Some Observations on Spore Infection of Hevea Stumps by Fomes lignosus (Klotzsch) Bres.

K. P. JOHN

Observations on spore production, spore viability and spore infection of stumps by Fomes lignosus, *recorded in part in annual reports of the Pathological Division of the R.R.I.M. between 1948 and 1955, are brought together with some more recent findings.*

Before the economic depression in the early thirties, the usual method of preparing land for replanting was by digging the whole area over to a depth of 18 to 24 inches, after winching out the trees and removing all roots from the soil, whether healthy or diseased (RUBBER RESEARCH INSTITUTE OF MALAYA, 1937). This method was very expensive and in later years it was the custom to dig over the disease patches only; in the healthy areas trees were jacked out and burned, any broken off lateral roots being left to rot away naturally. Occasionally healthy trees were merely felled a foot or two above ground level, to reduce replanting costs, the stumps remaining *in situ*. When *Fomes lignosus* infections were later traced to the stumps thus left in a replanting, it was thought that the stumps had either contained the causative agent in a dormant form or became infected by spores, although there was no direct evidence to support either hypothesis.

Opinions have differed in the past as to the production of spores by *F. lignosus* and their possible role in colonising stumps, so spreading white root disease. It had been reported, (NAPPER, 1932), both in Malaya and Ceylon that the fructifications of *F. lignosus* were either sterile or infrequently fertile. Napper found, however, that under certain climatic conditions fructifications produced large numbers of viable spores and deduced that wind dispersal of these spores could be a cause of infection. Nevertheless, when young trees of a replanting became infected in places where the old rubber stand had apparently been healthy, Napper continued to attribute such infections to dormant sources in the original stand, but pointed out that spore infection of the partially exposed and highly susceptible green root fragments could not be entirely disregarded (RUBBER RESEARCH INSTITUTE OF MALAYA, 1939).

However, in a field where incidence of *F. lignosus* was high during the early years after planting, the RUBBER RESEARCH INSTITUTE OF MALAYA (1950) was unable to detect the presence of dormant sources of infection on severed roots of 20-year-old trees, and this has been the author's experience since 1936.

In the past, rubber planted on land long used for sugar cane or cereal cultivation or abandoned to *lalang* (*Imperata cylindrica*) was entirely free from root diseases, but when these trees were felled and replanted, root disease (particularly that caused by *F. lignosus*) was common and was often traced to the stumps of the old stand. The logical inference was that these stumps had become infected by wind-borne spores.

The first direct evidence of the colonisation of stumps by spores of *F. lignosus* came in 1953, when an area of old rubber covered with a heavy growth of secondary jungle was cleared for planting, three years after felling. Two stumps that were found to be infected with the fungus were closely examined to find the direction of its spread; in both the infection had started at the cut surface. One of them had a heavy growth of rhizomorphs and fructifications at the top (33 in. above ground level) with no trace of

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the fungus on the roots. The other stump, which was dead down to the collar, had rhizomorphs growing downwards from the top and outwards along the lateral roots. Both cases clearly indicated that the infection had been spore borne and had originated at the cut surface.

The following observations and experiments were made to find out whether freshly cut roots or stumps could become infected by *F. lignosus* spores and if so under what conditions.

SPORE PRODUCTION

Regular collections of fructifications of *F. lignosus* were made from two estates in Selangor during the second half of 1949. Viable spores were produced in abundance from fructifications of all ages during the six months June to December. One, which was only an inch across, produced abundant spores; another, measuring 14×9 in. and more than six months old, riddled by boring beetles, still produced a good number of spores. For these observations the fructifications were kept in a damp chamber over glass slides, and it was noticed that most of the spores were discharged during the night, the heavy white deposit being easily visible to the naked eye. Spore production of these detached fructifications virtually ceased about 24 hours after collection.

