*J. nat. Rubb. Res.*, **11(1)**, 59-68

# *Phosphate Response Curves of Mycorrhizal* Hevea brasiliensis *in Two Sterilised Soils*

A.IKRAM*#, M.N.SUDIN* AND D.NAPI*

*Monoclonal* Hevea brasiliensis *GT 1 seedlings were grown with or without introduced arbuscular mycorrhizal fungi in steam-sterilised Segamat and Rasau series soils that had been fertilised with Christmas Island phosphate rock and a soluble P source ($KH_2PO_4$) at a wide range of applied phosphate levels. After 20 weeks, inoculated plants significantly out-yielded uninoculated plants in shoot and root growth despite infection of uninoculated plants by indigenous mycorrhizal fungi. The effectiveness of the phosphorus sources applied on shoot and root yields were not significant but affects mycorrhizal development and phosphorus contents in shoots. Plant growth was poor in steamed Segamat series soil due to release of biologically toxic nutrients, even at the higher rates of P applied. Under the conditions of the experiment, the main effect of P rates was significant only in Rasau series soil where increased shoot dry matter was obtained when plants were given the insoluble P source at 200 and 400 mg/kg or $KH_2PO_4$ at 100 and 200 mg/kg, than when P was not supplied.*

Mycorrhizas have a worldwide recognised value for plant survival and nutrient cycling in the ecosystem, contributing significantly to plant productivity in arable and plantation crops. The arbuscular mycorrhizal (AM) fungi infect roots of *Hevea brasiliensis* and stimulate growth and phosphorus (P) uptake in Malaysian soils[1-3]. Middleton[4] compared rock phosphates and superphosphates as sources of P for seedling rubbber in a pot experiment using a P-deficient soil and found that superphosphate gave results similar to that obtained with rock phosphate. At the end of 10 months, their plants displayed P-deficiency symptoms and calculations revealed that >90% of both fertilisers were fixed by soils. Since the mycorrhizal condition is the norm for most higher plants in natural soils, it is likely that Middleton's data had incorporated the effect of indigenous mycorrhizal fungi (IMF) on plant P uptake unless of course the experiments had been conducted in sterilised soil or solution culture. Indeed, efficient AM fungi stimulate the recovery of finely-ground rock phosphate from soil. Mycorrhizal experiments carried out using a series of phosphate fertiliser levels give an assessment of the mycorrhizal effect independent of P supply[5], and it is possible to select levels at which responses to mycorrhizal inoculation can be optimised. It is also expected that plants colonised by AM fungi will respond to a lower phosphate application than uninfected plants. In this paper, we examine the responses of mycorrhizal *H. brasiliensis* to two types of phosphates of different solubilities in two soils.

*Rubber Research Institute of Malaysia, P.O.Box 10150, 50908 Kuala Lumpur, Malaysia
#Corresponding author

## MATERIALS AND METHODS

### Experimental Design

The experiment was a factorial combination of the following treatments: (1) mycorrhizal inoculation (with, without); (2) P source (rock phosphate, soluble phosphate) and (3) seven rates of P application, in five replications.

### Soil

The two soils used had been described earlier (Ikram *et al* [6]). All soils were air-dried, sieved (<5mm) and steamed (100°C, 1.5 h) to eliminate IMF before filling into 10 cm diameter (30 cm height) cylindrical PVC containers to within 2.5 cm from the top. Both the heavy clay Segamat series (Tropeptic Haplorthox) and the sandy Rasau series (Typic Quartzipsamment) soils had pHs of <4.7 (1:2.5 water), were depleted in bases, low in total N and had Bray-II P levels of <10 mg P/kg soil. The Segamat series soil had a higher amount of manganese (Mn) and iron (Fe).

