J. nat. Rubb. Res., 3(1), 21-29

Studies on Sporulation, Pathogenicity and Epidemiology of Corynespora cassiicola *on* Hevea *Rubber*

K.H. CHEE*

Corynespora cassiicola *infects the leaves of* Hevea *rubber and causes rapid leaf fall. Isolates varied in their ability to produce spores and sporulation was best on potato sucrose agar. Conidial sporulation was highest when cultures were incubated in the dark for three days followed by a daily 2 h exposure to ultra-violet light or continuous light for three to six days. Field or single spore isolates varied greatly in cultural morphology and rate of sporulation, ranging from nil to 650 spores per square centimetre of agar surface. Conidia germinated within 4 h. Germ tubes arose more often from the end cells of the spores.*

Leaves are most susceptible to infection for up to four weeks. Hevea *clones differ in their degree of susceptibility, but immunity is common. Infection of leaf discs in the laboratory correlates well with field susceptibility. One hundred and thirty-seven clones were screened for resistance in both laboratory and field tests. Conidia from RRIC 103, the most susceptible clone, were not particularly aggressive. None of the several hosts tested was infected by the isolates from* Hevea. C. cassiicola *released its spores from 0800 h and attained a peak around noon; it gradually fell to a very low level in the evening and remained low throughout the early hours of the morning. There was no clear-cut seasonal pattern of spore release in relation to rainfall.*

Corynespora cassiicola [(Berk. & Curt.) Wei] is synonymous with *Corynespora melonis* [(Looke) Lindau], *Helminthosporium cassiicola* (Berk. & Curt.), *H. vignae* (Olive apud Olive, Bain & Lefebvre), *H. papayae* (H. Sydow) and *Cercospora vignicola* (Kawamura)[1]. This has been reported from Malaysia, Indonesia, Thailand, Philippines, Sri Lanka, India, Korea, Japan, China, Australia, Canada, Hungary, USSR, USA, Puerto Rico, Honduras, Cuba, Haiti, Barbados, Venezuela, Columbia, Brazil, Ivory Coast, Nigeria, Uganda and Tanyanyika. The fungus causes leaf spot, stem rot, fruit rot, root rot, seed rot, *etc.* and infects many plant parts of more than seventy hosts, including fruits, vegetables, perennial crops and ornamentals.

C. cassiicola was first recorded in Peninsular Malaysia in 1960 causing leaf spot on some iron-deficient nursery plants[2]. It was regarded as a minor disease in budwood nurseries on certain clones[3], in particular FX 25 and RRIC 52. *C. cassiicola* is generally more common and more severe in rubber nurseries and immature plantings than in mature fields. The severity of infection is markedly influenced by the susceptibility of the clone: the most susceptible clone in Malaysia is RRIC 103, followed by KRS 21, FX 25 and RRIM 725. In Indonesia, clones PPN 2058, PPN 2444 and PPN 2447 are highly susceptible, whereas GT 1 and RRIM 600 are moderately susceptible[4].

Although RRIC 103 originates from Sri Lanka, *C. cassiicola* was only discovered in that country on *Hevea* rubber in December 1985 by the Rubber Research Institute of Sri Lanka[5]. Since then, *Corynespora* leaf spot has become

*Rubber Research Institute of Malaysia, P.O. Box 10150, 50908 Kuala Lumpur, Malaysia

the most damaging disease in Sri Lanka, and affected plants have to be uprooted due to the lack of effective control measures[6]. *C. cassiicola* is also present on *Hevea* in Thailand, India[7] and Nigeria[8].

The fungus affects young as well as old leaves, particularly along the veins. It appears first as yellowish brown spots which enlarge into circular or irregular papery lesions about 2 mm in diameter and pale grey in colour. Young leaves, or portions of them where there are several lesions, may shrivel and dry up. As the leaves mature, a short length of the vein that borders the lesions is discoloured and becomes chocolate brown together with the ramifying smaller veins around it, giving the characteristic 'fish bone' appearance. Affected leaves gradually turn yellow in patches, before dropping. However, when leaf stalks are affected, the leaves fall while still often green, without lesions on the leaf blades. The severity and spread of *Corynespora* spp. are encouraged by water stress[9], poor soil fertility and inadequate or unbalanced nutrient supply[2,10].

