

RECENT DEVELOPMENTS IN THE CONTROL OF PORIA ROOT DISEASE

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Introduction

I propose to discuss with you today some of the results which we have obtained recently from experiments on the chemical control of Poria Root Disease. It is not my intention to give you a description of the disease, because I am sure most of you here are quite familiar with it. However, I would like to refer you to a survey conducted by us (Mulder and Redlich, 1962,) sometime ago on the occurrence of the disease in the up-country tea estates in Ceylon, in order to give you some idea of the background and purpose of the present investigations.

In 1961, we sent out a questionnaire to 112 estates, which had in the past reported *Poria*, and asked for statistics regarding the occurrence and control of the disease. The information we received is summarized in Table 1.

TABLE 1.—*A survey of Poria Root Disease in the up-country*

| | Serious | Minor | Total |
|--------------------------------------|---------------|------------|---------------|
| Number of estates (105) | 50 | 41 | 91 |
| Extent of <i>Poria</i> -infected tea | 735 acres | 42 acres | 777 acres |
| Area treated | 336 acres | 23 acres | 359 acres |
| Expenditure for the past 5 years | Rs. 2,000,000 | Rs. 59,000 | Rs. 2,059,000 |
| Average cost per acre | Rs. 6,000 | Rs. 2,500 | Rs. 5,700 |
| Recurrences in treated patches | 37 | 14 | 51 |
| New occurrences in old tea | 41 | 18 | 59 |

Altogether 105 estates responded to our circular, of which 91 reported the occurrence of either serious or minor attacks of *Poria* on their estates. The total infected area was 777 acres, 359 acres of which have already been treated in the past at an estimated cost of over 2 million rupees. The cost per acre of *Poria* treatment therefore works out to approximately Rs. 5,700. This is undoubtedly phenomenal!

And, what success have we achieved? I would say little. It will be seen that of the 91 estates that adopted some measures to control the disease 51 have reported recurrences in old treated patches. In addition, 59 estates have also observed new occurrences in old tea.

The picture you have just seen is in my opinion an understatement of the actual situation, because some estates (7) failed to answer our questionnaire, and the 14 estates that reported the absence of *Poria* have, according to our records, occasionally sent specimens of *Poria*-infected tea to the Institute for identification. Further, we have not taken into account the mid- and low-country estates, where we know definitely that a certain amount of disease is present. Be that as it may, it is still abundantly clear from this survey that, despite the large sum of money spent,

success in *Poria* control has been incomplete. It also shows that the conventional method of grubbing all diseased bushes together with a row or two of healthy bushes, deep forking and removing all roots above pencil thickness, is inadequate to meet the situation.

Other methods of control were, therefore, sought and the first choice was obviously by chemical means.

The use of chemicals for controlling soil-borne diseases has been known for a long time, although it never became established as a general practice for field crops, except on a limited scale. This is probably because most of the chemicals employed are still expensive to use on a large scale. A considerable number of volatile chemicals have been tested in the past as soil fumigants, but only a relatively few have been adapted for commercial use. Included are carbon disulphide, chloropicrin, methyl bromide, DD, PCNB, and Vapam. Today soil fumigation with chemicals is a more attractive alternative to heat sterilization for controlling soil-borne diseases, nematodes and soil insects.

Experimental

In the present investigations 4 chemicals were chosen initially to investigate their direct toxicity to *Poria hypolateritia*.

Laboratory Investigations

Segments of tea roots artificially infected with *P. hypolateritia* were exposed to the vapours of the 4 chemicals in sealed test tubes. After the segments had been exposed to the vapours for 72 hr., they were removed and the viability of the fungus determined by tissue transfers onto culture plates. The results are shown in Table 2.

