THE ROLE OF THE BASIDIOSPORE OF *RIGIDOPORUS LIGNOSUS* IN THE SPREAD OF WHITE ROOT DISEASE OF *HEVEA*

By

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SUMMARY

A study was made on the role of the basidiospores in causing new foci of infection. Spore inoculation of root piece and stem discs of Hevea *was successful* in vitro *but not seedlings in pots. Basidiospores do not colonize stumps of* Hevea *in the field, under natural conditions. In dry soil they lost viability very rapidly. There was no evidence of basidiospores colonizing field litter such as cover crop leaves, grass, plant debris and dried bark of* Hevea.

INTRODUCTION

White Root disease caused by *Rigidoporus lignosus* (Klotz.) Imazeki, is at present the most serious disease of rubber in Sri Lanka. The disease spreads mainly from old jungle or rubber stumps to the living roots of healthy trees by contact. A second source of infection can be through the basidiospores released from sporophores formed on colonized stumps of timber. There has been some uncertainty over the years regarding the role of the basidiospore in the spread of this disease. Petch (1927) surmised that airborne basidiospores of *R. lignosus* are capable of infecting stumps of jungle or rubber trees, thereby introducing the pathogen into previously disease free areas. Although Napper (1932a) thought that the fructifications of *R. lignosus* were either sterile or infrequently fertile, he found that under certain climatic conditions, fructifications produce a large number of viable spores and he deduced that wind dispersal of these spores could be the cause of infection. The early attempts to cause infection of stumps of *Hevea brasiliensis* (Muell-Arg.) in Malaysia using a basidiospore suspension of *R. lignosus* was unsuccessful (Newsam, 1962). However, John (1965) achieved some success when the inoculated stump surfaces were covered with soil. Later, Lim (1976) showed conclusively that the basidiospore can cause new centres of infection.

The Rubber Research Institute of Sri Lanka (RRISL) has studied all aspects of this disease in detail to develop methods for its biological control. This paper reports the results of studies on the role of the basidiospore in the spread of the disease.

MATERIALS AND METHODS

Spore inoculation

In vitro inoculations with basidiospores were carried out on moist autoclaved pieces of *Hevea* roots, approximately 1 cm diameter. The freshly collected sporophores were placed over the mouths of Erlenmeyer flasks containing autoclaved root pieces. These were left for 6 h, as it was found in preliminary experiments that sufficient numbers of spores are released in that time for successful inoculation. The flasks were then plugged with cotton wool and incubated at room temperature (RT 28°C$\pm$2°).

Sporophores were placed on freshly cut non-autoclaved discs of *Hevea* stems, approximately 10 cm diameter. Similar discs were also inoculated by placing 10 ml of spore suspension (10^4/ml) on the freshly cut surface. These were placed in clean polythene bags soon after inoculation and incubated at RT. A set of discs kept moist with 10 ml of distilled water, served as controls.

Spores were allowed to fall on dry autoclaved soil and the soil was adjusted to 50% moisture holding capacity (MHC), daily, upto thirty days. To test desiccation of spores in dry soil after different periods, autoclaved root pieces were introduced into the tubes at the time of adjusting the MHC. Observations were made on the development of hyphae on root pieces from germinated spores, where the visible mycelium would confirm spore germination and thereby indicate the viability of the spores after exposure to dry soil conditions.

In vivo inoculations were carried out using one year old seedlings of clone PB 86. These were uprooted carefully without injury to the root system and washed free of soil in running water. The root system of half the number of plants were injured by cutting away a portion of the tap root and the feeder roots with a flame sterilized pair of sharp scissors. Further injury was done on the surface of the tap root by shaving away the bark at a few points with a sterilized scalpel. The roots of these plants, with and without injury, were dipped in a viable spore suspension (10^4/ml) for periods of 16 and 24h. A spore suspension was added for 3 days to pots containing plants with injured roots. These were planted twenty per treatment, both in autoclaved and non-autoclaved soil and uprooted periodically after planting, to determine the percentage of infection.

