

OBSERVATIONS ON THE DEVELOPMENT OF THE  
SPOROPOHORE OF *RIGIDOPORUS LIGNOSUS*  
AND THE RELEASE AND GERMINATION  
OF BASIDIOSPORES

By

INDRANEE LIYANAGE, O. S. PERIES, A. de S. LIYANAGE,

AND

CHANDRIKA WETTASINGHE

SUMMARY

Sporophores of *Rigidoporus lignosus* are produced on infected stumps throughout the year and are freely available during rainy weather. They produce viable basidiospores from a very early stage of their development and continue to produce them in abundance at maturity. A bimodal pattern of spore release was observed with a peak early in the morning, when relative humidity (rh) was high and temperature low. When detached sporophores were subjected to varying conditions of rh, a large number of spores were released and they germinated at 24 h but thereafter the numbers released and germinating declined gradually with no spores being released at 96 h. Spore germination occurred in free water and at room temperature (RT  $28^{\circ}\text{C} \pm 2^{\circ}$ ), it was first observed 8 h after their release. Maximum germination occurred at RT, spore viability being adversely affected at  $15^{\circ}\text{C}$  and  $40^{\circ}\text{C}$ . Exposure to ultraviolet rays and sunlight reduced their viability.

INTRODUCTION

White root disease caused by *Rigidoporus lignosus* (Klotz.) Imazeki, is at present the most serious disease of rubber in Sri Lanka. In estates of the wet rubber growing districts, the area infected is estimated to be 8-10% (Liyanaige, 1978). Sporophores of *R. lignosus* which are often produced during rainy periods in tiers on dead stumps, fallen timber and roots of jungle or rubber trees (Sharples, 1936), release spores into the atmosphere under suitable conditions of temperature and humidity (Hilton, 1961). Lim (1977) observed that fruit bodies sporulate for up to about 18 months from inception and that maximum spore release occurs during the night.

The disease spreads from infected trees to healthy ones by root contact, although Lim (1977) proved that spores could also initiate infection. In view of the widespread occurrence of this disease in Sri Lanka, the Rubber Research Institute has initiated detailed studies on all aspects of the disease in order to develop methods for its biological control. This paper describes the development of the sporophore, and reports conditions for release of spores and their germination.

## MATERIALS AND METHODS

## Development of the sporophore

The rate of development of sporophores was studied from November 1978 to February 1979 by tagging them as soon as they appeared on rubber tree stumps. Their sizes were recorded weekly by carefully tracing them on paper and measuring the area. Records were also kept of daily rainfall and of the number of dry days during these studies.

## Collection of basidiospores

Basidiospores were collected by placing glass slides or McCartney bottles immediately beneath the hymenial surface of the middle region of sporophores of known age *in situ*, or of detached ones in the laboratory. Generally, spores were gathered for 1h, but when a large number of spores was needed for artificial inoculation, collections were made for up to 6 h. The collected spores were suspended in water and the concentration adjusted to  $10^4 \text{ml}^{-1}$ . For germination studies, drops of suspension were pipetted onto clean, sterile glass slides, spread to disperse spores and incubated for 24 h at room temperature (RT  $28^\circ \text{C} \pm 2$ ). For testing the effect of relative humidity (rh), sunlight and ultra-violet (uv) light, on germination, spores collected on slides were subjected to the required conditions directly, without suspending in water. Following the treatments, slides were sprayed with a fine mist of water and incubated at RT. A spore was considered to be germinated when the germ tube length exceeded its diameter.

## Spore release

To study the spore release pattern in the field, slides were exposed under several 6 wk old sporophores for 1h periods, consecutively for 3 days. The spores caught during each hour were assessed by staining with 0.5% cotton blue in lactophenol and counting in five random microscope fields (x200). Hourly variations in temperature and humidity were noted.

The pattern of spore release at different rh levels was also studied. Sporophores of approximately the same age were brought to the laboratory, slides were placed beneath them for 1 h and the release and viability of spores examined to ensure that viable spores were produced. Sporophores were then placed in desiccators containing saturated salt solutions (Solomon, 1951) to give values of 0, 52, 80 and 100% and kept there for 24 h periods for 4 days. After each 24 h period, spores were collected for 1 h on slides, in the manner described.