TABLE 2.—Direct action of soil fumigants on *Poria hypolateritia*

| Volume of fumigant (ml.) | DD | | | Vapam | | | Trapex | | | Formalin | | |
|--------------------------|----|----|----|-------|----|----|--------|----|---|----------|---|---|
| | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| 0.05 | v | v | v | v | v | v | v | v | v | v | v | v |
| 0.05 | v | v | v | v | v | v | v | v | v | v | v | v |
| 0.02 | NV | v | NV | v | v | NV | v | v | v | v | v | v |
| 0.40 | NV | NV | NV | v | NV | NV | v | NV | v | v | v | v |

v = Viable

NV = Non-viable

It is evident from Table 2 that of the 4 chemicals tested, namely DD, Vapam, Trapex and formalin, the first two appear very effective. Trapex shows some promise, whereas formalin appears to be completely ineffective in the gaseous phase.

Several other potentially good fumigants were not included in these tests, because of their hazardous nature to humans.

Pot Experiments in the Glass-house

Our next series of experiments were carried out in pots using DD, Vapam and Trapex.

Root segments colonized by *P. hypolateritia* were buried in fine, sandy loam in 9 inch diameter clay pots. One segment was placed vertically in each pot 3 inches below the surface. 10 ml. of the chemical was then injected at the centre of the pot and the hole closed immediately. The soil was then covered with polythene to prevent the escape of fumigant.

The viability of the fungus was determined by sowing *Tephrosia vogelii* seeds in the pots 2 months after fumigation. 6 months later, the *T. vogelii* plants were uprooted and the number of pots showing infected plants was recorded (Table 3).

TABLE 3.—Pot trials with soil fumigants for control of *Poria hypolateritia*

| Fumigant | No. of pots showing infected <i>T. vogelii</i> |
|----------|--|
| DD | 0 |
| Vapam | 4 |
| Trapex | 10 |
| Control | 16 |

No. of pots per treatment 16

All pots treated with DD were free of infection, while one-fourth of the pots treated with Vapam and 10 out of the 16 treated with Trapex contained infected *T. vogelii* plants. Thus tests in soil in the glass-house indicated that *P. hypolateritia* was more sensitive to DD than Vapam or Trapex. Figure 1 shows some pots with healthy and others with infected *T. vogelii* plants.

Fumigation Trials on Estates

The applicability of DD and Vapam to field conditions was next investigated.

Trials with DD

Trials were conducted on eight estates. A few of the larger *Poria* patches on these estates were chosen and cleaned by removing all remaining diseased and apparently healthy bushes both in the centre as well as on the periphery. The soil was then levelled and the patches divided into plots 20 ft. by 20 ft.; adjacent plots were separated by trenches 1 ft. wide and 3 ft. deep.

On 2 estates segments of tea roots and stumps naturally infected with *P. hypolateritia* were buried in each plot at random at different depths.

Fumigation was carried out at 3 dosage rates, viz. 1,000, 1,500 and 2,000 lb./acre, the DD being injected at 1 ft. staggered intervals, and 6 inches deep. After injection the holes were closed immediately by trampling hard with the heel, and the plots were covered with a heavy thatch of Guatemala grass (Figure 2). About 3 months after fumigation, when the DD fumes had escaped from the soil, *T. vogelii* was sown as an indicator crop on the plots in rows 2 ft. apart.

Except in one instance, all treatments were replicated six-fold, and there were 6 control plots on each estate.

Observations commenced 6 months after the sowing of *T. vogelii* and all obvious points of residual infection were mapped out monthly. At the end of a year, the *T. vogelii* was uprooted and the No. of plots showing infection, and the No. of points of infection in each plot were recorded. Table 4 shows the results of these fumigation trials.



Figure 1. Pots treated with various materials showing infected and healthy *Tephrosia Vogelii* plants.
(1) Control (2) DD (3) Trapex (4) Vapam



Figure 2. Fumigation with DD for control of *Poria* Root Disease in progress on Pundaluoya North Estate. Note fumigated area being covered with Guatemala grass loppings.