### Plants and Inoculum

Monoclonal GT 1 seedlings raised in trays of vermicullite for 21 days and selected for uniformity in height were planted into each pot. Mycorrhizal inocula comprised spores, infested soils and chopped (<0.5 cm) mycorrhizal root fragments of *Glomus manihot* Howeler, Sieverding & Schenck (MAN), *Entrophospora colombiana* Spain & Schenck (ENT), *Glomus clarum* Nicolson & Schenck (CLA) and *Scutellospora calospora* Nicolson & Gerdemann (CAL), added at a rate of 30 g inoculum/kg soil. The non-mycorrhizal control pots received filtered inoculum leachings (<38 m) to reintroduce a part of the same microflora.

### Fertilisers

The fertiliser rates used were 0, 25, 50, 100, 200, 400 and 600 mg P/kg soil (equivalent to 0, 32, 64, 127, 255, 509 and 1018 kg P/ha), on a dry soil basis. Soluble P was applied as $KH_2PO_4$ in solution, and the insoluble Christmas Island phosphate rock (15.8% total P; 7.53% citrate-sol P) weighed out and thoroughly shaken with soils in large plastic bags before potting. Basal nutrients containing elements essential for growth were also applied in solution and allowed to dry before application of the phosphate fertilisers. Complete details of nutrients and inoculation procedures were described earlier[3].

### Harvest and Measurements

Plants were watered daily by weight to maintain the soil at 70% field capacity. After 20 weeks, the plants were cut at soil level, and the shoots separated into stems and leaves before drying (80°C, 48 h) and weighing. Tissue P concentrations were determined on the dried shoot samples by standard analytical methods[7]. Roots washed free of soil were also dried and weighed after removing a subsample for staining. Procedures for treatment of roots and assessing mycorrhizal root colonisation (MRC) were given elsewhere[1].

### Statistics

Analyses of variance were conducted on the measured parameters and all treatment means were compared for significance at P = 0.05.

## RESULTS AND DISCUSSION

### Mycorrhizal Root Colonisation

In the current study, plants in the uninoculated (control) treatments were infected

by IMF as a result of inefficient soil sterilisation procedures. This was caused by the close packing of the soil bags in the autoclave during steaming. Hence, any growth enhancement effects from introduced AM fungi will be an underestimation of that possible when control plants remain totally uninfected. The mean percentage of roots infected was moderate (31%) in Rasau series but low (15%) in Segamat series soil. Differences in the percentage of roots infected between treatments given introduced mycorrhizal fungi and uninoculated plants naturally infected with IMF were only significant in Rasau series soil (*Table 1*).

Irrespective of inoculation, mycorrhizal development was also affected by the source and rates of P used in both soils. Heavy fertiliser applications are often detrimental to mycorrhizal development and function but in this experiment, there was a clear trend of increased MRC levels beyond 200 mg/kg of P applied in Segamat series soil and 50 mg/kg in Rasau series soil before being depressed at the highest level of P applied (*Table 2*).

**Shoot and Root Dry Weights**

Averaged over the source and rates of P, the main treatment effect of mycorrhizal inoculation on the dry weight of shoot was highly significant ($P<0.001$), indicating that growth of *H. brasiliensis* in both soils was stimulated by introduced AM fungi (*Figure 1*). The magnitude of shoot growth in Rasau series was twice that achieved in Segamat series soil, despite the erratic but substantial infection of roots of uninoculated plants by IMF (*Table 3*). The results are consistent with the view that selection of effective mycorrhizal species can still improve growth of plants in soils containing less effective IMF. Differences exist between AM fungi in stimulating plant growth and P uptake due to differences among isolates in the time taken to establish an early infection, in the spread of an active mycelial network in soil for uptake and to the rates of P inflow into the host root[8]. For both soils, the proportion of leaves in shoots of plants ranged from 33%–40% with the greater part of the biomass consisting of stems.

TABLE 1 EFFECT OF AM FUNGI INOCULATION ON MYCORRHIZAL ROOT COLONISATION (MRC) OF *H. BRASILIENSIS* IN TWO STEAMED SOILS*

| Inoculation treatment | MRC (%) | |
|---|---|---|
| | Rasau series | Segamat series |
| Inoculated | 36.8 a | 15.9 a |
| Uninoculated | 24.7 b | 13.8 a |
| S.E. (±) | 1 2 | 1.6 |
| L.S.D. (P<0 05) | 4.6 | NS |
| C.V. (%) | 32 | 47 |

*Means of 5 replicate pots; within each soil treatment, mean values not followed by common letters are significantly different ($P<0.05$).
(NS) not significant.
Data averaged over phosphate source and rate treatments.

