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Mycorrhizal Growth Responses of Pueraria phaseoloides *in Phosphorus-deficient Soils*

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The plantation cover legume, Pueraria phaseoloides, *was inoculated with arbuscular mycorrhizal fungi and grown in several phosphorus-deficient soils (six Ultisols, two Oxisols, an Entisol and a Spodosol) with or without a supply of phosphate fertiliser, in a glasshouse experiment. In most unsteamed soils, introduced fungi did not enhance plant growth despite the low inoculum potential of the indigenous endophytes. Irrespective of phosphate applied, growth increases from mycorrhizal inoculation were only achieved in steamed Bungor and Rengam and unsteamed Jambu series soils, or in unsteamed Rasau series soils with added phosphate. The results were discussed in terms of the varying phosphate-fixation capacities of the different soils, the effects of soil steaming and mycorrhizal dependency of* P. phaseoloides.

Cover legumes are an important component of modern rubber plantations that serve to control weeds, reduce soil evapotranspiration, control leaching, reduce root disease incidence, fix atmospheric nitrogen (N) and recycle nutrients and thereby improve growth of the main tree crop[1,2].

Enhanced growth of mycorrhizal plants in phosphorus (P)-deficient soils is attributed to P uptake and transport by external hyphae[3]. Such benefits to plant growth disappear when P supply is not limiting growth of non-mycorrhizal plants[4,5]. Infection by arbuscular mycorrhizas (AM) also confers a number of beneficial effects upon the growth of plants that include improved access to other immobile ions *e.g.* zinc (Zn), copper (Cu), and tolerances to extremes of shade, moisture, soil acidity, heavy metal concentrations as well as disease[3].

Tropical forage legumes and grasses normally form AM, and responses in growth and nutrition to introduced endophytes in P-deficient soils have been shown by many workers[6-14]. Phosphorus deficiencies in plants are common in strongly weathered soils of the tropics in which iron (Fe) and aluminium (Al) oxides with high P-sorption capacities predominate[15]. In these acid infertile soils, AM play an important role in nutrient uptake to achieve faster establishment of improved pastures, and to reduce P fertiliser losses due to P fixation. Indeed, the effect of AM inoculation and application of the less-soluble rock phosphate fertiliser application on many legumes have been the subject of many studies[16,17].

Pueraria phaseoloides is in widespread use as a cover legume in Malaysian plantations. The present study evaluates the effectiveness of introduced AM fungi on its growth at P levels used in commercial legume cultivation in a range of rubber-growing soils.

MATERIALS AND METHODS

The design was a complete factorial randomized block of the following treatments,

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in 4 replications, (1) AM inoculation: nil or inoculation with a mixed AM-fungal species of *Glomus manihotis, Glomus clarum, Glomus* sp.E_3[18], *Glomus macrocarpum, Glomus intraradices*, an unknown *Glomus* sp. (UGX), *Scutellispora calospora* and *Entrophospora colombiana* in equal ratios by weight. The choice of mixed inoculum was to eliminate problems of endophyte specificities for particular niches; (2) Phosphate application: nil or fertilised with 720 kg/ha of Christmas Island rock phosphate (CIRP; 15.8% total P, 7.34% citrate-sol P). Under actual estate practice, this is the only fertiliser used to establish cover legumes[19].

The soils (0–15 cm depth) represent a range of parent materials: Marang, Bungor and Apek series derived from shale; Rengam and Lancang series from granite; Prang series from schist; Rasau series from sub-recent alluvium; Jambu series from old beach sands; Kaki Bukit series from limestone and Sg Mas series from serpentinite *(Table 1)*. The topsoil texture ranged from sandy (Rasau and Jambu) to sandy clay (Lancang), sandy loam (Marang), sandy clay loam (Rengam, Sg Mas, Bungor, Apek and Kaki Bukit) to clayey (Prang). Methods of soil analyses were as described by RRIM[20]. Except for Sg Mas, Kaki Bukit, Rasau and Jambu series, all soils had pHs (1:2.5 soil: water ratio) of <4.7. All the soils were depleted in bases and had available P levels of <10 mg P/kg soil. The shale-derived soils generally had high levels of exchangeable Al. Sg Mas and Kaki Bukit series showed high levels of total manganese (Mn) due to the nature of their parent materials.