Freshly cut stumps were placed alongside infected stumps bearing numerous sphorophores, in the field, so that the spores released could settle on the freshly cut surface of stumps.

Results

Spore inoculation

Basidiospores when allowed to settle on autoclaved root pieces in Erlenmeyer flasks, produced the characteristic mycelium of *R. lignosus* in about 10—14 days (Fig. 1) in eight out of the ten flasks. In some of these, rhizomorph production was evident after about 4 weeks. A slight discolouration in the internal tissue was also noticed in a few of the pieces indicating penetration of the hyphae into the wood tissue. These cultures could not be maintained free of contamination for periods longer than three months.

Spore inoculations of the stem discs also gave positive results (Fig. 2). Although the discs were not autoclaved 90% of the inoculations were successful. A thick mat of fungal mycelium was seen much earlier than with the autoclaved root pieces. The discs used as the control were contaminated most commonly with *Aspergillus* and *Penicillium* spp., a few days after the inoculation. Although the basidiospores germinated and produced mycelial growth on the inoculated surface of discs, many other types of fungi appeared on the non-inoculated surface of the same discs. This resulted in contamination of the inoculated surface within about 4 weeks of inoculation.

Spores when dusted on to dry autoclaved soil, lost their viability very rapidly. The longest period of desiccation after which mycelial growth was recorded on root pieces was 24h in dry soil and there too the success was only 10%. The spores left for longer periods: 48, 72, 96, 120 and 144h did not germinate and cause infection of autoclaved root pieces.

Ten infected stumps exposed to natural conditions in a mature field, produced numerous sporophores releasing a large number of viable spores. Freshly cut stumps placed alongside the stumps with sporophores were not infected, although exposed for 1 year. Wood chips taken from such exposed stumps at various positions did not produce the fungus on malt agar.

The plants subjected to spore inoculations and then grown in autoclaved or non-autoclaved soil did not produce foliar symptoms of the disease. Also, there was no mycelial growth on the roots, three and six months after inoculation. These plants were then left for a further

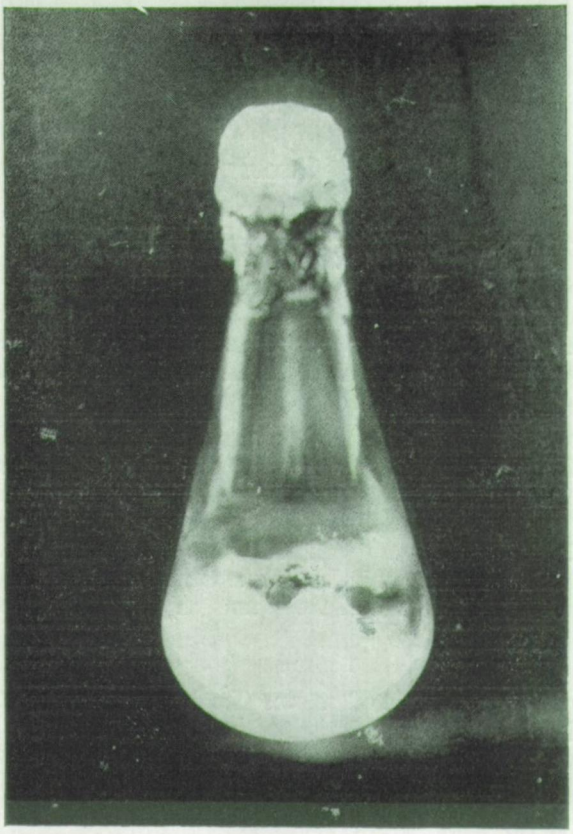

Fig. 1. *Fungal growth on autoclaved root pieces, 3 weeks after dusting with basidiospores.*

