

STUDIES ON THE SPREAD OF WHITE ROOT DISEASE CAUSED BY *RIGIDOPORUS LIGNOSUS* IN SRI LANKA

By

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SUMMARY

The rate of spread of the fungus in the field varied from 1.9 to 10.6 m (av. 4m) per year. The spread was generally low in the dry, compared to wet areas. Infection spreads more often within the planting row than between rows. New infections were generally noted in the vicinity of previous attacks. More laterals are present in the 0-45 cm depth of soil than in deeper layers. Infection was mostly observed on the laterals, in the first 30 cm depth. On the tap root, infections generally spread deep and have been recorded at 186 cm depth. In a majority of the collar infected trees, the tap root was infected, severely.

The size of the food base is important in the initiation and the spread of the disease. Only a few plants were infected when large infected stumps were buried near plants but even small pieces of inoculum caused infection when placed in contact with the roots and in the planting hole itself.

Infected stumps and large laterals remain viable for more than 30 months in the soil, although some decay completely. There was no obvious difference between the rate of decay under various covers during the experimental period but the viability was lost rapidly under a cover of Mimosa.

The production of rhizomorphs was greater in bare soil than in soils collected beneath different covers.

INTRODUCTION

Root diseases of *Hevea* originate in the jungle, and woody trees of a number of species belonging to many families are known to be their natural hosts. When jungles are felled for cultivation, the disease surviving in the boles and roots of previously infected trees, remain in the soil and pass on the infection to cultivated trees through root contact.

The concept of inoculum potential was first described by Bancroft (1912) who observed that a substantial food base in the form of infected timber was necessary to establish infection in rubber plants. Petch (1921, 1928) also made similar observations in the field. De Jong (1933) found that *R. lignosus* could infect young seedlings of *H. brasiliensis* with natural or artificial inocula 3 and 1.8 cu. in. in size, respectively. Alston (1953) showed that at least 5 cu. in. of infected timber was essential to cause infection of young nursery plants. Peries (1965) found that the fungus becomes self propagating and independant of the external food base only when a sufficient volume of the root is infected to take the place of the external food base. John (1966) showed that the extent of infection was directly related to the size of the inoculum.

R. lignosus may grow *in vitro* at 1.6 cm or more per day (Fox, 1965; Liyanage, Liyanage and Peries, 1977). Bouychou (1966) reported that rhizomorphs grow 2.3m a year and Fox (1971) that they can spread 3.7m a year, in the field.

The beneficial effects of creeping leguminous covers in reducing the spread of the disease is well known (Birkinshaw, 1923; Napper, 1932a; Hutchinson, 1961; Fox, 1961b; 1965a; 1971; 1977). The causal fungus can persist for up to 4 years in roots 3 in. in diameter but under cover crops it was no longer viable after 12–15 months (John, 1960).

This study was made to find out the rate of spread of the disease, with special reference to cover crops, and the effect of the latter on the decay of inoculum, under conditions in Sri Lanka.

MATERIALS & METHODS

Spread

Natural

Five apparently healthy mature trees of different ages, on the perimeter of an infected patch were selected at random from each site to study the pattern of root formation and the vertical and horizontal spread of the fungus. The soil around the plants was excavated and the lateral roots were traced to their ends, with minimum damage to them. The number of laterals in the first 0–45 cm and 45–90 cm were counted and their lengths measured. The depths at the origin and the end of each lateral were recorded, together with the spread of infection from the collar and from the end of the root and maximum depth at which infection was seen on each. The length of the tap root, the extent to which it was colonized externally, and the state of decay of the laterals and the tap root were all noted. Chips of wood removed from some of the infected stumps were planted on 2% Malt agar (MA) to determine the extent of fungal colonization of the wood.

The rate of spread of the disease was studied in several mature areas, infected by *Rigidoporus*, by mapping out the infections periodically, after collar examination of the trees. Detailed records were kept of the initial mapping and re-examinations after 12 and 24 months.