This paper describes experiments to study the sporulation, pathogenicity, epidemiology and host reaction of *Corynespora cassiicola* on *Hevea* rubber.

MATERIALS AND METHODS

The isolates used in the study were mostly obtained from leaves of *Hevea* rubber at the RRIM Experiment Station in Sungei Buloh, Selangor. The two isolates from papaya leaves were from the RRIM Experiment Station, Sungei Buloh and from Tanjung Malim, Selangor. The fungus is known for its reluctance to produce spores in culture[11]. Hence, as spores of *C. cassiicola* were required for pathogenicity studies, investigations were carried out to determine the best nutrient medium as well as the effect of light on sporulation.

The isolates were grown on petri dishes containing potato dextrose agar (PDA), cornmeal agar (CMA) and potato sucrose agar (PSA, prepared fresh from 100 g potato and 5 g sucrose per litre). Three dishes were used for each experiment which was repeated once. To determine the effect of light, cultures were incubated at 26°C in the dark for three days, followed by three days under different light treatments:

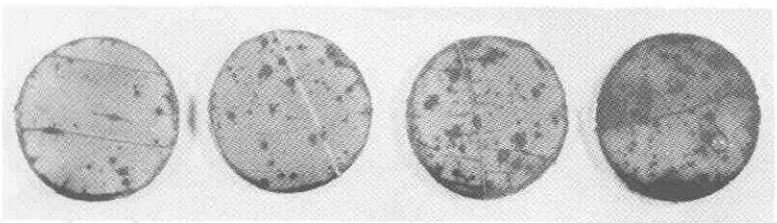
- Daily 2 h of fluorescent light (220 lux per day)
- 2 h of ultra-violet light (3650 Å)
- Alternate light and dark for 12 h each
- Continuous light
- Continuous dark.

Agar plugs were then cut from the edge of each colony with a 1 cm diameter cork borer, the spores removed by shaking the plugs in water were counted in a haemocytometer, and expressed as the number per square centimetre of agar surface. Three plates were used in each test and each test was carried out three times. Spore germination was tested by incubating drops of spore suspension on microscopic slides in petri dishes lined with moist paper.

The size of the spores was measured from one hundred spores caught in a Burkard spore trap placed near infected trees.

For the infection studies, seedlings of six host plant species known to be susceptible to *C. cassiicola* were grown in polybags under shade and inoculated by spraying spores of the fungus on the leaves. The inoculum typically contained 7×10^4 spores per millilitre. Inoculated plants were bagged for 24 h to maintain high humidity. Lesions were noted for over a period of four weeks and a positive infection was confirmed by successful re-isolation of the fungus.

In the inoculation of *Hevea* leaf discs, discs were cut from mature green leaves using a 1.5 cm diameter cork borer. Ten discs (abaxial surface facing upwards) were floated on distilled water in a petri dish, and inoculated by spraying a spore suspension and incubating at 28°C under daily alternate light and dark for six days. The severity of infection on the leaf discs was scored from 0 (no infection) to 3 (severe) based on the symptom guide shown in *Figure 1*.



1 2 3 Highly susceptible clones such as RRIC 103 and FX 25

Figure 1. The different severity of infection of leaf discs on different rubber clones. The picture is used as a guide for assessment of clonal susceptibility in the laboratory.

Field susceptibility of *Hevea* clones was assessed on nursery plants 3–5 m high and similarly scored 0 to 3 in which 0 = immune; 1 = one to five leaves infected; 2 = six to ten leaves infected; and, 3 = more than ten leaves infected.

