Journal of the Rubber Research Institute of Sri Lanka, (1997) **80**, 20-29

ECONOMICAL AND LESS HAZARDOUS FUNGICIDE FOR THE MANAGEMENT OF WHITE ROOT DISEASE

R Jayaratne, C K Jayasinghe and P C Wettasinghe

(Accepted 16 January 1997)

SUMMARY

Rigidoporus lignosus, the causal agent of white root disease is the most destructive root pathogen of *Hevea brasiliensis* in Sri Lanka. Presently 5-8% of the cultivated lands are affected with this deadly disease and are under bare patches.

The recommended fungicides in Sri Lanka, Pentachloro nitrobenzene (PCNB) and Pentachloro phenol (PCP), were prohibited due to high mammalian toxicity and the fungicides of the triazole family which are being recommended in other countries such as Malaysia and Ivory Coast are not acceptable to Sri Lankan rubber growers due to the high costs of these chemicals.

The observations of experiments carried out with less toxic chemicals revealed that 8-10% phenol in bituminous base could be used as an effective fungicidal "Collar protectant" in the management of white root disease in Sri Lanka. In this paper the economics and the toxicological impact on the environment are also discussed.

Key words: "collar protectant", fungal colony, phenol, *Rigidoporus lignosus*

INTRODUCTION

The introduction of the *Hevea brasiliensis* to the East from the South American region well over 100 years ago probably had a greater long term impact on the overall economic development of the region than any other single factor. Since then the number of recognised pests and diseases also increased rapidly and in turn, had their impact on the economics of the industry. Out of the diseases the root diseases have caused more losses than all other pests and diseases combined. Out of the root diseases the worst has been the white root disease, first ascribed to *Fomes semitostus* Berk by Ridley (1904) and later identified as *Fomes lignosus* Klotzsch by Lloyd (1912). The fungus is now reffered to as *Rigidoporus lignosus* (Klotzsch) Imazeki.

In Sri Lanka, the white root disease caused by *Rigidiporus lignosus* is still the major root disease in rubber plantations. It was reported that 5-8% of the cultivated rubber lands are under bare patches in Sri Lanka due to this deadly disease (Liyanage, 1977). The rate of the spread of the fungus in the field varies from 1.9 to 10.6 m (average 4 m) per year resulting about 2% mortality per annum (Liyanage, 1979). Despite the severity of the losses they cause, root diseases have always attracted less attention than the others probably because of their unseen and insidious nature.

Control of this pathogen is based on several principals viz. cultural, biological and chemical methods and these aspects were discussed in detail by Peries *et al.* (1965), Liyanage (1984), Tran (1985), Tan (1990) and Anon (1994).

The development of the first collar protecive dressings for the use against *R. lignosus* was described by Fox (1965). In the recent past the most widely used fungicide in Malaysia and Sri Lanka was the Pentachloro nitrobenzene (PCNB) (Fox 1966, Liyanage, 1984). However, in 1989 the use of PCNB was completely banned in Sri Lanka after the discovery of carcinogenic contaminants. This was subsequently replaced by Pentachloro phenol (PCP) (Jayasinghe *et al.*, 1995) which too comes under highly toxic group of chemicals and Jayasinghe *et al.* (1996) pointed out the necessacity of the development of a less toxic chemical for the management of white root disease.

As this technique was labour intensive. Pathologists in Cote d'Ivoire (Tran, 1985) and Malaysia (Ng & Yap, 1990; Tan, 1990) initiated research programmes to develop soil drenching fungicides in the management of white root disease.

Further, workers in Africa and Eastern Hemisphere evaluated 10 chemicals, namely hexaconazole (Tan, 1990; Lam and Chin, 1993, Tran, 1994), myclobutanil (Tan, 1990), propiconazole (Tan, 1990 & Chan *et al.*, 1991). terbuconazole (Tan, 1990), triadimefon (Ng & Yap, 1990; Tan, 1990; Chan *et al.*, 1991) triadimenol (Ng & Yap, 1990; Tan, 1990, Tran, 1994) and tridemorph (Tran, 1985) dimiconazole. cyproconazole and brumuconazole (Tran, 1994) with various success during the last decade. Unfortunately, the high cost involved in purchasing these chemicals prevented there wide usage in most of the natural rubber producing countries.

