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SOIL AND PLANT MANIPULATION FOR THE CONTROL OF WHITE ROOT DISEASE OF HEVEA

by

O. S. Peries

INTRODUCTION

White root disease, caused by the fungus, *Rigidoporus lignosus* (Klozsch) Imazeki, is the most important disease of the rubber tree, *Hevea braziliensis* Muell. Arg. in Sri Lanka, at present. Some 8–10% of the area planted with rubber in the wet low country area lies fallow at any time; as the trees planted in those areas have been killed by the disease (Liyanage, 1977). The economic outcome of this is that the owners of the rubber land and the country lose about 10% of the income, which otherwise would have been produced by that cultivated land. Therefore, the Rubber Research Institute of Sri Lank (RRISL) has laid special emphasis on the development of suitable and economical methods of controlling this disease.

It is essential to have detailed information on the biology of the casual fungus, its mode of infection, survival in the soil and the effects of soil conditions on disease incidence and spread to devise effective methods of controlling a fungal root disease. This vital information has been painstakingly gathered through a series of carefully planned and executed experiments done by the RRISL (Peries, 1962–1967; Liyanage, 1976–1983).

The pathogen, *R. lignosus*, is a weak parasite, which lives on dead organic matter, a food base, drawing its energy from that source, while it establishes infection on a living root. (Peries *et al.* 1965). This food base must be of a certain minimum size, for the fungus to draw sufficient energy for infection (Alston, 1954; Liyanage and Peries, 1983). Therefore one method of eradicating the disease is to ensure that all infected root debris, down to a minimum size, is removed at the time of uprooting the old stand and preparing the land for replanting. Preparing a 100% clean planting site is difficult, if not impossible; so that other, supplementary methods, of reducing the risk of infection must be sought.

White root disease infection usually takes place by root contact (Petch, 1921); therefore, if root debris from the old stand is encouraged to rot rapidly, then there is a good chance that the new stand can escape infection by "disease avoidance." This can be done by spraying various chemicals, on rubber stumps and roots, that will hasten the rate of wood rotting or growing

cover crops, under which the same result can be achieved. Of course, if an area can be left fallow after one planting cycle, before the next crop is planted, then adequate time can be allowed for the plant debris from the previous crop to rot, before the next crop is planted. However, the economics of such a technique would be the most important barrier to its adoption; as the income from the land will be nil, during the fallow period.

Another method of reducing disease incidence is to kill the pathogen with a fungicide or, as this is a soil borne disease, by some biological manipulation of the soil (Peries 1970). The latter method can be expected to be more successful, as many fungicides tend to be inactivated on contact with the soil. It would certainly be more economical as most methods of soil amendment would be cheaper than the cost of fungicides and their application in these days.

It is an important characteristic of *R. lignosus* that this fungus grows superficially over the root surface for a considerable distance, and infects it about 25–30 cm behind the limit of the visible fungus. Therefore, if a fungicide or fungistat can be found to stop the growth of the fungus on the root surface, then root infection can be prevented and the food base can be expected to rot in the meantime. Thus depending on the period for which the fungicide remains effective, infection can be eradicated (Liyanage and Peries, 1983).

This paper describes the results of recent experiments on the effect of soil amendment with sulphur on disease control and the use of prophylactic fungicides to protect the roots from infection for limited periods, while the food bases which harbour the fungus until the latter has established an infection of the living host roots, rot away thus losing their ability to transfer infection on to healthy roots. It has been found that the addition of 114 g. sulphur as a surface application on a 1 sq. m. area around the plant reduces pH of the soil and alters the population of soil microorganism leading to the predominance of *Trichoderma*, *Penicillium* and *Aspergillus* species (Peries 1962–67). These fungi, antagonistic to *R. lignosus*, smother its growth and cause lysis of its mycelium and inhibition of its growth. These results are discussed and their significance in the development of a biological method of controlling the disease are shown here.

MATERIALS AND METHODS

The growth response of *R. lignosus* to the pH of the medium was studied in 9 cm petri dishes containing 10 ml of 2% Malt Agar (MA), at different pH values, adjusted by using 0.1 N HCI or KOH, after autoclaving, Inoculum disks, 7 mm in diameter, taken from the leading edge of a 8-day old culture

of the fungus were placed in the centre of the petri dish, and incubated at room temperature (RT), which was 28° C $\pm$ 2. Measurements of growth were made daily, two per dish, at right angles to each other.

