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Effect of Humidity and Temperature on the Development of South American Leaf Blight (Microcyclus ulei) *of* Hevea brasiliensis

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Dry conidia of Mycrocyclus ulei *require 6–8 h of high humidity immediately after deposition to infect young leaves of* Hevea brasiliensis. *After inoculation high disease intensity was observed on plants incubated at 19°C–22°C or 23°C–25°C, but little infection developed at 26°C–29°C and none at 30°C–32°C. Lesion development was optimum at 23°C–25°C. Conidial sporulation occurred, between 19°C and 28°C and was increased by high humidity, especially in the range 23°C–25°C.*

South American leaf blight (SALB) caused by *Microcyclus ulei* (P. Henn.) Arx is a serious impediment to the growth of *Hevea brasiliensis* Muell. Arg. The disease is presently confined to South America and is absent from South East Asia where most of the world's rubber is planted. Studies on the biology of the fungus, such as growth and conidial sporulation in culture, conidial germination and ascospore discharge, show that its optimum temperature for development is 23°C or less[1,2]. This leads to speculation that SALB may not be as damaging in South East Asia as in South America because the average temperature throughout lowland Malaysia, for example, is above 23°C. This paper describes experiments designed to test the effects of temperature and humidity on the infection, disease development and sporulation of SALB on living plants.

MATERIALS AND METHOD

Brown budded stumps of clone FX 3864 were grown in 25 × 55 cm polyethylene bags kept in a partially covered and well illuminated concrete enclosure at ambient conditions (RH, 50%–90%; night temperature, 17°C–22°C; day temperature, 23°C–28°C) in Itabuna, in the state of Bahia, Brazil, in the cooler months of May to October 1983. Temperatures were recorded from thermometers hanging on the budded stumps six times during a daily 24 h period and from a thermohygrograph placed nearby. Plants were individually watered daily. Unless otherwise stated, inoculation was carried out on seven- to ten-day-old leaves and the whole plant immediately covered with a polyethylene bag.

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The inoculum used was field conidia collected from clone FX 3864 and clone FX 2261 in Estacao Experimental Djalma Bahia (EDJAB). Inoculation was done either by dry deposition of conidia on the leaves or spraying with a conidial suspension. In dry deposition, a camel hair brush was used to pick up conidia from diseased leaves and brush them onto the under-surface of the young leaves. Spraying was by means of an Atomist Atomiser containing 10^6 spores per millilitre. The spray was directed to the lower surface of the leaves.

The various temperatures used in the experiments referred to those at night and were obtained by placing the bagged plants at an appropriate distance (2m or more) from a 800 watt 'Termo Krik' home radiator. The radiator was switched on nightly from 2100 h to 0700 h to maintain the air temperature within the desired range. The temperature of bagged plants was 3°C higher than unbagged plants, but this discrepancy was corrected by positioning bagged plants further from the radiator. High humidity is 90%-100% and low humidity that at ambient conditions. The method of Shipton and Brown[4] was used to clear and stain the leaves for microscopic examination of leaf lesions.

Effect of Humidity on Infection

Immediately after inoculation, each plant was covered and tied with a polyethylene bag and instant high humidity was induced by injecting 50 ml warm water (50°C) into the bottom of each bag. The bags were removed after 0, 2, 4, 6, 8 10 and 24 h. The plants were then maintained at ambient temperature for seven days before lesions were counted from ten leaves on two plants.

Effect of Temperature on Infection

The plants were inoculated in the evening with a conidial suspension and incubated at 19°C–22°C, 23°C–25°C, 26°C–29°C and 30°C–32°C for 10 h before the bags were removed. Lesions on the leaves were counted after seven days.

Effects of Humidity and Temperature on Disease Development

Plants were inoculated and covered as described above and held at ambient temperature for 48 h to allow the infection to become established. After this pre-treatment, polyethylene bags were removed from each set of two plants to expose them to ambient humidity at 19°C–22°C, 23°C–25°C and 26°C–28°C for 10 h nightly. The second lot of plants was covered with polyethylene bags for seven days at almost the same temperatures. Diseased leaves, sampled on the seventh and fourteenth day after inoculation, were cleared and stained for microscopic examination. The diameters of thirty well defined lesions were measured in each treatment consisting of ten leaves from two plants.