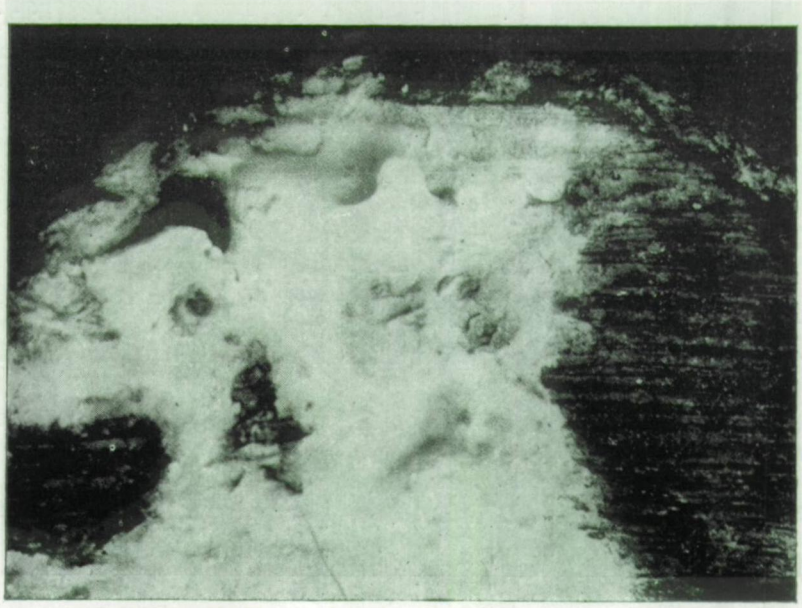

Fig. 2. *A thick mat of fungal growth on freshly cut stem discs inoculated with a suspension of basidiospores.*

period of 6 months but were free of disease. However, of the twenty injured plants inoculated by pouring a spore suspension for 3 days, one plant had rhizomorphs on its root system. This did not show typical symptoms of White Root disease but had only mycelial strands similar to *R. lignosus*. This also did not fulfil Koch's postulates.

Discussion

Opinions have differed in the past regarding the role of basidiospores in the spread of white root disease. Successful inoculations were possible in the laboratory under sterile conditions, and also with freshly cut wood discs when exposed to inoculum in the laboratory. However, it was not possible to cause infection of stumps in the field by allowing the freshly cut stumps to remain in close proximity with stumps having a large number of sporophores. This method of inoculation was considered as the ideal, as it simulates field conditions.

An attempt was made to infect field litter such as cover crop leaves, grass, plant debris and dried bark of *Hevea* with basidiospores but there was no evidence of spores colonizing litter. Basidiospores of *R. lignosus* did not infect the roots of seedlings in the pot experiment. This could probably be due to the presence of competitive micro-organisms under field conditions but under sterile and semi-sterile conditions in the laboratory the competitive effect was minimal. It would also be postulated that physical conditions within the plant such as moisture content or temperature are often unsuitable. Also plants might differ considerably in resistance to invasion.

Spore infection, long postulated as an effective means of spread by the pathogen has now been confirmed in artificial inoculation of stumps felled during normal hand clearing by Lim (1977). He observed that infection was not achieved *in vitro* on stumps and root sections, however, he has demonstrated that infection occurs readily by inoculating the cut surfaces of freshly felled stumps left exposed and to die slowly following clearing of rubber. He achieved 50% success by inoculating stumps with 25 ml of a spore suspension containing 4×10^6 spores per ml. However, in the uninoculated stumps in the same experimental area, 18.7% infection was noted, suggesting that there may have been some infected material lying in contact with the rubber stumps.

These studies show that, in Sri Lanka, although fructifications produce viable basidiospores which can infect *Hevea* stem discs and root pieces *in vi ro*, it is unlikely that under field conditions basidiospores could cause new foci of infection. The spread of the disease in Sri Lanka, therefore could be assumed to be mainly through contact with the infected root debris.

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