## RESULTS

## Development of the sporophore

Sporophores were collected throughout the year at Dartonfield Estate. They were freely available, particularly during the two wet seasons caused by the South-West and the North-East monsoons. Both very small ( $1 \text{cm}^2$ ) and large ( $100 \text{cm}^2$ ) sporophores produced spores in large numbers, even though some of the old sporophores were infested with insects.

Several attempts were made to study the development of sporophores from their inception to maturity but there were several difficulties. Handling caused them to shrivel and fall. Also, during dry periods they dried up and eventually fell off stumps suggesting

that they grow better during wet weather. Those tagged for the experiment grew to a mean size of 83.7 cm<sup>2</sup> after which they dropped off because of handling or exposure to dry weather. Only those that were covered and shaded by weeds or a cover crop grew. They then grew rapidly for about 7wk, more than doubling in size every week, after which growth slowed. Growth was most rapid during periods of evenly distributed high rainfall and was slow when there were several dry days in the week. During dry weather periods, spores were produced but in small numbers (Table 1).

Table 1. *The rate of growth of sporophores and number of spores released*

	Wk after appearance	Mean size (cm <sup>2</sup> )	Total rainfall (mm)	No. of dry days	Mean no. spores
November 1978	0	0.13	178.8	0	10.2
	1	0.65	68.90	0	10.6
	2	1.81	105.10	0	11.3
	3	5.74	5.40	3	11.9
	4	7.94	137.50	0	13.5
December 1978	5	13.81	51.20	0	25.7
	6	28.32	54.2	0	36.8
	7	40.83	6.50	4	39.0
	8	52.00	67.0	2	41.0
January 1979	9	61.23	133.90	3	52.0
	10	69.31	0.0	7	60.2
	11	73.52	27.8	6	69.1
	12	77.81	18.1	4	72.3
February 1979	13	80.30	2.0	6	70.2
	14	83.72	11.8	6	56.3
	15	83.72	28.2	5	31.9
	16	83.72	15.1	5	19.2

Sporophores produced viable spores from a very early age, the number increasing as they grew. Medium sized sporophores and their mid-regions released more spores, with a higher percentage of germination than the very small and very large ones (Tables 2 and 3). Germ tube length also followed a similar pattern.

Table 2. *Release and germination of basidiospores from sporophores of different sizes*

Age (wk)	Mean cap area (cm <sup>2</sup> )	No. spores	Germination %	Mean germ tube length (μm)
2	38.7	13.8	13.2	53.6
8	103.2	141.2	65.2	170.4
14	225.8	4.0	28.8	101.6

Table 3. *Release and germination of basidiospores from different areas of the sporophore*

Region of Sporophore	Mean no. spores released	Germination. (%)	Mean germ tube length ( $\mu\text{m}$ )
Growing edge	2.0	11.5	67.2
Middle	34.5	72.7	187.2
Basal	10.2	20.0	69.6

### Spore release

The rhythm of spore release was monitored during 3-day periods, and observations made during November, 1979 when the temperature ranged from 23.0°C to 27°C mean temperature was 23.3°C to 25°C and mean rh 81% to 93%, are presented here. Two distinct peaks were evident, one between 0300 - 0500 h, the other less pronounced, between 1700 - 2000 h (Fig. 1). The peaks generally coincided with high rh and low temperature. There was no time of day when spore release ceased completely.

