

GROWTH RESPONSE OF WATTLE (*ACACIA MEARNSII*) SEEDLINGS TO PHOSPHORUS FERTILISATION AND INOCULATIONS WITH *GLOMUS DESERTICOLA* AND *RHIZOBIUM* SP. IN NON-STERILE SOIL

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UDAIYAN, K., SUGAVANAM, V. & MANIAN, S. 1997. Growth response of wattle (*Acacia mearnsii*) seedlings to phosphorus fertilisation and inoculations with *Glomus deserticola* and *Rhizobium* sp. in non-sterile soil. The effect of phosphorus (P) fertilisation and inoculation with the vesicular-arbuscular mycorrhizal (VAM) fungus *Glomus deserticola* and *Rhizobium* sp. was studied in containerised seedlings of wattle (*Acacia mearnsii*). The non-sterile soil used was amended with tricalcium phosphate and superphosphate at the rates of 0, 50, 100, 150, 200 and 250 mg kg⁻¹ soil. The seedlings inoculated with *G. deserticola* alone but receiving no P fertiliser were comparable in growth to those uninoculated seedlings which received 150 mg kg⁻¹ soil of tricalcium phosphate and 100 mg kg⁻¹ soil of superphosphate. *Rhizobium* inoculated wattle seedlings which received no P fertiliser were similar in size to those of uninoculated seedlings fertilised with 200 and 150 mg kg⁻¹ soil of tricalcium phosphate and superphosphate respectively, whereas the seedlings coinoculated with *G. deserticola* and *Rhizobium* sp. without added P fertiliser were more than equivalent in growth to uninoculated control seedlings fertilised with 250 mg kg⁻¹ soil of tricalcium phosphate or superphosphate. P fertilisation invariably increased tissue P concentration in the seedlings, reduced mycorrhizal root colonisation but markedly stimulated nodulation. The highest nutrient (N,P,K) concentrations in the plant tissues were recorded in the seedlings coinoculated with *G. deserticola* and *Rhizobium* sp. when compared with individual inoculations. Phosphorus utilisation efficiency decreased with increasing P fertiliser doses and inoculations with *G. deserticola* and *Rhizobium* sp.

Key words: *Acacia mearnsii* - phosphate fertilisers - *Glomus deserticola* - *Rhizobium* sp.
- VAM root colonisation - nodulation - phosphorus utilisation efficiency

UDAIYAN, K., SUGAVANAM, V. & MANIAN, S. 1997. Tindak balas pertumbuhan anak benih wattle terhadap pembajaan dan penginokulan fosforus dengan *Glomus deserticola* dan *Rhizobium* sp. dalam tanah yang tidak disteril. Kesan pembajaan fosforus (P) dan penginokulan dengan kulat mikoriza vesikular-arbuskular (VAM), *Glomus deserticola* dan *Rhizobium* sp. dalam anak benih tabung wattle (*Acacia mearnsii*) dikaji. Tanah yang tidak disteril diubah dengan trikalsium fosfat dan superfosfat pada kadar 0, 50, 100, 150, 200 dan 250 mg kg⁻¹ tanah. Anak benih yang diinokulat dengan

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G. deserticola sahaja tetapi tidak menerima baja-P didapati setanding dengan pertumbuhan anak benih yang tidak diinokulat yang menerima 150 mg kg⁻¹ fosfat trikalsium dan 100 mg kg⁻¹ tanah superfosfat. Anak benih wattle yang dinokulat dengan *Rhizobium* yang tidak menerima baja-P didapati sama saiznya dengan anak benih yang tidak diinokulat yang dibaja dengan 200 dan 150 mg kg⁻¹ fosfat trikalsium dan superfosfat sementara anak benih yang dikoinokulat dengan *G. deserticola* dan *Rhizobium* sp. tanpa ditambah baja-P didapati lebih daripada setara dalam pertumbuhan berbanding anak benih yang tidak diinokulat yang dibaja menggunakan 250 mg kg⁻¹ fosfat trikalsium atau superfosfat. Pembajaan-P secara tetap menambahkan kepekatan tisu P dalam anak benih yang mengurangkan pengkolonian akar mikoriza tetapi dengan jelas merangsang penodulan. Kepekatan nutrien didapati tertinggi (N,P,K) dalam tisu tumbuhan dicatatkan dalam anak benih yang diinokulat dengan *G. deserticola* dan *Rhizobium* sp. jika dibandingkan dengan peninokulan individu. Kecekapan penggunaan fosforus berkurang dengan bertambahnya dos baja-P dan peninokulan dengan *G. deserticola* dan *Rhizobium* sp.

