

Associative Influence of Soluble Phosphate, Rock Phosphate and Arbuscular Mycorrhizal Fungus on Plant Growth and Phosphorus Uptake of Three Tropical Legumes

B. D. Ahiabor^{1*} and H. Hirata²

^{1,2}Laboratory of Plant Nutrition, Department of Biological Production, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo, Japan

¹Present address: CSIR-SARI, P. O. Box 52, Tamale, Ghana

*Corresponding author

Abstract

Cowpea, pigeonpea and groundnut were grown in the glasshouse in a gamma ray-sterilized Andisol (Melanudand) subsoil (Bray 1-P: 1.04 $\mu\text{g P g}^{-1}$; Bray 2-P: 1.91 $\mu\text{g P g}^{-1}$) and inoculated with or without mycorrhiza, *Glomus etunicatum* (Ge). They were fertilized with South African rock phosphate (EPL 86) and 20 mg of readily soluble phosphate (SP). KH_2PO_4 was also used as starter fertilizer and its effect on utilization of the rock phosphate-P for growth by the legumes was investigated. Shoot dry weight of cowpea was unaffected by mycorrhiza only treatment but those of groundnut and pigeonpea were increased. Rock phosphate (RP), however, failed to increase the shoot growth of the legumes irrespective of mycorrhizal treatment. Total shoot dry weight and total P content increases of pigeonpea and groundnut plants receiving the SP + Ge and tripartite (RP + SP + Ge) treatments were mainly due to the combined roles of SP and Ge since there were no significant differences between these treatments. This suggests a lack of any RP role in such responses. In cowpea, however, the tripartite application also enhanced the total shoot dry matter production and, in addition, specifically increased the dry weight and P content of its vegetative parts. The effects of the tripartite treatment on the total shoot dry weight of cowpea and the P content of its vegetative organs were synergistic, with mycorrhiza playing a strong role, mediated through the well-developed root system and the improved nodulation. The treatment also induced additive responses of sporulation on cowpea, the mycorrhizal association in cowpea greatly enhanced the extensity of root ramification into the soil thereby enabling the roots to extract P from the sparingly soluble RP. In pigeonpea and groundnut, however, no significant priming effect on growth and RP-P utilization due to the starter SP was observed. These findings suggest that in some agricultural crop plants root access to rock phosphate-P could be enhanced through simultaneous mycorrhization and application of small doses of a readily soluble phosphate fertilizer as a primer.

Introduction

An important observation on rock phosphate (RP) research, especially in the tropics, is the role of plant-available phosphorus in the very early stages of plant development (Hammond *et al.*, 1986). One of the major limitations of finely-ground RP is its inability to satisfy this early requirement because of its slow rate of dissolution. To overcome this, it is often recommended that the RP be applied several weeks or months prior to planting so that a greater portion of it is

dissolved by the time of planting. This approach is effective, however, only when practised in flooded systems which can increase the availability of P in the reaction products (Kanabo & Gilkes, 1988), or when the P retention capacity of the soil is low so that the dissolved P remains available to the plant throughout the incubation period. Unfortunately, in tropical soils fertilizer P can be fixed into forms unavailable to plants by Fe and Al oxides commonly found in such soils (Sample *et al.* 1980; Abekoe &

Sahrawat, 2001).

One practice that has been observed to effectively increase the utilization of unacidulated RP is to satisfy the early requirements of the crop with soluble P and then rely on the unacidulated RP in the soil to provide for the subsequent P requirements (Chien *et al.*, 1987a). Unavailability of P from slightly reactive RP may be one of the reasons for low crop yields in P-deficient soils fertilized with RP. However, with the ever-increasing need to reduce farm expenditure, RP, which is about three times less than the price of single superphosphate (Nye & Kirk, 1986), is often used on acid soils that naturally have low P levels. Strategies to optimize the agronomic effectiveness of such low-price RPs would, therefore, be beneficial to the resource-poor peasant farmers of the tropics.

Legume plants in association with *Rhizobia* are known to fix atmospheric nitrogen. This symbiotic N_2 -fixation, which initiates a chain of reactions leading to an increased availability of rock phosphate-P, is dependent on photosynthate supply and on the availability of phosphate (Nyatsanga & Pierre, 1973). Danso (1992) and Mortimore *et al.* (1997) observed that the process of biological N_2 -fixation by cowpea nodules requires large amounts of P, and that its availability is a primary constraint to N_2 -fixation and, therefore, the N economy of many tropical ecosystems. Nyatsanga & Pierre (1973) proved that with alfalfa and soybean, the use of biologically fixed N acidifies the soil. However, before symbiotic nitrogen fixation can occur, photosynthates and nutrients must be made available by the host plant for nodule formation and for the build-up of a population of rhizobium bacteroids in these nodules. Hence, before

legumes can solubilize RP through acidification, a certain quantity of P must be available for the formation of the N_2 -fixing apparatus. In practical terms, this means that a small quantity of easily available P in the form of a starter fertilizer might be needed for a priming action in order to allow legumes to create conditions under which they can acidify their rhizosphere soil and thus solubilize and mobilize sparsely soluble rock phosphates. The addition of small amounts of soluble phosphates to act as a starter dose until P from the rock phosphate becomes available can, therefore, be useful in increasing the effectiveness of these rocks (Chien *et al.*, 1987a; Tiwari, 1979). A starter soluble P fertilizer will promote root development to a sufficient degree so that during the course of the growing season the crop can benefit not only from the soluble P provided but also from the RP to a much greater degree than would, otherwise, have occurred (Hammond *et al.*, 1986).