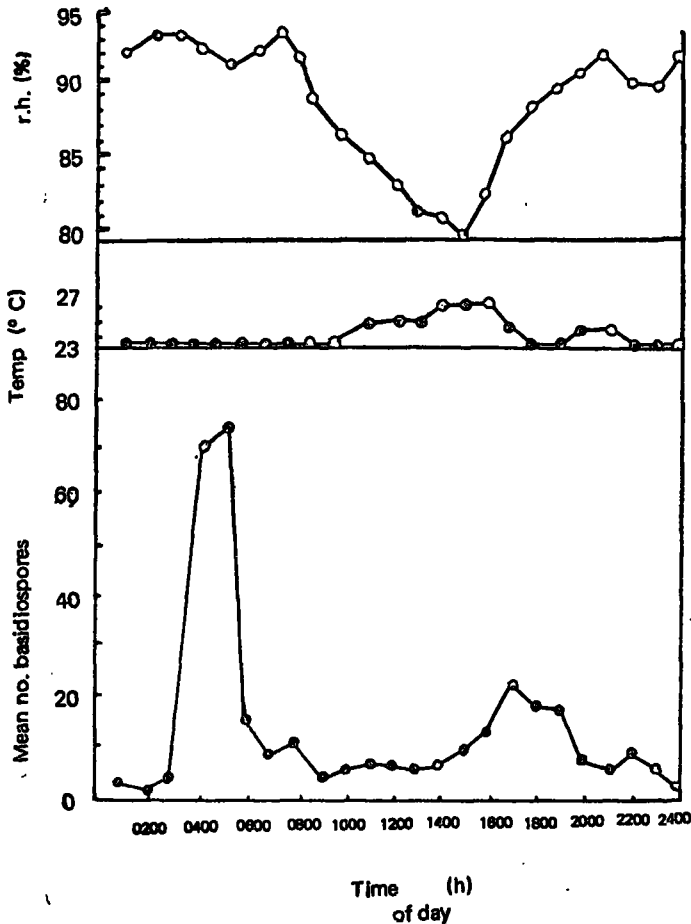


Fig. 1. Diurnal rhythm of spore release in *R. lignosus* in relation to variation in rh and temperature

In controlled rh experiments the number of spores released, the proportion that germinated and the germ tube length all differed significantly at different humidities (Table 4). At all humidities the maximum number of spores were released during the first hour of a 24 h period. The numbers decreased rapidly after 48 h and after 96 h exposure: no further spores were released. No spores were released at 0% rh. Germination and germ tube length varied directly with rh. Spores collected after 72 h at 52% rh did not germinate. The maximum germ tube length was recorded at 100% rh and the lowest at 52%. There was a significant decrease in germ tube length with increase in the period of exposure to low rh.

Table 4. *Percentage germination and germ tube length of basidiospores exposed to different humidities for various periods*

Period (h)	rh	Mean no. spores released	Germination %	Germ tube length ( $\mu\text{m}$ )
0	0	69.8	62.5	50.1
	0	0	0	0
24	52	14.6	32.3	6.3
	80	65.2	65.7	45.9
	100	67.8	68.3	49.8
48	0	0	0	0
	52	7	18.2	3.2
	80	51.8	49.7	35.6
	100	57.5	53.5	42.7
72	0	0	0	0
	52	2.4	0	0
	80	9.8	25.1	18.2
	100	9.9	25.8	23.6
96	0	0	0	0
	52	0	0	0
	80	0	0	0
	100	0	0	0
LSD (P. = 0.01)		6.07	3.84	1.85

#### Spore germination

Basidiospores began to germinate 8-10 h after their release at RT and the proportion germinating increased, up to 24 h under the test conditions. By 48 h extensive growth of the germ tube was observed, often with secondary branching. Spores deposited on slides germinated more rapidly when a water film was added than those shed into free water. Those shed on dry glass slides and left in the laboratory at RT failed to germinate.

RT ( $28^{\circ}\text{C} \pm 2^{\circ}\text{C}$ ) was optimum for germination (58.7%) which was slightly reduced at  $30^{\circ}\text{C}$  (Fig. 2). Germination was significantly less at both  $35^{\circ}\text{C}$  and  $25^{\circ}\text{C}$  and at  $15^{\circ}\text{C}$  and  $20^{\circ}\text{C}$  hardly occurred at all. Germ tubes were also longest at RT.