This investigation addresses the above issues and reports on the results of a series of experiments carried out to develop a safe and economical fungicide in the management of white root disease.

MATERIALS AND METHODS

1. Inoculum

Rigidoporus lignosus isolate DF (isolated from Dartonfield Estate) obtained from *Hevea* roots was used *in vitro* experiments.

2. Fungicides

Effectiveness of Phenol (C_6H_5OH) as a fungicide was evaluated *in vitro* and *in vivo* experiments using following techniques.

Management of white root disease

In vitro ***screening***

(1). Well method (Vincent, 1927)

(A) Four wells were cut along the diagonals of Malt Agar plates at a distance of 2.5 cm from the center on opposite sides using a No. 3 Cork borer (6.5 mm diameter). One ml of the phenol (Park Scientific Limited, U.K.) 94.1% samples of concentrations 2%, 4%, 8% and 10% (w/v in sterile distilled water) were poured into wells. The center of the plates was inoculated with a 5 mm diameter agar plug cut out from the periphery of actively growing 7 day old cultures of *Rigidoporus lignosus*. Plates were incubated under normal light and dark regime at 28 ± 2°C for 8 days. Wells filled with distilled water served as controls. Each treatment was replicated for 6 times. Observations were made for the presence or absence of growth inhibition and the length of the inhibition zones was recorded.

(B) Two concentrations of phenol *viz*. 8% and 10% were further screened in a bituminous base against *Rigidoporus lignosus* using the same well method described above. One ml test samples in bituminous base were introduced into the wells. Bituminous base without phenol served as the control. Each treatment was replicated 6 times.

(2). Painting of root pieces method described by Fox (1977)

Eight percent and 10% Phenol in a bituminous base were further tested against *Rigidoporus lignosus* using the method described by Fox (1977). Phenol in bituminous base was applied on 10 cm long and 1 cm diameter pieces of healthy rubber roots obtained from mature rubber trees, leaving 2 cm length free of the chemical. Prior to application, root pieces were washed free of soil and autoclaved at 15 psi for 1 h. The untreated end of the wood piece was then implanted to a 7 day old cultures of *R. lignosus* grown on 10 ml of Malt Agar in boiling tubes. Each treatment was replicated for 7 times and root pieces without application of fungicides and with bituminous base without any chemicals served as the controls. The length of the treated area colonized by the fungus was measured 6 days after incubation under normal light and dark regime at room temperature (28 ± 2°C).

In vivo ***screening***

(A) Curative trials - on Artificially infected plants

Thirty RRIC 100 plants were established in a nursery at Dartonfield estate which about 6 months old were inoculated using the *Hevea* roots naturally infected with *R. lignosus*. Inoculation was done by burrying 4 infected root pieces at a depth of 6-10" below the ground level closer to the main root of each plant. After about six months the collar examination was done to determine the level of establishment of the disease on root system. Plants which had considerable disease incidence (2-3 score, see Table 4) were treated with 10% phenol containing "Collar Protectant" in the usual conventional method of treatment. The infected plants, a) treated with bituminous base only, and b) without any treatment served as the controls. The development of infection was monitored regularly by examining the collar region of these plants upto 12 months.

(B) Curative and preventive trials on naturally infected plants

Naturally infected 3-4 year old plants at Talduwa Estate, Avissawella were treated with 8 and 10% phenol based "Collar protectant". The treated plants showed varying degrees of infection at the time of treatment. Two adjoining trees on either side of an infected tree were treated with 8% concentration as a preventive measure. Three trees without any treatment served as controls.

(C) Determination of the possible mechanism of inhibitory action of phenol

Two effective concentration of phenol. 8 and 10% were incorporated into malt agar plates. The centre of these plates was inoculated with a 5 mm diameter agar plug cut out from the pheriphery of actively growing 7 day old cultures of *Rigidoporus lignosus*. Another set of plates with distilled water served as the controls.

When the control plates were fully grown, the centre inoculated Agar plug from the phenol incorporated plates were transferred on to fresh malt agar plates asceptically and incubated for about 7 days at room temperature until the controls were fully grown again. The growth of fungal colony was observed at this stage.