TABLE 4.—Results of fumigation trials with DD for control of *Poria hypolateritia* on estates

| Treatment | Estates | No. of infected plots | No. of points of infection |
|---------------------|-----------------|-----------------------|----------------------------|
| 1,000 lb. per acre | Dunsinane | 5/8 | — |
| | Le Vallon | 4/6 | 15 |
| | Loolecondera | 4/6 | 15 |
| | Spring Valley | 1/6 | 1 |
| | Mousa Ella | 2/6 | 3 |
| | Mattakelle | 3/6 | 6 |
| | Bunyan | 6/6 | 33 |
| | North Punduloya | 3/6 | 21 |
| | | | <u>28/50</u> |
| 1,500 lb. per acre | Spring Valley | 3/6 | 12 |
| | Mousa Ella | 1/6 | 3 |
| | Mattakelle | 2/6 | 10 |
| | Bunyan | 5/6 | 24 |
| | North Punduloya | 3/6 | 23 |
| | | <u>14/30</u> | <u>72</u> |
| 2,000 lb. per acre | Dunsinane | 0/6 | 0 |
| | Le Vallon | 1/6 | 2 |
| | Loolecondera | 1/6 | 1 |
| | Spring Valley | 0/6 | 0 |
| | Mousa Ella | 0/6 | 0 |
| | Mattakelle | 0/6 | 0 |
| | Bunyan | 2/6 | 3 |
| | North Punduloya | 1/6 | 1 |
| | | <u>5/48</u> | <u>7</u> |
| Control (untreated) | Dunsinane | 4/4 | — |
| | Le Vallon | 6/6 | 44 |
| | Loolecondera | 5/6 | 20 |
| | Spring Valley | 5/6 | 24 |
| | Mousa Ella | 5/6 | 24 |
| | Mattakelle | 5/6 | 14 |
| | Bunyan | 6/6 | 55 |
| | North Punduloya | 5/6 | 50 |
| | | <u>41/46</u> | <u>231</u> |

It is clear from the results that the best control is obtained by applying DD at the rate of 2,000 lb. (170.6 gallons) per acre. 1,000 and 1,500 lb. per acre do not appear to give effective control.

Several other treatments were also tested on these estates, but the results are not presented here. Included were 1,000 and 2,000 lb. injected at 12 and 24 inches, 1,500 lb. at 12 inches, and 3,000 lb. at 24 inches.

In a few instances the total dosage was divided into two and injected at 2 levels, namely, 6 and 12 inches or 12 and 24 inches. These treatments were on the whole inferior to fumigation at the surface.

In one trial, fumigation with DD (1,000 lb./acre) was followed by inoculation with a spore suspension of *Trichoderma viride*, a fungus known to be antagonistic to *P. hypolateritia*. This resulted in slightly better control than fumigation alone but the difference was not appreciable.

Field Experiments

In conjunction with the field trials, some field experiments were also carried out in order to study, in greater detail, certain aspects of soil fumigation, in particular the effect of depth of injection of DD on *Poria* control.

In one experiment, segments of tea roots 6 inches long and 1 inch in diameter were inoculated with *P. hypolateritia* and were incubated for 3 months to permit the pathogen to penetrate the wood.

Five rectangular pits, 6 ft. long, 4 ft. wide and 4 ft. deep, were dug, and holes just over an inch in diameter and 1 ft. long were bored on the side walls of each pit. These holes were arranged in 6 horizontal rows 1/2, 1, 1½, 2, 2½, and 3 ft. below the surface, and in each row there were 5 holes spaced 1 ft. apart. An infected root segment was then inserted at the distal end of each hole and packed solidly with soil. Figure 3 shows one of these pits. Finally, the pits were filled with the loose soil and compacted well.

DD was injected at two depths, at 6 inches on one side of the pit and at 12 inches on the other, at the rate of 2,000 lb./acre. 4 pits were fumigated in this manner and one was left unfumigated and served as a check.

After 3 months, the pits were opened and all buried root segments retrieved. The viability of the fungus in the root segments was determined by incubation in moist chamber, doubtful cases being confirmed by tissue cultures from the inside of the wood.

The results obtained are given in Table 5.