Furthermore, the effects of added phosphates can be increased by inoculation with arbuscular mycorrhizal (AM) fungi as it has frequently been shown that mycorrhizal plants are much more efficient at utilizing rock phosphate fertilizers applied to the soil than non-mycorrhizal plants (Hall, 1975; Mosse *et al.*, 1976; Murdoch *et al.*, 1967). It is, however, well established that high rates of application of soluble P fertilizers to soils greatly reduce the growth benefits from mycorrhizal infection (Baylis, 1967; Hall, 1977; Murdoch *et al.*, 1967). Thus, mycorrhizal associations on plants can play an important role in the P nutrition of such plants in soils having low available phosphorus. Tinker (1980) has shown that plants infected with AM fungi produce greater plant growth and increased P uptake

in soils deficient in P.

The objective of this study was to investigate whether the addition of a small quantity of an easily soluble phosphate could exert a priming effect that could lead to the enhancement of the availability of the rock phosphate-P to, and its consequent utilization by, three tropical legumes (cowpea, pigeonpea and groundnut) inoculated with the arbuscular mycorrhizal fungus, *Glomus etunicatum* in an Andisol (Melanudand) subsoil.

Materials and methods

Growth medium

Air-dry subsoil (100 cm depth) samples (to represent a low P soil) of an Andisol (Melanudand) from Tokyo University of Agriculture and Technology farm at Fuchu (35° 40' N, 139° 30' E) were used. The available P content of the soil was 1.04 $\mu\text{g P g}^{-1}$ (Bray 1) and 1.91 $\mu\text{g P g}^{-1}$ (Bray 2). The pH of the soil (pH 4.0; 1:2.5 soil: water) was adjusted to pH 6.0 (1:2.5 soil: water) using lime (1.6 g CaMgO per pot, determined by lime requirement test). The soil samples (< 2 mm) were sterilized by gamma-ray irradiation (1.5 Mrad) and, prior to loading into 12.4 cm-diameter Wagner pots (900 g air-dry soil per pot), nutrient amendments (mg pot⁻¹) were applied as follows: N (urea) 150; K (K₂SO₄, KCl) 100; Fe (FeCl₃·6H₂O) 25; Cu (CuCl₂·2H₂O) 5; Mn (MnCl₂·4H₂O) 15; Mo {(NH₄)₆Mo₇O₂₄·4H₂O} 5; Zn (ZnSO₄·7H₂O) 50; B (H₃BO₃) 15; Co (CoCl₂·6H₂O) 2.5. Solutions of these salts were mixed thoroughly with the bulk soil, packed in polyethylene bags, and then placed in the pots. When amending the soils with K (as KCl and K₂SO₄), the amount of K from KH₂PO₄ in the 20 mg SP-P treatments was considered in order to homogenize all

the pot soils.

Phosphate fertilizers

A water-soluble phosphate (SP) in the form of KH₂PO₄ and South African rock phosphate (RP) {EPL 86; 17.2% total P and 2.1% citrate (2% soluble P)} were used. Phosphorus fertilizer treatment combinations adopted were 0 mg P, 20 mg SP-P, 200 mg RP-P and 20 mg SP-P + 200 mg RP-P per pot. The 200 mg RP-P was based on 2% citrate-soluble P. The mode of application of these fertilizer materials are illustrated in Fig. 1.

Plant culture

Seeds of cowpea (*Vigna unguiculata* L. cv. Kuromame), pigeonpea (*Cajanus cajan* L. Millsp. cv. ICPL 86009) and groundnut (*Arachis hypogaea* L. cv. Nakateyutaka), ranging from 0.15 to 0.18 g, 0.1 to 0.13 g and 1.0 to 1.2 g, respectively, were surface-sterilized by immersion in 0.5 g l⁻¹ NaOCl for 4 min before nursing on a vermiculite bed at room temperature (25 °C). Germinated seeds with approximately 0.5 cm radicle length were transplanted at two per pot and were later thinned to one plant per pot. At transplanting, the pregerminated seeds of cowpea and pigeonpea were dipped for 30 min in suspensions of the rhizobia NOKO 704 and NOKO 705, respectively (Ahiabor & Hirata, 1995). Groundnut was, however, inoculated with NC 92. The plants were cultivated in the glasshouse under ambient conditions to reach 70 (cowpea), 127 (pigeonpea) and 104 (groundnut) days, during which time the pot soil moisture regime was maintained at 60% of the maximum water-holding capacity by weighing each pot and then adding distilled water to attain the required weight.

