

**EFFECT OF VA MYCORRHIZAE (*GIGASPORA MARGARITA*) ON
WHITE ROOT DISEASE RESISTANCE IN
PUERARIA PHASEOLOIDES AND
HEVEA BRASILIENSIS SEEDLINGS**

by

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ABSTRACT

White root disease caused by the fungus Rigidoporus lignosus Muell Arg. is a serious problem in rubber plantations of Sri Lanka. It is now clear that certain root disease severity can be influenced by VA mycorrhizae. Effect of VA mycorrhizae (Gigaspora margarita), on the development of white root disease on Hevea seedling were tested.

Inoculations with mycorrhizal inoculum and Rigidoporus inoculum were done at three different times.

Gigaspora margarita inhibited the infection of roots of Hevea seedlings by R. lignosus up to 240 days. Mycorrhizal P. phaseoloides roots also showed a marked reduction in Rigidoporus infection upto about 60 days, thereafter the inhibitory effect ceased.

INTRODUCTION

Vesicular arbuscular (VA) mycorrhizae is of particular interest because of its universal existence throughout the Plant Kingdom. Enhancement of plant growth by VA mycorrhizal fungi results mainly from an improvement in the ability of the mycorrhizal root system to take up phosphorus from the soil, although other less important factors may also be involved. It has also been found that root disease severity can be influenced by VA mycorrhizae. It has been reported that disease severity can be increased, decreased or unaffected by the presence of VA mycorrhizal fungi (Ross, 1972; Baltruschat *et al.* 1972; Daft *et al.* 1972; Kellam *et al.* 1977; Rancadori *et al.* 1977; Schenck *et al.* 1978; Zak, 1964).

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White root disease caused by the Basidiomycete fungus *Rigidoporus lignosus* is the most serious disease affecting rubber in Sri Lanka. It causes damage both to immature and mature rubber. Usually in replanted areas the incidence of the disease reaches a peak in about 2-5 years and thereafter it declines, it becomes a problem in mature rubber, only if the disease is neglected during the early stages of the growth of rubber. Presently about 4-8% of the total hectareage planted with is affected with the disease.

Treatment of white root disease has to be done in three stages viz. preplantings planting and postplanting. Therefore, it is a very laborious and expensive process. If certain species of VA mycorrhizae can inhibit the development of *Rigidoporus* infection at early stages of growth of rubber plants, it is possible to control it easily by introducing the effective fungal species probably into seedling nurseries or to polybag plants.

The purpose of this study was to investigate the effect of *Gigaspora margarita* an exotic species to our soils, on the development of white root infection in rubber roots and in *P. phaseoloides* which is grown as a ground cover to improve the soil conditions.

MATERIALS AND METHODS

Lateritic clay soil (pH 6.2, 1 : 5 distilled water), deficient in available phosphorus (3.33^ug/g, NH₄F/HCl extractable P) was used for both experiments. The soils were sieved through 1 cm. wiremesh in order to remove stones and gravel. The soil was mixed with sand in the ratio 1:1 before autoclaving for 5 h. Small plastic pots of 15 cm. diameter were used for *Pueraria* plants, and medium size cement pots 25 cm diameter were used for *Hevea* plants.

Sand containing roughly equal amounts of spores and root pieces obtained from the stock cultures maintained in the green-house were used as the mycorrhizal inoculum. Non-mycorrhizal pots received twice filtered (Whatman No. 6) washings from stock culture sand to equalize the microbial populations in both treatments. *Rigidoporus lignosus* inoculum was prepared by growing the fungus on wood dowels (15 X 15 X 15 mm) under asptic conditions. Eight infected wood dowels per pot were used as the inoculum. Control pots were added with non infected dried wood dowels.

P. phaseoloides plants were established in pots by sowing acid treated seeds inoculated with *Rhizobia* (CB 756). *Hevea* seedlings were also established by germinating the seeds of clone (PB 86) in the pots. When the plants were well established they were thinned down to 12 seedlings for *P. phaseoloides* and 4 seedlings of *Hevea* per pot.

Inoculation with mycorrhizae was done by adding the inoculum in a thin layer placed 3–4 cm. below the surface of the soil. Inoculation with *R. lignosus* was done by burying eight pieces of wood dowels infected with the fungus 3–4 cm below the surface of the soil. Inoculation with either mycorrhizae or *Rigidoporus* was done at three different times.

- (A) both inocula together
- (B) Mycorrhizal inoculation, after 50 days of plant growth.
- (C) *Rigidoporus* inoculation, after 50 days of plant growth.

All the pots were arranged inside the green-house according to a randomized block design. Each treatment was replicated four times. Plants were supplied with Jensen's nutrient medium without phosphorus (Vincent, 1970) once a month.