TABLE 5.—Effect of depth of injection of DD on *Poria* control.
Viability of *P. hypolateritia* in infected tea roots buried at several depths

| Treatments | Root depth (feet) | | | | | |
|---------------------------------|-------------------|-------|-------|-------|-------|-------|
| | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 | 3.0 |
| A. 2,000 lb. per acre at 6 in. | 0/20 | 0/20 | 10/20 | 18/20 | 18/20 | 20/20 |
| B. 2,000 lb. per acre at 12 in. | 12/20 | 10/20 | 13/20 | 17/20 | 17/20 | 20/20 |
| C. Control (untreated) | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 | 10/10 |

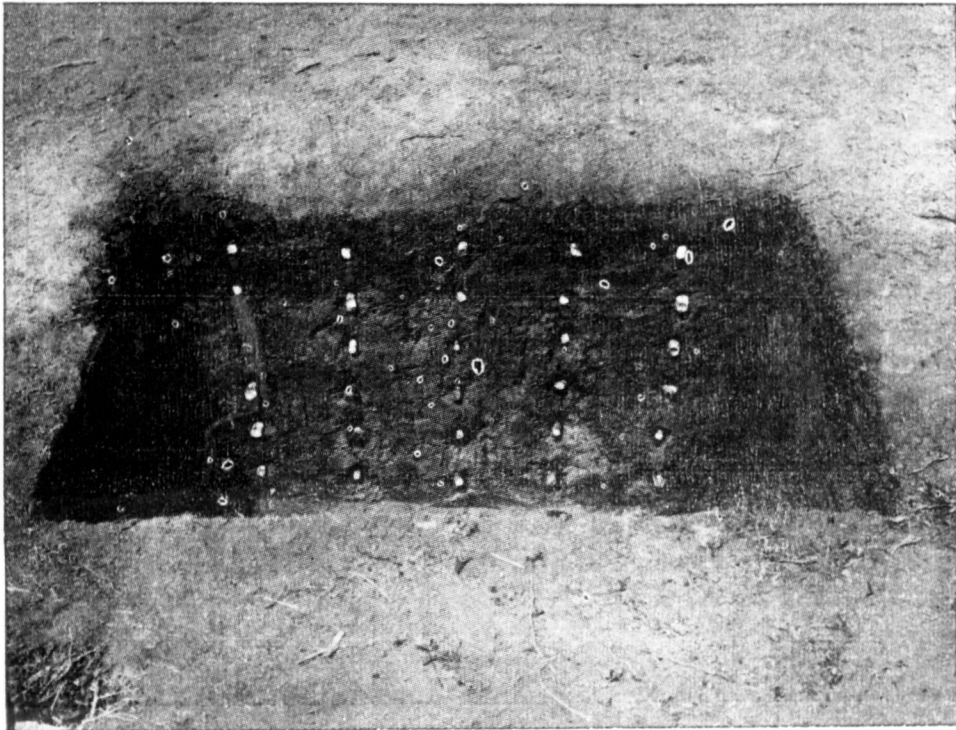


Fig 3. Pit trial to test the efficacy of DD in controlling Poria Root Disease. Picture shows one of the pits used in the trial. Note the 6 horizontal rows of holes made on one of the side-walls for placement of infected root segments prior to fumigation. For details see text.

