

Studies on Epidemiology of Pink Disease and the Effect of Temperature on Mycelial Growth of Corticium salmonicolor of Hevea Rubber

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The effects of temperature on mycelial growth and the relationship between rainfall, temperature and disease incidence were studied. The optimum temperature for the growth of the C. salmonicolor was 28° C. The minimum and maximum temperatures for fungal growth were 5° C and 40° C, respectively. In the field there was an exponential reduction of basidiospores caught after rainfall. Studies carried out for 22 months in the infected field showed that basidiospores played an important role in the incidence of pink disease.

Corticium salmonicolor (Berk. & Br.) is synonymous with *Pellicularia salmonicolor* (Berk & Br) Dastur, *Botryobasidium salmonicolor* (Berk & Br) Venkatarayan, *Phanerochaete salmonicolor* (Berk & Br.) Julich¹. This fungus cause damage to important economic crops such as *Hevea*², *Eucalyptus*³, apples⁴⁻⁵ and cocoa⁶.

Pink disease is presumably transmitted by basidiospores and conidia¹. Christians *et al*⁷ showed that basidiospores play an important role in the incidence of pink disease in cocoa. Previous studies had shown that environmental factors such as rainfall is important in basidiospore release⁸⁻⁹. The severity of infection depended on the pattern of rainfall¹⁰. In *Eucalyptus* plantations in India, the disease was not effective in areas receiving less than 200 cm of rainfall per year³. Christians *et al*.⁷

observed that *C. salmonicolor* in the infected cocoa branch was stimulated by sporadic rain while heavy and regular rainfall induced the formation of corticium stage of the fungus which can cause new infection. Temperature also plays an important role in pink disease development. In Himachal Pradesh, India, the maximum incidence and development of pink disease on apple was observed during the month of July–August with average atmospheric temperature of 26°C and 27°C¹¹.

There are only few reports on the biological aspects of the fungus and the epidemiology of the disease even though the disease is present in most rubber growing countries. This paper describes the effects of temperature on mycelial growth, the relationship between rainfall with basidiospore release and disease incidence.

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MATERIALS AND METHODS

Isolates of *C. salmonicolor*

The isolates used in the study were obtained from rubber collected from various states in West Malaysia. Isolate Cort. 1 was from Kedah, Cort. 2 and Cort. 6 from Selangor, Cort. 3 and Cort. 4 from Pahang while Cort. 5 was from Perak. The isolates were maintained on malt extract agar (MEA) at 21°C under fluorescent light.

Effect of Temperature on Mycelial Growth

Inoculum disks of six mm diameter from the margin of 5-day-old cultures were placed invertedly on MEA in 9 cm petri dishes and were incubated in light at different temperatures (5°C, 10°C, 21°C, 23°C, 25°C, 28°C, 31°C, 35°C and 40°C). There were five replicates per treatment. The diameter of each colony was measured after incubation for four days. Mean colony diameter of different isolates was determined after subtracting the initial diameter of inoculum plug.

Epidemiology

The experiment was conducted in a three-and-a-half-year-old rubber plantation (PB 260 clone) in Tanjung Malim, Perak. A Burkard spore trap was placed about 3.15 m from an infected tree with disease lesion about 3 m from the ground. The orifice of the spore trap which is about 2.2 m from the ground faced the infected tree. The volume of air drawn through the trap was 10 l/min (0.6 m³/h). All the pink disease infected trees (except the tree being examined) located within 30 m radius were treated with Calixin Ready-mixed®. This was to minimise interference from basidiospores from the surrounding infected trees.

The drum containing Melinex tape, thinly coated with a mixture of phenol-wax-vaseline (1:19:180; w/w/w) was changed every seven days at 12.00 noon. The Melinex tape was cut into seven strips, each representing a day of trapping of spores. The number of spores per hour was estimated by counting spores after the strips was marked using a sharp knife with the aid of perspex block provided by Burkard. Basidiospores were stained with trypan blue before counting under a microscope (*Figure 1*).

An automatic weather station was used to record rainfall. The experiment was conducted for 28 days.

In another trial at another estate in Tanjung Malim, Perak, a Burkard spore trap was placed in the centre of a block (56 ha) of 5-year-old trees (PB 260 clone). The orifice was 3.5 m from the ground and the trap was allowed to swing freely. One hundred trees including the pink disease infected trees were selected within 30 m radius from the spore trap. The trees were monitored weekly for new pink disease incidence (proportion of trees with symptoms of pink disease in relation to the total number of observed trees). Spore trapping was carried out for nearly two years (22 months) from 1992 to 1993. The same method of counting of spores on the Melinex tape mentioned above was used.