Effects of Humidity and Temperature on Sporulation

Young leaves of clone FX 2261 budded stumps were sprayed with a conidial suspension and covered with polyethylene bags for 24 h. The inoculated plants were incubated at nightly temperature of 23°C–25°C for five days to allow the disease to become well established. On the sixth day onwards, plants which were still covered with bags were incubated at 19°C–22°C, 23°C–25°C or 26°C–28°C. Five pieces of diseased leaves measuring 1 cm^2 were cut from each plant at 1000 h on the seventh, fourteenth and twenty-first day after inoculation. The leaf pieces were submerged individually in test tubes containing 2 ml lactophenol. The tubes were agitated by a Phoenix agitator for 3 min at the highest speed. Conidia were counted wtih a double Neubauer ruling haemocytometer.

RESULTS

Effect of Humidity on Infection

Dry conidia required at least 6–8 h of high humidity to initiate a lesion *(Figure 1)*. As a

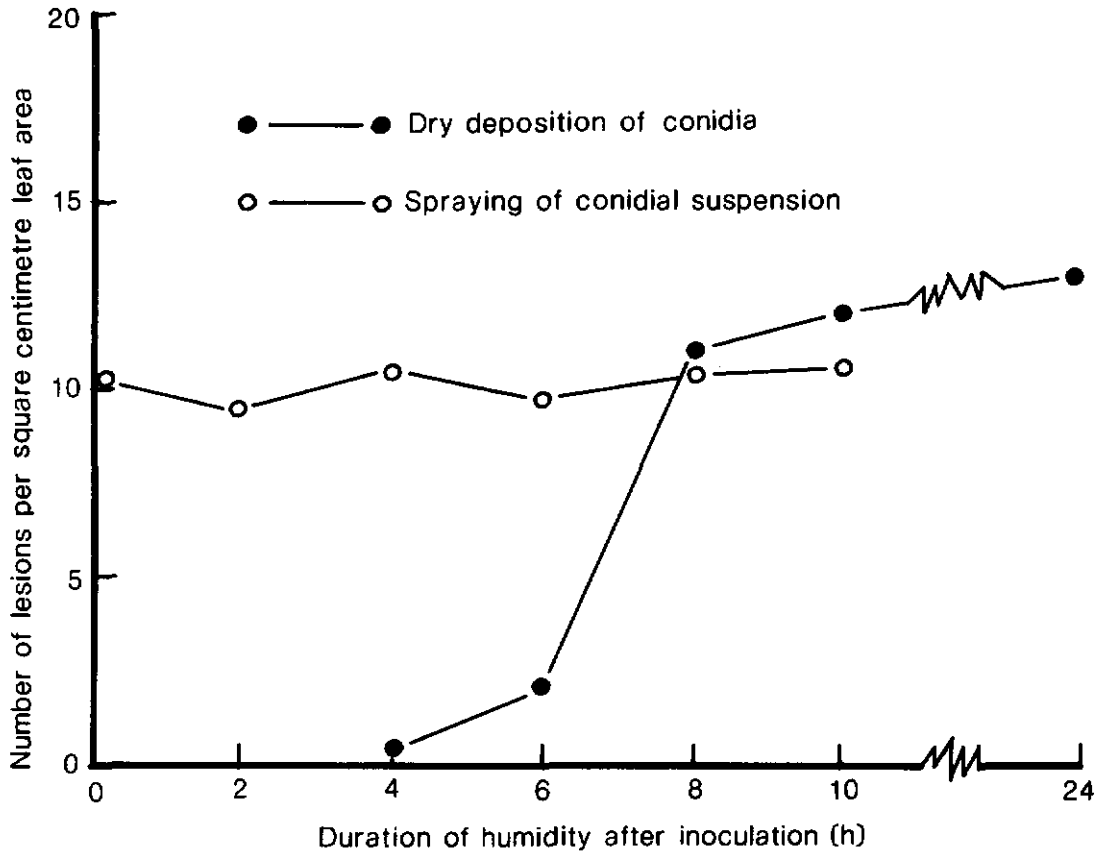

Figure 1. Number of disease lesions per square centimetre of leaf area on plants (clone FX 3864) incubated under high humidity for different periods of time after inoculation by dry deposition of conidia and spraying of conidial suspension.

result, no lesions occurred on leaves incubated at high humidity for only 2 h or 4 h. When incubated at high humidity for 6 h or 8 h, an average of one and eleven lesions respectively developed per square centimetre of leaf area ($LSD_{0.05}$ = 2.59**). There was no significant difference at the 5% probability level between the number of lesions on leaves incubated for 8, 10 or 24 h.