Artificial

Four field experiments were laid down to study the spread of the disease under artificial conditions, in relation to the following:

Size and position of food base in planting row

A 5-acre block of the Rubber Research Institute Estate was planted with the clone PB 86 at a spacing of 5.1 × 3.0 m. Infected stumps and large laterals 12.7–7.6 cm diameter containing viable inoculum of *R. lignosus* were buried in the planting row at two distances (60 and 180 cm) from the planting point and at two depths (30 and 90 cm). The spread of infection from these sources was recorded in the first year on the basis of foliar symptoms and from the second year onwards, by collar examination of trees.

Size and position of food base in planting hole

Four types of inoculum *viz.* large and small laterals: 5.0 cm and 2.5 cm diameter, respectively, four pieces of each; small and very small roots: 1.2 cm and 0.6 cm diameter, 6 & 8 pieces of each, respectively, were buried in the planting hole at the time of planting.

In all cases the length of the roots was 30 cm. These were placed at the edge of the planting hole at its four corners, at a depth of 30 cm.

Contact of food base with laterals

The root system of four, 7-year old PB 86 trees were exposed and infected root pieces of 0.6 cm diameter were tied with wire on to four lateral roots at a distance of 1 m from the collar and the roots were covered with soil soon after the treatment. This treatment was replicated thrice to allow for roots to be exposed 3, 6 and 9 months after placement of the inoculum. A similar number of trees and roots were inoculated following the same method, using infected root pieces of 2.5 cm; 5 cm and 7.5 cm diameter.

Effect of covers

Soil samples were collected from plots under different cover crops viz. *Pueraria phaseoloides*, *Centrosema pubescence*, *Desmodium ovalifolium*, *Calapogonium muconoides*, *Mimosa invisa*, *Stylosanthus guyanensis*, naturals and bare soil, from Parambe Estate (Parambe soil series), at two depths 0—3 cm and 30—33 cm. The soil was air dried for 24 h and sieved through a 3mm mesh sieve. The moisture holding capacity (MHC) and pH of the soil were determined and 30 g of dry soil was placed in a 9 cm petri dish and the moisture adjusted to 50% MHC. Inoculum discs, 11 mm diameter, taken from the growing edge of a 6-day old *R. lignosus* culture grown on 2% MA were placed at the centre of each petri dish on a flamed cover slip, 22 mm diameter with the inoculum downwards. When the disc was placed directly on the soil, the agar disc was contaminated and the inoculum was either destroyed or the fungal growth inhibited. The petri dishes were sealed with adhesive tape in order to retain the desired moisture level over the experimental period. The plates were incubated at room temperature for 3 weeks (RT 28° ± 2° C) after which the number and length of distinct and indistinct rhizomorphs were recorded. Each treatment was replicated six times.

DECAY OF INOCULUM UNDER DIFFERENT COVERS

Different cover crops viz. *P. phaseoloides*, *C. pubescence*, *D. ovalifolium*, *C. muconoides*, *M. invisa*, *S. guyanensis* and naturals were established in the various soil series viz. Agalawatta, Boralu, Parambe and Homagama. Pieces of infected *Hevea* roots, 25 cm long and 7.5, 5.0 and 2.5 cm in diameter were used as the three different sizes of inoculum. The fresh weight and the density of these were determined. Eight sets of inoculum were used in each experimental plot and the decay was assessed 3, 6 and 9 m after placement of the inoculum. The experiment was replicated four times in each soil series.

RESULTS

Spread

Natural

It was generally observed that in *Hevea* there are more laterals present between 0-45 cm soil depth than in deeper layers. The laterals extend at shallow depths to intertwine with roots of adjoining trees but their spread in the deeper layers appeared to be limited.