In epidemiology studies, a Burkard spore trap was placed in the centre of a group of RRIC 103 trees at the RRIM Experiment Station in Sungei Buloh. The trap was operated for two years from 1985 to 1987. Meteorological data was obtained from the Experiment Station itself.

RESULTS

Infection and Leaf Fall

Infection by *C. cassiicola* occurred in intervenial areas of the leaves. Up to thirteen lesions were observed without causing the leaf to fall; but more commonly the leaves turned yellow and fell when there were less than six lesions on the intervenial area. When infection occurred on the lateral veins, the spread of the infection was rapid and leaf fall ensued. Leaf fall also occurred rapidly when infection was on the main vein, even though there might be only one lesion. Leaves did not fall if lesions on the lamina were cut out with a cork borer or painted with 5% mancozeb.

Conidial Production and Germination

Of the three media tested for sporulation when the culture was incubated in the dark for three days followed by continuous light for three days at 26°C, PSA produced significantly more conidia (313 per square centimetre) than PDA (175 per square centimetre) and CMA (162 per square centimetre).

Eighteen isolates were grown on PSA and similarly incubated. The culture differed in morphology *(Figure 2)* and there was great variation in the ability of the isolates to produce conidia, ranging from 6 to 644 conidia per square centimetre (LSD 93.2). Surprisingly, the isolates from the highly susceptible clone RRIC 103 had the weakest sporulation. Twenty-one single-spore isolates from one culture were tested for conidial production. They varied greatly in colony size and colour, in addition to the variability of the spore septae (0–13), and numbers of spores produced (31–619 per square centimetre of agar surface, LSD 26.9).

When isolates were incubated in the dark for three days followed by incubation in the light for a further three, six or nine days, there was significantly more sporulation after six days (average 630 spores per square centimetre) than after three days (average 211 spores per square centimetre) or after nine days (average 169 spores per square centimetre).

Sporulation was greatest with 2 h daily exposure to ultra-violet light, and progressively less with continuous light, alternate light and dark, 2 h daily of fluorescent light and continuous dark *(Table 1)*. Daily scraping of the

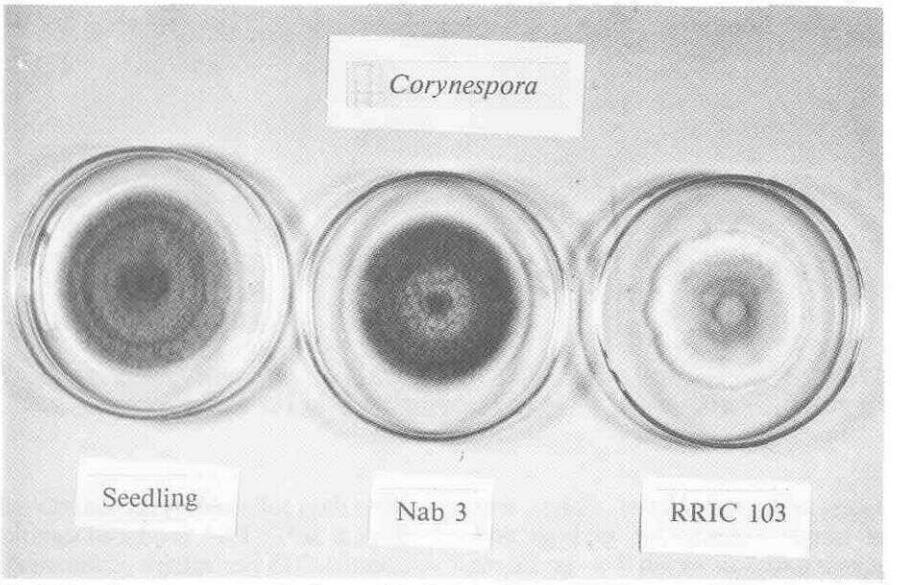


Figure 2. Variation in colony morphology of isolates of Corynespora cassiicola *from* Hevea *rubber.*

TABLE 1. EFFECT OF LIGHT ON THE SPORULATION OF *CORYNESPORA CASSIICOLA* ON AGAR MEDIUM

Treatment	No. of conidia/cm^2
Ultra-violet light 2 h daily	704
Continuous light[a]	313
Alternate light[a] and dark	232
Light[a] 2 h daily	188
Continuous dark	27
$LSD_{5\%}$	107.24

[a]Fluorescent light, 220 lux for 12 h

aerial mycelium of the culture did not increase sporulation.