All the soils were air-dried and sieved (<4 mm) before being filled into 11-cm diameter plastic pots. One set of soil was steam-sterilised (100°C, 1.5 h) to eliminate indigenous mycorrhizal fungi (IMF) whereas another was left unsterilised. Mycorrhizal inoculum consisted of mixtures of chopped (0.5 cm) roots and infested soils from *Calopogonium caeruleum* pot cultures of the various single-strain fungal species (10 g/pot) , localised in a band 3 cm below the soil surface. Uninoculated control plants received similar amounts of autoclaved soil:root mixtures representing all the AM-fungal species and filtered (Whatman No.1) inoculum leachings to reintroduce a part of the same microflora. For the applied P treatments, the soils were incubated with equivalent amounts of CIRP (0.69 g/pot) before use.

Surface-sterilised and 3-day-old pre-germinated *P. phaseoloides* seeds were planted (4 plants/pot) and inoculated with a suspension (5.0 ml) of effective bradyrhizobia (RRIM 968) after 7 days to ensure nodulation. Plants were watered daily, fed with minus N and P nutrient solution[21] weekly, and the soils maintained at field capacity throughout the experiment. The plants were grown in the glasshouse under natural daylight where average maximum and minimum temperatures during the period were 38°C and 25°C, respectively. Relative humidity ranged from 42%–92%. Maximum photo-synthetic photon flux density (PPFD) measured with a LICOR LI-185B Quantum Photometer varied from 62-69 W/m^2.

At harvest (42 days after sowing), shoots were cut at ground level, oven-dried (80°C, 48 h) and weighed. Roots were washed, cut into 4 mm segments and subsampled for mycorrhizal root colonisation after initial clearing in KOH and staining with lactophe-noltrypan blue on grid-line intersects[22,23] Mycorrhizal root colonisation is the quan-titative assessment of colonised root lengths over total root lengths determined on a mini-mum of 100 root segments under the dissecting microscope.

An analyses of variance was conducted on all the measured variables and for any soil, the

TABLE 1. SOIL PROPERTIES

Soil series	Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)	pH	Org. C (%)	Total N (%)	P (p.p.m.) Total	P (p.p.m.) Avail	Exch. K (m.e.%)	Exch. Ca (m.e.%)	Exch. Mg (m.e.%)	Total Mn (p.p.m.)	Exch. Al (p.p.m.)	Fe (%)	C.E.C. (m.e.%)
Sg Mas	10.5	21.5	17.3	32.8	5.8	1.81	0.16	371	5	0.19	3.27	2.93	5690	52	22.19	12.27
Jambu	93.3	4.3	0.1	1.9	4.9	1.37	0.11	42	7	0.04	1.16	0.26	32	35	0.03	2.60
Rasau	49.9	36.4	11.9	1.3	5.2	1.51	0.15	101	7	0.07	0.26	0.12	19	83	0.22	3.61
Kaki Bukit	17.5	20.0	23.2	36.6	5.1	1.19	0.15	181	6	0.79	2.36	2.10	5598	38	4.26	11.56
Rengam	59.6	7.5	2.8	28.6	4.4	2.37	0.26	175	9	0.16	0.44	0.28	51	171	1.50	5.42
Lanchang	22.5	14.1	4.5	57.4	4.6	2.13	0.18	617	5	0.14	0.41	0.13	263	151	4.00	8.04
Marang	23.2	50.3	14.4	14.0	4.7	0.78	0.10	62	6	0.09	0.48	0.25	21	164	0.37	3.91
Bungor	11.8	48.2	9.6	23.6	4.1	1.01	0.11	153	7	0.10	0.30	0.19	44	451	1.98	5.05
Prang	18.7	8.6	12.0	52.3	4.4	2.12	0.20	599	5	0.16	0.31	0.17	473	146	10.72	9.20
Apek	8.7	12.9	37.5	34.9	4.5	1.25	0.11	62	6	0.26	1.42	0.55	44	258	0.34	8.67