TABLE 2. EFFECT OF PHOSPHATE RATES ON MYCORRHIZAL ROOT COLONISATION (MRC) OF *H. BRASILIENSIS* IN TWO STEAMED SOILS*

| P rates (mg/kg soil) | MRC (%) | |
|---|---|---|
| | Rasau series | Segamat series |
| 0 | 21.1 d | 8.1 c |
| 25 | 26.2 cd | 9.5 c |
| 50 | 37.1 a | 9.4 c |
| 100 | 35.2 ab | 8.1 c |
| 200 | 33.8 ab | 17.7 b |
| 400 | 32.4 a–c | 25.6 a |
| 600 | 29.1 b–c | 27.5 a |
| S.E. (±) | 2.2 | 1.6 |
| L.S.D. (P<0.05) | 3.1 | 4.3 |

*Means of 5 replicate pots; within each soil treatment, mean values not sharing common letters are significantly different (P<0.05). Data averaged over mycorrhizal inoculation and phosphate source treatments.

TABLE 3. EFFECT OF AM FUNGI INOCULATION ON MEAN SHOOT AND ROOT DRY WEIGHTS OF *H. BRASILIENSIS* IN TWO STEAMED SOILS*

| Treatment | Shoot dry wt. (g/plant) | | Root dry wt. (g/plant) | |
|---|---|---|---|---|
| | Rasau series | Segamat series | Rasau series | Segamat series |
| Inoculated | 11.8 a | 5.8 a | 6.3 a | 2.6 a |
| Uninoculated | 9.0 b | 4.0 b | 4.0 b | 1.7 b |
| S.E. (±) | 0.4 | 0.3 | 0.2 | 0.2 |
| L.S.D. (P<0.05) | 1.1 | 0.9 | 0.7 | 0.4 |
| C.V. (%) | 33 | 55 | 38 | 60 |

*Means of 5 replicate pots; within each soil type, mean values not sharing common letters are significantly different (P<0.05). Data averaged over phosphate source and rate treatments.