Temperature and relative humidity were recorded using a thermohygrograph (Saito, Japan) and the rainfall data were obtained from the management of the estate.

RESULTS

Effect of Temperature on Mycelial Growth

The optimum temperature for mycelial growth of *C. salmonicolor* isolates was 28°C.

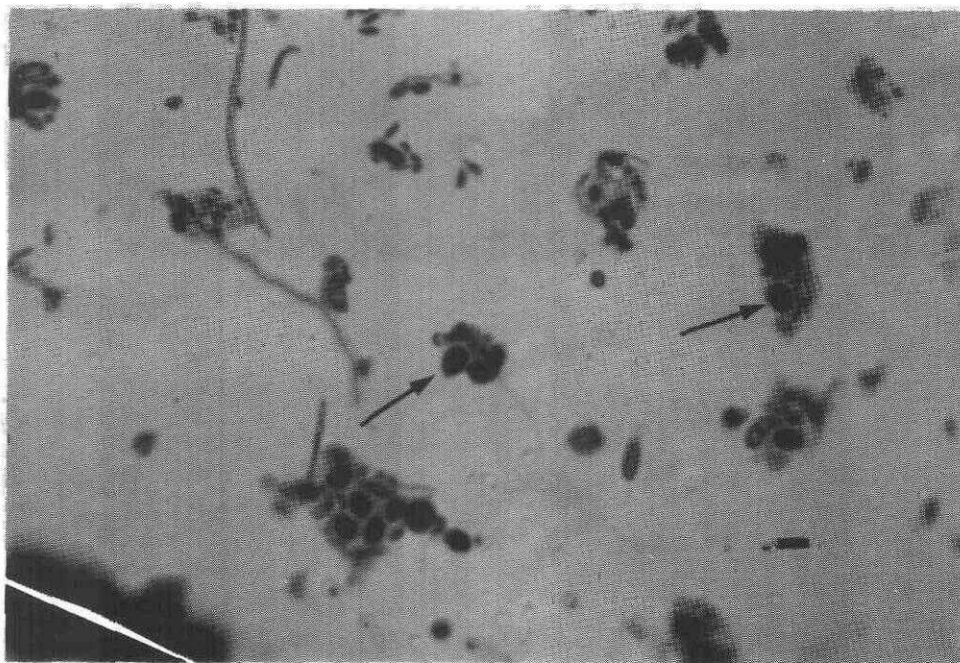


Figure 1. Basidiospores on Melinex tape from spore trap (1 bar = 10 μ m).

At 5°C and 40°C, the growth was negligible as these were the minimum and maximum temperatures for the growth of the fungus, respectively. The measurable growth was within a range of 10°C–35°C (Table 1a). At 28°C, the growth of isolate Cort.1 was significantly slower than other isolates (Table 1b).

Epidemiology

Rainfall was recorded for 22 days out of the 28 days trial period. More basidiospores were caught during rainfall (Figure 2) and the number of basidiospores released, decreased exponentially after rainfall as shown in the transformed model below:

$$Y' = 4.103 - 0.21X \quad \dots 1$$

where Y' is the Ln Y whereby Y is the number of basidiospores caught per hour
 X is the duration during ($x = 0$ h) or after rainfall ($x = 1, 2, 3, \dots, 15$ h)

Basidiospores were caught even 15 hours after rainfall.

Studies carried out for 22 months had shown that under the rubber canopy, only minimal fluctuations in the minimum and maximum temperatures were observed. The minimum and maximum temperatures were between 21°C–25°C and 28°C–33°C, respectively. In 1992 and 1993, high percentage rainy days occurred