Viability of Spores After Storage

In the same year (1949) spores from fresh fructifications were collected overnight on $\frac{3}{4}$ in. square cover slips and stored either at laboratory temperatures (26°C to 32°C) or in a refrigerator at 5°C. An attempt was made to determine their viability by adding a drop of sterile water to each cover slip and examining after a further 24 hours in a damp chamber, but uniform germination was not obtained; only a few spores germinated at the periphery of the water drop. Only slightly better results were obtained when the cover slips were lightly sprayed with sterile water, the tendency being still for the spores to germinate only at the periphery of water droplets. This is in agreement with Tompkin's findings reported by GOTTLIEB (1950) that some spores germinate equally well or better in a saturated atmosphere than when floating in water and hardly germinate if submerged. Inverting the dry cover slips on to a corn-meal agar surface in petri dishes, however, gave uniform and good germination in less than 24 hours. NAPPER (1932) had also used corn-meal agar for spore germination and found it quite satisfactory. FOX (1960) obtained good germination with spores streaked on to the surface of tap-water agar.

As a general rule the germination rate fell off rapidly from the third to the fifth day in the laboratory, and from the second to the fourth day in the refrigerator. Differences due to the source of the spores were more pronounced; while nearly all spores from some fructifications germinated, only a few did so from others.

ARTIFICIAL INOCULATION

Artificial Inoculation Experiments with Cut Roots and Stumps in Pots

Seven freshly-cut, healthy root sections, surrounded by a little soil which was collected from under actively growing fructifications of *F. lignosus* and passed through a $\frac{1}{25}$ in. mesh sieve to exclude root fragments, were placed in three pots of sterilised soil. The pots were kept watered in the laboratory, for four months. Isolations made from the root sections failed to reveal *F. lignosus* but one root produced *Poria hypobrunnea* Petch.

Sections of fresh healthy roots were buried vertically, with one end exposed, in six pots containing sterilised soil. Fresh fructifications were placed over them, supported by a fine-mesh sieve, and left there for 48 hours. The pots were kept watered in the laboratory for four months but no infection by *F. lignosus* developed.

Three to five short sections of healthy roots were buried horizontally in sterilised soil at a depth of four inches in each of five 10-inch pots; one root section, in contact with the others, was placed vertically so as to protrude above soil level. For two months the pots were kept under actively growing fructifica-

tions of *F. lignosus* in the field, then taken to the laboratory and kept watered for a further two months. In two of the five pots white mycelium (confirmed by isolations as *F. lignosus*) was present on the surface of all roots, and one of them exhibited a rot typical of *F. lignosus*.

Three six-month-old rubber seedlings growing in soil in three pots were cut near soil level and kept under actively sporing fructifications of *F. lignosus* in a damp chamber for 72 hours, after which they were kept in the laboratory and watered. None of the stumps had become infected when examined three months later.

In 1950 *F. lignosus* was induced to grow and produce rhizomorphs on short lengths of freshly cut decorticated young rubber stems that were inoculated with spores and incubated in a moist chamber. Although the fungus did not penetrate the wood, the finding considered in conjunction with those recorded above lends some support to the suggestion that the stumps of a healthy rubber tree may become invaded by *F. lignosus* through infection of its cut surface by airborne spores. It is interesting to compare the findings of FOX (1960) of extensive penetration of the wood leading to a typical *F. lignosus* rot in lengths of tap roots of young trees surface-sterilised and kept under aseptic conditions but inoculated with a large volume of a pure culture of the fungus.

Artificial Inoculation of Stumps in the Field

In 1955, in an attempt to infect cut surfaces of tree stumps with spores, thirty-one six-year-old budded trees due for thinning in Field 48 on the Experiment Station were felled a few inches above ground level and the cut surfaces were sprayed with a heavy suspension in distilled water of spores of *F. lignosus* obtained in the laboratory from fresh fructifications growing on a stump kept permanently wet. After inoculation the stumps were covered in various ways—with brown paper, soil, mulch, or a combination of these —while two inoculated stumps were left uncovered. At the same time a similar spore suspension was applied to wounded lateral roots of eight other stumps, from five of which the wounded roots were also severed, the roots being covered with soil but the stumps left exposed.