Several field experiments were laid down where sulphur was added to the soil at the rate of 114 g. and 228 g per m^2 and gently forked in. Soil samples were collected from treated and control areas, before treatment and at regular intervals thereafter, at depths of 0–3 cm, 15–18 cm and 30–33 cm, and the pH recorded. The fungal population of the soil, at different depths before and 2, 6 and 10 weeks after the addition of sulphur was determined by the soil dilution plate method (Brierley *et al.* 1927).

The growth of the pathogen at different pH levels, the soil reaction to the addition of sulphur and the effect of sulphur on the soil fungal population were also studied in petri dishes and pots containing soil. In these experiments 2 cm agar discs of the fungus were placed on soil petri dishes and in flower pots. The pH of the soil was adjusted to the desired level with 0.1 N HCI or KOH, or the soil was treated with the equivalent amount of sulphur as in the field experiments. The inoculum discs were recovered and examined at regular intervals and the change in soil microflora recorded as in the field studies.

Different species of soil fungi were also grown separately and as paired cultures with *R. lignosus* in petri dishes on agar. These plates and slides made from the cultures, were examined under the microscope to assess the changes taking place.

Different fungicides, at various concentrations were incorporated in a grease base and applied to the healthy parts of the root system of already infected plants, after cutting out the infected areas. The same fungicides were applied to the tap root and main laterals of healthy trees to a distance of about 60 cm in each case; the selected trees being adjacent to infected trees and likely to be infected under normal field conditions.

A series of field experiments were carried out on heavily infected and disease-free clearings in the wet rubber planting districts and slightly affected areas in dry districts to test the efficacy of tree stump poisons viz : urea, borax and 2, 4, 5 - T in diesolene. In the control areas, trees were uprooted and burnt or the stumps were left untreated (Liyanage and Peries, 1983).

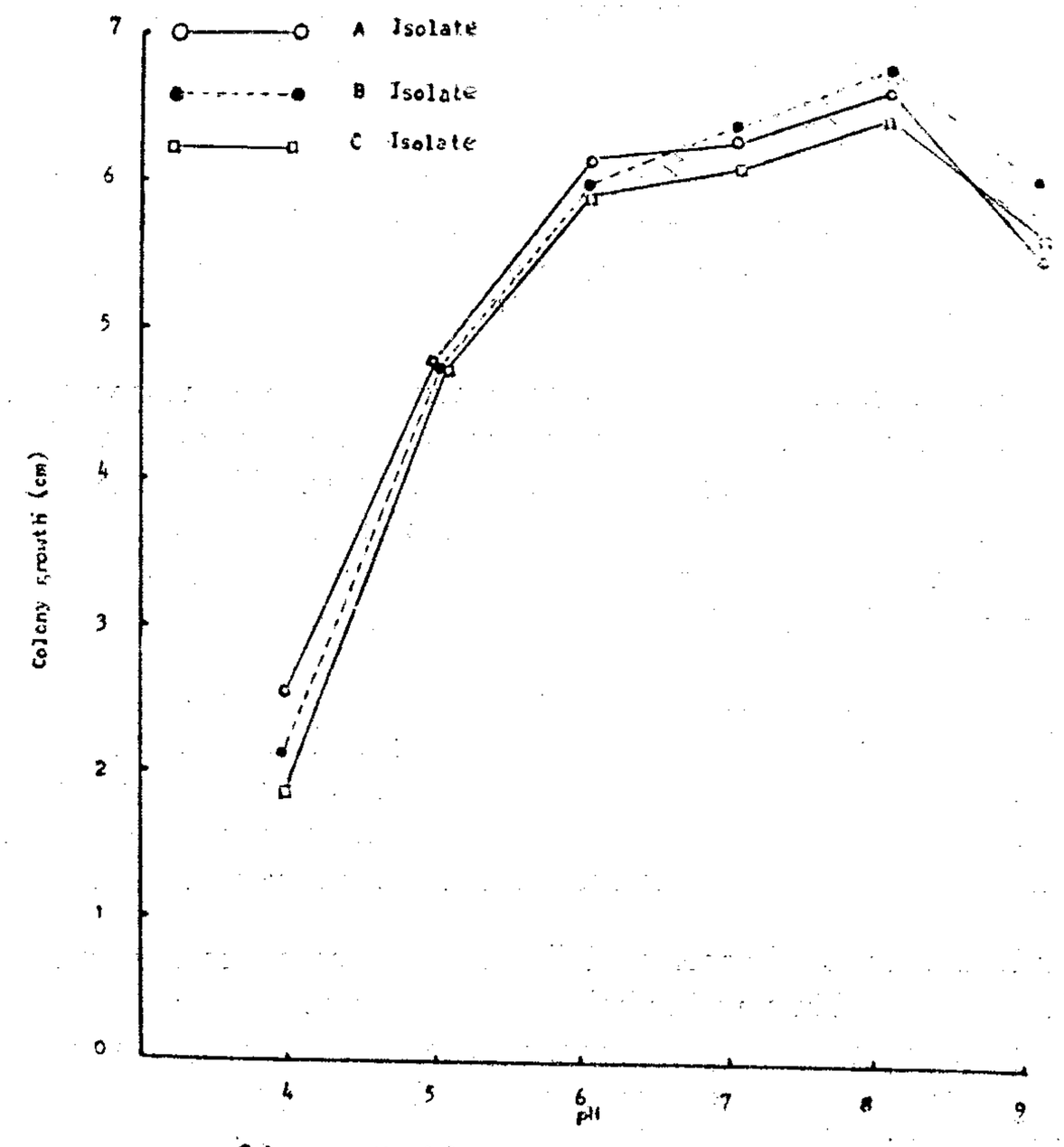
Standard lengths of roots of different sizes, both healthy and infected (Fig. 1), were buried in planting holes, others in areas that were clean weeded or had a good cover of different legumes or naturals (a mixture of grasses and legumes). The buried roots were dug up and inspected at regular intervals, to assess their stage of decay, under various conditions.