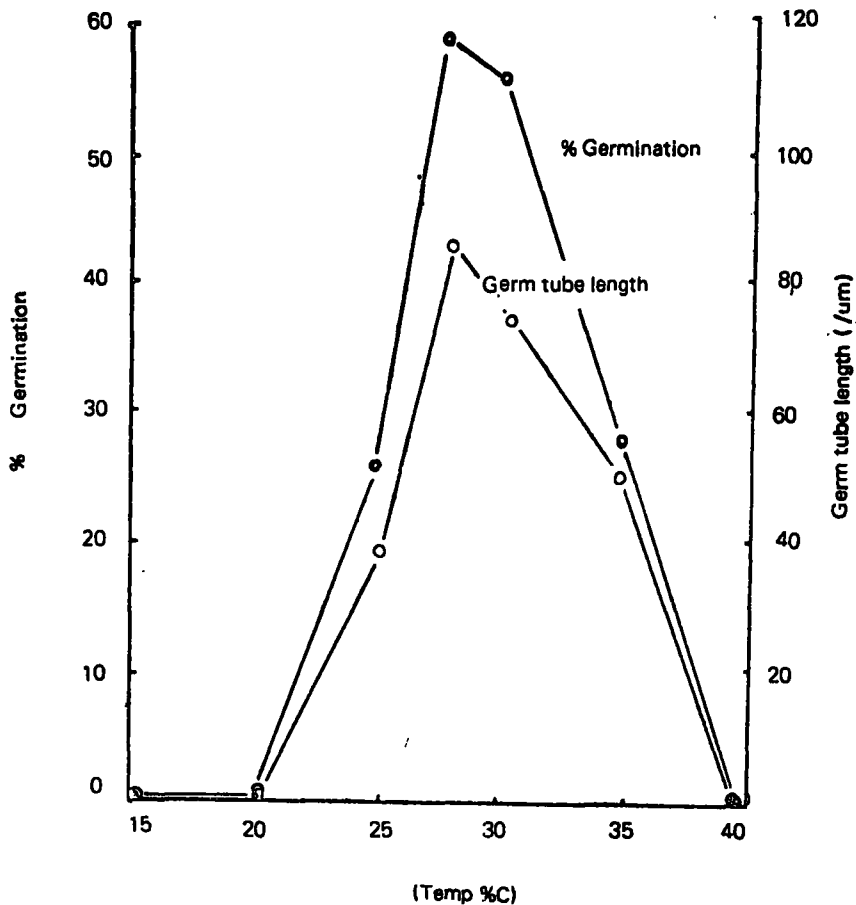


Fig. 2. The effect of temperature on germination of basidiospores : 0 - 0 percentage germination, ● - ○ germ tube length after 24 h.

Spores shed on to dry glass slides germinated only at 100% rh when a film of condensed water was formed. However, when spores were subjected to different humidities and then transferred to suitable conditions, those kept at 52%, 80% and 100% rh germinated but not those at 0% rh (Table 4).

Direct sunlight, UV light and RT all reduced germination markedly over a 90 min exposure period. After only 10 min exposure to sunlight and UV germination was only 15 - 25% and all spores were killed by 50 min (UV) and 70 min (sunlight) exposure.