Conidia were found on both the abaxial and adaxial surfaces of leaves of all ages. In general, there were more conidia on the adaxial surface of leaves at their pale-green stage than on older leaves. The average size of the conidia of *C. cassiicola* from *Hevea* rubber was 64.4 × 5.52 mμ (range 23.4 to 132.6 × 2.6 to 7.8 mμ).

Conidia of *C. cassiicola* germinated within 4 h. The germinated spores produced one or several germ tubes between the septae, but more often germ tubes arose from the end cells of the spores *(Figure 3).*

Pathogenicity

Healthy leaves of various ages of clone FX 25 in the nursery were sprayed in the evening with a suspension containing 7 × 10^4 spores per millilitre of *C. cassiicola,* and infection was assessed two weeks later. Infection occurred readily on leaves up to four weeks from bud-burst, but older leaves were also infected.

Conidia from four isolates, including one from clone RRIC 103, were inoculated on leaf discs of clone FX 25 (susceptible) and RRIM 600 (resistant). Different isolates varied in pathogenicity, causing slight to moderate infection on FX 25 and very little or no infection on RRIM 600, depending on the isolate used. However, infection of FX 25 caused by the isolate from RRIC 103 was between slight and moderate, indicating that this isolate (from the most susceptible clone) was not particularly aggressive.

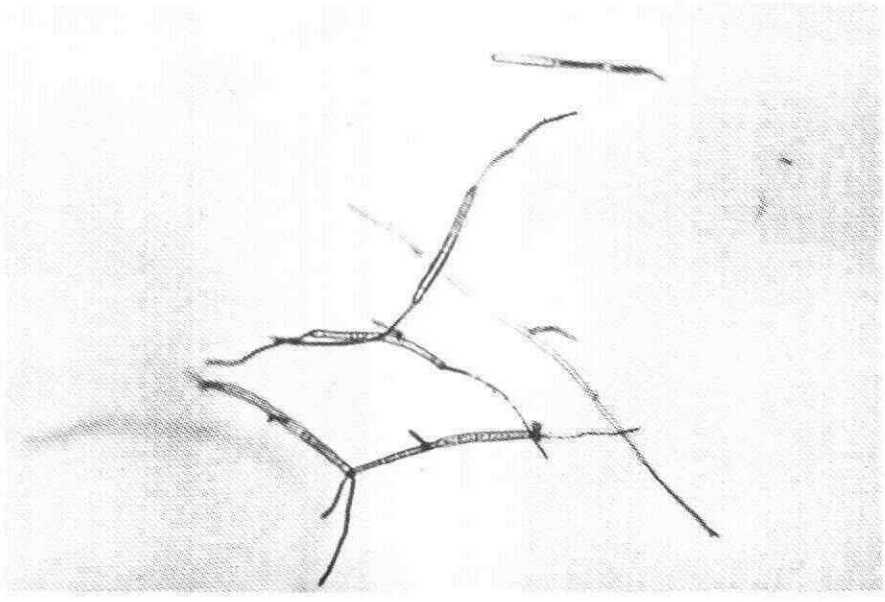


Figure 3. Germinating spores of Corynespora cassiicola.

No infection occurred when conidia of the rubber isolates were inoculated onto papaya, tomato, lettuce, soya bean, cocoa and oil palm. Similarly, the isolate from papaya infected papaya only.

Leaf discs of 137 clones were inoculated in the laboratory and their average disease scores together with their field disease assessments are given in *Table 2*. The results obtained with the two methods were similar. It is also noteworthy that most RRIM clones were either immune or resistant to *C. cassiicola*.