Introduction

Microorganisms in the rhizosphere may increase or decrease the absorption of inorganic nutrients by plant roots and there appears to be significant interactions between them. It has been clearly shown that vesicular-arbuscular mycorrhiza (VAM) can improve plant growth through increased uptake of phosphorus (P), especially in low-fertile soils (Safir *et al.* 1972, Mosse *et al.* 1973). Several studies in recent years have exposed the beneficial interaction between VAM fungi and *Rhizobium* sp. on leguminous plants (Jasper *et al.* 1988, 1989, Reena & Bagyaraj 1990). Nodulation and subsequent nitrogen fixation by leguminous plants require an optimum level of P in the host tissue (Hayman 1986, Marschner 1986). It is also known that heavy application of P fertiliser can inhibit the percentage infection of roots by VAM fungi (Siqueira *et al.* 1984, Thomson *et al.* 1986). However, when the initial concentration of P is extremely low, small additions may favour infection (Sanders & Tinkers 1973, Schubert & Hayman 1986).

Restoration and maintenance of soil fertility is a basic and critical problem, especially in the tropics where nutrient deficient soils are frequent. Such soils are reclaimed through afforestation programmes with multipurpose forest tree species which require large quantities of good quality seedlings. If commercial applications of mycorrhizal fungi are to become a reality, it must be demonstrated that mycorrhizal fungi can economically substitute P fertiliser. Hence, the objectives of the present study were to (a) examine the growth benefits of phosphorus fertilisation and inoculations with the mycorrhizal fungus *Glomus deserticola* and *Rhizobium* sp. on wattle (*Acacia mearnsii* De Wild.) seedlings, (b) equate the inoculation benefits of the seedlings with the fertilisation doses of tricalcium phosphate and superphosphate, and (c) relate the tissue nutrients concentrations with VAM root colonisation, sporulation and nodulation.

Materials and methods

Soil

Sandy loam collected from the experimental fields of the Bharathiar University, Coimbatore, India, had a pH of 8.0 (1:1, soil:water), electric conductivity 0.2 mS cm⁻¹, nitrogen 10.9 mg kg⁻¹, phosphorus 0.5 mg kg⁻¹ and potassium 24.2 mg kg⁻¹. The total N and available P were determined respectively by the micro-Kjeldahl's method and the molybdenum blue method of Jackson (1973). Exchangeable K was extracted from the soil in an ammonium acetate solution (pH 7.0) and measured with a digital flame photometer (Jackson 1973). The soil had a natural VA mycorrhizal population (*Acaulospora scrobiculata*, *Glomus fasciculatum*, *G. geosporum* and *G. mosseae*) of 72 spores 100 g⁻¹ dry soil.

Phosphorus fertilisation

Phosphorus was applied in the form of commercial acid soluble tricalcium phosphate [$\text{Ca}_3(\text{PO}_4)_2$] and water soluble superphosphate [$\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$] at the rate of 0, 50, 100, 150, 200 and 250 mg kg⁻¹ soil and uniformly mixed. One milligram of tricalcium phosphate and superphosphate contains 0.61 and 0.81 mg of phosphate respectively.

Plant material

Wattle (*Acacia mearnsii*) seeds were procured from the Institute of Forest Genetics and Tree Breeding, Coimbatore, India. Healthy, uniform seeds were surface sterilised in 5% H₂O₂, treated with boiling water for 30 s, then soaked in water for about 24 h and sown in 10 × 15 cm polythene bags, each containing 3 kg unsterilised soil. Initially, three seeds were planted per bag but the seedlings were thinned to one on the tenth day.

Inoculum

The VAM fungus *Glomus deserticola* Trappe, Bloss & Menge, isolated and recorded as a dominant species in the rhizosphere soil samples of *Acacia mearnsii* found growing in the plantation forest of Kodaikanal, Tamil Nadu, India, was multiplied in pot culture in the roots of *Sorghum vulgare* and the soil containing the extramatrical hyphae, chlamydo spores and infected root segments served as mycorrhizal inoculum. Fifty gram inoculum containing c. 12 500 infective propagules, based on a most probable number estimate (Porter 1979) was placed in each polythene bag about 2 cm below seed level.

Fresh nodules of wattle (*Acacia mearnsii*) were collected from the plantation forest, Kodaikanal, Tamil Nadu, India. *Rhizobium* sp. was isolated and maintained in yeast extract mannitol broth. Ten millilitre broth was added to each polybag.

Experimental design

The experimental design was $4 \times 2 \times 6$ factorial consisting of 4 levels of endophyte inoculations, 2 phosphate sources and 6 levels of P fertiliser doses. Thus in all there were 48 treatments and each treatment was replicated 5 times. The treatments were arranged in a completely randomised block design.