Fig. 1 Dead Hevea root, infected by R. lignosus

RESULTS

Reaction of Growth Medium

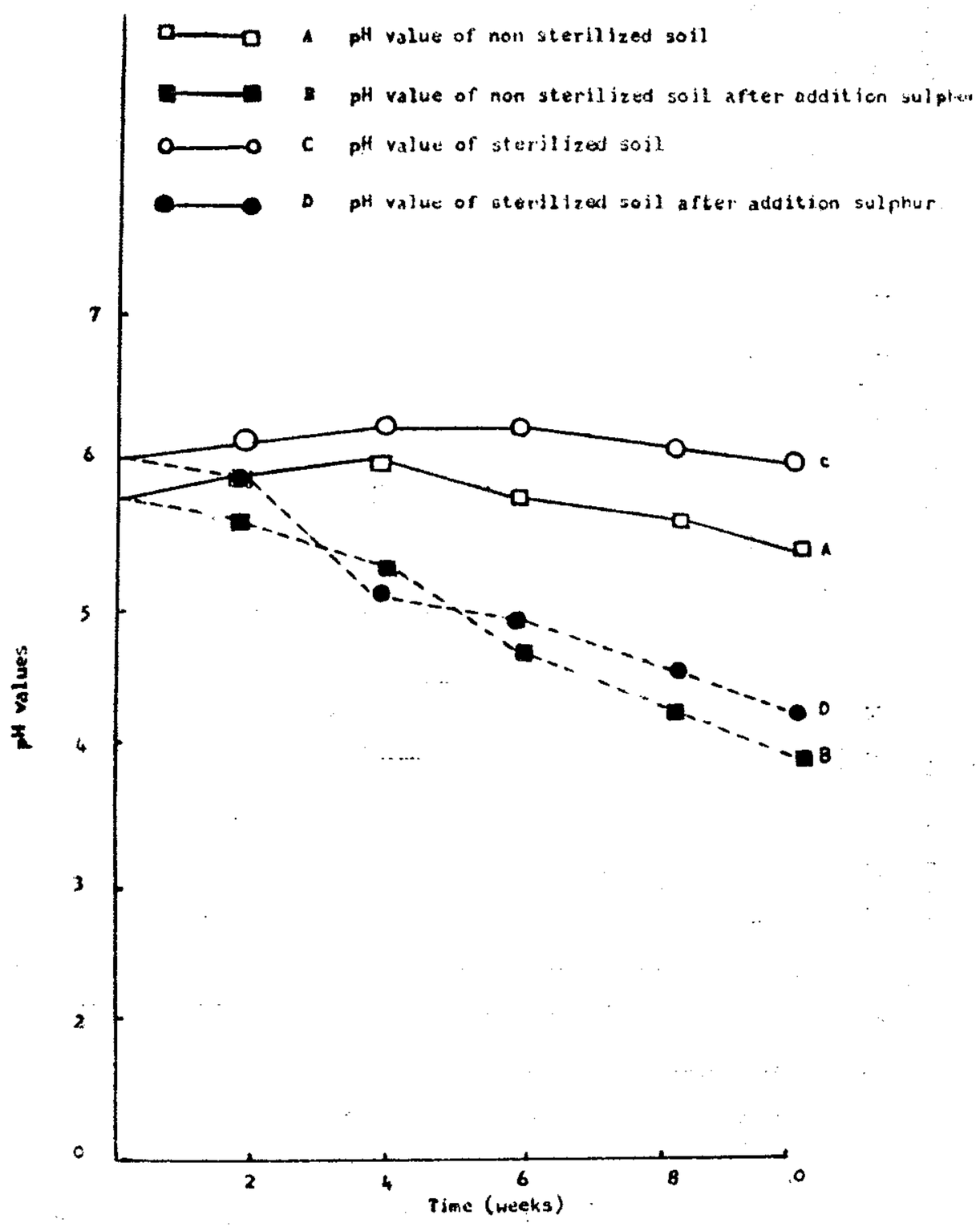
It was found that *R. lignosus* grows best at about pH 8 (Fig. 2). The growth at pH 4 was poor, it improved with increasing pH, but at pH 9 it tailed off again and at pH 11 there was hardly any growth. The response to pH on soil and in pots was very similar. All cultures behaved in the same manner; so that it can be concluded that the pathogen prefers a slightly alkaline medium for growth.