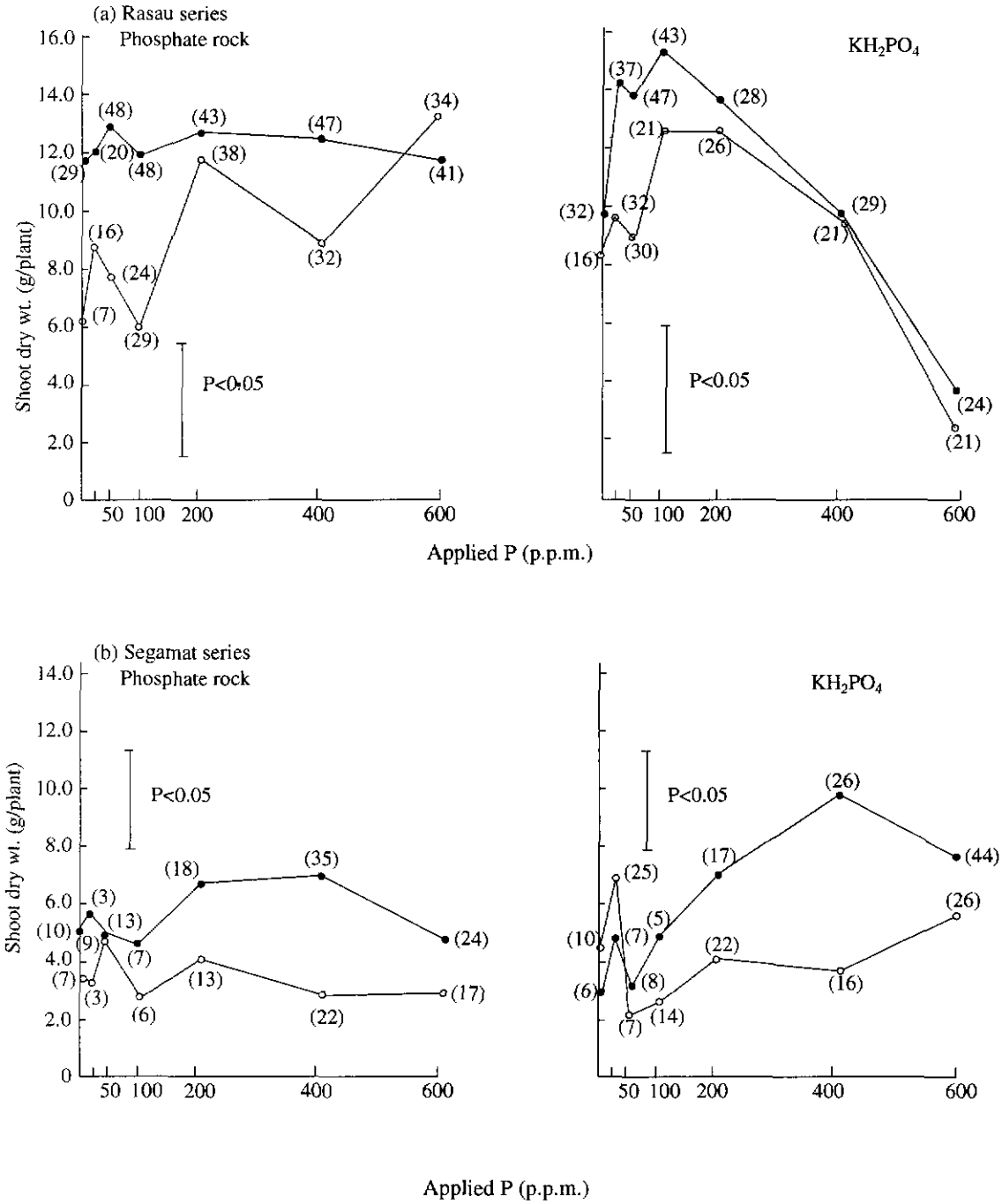


*Figure 1. Effect of AM fungi inoculation and phosphorus application on shoot dry weights of* H. brasiliensis *in two soils (•, inoculated; ∘, uninoculated). Vertical bars represent LSD values at P=0.05. Mycorrhizal root colonisation values given in brackets.*

TABLE 4. INTERACTIONS BETWEEN PHOSPHATE SOURCE AND RATES IN AFFECTING SHOOT DRY WEIGHTS IN TWO STEAMED SOILS*

| P rate (mg/kg) | Shoot dry wt. (g/plant) | | | | | |
|---|---|---|---|---|---|---|
| | Rasau series | | P | Segamat series | | P |
| | PR | SP | mean | PR | SP | mean |
| 0 | 8.9 d | 9.1 cd | 9.0 CD | 4.3 bc | 3.7 bc | 4.0 |
| 25 | 10.4 b–d | 11.9 a–d | 11.2 A–C | 4.7 a–c | 5.9 ab | 5.3 |
| 50 | 10.3 b–d | 11.3 a–d | 10.8 A–C | 5.4 ab | 2.9 c | 4.1 |
| 100 | 8.9 d | 13.9 a | 11.4 AB | 3.9 bc | 4.0 bc | 4.0 |
| 200 | 12.1 a–c | 13.1 ab | 12.6 A | 5.4 ab | 5.6 ab | 5.5 |
| 400 | 10.6 b–d | 9.6 cd | 10.1 BC | 5.1 a–c | 6.9 a | 6.0 |
| 600 | 12.4 a–c | 3.1 e | 7.8 D | 4.1 bc | 6.7 a | 5.4 |
| S.E. (±) | 1.1 | | 0.8 | 0.8 | | 0.3 |
| L.S.D. (P<0.05) | 3.0 | | 2.1 | 2.4 | | NS |

*Means of 5 replicate pots; within each soil treatment, mean values across rows and columns (phosphate source X rate interaction) and within a column (phosphate mean) not followed by common letters are significantly different (P<0.05). PR, phosphate rock; SP, soluble phosphate; NS, not significant.
Data averaged over VA fungi inoculation treatments.