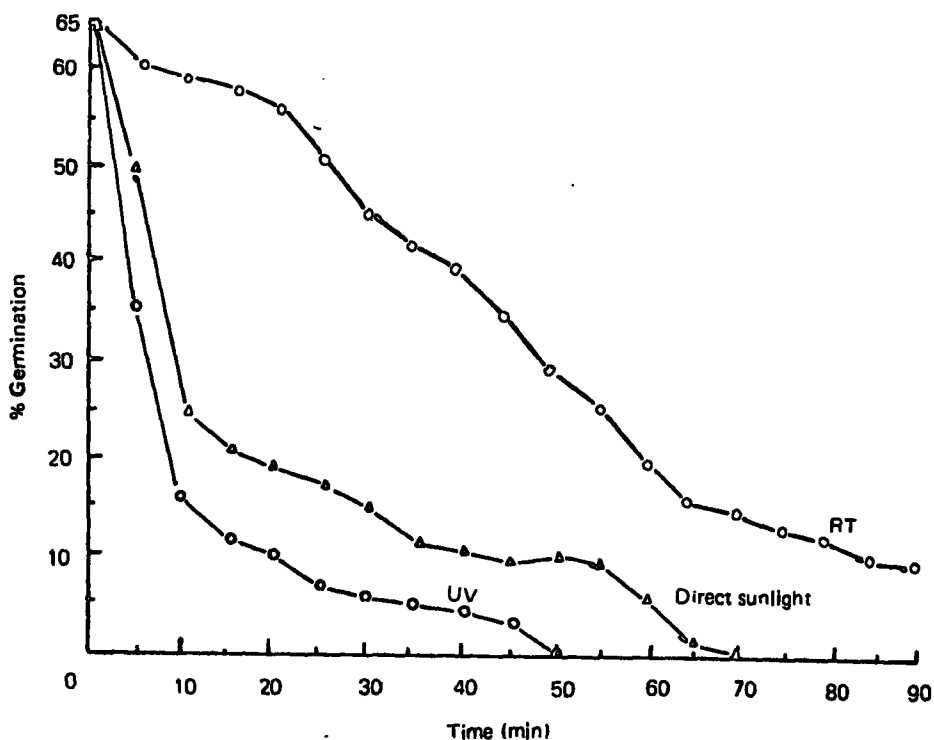


Fig. 3. The effect of exposure of basidiospores to direct sunlight, RT and UV light, on their germination

#### DISCUSSION

Sporophores are produced in abundance during rainy weather when it is also relatively cool and humid. Lim (1977) encouraged prolific sporulation artificially by keeping stumps with sporophores in a covered shed maintained at a high rh and a temperature slightly lower than RT.

Small sporophores, produce few spores, presumably because the hymenial tubes are short and contain few basidia. These gradually become longer and eventually produce more spores as the sporophore enlarges. The mid regions of medium-sized sporophores generally released a large number of spores which germinated well. This is possibly because most of a very large sporophore is senile whilst the medium sized ones used in the experiments were possibly at a peak of spore production.

Among the basidiomycetes are drought tolerant types that cease to discharge spores under dry conditions. However, they remain alive, rapidly absorb water when wetted by rain and then soon start to liberate spores, as do some gelatinous fungi (Ingold, 1970). In contrast, sporophores of *R. lignosus* become dehydrated and curl up when subjected to dry weather. Spore numbers released are also drastically reduced and the sporophores

do not absorb water and release spores on being re-wetted.

There are a few basidiomycetes in which the sporophores not only survive under very dry conditions but also continue to liberate spores, *Fomes fomentarius* (L. ex Fr.) Kickz., and *Ganoderma appalantum* (Pers. ex Wallr.) Pat. are examples (Buchwald & Hellmers, 1946). A large active specimen of *F. fomentarius* detached in May from a beech trunk discharged spores copiously at the beginning but ceased to shed any after 2 days in the laboratory (Ingold, 1970). In the present studies also, mature detached sporophores discharged large numbers of spores in the first day in the laboratory but ceased to discharge on the fourth day, suggesting that the sporophore itself does not have a sufficient water reserve for continued spore discharge. However, in the field, partially dried, intact sporophores, released viable spores in large numbers, presumably deriving the necessary water from the host.

High rh coupled with low temperature appears to favour spore release in *R. lignosus* with a conspicuous early morning peak, and a smaller peak in the evening. These peaks coincided with times when the rh was high and temperature low. Spore release in Basidiomycetes shows different diurnal patterns (De Groot, 1968; Hirst, 1953; Pady, Kramer & Wiley, 1963; Haard & Kramer, 1970). When detached sporophores of *R. lignosus* were artificially subjected to different humidities no spore discharge occurred at 0% rh. Patton & Myren (1970), however, have observed good release in detached sporophores of *Polyporus tomentosus* for 2 days at rh near 0% and for over 5 days between 80% and 100%.

The pattern of airborne spore release is often determined using a Hirst spore trap. Although this trap was operated on several occasions in the vicinity of sporophores, a very few spores were trapped only in the night time. This may be because the sporophores, generally grow close to the ground and they discharge spores at this level. During the night in fair weather, there is often a boundary layer of still air several cm thick next to the ground. Spores discharged into this zone would soon fall to the ground and not be available to a Hirst trap operating at the standard height of 1 m. During the day, with the onset of turbulent conditions and the reduction of the boundary layer to 1 mm or less, discharged spores would be brought into the trapping region, resulting in a periodicity which is independent of the spore liberation process (Ingold, 1970).