Colony growth of R. lignosus at different pH levels.

Soil Amendment

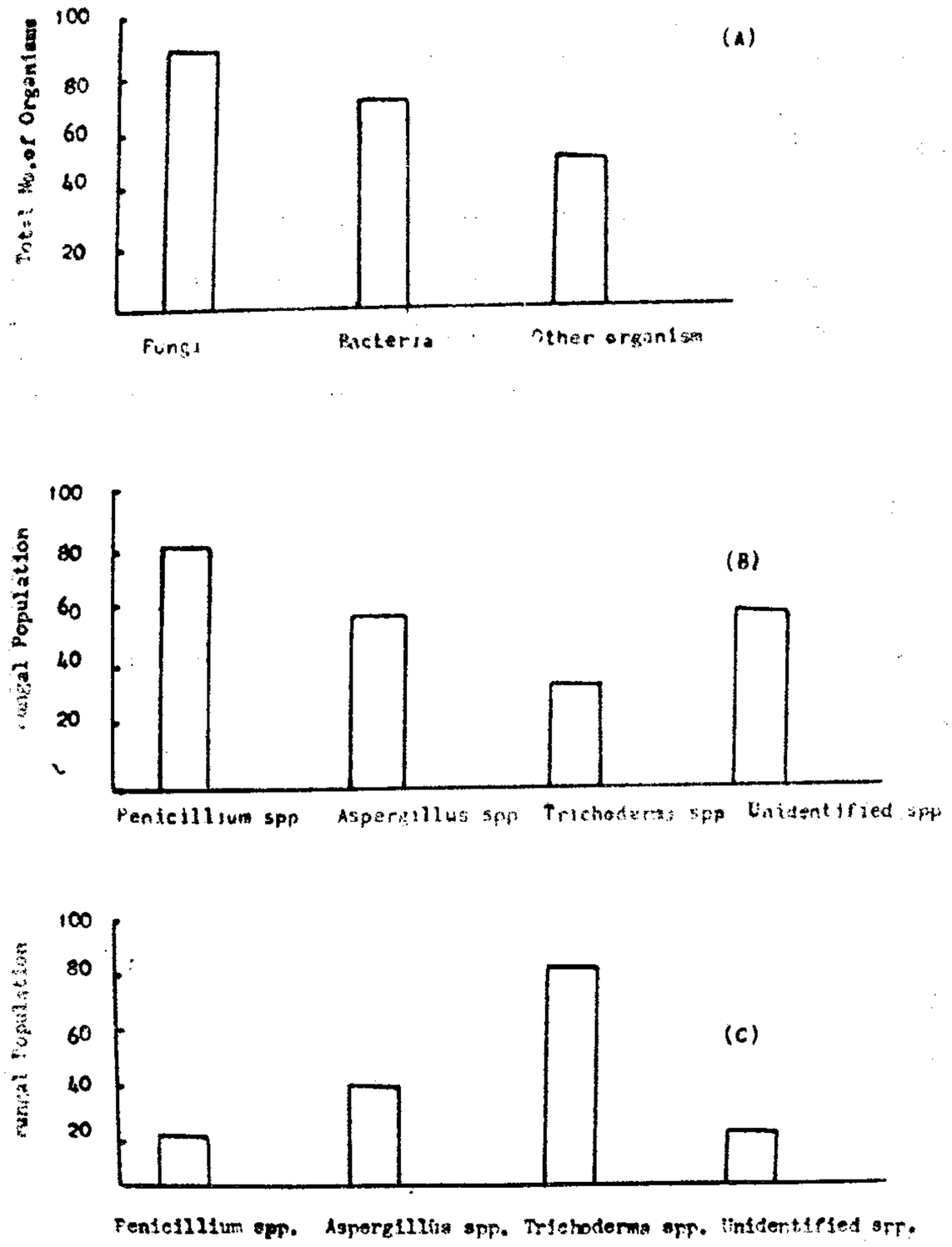
The pH of the soil, at all depths tested, fell sharply when sulphur was added. The lowest pH was recorded 6 weeks after the application of sulphur, and this level was maintained for a few weeks, after which it recovered to some extent; but the soil reaction continued to be more acidic than that of the control, and around pH 4.0 - 4.2 for almost 1 year. The reaction of sterilised soil to the addition of sulphur as compared to non-sterile soil, was interesting (see Fig. 3) in that the pH fell irrespective of sterilization, when sulphur was added.