When conidia were applied to the leaves by spraying, about ten lesions occurred per square centimetre leaf area, irrespective of whether the polyethylene bags were covering the plants for 0, 2, 4, 6, 8 or 10 h ($LSD_{0.05} = 3.69^{NS}$) *(Figure 1).*

Effect of Temperature on Infection

The mean numbers of diseased lesions per square centimetre leaf area of plants incubated at 19°C–22°C, 23°C–25°C, 26°C–29°C, or 30°C–32°C were 10.1, 10.4, 1.4, and 0 respectively. The disease intensity was thus similar on plants incubated at 19°C–22°C or 23°C–25°C. Incubation at 26°C–29°C reduced the numbers of lesions significantly ($LSD_{0.05} = 2.62$). There was no infection at

30°C–32°C. There were no lesions on any of the control (uninoculated) plants.

Effects of Humidity and Temperature on Disease Development

The mean diameters of lesions measured on the seventh day after inoculation on clone FX 3864 are shown in *Table 1*. Lesions developing at 23°C–25°C were significantly larger than those at 19°C–22°C or 26°C–28°C. High humidity was not necessary for development of the lesions. However, it enhanced early sporulation (one week from inoculation) of the well developed lesions on plants incubated at 23°C–25°C. Lesions on plants kept at low humidity at 19°C–22°C or 26°C–28°C produced a negligible number of conidia two weeks after inoculation, while more profuse sporulation occurred on plants incubated at 23°C–25°C.

The mean diameter of lesions on plants incubated at low humidity at 23°C–25°C was significantly larger than that held at about the same temperature in polyethylene bags.

Effects of Humidity and Temperature on Sporulation

The numbers of conidia per square centimetre leaf area produced on plants of clone FX 2261 incubated under different humidities and temperatures at one, two and three weeks after inoculation are shown in *Figure 2* ($LSD_{0.05} = 2.02$, 6.81 and 3.02 respectively). During the sporulation period, diseased plants with well developed lesions incubated at 19°C–28°C under high humidity produced significantly more conidia than those incubated at the same temperatures but under low humidity. The fungus sporulated within a week of inoculation if held at high humidity at 19°C–28°C. Conidial sporulation was highest two weeks after inoculation irrespective of humidity. Pycnidia began to form on leaves incubated under high humidity at 19°C–28°C for three weeks.

Under both high and low humidity conditions, the plants incubated at 26°C–28°C produced more conidia than those incubated at 19°C–22°C or 23°C–25°C. This was partly due to differences in leaf age at the time of inoculation resulting in different lesion sizes. Leaf flushes used for tests at 26°C–28°C were seven days old while those at 19°C–22°C and 23°C–25°C were ten days old. Younger leaves produced bigger lesions (mean 7.5 mm diameter) than the older leaves (mean 1.5 mm diameter). Leaf age seems to have a greater effect over temperature on lesion development.

TABLE 1. EFFECT OF TEMPERATURE AND HUMIDITY ON LESION SIZE AND SPORULATION OF *M. ULEI* ON *H. BRASILIENSIS*, CLONE FX 3864

Treatment		Average lesion diameter 7 days after inoculation (μm)	Sporulation	
Temperature (°C)	RH		7 days after inoculation	14 days after inoculation
19–22	Low	211	No	Yes
19–22	High	176	No	–
23–25	Low	484	No	Yes
23–25	High	364	Yes	–
26–28	Low	213	No	Yes
26–28	High	207	Yes	–
		$LSD_{0.05} = 50.91$		