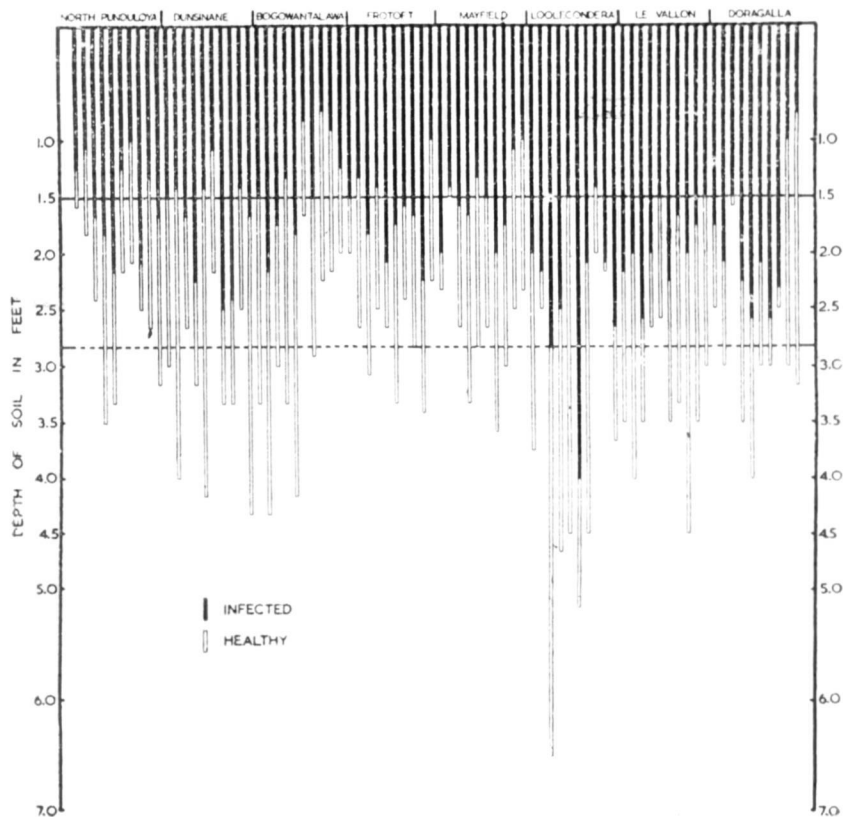


Figure 4. A diagrammatic representation of the vertical distribution of *P. hypolateritia* mycelium on the root surfaces of diseased tea bushes. Picture shows the depth of the root system of 80 diseased bushes and the portion colonized by the fungus.

It is evident from Table 5 that when injection is carried out at 6 inches, 100% control can be obtained only in the top 1 ft.; at 1.5 ft. the control obtained is only 50%, whereas at 2 ft. and below control is poor. On the other hand, with injection at 1 ft. the overall control is inferior to injection at 6 in. The control in the top 1.5 ft. is significantly reduced and there is no marked improvement in the control below 1.5 ft. It thus appears that there is no clear advantage in placing the fumigant deeper, because in doing so the control at the surface is decreased and at the same time there is no increase in the control obtained in the lower layers of the soil.

Effect of forking on fumigation

Attempts to obtain better penetration of the fumigating gases by forking the top soil with a 16 in. fork did not show any significant improvement.

In an experiment conducted at Mattakelle estate, 2 series of 10 plots each were taken and one series was deep forked, while the other was left unforked. Five of the plots in each series were then fumigated at 6 in., and the other 5 at 12 in., (2,000lb./acre).

Before fumigation, infected root segments were buried in all plots at 3 different depths, 6, 12 and 18 in. There were 5 root segments at each depth in every plot. 3 months after fumigation all root segments were recovered and the viability of the fungus determined as before. The results are shown in Table 6.

TABLE 6.—*Effect of deep forking and depth of injection of DD on Poria Control Viability of P. hypolateritia in infected root segments buried at different depths*

| Treatments | Root depth (feet) | | |
|--------------------------------------|-------------------|------|------|
| | 0.5 | 1.0 | 1.5 |
| A. Fumigation at 6 inches, forked | 1/25 | 6/25 | 8/25 |
| B. Fumigation at 6 inches, unforked | 0/25 | 0/25 | 4/25 |
| C. Fumigation at 12 inches, forked | 3/25 | 3/25 | 6/25 |
| D. Fumigation at 12 inches, unforked | 9/25 | 1/25 | 2/25 |

The overall control obtained in this experiment was better than in the previous one at both levels of injection. Again, injection at 6 in. appeared to be superior to injection at 12 in. Increased injection depth therefore does not appear to give increased control. On the contrary, as seen earlier, it results in inferior control at the surface.