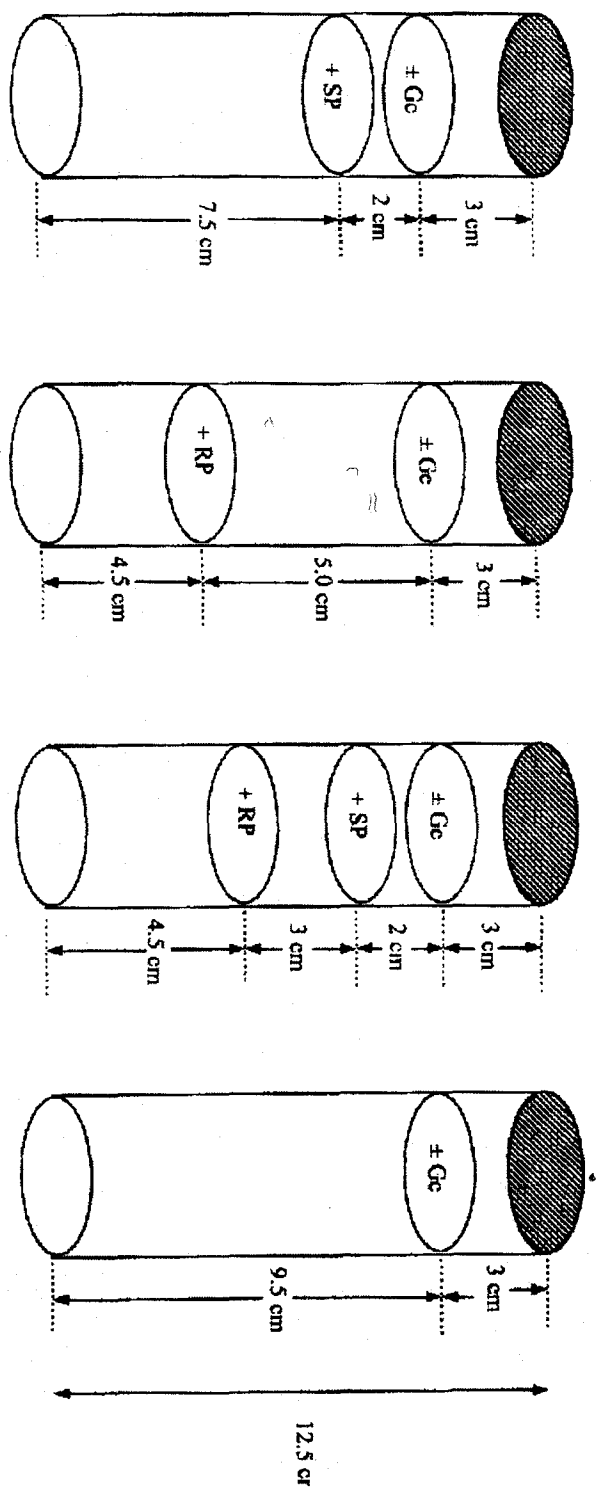


Fig. 1. The illustration of the pot treatment combinations used. + and - respectively indicate the presence or absence of the particular treatment.
Ge = *Glomus etunicatum*; SP = 20 mg $\text{KH}_2\text{PO}_4\text{-P}$; RP = 200 mg South African Rock Phosphate-P.

AMF inoculations

One week before planting, the soils (in three replicates) were inoculated with 100 spores of *Glomus etunicatum* (denoted in all treatment descriptions in figures and tables as 'Ge') which were isolated locally from the field (Ahiabor & Hirata, 1995) by placing them in a layer 3 cm below the soil surface (Fig. 1). Control pots (no inoculation with Ge spores) were also set up. The surface of the spores was sterilized by exposing them to 50 g l⁻¹ N-Chloro-p-toluene-sulfonamide sodium salt (Chloramine-T) and 0.25 g l⁻¹ Streptomycin sulfate for 15 min and 20 min, respectively (Mosse, 1981). The antibiotics were washed off the spores with several changes of sterilized distilled water. The germination rate (25 °C; 2 weeks) of the spores was 70% in a film of water. All treatments received a filtrate of the mycorrhizal inoculum (collected through No. 2 Whatman filter paper) to reduce differences in the other soil microorganisms.

Plant harvest

At the respective harvests, plant tops were detached at the base of the stem and the roots separated from the soil by gently shaking the bulk soil in a plastic bowl. Detached root segments resulting from the shake were carefully picked with a pair of forceps and pooled with the bulk root and then gently washed on a 2-mm mesh sieve under a jet of tap water. After nodules were removed with a pair of forceps, the length of the roots was measured with the Comair Root Length Scanner. Dry weights of nodules, roots, stem plus leaves, and pods were measured after drying in a forced-air oven at 80 °C for 48 h. Specific root length (SRL), an expression of the degree of root

fineness, was calculated as the ratio of root length (in metres) to root dry weight (in grammes).

Shoot phosphorus analysis

Dried shoot parts were homogenized into fine particles in a mechanical grinder and about 0.5 g aliquots were digested in concentrated H₂SO₄ using 300 g l⁻¹ H₂O₂ as an oxidant. Phosphorus in the digests was determined colorimetrically (Murphy & Riley, 1962; modified by Watanabe & Olsen, 1965).

Estimation of mycorrhizal colonization rate

The extent of AMF colonization of roots (i.e. per cent root length colonized) was assessed by the gridline-intercept method (Giovanetti & Mosse, 1980). About 3 g of 2-mm diameter root samples per treatment were cut into approximately 2-cm pieces with a pair of scissors and mixed thoroughly. These were cleared and stained (on a hot plate at 90 °C) with 100 g l⁻¹ KOH solution and 0.5 g l⁻¹ trypan blue in lactoglycerol (modified from Kormanik & McGraw, 1984), respectively. Both cowpea and pigeonpea roots took 60 min to clear and 20 min to stain whereas groundnut roots took 3 h and 40 min, respectively, since the cytoplasm and nucleus of the latter were quite resistant to clearing (Ahiabor & Hirata, 1994; 1995).

Measurement of AMF sporulation

Tap water (100 ml) was added to 5 g samples of the air-dry post-harvest soil and sonicated at 100 watts for 2 min. The soil suspension was leached through a 0.053 mm-mesh sieve to trap the spores on the sieve. The spores were retrieved by sucrose {60% (w/v) sucrose solution} density-

gradient centrifugation (modified from Daniels & Skipper, 1984), and counted in a film of water under a stereomicroscope at 40× magnification.