In *Pueraria* plants the development of infection in roots due to both inocula were assessed after 25, 30, 35, 40, 45, 60 and 150 days. In rubber plants the percentage infection due to both inocula was assessed after 240 days. Percentage mycorrhizal infection was determined after staining the roots with trypan blue (Phillips and Hayman, 1970) using the grid line intersect method (Giovannetti *et al* 1980). *Rigidoporus* infection percentages were obtained visually for *P. phaseoloides* plants and by measuring the infected root length in rubber plants, where white rhizomorphs can be seen clearly on infected roots.

RESULTS

In *P. phaseoloides* plants the development of VA mycorrhizal infection was much faster than the development of *Rigidoporus* infection (Tables 1 and 2).

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Table 1a. Mean mycorrhizal percentage root length infected in Pueraria plants.

Treatment	25 Days	30 Days	35 Days	40 Days	45 Days	60 Days	150 Days
MR ₀ P ₁	33	40	49	67	62	64	70
MR ₀ P ₂	25	38	47	62	57	59	61
M ^a R ^b P ₁	36	46	60	62	63	62	64
M ^a R ^b P ₂	32	55	39	52	45	59	61
M ^b R ^a P ₁	—	—	—	—	—	34	42
M ^b R ^a P ₂	—	—	—	—	—	22	40
MRP ₁	27	30	37	50	56	58	65
MRP ₂	31	38	37	45	45	51	59

M — Mycorrhizal inoculum

R — *Rigidoporus* inoculum

O — No inocula

a — Introduced first

b — Introduced after 50 days of plant growth.

P₁ — Without added phosphorus

P₂ — With added phosphorus at the rate of 600/ug/g soil as super phosphate.

Table 1 (b). *Mycorrhizae (%) — Angular transformed data*

MR Treatment	Days after treatment																	
	25			30			35			40			45			150		
	P ₁	P ₂	Mean	P ₁	P ₂	Mean	P ₁	P ₂	Mean	P ₁	P ₂	Mean	P ₁	P ₂	Mean	P ₁	P ₂	Mean
	(LSD = 4.62) (5%)			LSD = *NS (LSD = 20.5)			LSD = *NS (LSD = 6.54)			LSD = *NS (LSD = 15.9)			LSD = *NS (LSD = 10.8)			LSD = *NS (LSD = 5.98)		
MR ₀	35.1	30.0	32.5	38.7	38.2	38.5	44.4	43.8	44.1	55.0	52.0	53.5	51.5	49.5	50.5	57.4	51.9	54.7
M ^a R ^b	37.3	34.6	35.9	42.8	47.9	45.4	51.1	38.9	45.0	52.0	46.5	49.2	52.7	43.4	48.1	53.4	51.7	52.5
M ^b R ^a	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	40.2	39.1	39.6
MR	30.9	34.0	32.5	28.0	38.3	33.2	37.4	37.4	37.4	45.4	54.4	49.9	48.5	42.1	45.3	54.1	50.4	52.2
Mean	34.4	32.9		36.5	41.5		44.3	40.0		50.8	51.0		50.9	45.0		51.3	48.3	

. NS — Not Significant at (5%) Level.

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Table 2a. Mean percentage *Rigidoporus* infected root length in *Pueraria* plants

Treatment	25 Days	30 Days	35 Days	40 Days	45 Days	60 Days	150 Days
M ₀ RP ₁	6	16	22	16	37	37	30
M ₀ RP ₂	9	15	20	30	22	43	31
M ^a R ^b P ₁	—	—	—	—	—	15	26
M ^a R ^b P ₂	—	—	—	—	—	9	39
M ^b R ^a P ₁	19	17	14	25	29	26	29
M ^b R ^a P ₂	14	14	21	29	10	5	29
MRP ₁	6	4	8	9	12	8	31
MRP ₂	Nil	2	5	4	5	9	41

In *P. phaseoloides* plants, presence of mycorrhizal fungi has an inhibiting effect on *Rigidoporus* infection development upto about 60 days of growth. After 150 days of growth this effect was not seen. Both infections occur simultaneously without having much effect on the other. No infections were observed on control plants having M₀ and R₀ treatments.

In rubber plants the *Rigidoporus* infection in tap root is significantly greater when compared with that of feeder roots. However, presence of VA mycorrhizae in feeder roots, has completely inhibit the development of *Rigidoporus* infection in both tap root as well as on feeder roots even after 240 days (M^aR^b treatmnt).

At high level of phosphorus (P₂) mycorrhizal plants have a significantly higher *Rigidoporus* infection (Tables 3 & 4).