The condition of the stumps and roots six months after inoculation is presented in *Table 1*. Where colonisation had occurred the fungus had travelled superficially 8 in. to

TABLE 1. RESULTS OF INOCULATION OF STUMPS AND ROOTS WITH SPORES OF F. LIGNOSUS

Treatment	Number treated	Number infected with				
		F. lignosus	*F. noxius*	*U. zonata*	*S. repens*	*P. hypobrunnea*
Stumps						
Brown paper	4	–	–	–	–	1
Brown paper+soil	6	3	–	–	–	1
Brown paper+mulch	7	–	1	–	–	2
Mulch	4	–	–	1	–	3
Mulch+soil	4	2	–	–	–	1
Soil	4	2	–	–	–	–
Uncovered	2	–	–	1	–	1
Roots						
Wounded+soil	3	1	–	–	1	1
Severed and wounded +soil	5	1	–	1	–	3

42 in. down the tap root and 2 in. to 36 in. out along the lateral roots. Some of the infected stumps were dead to the extent of only a few inches from the top, but the majority of them were wholly dead above ground and penetration had progressed down the tap root and along the laterals for varying lengths, with a maximum of 24 in. *F. lignosus* was successfully re-isolated from all infected stumps and roots and one year after inoculation the stumps produced fructifications in abundance.

DISCUSSION AND CONCLUSION

There is no evidence to support the suggestion that early infections of *F. lignosus* remain dormant in the roots of mature healthy trees to become active when the trees are felled. In fact minor infections are cut off by gum barriers and the infected wood becomes penetrated by feeding roots and saprophytic fungi, the tissue eventually being converted to humus while callus seals off the wound. Healed root lesions invariably have some discoloured wood which is usually sterile, beneath the callus growth, though occasionally a fungus or bacterium can be found there. If, however, an infection was present on the roots at the time of felling a tree, it would be expected to spread quickly on the moribund stump.

Laboratory experiments on spore infection of cut roots and stumps in pots always failed; the only success was obtained in an experiment with cut roots in pots which were left in the field for 2 months, continually exposed to fresh spore infection.

In the field experiment it is noteworthy that infection by *F. lignosus* took place only where there was a soil covering of the stumps or wounded roots, whether or not there was also a mulch or brown paper covering. Whereas a few of the stumps covered with soil were found to be partially exposed six months after the treatments, all the stumps covered only with mulch or brown paper were by that time fully exposed, the covering having disintegrated, and no infection by *F. lignosus* had taken place in any of them. The soil placed over the stumps had apparently helped in some way to maintain conditions suitable for spore colonisation. It is relevant to add that over a hundred untreated stumps of thinned out trees in this area did not become infected by *F. lignosus* though most of them were colonised by *P. hypobrunnea.*

The results incidentally show that stumps are liable to spore infection by *Fomes noxius* Corner, *Ustulina zonata* (Lev.) Sacc., *Sphaerostilbe repens* Berk. *et* Br., and *P. hypobrunnea* in addition to common wood-rotting saprophytes, some of which have been named in a previous paper (NEWSAM *et al.*, 1961). The failure of *F. lignosus* to colonise all inoculated stumps or roots may have been due to its inability to compete with the other organisms. Evidence of mutual antagonism or exclusiveness was found in stumps invaded by *F. lignosus*, *F. noxius* and *P. hypobrunnea.* Antagonism has been exploited by RISHBETH (1963) who has found that inoculating pine stumps with oidia of the wood-rotting basidiomycete *Peniophora gigantea* (Fr.) Masee gave good protection against *Fomes annosus* Fr., checking the advance of the latter in tissues infected at the time of felling or to some extent replacing it.

There is ample direct and indirect evidence to show that spore infection of stumps by *F. lignosus* is of common occurrence, though the conditions favouring infection are not clear. Of course infection will depend on the availability of spores in abundance, their viability, virulence and the condition of the stumps. There is no doubt that the incidence of *F. lignosus* in a replant where the old stand has been free from disease is brought about by spore infection of the stumps of felled trees of the old stand and, later, of those of thinned out trees of the new stand. It is evident that protection of stumps against spore infection is an important aspect of root disease control.

