J. Rubb. Res. Inst. Malaysia, 33(1), 54–58

SHORT COMMUNICATION

Growth Responses of Calopogonium caeruleum *to Vesicular-arbuscular Mycorrhizal Fungi Inoculation*

A. IKRAM, A.W. MAHMUD AND D. NAPI

Inoculation with vesicular-arbuscular mycorrhizal fungi improved growth of pot-grown Calopogonium caeruleum *over a range of P fertiliser applications in the absence or presence of indigenous mycorrhizal fungi. In sterilised soils, the experimental evidence indicates that as much as 2.1 times more P was required by uninoculated plants to achieve similar dry weight yields of* Glomus fasciculatum-*inoculated plants.*

Plants infected by vesicular-arbuscular mycorrhizal fungi (VAMF) generally show increased growth and phosphorus (P) uptake in P-deficient soils[1, 2, 3]. Consequently, the benefit of mycorrhizal inoculation in agriculture lies in its more efficient utilisation of phosphate fertilisers. Legumes are particularly responsive because of the demand for P in maximising growth, nodule formation and nitrogen (N)-fixation[4,5]. This paper attempts to demonstrate the potential of two introduced VAMF to increase growth of the plantation legume *Calopogonium caeruleum* in pot experiments.

MATERIALS AND METHODS

The experiment was a factorial combination of mycorrhizal inoculation (two VAMF and an uninoculated control), P application (five levels) and soil treatments (sterilised, 100°C, 1.5 h and unsterilised), in three replications. The soil used was a sandy Entisol (Typic Quartzipsamment) collected from RRIM Experiment Station in Sungai Buloh (*Field 54B*, 0–20 cm) with properties as follows: pH 4.5 (1:2.5 weight/volume soil:H_2O), 1.91% organic C, 0.21% total N and 7 p.p.m. available P (Bray II). Basal nutrients were added in solution (final concentrations; Mg, 12 p.p.m.; K, 72 p.p.m.; Cu, 1.27 p.p.m.; Zn, 1.14 p.p.m ; and Mo, 0.06 p.p.m.) uniformly to all pots.

The insoluble P source used, Christmas Island rock phosphate (CIRP, 15.8% total P, 7.34% citrate-sol P, 70% passing through a 125 μm sieve) was mixed thoroughly throughout the soil at 0, 28, 57, 85 and 113 kg P per hectare on a pot surface-area basis. The highest rate of CIRP application was therefore that recommended for traditional sowing of legume covers[6]. The soils were filled into 8.5 cm diameter plastic pots (450 g soil) and maintained at field capacity for the duration of the experiment.

COMMUNICATION 764

Fungal inoculum consisted of soil-root mixtures (10 g) from three-month-old pot cultures (*C. caeruleum*, as host) of *Glomus macrocarpum* Tul. and Tul[7] and *Glomus fasciculatum* (Thaxter sensu Gerd.) Gerd. and Trappe[7] placed under the seedlings at planting. Root infection of these were 54% and 100%, and spore numbers, 36 per gramme and 107 per gramme soil respectively, for *G. macrocarpum* and *G. fasciculatum*. The uninoculated control plants received 2 ml of filtered soil sievings to ensure common contaminating microflora.

The sown plants were four-day-old surface-sterilised pre-germinated seedlings (two per pot) inoculated with a heavy suspension (2 ml) of the effective *Rhizobium* sp. strain RRIM 77[8] to ensure nodulation. Plants were grown in an open glasshouse (mean temperatures, 28.5°C/21.5°C day/night) and harvested at eight weeks for dry weight and tissue P concentrations. The incidence of root infection (percentage infection) was determined on 100 one-cm segments randomly selected from a composite sample of each replicate after initial clearing (KOH) and staining in lactophenol-trypan blue[9].