Experimental design and statistical analysis

For each crop, the pots were arranged to conform to the randomized complete block design consisting of eight treatments at three replications per treatment. Analysis of variance was used to analyze all data, and mean comparisons were made using Duncan's Multiple Range Test (DMRT) at $P = 0.05$.

Results and discussion

The extent of *G. etunicatum* colonization of plant roots (i.e. colonization percent) ranged between 14% and 50%. With each treatment, comparable levels of colonization were observed in all the three legumes (Fig. 2). Though the combination of SP and RP produced significant increases in root colonization when compared to Ge treatment (+ Ge), only in cowpea did RP contribute to this increase. In cowpea, mycorrhizal tripartite treatment (RP + SP + Ge) yielded about 65% increase over Ge+SP treatment and 127% increase over Ge+RP treatment, respectively (Fig. 2a). In all the legumes, the presence of a small dose of SP enhanced root colonization by *G. etunicatum* but RP did not. Elsewhere, however, rock phosphate significantly increased AM colonization of roots of maize (Asmah, 1995) and *Leucaena* (Manjunath *et al.*, 1989). The low level of soluble phosphate applied in this study did not depress mycorrhizal formation in these plants. AM colonization can be suppressed at higher P levels (Mosse, 1981; Stribley *et al.*, 1980), possibly by

increasing P concentration in roots (Menge *et al.*, 1978). The absence of depression of AM colonization by SP in this study was probably because P concentrations in the soil solution and in the roots were still lower than those inhibitory to AM colonization.

In all legumes, SP and RP combination significantly raised spore production (Fig. 3). These increases were synergistic with that in cowpea being the result of the RP addition. No RP role could be implicated in the case of pigeonpea and groundnut. In cowpea, however, a remarkable sporulation was also observed when the starter SP was applied alone (Fig. 3a). Mycorrhizal inoculations produced significant growth (shoot dry weight) increases in pigeonpea and groundnut (Table 1) but in cowpea this mycorrhizal growth enhancement occurred in only those treatments containing SP (Table 1). Although shoot growth was enhanced by SP in all mycorrhizal plants, only in cowpea did the co-applied RP contribute to this growth enhancement.

Reports on growth responses of plants, whether mycorrhizal or not, to additions of rock phosphate have been erratic. Mosse *et al.* (1976) reported that dry weights of onion and clover were increased by mycorrhizal infection but not by added rock phosphate with or without mycorrhiza. In this study, without P application (from SP or RP), the shoot dry weights of cowpea and pigeonpea were not affected by mycorrhizal infection but that of groundnut was increased (Table 1). In agreement with Mosse *et al.* (1976), whether plants were mycorrhizal or not, their shoot dry weights were not increased by RP treatment alone.

It was observed that in cowpea especially, the enhanced sporulation in the tripartite treatment highly correlated with both the

