factor for optimizing fertilizer efficiency, not only of soluble phosphate but also of rock phosphates, in the production of high-value perennial crops like micropropagated oil palms in tropical soils.

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