Epidemiology

Analysis of spore trap data for two years showed that spore release began at 0800 h and attained a peak around noon as observed by other workers[12]. It then fell to a very low level until the following sunrise *(Figure 4)*. The seasonal effect of spore release in relation to the amount of rainfall and number of rainy days was not readily apparent, but the indication was that fewer spores were caught during wet weather *(Figure 5)*. High spore counts were often encountered on fine days preceded by a day of wet weather.

DISCUSSION

Malaysia is fortunate that clones bred here have until now apparently been resistant to *Corynespora* leaf spot. However, on certain introduced clones such as RRIC 103, the disease causes perpetual leaf fall, retardation of growth and even death. The fungus infects both young and old leaves, in contrast to the two fungal pathogens causing secondary leaf fall — *Colletotrichum gloeosporioides* and *Oidium heveae* which attack only the young leaves. Chemical control of *Corynespora* leaf spot is likely to be much more difficult than that of secondary leaf fall, as the disease occurs throughout the year and on leaves of all ages. Further, our limited field trials show that to be effective, uniform and adequate spray coverage of leaves is critical — a condition not easily met in plantation practice. This is necessary because the fungus produces a toxin[13] which induces leaf abscission, so that one infection point on the petiole or main vein is sufficient to cause

TABLE 2. LABORATORY AND FIELD SUSCEPTIBILITY OF *HEVEA* CLONES TO *CORYNESPORA CASSIICOLA*

Clone/code	Laboratory score	Field susceptibility	Clone/code	Laboratory score	Field susceptibility
RO/1/2-47	3	3	MT/1/21-24	0	0
RO/1/10-54	1	1	MT/1/24-27	1	1
RO/1/11-55	0	0	MT/1/39A-38	2	2
RO/1/24-61	2	2	MT/1/40A-40	3	2
RO/1/25-62	0	0	MT/C/2-10/54	1	2
RO/1/61-85	2	3	MT/C/11-9/10	1	1
RO/1/81-96	0	0	MT/C/11-9/66	0	0
RO/1/108-111	0	0	MT/IT/10-19	3	3
RO/C/8-24/97	1	0	MT/IT/14-30/170	0	0
RO/C/8-24/223	1	0	MT/IT/14-30/137	0	0
RO/C/8-24/293	2	1			
RO/CM/10-44/780	3	2	7/02/81-1/15	2	1
RO/JP/3-22/42	2	1	7/02/81-1/55	0	0
RO/JP/3-22/89	0	0			
RO/JP/3-22/109	0	0	BPM 1	0	0
RO/JP/3-22/146	0	0	BPM 3	1	1
RO/JP/3-22/153	0	0	BPM 22	2	2
RO/JP/3-22/169	0	0	BPM 24	1	1
RO/JP/3-22/186	0	0	BPM 26	2	2
RO/JP/3-22/189	0	0			
RO/JP/3-22/392	0	0	Nab 17	1	0
RO/PB/1-2/51	0	0			
RO/PB/2-3/49	0	0	PB 235	0	0
RO/PB/2-3/53	0	0	PB 242	0	0
RO/PB/2-3/78	3	3	PB 245	0	0
RO/PB/2-3/82	1	1	PB 255	1	0
RO/PB/2-2/118	0	0	PB 259	0	0
RO/PB/2-3/186	0	0	PB 260	0	0
RO/PB/2-3/245	0	0	PB 274	0	0
			PB 280	0	0
AC/I/14-6	2	1	PB 28/59	0	0
AC/I/24-10	0	0	PB 310	1	0
AC/F/5-21/100	1	1	PB 312	1	0
AC/F/5-21/108	0	0	PB 314	1	0
AC/F/5-21/203	2	2	PB 324	1	0
AC/AB/15-54/580	1	0	PB 326	1	0
			PB 328	0	0
			PB 330	0	0

TABLE 2. LABORATORY AND FIELD SUSCEPTIBILITY OF *HEVEA* CLONES TO *CORYNESPORA CASSIICOLA* (CONTD.)