Harvesting and measurement

Plants were harvested after 120 days and their dry weights were recorded. Root subsamples (10 segments of 1 cm length seedling¹) were processed for microscopic observation following the procedure of Phillips and Hayman (1970) and the percentage mycorrhizal infection was determined by the root slide technique of Read *et al.* (1976). The dried plant material (shoot and root) was ground and used for the analysis of N, P and K. Total Kjeldahl nitrogen was detected on a Kjeltac Auto Analyser (1030); phosphorus determination was done by the vanadomolybdate phosphoric yellow colour method and potassium content was determined by flame photometer (Jackson 1973). Phosphorus utilisation efficiency (PUE) was calculated using the formula of Siddiqui and Glass (1981) : $PUE = \text{plant dry weight} / P \text{ content}$.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) and the means were separated by Duncan's new multiple range test ($p < 0.05$). Regression analyses were performed between phosphate fertiliser concentration and plant variables.

Results

Growth and biomass

Growth in wattle seedlings as measured by root and shoot dry weights were invariably enhanced by P fertilisation and microbial inoculation. Only the coinoculated seedlings with *G. deserticola* and *Rhizobium* sp. recorded significantly ($p < 0.05$) higher growth than their uninoculated control seedlings (Tables 1 and 2). *Glomus deserticola* inoculated wattle seedlings grown with (0) fertiliser P were similar in growth to those of control seedlings fertilised with 150 mg kg⁻¹ soil of tricalcium phosphate or 100 mg kg⁻¹ soil of superphosphate. Similarly, the growth equivalent of *Rhizobium* inoculation in wattle seedlings was 200 mg kg⁻¹ soil of tricalcium phosphate or 150 mg kg⁻¹ soil of superphosphate. However, the seedlings coinoculated with *G. deserticola* and *Rhizobium* sp. in (0) fertiliser P showed the highest growth benefit which was equivalent to those of the control seedlings with 250 mg kg⁻¹ soil of tricalcium phosphate or superphosphate (Tables 1 and 2).

Table 1. Effects of tricalcium phosphate on the biomass, root colonisation and nodule dry weight of *Acacia mearnsii* inoculated with *Glomus deserticola* alone, *Rhizobium* sp. alone and *G. deserticola* + *Rhizobium* sp.

Parameter	Treatment	Ca ₃ (PO ₄) ₂ (mg kg ⁻¹ soil)											
		0		50		100		150		200		250	
Root dry weight (mg plant ⁻¹)	Control	54	D	58	CD	62	BC	65	ABC	68	AB	70	A
			c		b		b		b		b		b
	<i>G. deserticola</i>	63	B	68	B	70	B	76	AB	86	A	90	A
			b		b		b		b		b		b
Shoot dry weight (mg plant ⁻¹)	<i>Rhizobium</i> sp.	68	C	70	BC	72	BC	89	AB	93	A	100	A
			ab		b		ab		ab		b		ab
	<i>G. deserticola</i> + <i>Rhizobium</i> sp.	75	C	115	BC	119	BC	135	AB	142	AB	178	A
			a		a		a		a		a		a
Root colonisation (%)	Control	176	D	223	C	262	BC	281	AB	290	AB	303	A
			c		b		b		b		b		b
	<i>G. deserticola</i>	275	B	280	B	293	B	323	AB	369	A	380	A
			b		b		b		b		b		b
Nodule dry weight (mg plant ⁻¹)	<i>Rhizobium</i> sp.	280	C	285	BC	288	BC	290	B	293	AB	300	A
			ab		b		b		b		b		b
	<i>G. deserticola</i> + <i>Rhizobium</i> sp.	325	C	943	B	965	B	1226	AB	1359	AB	1508	A
			a		a		a		a		a		a
Root colonisation (%)	Control	22.3	A	15.5	AB	13.3	BC	10.7	BC	8.5	C	8.2	C
			c		c		c		c		c		c
	<i>G. deserticola</i>	60.7	AB	62.3	A	58.4	AB	51.2	AB	48.9	B	32.1	C
			ab		ab		ab		ab		ab		ab
Nodule dry weight (mg plant ⁻¹)	<i>Rhizobium</i> sp.	31.5	AB	30.2	AB	33.5	A	28.4	AB	25.0	BC	20.6	C
			bc		bc		bc		bc		bc		bc
	<i>G. deserticola</i> + <i>Rhizobium</i> sp.	83.4	A	80.1	A	75.4	AB	68.4	BC	63.3	C	64.5	bc
			a		a		a		a		a		a
Nodule dry weight (mg plant ⁻¹)	Control	2.4	D	4.2	CD	5.4	ABC	4.5	BC	6.3	AB	7.0	A
			b		b		b		b		c		c
	<i>G. deserticola</i>	3.2	D	4.1	CD	6.8	BCD	8.2	ABC	10.0	AB	12.3	A
			b		b		b		b		bc		b
Nodule dry weight (mg plant ⁻¹)	<i>Rhizobium</i> sp.	5.6	C	8.2	BC	12.5	AB	12.8	AB	13.3	AB	14.0	A
			b		ab		a		a		ab		ab
	<i>G. deserticola</i> + <i>Rhizobium</i> sp.	8.8	C	10.6	C	12.3	BC	15.7	Ab	17.0	AB	18.0	A
			a		a		a		a		a		a

Values within rows (capital letters) and columns (small letters) followed by the same letters are not significantly different ($p < 0.05$: Duncan's multiple range test).

