

## THE USE OF SULPHUR FOR THE CONTROL OF WHITE ROOT DISEASE CAUSED BY *RIGIDOPORUS LIGNOSUS*

By

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### ABSTRACT

The effect of sulphur as a soil amendment for the control of white root disease caused by *Rigidoporus lignosus* in immature rubber plantations was studied. The change in the pH and the population of fungi in soil after amendment with sulphur was recorded. *Trichoderma* spp., was the dominant fungus in the surface layers of soil after the addition of sulphur ; but *Penicillium* spp., were more common at 15 and 30 cm depth. Some of the *Trichoderma* spp., and *Penicillium* spp., caused lysis of *Rigidoporus* hyphae when grown together with the latter.

### INTRODUCTION

The Rubber Research Institute of Sri Lanka recommends the use of sulphur as a soil amendment for the control of white root disease (WRD) caused by *Rigidoporus lignosus* (Peries, Munasinghe and Samaraweera, 1964 ; Peries and Samaraweera, 1965). The role played by sulphur in the control of WRD was not understood although Peries *et al* (1964) observed that the pH of the soil was reduced on the addition of sulphur with an accompanying increase in the population of *Trichoderma* spp., in the soil. It was important to understand the basis of the action of sulphur in WRD control. Therefore, the soil reaction and the variation in the fungal population of the soil, on the addition of sulphur was studied. The changes in pH and the fungi that occur in the soil after amendment with sulphur are discussed.

### MATERIALS AND METHODS

In a field experiment laid down in 1977, sulphur at the rate of 114 g per square meter was added to a 5 m x 5 m patch of vacant land which previously had a cover of *Pueraria phaseoloides*. Sulphur was sprinkled on the patch and gently forked in, on the undisturbed soil. Soil samples were collected at depths 0 - 3, 15 - 18 and 30 - 33 cm before the addition of sulphur and periodically after the application of sulphur and the change in pH was noted. The fungal population before and 6 weeks after the addition of sulphur was determined by the soil dilution plate method.

The optimum pH required for the growth of two isolates of *R. lignosus* from *Hevea* and isolates of *Rigidoporus* obtained from Cacao, Cinnamon, Tamarind, Manihot, Albizzia and Monstera was tested. Inoculum discs, 7 mm diameter, taken from the periphery of a 7-day-old culture of *R. lignosus* from various hosts, grown in 9 cm petri dishes, each containing 10 ml of 2% malt agar (MA), were used in the experiment. The discs were placed in the centre of the petri dishes, containing MA adjusted to pH values ranging from 3 to 11. The medium was brought to the required pH, by adding 0.1N HCl or 1N NaOH, after autoclaving.

In 1978, an extensive field experiment was laid down in seven estates in various districts, using a higher amount of sulphur (228g) per planting hole to determine the change in the pH and the period over which the activity of sulphur would persist. A control

using the standard dosage (114g) was also used. In this experiment the planting holes were cut to the standard size and the holes filled after about a month to simulate field conditions. These were then left for a month and sulphur sprinkled on the surface. The pH was recorded at two depths (0 - 10 cm) and (30 - 40 cm), every month.

#### RESULTS AND DISCUSSION

The experiment conducted in 1977, showed that after amendment of soil with sulphur, the pH decreased sharply at all three depths. However, the pH at a depth of 0 - 3 cm remained low for at least 4 weeks longer than for the deeper layers. This may have been because the sulphur did not get in to the deeper layers rapidly, as the soil was not disturbed. At all three depths the lowest pH was recorded about 6 weeks after addition of sulphur. At 15 and 30 cm depths sharp increases in the pH were noted after 8 weeks, thereafter up to 40 weeks the pH remained within the range 4.0 to 4.2. In the surface layer and at a depth of 15 cm, the pH reached almost the original level after 44 weeks, while at 30 cm the pH remained lower than the original level even at 44 weeks. The drop in pH, in the field, after addition of sulphur, under field conditions, is much less than that recorded in the pot experiments. This has to be expected as the conditions in the field are different. However, in the field the pH remains at a lower level than in the control, for nearly 1 year (Fig. 1).