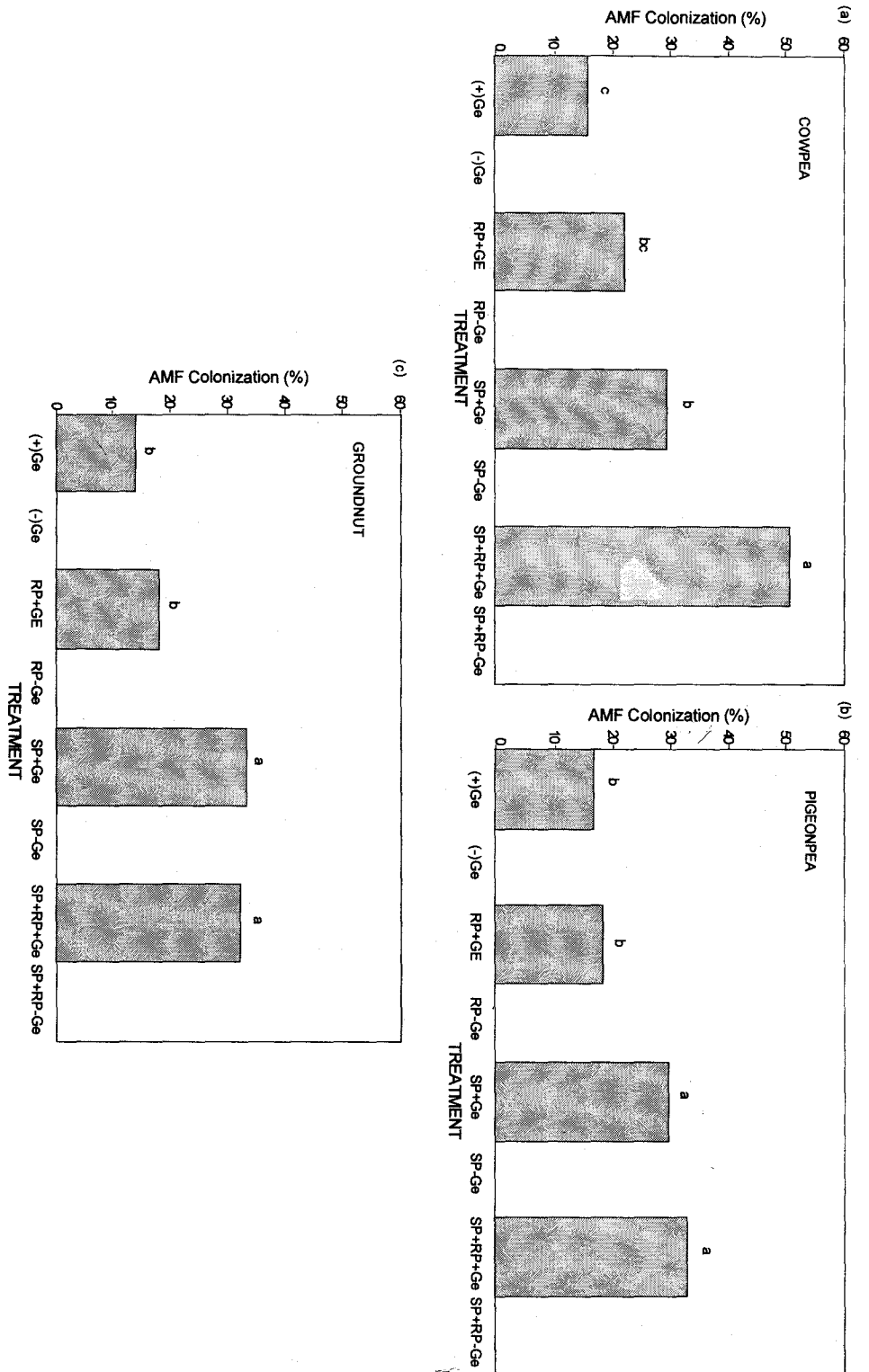


Fig. 2. Extent of AMF fungal colonization of roots of cowpea (a), pigeonpea (b) and groundnut (c) fertilized with rock phosphate and soluble phosphate, and inoculated with *Glomus etunicatum* in an Andisol subsoil.

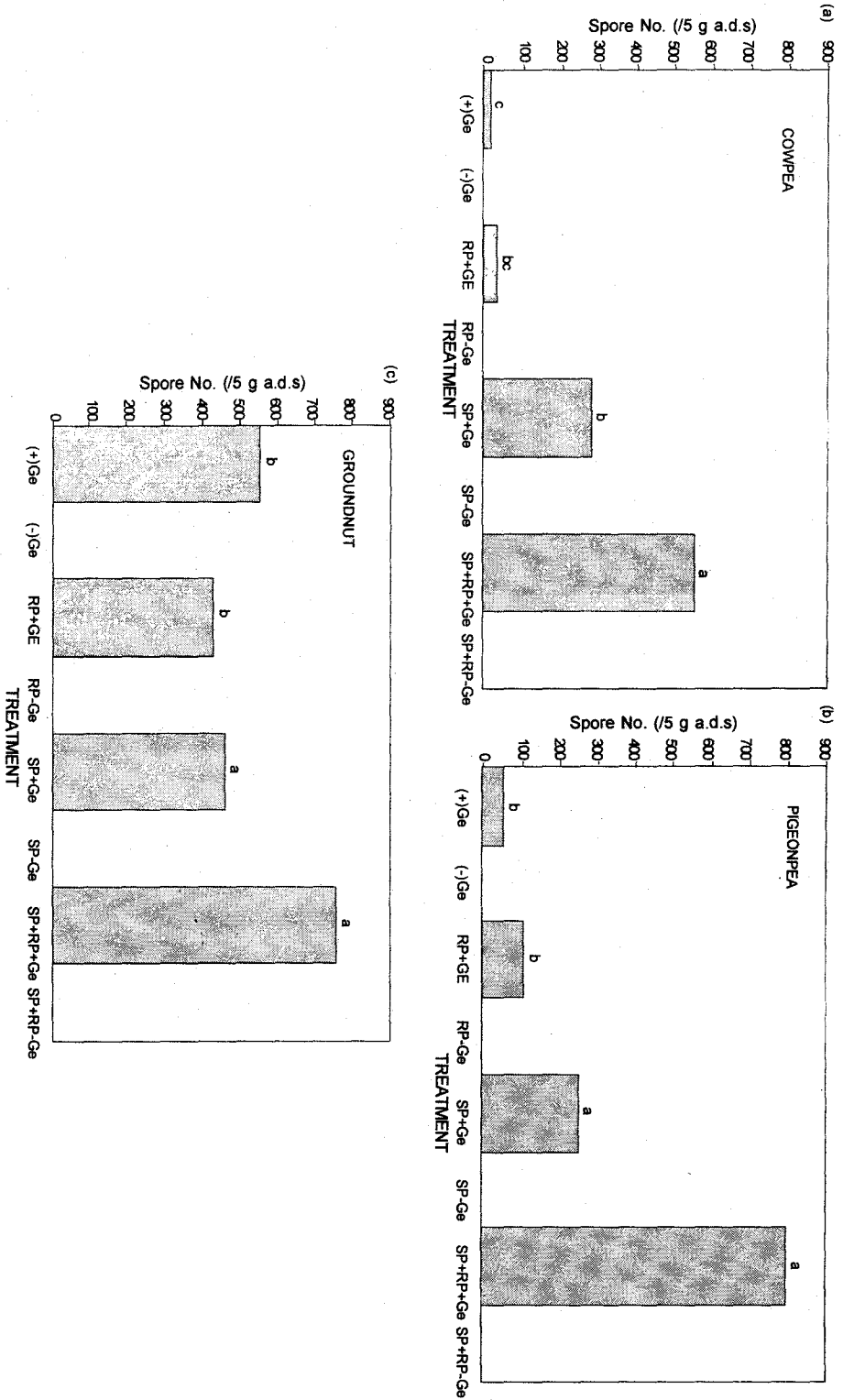


Fig. 3. Sporulation of *Glomus etunicatum* on cowpea (a), pigeonpea (b) and groundnut (c) fertilized with rock phosphate and soluble phosphate in an Andisol subsoil.