Table 2. Effects of superphosphate on the biomass, root colonisation and nodule dry weight of *Acacia mearnsii* inoculated with *Glomus deserticola* alone, *Rhizobium* sp. alone and *G. deserticola* + *Rhizobium* sp.

Parameter	Treatment	Ca ₃ (H ₂ PO ₄) ₂ (mg kg ⁻¹ soil)											
		0		50		100		150		200		250	
Root dry weight (mg plant ⁻¹)	Control	54	D	60	BC	63	BC	68	AB	69	AB	76	A
	<i>G. deserticola</i>	63	D	71	CD	76	BCD	86	BC	95	AB	112	A
	<i>Rhizobium</i> sp.	68	D	72	CD	76	BCD	91	BC	95	B	120	A
	<i>G. deserticola</i> + <i>Rhizobium</i> sp.	75	C	132	B	134	B	143	AB	150	AB	175	A
Shoot dry weight (mg plant ⁻¹)	Control	176	D	268	B	274	B	283	AB	307	AB	330	A
	<i>G. deserticola</i>	275	D	320	CD	345	BCD	360	BCD	432	AB	450	A
	<i>Rhizobium</i> sp.	280	D	342	CD	368	BCD	443	AB	460	AB	475	A
	<i>G. deserticola</i> + <i>Rhizobium</i> sp.	325	C	927	B	1047	B	1232	AB	1690	A	1725	A
Root colonisation (%)	Control	22.3	A	13.2	B	12.8	B	10.5	B	8.6	B	6.5	B
	<i>G. deserticola</i>	60.7	A	58.2	A	50.7	AB	43.2	ABC	36.5	B	28.5	C
	<i>Rhizobium</i> sp.	31.5	A	27.2	ABC	25.0	ABC	22.7	BCD	18.7	CD	15.3	D
	<i>G. deserticola</i> + <i>Rhizobium</i> sp.	88.4	A	75.4	AB	68.2	BC	65.3	BC	50.2	D	55.2	CD
Nodule dry weight (mg plant ⁻¹)	Control	2.4	C	6.2	BC	6.8	BC	8.0	AB	10.6	AB	12.6	A
	<i>G. deserticola</i>	3.2	C	6.2	C	8.3	C	10.0	BC	16.4	AB	18.3	A
	<i>Rhizobium</i> sp.	5.6	C	12.3	BC	18.6	AB	20.0	AB	22.5	A	24.8	A
	<i>G. deserticola</i> + <i>Rhizobium</i> sp.	8.8	C	16.4	C	18.3	BC	22.6	ABC	28.5	AB	30.6	A

Values within rows (capital letters) and columns (small letters) followed by the same letters are not significantly different ($p < 0.05$; Duncan's multiple range test).

Table 3. Effects of tricalcium phosphate on the tissue nutrients concentrations of *Acacia mearnsii* inoculated with *Glomus deserticola* alone, *Rhizobium* sp. alone and *G. deserticola* + *Rhizobium* sp.

Parameter	Treatment	Ca ₃ (PO ₄) ₂ (mg kg ⁻¹ soil)											
		0		50		100		150		200		250	
Nitrogen (%)	Control	1.2	C	2.05	BD	2.60	AB	2.68	AB	2.75	AB	3.26	A
	<i>G. deserticola</i>	2.63	C	2.70	C	2.78	BC	2.80	BC	2.95	AB	3.75	A
	<i>Rhizobium</i> sp.	2.83	D	2.90	CD	3.11	BCD	3.26	ABC	3.47	AB	2.96	A
	<i>G. deserticola</i> + <i>Rhizobium</i> sp.	3.26	C	3.31	C	3.42	C	3.57	BC	4.03	AB	4.21	A
Phosphorus (%)	Control	0.17	C	0.20	BC	0.22	BC	0.30	AB	0.36	A	0.40	A
	<i>G. deserticola</i>	0.30	D	0.45	CD	0.48	BC	0.54	ABC	0.64	AB	0.70	A
	<i>Rhizobium</i> sp.	0.23	D	0.36	C	0.40	BC	0.43	ABC	0.50	AB	0.52	A
	<i>G. deserticola</i> + <i>Rhizobium</i> sp.	0.50	D	0.63	C	0.75	BC	0.84	AB	0.93	AB	0.97	A
Potassium (%)	Control	0.98	D	1.15	D	1.20	CD	1.25	BC	1.43	B	1.68	A
	<i>G. deserticola</i>	1.23	C	1.28	C	1.34	C	1.46	BC	1.78	AB	1.97	A
	<i>Rhizobium</i> sp.	1.45	C	1.65	BC	1.73	BC	1.86	B	1.98	AB	2.08	A
	<i>G. deserticola</i> + <i>Rhizobium</i> sp.	1.78	C	1.82	C	1.96	C	2.15	B	2.32	AB	2.56	A