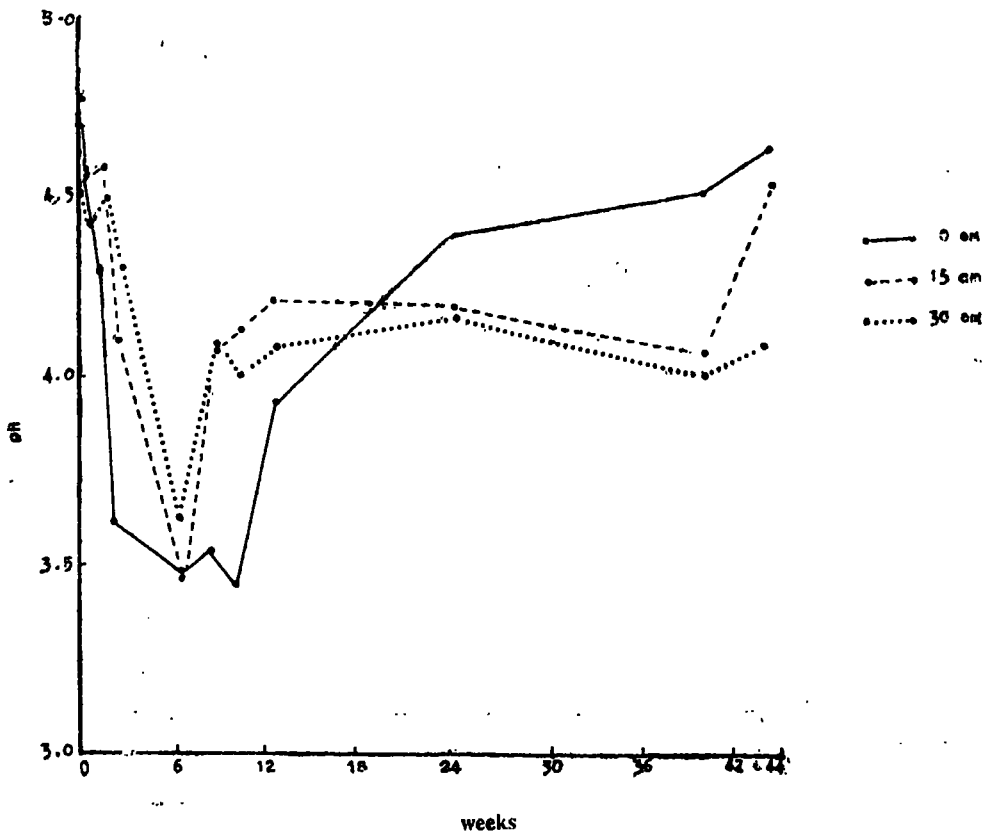


Fig 1. The progressive change in pH after addition of sulphur to soil

The fungal population was highest in the surface layers and there was a gradual reduction in the number of fungi with increase in depth. *Trichoderma* sp., was the predominant fungus in the surface layers after amendment with sulphur, their number being almost double that in untreated soil. However, the number of *Penicillium*, *Aspergillus* and unidentified species of fungi declined. At both 15 and 30 cm there was a sharp increase in the number of colonies of *Penicillium* spp., with the number of colonies of *Trichoderma* spp., remaining almost unchanged (Fig. 2).