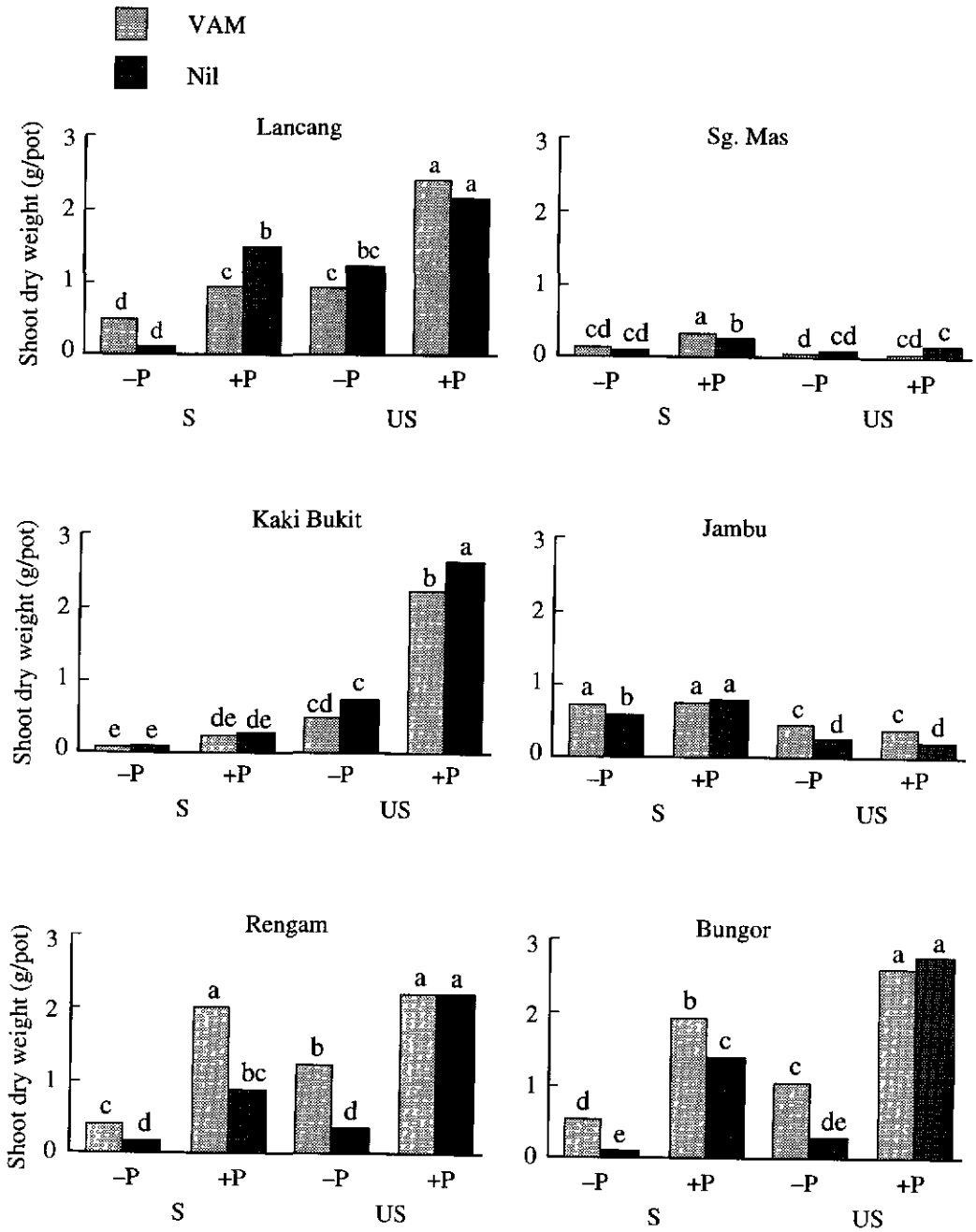

Figure 1. Effect of mycorrhizal inoculation and applied phosphate (–P, +P) on shoot dry weight of P. phaseoloides *in steamed (S) and unsteamed (US) soils. For any soil, treatments with the same letters are not different (P<0.05).*

interaction sum of squares used to compare differences between treatment means at P=0.05.

RESULTS

Shoot Dry Weights

In all steamed except Jambu and Kaki Bukit series soils, adding P to both AM-inoculated or uninoculated plants alleviated P-deficiency and thereby improved growth *(Figure 1)*.

In steamed Rengam, Bungor and Rasau series soils, AM fungi stimulates growth of *P. phaseoloides* in soils with or without added P. For both Bungor and Rengam series, the early growth of plants appeared stunted in steamed soils. In unsteamed Bungor and Rengam series soils, AM-inoculated plants were not different from the uninoculated controls when P was added. In unsteamed Rasau series soils, AM improved shoot growth only when P was added.