Values within rows (capital letters) and columns (small letters) followed by the same letters are not significantly different ($p < 0.05$; Duncan's multiple range test).

Table 4. Effects of superphosphate on the tissue nutrients concentrations of *Acacia mearnsii* inoculated with *Glomus deserticola* alone, *Rhizobium* sp. alone and *G. deserticola* + *Rhizobium* sp.

Parameter	Treatment	Ca ₃ (H ₂ PO ₄) ₂ (mg kg ⁻¹ soil)											
		0	50	100	150	200	250						
Nitrogen (%)	Control	1.20	C	2.15	BC	2.68	AB	2.70	AB	2.80	AB	3.23	A
			b		c		b		b		b		c
	<i>G. deserticola</i>	2.63	C	2.75	BC	2.88	BC	2.90	BC	2.98	B	3.53	A
			a		bc		b		b		b		bc
Phosphorus (%)	<i>Rhizobium</i> sp.	2.83	C	2.90	C	2.97	BC	3.21	BC	3.35	B	3.87	A
			a		ab		b		b		b		ab
	<i>G. deserticola</i> + <i>Rhizobium</i> sp.	3.26	C	3.45	BC	3.83	AB	3.88	A	3.90	A	3.95	A
			a		a		a		a		a		a
Potassium (%)	Control	0.17	C	0.23	BC	0.32	ABC	0.30	AB	0.42	A	0.46	A
			b		c		b		b		c		c
	<i>G. deserticola</i>	0.30	C	0.47	BC	0.52	BC	0.63	AB	0.72	AB	0.83	A
			b		b		b		b		ab		b
Potassium (%)	<i>Rhizobium</i> sp.	0.23	C	0.38	BC	0.43	B	0.48	B	0.63	AB	0.68	A
			b		bc		b		b		bc		bc
	<i>G. deserticola</i> + <i>Rhizobium</i> sp.	0.50	D	0.68	CD	0.83	BC	0.98	AB	0.99	AB	1.26	A
			a		a		a		a		a		a
Potassium (%)	Control	0.98	D	1.23	CD	1.36	CD	1.48	BC	1.69	AB	1.83	A
			c		c		c		c		b		b
	<i>G. deserticola</i>	1.23	D	1.38	CD	1.48	BCD	1.63	ABC	1.82	AB	1.97	A
			bc		bc		bc		bc		b		b
Potassium (%)	<i>Rhizobium</i> sp.	1.45	D	1.70	CD	1.87	BC	1.94	ABC	1.98	AB	2.27	A
			b		ab		ab		ab		b		b
	<i>G. deserticola</i> + <i>Rhizobium</i> sp.	1.78	B	1.93	B	1.97	B	2.03	B	2.63	A	2.95	A
			a		a		a		a		a		a

Values within rows (capital letters) and columns (small letters) followed by the same letters are not significantly different ($p < 0.05$: Duncan's multiple range test).

VAM root colonisation and rhizobial nodulation

VAM root colonisation generally decreased with increasing doses of P fertilisation. The wattle seedlings receiving comparable doses of P fertilisers exhibited higher VAM root colonisation when inoculated with either *G. deserticola* or *Rhizobium* sp., the former being more favourable (Tables 1 and 2). Nodular biomass increased with increasing P fertiliser doses. They were further increased by inoculations with endophytes, with a maximum in dual inoculated followed by rhizobial inoculated seedlings. A negative relationship was observed between P fertiliser doses and VAM colonisation (Table 5).

Tissue nutrient concentrations

The concentrations of plant tissue nutrients (N, P, K) increased with P fertiliser doses (Tables 3 and 4). Endophyte inoculations also promoted tissue nutrient concentrations, the dual inoculation giving the highest benefit. Phosphorus utilisation efficiency decreased with increasing P fertiliser doses and inoculations with *G. deserticola* and *Rhizobium* sp. The dual inoculation resulted in low phosphorus utilisation efficiency followed by *G. deserticola* inoculation (Figure 1).