TABLE I
 Dry weights of nodules (mg/plant), stem plus leaves (g/plant) and total shoot (g/plant) of cowpea, pigeonpea and groundnut fertilized with rock and soluble phosphates (RP, SP) and inoculated with *Gliomus etunicatum* (Ge) in an Andisol subsoil

Treatment	Cowpea			Pigeonpea			Groundnut					
	Nodule	Stem+leaves	Pod	Total shoot	Nodule	Stem+leaves	Pod	Total shoot	Nodule	Stem+leaves	Pod	Total shoot
+Ge	2.0 b*	0.957e	nd **	0.957e	1.030b	1.853bc	nd	1.853bc	nd	5.238b	3.298bc	8.536b
-Ge	0.667b	0.739e	nd	0.739e	nd	0.486c	nd	0.486c	nd	3.620bc	2.131d	5.752cd
RP+Ge	2.330b	1.201de	nd	1.201de	5.300b	2.572b	nd	2.572b	nd	4.58 bc	3.940b	8.521b
RP-Ge	2.000b	0.829e	nd	0.829e	1.710b	0.548c	nd	0.548c	nd	3.013c	1.396e	4.41d
SP+Ge	7.000b	2.789b	1.846a	4.635b	40.700a	10.662a	nd	10.884a	35.667 a	8.486a	5.196a	13.683a
SP-Ge	5.330b	2.020c	0.274c	2.294c	6.240b	3.267b	nd	3.267b	nd	4.766bc	2.824cd	7.59bc
RP+SP+Ge	30.670a	5.276a	1.275b	6.551a	42.410a	11.708a	nd	11.737a	45.333 a	8.166a	5.254a	13.42a
RP+SP-Ge	4.670b	1.731cd	0.047c	1.778cd	3.720b	2.169b	nd	2.169b	nd	3.752bc	1.881de	5.632d

*In a column, values (means of three replicates) with similar letter(s) are not significantly different at P = 0.05 by Duncan's Multiple Range Test (DMRT).
 **nd = not detected

remarkable growth experienced with this treatment ($R=0.96$; $n=8$) and the extensive AMF colonization ($R = 0.92$; $n = 8$). In pigeonpea too, the relatively high positive correlations between sporulation and both colonization ($R = 0.82$; $n = 8$) and plant growth ($R = 0.76$; $n = 8$) suggests that the increased sporulation (Fig. 3b) resulting from the dual P fertilizer addition was due to an enhanced *G. etunicatum* proliferation (Fig. 2b) and plant growth. As groundnut lacked such significant correlations ($R = 0.12$ and 0.37 , respectively; $n = 8$), the cause of the remarkable sporulation in the dual fertilizer treatment (Fig. 3c) could not be explained with the available data and, therefore, may have been caused by some undetermined factor(s).

The addition of SP (whether singly or with RP) greatly increased nodulation in both mycorrhizal pigeonpea and groundnut but in mycorrhizal cowpea, only when co-applied with RP did it synergistically stimulate nodulation (Table 1). Mycorrhizal inoculations accelerated root dry matter production in RP- and SP-fertilized pigeonpea and groundnut (Table 2) but in cowpea only when these P sources were combined was this promotion (which was even synergistic with respect to the phosphate fertilizers) observed. Inoculations with the AM fungus enhanced root elongation (total root length) in all legumes only with SP treatment (Table 2). The combination of RP and SP again synergistically increased the root length of mycorrhizal cowpea. Specific root length (an index of root fineness) was generally greater in mycorrhizal than nonmycorrhizal cowpea while the reverse tendencies were observed for pigeonpea and groundnut (Table 2).

Total shoot P accumulation in all plants was significantly high with starter SP application in the presence of mycorrhiza (Table 3), especially in cowpea which showed no mycorrhizal response unless a starter SP was present. However, in the absence of mycorrhiza (-Ge *vs* SP-Ge and RP-Ge *vs* RP+SP-Ge), starter SP did not produce any total shoot-P uptake response in groundnut unlike in cowpea and pigeonpea. In all legumes, the addition of the starter SP fertilizer and mycorrhization induced a shoot (stem plus leaves) P-uptake response to the rock phosphate (Table 3). Only in the tripartite treatment did RP induce a significantly high P-uptake response in those organs of cowpea. In both mycorrhizal cowpea and pigeonpea, application of SP significantly decreased the concentration of P in stem plus leaves whereas in groundnut this parameter remained unchanged regardless of treatment (Fig. 4a, b and c). The starter SP, however, produced significant increases in the P concentration in the pods of mycorrhizal groundnut irrespective of RP application.

Since there was no positive mycorrhiza effect on shoot-P concentration of RP-fertilized pigeonpea and groundnut (Fig. 4), the increased total-P uptake in such plants (Table 3) resulted from increased dry matter accumulation (P uptake is a product of concentration and dry weight) in response to mycorrhizal inoculations and not to improved P nutrition. Though an increase in the concentration and/or the content of P in plants is the most often described response to AMF (Krishna & Bagyaraj, 1984; Packovsky & Fuller, 1986), it has often been reported that mycorrhizal infection also increases the concentration of nutrients other than P, which can induce increased