Table 2 (b). Rigidoporus (%) — Angular transformed data

MR Treatment	Days after treatment																																			
	25			30			35			40			45			60																				
	P ₁	P ₂	Mean	P ₁	P ₂	Mean	P ₁	P ₂	Mean	P ₁	P ₂	Mean	P ₁	P ₂	Mean	P ₁	P ₂	Mean																		
	(LSD = 20.4) (5%)			LSD = *NS			(LSD = 26.9)			LSD = *NS			(LSD = 21.7)			LSD = *NS			(LSD = *NS)			LSD = 10.39			(LSD = *NS)			(LSD = 7.8)			(LSD = *NS)			LSD = 12.2		
MoR	7.32	10.3	8.8	17.3	18.1	17.7	24.5	23.2	23.9	18.3	34.9	26.6	37.7	24.9	31.3	31.2	34.0	32.6																		
M ^a R ^b	—	—	—	—	—	—	—	—	—	0.0	0.0	0.0	0.0	0.0	0.0	19.7	17.0	18.4																		
M ^b R ^a	24.9	16.0	20.4	20.9	15.9	18.4	21.7	23.6	22.7	28.6	19.4	24.0	16.7	16.7	16.7	29.2	11.9	20.6																		
MR	7.5	0.0	3.8	9.7	6.9	8.3	11.3	9.4	10.3	14.9	9.4	12.2	15.5	11.4	13.4	12.3	17.4	14.8																		
Mean	13.2	8.8		16.0	13.6		19.2	18.7		15.5	15.9		17.5	13.2		23.1	20.1																			

* NS — Not Significant at (5%) Level

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Table 3. *Mean Rigidoporus infection in the tap root and feeder roots of rubber after 240 days.*

Treatment	Mean infection in tap roots (%)	Mean percentage infection in feeder roots
M ₀ RP ₁	71	7
M ₀ RP ₂	54	13
M ^a R ^b P ₁	Nil	Nil
M ^a R ^b P ₂	Nil	Nil
M ^b R ^a P ₁	51	14
M ^b R ^a P ₂	68	16
MRP ₁	50	16
MRP ₂	69	11
S.E. ±	3.50	1.98
LSD (5%)	10.48	5.92

Table 4. *Mean mycorrhizal infection in feeder roots of rubber after 240 days.*

Treatment	Mean (%)
MR ₀ P ₁	74
MR ₀ P ₂	47
M ^a R ^b P ₁	46
M ^a R ^b P ₂	20
M ^b R ^a P ₁	45
M ^b R ^a P ₂	14
MRP ₁	45
MRP ₂	30
S.E. ±	5.02
LSD (5%)	10.66

At higher level of phosphorus the mycorrhizal percentage infection was significantly less.

Mycorrhizal plants without *Rigidoporus* infection showed a higher dry weight when compared with the *Rigidoporus* inoculated plants (Table 5).

Table 5. Mean shoot dry weight of plants (g/pot)

Treatment	Mean dry weight (g)
M ₀ R ₀	7.56
M ₀ R	6.84
M ^a R ^b	7.74
M ^b R ^a	7.09
MR	6.02
S.E ±	0.41
L.S.D. (1%)	1.24

DISCUSSION

Control of white root disease is a very expensive and laborious process. Biological control methods are the most suitable to combat this disease. Several studies indicate that root infection by vesicular-arbuscular mycorrhizae influence diseases caused by soilborne fungi (Ross, 1972, Schenck, *et al.*, 1977).

Results from both experiments indicate that *G. margarita* has some inhibitory effect on the development of white root disease. In *P. phaseolides* plants this inhibitory effect does not seem to exist after about 150 days, but in rubber plants this effect is there even after 240 days of growth. Therefore, it is worthwhile finding out how long this effect can be on rubber roots. The possibility of exploring VA mycorrhizal types as a method of biological control against white root disease depends on the period of inhibition. Tolerance to white root disease caused by *G. margarita*, appears to be due to the ability of mycorrhizal roots to absorb more phosphorus and possibly other minerals. Also, the possibility that *G. margarita* produces some antibiotics against *Rigidoporus* or induce phytoalexin production in plant roots cannot be excluded. In *Pueraria* plants, the mechanism that caused to overcome

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the inhibitory effect after a certain period is not very well understood. However, if this effect is entirely due to nutritional superiority, associated with infection by the mycorrhizal fungus, it will not be possible to overcome the inhibitory effect after a certain period. Therefore, it is very likely that some antibiotics or production of phytoalexins are involved. However, a series of experiments are needed to check this hypothesis.

Therefore, if mycorrhizal rubber roots (*G. margarita*) can maintain this inhibitory effect against white root disease for a long period (1 - 2 years) it will be possible to control white root disease biologically at a very much lower cost. These mycorrhizal fungi can be introduced into seedling nurseries probably after sterilization of soil (Fumigation with CH_3Br) to reduce the competition with naturally occurring population of VA mycorrhizae.

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