In steamed Marang, Apek and Jambu series soils, plants were not responsive to AM only when P was applied. In unsteamed Marang and Apek series soils, AM appeared ineffective with or without added P. However, in unsteamed Jambu series soils, AM improved shoot yield with or without added P.

The early growth of plants in steamed Kaki Bukit, Lancang and Prang series soils also appeared stunted. In steamed Prang and Sg Mas series soils, AM-inoculated plants were better than the uninoculated controls when P was applied. In unsteamed-soils, AM inoculation would not increase growth further with or without added P. Irrespective of P added, AM inoculation was also ineffective in steamed or unsteamed Kaki Bukit and Lancang series soils. In steamed Lancang series soils, AM plants performed much less than the controls when P was added.

Mycorrhizal Root Colonisation

Uninoculated plants in all steamed soils were devoid of mycorrhizal infection *(Table 2)*. Uninoculated plants grown in Jambu and Prang series soils had low levels of infection which may have arisen from contamination. Applied P significantly improved mycorrhizal root colonisation in steamed Jambu, Rasau, Lancang and Marang series soils.

For most of the unsteamed soils except Prang, Rasau and Lancang, uninoculated plants were also devoid of infection indicating absence of indigenous mycorrhizal fungi (IMF). In five out of eight soils, infection levels due to introduced AM in steamed soils were significantly greater ($P<0.05$) than in unsteamed soils. For all unsteamed soils except Jambu series, the effect of applied P in affecting mycorrhizal root colonisation was not significant.

DISCUSSION

The results demonstrate the limited usefulness of AM inoculation in stimulating growth of *P. phaseoloides* in most of the unsteamed soils studied, despite the low infection potential of IMF. They also indicate that laboratory measurements of initial soil P contents offer no valid guide to the crop's responsiveness to AM, since all the soils used had varying phosphate-fixation capacities that affect the availability of P for plant uptake and dry matter production. Further, the dissolution of rock phosphates in these soils may vary, depending on important variables such as pH, rate of acid excretion by roots and size of the phosphate particles[24]. Oxides and hydroxides of Fe and Al are often invoked in explaining the different P sorption characteristics of acid soils[25] . Although P is the fourth macronutrient after N, potassium (K) and sulphur (S) in order of amounts needed by plants, it ranked second only to N in terms of growth limitation. In