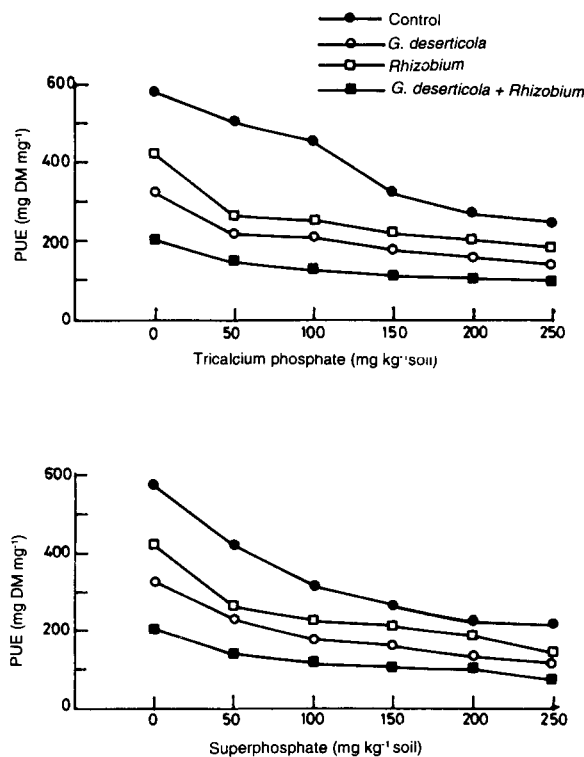


Figure 1. Effect of tricalcium phosphate and superphosphate on the phosphorus utilisation efficiency (PUE) of *Acacia meurnsii* inoculated with *Glomus deserticola* alone, *Rhizobium* sp. alone and *G. deserticola* + *Rhizobium* sp.

Table 5. Regression analysis between applied phosphate and root colonisation

Treatment	Tricalcium phosphate	Superphosphate
Control	$Y = 19.80 - 0.05 X$ ($r^2 = 0.35$)	$Y = 19.14 - 0.05 X$ ($r^2 = 0.32$)
<i>G. deserticola</i>	$Y = 33.57 - 0.04 X$ ($r^2 = 0.50$)	$Y = 61.98 - 0.13 X$ ($r^2 = 0.44$)
<i>Rhizobium</i> sp.	$Y = 65.86 - 0.10 X$ ($r^2 = 0.56$)	$Y = 31.17 - 0.06 X$ ($r^2 = 0.41$)
<i>G. deserticola</i> + <i>Rhizobium</i> sp.	$Y = 83.31 - 0.86 X$ ($r^2 = 0.58$)	$Y = 81.80 - 0.12 X$ ($r^2 = 0.52$)

Discussion

Tropical soils, in general, are characterised by low availability of phosphates and the predominance of Fe and Al oxides with high P sorption capacities (Fox 1978). Additionally, P applied to these soils as water-soluble fertilisers is quickly converted to forms not available to plants (Fox & Searle 1978). Under these conditions and where rock phosphate is readily available, it is better to utilise less soluble and less expensive P sources in association with microorganisms that can either solubilise P or enhance its absorption from slowly soluble forms, than expensive soluble P fertilisers (Manjunath *et al.* 1989).

In the present study, application of phosphorus fertilisers enhanced growth and biomass in wattle seedlings and growth exhibited a linear relationship with fertiliser dosage. P fertilisation is already known to enhance the growth and biomass of the *Acacia* spp. (Jasper *et al.* 1989). The soil used in the experiments was extremely poor in P content (0.5 mg kg⁻¹ soil) and any amount of added P will naturally benefit the P starved seedlings. Vesicular-arbuscular mycorrhizas are known to promote plant growth and phosphorus uptake (Mosse 1973, Gerdemann 1975). When inoculated individually, *G. deserticola* substituted up to 150 and 100 mg kg⁻¹ soil of tricalcium phosphate or superphosphate respectively and more than 250 mg kg⁻¹ soil of tricalcium phosphate or superphosphate when coinoculated with *Rhizobium* sp. Similar mycorrhizal effects have also been reported in sour orange and Troyer citrange (Menge *et al.* 1978) and *Acacia* spp. (Jasper *et al.* 1988, 1989).

Applications of phosphorus fertilisers enhance nodulation and nitrogen-fixing capabilities of legumes (Crush 1974, Mosse *et al.* 1976). In the present study the nodular biomass increased with fertiliser doses, which were further increased by endophyte inoculations. The response of seedlings to mycorrhizal and rhizobial inoculation was greater when supplemented with high doses of P suggesting that an adequate supply of P is essential for biological nitrogen fixation (Gardner *et al.* 1984). It has been well established that bacteria improve plant growth by producing plant hormones (Brown 1974) which also play a key role in the infection mechanism of *Rhizobium* (Nutman 1965). Plant growth regulators may indirectly influence VAM fungi by affecting root growth since VAM fungal colonisation has been reported to be dependent on root growth rate (Harley 1969). Thus, it can be concluded that plant hormones may be indirectly involved in mycorrhizal formation. Whatever the mechanism, the present study emphasises

that dual inoculation with mycorrhizal fungus and rhizobia aids the growth of wattle seedlings despite the presence of native symbionts.