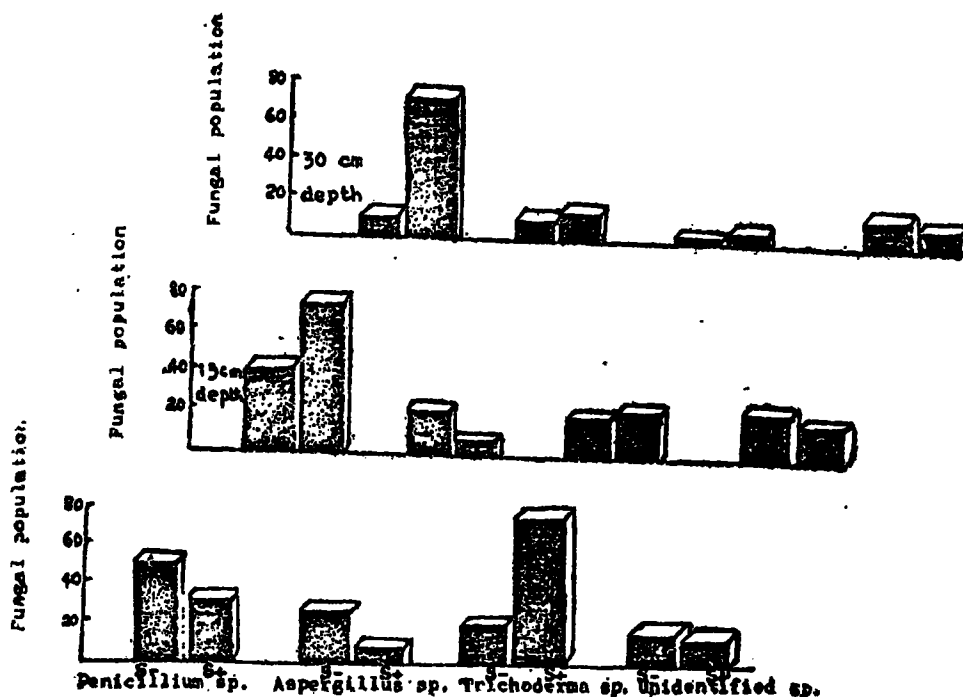


Fig. 2. Fungal population in soil before and 3 weeks after addition of sulphur

Selected isolates of *Penicillium* and *Trichoderma* were tested for antagonism to *R. lignosus* by pairing them with the latter on MA, on slide cultures. It was observed that some of them caused lysis of *Rigidoporus* hyphae under laboratory conditions (Fig. 3 and 4).

The optimum pH for isolates of *R. lignosus* was found to be around 8. At pH 3 the growth was significantly reduced. The isolates of *Rigidoporus* obtained from other hosts too behaved in this manner. At pH 4 the colony growth was less than half that at the optimum pH of 8 (Table 1). Therefore, if the soil pH could be maintained at a level below pH 4, the growth of the fungus in soil could be significantly reduced.



**Fig 3.** *Trichoderma* sp. grown in paired culture with *R. lignosus* on an agar coated slide. Note the darkly stained hyphae of *Trichoderma* sp. and the disintegrating hyphae of *R. lignosus*.



**Fig 4.** *Penicillium* sp. grown in paired culture with *R. lignosus* on an agar coated slide. Note the darkly stained hyphae of *Penicillium* sp. and lysis of *R. lignosus* hyphae (arrowed)

Table 1. Mean colony diameter (mm) of *Rigidoporus* isolates from different hosts, on the 5th day after inoculation on MA at different pH levels

pH Isolates	3	4	5	6	7	8	10	11
<i>Hevea</i> (B-avirulent)	11.0	24.5	41.7	53.3	59.2	62.7	44.5	44.9
<i>Hevea</i> (H-virulent)	36.7	53.2	70.0	69.7	71.0	76.0	55.5	43.5
Cinnamon	9.8	19.3	39.0	46.3	49.5	49.5	39.3	40.0
Cacao	12.0	26.2	41.3	45.8	49.5	52.2	41.2	42.8
Albizzia	0	18.0	39.0	41.3	37.3	40.2	40.8	42.2
Manihot	0	13.3	22.5	32.2	30.7	39.5	26.3	27.2
Tamarind	0	26.2	16.2	37.7	42.8	42.7	48.3	36.2
Monstera	0	20.2	39.3	49.3	46.5	55.2	40.5	44.0