Maximum germination of basidiospores occurred at  $28^{\circ}\text{C} \pm 2^{\circ}$  and 100% rh. Exposure of spores to UV radiation and direct sunlight for a period of 10 min caused a sharp reduction in their germination. The major peak of spore liberation occurs between 0300 and 0600 h. These spores are ready for germination about 8 h after release, between 1100 and 1400 h. Usually, at this time the atmospheric humidity is low and temperature is high; these conditions being unfavourable for maximum germination. This may be a reason why basidiospores do not play a more important role in the distribution of the fungus.

Sections of stumps were readily colonized by spores under laboratory conditions but this did not occur in the field, presumably because sunlight, temperature and humidity conditions were unfavourable. John's (1965) success in infecting *Hevea* stumps with spore suspensions in the field, by covering the inoculated surfaces with soil, probably depended on a cool moist environment, where the spores were protected by the soil cover.

Although sporophores produce large numbers of viable basidiospores which can infect *Hevea* stem discs and root pieces *in vitro*, the limited role of basidiospores in causing infection, observed under local conditions, indicates that the disease generally spreads by other means, thought to be by root contact over short distances. However, the possibility that the disease can be carried over long distances by basidiospores cannot be discounted.

## REFERENCES

- BUCHWALD, N. F. and HELMERSE, E. (1946). Fortsatte lagttagelses over spore faeldning has Tendersuamp (*Polyporus fomentarius*). *Friesia* 3, 212 - 216.
- DE GROOT, R. C. (1968). Diurnal cycles of airborne spores produced by forest fungi. *Phytopathology* 58, 1223 - 1229.
- HAARD, S. M. & KRAMER, C. L. (1970). Periodicity of spore discharge in the Hymenomyces. *Mycologia* 62, 1145 - 1169.
- HILTON, R. N. (1961). Sporulation of *Fomes lignosus*, *Fomes noxius* and *Ganoderma pseudoferreum*. Proceedings of the Natural Rubber Research Conference, Kuala Lumpur 1960. *Rubber Research Institute of Malaysia*, 496 - 502.
- HIRST, J. M. (1953). Changes in atmospheric spore content : diurnal periodicity and the effects of weather. *Trans. Br. mycol. Soc.* 36, 375 - 393
- INGOLD, T. (1970). Fungal spores and their liberation and dispersal, 107 - 129. Clarendon Press, Oxford.
- JOHN, K. P. (1965). Some observations on spore infection of *Hevea* stumps by *Fomes lignosus* (Klotzsch) Bres. *Jl. of Rubb. Res. Inst. Malaysia* 19, 17 - 21.
- LIM, T. M. (1977). Spore production, germination and infection in *Rigidoporus lignosus* on *Hevea*. *Journal Sains Pusat Penyelidikan Getah Malaysia* Jilid 1, Bilangan 1, 15 - 19.
- LIYANAGE, A. de S. 1978. Review of the Plant Pathology Department. *Ann. Rev. Rubb. Res. Inst., Sri Lanka* 1977, 66 - 98.
- PADY, S. M., KRAMER, C. L. and WILEY, B. J. (1963). Materials, methods and general results of diurnal studies. *Mycologia* 54, 168 - 180.
- PATTON, R. F., and MYREN, D. T. (1970). *Root rot induced by Polyporus tomentosus in pine and spruce plantations in Wisconsin*. In: Toussoun, T. A. Bega, R. V. and Nelson P. E. (eds.), *Root diseases and soil-borne pathogens*, 167 - 170. Univ. California Press, Berkeley - Los Angeles - London.
- SHARPLES, A. (1936). *Diseases and pests of rubber tree*. Macmillan and Co, London, 167 - 170.
- SOLOMON, M. E., (1951) . Control of humidity with potassium hydroxide, sulphuric acid and other solutions. *Bulletin Environmental Research* 42, 543 - 554.