Clone/code	Laboratory score	Field susceptibility	Clone/code	Laboratory score	Field susceptibility
PR 302	0	0	RRIM 913	1	1
PR 305	1	1	RRIM 914	0	0
PR 306	0	0	RRIM 915	0	0
PR 307	0	0	RRIM 916	0	0
PR 309	1	1	RRIM 917	0	0
			RRIM 918	0	0
RRIC 100	0	0	RRIM 919	0	0
RRIC 101	0	0	RRIM 920	0	0
RRIC 102	0	0	RRIM 921	0	0
RRIC 110	1	1	RRIM 922	1	0
			RRIM 923	1	1
RRIM 623	1	0	RRIM 924	1	0
RRIM 701	0	0	RRIM 925	0	0
RRIM 728	2	2	RRIM 926	0	0
RRIM 729	0	0	RRIM 927	1	0
RRIM 802	0	0			
RRIM 803	0	0	IAN 873	3	3
RRIM 805	0	0			
RRIM 806	1	1	PM 8	1	1
RRIM 807	1	1	PM 10	0	0
RRIM 808	0	0	PM 251	0	0
RRIM 809	1	2			
RRIM 810	0	0	PC 10	0	0
RRIM 901	1	0	PC 11	1	0
RRIM 902	0	0	PC 20	0	0
RRIM 903	1	1	PC 25	0	0
RRIM 904	0	0	PC 28	1	1
RRIM 905	1	0	PC 37	2	2
RRIM 906	1	1	PC 41	1	1
RRIM 907	0	0	PC 42	3	3
RRIM 908	0	0	PC 45	0	0
RRIM 909	0	0	PC 51	1	0
RRIM 910	0	0	PC 53	1	1
RRIM 911	0	0	PC 54	3	2
RRIM 912	0	0	PC 55	0	0
			PC 57	1	0
			PC 71	1	1
			PC 72	1	0
			PC 92	0	0