Levels of P that result in similar growth of uninoculated wattle seedlings to that of seedlings inoculated with *G. deserticola* appear to inhibit root colonisation by *G. deserticola*. The decreasing root colonisation was also related to the proportionately increasing concentrations of tissue phosphorus. Sanders (1975) and Menge *et al.* (1978) opined that phosphorus in plant tissues suppresses mycorrhizal development while P levels in soil do not affect. The addition of P to soil in all treatments increased the nutrient content of wattle seedlings. Similar effects have been reported in faba beans by Ishac *et al.* (1994). Coinoculation of *G. deserticola* and *Rhizobium* sp. resulted in higher nutrient accumulation compared to individual inoculations. Manjunath *et al.* (1984) reported that the colonisation with efficient mycorrhizal fungi significantly improved phosphorus nutrition and consequently nodulation and nitrogen fixation by rhizobia.

Mycorrhizal inoculation influences phosphorus utilisation efficiency. Stribley *et al.* (1980) have shown that for a given dry weight, mycorrhizal plants contain more phosphorus than non-mycorrhizal plants, which suggests that mycorrhizal plants are less efficient in the utilisation of phosphorus for the production of dry matter. In the present study increasing P fertilisers and *G. deserticola* and rhizobial inoculations decreased P utilisation efficiency. This suggests a temporal displacement of P acquisition from its utilisation in growth. The results of the present study suggest that the microbial inoculation along with the sparingly soluble phosphorus source is suitable for maximum growth and improvement of wattle seedling quality.

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References

- BROWN, M.E. 1974. Seed and root bacterization. *Annual Review of Phytopathology* 12: 181-198.
- CRUSH, J.R. 1974. Plant growth responses to vesicular-arbuscular mycorrhiza. VII. Growth and nodulation of some herbage legumes. *New Phytologist* 73 : 743 - 752.
- FOX, R.L. 1978. Studies on phosphate nutrition in the tropics. Pp. 169-187 in Andrew C.S. & Kamprath E.R. (Eds.) *Mineral Nutrition of Legumes in Tropical and Subtropical Soils*. CSIRO, Canberra, Australia.
- FOX, R.L. & SEARLE, P.G.E. 1978. Phosphate adsorption by the soils of the tropics. Pp. 97-119 in Drosdrof, M. (Ed.) *Diversity of Soils of the Tropics*. American Society of Agronomy, Madison, Wisconsin, United States of America.
- GARDNER, I.C., CLELLAND, D.M. & SCOTTS, A. 1984. Mycorrhizal improvement in non-leguminous nitrogen fixing associations with particular reference to *Hippophae rhamnoides* L. *Plant and Soil* 8 : 189 - 200.
- GERDEMANN, J.W. 1975. VA mycorrhiza. Pp. 575-591 in Torrey, J.G & Clarkson, D.T (Eds.) *Development and Function of Roots*. Academic Press, London, United Kingdom.