The progressive change in pH after addition sulphur

The total fungal population was highest, close to the surface, and there was a distinct decrease in the number and variety of fungi present at increasing

depths of soil. There was a significant change in the pattern of fungi present in the soil, when it was treated with sulphur. The dominant fungus in sulphur treated soil was *Trichoderma* spp , their number being almost doubled. On the other hand the numbers of *Penicillium* spp., *Aspergillus* spp. and unindentified fungi, dropped significantly (see Fig. 4).



A. Soil micro-organism population

B. Fungal population before addition sulpher

C. Fungal population four week after addition sulpher

Fig. 4. Change in soil fungal population after the addition of sulphur

Species of *Trichoderma, Penicillium* and *Aspergillus* were grown on agar plates as paired cultures with *R. lignosus*. When these plates were carefully

examined and slides made from them were studied under the microscope, it was found that some *Trichoderma spp.*, grew over the pathogen smothering the latter and others caused lysis of *R. lignosus* hyphae (Figs. 5 and 6). Most of

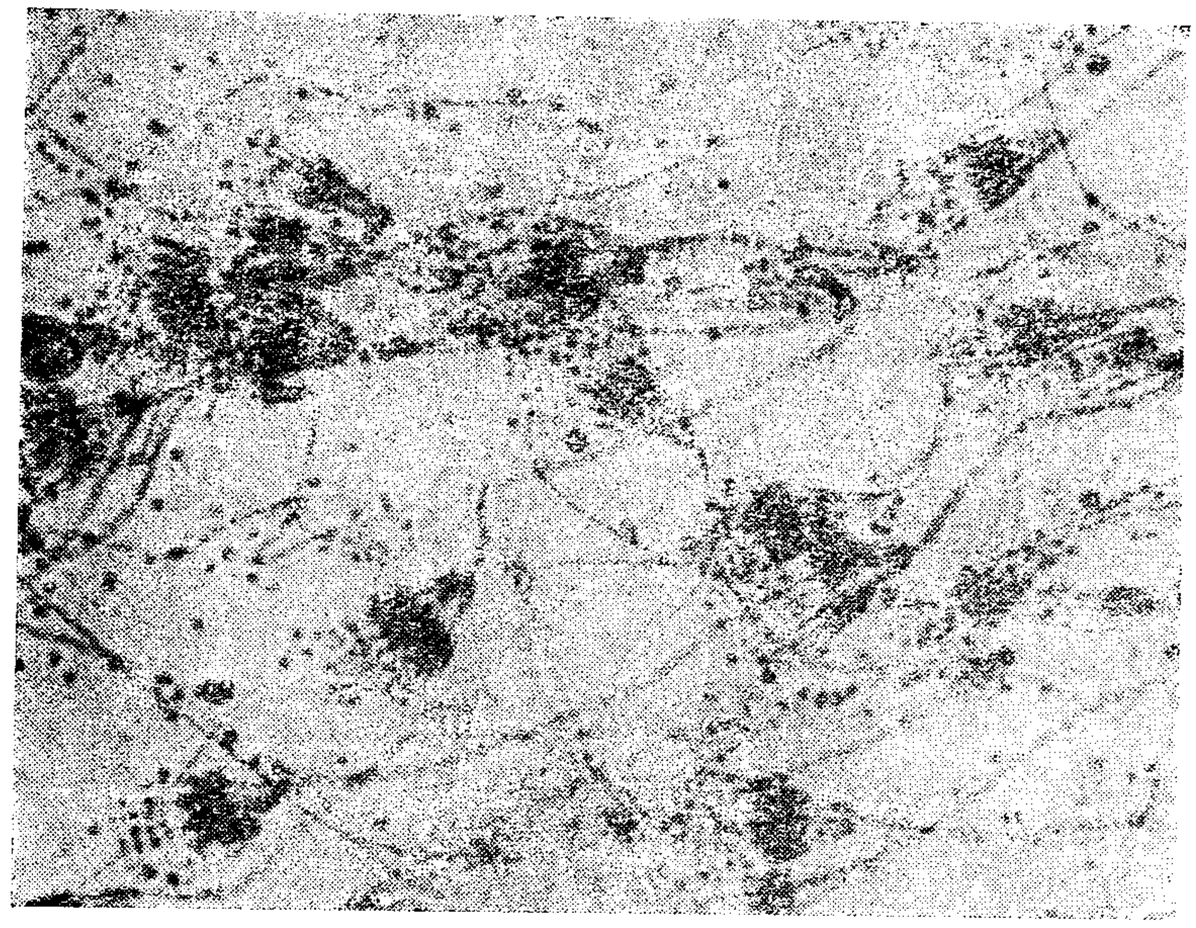


Fig. 5. R. lignosus hyphae smothered by Trichoderma spp. after addition of sulphur to the soil.

Fig. 6. R. lignosus hyphae (vacuolated parasitised by hyphae of *Trichoderma* spp, (dark hyphae).

the *Penicillium* spp. inhibited or prevented the growth of the pathogen on petri dish cultures, showing definite antagonistic zones as areas free of both fungi. Some species of *Penicillium* caused lysis of hyphae of *R. lignosus*, just as the *Trichoderma* spp., do; however, this is less common with the *Penicillia* than with the *Trichoderma* spp.