TABLE 1. GROWTH OF *C. SALMONICOLOR* ON MALT EXTRACT AGAR (MEA)

a) GROWTH AT DIFFERENT TEMPERATURES

Temperature	Mean colony diameter (cm)					
	Cort.1	Cort.2	Cort.3	Cort.4	Cort.5	Cort.6
5° C	0 a ¹	0 a	0 a	0 a	0 a	0 a
10° C	0.30 c	0.57 d	0.42 d	0.56 c	0.44 c	0.37 d
21° C	4.35 b	5.66 b	5.95 b	5.45 b	4.95 b	5.25 b
23° C	5.37 e	6.17 f	6.17 f	5.99 e	5.47 d	5.91 e
25° C	5.61 f	6.90 g	6.84 g	6.68 f	6.53 e	6.33 f
28° C	6.07 g	7.46 h	7.05 h	7.23 g	7.17 f	7.33 g
31° C	4.41 b	4.80 c	5.13 c	5.41 b	5.05 b	3.28 c
35° C	0.20 d	0.43 e	0.26 e	0.25 d	0.53 c	0.38 d
40° C	0 a	0 a	0 a	0 a	0 a	0 a

b) GROWTH AT 28° C

Isolate	Mean colony diameter (cm)
Cort.1	6.07 a ¹
Cort.2	7.46 e
Cort.3	7.05 b
Cort.4	7.23 cd
Cort.5	7.17 bc
Cort.6	7.33 de

¹Mean on the same column followed by the same letter are not significantly different at 5% level of probability by Duncan's New Multiple Range Test.

in April–May and November–December. The month of June, 1992 was dry (< 20% rainy days). The monthly exposure of high relative humidity (RH ≥90%) under the rubber canopy was between 10 h–20 h (*Figure 3*).

Generally, the concentration of basidiospores in the air increased from March, 1992 to December, 1993. High concentration of basidiospores were caught in March and November, 1992. In 1993, high concentration

of basidiospores occurred from March to July and November to December. In general, the incidence of disease increased with the increase in concentration of basidiospores in the air as indicated by the increased incidence from March, 1992 until December, 1993 especially during March to July 1992, November to January 1993, June–July and October to December, 1993. No new disease incidence was observed during July–September, 1992 and

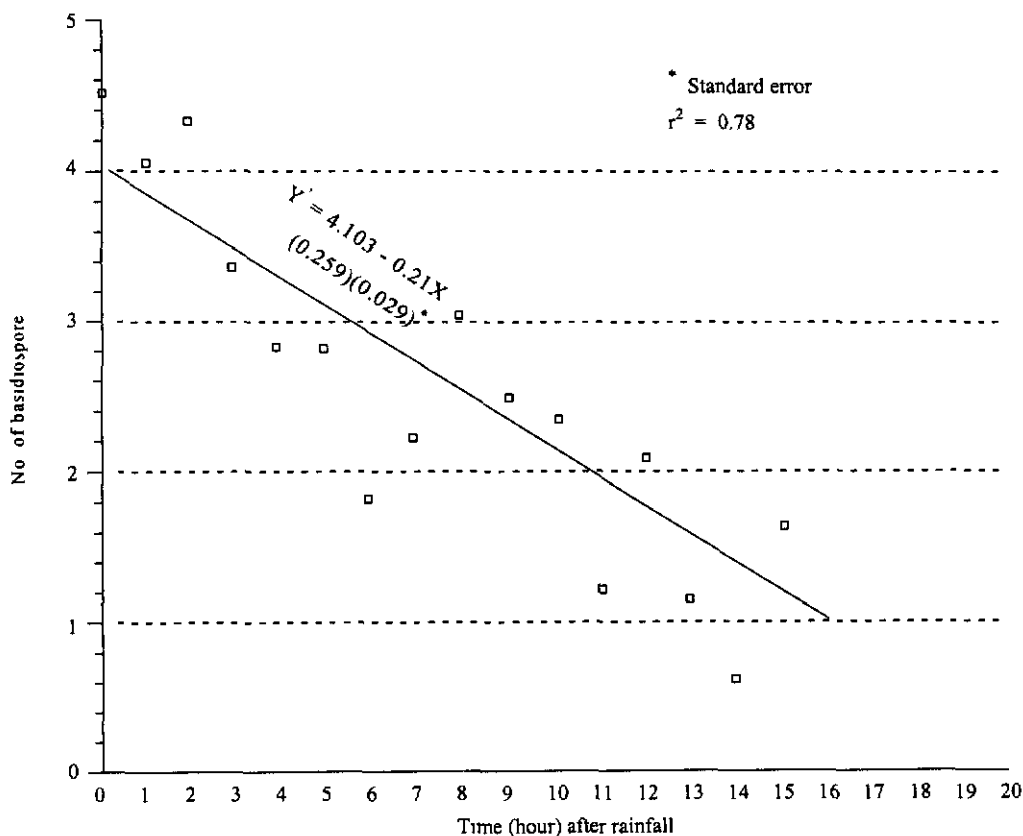


Figure 2. Regression line between time (during and after rain) and number of basidiospores per h.