In general, shoot and root growth responded similarly to applied P (*Figure 2*). In either soils, the main effect of mycorrhizal inoculation on root weights was highly significant (P<0.001) but the main effect of P source was not.

Irrespective of mycorrhizal inoculation, the main effect of the rates of P was significant for shoot weights only in Rasau series soil (P<0.001) where increased dry matter was obtained when plants were given the insoluble source of P at 200 and 600 mg/kg , or $KH_2PO_4$ at 100 and 200 mg/kg, than when P was not applied (*Table 4*). As in the case of shoot growth, the effect of the rates of P on root growth was only significant in Rasau series soil but this was mainly due to depressed yields at the two highest levels of soluble P applied (*Table 5*). The varying P application rates of the soluble and insoluble sources of P on plant growth were due to the varying P-fixation capacities of the soils used, and this in turn affects the availability of P for plant uptake and dry matter production. Labile P fixed by the relatively higher amounts of oxides and hydroxides of iron (Fe) and aluminium (Al) in the Segamat series soil could explain their greater P-fixation. The dissolution of phosphate rocks in acidic Malaysian soils is greatly enhanced in soils with a high percentage of silt and clay and with a high P-fixation capacity[9].

Plant growth in the steamed Segamat series soil was severely limited even when AM fungi inoculation is known to relieve the depressed growth of plants in soils that were steamed[10,11]. This could be due to the changed chemical conditions of the soil from release of biologically toxic nutrients. Many of the plants (*ca.* 16%) growing in this soil were devoid of