RESULTS AND DISCUSSION

Growth responses to VAMF inoculation were evident at most rates of fertiliser P applications *(Figure 1)*. In sterilised soils, the inoculant fungi *G. fasciculatum* increased tops dry weight (DW) and P concentrations over the entire fertiliser range. Despite the significant growth increase, infection of *C. caeruleum* roots by *G. macrocarpum* was however not apparent *(Table 1)*. This could perhaps be due in part to the effects of plant growth-regulating substances similar to that known for *G. mosseae*[10] and to the organic matter introduced as inoculants. Uninoculated plants grew poorly in sterilised soil, indicating the dependence of an effective mycorrhizal association for normal growth of this legume. It is not known why *G. macrocarpum* failed to survive in sterilised soil. In unsterilised soils, the results were less predictable because of the presence of indigenous mycorrhizal fungi (IMF). Although no attempts were made to estimate the numbers of infective propagules of IMF in the unsterilised soil, root infection at the end of the experiment (range, 7%–67%) indicated that it was reasonably well-supplied with IMF. To distinguish the introduced VAMF from the IMF within plant root would be difficult[11], and thus the infection levels recorded for the introduced endophytes in unsterilised soils were therefore the total infection of both the inoculant VAMF and IMF. Both the introduced VAMF produced greater tops DW and P concentrations than the controls at the lower (28 kg P per hectare) and the highest (113 kg P per hectare) levels of applied P. Maximum yields by uninoculated plants were achieved at 57 kg P per hectare but higher yields were possible with *G. macrocarpum* at 28 kg P and 57 kg P per hectare. In contrast, the experimental evidence suggests that plant growth response to *G. fasciculatum* in both sterilised and unsterilised soils would require higher rates of P applications. These observations thus emphasise the importance of the three-way interaction among soil, plant and fungus in determining the overall growth response of the plant to VAMF[3,12].