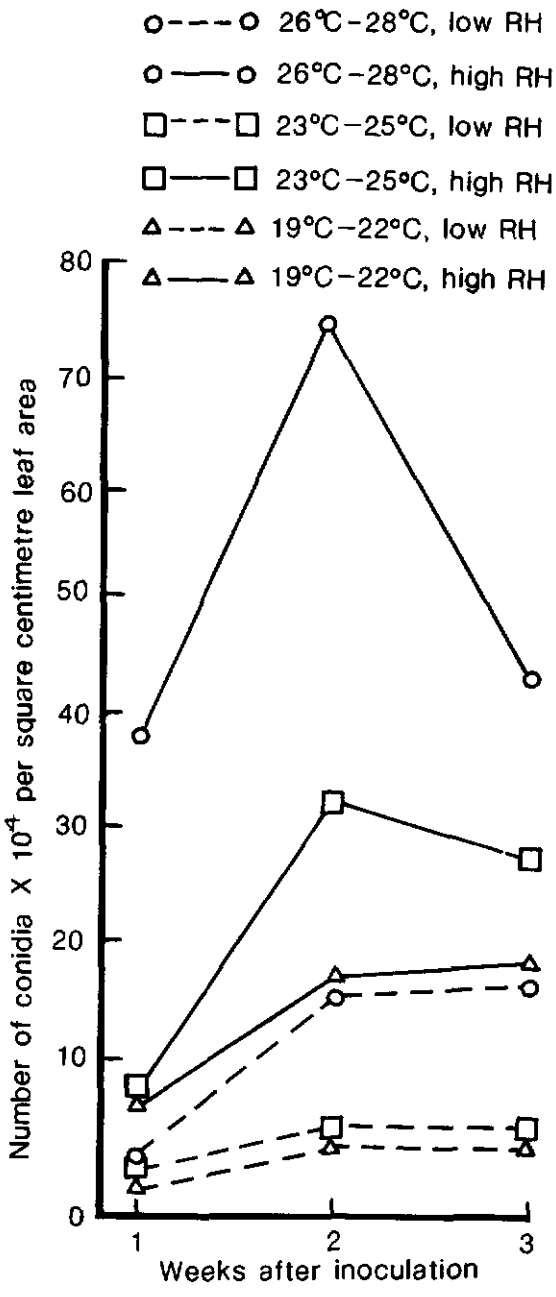

Figure 2. Number of conidia per square centimetre of leaf area on plants (clone FX 2261) incubated under different humidities and temperatures at one, two and three weeks after inoculation.

DISCUSSION

Inoculating plants under controlled humidity and temperature has shown that infection of *H. brasiliensis* by *M. ulei* is profoundly affected by these two environmental parameters. The optimum temperature for infection and disease development was 23°C-25°C. Temperatures up to 28°C did not prevent infection but sporulation was curtailed. Temperatures below 23°C resulted in less infection, but low temperature indirectly enhanced sporulations by inducing dew formation. Dry conidia required at least 6 h of high humidity to initiate a lesion, confirming Langford's observation[3] that a moist period of approximately 8 h was essential for conidia to infect the leaves of potted plants. The present study showed that the amount of infection was increased somewhat by extending the period of high humidity to 10 h or more. Langford[3] also observed an increase in infection after 10 h high humidity. In practically all areas suitable for rubber cultivation, rain or dew of sufficient duration to keep the foliage wet for 10 h or more occurs frequently. It seems unlikely, therefore that climatic conditions dry enough to inhibit SALB will be found suitable for rubber cultivation. Indeed, dry weather has not curbed SALB development in plantings of susceptible clones in any areas studied by Langford in Costa Rica and Panama[3]. Especially misleading has been temporary escape from SALB in areas where a long dry season has retarded, but not inhibited, severe disease damage.