The antagonism of *Trichoderma* spp. to *R. lignosus* was confirmed in soil plates and pot experiments, where it was noted that the pathogen did not grow on sterilized soil inoculated with *Trichoderma* spp. Robust colonies of *R. lignosus* always disintergrated and died when they were placed on soils where *Trichoderma* spp. were dominant.

The pathogen did not grow out of inoculum plugs transferred from such plates to sterile agar culture plates.

Rate of Wood Rot

Small roots (0.6 cm diameter) rotted out fast and lost their ability to infect living roots, in 6 months when infected material (Fig. 1) was buried in planting holes. However, larger lateral roots, 1, 2, 2.5 and 5.0 cm in diameter, caused 20%, 40% and 80% infection, respectively, within 1 year of being placed in planting holes. Most of the 2.5 and 5.0 cm diameter pieces of inoculum remained viable for over 1 year, showing that they had not rotted out at that stage. Later studies have shown that large pieces of infected wood do not disintegrate even at the end of 18 months (Liyanage, Peries and Liyanage, 1983).

The experiments on stump poisons showed that 2, 4, 5—T in dieselene induced significantly faster decay of rubber stumps than the other chemicals used in these studies i.e., urea and borax. The protection of cut surfaces with creosote, after application of 2, 4, 5—T, was tested as a means of preventing the infection of stumps with spores of the pathogen. However this study did not provide any useful result as none of the control stumps (untreated) were infected at the end of the study. Poisoning the stumps also did not eliminate the disease from infected stumps, and such disease sources transmitted the disease to new plants, irrespective of the treatment to which they were subjected.