In certain instances, when soil conditions are favourable *Hevea* tap roots were traced to a depth of 262 cm. Once the tap root is infected, the vertical spread is quite rapid and infection was recorded at depths of 163 and 186 cm. In almost all the trees examined, a majority of the laterals were decayed with the infected tap root remaining. However, in some of the trees although collar infection was apparent, some laterals were free of infection while in others the infection had spread almost throughout their length. The infection was commonly seen to extend from the collar to the edge of the laterals rather than from the tip of the lateral towards the collar. In some instances infected laterals were seen just below the soil surface. Laterals in the deeper layers (79 cm) were also infected, although the mean depth at which infection was most common was between 0—30 cm. In one instance the infection on the laterals had even spread down to a depth 249 cm (Table 1). It was also observed in a few cases that fungal mycelium had grown to a height of 12 cm above ground level.

TABLE 1. VERTICAL AND HORIZONTAL SPREAD (CM) OF *R. Lignosus* IN MATURE *Hevea* TREES SHOWING FOLIAR SYMPTOMS

<i>Estate & year of planting</i>	<i>Tap root length</i>	<i>Vertical spread on tap root</i>	<i>Spread on laterals from collar</i>	<i>Spread on laterals from tip</i>	<i>Depth of infection on laterals</i>	<i>Range of depth of infection on laterals</i>
Peenkanda (1956)	177	163	105	24	31	16—65
Elston (1953)	108	108	161	96	23	13—50
Padukka (1953)	115	91	153	58	21	8—33
Urumutta (1959)	107	91	158	105	21	5—79
Nakiyadeniya (1954)	148	135	all laterals fully decayed @			
Galewatta (1953)	147	104	107	0	27	4—52
Kiriwanaketiya (1954)	178	103	155	0	31	9—61
Parambe (1955)	262	186	193	310	31	18—46
Ambadeniya (1959)	131	134	all laterals fully decayed*			
Panawatta (1949)	205	142	71	0	43	7—249
The clone was PB 86 on all estates except at Panawatta where it was a polyclone area.				@ Mean No. of laterals	13	
				* Mean No. of laterals	10	

Percentage infection of laterals was highest at 0—30 cm depth at all sites, remarkably lower at 31—60 cm and lowest at 61—90 cm (Table 2).

TABLE 2. DISTRIBUTION OF INFECTED LATERALS AT DIFFERENT DEPTHS SHOWN AS A PERCENTAGE OF THE TOTAL NUMBER OF INFECTED LATERALS RECORDED

<i>Estate</i>	<i>Depth</i>		
	0—30 cm	31—60 cm	61—90 cm
Urumutta	...	75.0	12.5
Elston	...	81.8	18.2
Kiriwanaketiya	...	60.0	20.0
Parambe	...	66.6	33.4
Padukka	...	83.4	16.6
Peenkande	...	58.4	25.0
Panawatte	...	44.0	28.0
Galewatte	...	58.0	42.0
Mean	...	65.9	24.4

The rate of spread of infection varied at different locations, under field conditions. The wet locations (e.g. Moraliyoa Estate and Kiriwanaketiya Estate) had a higher rate of spread when compared to the dry locations (Gölanda Estate, see Table 3). In most instances healthy

trees close to infected trees which are either dead or showing foliar symptoms got infected. The healthy trees that came in contact with infected stumps which are not fully rotted also were infected.

TABLE 3. RATE OF SPREAD OF INFECTION IN MATURE *Hevea*

<i>Estate, Clone and year of planting</i>	<i>Patch no.</i>	<i>Spread(m) in 1 year</i>	<i>Range of spread</i>
Moralioya RRIC 45 (1960)	1	4.6	2.2 — 8.7
Moralioya PB 86 (1959)	2	4.4	2.5 — 7.5
Kiriwanaketiya RRIC 52 (1962)	1	6.3	3.8 — 10.6
Kiriwanaketiya PB 86 (1960)	2	3.7	2.6 — 5.0
Golinda PB 86 (1959)	1	2.5	1.9 — 4.0
Golinda PB 86 (1959)	2	3.8	2.8 — 6.3
Golinda PB 86 (1959)	3	3.1	3.1 — 4.1
Golinda PB 86 (1959)	4	3.7	3.4 — 3.8
		Mean	4.0