L S D (P = 0.01) = 3.7

In the field experiments conducted in 1978 where a large number of soil samples were used from several experimental sites, a sharp drop in the pH was recorded in the first month itself and this reduction was maintained for nearly 18 months in all estates, except in one, where the pH had reverted almost to the original level, by this time. It was also noted that, generally the drop in pH was highest in the surface layers of the soil. The change in pH after the addition of 114 and 228g followed a similar pattern, but the difference in pH recorded for the single and the double dose was not marked although the latter gave a slightly lower pH (Table 2). The data recorded after 24 months indicated that the soil had reverted to the original pH in most estates. This indicates that an increase in quantity of sulphur is unwarranted.

Table 2. The effect of sulphur on the pH of soil at various locations

Estate & District	Soil type	pH before application of sulphur	pH after 12 months				pH after 18 months			
			228 g sulphur		114 g sulphur		228 g sulphur		114 g sulphur	
			U	L	U	L	U	L	U	L
Dartonfield (Kalutara)	Agalawatta	4.2	3.3	3.6	3.4	3.7	4.2	4.2	4.2	4.2
Doloswela (Ratnapura)	Ratnapura	4.9	3.3	3.4	3.4	3.7	3.4	3.8	3.8	3.9
Golinda (Kegalle)	Boralu	4.7	3.2	3.4	3.4	3.6	3.8	3.8	3.8	3.9
Moralioya (Kelani valley)	Parambe	4.4	3.3	3.5	3.3	3.7	3.6	3.6	3.9	3.8
Muwankanda (Kurunegala)	Agalawatta	4.4	3.6	3.9	3.9	3.9	3.7	3.8	4.0	4.0
Padukka (Colombo)	Parambe	4.4	3.3	3.6	3.6	3.7	3.7	3.7	3.7	3.7
Peenkande (Ratnapura)	Agalawatta	4.5	3.4	3.5	3.5	3.6	3.8	3.8	3.8	3.9
Stokesland (Galle)	Boralu	4.5	3.4	3.5	3.6	3.7	3.8	3.8	3.9	3.9
	Agalawatta	4.7	3.4	3.7	3.5	3.7	3.9	3.9	4.0	4.0
	Agalawatta	4.3	3.5	3.8	3.7	3.8	3.8	3.9	4.0	4.0

The pH of untreated soil (control) was recorded in each estate, on every sampling date, and was more or less constant.

U = upper layer (0 — 10 cm)  
L = lower layer (30 — 40 cm)

These results prove that the effective action of sulphur could remain for approximately 1 year. Therefore, it is justifiable to apply a second dose of sulphur at the end of the first year.

Experiments on the decay of inoculum in the field, which have been conducted subsequently, indicate that only 35% of the stumps and large laterals decay fully when left in the field for 1½ years while 45% and 20%, respectively, were partially and slightly decayed at this time ; with the fungus retaining its viability in this material. Assessments taken after 2½ years have shown that most of the inoculum had completely decayed at this stage while only 25% of the stumps and 6% of the large laterals were partially decayed and retained their viability. The fact that a majority of the inoculum is non-viable after 2½ years, suggests that the incidence of the disease could be reduced considerably by protecting the new plantation with sulphur in its first 2 years.

#### REFERENCES

- PERIES, O. S., MUNASINGHE, H. L. AND SAMARAWEEERA, S. K. (1964). Review of the Plant Pathology Department. *Ann. Rev. Rubber Res. Inst. Ceylon* 1963, 60 - 62.
- PERIES, O. S. AND SAMARAWEEERA, S. K. (1966). Review of the Plant Pathology Department. *Ann. Rev. Rubber Res. Inst. Ceylon* 1965, 44 - 58.