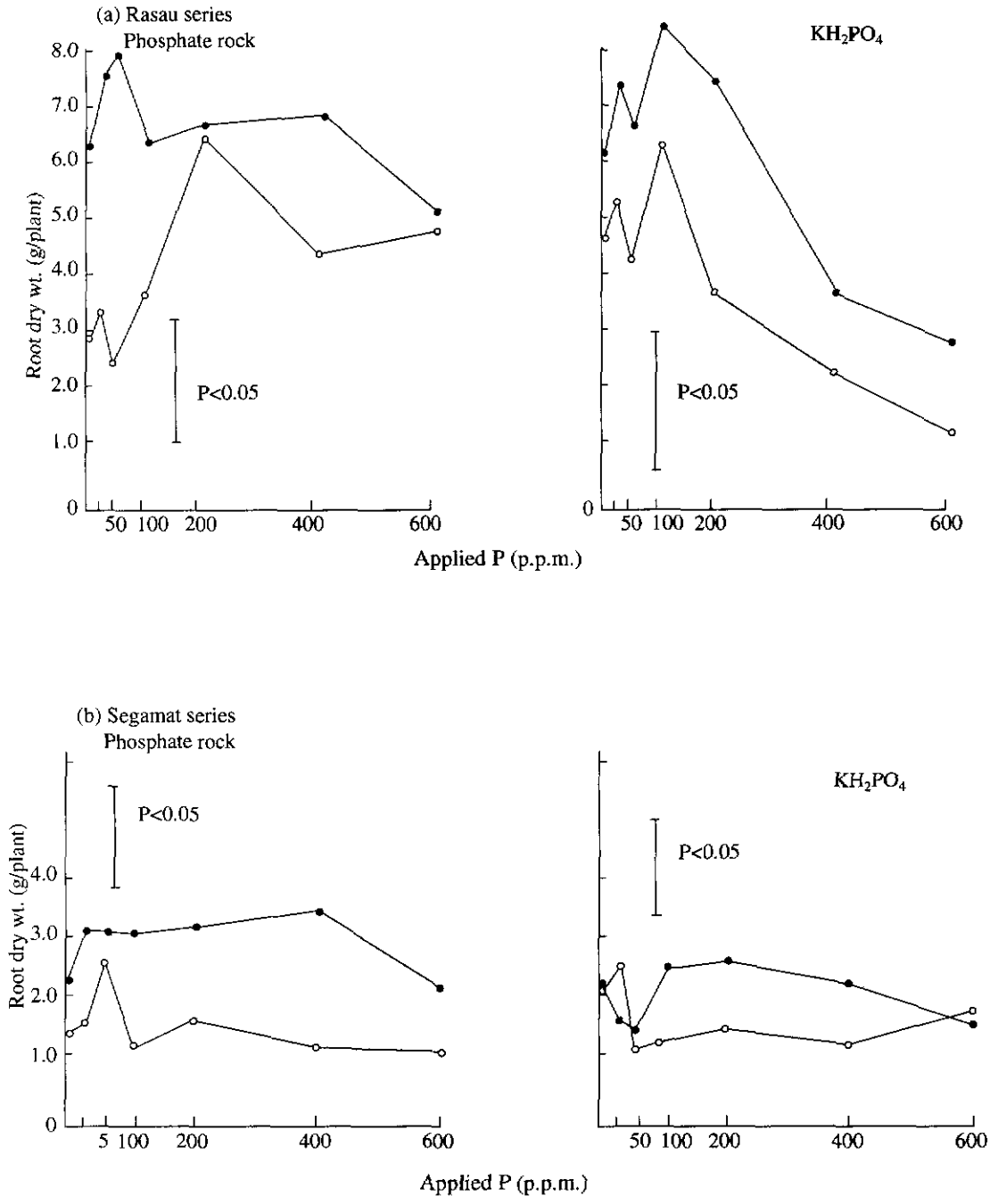


*Figure 2. Effect of AM fungi inoculation and phosphorus application on root dry weights of* H. brasiliensis *in two soils (•, inoculated; ○, uninoculated). Vertical bars represent LSD values at P=0.05.*

TABLE 5 INTERACTION BETWEEN PHOSPHATE SOURCE AND RATES IN AFFECTING ROOT DRY WEIGHTS OF *H BRASILIENSIS* IN STEAMED RASAU SERIES SOIL*

| P rate (mg/kg) | Root dry weight (g/plant) PR | SP | P mean |
|---|---|---|---|
| 0 | 4.6 cd | 5.4 bc | 5.0 AB |
| 25 | 5.5 bc | 6.3 a–c | 5.9 A |
| 50 | 5.2 bc | 5.5 bc | 5.4 AB |
| 100 | 5.1 bc | 7.4 a | 6.2 A |
| 200 | 6.5 ab | 5.5 bc | 6.0 A |
| 400 | 5.4 bc | 3.0 de | 4.2 BC |
| 600 | 5.0 bc | 2.1 e | 3.5 C |
| S.E. (±) | 0.6 | | 0.4 |
| L.S.D. (P<0.05) | 1.7 | | 1.2 |

*Means of 5 replicate pots; mean values across rows and columns (phosphate source X rate interation) and within a row (phosphate mean) not sharing common letters are significantly different (P<0.05).
PR, phosphate rock; SP, soluble phosphate.
Data averaged over VA fungi inoculation treatments.

leaves at harvest. Apart from the elimination of IMF, sterilisation alters the structure and physiological properties of soil by releasing nutrients both beneficial *e.g.* nitrogen (N) and P, or toxic *e.g.* free Mn, that affect growth of plants[12–14] In an earlier experiment using the same steamed soils[6], the extremely high levels of available N (ammonium and nitrate ions) released from steaming the clayey Segamat series soil suppressed early growth and nodule function of the legume *Pueraria phaseoloides*. The organic P released would be fixed by the inorganic components of soil and would not be available for plant growth. Toxic levels of Al and Mn in soil solution under acid conditions may also reduce plant growth, and a Mn-induced Fe-deficiency in rubber and legumes is commonly observed in glasshouse experiments[15,16]. In the current study, the very high levels of Mn in soil solution could cause Mn toxicity and stunt plant growth.