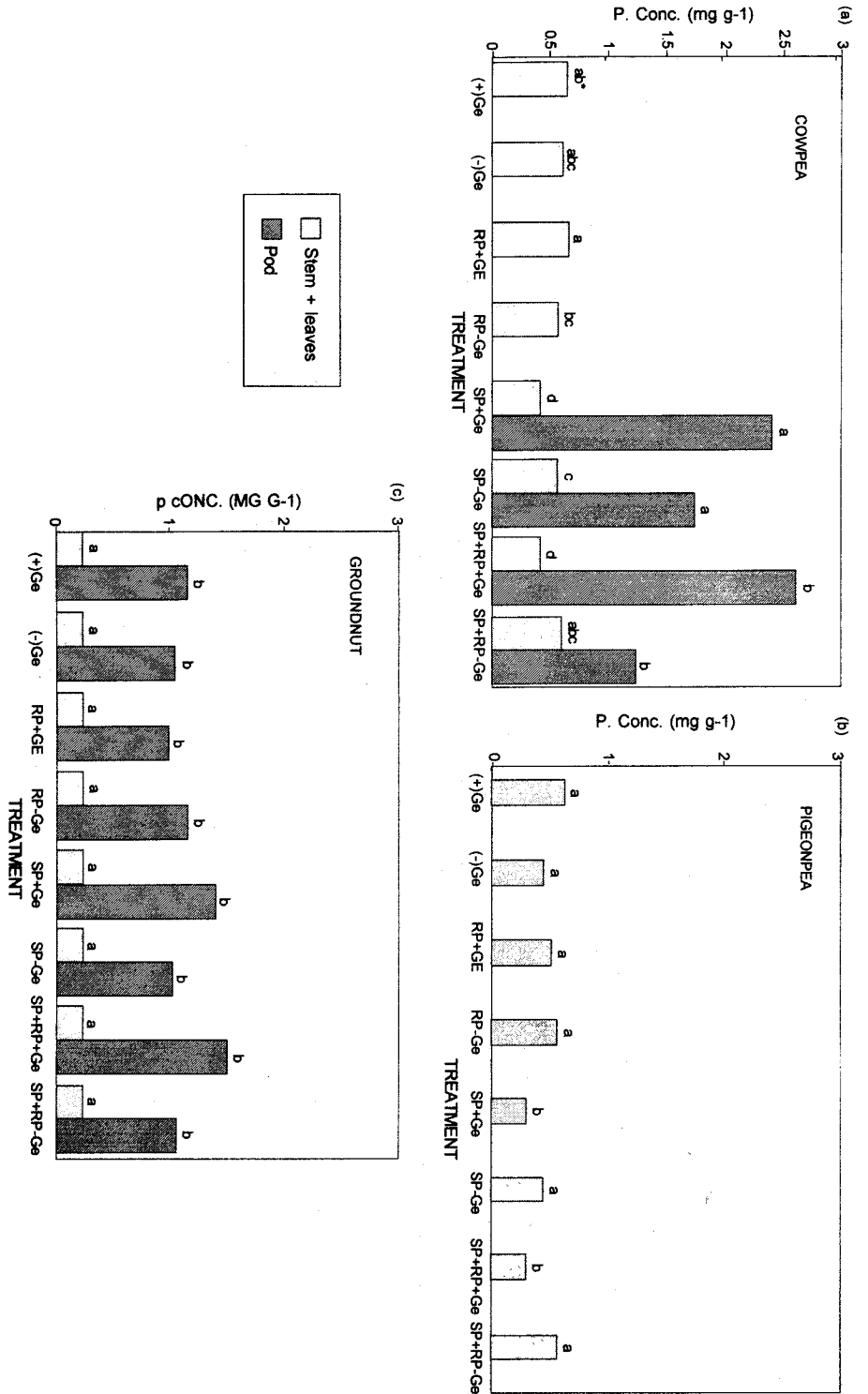


Fig. 4. Phosphorus concentrations in the stem plus leaves and pods of cowpea (a), pigeonpea (b) and groundnut (c) fertilized with rock phosphate and soluble phosphate, and inoculated with *Glomus etunicatum* in an Andisol subsoil.

Root dry weights (g/plant), root lengths (m/plant) and specific root lengths (S.R.L.) (mg of cowpea, pigeonpea and groundnut fertilized with rock and soluble phosphates (RP, SP) and inoculated with *Glomus etunicatum* (Ge) in an Andisol subsoil

Treatment	Cowpea			Pigeonpea			Groundnut		
	Root dry wt	Root length	S.R.L	Root dry wt	Root length	S.R.L.	Root dry wt	Root length	S. R. L.
+Ge	0.203d*	51.02cd	241.36bcd	0.748cd	144.47c	193.66b	1.596b	165.17cd	105.47b
-Ge	0.13d	23.56d	180.97cd	0.179d	37.92c	219.09ab	1.046c	152.07d	146.79a
RP+Ge	0.30cd	85.07cd	308.20ab	0.967c	173.98c	190.72b	1.541b	143.01de	92.55b
RP-Ge	0.135d	19.50d	144.59d	0.245d	35.82c	146.96b	0.969c	105.97e	109.60b
SP+Ge	0.51b	205.65b	418.66a	4.316a	728.62a	167.42b	2.253a	239.94a	107.37b
SP-Ge	0.50bc	104.65c	205.88bcd	1.67b	484.35b	304.88a	1.341bc	194.05bc	144.70a
RP+SP+Ge	0.953a	280.64a	291.62bc	4.589a	707.44a	152.73b	2.153a	220.51ab	102.39b
RP+SP-Ge	0.42bc	123.65c	285.94bc	0.952c	203.73c	215.87ab	1.036c	163.50cd	159.71a

*In a column, values (means of three replicates) with similar letter(s) are not significantly different at $P = 0.05$ by Duncan's Multiple Range Test (DMRT).