0, 1, 2, 3 — Immune, slight, moderate and severe, respectively

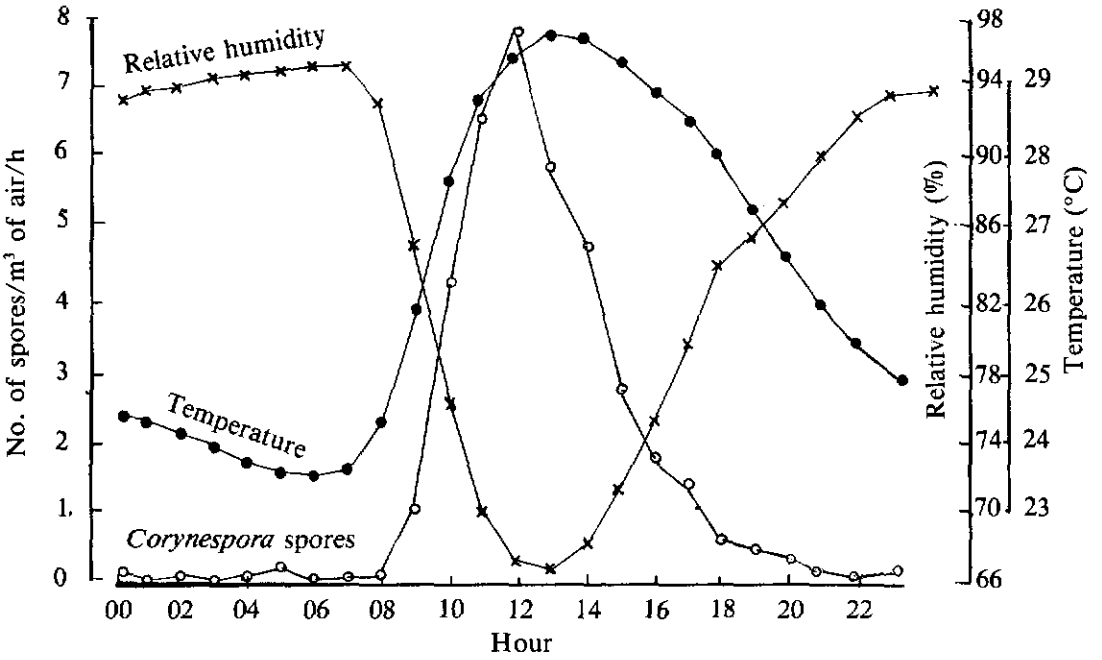


Figure 4. Diurnal periodicity of spore liberation in Corynespora cassiicola.

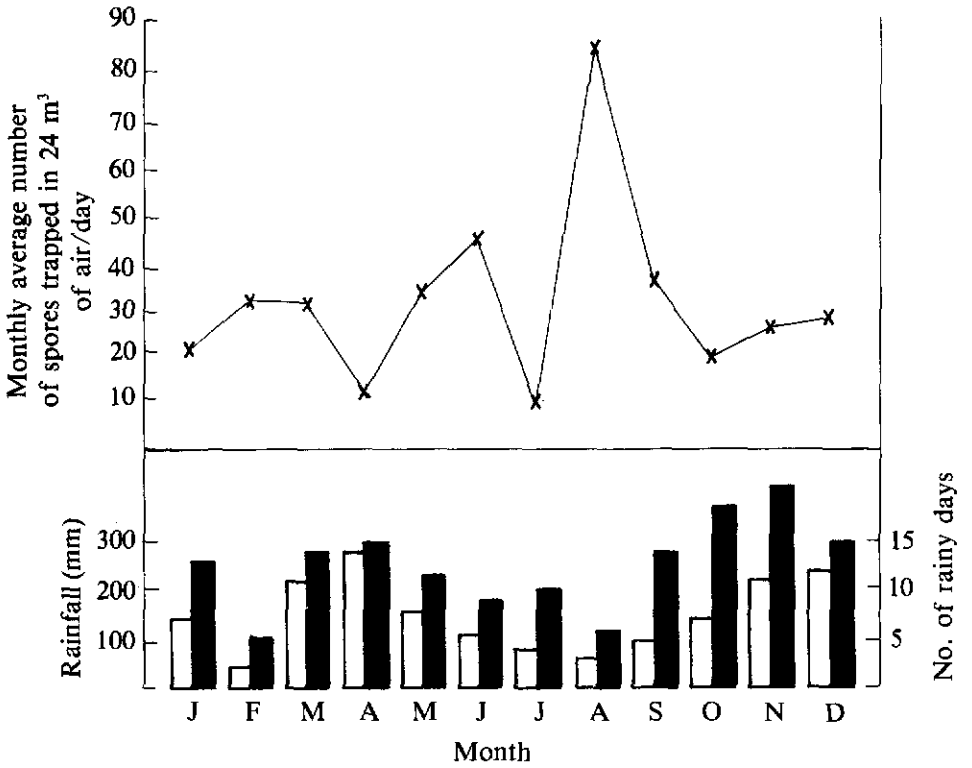


Figure 5. Seasonal spore liberation in Corynespora cassiicola.

leaf fall. Several chemicals including chlorothalonil, benomyl, triademefon and tridemorph when applied prophylactically appear to be effective against the disease. It would seem likely that the only practical method of combating *Corynespora* leaf spot on *Hevea* is to avoid planting susceptible clones. This requires susceptible clones to be detected early during the breeding and selection process. To this end, many of the important clones in Malaysia have been tested *(Table 2)*. In view of the seriousness of the disease and the extended period of fungicide applications if chemical control is considered, existing clones highly susceptible to the disease may have to be uprooted. For young plantings, crown budding with resistant clones warrants serious consideration.