Langford[3] observed that susceptible trees were completely defoliated by SALB during the hot months in Panama (average 29°C, maximum 36°C and minimum 21°C); and neither was SALB checked by low temperature in Costa Rica (average 21°C, maximum 29°C and minimum 11°C). The minimum night temperature in most rubber growing areas in Asia often falls within the optimum range for the development of SALB *(Figure 3).*

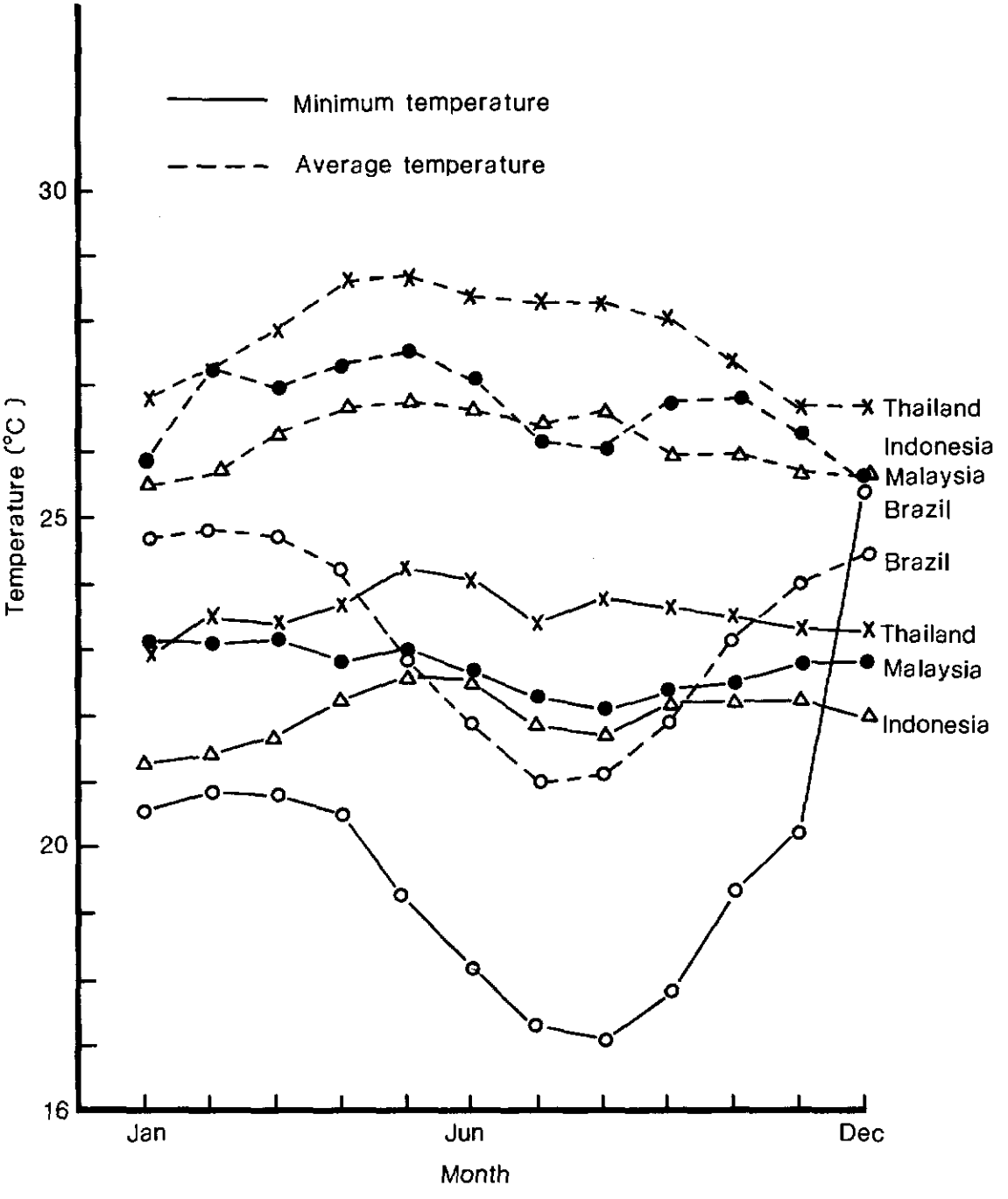

Figure 3. Mean ten years minimum and average temperatures for Itabuna (Brazil), Johore (Malaysia), Hat Yai (Thailand) and Medan (Indonesia).

Temperature and humidity conditions would appear to be favourable to the spread of the disease should the fungus arrive in South East Asia. Prevention of entry of the disease should once again be stressed.

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