- HARLEY, L.L. 1969. *The Biology of Mycorrhiza*. Leonard Hill, London, United Kingdom. 334 pp.
- HAYMAN, D.S. 1986. Mycorrhizae of nitrogen-fixing legumes. *MERCEN Journal of Applied Microbiology and Biotechnology* 2: 121-145.
- ISHAC, Y.Z., ANGLE, J.S., EL-BOROLLOS, M.E., EL-DEMERDASH, M.E., MOSTAFA, M.L. & FARES, C.N. 1994. Growth of *Vicia faba* as affected by inoculation with vesicular-arbuscular mycorrhizae and *Rhizobium leguminosarum* bv. *viciae* in two soils. *Biology and Fertility of Soils* 17: 27 - 31.
- JACKSON, M.L. 1973. *Soil Chemical Analysis*. Prentice Hall (India), New Delhi, India. 498 pp.
- JASPER, D.A., ROBSON, A.D. & ABBOTT, L.K. 1988. Revegetation in an iron-ore mine – nutrient requirements for plant growth and the potential role of vesicular-arbuscular (VA) mycorrhizal fungi. *Australian Journal of Soil Research* 26 : 497 - 507.
- JASPER, D.A., ABBOTT, L.K. & ROBSON, A.D. 1989. Acacias respond to addition of phosphorus and inoculations with VA mycorrhizal fungi in soils stockpiled during mineral sand mining. *Plant and Soil* 115 : 99 - 108.
- MANJUNATH, A., BAGYARAJ, D.J. & GOPALA GOUDA, H.S. 1984. Dual inoculation with VA mycorrhiza and *Rhizobium* is beneficial to *Leucaena*. *Plant and Soil* 78 : 445 - 448.
- MANJUNATH, A., HUE, N.V. & HABTE, M. 1989. Response of *Leucaena leucocephala* to vesicular-arbuscular mycorrhizal colonization and rock phosphate fertilization in an oxisol. *Plant and Soil* 114 : 127 - 133.
- MARSCHNER, H. 1986. *Mineral Nutrition of Higher Plants*. Academic Press, London, United Kingdom. 676 pp.
- MENGE, J.A., LABANAUSKAS, C.K., JOHNSON, E.V.L. & PLATT, R.G. 1978. Partial substitution of mycorrhizal fungi for phosphorus fertilization in the greenhouse culture of citrus. *Soil Science Society of America Journal* 42 : 926 - 930.
- MOSSE, B. 1973. Advances in the study of vesicular-arbuscular mycorrhizae. *Annual Review of Phytopathology* 11 : 171 - 176.
- MOSSE, B., HAYMAN, D.S. & ARNOLD, D.J. 1973. Plant growth responses to vesicular-arbuscular mycorrhiza. 5. Phosphate uptake by three plant species from P-deficient soils labelled with ³²P. *New Phytologist* 72 : 808 - 815.
- MOSSE, B., POWELL, C.L. & HAYMAN, D.S. 1976. Plant growth responses to vesicular-arbuscular mycorrhiza. 9. Interactions between VA mycorrhiza, rock phosphate and symbiotic nitrogen fixation. *New Phytologist* 76 : 331 - 342.
- NUTMAN, P.S. 1965. The relation between nodule bacteria and the legume host in the rhizosphere and in the infection process. Pp. 231 - 247 in Baker, K.F. & Synder, W.C (Eds.) *Ecology of Soil Borne Plant Pathogens*. University of California, Berkeley, United States of America.
- PHILLIPS, J.M. & HAYMAN, D.S. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society* 55 : 158 - 161.
- PORTER, W.M. 1979. The most probable number method for enumerating infective propagules of vesicular-arbuscular mycorrhizal fungi in soil. *Australian Journal of Soil Research* 17 : 515 - 519.
- READ, P.J., KOUCHKI, H.K. & HODGRON, J. 1976. Vesicular-arbuscular mycorrhizae in natural vegetation system. The occurrence of the infection. *New Phytologist* 77 : 641 - 653.
- REENA, J. & BAGYARAJ, D.J. 1990. Growth stimulation of *Tamarindus indica* to selected VA mycorrhizal fungi. *World Journal of Microbiology and Biotechnology* 6: 59 - 63.
- SAFIR, G.R., BOYER, J.S. & GERDEMANN, J.W. 1972. Nutrient status and mycorrhizal enhancement of water transport in soybean. *Plant Physiology* 49 : 700 - 703.
- SANDERS, F.E. 1975. The effect of foliar-applied phosphate on the mycorrhizal infections of onion roots. Pp. 261-276 in *Endomycorrhizas*. Academic Press, London, United Kingdom.
- SANDERS, F.E. & TINKER, P.B. 1973. Phosphate flow into mycorrhizal roots. *Pesticide Science* 4 : 385 - 394.
- SCHUBERT, A. & HAYMAN, D.S. 1986. Plant growth responses to vesicular-arbuscular mycorrhiza. XVI. Effectiveness of different endophytes at different levels of soil phosphate. *New Phytologist* 103 : 79 - 90.
- SIDDIQUI, M.Y. & GLASS, A.D.M. 1981. Utilization index: a modified approach to the estimation and comparison of nutrient utilization efficiency in plants. *Journal of Plant Nutrition* 4 : 289 - 302.

- SIQUEIRA, J.O., HUBBELL, D.H. & VALLE, R.R. 1984. Effects of phosphorus on formation of the vesicular-arbuscular mycorrhizal symbiosis. *Pesquisa Agropecuaria Brasilia* 19 : 1465 - 1474.
- STRIBLEY, D.P., TINKER, P.B. & SNELGROVE, R.C. 1980. Effect of vesicular-arbuscular mycorrhizal fungi on the relations of plant growth, internal phosphorus concentration and soil phosphate analyses. *Journal of Soil Science* 31: 655 - 672.
- THOMSON, B.D., ROBSON, A.D. & ABBOTT, L.K. 1986. Effects of phosphorus on the formation of mycorrhizas by *Gigaspora calospora* and *Glomus fasciculatum* in relation to root carbohydrates. *New Phytologist* 103 : 751 - 765.