There was a linear effect of CIRP on tops DW of both inoculated and uninocu-

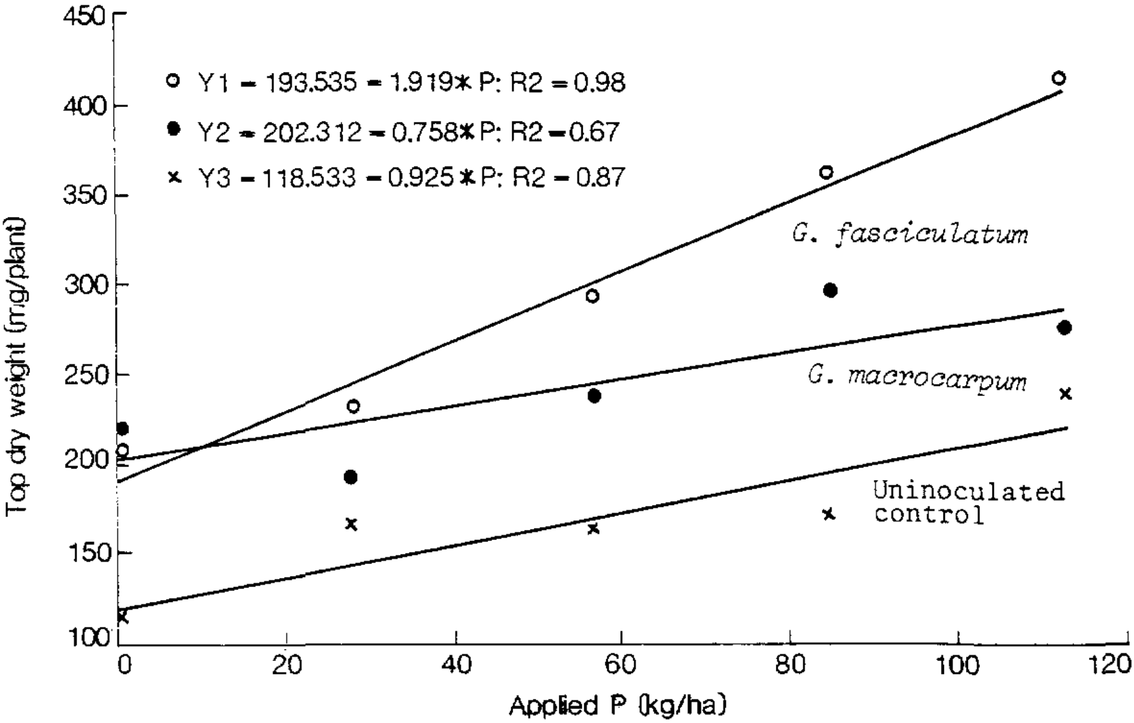

Figure 1. Response curves of C. caeruleum *to vesicular-arbuscular mycorrhizal fungi inoculation and Christmas Island rock phosphate application in sterilised soil.*

lated *C. caeruleum* in sterilised soils, but in unsterilised soils the effect varied from linear to cubic. It is likely that such irregularities were due to the presence of IMF, producing linear *(G. fasciculatum)*, quadratic and cubic *(G. macrocarpum)*, and quadratic (uninoculated) responses on tops DW. The model produced in this work is therefore confined to the response of *C. caeruleum* grown in sterilised soil. Over the fertiliser ranges tested, the responses can be described by the following regression equations:

$$Y_1 = 193.535 + 1.919\,P$$

$$r^2 = 0.98$$

for *G. fasciculatum*

$$Y_2 = 202.312 + 0.758\,P$$

$$r^2 = 0.67$$

for *G. macrocarpum*

$$Y_3 = 118.533 + 0.925\,P$$

$$r^2 = 0.87$$

for uninoculated treatments

where Y_1, Y_2 and Y_3 are the dry weights and P, the rates of phosphorus applied. The coefficients of P for mycorrhizal treatments over that of uninoculated plants estimate the relative effectiveness of P for tops DW[13] *i.e.* the ratios were 2.075 and 0.820 for *G. fasciculatum* and *G. macrocarpum* respectively. On this basis, uninoculated plants in sterilised soils required 2.1 times as much P as *G. fasciculatum*-inoculated plants to achieve the same DW yield of tops.

TABLE 1. EFFECT OF VAMF INOCULATION AND P APPLICATION ON TOPS DRY WEIGHTS, TOPS P CONCENTRATIONS AND ROOT INFECTION OF *C. CAERULEUM* GROWN IN STERILISED AND UNSTERILISED SOILS

Item	Sterilised soil					Unsterilised soil				
	No P	28 kg P/ha	57 kg P/ha	85 kg P/ha	113 kg P/ha	No P	28 kg P/ha	57 kg P/ha	85 kg P/ha	113 kg P/ha
Tops DW (mg/plant)										
G. macrocarpum	221	193	238	296	277	426	627	603	503	553
G. fasciculatum	208	233	293	362	414	442	512	512	517	590
Uninoculated	115	161	165	172	240	262	394	519	501	449
Tops P concentration (%)[a]										
G. macrocarpum	0.13	0.07	0.13	0.09	0.08	0.09	0.17	0.16	0.19	0.21
G. fasciculatum	0.10	0.14	0.18	0.19	0.17	0.12	0.12	0.19	0.20	0.18
Uninoculated	n.d.	0.07	0.08	0.10	0.08	0.08	0.09	0.16	0.11	0.11
Infection[b] (%)										
G. macrocarpum	0	0	0	0	0	33	25	23	46	56
G. fasciculatum	27	30	69	70	46	36	56	69	69	48
Uninoculated	0	0	0	0	10[c]	7	17	33	67	19
Standard error of mean										
P level			19					27		
Mycorrhiza			15					21		
L.S.D. (5%)										
P level			54					77		
Mycorrhiza			42					60		

n.d. = not determined
[a]Total of three replications, as % DW
[b]Means of three replication
[c]Contamination in one replicate pot

It is accepted that the potential to increase crop yields through VAMF inoculation is greatest in soils of low fertility containing a small and inefficient IMF population[5]. There is support for this contention in this study, where plant growth and P uptake responded to inoculation with VAMF when uninoculated plants were colonised by IMF. The experiment was done once but further work is in progress using this and other soils under rubber to check on the generality of the responses to the foreign endophytes.