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TABLE 1. DETAILS OF EXPERIMENTS

Site and experiment	Soil type	Planting distance, ft	Plot size	Trees per acre: Initial	Trees per acre: Final	Planting material and date of planting/budding	Design	Treatment levels in lb per tree per annum of (1) Ammonium sulphate (2) Christmas Island rock phosphate (3) Potassium chloride
Experiment F Flemington Estate	Selangor series coastal alluvium	Straight line planting—20×10	0.54 acre	112	78	See text. Planted Oct. 1949 budded Oct.–Nov. 1950	3×2×2 NPK factorial: three replicates in each planting system	(1) 0, 2, 4 (2) 0, 1½ (3) 0, ½
		Double row hedge planting—60×5×5	0.36 acre	130	74			First application: March 1957
Experiment SG R.R.I.M. Experiment Station, Sungei Buloh	Rengam series sandy clay loam granite-derived	60×4½	0.61 acre	120	117	Tjir 1, BR 2, PB 49, AVROS 157, RRIM 509 Planted 1949 budded May–July 1950	3^3 NPK factorial single replicate	(1) 0, 2, 4 (2) 0, 1½, 3 (3) 0, ½, 1 (3) 0, ½, 1 First application: April 1957
Experiment SP Sepang Estate	Seremban series loam schist-derived	22×11	1.00 acre	115	115	PB 86 Planted 1948 budded Sept.–Nov. 1949	3^3 NPK factorial single replicate	(1) 0, 2, 4 (2) 0, 1½, 3 (3) 0, ½, 1 First application: May 1959

TABLE 2. EXPERIMENT F: EFFECT OF AMMONIUM SULPHATE ON SEED PRODUCTION (NUMBER OF SEED PER TREE)

(Total seed fall as percentage of control in parenthesis)

Type of planting	Treatment	PEAK SEED-FALL COLLECTIONS										Total
		1957 2.8–30.8	1958 25.8–22.9	1959 11.2	1959 29.10–9.12	1960 —	1961 19.1–28.2	1961 22.8–27.9	1962 4.9–29.9	1963 9.2–16.3	1963 2.9–7.10	
Straight line	No. of collections	3	4	1	6		11	6	4	6	6	
	n_0	15.9	38.8	17.0	31.5	—	75.2	94.1	53.9	56.6	69.1	452.1 (100)
	n_1	14.2	39.9	20.4	47.1	—	91.1	82.5	51.3	77.0	81.1	504.6 (112)
	n_2	13.2	44.1	27.0	57.6	—	113.9	82.2	51.5	120.0	82.8	592.3 (131)
	Sig. of $n_2 - n_0$ effect	*		**	***		**			***		
	s.e.	±0.85	±4.22	±2.26	±3.92		±8.92	∓13.44	±4.19	±8.21	±7.02	
	Min. 5% sig. diff.	2.5	12.4	6.6	11.5		26.2	39.4	12.3	24.1	20.6	

		TOTAL ANNUAL COLLECTIONS							
		1957	1958	1959	1960	1961	1962	1963	
Straight line	No. of collections	11	10	19	2	22	7	22	
	n_0	21.9	44.1	102.5	7.3	188.6	61.7	151.4	577.5 (100)
	n_1	22.2	46.8	126.1	6.0	196.0	59.8	185.6	642.5 (111)
	n_2	18.8	53.5	153.9	5.8	221.2	59.1	238.1	750.4 (130)
Hedge	n_0	16.7	27.9	109.7	2.3	146.3	26.7	131.5	461.1 (100)
	n_1	17.0	33.0	114.9	2.8	174.5	29.4	178.3	549.9 (119)
	n_2	15.1	36.0	133.9	2.5	175.7	27.7	199.8	590.7 (128)

Effects non-significant except where indicated: ***P < 0.001, **P < 0.01, *P < 0.05

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Rubber Research Institute of Malaya
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