Artificial

Size and position of food base in planting row

None of the plants showed foliar symptoms due to *Rigidoporus* infection after 8 months. After 1½ years only two plants were dead due to the infection and only one showed foliar symptoms. At this stage all the plants that were lying on either side of the introduced inoculum were collar examined. This revealed two more plants which were collar infected but not showing any foliar symptoms. Upto this time, out of a total of 480 plants only five were infected and the infection had originated either from stumps placed at the centre of two planting points (180 cm away) or the large laterals placed 60 cm and 180 cm away from the planting point. Infection was seen only in plants adjacent to the inoculum indicating that in 1½ years the infection had not spread to the plants in the row, through contact. Thirty five percent of the inoculum was fully decayed and not viable after 1½ years while 45 and 20% respectively were partially and slightly decayed, but infective at the same time.

Size and position of food base in planting hole

Large inocula caused 20% infection but small roots did not cause any infection in the first 6 months. At this stage the inoculum remained viable except in the smallest roots (0.6 cm diameter), which were completely decayed leaving only a few pieces of bark in some instances.

After 1 year 80, 40 and 20% infection was recorded with inocula of diameter 5.0, 2.5 and 1.2 cm, respectively. At this stage most of the 5.0 cm and a few of the 2.5 cm size inocula remained viable and were only partly decayed (Table 4).

TABLE 4. PERCENTAGE INFECTION OF BUDDED STUMPS, SIX MONTHS AFTER INTRODUCING THE INOCULUM IN THE PLANTING HOLE

<i>Inoculum diameter (cm)</i>	<i>% Infection</i>
0.6	0
1.2	20
2.54	40
5.0	80

Contact with laterals

Only external growth of rhizomorphs was seen on some of the inoculated roots after 3 months. There was no internal infection in any of the roots, even those inoculated with the largest inocula (7.5 cm diam.). However, the fungus grew profusely on the surface even from the smallest inoculum pieces. After 6 months there was no increase in the number of roots showing external symptoms, but internal spread of the fungus was recorded at this stage.

As expected, the larger pieces of inoculum produced more fungal activity *e.g.* a piece of inoculum 7.5 cm diam. caused an external growth of mycelium 178 cm long and an internal spread of infection to a distance of 54 cm. In a few instances the edge of the laterals was also decayed due to infection (Table 5).

TABLE 5. DEVELOPMENT OF DISEASE ON INOCULATED ROOTS, MEAN SPREAD ON LATERALS (CM)

Inoculum diameter (cm)	3 months after inoculation		6 months after inoculation		
	Towards collar	Away from collar	Towards collar	collar	Away from collar
	External infection	External infection	External infection	Internal infection	External infection
1.0	21.7	51.0	0	0	0
	0	0	0	0	0
	0	0	40.5	23.0	0
	0	0	20.0	0	0
2.54	0	0	22.0	0	*
	0	0	0	0	0
	0	0	0	0	0
	79.5	30.0	0	0	0
5.0	79.0	96.0	53.3	0	45.0
	64.3	53.5	70.0	0	30.0
	59.0	68.0	0	0	0
	0	0	128.0	18.0	61.0
7.5	0	0	178.0	54.0	*
	61.0	74.0	66.5	28.5	*
	0	0	88.5	0	0
	76.7	52.0	0	0	0

There was no internal infection at all after 3 months and away from the collar after 6 months.

* Part of the lateral decayed.

Small inoculum pieces 1 cm diameter caused infection in 15.4% of roots and with the largest size (7.5 cm) 30% of the roots were infected (Table 6).

TABLE 6. PERCENTAGE INFECTION OF LATERAL ROOTS OF 7 YEAR OLD *Hevea* TREES AFTER ARTIFICIAL INOCULATION

Inoculum diameter (cm)		Percentage infection after inoculation				
		3 months		6 months		
		External	Internal	External	Internal	
1.0	(24 cu.cm)	...	28.6	0	23.0	15.4
2.5	(100 cu.cm)	...	16.7	0	7.7	0
5.0	(200 cu.cm)	...	50.0	0	41.7	8.3
7.5	(400 cu.cm)	...	30.8	0	40.0	30.0

Effect of covers

Generally, the pH of surface soils under covers was lower than that of bare soils, except where naturals and *C. pubescens* grew. At greater depth, however, there was no marked difference in soil pH under different covers but it was generally lower than in the surface soil. The maximum number of rhizomorphs were produced on soils collected from the surface of a clean weeded bare area. However, more rhizomorphs grew on soils taken from under *P. phaseoloides* and *C. muconoides* than other covers. A similar pattern was observed on soils taken at 30 cm depth. The growth of rhizomorphs showed a high degree of variability on soils collected at both depths (Table 7).