There was no marked differences between the amount of control obtained in the forked and unforked plots, when injected at 12 in. On the other hand, there was a strong indication that control was less if plots were forked and injected nearer the surface. This is probably because the fumigating gases are not held long enough at the surface to kill the pathogen. It thus appears that loosening the top 16 in. of the soil will not improve matters unless the porosity of the surface layer is decreased by rolling, or by watering, or by some other manner of sealing.

Trials with Vapam

In a trial at St Coombs, Vapam was tested at the rate of 100, 150 and 200 gallons per acre. Each dosage was applied at two depths, viz. 12 and 24 in., to 4 replicates 10 ft. by 15 ft. Assessment for residual infection was made by planting out *T. vogelii*. The results are shown in Table 7.

TABLE 7.—*Relative effectiveness of different rates of Vapam in controlling Poria hypolateritia*

| Treatment (gal./acre) | Depth of injection (in.) | No. of points of infection | No. of infected plots |
|-----------------------|--------------------------|----------------------------|-----------------------|
| 100 | 12 | 32 | 2/4 |
| 150 | „ | 31 | 4/4 |
| 200 | „ | 2 | 2/4 |
| 100 | 24 | 27 | 4/4 |
| 150 | „ | 28 | 3/4 |
| 200 | „ | 31 | 4/4 |
| Control | — | 16 | 4/4 |

In this trial only one treatment showed any promise. Under the conditions of the trial, the use of 200 gallons of Vapam seems necessary to obtain maximum benefits. Clearly more field trials are necessary to assess fully the effectiveness of this product.

Discussion

In field trials conducted over a 2-yr. period DD was found to be effective in controlling *Poria* Root Disease when applied to the soil at the rate of 2,000 lb. per acre. Lower rates of application, from 1,000-1,500 lb. per acre were ineffective, whereas a larger rate, namely 3,000 lb./acre, did not show significantly increased control. The results of field experiments, however, indicate that 2,000 lb. or 170 gallons of DD applied 6 inches deep can kill *Poria* effectively only up to a depth of 18 in. When the same quantity of fumigant was applied 12 in. deep, the surface was less effectively fumigated and there was no significant improvement in the fumigation below 18 in.

For complete destruction of *Poria* it will be necessary for the DD fumes to penetrate all infested soil and to be held there in sufficient concentration for a certain length of time. Our observations indicate that the depth of infested soil is on the average not more than 30 in.

80 infected bushes in an advanced stage of defoliation were dug out with their roots intact, and the depth of the root system and the vertical distribution of the mycelium on the roots were measured. The results are shown diagrammatically in Figure 4.

It appeared from the results that the fungus was confined mainly to the top 30 in. of the soil, even though in many instances the root system extended deeper. The mean depth of the root system was 3 ft. (range 1 ft. 6 in.-6 ft. 6 in.), and the mean vertical distribution of *P. hypolateritia* 1 ft. 9 in.

Thus for effective destruction of *Poria* inoculum, one should aim at thorough fumigation of at least the top 30 in. of the soil. The present studies indicate that DD can kill *Poria* effectively up to a depth of 18 in. How are we going to deal with this layer of soil between 18 and 30 in. in depth, where most of the infection probably occurs on the deep-lying tap roots?

I would suggest that the best way to deal with this layer between 18 and 30 in. is to leave it alone and allow sufficient time for the inoculum to perish on its own. *Poria* mycelium requires a food base to feed on and in the absence of other suitable substrates the mycelium in infected roots will die soon after the food reserves in them are exhausted. At present we have no idea how long this will take, but longevity tests now in progress should give us an answer in an year or two.

However, there are indications from this experiment that *Poria* mycelium established in tea roots up to a diameter of 3 cm. can remain viable for only 2-3 years. This would imply that one would have to put off replanting after fumigation by the same period in order to ensure elimination of most of the deep-lying inoculum.

It would certainly be unwise to keep any tea soil exposed and I therefore suggest planting up all fumigated areas with *T. vogelii*. In addition to serving as an interim crop, it would also help to detect any superficial infections left over. After a year under *T. vogelii*, tea could then be planted, thus giving the new tea roots at least 2 years to reach the 18 in. 'zone'.