RESULTS AND DISCUSSION

***In vitro* screening**

Table 1 shows that the concentrations above 5% are highly effective in inhibiting the growth of the fungus *Rigidoporus lignosus*. This was further confirmed by the well method in 2nd experiment (Table 2 and Fig. 1) and the root pieces painting technique (Table 3 and Fig. 2) using bituminous base.

Table 1. *Growth of* Rigidoporus lignosus *colonies after 4 days of incubation (Mean value of 6 replicates)*

Phenol Concentration (%)	Mean growth of *R. lignosus* (cm)
2	1.38 ± 0.036
5	0.18 ± 0.015
8	Nil
10	Nil
control	2.46 ± 0.019

Control - Distilled water
2.5 cm is the maximum growth between the well and the center of the plate.

Table 2. *Growth of* Rigidoporus lignosus *after 4 days of inoculation (cm) (Mean value of 6 replicates)*

Phenol concentration (%)	Mean growth of *R. lignosus* (cm)
Control I	2.5 ± 0.00
Control II	2.2 ± 0.019
8	Nil
10	Nil

Control I - Distilled water, Control II - Bituminous base.
2.5 cm is the maximum growth between the wells and the center of plate.

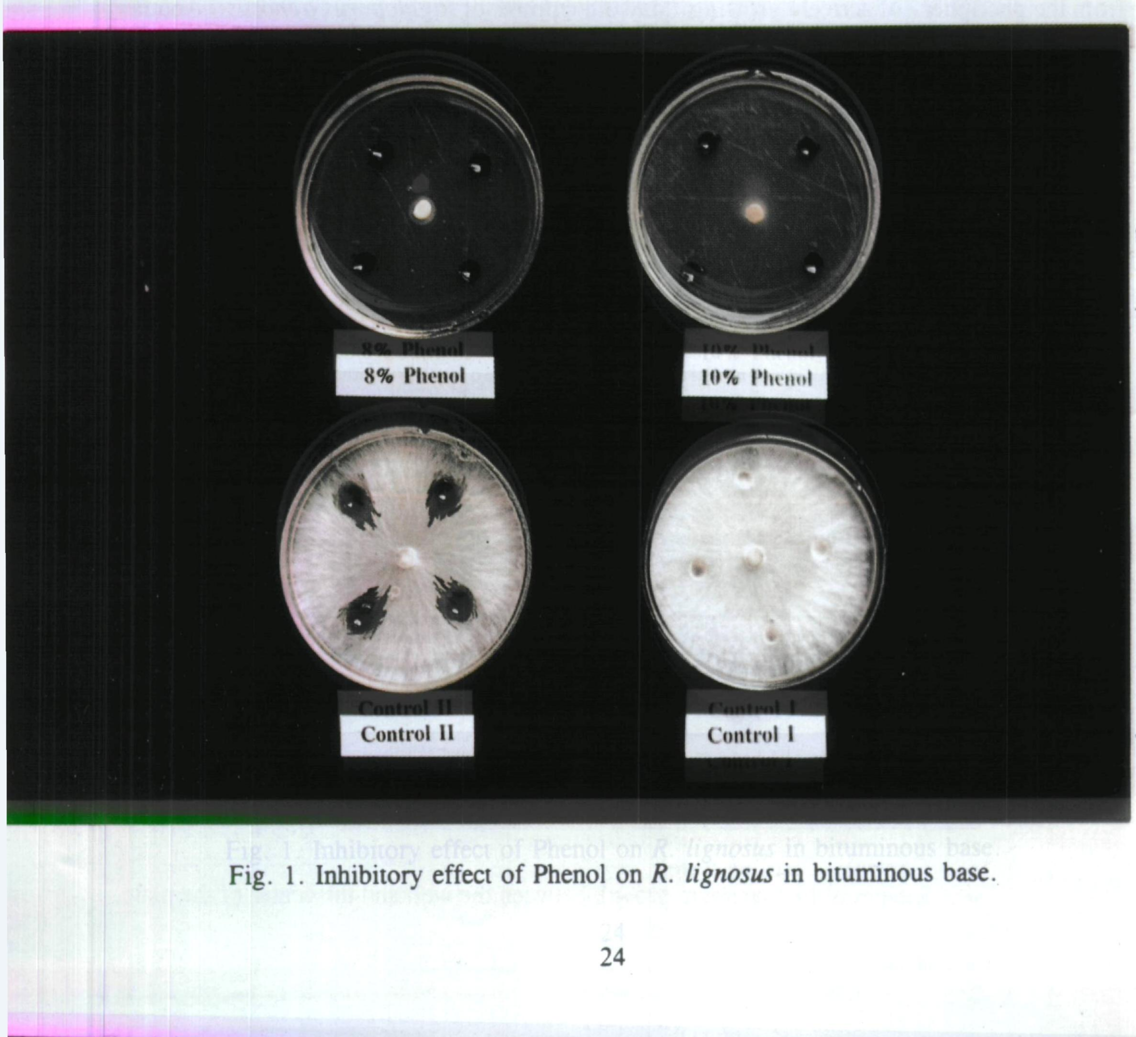

Fig. 1. Inhibitory effect of Phenol on *R. lignosus* in bituminous base.

Table 3. *Growth of* Rigidoporus lignosus *on rubber root pieces after 10 days of incubation (cm). (Mean value of 7 replicates).*

Phenol concentration (%)	Mean growth of *R. lignosus* (cm)
Control I	7.5 ± 0.00
Control II	3.72 ± 0.056
8	Nil
10	Nil

Control I - Distilled water, Control II - Bituminous base.
7.5 cm is the maximum length of a root piece.