ACKNOWLEDGEMENT

The authors thank Drs B. Mosse and D.S. Hayman (Rothamsted Experimental Station, Harpenden) for the original supply of *G. fasciculatum* strain E3; and Encik Loke Lai Yuen, Cik Susan Wong and Encik Mat Sedar Tamyiz for continued technical assistance.

Rubber Research Institute of Malaysia
Kuala Lumpur *September 1984*

REFERENCES

1. MOSSE, B. (1973) Advances in the Study of Vesicular-arbuscular Mycorrhiza. *Ann. Rev. Phytopath.*, **11**, 171.

2. MOSSE, B. (1978) Vesicular-arbuscular Mycorrhiza Research for Tropical Agriculture. *Res. Bull. 194.* Hawaii Institute of Tropical Agriculture and Human Resources, University of Hawaii.

3. MOSSE, B. AND HAYMAN, D.S. (1980) Mycorrhiza in Agricultural Plants. *Tropical Mycorrhiza Research (Mikola, P. ed.)*, p. 213. Oxford University Press.

4. ABBOTT, L.K., ROBSON, A.D. AND PARKER, C.A. (1976) Double Symbiosis in Legumes – the Role of Mycorrhizas. *Soil Microbiology and Plant Nutrition (Broughton, W.J., John, C.K., Rajarao, J.C. and Lim, B. ed.)*, p. 176. Kuala Lumpur: University of Malaya Press.

5. MOSSE, B. (1977) The Role of Mycorrhiza in Legume Nutrition on Marginal Soils. *Exploiting the Legume-Rhizobium Symbiosis in Tropical Agriculture (Vincent, J.M., Whitney A.S. and Bose, J. ed.)*, p. 275. *Proc. NifTAL Workshop, Kahului, Muai, Hawaii 1976.*

6. RUBBER RESEARCH INSTITUTE OF MALAYA (1972) Cover Management in Rubber. *Plrs' Bull. Rubb. Res. Inst. Malaya No. 122*, 170.

7. GERDEMANN, J.W. AND TRAPPE, J.M. (1974) The Endogonaceae in the Pacific Northwest. *Mycologia Mem.*, **5**, 1.

8. IKRAM, A. (1983) *Rhizobium* Inoculation of *Calopogonium caeruleum. Soil Biol. Biochem.*, **15**, 537.

9. PHILLIPS, J.M. AND HAYMAN, D.S. (1970) Improved Procedures for Clearing Roots and Staining Parasitic and Vesicular-arbuscular Mycorrhizal Fungi for Rapid Assessment of Infection. *Trans. Br. Mycol. Soc.*, **55**, 158.

10. BAREA, J.M. AND AZCON-AGUILAR, C. (1982) Production of Plant Growth-regulating Substances by the Vesicular-arbuscular Mycorrhizal Fungus *Glomus mosseae. Appl. Environ. Microbiol.*, 43, 810.

11. ABBOTT, L.K. AND ROBSON, A.D. (1978) Growth of Subterranean Clover in Relation to the Formation of Endomycorrhizas by Introduced and Indigenous Fungi in a Field Soil. *New Phytol.*, **81**, 575.

12. HAYMAN, D.S. (1983) The Physiology of Vesicular-arbuscular Endomycorrhizal Symbiosis. *Can. J. Bot.*, **61**, 944.

13. PAIRUNAN, A.K., ROBSON, A.D. AND ABBOTT, L.K. (1980) The Effectiveness of Vesicular-arbuscular Mycorrhizas in Increasing Growth and Phosphorus Uptake of Subterranean Clover from Phosphorus Sources of Different Solubilities. *New Phytol.*, **84**, 327.