1993. However, in the dry month (June 1992), new disease incidence was observed (Figure 3).

The relationship between disease incidence with basidiospores in the air, rainy days, and maximum temperature can be presented by the model:

$$Y = 30.2444 + 0.0702SP + 0.0927PERRD(-1) - 0.8233MT + 0.496TREND \quad \dots 2$$

(2.25)*
(1.73)
(-1.41)
(5.00)

$r^2 = 0.735$

where Y is the disease incidence
 SP is the spore
 $PERRD(-1)$ is the rainfall in the previous month
 MT is the maximum temperature
 $TREND$ is the trend

* the number in the bracket represents t-test.

Time Series Analysis showed that basidiospores in the air was significantly [5% ($t > 2.07$)] related with pink disease incidence. However, there is a tendency that the other

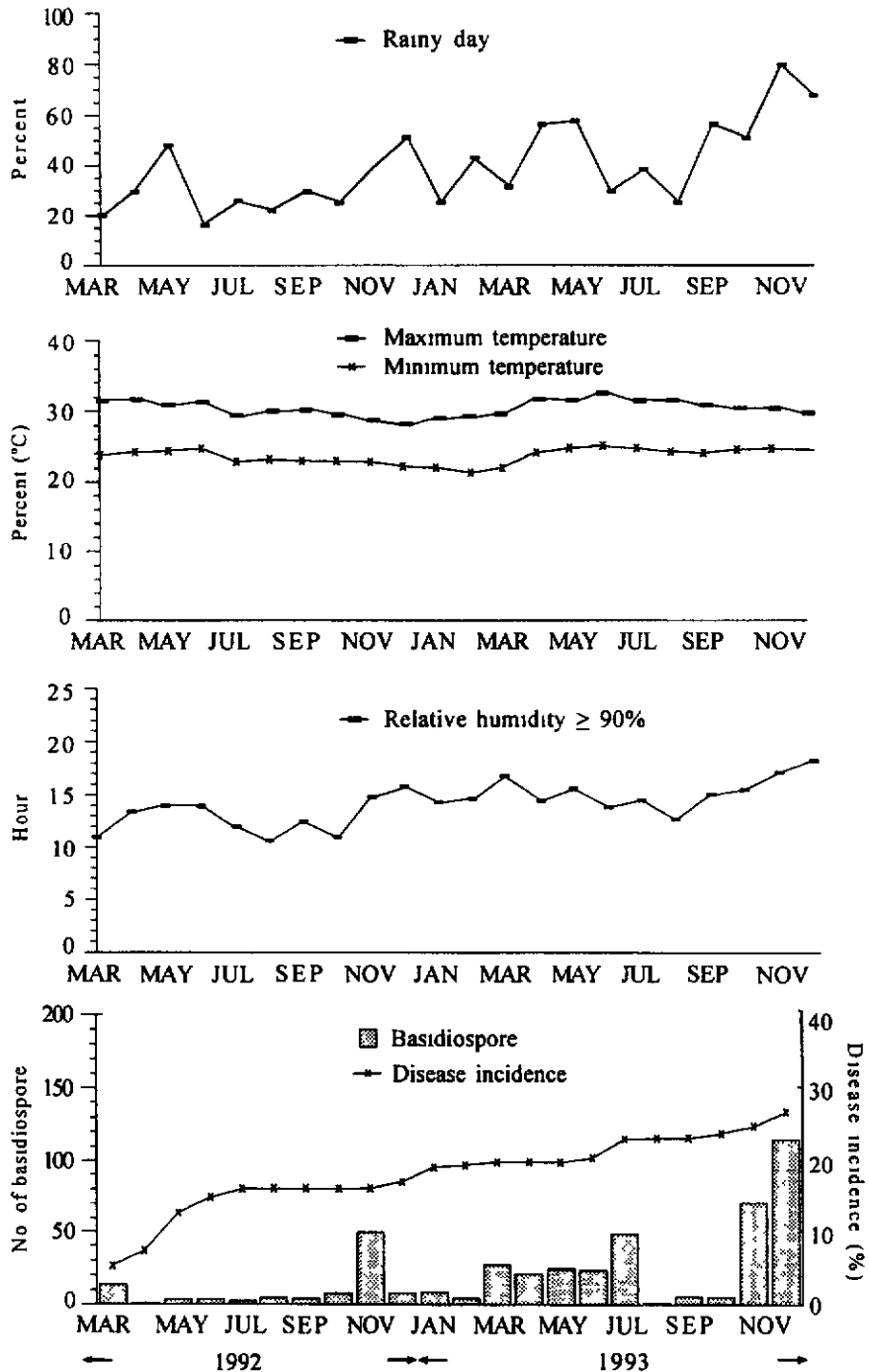


Figure 3 Profile of monthly average of number of basidiospores, disease incidence and weather measurements.