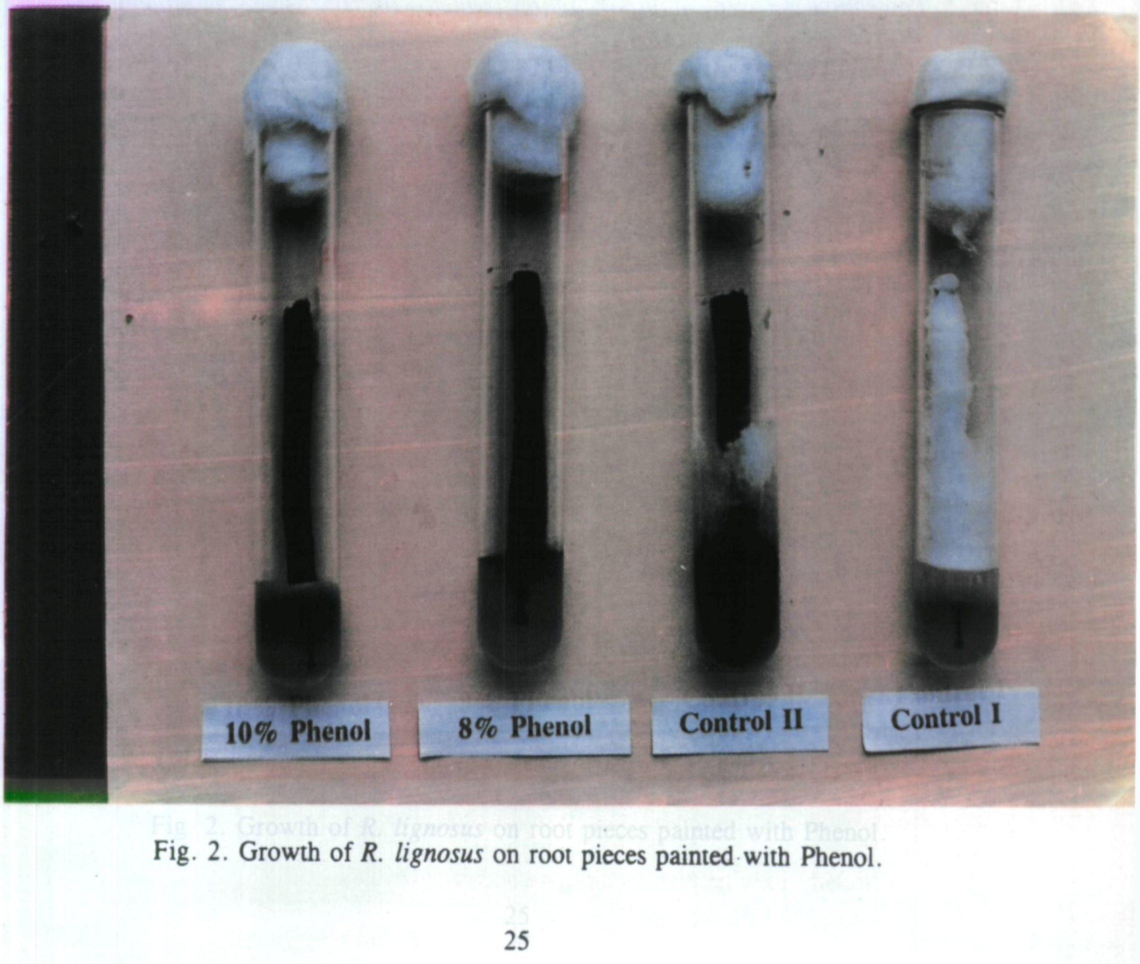

Fig. 2. Growth of *R. lignosus* on root pieces painted with Phenol.

In vivo screening

Assessments taken on artificially inoculated plants showed that phenol at 10% concentration in bituminous base is very effective in controlling the infected plants (Table 4). The recovery percentage was 100% for treated plants while all untreated plants died within 6 months of inoculation.

Assessments taken at 3 monthly intervals on plants treated in the field too showed that 10% Phenol is a very effective curative agent for white root disease. However. treatment success was reduced significantly when plants were severely affected (disease score of 4). Therefore as previously stated by many workers. (Fox 1966. Tan 1990. Ng and Yap 1990. Chan *et al.* 1991. Peries 1965) the early detection of the disease is vital for the success of chemical control of white root disease. Table 5 shows the Percentage Recovery of naturally infected field plants treated with 8 and 10% phenol in bituminous base. The plants treated with 8% and 10% phenol did not develop any infection even 3 years after the treatment.

Table 4. *Percentage recovery of nursery plants treated with 10% phenol in bituminous base*

Fungicide in bituminous	*Disease severity index (from collar inspection)	Total number of trees treated	% recovery after 10 months
10% Phenol	2	8	100%
	3	8	100%
Normal candarsan	2	5	Nil
(bituminous base)	3	4	Nil
Only cleaning of the	2	2	Nil
roots	3	3	Nil

* Disease severity index

1. Collar slightly affected. no foliar symptoms.
2. Collar half circumference rotted with light foliar symptoms.