TABLE 7. PRODUCTION OF RHIZOMORPHS OF *R. Lignosus* ON SOILS COLLECTED FROM PLOTS OF DIFFERENT COVER CROPS AT TWO DEPTHS, 0 AND 30 CM

Cover crop	No. distinct rhizomorphs		Mean length distinct rhizomorphs(cm)	
	Actual mean	Log. transformed mean	0cm	30 cm
<i>Centrosema pubescense</i>	0	-0.69	0	0
<i>Stylosanthus guyanensis</i>	0	-0.69	0	0
<i>Mimosa invisa</i>	0.33	-0.37	12.08	5.33
<i>Desmodium ovalifolium</i>	0.42	0.33	0	33.17
Naturals	0.67	0.12	0	40.65
<i>Pueraria phaseoloides</i>	1.17	0.08	21.62	16.92
<i>Calapagonium muconoides</i>	1.13	0.11	13.15	16.08
Bare soil	4.58	1.60	55.87	71.42
LSD at 5%	—	0.49		24.62

Analysis of variance of the number of distinct rhizomorphs showed no significant difference between the two depths. Therefore, the number of rhizomorphs for both depths have been taken together in deriving the mean.

DECAY OF INOCULUM UNDER DIFFERENT COVER CROPS

Under a cover of *Mimosa invisa* the decay and the viability were less at 6 months, compared to other cover crops. However, after 9 months most inocula used in the experiment were not viable (Table 8).

TABLE 8. PERCENTAGE VIABILITY AND PERCENTAGE DECAY OF INFECTED ROOT PIECES 6 MONTHS AFTER PLACEMENT UNDER DIFFERENT COVER CROPS

Cover crop	Inoculum size	Mean viability %	Mean decay %
<i>Centrosema</i>	100	53.3	58.3
	200	25.0	33.3
	400	41.7	33.3
<i>Calapagonium</i>	100	31.2	50.0
	200	18.7	41.7
	400	25.0	41.7
<i>Mimosa</i>	100	12.5	25.0
	200	6.2	18.7
	400	25.0	31.2
<i>Stylozanthus</i>	100	18.7	50.0
	200	37.5	50.0
	400	43.7	31.2
<i>Pueraria</i>	100	18.7	43.7
	200	37.5	31.2
	400	43.7	50.0
<i>Desmodium</i>	100	25.0	68.7
	200	37.5	31.2
	400	62.5	37.5
Naturals	100	12.5	43.7
	200	31.2	31.2
	400	31.2	25.0

DISCUSSION

In the present studies, conducted in several infected patches under varying soil and rainfall conditions, the rate of spread of *R. lignosus* under natural conditions varied from about 1.9 m to 10.6 m (average 4 m) per year. This is higher than that recorded by other workers (Bouychou, 1966; Fox, 1971). Fungal growth seems to be slower in dry than in wet districts.

This study clearly showed that the infection did not spread in concentric circles. This is because the rate of spread was faster within, than between rows. The planting distances in the trial area resulted in the plants within the row being closer to one another than those between rows; therefore, root contact within the row was more frequent. Infection was more common in the vicinity of diseased trees although this relationship was not clear in all cases. This may result from differences in the rate of root invasion of individual trees, their inherent resistance, and to spontaneous recovery as observed by many workers (Reydon, 1931; De Jong, 1933; Newsam, 1962; Fox, 1965a).