TABLE 3

Phosphorus content (mg/plant) of the shoots of cowpea, pigeonpea and groundnut fertilized with rock and soluble phosphates (RP, SP) and inoculated with *Glomus etunicatum* (Ge) in an Andisol subsoil

Treatment	Cowpea			Pigeonpea			Groundnut		
	Stem+leaves	Pod	Total shoot	Stem+leaves	Pod	Total shoot	Stem+leaves	Pod	Total shoot
+Ge	0.657cd*	nd**	0.657c	0.884b	nd	0.884b	1.270bc	3.624b	4.894b
-Ge	0.476d	nd	0.476c	0.207c	nd	0.207c	0.741d	2.313cd	3.054cd
RP+Ge	0.856bc	nd	0.856c	1.094b	nd	1.094b	1.064cd	3.880b	4.944b
RP-Ge	0.473d	nd	0.473c	0.241c	nd	0.241c	0.700d	1.566d	2.266d
SP+Ge	1.123b	4.225a	5.348a	2.823a	nd	2.823a	1.697ab	7.267a	8.964a
SP-Ge	1.132b	0.684b	1.186b	1.376b	nd	1.376b	0.992cd	2.939bc	3.931bc
RP+SP+Ge	2.179a	3.274a	5.453a	2.969a	nd	2.969a	1.900a	7.112a	9.012a
RP+SP-Ge	1.100b	0.171b	1.271bc	0.954b	nd	0.954b	0.914cd	1.925cd	2.839cd

*In a column, values (means of three replicates) with similar letter(s) are not significantly different at $P=0.05$ by Duncan's Multiple Range Test (DMRT).

**nd = not detected

biomass production. Ahiabor & Hirata (1994) concluded that high concentrations of Ca and K in shoots of mycorrhizal groundnut and pigeonpea, respectively, accounted for their increased growth.

Among the three legumes, only cowpea possibly utilized the rock phosphate for shoot growth in the presence of mycorrhiza and a starter soluble phosphate fertilizer. For this legume, there was no effect of P source on colonization (i.e. there was no difference in AM colonization between plants grown under the superphosphate and rock phosphate treatments). The effect of P source is, however, evident when pigeonpea and groundnut are considered. Increases in total shoot growth, nodulation, root biomass and total P-uptake were not due to the RP component of the dual fertilization in mycorrhizal pigeonpea and groundnut. In mycorrhizal cowpea, however, the enhanced root production, shoot growth and nodulation were the collective result of the two P fertilizers. Furthermore, the efficient uptake of P in the vegetative parts of dually fertilized mycorrhizal cowpea may induce a higher root growth leading to an enhanced exploitation of the RP. The synergistic effect of the tripartite treatment on nodulation, root and shoot dry weights of cowpea, as well as the P content of its vegetative organs suggests a strong mycorrhizal role in the synergism which may have been mediated possibly through an accelerated P uptake.

Increased root fineness is an indication of an enhanced nutrient acquisition at low nutrient availability. Hence, the generally finer root architecture observed in mycorrhizal cowpea (Table 2), coupled with its increased root length in the tripartite treatment may have conferred on this crop

the ability to utilize the sparingly soluble RP-P for shoot dry matter (especially the vegetative parts) production. The positive shoot growth response of cowpea to RP application may also have been due to the effects of a greater mycorrhizal activity (Fig. 2a and 3a) and enhanced root growth on some unmeasured factor(s) other than a favourable P nutrition. For the same root volume, a much greater surface would be exposed by cowpea than by groundnut and pigeonpea plants. The smaller size would also increase the number of soil spaces into which these fine roots would penetrate. Root surface area, rate of root growth, root fineness (high S. R. L. values) and quantity of roots are believed to be involved in the efficiency of roots in absorbing phosphate in the soil. This confirms the general opinion that crops with small root diameters (i.e. high S. R. L. values) or well-developed fibrous roots are best suited to utilize P from rock phosphates.

Alternatively, the enhanced P uptake by cowpea in the tripartite treatment may have possibly resulted from the utilization of the SP, promoted by the markedly developed root system and the extensive AMF colonization. In a soil situation, diffusion of phosphorus to the root could be the limiting factor to supply. Cowpea, which is active in exploring for phosphorus may maintain a sufficient continuous uptake (not necessarily an increased one) to ensure supply, particularly in early growth, thereby increasing the efficiency of the use of the phosphorus gained. It should, however, be noted that mycorrhizal effects on plants in the limited confines of a pot may be quite different from those in the field where patterns of root and mycorrhiza fungal mycelial growth will be quite different. A

field experiment (an ecophysiological approach) is, therefore, recommended to corroborate the above interesting observations in cowpea even though the results of this study give a strong indication of the phenomenon that might pertain in the field. Further work also needs to be done to clearly ascertain the source(s) of the absorbed P in the dually fertilized mycorrhizal cowpea plant observed in this experiment.

Conclusion

The use of a starter soluble phosphate fertilizer in combination with AM fungi holds promise as an efficient means of utilizing indigenous rock phosphate resources for agricultural purposes. Since almost all agricultural crops are mycorrhizal (native or inoculated), rock phosphate materials are cheap and chemical fertilizers are usually expensive beyond the reach of the ordinary resource-poor farmer, this technology will help reduce the cost of production and increase food production. This will lead to increase in both food security and net farm-household income, thereby alleviating poverty. With most farmers already familiar with rock phosphate as a P-fertilizer material and also AM fungi being inherent in all agricultural soils, the prospects of practical adoption of this technology are high.

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