3. Collar badly rotted with severe foliar symptoms.
4. Collar completely rotted with fully wilted leaves.

Mode of inhibitory action of phenol

There was no fungal growth in both concentration of phenol even after 10 days of incubation.

This suggests that the mode of action of phenol is fungicidal for *Rigidoporus lignosus*.

Table 5. *Recovery of naturally infected plants treated with 10% Phenol in bituminous base*

	Pre-treatment assessment No.of trees	After 3 months	After 6 months	After 9 months	% Recovery after 12 months
Disease severity index	*1 2 3 4	1 2 3 4 0 D	1 2 3 4 0 D	1 2 3 4 0 D	
No.of trees treated/recovered	1 4 5 3	2 5 1 1 2 3	1 1 - - 6 6	- - - - 7 6	70%

* Disease severity index

0. Collar free from infection.
D. Dead plants
1. Collar slightly affected no foliar symptoms
2. Collar half circumference rotted with mild foliar symptoms.
3. Collar badly rotted with severe foliar symptoms.
4. Collar completely rotted with fully wilted leaves.

@ Plants with index 4 were not considered for % recoveries, as generally these are beyond recovery.

Economics and impact on the environment

Although in recent times substantial emphasis was made to recommend soil drenching chemicals, the cost of these products is the key factor in prohibiting their use. Eventhough the labour wages are rapidly increasing, the convential brush application of "Collar Protectant" is a much cheaper method of treatment in Sri Lanka. The cost involved in the treatment of a 2-3 year old plant will be around Rs.55/= (US $ 1) whereas the cost of drenching with Bayleton or Bayfidan will be around Rs.250/= (US $ 4.8).