C. cassiicola is very variable in cultural morphology, growth rate and sporulation[11,14]. Sporulation occurred only with certain isolates in certain media incubated under a few hours of light each day. Contrary to findings by other workers[15], scraping of the mycelium from the culture did not enhance sporulation. The number of spore septae varied[14], as did the size of the spores.

Since the *Hevea* isolates did not cross-infect other hosts, nor did the papaya isolate infect *Hevea,* it is possible that isolates are host-specific. However this aspect needs to be tested on other host plants.

In the nursery, GT 1 and RRIM 600 plots adjacent to the very susceptible clone FX 25 were slightly affected and some leaves on the lower whorls had multiple lesions. Another clone, PB 86 also planted next to FX 25 was not affected. Pathogenicity studies did not show the fungus isolated from GT 1 or RRIM 600 to be different from that of FX 25.

ACKNOWLEDGEMENT

The author wishes to thank Encik Arifin bin Khalid and Encik Chew Beng Hock for technical assistance.

REFERENCES

1. WEI, C.H. (1950) Notes on *Corynespora. Mycol. Pap. Commonwealth Mycological Institute No. 34,* 10.
2. NEWSAM, A. (1961) Pathology Division. *Rep. Rubb. Res. Inst. Malaya 1960,* 63–70.
3. RUBBER RESEARCH INSTITUTE OF MALAYSIA (1975) *Corynespora* leaf spot. *Plrs' Bull. Rubb. Res. Inst. Malaysia No. 139,* 84–86.
4. TEOH, C.H. (1985) Private Communication. Harrisons Malaysian Plantations Berhad, Prang Besar Research Station, Kajang, Selangor, Malaysia.
5. WHEELER, B.J. (1985) Private Communication. Imperial College, University of London, UK.
6. LIYANAGE, A.S. (1987) Private Communication. Rubber Research Institute of Sri Lanka, Sri Lanka.
7. RAMAKRISHNAN, T.S. AND PILLAY, P.N.R. (1961) Leaf Spot of Rubber caused by *Corynespora cassiicola* (Berk. & Curt.) Wei. *Rubb. Bd Bull.,* **5,** 52–53.
8. AWODERU, V.A. (1969) A New Leaf Spot of Para Rubber *Hevea brasiliensis* in Nigeria. *Pl. Dis. Reptr,* **53,** 406–408.
9. HERVERT, V. AND KAZDA, V. (1976) Water Deficit in Cucumber *(Cucumis sativus)* infected by *Corynespora melonis (C. cassiicola). Acta. Inst. Bot. Acad. Sci.,* Slov B.1, 257–263.
10. RAO, B.S. (1975) *Maladies of* Hevea *in Malaysia, 108 pp.* Kuala Lumpur: Rubber Research Institute of Malaysia.
11. DUARTE, M.L.R., ASANO, S. AND ALBUQUERQUE, F.C. (1983) Comparative Study of the Morphological and Physiological Characteristics of two *Corynespora cassiicola* Isolates. *Fitopathologia Brasileira,* **8,** 205–214.
12. NAIR, C.M. AND RAJ, J.S. (1966) Diurnal Periodicity of Spores Liberation in *Corynespora cassiicola* (Berk. & Curt.) Wei. *Agric. Res. J. Kerala,* **4,** 54–56.
13. ONESIROSAN, P., MABUNI, C.T., DURBIN, R.D., MORIN, R.B., RICH, D.H. AND ARNY, D.C. (1975) Toxin Production by *Corynespora cassiicola. Physiol. Pl. Path.,* **5,** 289–295.
14. SPENCER, J.A. AND WALTER, H.J. (1969) Variations in Certain Isolates of *Corynespora cassiicola. Phytopathology,* **59,** 58–60.
15. ONESIROSAN, P., ARNY, D. AND DURBIN, R.D. (1975) Increasing Sporulation of *Corynespora cassiicola. Mycopathologia,* **55,** 121–123.