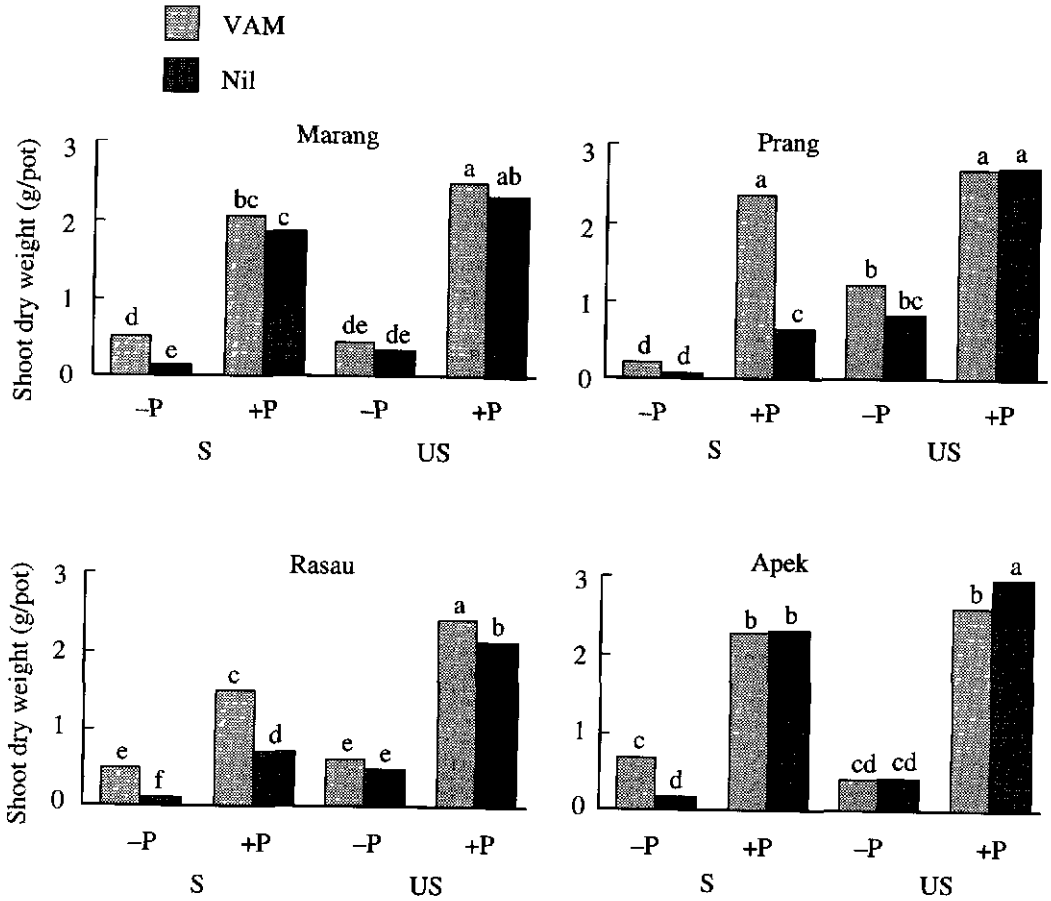

Figure 1 (Contd). Effect of mycorrhizal inoculation and applied phosphate (–P, +P) on shoot dry weight of P. phaseoloides *in steamed (S) and unsteamed (US) soils. For any soil, treatments with the same letters are not different (P<0.05).*

TABLE 2. EFFECT OF AM-FUNGI INOCULATION AND PHOSPHATE APPLICATION ON MYCORRHIZAL ROOT CONLONISATION (%) OF *P. PHASEOLOIDES* IN STEAMED AND UNSTEAMED SOILS*

AM fungi	Applied phosphate	Soil series: Sg Mas	Jambu	Rasau	Kaki Bukit	Rengam	Lanchang	Marang	Bungor	Prang	Apek
(a) Steamed											
AM	–P	0 b	16.7bc	2.0b	0 b	42.2a	2.7b	28.2b	27.9ab	0.6cd	29.0a
	+P	0.8b	24.9a	16.0a	0.9b	57.2a	28.1a	32.7a	55.0a	18.8bc	38.3a
Nil	–P	0 b	0 c	0 c	0 b	0 c	0 c	0 d	0 d	1.1cd	0 b
	+P	0 b	7.6b	0 c	0 b	0 c	0 c	0 d	0 d	0 d	0 b
(b) Unsteamed											
AM	–P	4.4a	0.9bc	9.5a	3.5ab	19.3b	13.5a	4.0c	5.2c	7.1ab	8.5b
	+P	0 b	1.0bc	8.6a	19.0a	15.4b	19.7a	0 d	24.7bc	19.9a	3.1b
Nil	–P	0 b	0 c	11.7a	0 b	0 c	0.5bc	0 d	0 d	5.1ab	0 b
	+P	0 b	0 c	6.8a	0 b	0 c	1.1bc	0 d	0 d	3.5a-c	0 b

*Means for each soil not followed by similar letters are significantly different ($P<0.05$), on an ln $(X+1)$ transformed basis

tropical legumes, P-deficiency reduces root growth and development and delay initiation of nodules and N_2 fixation[26].

There appeared to be two broad groups of soil in terms of plant growth. One group comprises Sg Mas, Jambu, Rasau and Kaki Bukit series soils where shoot yields for both steamed and unsteamed soils were relatively lower than yields in the remaining soils. In the other group, growth increases from introduced AM fungi were possible in unsteamed Bungor and Rengam series soils when P was not applied, or in steamed soils with or without applied P. In the first group, growth increases from introduced AM fungi were also possible in steamed Sg Mas series soils with P applied. Soil sterilisation can have other effects apart from the elimination of IMF. It alters the structure and physico-chemical properties of soil by releasing nutrients both beneficial (N and P) and toxic (free Mn) that affect growth of plants[27,28]. The organic P released could be fixed by the inorganic components of soil and would not be available for plant growth. Thus, in most soils except Kaki Bukit series, AM inoculation relieved the depressed growth of plants in soils that were steamed. Colonisation by introduced AM fungi in unsteamed Marang series soils were possibly too low to cause a discernible shoot yield increase in treatments without P added.