Replacement of Pentachloro Phenol (PCP) which is classified as in the group "Highly Hazardous" according to the WHO classification (LD_{50} - For rats by oral is 50 mg/kg) with phenol which is a much less toxic chemical (LD_{50} - For rats by oral is 530 mg/kg) may have a big impact on the environmental pollution problem. Use of these chemicals such as PCNB and PCP extensively in rubber plantations will undoubtly contaminate the underground water courses. People living in these estates (labourers) totally depend on the underground water for their living through the use of wells. Therefore it is timely to look for less toxic chemicals or biological control methods to be used in controlling this menace disease in rubber plantations of Sri Lanka.

ACKNOWLEDGEMENT

The authors are very thankful to Mr Wimal Amaratunge for preparing the photographs and Mrs P Amarasekera for helping with word processing.

REFERENCES

Anon (1994). White root disease. Identification and treatment of diseases of *Hevea brasiliensis*, International Rubber Research and Development Board, Hertford, U.K. pp 1-9.

Chan, W H, Wong, C P and Wong, C C (1991). Control of white root disease in immature rubber with three systemic fungicides. *The Planter* **67**, 251-265.

Fox, R A (1965). Formulations of collar protectant dressings for use against *Fomes lignosus. Rubber Research Institute Malaya Research Archive* **50**, 11.

Fox, R A (1966). White root disease of *Hevea brasiliensis* collar protectant dressings. *Journal of the Rubber Research Institute of Malaya* **19**, 231-241.

Jayasinghe, C K, Jayasuriya, K E, Fernando, T H P S (1995). Penta Chloro Phenol - An effective and economical fungicide for the management of white root disease caused by *Rigidoporus lignosus* in Sri Lanka. *Journal of the Rubber Research Institute of Sri Lanka* **75**(1), 61-70.

Lam, C H and Chin, S B (1993). Hexaconazole (Anvil 5 sc), a cost effective fungicide for controlling white root disease in immature rubber. *The Planter* **69**, 465-474.

Liyanage, A de S (1977). Review of the Plant Pathology Department. *Annual Review of the Rubber Research Institute of Sri Lanka* 66-98.

Liyanage, A de S (1979). Review of the Plant Pathology Department. *Annual Review of the Rubber Research Institute of Sri Lanka* 65.

Liyanage, A de S (1984). Root diseases. in "*Practical Guide to Rubber Planting and Processing*" (A de S Liyanage and O S Peries Eds.). pp 136-148. Rubber Research Institute of Sri Lanka, Agalawatta. Sri Lanka.

Lloyd, G C (1912). *Mycol. Notes* No.38. 519.

Ng, K J and Yap, T H (1990). The effect of triadimefon and triadimenol for controlling white root disease of rubber. *Proceedings of 3rd International Conference on Plant Protection in the Tropics*, Malaysia 2. 31-35.

Peries, O S, Fernando, D M and Samaraweera, S K (1965). Control of white root disease (*Fomes lignosus*) of *Hevea brasiliensis*. *Journal of the Rubber Research Institute of Ceylon* **41**, 81-89.

Ridley, H N (1904). Parasitic fungi on *Hevea brasiliensis*. *Agric. Bull. Straits Settlements* No.3, 173-175.

Tan, A M (1990). The present status of fungicide drenching on the control of white root disease of rubber. *Proceeding of International Rubber Research and Development Board Symposium on root diseases of Hevea*, China, 47-54.

Tram Van Canh (1985). Use of calixin and sandofan F against white root disease and black stripe of *Hevea brasiliensis*. *Proceedings of International Rubber Conference*, Kuala Lumpur 3: 222-237.

Tram Van Canh (1994). Use of fungicides from the triazole family to reduce the impact of *Rigidoporus lignosus*, causal agent of white root disease on rubber (*Hevea brasiliensis*). *Proceedings of the International Rubber Research and Development Board Symposium on Diseases of Hevea* held in Cochin, India 85-90.

Vimcemt, J M (1927). Distortion of fungal hyphae in the presence of certain inhibitors. *Nature* 159, 850.

(Received 14 October 1996)