two variables, maximum temperature and rainfall in the previous month, contributed to the incidence of pink disease since the two variables are significant at 20% ($t > 1.32$) and 10% ($t > 1.72$) respectively with pink disease incidence. Provided all the variables are constant, increase of temperature will decrease pink disease incidence. However, an increase in rainy days of previous month will increase the incidence of pink disease. The other parameter, relative humidity, more or equal to 90% (RH \geq 90%) was not significant.

DISCUSSION

There are three cardinal temperatures which affect the mycelial growth of a fungus *i.e.* the minimum, maximum and optimum temperatures¹². Since the minimum and maximum temperatures for growth of *C. salmonicolor* isolates obtained from Malaysia were 5°C and 40°C, respectively while the optimum temperature was 28°C, the fungus is in the Mesofil group. Verma and Munjal¹¹ stated that 25°C was the best temperature for growth of *C. salmonicolor* and no growth was observed at 5°C. Luz¹³ stated that the optimum temperature for *C. salmonicolor* isolate from cocoa in Brazil was between 23°C–26°C, and 35°C completely inhibited fungal growth. This study showed that the optimum temperature for mycelial growth of *C. salmonicolor* in Malaysia is higher than in other countries.

As the growth at temperatures between 5°C–10°C and 35°C–40°C were not evaluated, the 'minimum' and 'maximum' growth temperatures for *C. salmonicolor* may be within those figures. However, the minimum and maximum temperature for the growth of the

fungus may not be less than 5°C or more than 40°C.

The release of basidiospores of *C. salmonicolor* in cocoa was during and after rainfall and lasted 13.5 h after rainfall⁷⁻⁸. Many basidiospores of *C. salmonicolor* from the pink disease lesion in the rubber plantations were also released during rainfall period and reduced exponentially after rainfall. Basidiospores were still released 15 h after rainfall since the basidiospores could be caught during that particular hour. This indicated that high inoculum density of basidiospores was available during and immediately after rainfall. This may explain why the incidence of pink disease normally occur during rainfall period.

The temperature under the canopy of rubber trees in Malaysia was between 21°C–33°C. This range of temperatures is suitable for the growth of the pink disease fungus as the optimum temperature for growth was 28°C. This temperature is also suitable for the germination of basidiospores which was within 18°C–32°C¹⁴. Christians *et al.*⁷ mentioned that even during the dry season, development of pink disease symptoms can still be observed in cocoa plantations. This observation can also be seen in the rubber plantations whereby new incidences of pink disease were observed in June 1992. Eventhough the month of June was considered a dry month, there were still sporadic rains during the month. The role of sporadic rain on pink disease in cocoa trees had been mentioned by Christians *et al.*⁷ The sporadic rain probably not only stimulated active infection but also inactive infections (dormant) in cocoa. Similarly, this may occur in rubber trees as well thus creating new disease incidence. The rainy days which occur during

the previous months (May) also provide conducive environment for the growth of the fungus. Hilton² mentioned that few months were needed for the pathogen to kill the stem of a rubber tree and a longer time was required during dry weather. Although, the disease can develop during dry period, more pink disease occurred during the wet period.

It has been reported that high relative humidity due to intercultivation or heavy weed growth created ideal micro-climatic conditions for the development of pink disease in Eucalyptus³. High relative humidity is always present under rubber canopy (RH \geq 90%) whether on dry or wet months. However, it was observed that high relative humidity (RH \geq 90%) was not a critical factor governing incidence of pink disease in rubber as compared to rainfall.

Harrison *et al.*¹⁵ has used spore trapping as a guide to initiate control of potato early blight caused by *Alternaria solani*. The use of spore trapping to determine the presence of basidiospores as an early indicator to initiate control will be advantageous as the corticium stage which produces the basidiospores was the most destructive¹⁶. However, the practise is not practical for small rubber planters.

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