A closer examination of growth in the steamed Rasau series soil revealed that responses to the phosphate rock applied were due to uninoculated plants, whereas plants inoculated with introduced AM fungi were unaffected over the entire fertiliser range (*Figure 1*). When plants were given the soluble P source, a drastic decline in shoot yields occurred beyond 200 mg/kg due to luxury uptake of P and toxic concentrations of P in plant tissue. At the two highest levels of $KH_2PO_4$ applied, P in shoot concentrations exceeded 0.35% (*Table 6*).

**Phosphorus in Shoot**

In his experiments, Middleton[4] did obtain larger plants when superphosphate was applied as pockets into soil but not when mixed with soil or broadcasted. In this study, the effect of the sources of different solubilities was only significant in the uptake of P where in both soils,

TABLE 6. PHOSPHORUS CONTENT IN SHOOT OF *H. BRASILIENSIS* AT SEVEN RATES OF APPLIED PHOSPHATES IN TWO STEAMED SOILS*

| P rates (mg/kg) | P in shoot (mg/plant) | | | | | |
|---|---|---|---|---|---|---|
| | Rasau series | | P | Segamat series | | P |
| | PR | SP | mean | PR | SP | mean |
| 0 | 5.1 (0.060)de | 4.2 (0.046)c | 4.6 C | 3.6 (0.177)d | 3.8 (0.125)cd | 3.7 C |
| 25 | 5.6 (0.052)de | 7.1 (0.063)de | 6.4 C | 5.1 (0.116)cd | 5.6 (0.089)cd | 5.3 BC |
| 50 | 7.6 (0.069)de | 6.1 (0.053)de | 6.9 C | 4.0 (0.082)cd | 3.3 (0.124)cd | 3.7 C |
| 100 | 5.3 (0.053)de | 8.1 (0.058)de | 6.7 C | 4.2 (0.109)cd | 4.6 (0.120)cd | 4.4 BC |
| 200 | 8.0 (0.072)de | 14.7 (0.120)b | 11.4 B | 5.0 (0.208)cd | 6.5 (0.170)c | 5.8 B |
| 400 | 7.4 (0.070)de | 29.4 (0.359)a | 18.4 A | 5.1 (0.120)cd | 10.7 (0.226)b | 8.0 A |
| 600 | 9.5 (0.079)cd | 14.3 (0.479)bc | 11.9 B | 5.3 (0.172)cd | 14.1 (0.253)a | 9.7 A |
| S.E. (±) | 1.8 | | 1.2 | 1.0 | | 0.7 |
| L.S.D. (P<0.05) | 4.9 | | 3.5 | 2.7 | | 1.9 |
| C.V. (%) | 58 | | | 52 | | |

*Means of 5 replicate pots; shoot P concentrations (%) in brackets. Within each soil treatment, mean values across rows and columns (phosphate source X rate interaction) and within a column (phosphate mean) not sharing common letters are significantly different (P<0.05).
PR, phosphate rock; SP, superphosphate.
Data averaged over AM fungi inoculation treatments.

the P contents in shoots were greatly increased by the higher rates of $KH_2PO_4$ applied (200–600 mg/kg in Rasau; 400 and 600 mg/kg in Segamat series soil) but not with phosphate rock than when P was not supplied (*Table 6*). However, the main effect of mycorrhizal inoculation in increasing P uptake was only significant in Rasau series soil.

We conclude that under the conditions of this study, the effectiveness of the two different P sources applied in increasing shoot and root dry weights of rubber in steam-sterilised soils was similar when using the entire growth-response curve.

ACKNOWLEDGEMENTS

We thank S. Sarojini, Rahim Ali, Karim Jaafar and Ismail Abd. Rahman for capable technical assistance.