In collar infected trees the tap root was invariably severely infected although only some laterals were infected occasionally. Therefore, it is essential that, in an area where *Rigidoporus* infection is present, tree stumps should be eradicated together with the entire tap root. It is important, to note that, in a majority of cases, the infection had spread from the collar outward to the laterals, rather than the other way. Therefore, apparently the collar gets infected from an external food base either direct or through a lateral, which readily decays. The infection then spreads from the diseased collar region to the remaining laterals.

Artificial field inoculations indicated that the infection spreads more commonly from the point of contact on a lateral towards the collar than towards the tip of that lateral. This may be because the tap root has larger reserves of starch than the ends of the laterals and the fungus grows along the medullary rays towards a food source (Peries and Irugalbandara, 1973).

It has always been surmised that the fungus can remain viable in large stumps for several years, but no data were available to date on duration of fungal survival in infected root pieces either buried in soil or lying under cover crops. This is a point of considerable importance in relation to root disease control in a replanting, particularly after clearing by mechanical methods which could leave infected root fragments widely distributed in soils. In this study fragments of small roots (approx. 24 cm³) buried in the planting hole, decayed in 6 months without causing infection of the new plants. This may be due to the root system of the plants not coming into contact with the source of infection. It was noted that only small roots (0 - 3mm diam.) were formed upto the time the plant is 6 months old and that these were generally confined to the 0 - 15 cm layer of soil. In these experiments the inoculum was buried at a depth of 30 cm, which is deeper than the early rooting zone. Other field experiments indicated that inoculum of similar size (24 cm³) can cause superficial growth of mycelium on roots of 7-year old plants, in the first 3 months if they were tied to the roots, this progresses to internal infection in 6 months; but after 9 months, only a few plants were infected and those too were from the larger pieces of inoculum used. This clearly showed that, although small pieces of inoculum can bring about superficial infection, the fungus becomes self propagating only when a sufficient volume of the root is infected to take the place of the external food base which decays in time. Similar observations have been made by Peries (1965), and Altson (1953) who concluded from seedling inoculations that only diseased material larger than 2 or 3 cm diam. (30 - 50 cm³) can cause infection and therefore, need be eradicated.

In the present investigation, inoculum pieces 2.5 cm (approx. 100 cm³) and 5.1 cm (approx. 200 cm³) in diameter remained viable for nearly 1½ years. Some of the stumps and the large laterals (approx. 400 cm³) remained viable for over 2½ years. John (1958) found that root sections from 12 - 61 cm³ used as inocula were partially or completely disintegrated after

burial in the soil for 1 year. Special attention must be paid to stumps and large laterals as they remain viable for long periods, during which the root system of the new plants is fairly extensive. The current studies have shown that the roots begin to grow out of the planting hole in 6 months and in 9 months 75% of the small laterals had grown beyond the perimeter of the planting hole.

The beneficial effects of creeping leguminous covers in reducing disease incidence as shown here, have been extensively reviewed (Hutchinson, 1961; Fox, 1961b; 1965a; 1971; 1977). Napper (1932a) reported that the overall incidence of *R. lignosus* with three different leguminous cover plants was half that where the ground was clean weeded. In the present studies, *R. lignosus* inoculum was not viable after 9 months under cover plants. Decay of wood was slow but fungal viability was lost rapidly under *Mimosa* spp. Although a change in the density of the inoculum would give a good estimation of the decay, it was not possible to get accurate values for density; because in the process of decay, particles of soil and leaf debris got collected within the crevices of decaying wood giving erroneous results. Therefore, decay was assessed on visual observation.

Fox (1965a, 1977) found greater growth inhibition, mycelial lysis and inoculum destruction of *R. lignosus* by soil under two covers as compared with the soil from a bare or clean weeded area. In this study it was observed that rhizomorph production was greater in bare soils than in soils under different covers. It appears that ground covers condition the soil to favour the development of micro-organisms antagonistic to root parasites, thus militating against the activity of the pathogen. This desirable action of creeping legumes should be exploited for root disease control too, quite apart from the other multifarious benefits of legume covers. It would be of special benefit where the method of clearing the old stand has been poor and left behind infected material in the soil.

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