In unsteamed Apek series soils supplied with P, shoot dry weight yields of *P. phaseoloides* with introduced AM fungi were significantly lower than that of uninoculated plants. This is not surprising for it is known that AM fungi can cause growth depressions in plants when P was not limiting growth, either from toxic concentrations of P in plant tissue[29] or to competition between plant host and fungus from photosynthetically-derived carbon compounds[30-33]. The results appeared odd for unsteamed Jambu series soils. Steaming may have caused a flush of organic matter decomposition producing artificially high levels of ammonium and nitrate ions to influence plant growth and to suppress nodule formation and N_2-fixation of pot-grown plants. Alternatively, the improved growth of all plants in the steamed soils could presumably be due to control of soil-borne pathogens that were removed by steaming.

Prang and Sg Mas series soils are Oxisols with high P-sorption capacities. The higher Fe and Al status in these soils would explain their greater P-fixation and the plant's responsiveness to introduced AM in steamed soils when P was supplied. However, there were no responses to AM inoculation in unsteamed soils. Root systems of plants vary considerably in the Al ion concentration they can tolerate. A high Al ion concentration within the free space of roots may prevent it from taking up P. High levels of available Fe can depress the uptake of Mn by crops. Both Mn deficiency and toxicity are widespread in agricultural practice[34]. Sg Mas and Kaki Bukit series soils contained excessively high levels of total soil Mn that could cause Mn toxicity and stunt plant growth. An increase of Mn availability can result from the killing-off of Mn-oxidising micro-organisms by sterilisation or under waterlogged conditions where reducing processes dominate. The uptake of Mn by crops are however affected by cation competition with Mg and Ca, and the heavy metals Fe, Cu and Zn, and excessive uptake of Mn *e.g.* from the limestone-derived Kaki Bukit series soil, can induce Fe deficiency in *P. phaseoloides*. Higher levels of Mn, along with other metals (Al, Zn, Cu) and H-ions in soil solutions are also known to reduce mycorrhizal colonisation[35,36]. In unsteamed Rasau series soils, colonisation by IMF did not enhance growth of uninoculated plants. However, better plant growth when P was supplied suggested a better utilisation of P by introduced AM fungi.

There could also be other reasons to explain why *P. phaseoloides* may not be highly responsive to AM fungi in the various soils. Compared to many other tropical forage legumes, *P. phaseoloides* has been ranked as least mycorrhizal dependent[13]. Gerdemann[37] advanced the concept of mycorrhizal dependency which states the degree a plant is dependent on the mycorrhizal condition to produce its maximum growth yield at a given level of soil fertility. Of the 18 tropical forage legumes and 6 grass species that Saif[13] tested in a sterilised low-P Colombian Oxisol, a great variation in dependence on mycorrhizas was observed although all plants showed increased growth and nutrient uptakes from AM inoculation. Of the legumes tested (species of *Zornia, Centrosema, Leucaena, Macroptilium, Stylosanthes, Desmodium, Arachis, Pueraria*), *Zornia* sp. was most dependent on AM, followed by *Centrosema* sp. The mycorrhizal dependency of *P. phaseoloides* approximates that of *Desmodium ovalifolium* or the grass *Panicum maximum*, which are least dependent. Differences in mycorrhizal dependencies were attributed to root hair lengths, root production, root fibrousity and root geometry. Another factor is that the mixed AM inoculum may have its components reacting differently in the different soils. Other workers have reported that individual soils encouraged the development of certain mycorrhizal fungi more than others[38,39]. Furthermore, there is a wide variation in the ability of AM to stimulate plant growth[40,41]. In this study, the infectivity and effectiveness of the different endophytes in the mixed inocula on *P. phaseoloides* are not known. Hence, careful selection of effective single-strain AM species can still improve growth of plants in soils containing ineffective IMF.

Although there are doubts in this study on the abilities of AM to enhance shoot yields of *P. phaseoloides* in most of the soils studied, the short-term pot experiment is still no substitute for a long-term field experiment. Ideally, P-response curves from a series of levels of rock phosphates or super-phosphates should have been attempted, from where it would be possible to select the fertiliser levels at which responses to AM fungi could be optimised compared to that obtainable from fertiliser application alone[4,42]. It is then expected that plants colonised by AM will respond to lower P applications than uninfected plants. It is concluded that AM stimulation of *P. phaseoloides* in unamended tropical soils could only be achieved in limited situations, depending on soil P-fixation capacities, the type of AM species used, the levels of trace elements affecting mycorrhizal colonisation and plant growth, the supply of P fertiliser, and their interactions.

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