*Date of receipt: April 1996*
*Date of acceptance: June 1996*

REFERENCES

1. IKRAM, A., MAHMUD, A.W., GHANI, M.N., IBRAHIM, M.T. AND ZAINAL, A.B. (1992) Field Nursery Inoculation of *Hevea brasiliensis* Muell.Arg. Seedling Rootstock with Vesicular-Arbuscular Mycorrhizal (VAM) Fungi. *Pl. Soil*, **145**, 231.

2. IKRAM, A., MAHMUD, A.W. AND OTHMAN, H. (1993) Growth Response of *Hevea brasiliensis* Seedling Rootstock to Inoculation with Vesicular-arbuscular Mycorrhizal Fungal Species in Steam-sterilised Soil. *J. nat. Rubb Res.*, **8(3)**, 231.

3. IKRAM, A., JENSEN, E.S. AND JAKOBSEN, I. (1994) No Significant Transfer of N and P from *Pueraria phaseoloides* to *Hevea brasiliensis via* Hyphal Links of Arbuscular Mycorrhiza. *Soil Biol. Biochem.*, **26**, 1541.

4. MIDDLETON, K R (1960) A Comparison of Rock Phosphate and Superphosphate as Sources of Phosphorus for Seedling Rubber *J Rubb Res Inst Malaya*, **16**, 139

5 ABBOTT, L K AND ROBSON, A D (1977) Growth Stimulation of Subterranean Clover with Vesicular Arbuscular Mycorrhizas *Aust J Agr Res* , **28**, 639.

6 IKRAM, A , SUDIN, M N AND JENSEN, E S (1994) Symbiotic $N_2$-Fixation of *Pueraria phaseoloides* as Influenced by Arbuscular Mycorrhizal Fungi and Applied Phosphate. *J nat Rubb Res* , **9**, 258.

7 RUBBER RESEARCH INSTITUTE OF MALAYSIA (1970) *Manual of Laboratory Methods of Plant Analysis*. Analytical Chemistry Division, Rubber Research Institute Malaysia, Kuala Lumpur. 68p

8 JAKOBSEN, I., ABBOTT, L K. AND ROBSON, A D (1992) External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* I. Spread of hyphae and phosphorus inflow into roots *New Phytol* , **120**, 371

9 LAU, C H AND MAHMUD, A.W. (1992) Dissolution of Phosphate Rocks in Malaysian Soils under Rubber and Their Availability to Plants *J nat Rubb Res* , **7(1)**, 71.

10. IKRAM, A , MAHMUD, A.W AND NAPI, D (1987) Effects of P-Fertilization and Inoculation by Two Vesicular-Arbuscular Mycorrhizal Fungi on Growth and Nodulation of *Calopogonium caeruleum Pl Soil*, **104**, 195.

11. IKRAM, A , SUDIN, M N AND JENSEN, E S (1995) Symbiotic $N_2$-Fixation of *Pueraria phaseoloides* as Influenced by Arbuscular Mycorrhizal Fungi and Applied Phosphate *J nat Rubb Res* , **9(4)**, 258.

12. BOWEN, G D AND ROVIRA, A D (1961) Plant Growth in Irradiated Soil *Nature*, **191**, 936.

13 SALONIUS, P O , ROBINSON, J B AND CHASE, F E (1967) A Comparison of Autoclaved and Gamma Irradiated Soils as Media for Microbial Colonization Experiments. *Pl Soil*, **27**, 239

14. SMITH, F A AND SMITH, F E (1981) Mycorrhizal Infection and Growth of *Trifolium subterraneum* Use of Sterilised Soil as a Control Treatment. *New Phytol* , **88**, 299

15 BACHIK, A.T , RAVEENDRAN, N , WONG, S P AND PUSHPARAJAH, E (1984) ·Manganese Toxicity Symptoms in *Hevea Proceedings, International Conference on Soils and Nutrition of Perennial Crops (Bachik, A T and Pushparajah, E eds)*, 67 Kuala Lumpur: Malaysian Society of Soil Science.

16. IKRAM, A (1995) Mycorrhizal Growth Responses of *Pueraria phaseoloides* in Phosphorus-deficient Soils. *J nat Rubb Res* , **10(1)**, 26.