If on the other hand, a particular planter intends rehabilitating his land before replanting, the ideal time to do so would be after fumigation. *Guatemala* grass or *mana* grass could be planted 3 months after fumigation and grown for 1 or 2 years as required.

Tea has been planted in two of our trials; on one estate it is 2-years old and so far we have had no casualties on the plots treated with 2,000 lb. of DD.

I would like to mention here that many of the *Poria* patches on some estates have been in this condition for several years. The chances of *Poria* mycelium remaining alive in the centre of these patches is therefore very remote and in many cases one would expect to find very little inoculum even on the perimeter. In fact, most of these patches may even be completely free from *Poria*.

In most of these cases therefore an enterprising planter can by careful examination of his patches confine his treatment only to the perimeter or only to those regions where inoculum is suspected, thus effecting a considerable saving. This will, of course, mean taking a risk, but I think it is justifiable.

I would also like to take this opportunity to emphasise the need for thorough demarcation of the *Poria* patch before fumigation. We have observed in several instances that on fumigated patches most of the residual infections have been invariably present on the perimeter. The old adage "of the diseased bush plus a row or two of healthy bushes" still applies and special attention should therefore be given to the perimeter.

We have also observed that there is a tendency to neglect *Poria* cleaning in new clearings, because very often only single bushes are affected. This is a very serious matter. It is comparatively easy to control *Poria* when the tea is young, and one should therefore watch all new clearings carefully, especially if the tea is planted on old *Poria* patches or if the land was originally under jungle, and all infections should be dealt with then and there.

Well, gentlemen, that is the advice we can offer you at the moment. We will, however, continue our investigations and look for better and cheaper materials and improved methods of application.

We have also 10 large field trials with DD in progress on estates. If these trials also show the same encouraging results we have seen earlier, we will issue definitive recommendations on the use of DD for *Poria*-control before the year is over.

Now, a word or two on the cost of fumigation. The present price of DD, if bought in large drums (47 gallons), is Rs. 11/50 per gallon. For fumigation at the rate of 2,000 lb./acre, one would require approximately 170 gallons, the cost of which would be Rs. 1,955/-. We reckon that about 20 labourers would be required to fumigate an acre and the cost of labour would be about Rs. 50/-. The total cost therefore works out to just over Rs. 2,000/- for an acre.

Poria control by fumigation with DD would therefore result in a considerable saving in money and labour. The saving in labour could be of tremendous advantage to those estates which are handicapped in this respect.

Concluding Remarks

In concluding, I would like to refer briefly to two other aspects of work that have been commenced recently. Firstly, in a search for sources of resistance or immunity in tea clones to *Poria* Root Disease, 23 clones have been inoculated in a pot trial and are under observation. A few clones have already shown susceptibility to the disease. When this trial is finished, we hope to screen more clones.

You would have noticed that sometimes healthy bushes are present in the centre of very old *Poria* patches. It is not certain whether these bushes have merely escaped infection or whether they are inherently resistant to the disease. This point is being investigated.

Secondly, the role of *T. viride* in soil fumigation is also being examined. We have noticed constantly that *T. viride* developed on infected roots in which *Poria* had become non-viable following fumigation with DD. *T. viride* has also been found to be antagonistic to *P. hypolateritia* in agar cultures. Similar observations have been made by other workers, too. Bliss (1951) considers the destruction of *Armillaria mellea* by carbon disulfide (CS₂) as entirely due to the antibiotic action to *T. viride*. Recently, Darley and Wilbur (1954) have suggested that at the dosage used CS₂ could be directly toxic to *A. mellea*. These points are also under investigation.

Finally, I would like to take this opportunity to thank the Superintendents of those estates who have co-operated with us in carrying out the fumigation trials. My thanks are also due to Dr Mulder, former Pathologist, for initiating some of the field trials, and to Mr. W. W. Redlich for technical assistance.

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