Decay of infected roots was accelerated when they were placed under a pure legume cover, as compared to clean weeded areas and mixed covers of grasses and legumes. The activity of this fungal pathogen was also found to be considerably reduced, when it was grown on soils taken from under a pure cover of *Pueraria phaseoloides;* but this result was inconsistent, as the fungus grew well on different soil types, taken from under the same cover crop.

Fungicides

These studies confirmed the results obtained by Fox (1966) that 2% pentachloro-nitropenzene (PCNB), incorporated in a grease base was the best material for use for the protection of the tap root and the main laterals from infection, or a period of 1–2 years, while the food bases rotted. PCNB is a fungistat rather than a fungicide; however, it prevents the superficial growth of the pathogen on the root surface, when it is applied on the root. This precludes infection, as *R. lignosus* grows superficially over the root for a considerable distance, before it penetrates and infects the root, some 30 cm. behind its leading edge of growth on the surface of the root. The effect of the PCNB was found to last for up to 30 months, which is sufficient time for all but the largest pieces of inoculum to rot in the field, and thus become non-infective.

DISCUSSION

An economical method of controlling white root disease can be formulated by the consideration and evaluation of the results of the studies presented here. This would entail a great deal of care at the time of uprooting the old stand, in order to ensure a relatively disease free-seed bed. These studies have shown that the smaller pieces of inoculum (0.6cm in diameter) decay rapidly, lose their infective ability and do not cause infection, even if they are buried in the planting hole. Therefore, the removal of the larger pieces of infected material from the soil, at the time of replanting, is essential and is the first step in soil manipulation.

Treating the soil with sulphur not only reduces its pH, but alters the soil microbiological balance significantly. The resulting state, with an acidic soil and a predominance of *Trichoderma* spp. in it, militates against the growth of *R. lignosus*. This pathogen grows best in an alkaline medium, and the Trichodermas are antagonistic to it, so the amendment of the soil with sulphur, will assist in disease control in two ways. However, sulphur is an expensive raw mateial now, and it should be used only in areas, where it is essential i.e. in the areas where the diseasse is known to exist. Thus demarcation of infected areas in the old stand and treatment of these areas alone, at the time of replanting is the ideal to aim at. Sulphur should be used at the rate of 114 g per m^2 as larger quantities have been shown to be uneconomical.

Poisoning of the cut stumps of trees, with 2, 4, 5-T helped to kill roots rapidly and facilitated their invasion by saprophytes, which have a high competitive ability. Stump poisoning should be done only in areas where the infection is absent. It should not be carried out in heavily infected areas, as poisoning does not eradicate the disease, it merely facilitates decay, and can be considered another method of soil manipulation to control the disease.

Rubber wood generally decays more rapidly under a pure cover of legumes than in clean weeded areas or those with mixed covers. Therefore, the maintenance of a good legume cover should be a high priority investment for root disease control, apart from any other consideration of its merits. The more rapid the decay of the wood (food bases) the less the chance of infectiom of the new stand; as the sources of inoculum are destroyed along with the wood. Cover crops also help to dissipate the inoculum potential of the fungus, by providing the latter with a great deal of extra root material to infect, in place of the host roots. Such infection of non-host roots leads to rapid loss of infective ability by the pathogen, thus adding to the utility of the legume cover in the context of disease control.

The use of a prophylactic fungicide is the final step and is called for only when a plant is infected. The diseased plant is treated by cutting out all infected parts and, to ensure that the root is protected from re-infection until the food base rots out, destroying its potential as an infective organ, the fungicide is applied to the roots and it merely protects the roots until the external food hase rots out in the soil. Therefore, here the plant is mainpulated and merely given time to provide the conditions for decay of the inoculum. When that is achieved, there is no further risk of infection.

In conclusion, the most economical method of controlling white root disease is to ensure that all disease inoculum is removed at the time of uprooting the old stand. Then to treat all known infected areas with 114 g of sulphur, and grow a good legume cover over the whole area. If any plant in the new stand is infected, it is treated by removing foot bases and cutting out all infected roots, followed by the treatment of the tap root and laterals with 2% PCNB in a grease base. This scheme of treatment will ensure more than 95 per cent protection of new plants from this disease in replanted areas.

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