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Leaf Blight Disease of Coconut 1. Studies on *Pestalozzia palmarum*

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ABSTRACT

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The cultural behaviour of *Pestalozzia palmarum* Cooke was studied on different agar media. Temperature-growth studies indicated that the colony growth and sporulation of the fungus were best at 25°C. The fungus grew best on Potato Dextrose Agar. The optimum temperature for the germination of conidia was 25°C. Germination was reduced with the increase of concentration of conidia.

It is doubtful whether *P. palmarum* is the primary cause of leaf blight of coconut in view of its inability to readily infect healthy coconut leaves.

INTRODUCTION

Leaf blight disease of Coconut, *Cocos nucifera* L., is common in coconut growing areas of the world. Its importance can, however, vary. In certain countries this disease can cause considerable damage, particularly in young plantations (Briolle, 1968).

Since the first record of the disease, the fungus *Pestalozzia palmarum* Cooke has been cited as the primary causal agent of leaf blight in many countries (Anon., 1961, 1964; Child, 1974; McPaul 1962; Shaw, 1965). However, basic knowledge of this fungus is lacking.

This paper describes studies on the effect of temperature on colony growth, germination of conidia and sporulation. Experiments were also carried out to find a suitable artificial medium for growing the fungus in the laboratory.

MATERIALS AND METHODS

Inoculum

Coconut leaves showing symptoms of leaf blight were first collected from diseased palms at Bandirippuwa Estate, Lunuwila. To prepare a culture of the fungus leaf pieces (about 25 mm) bearing lesions were surface sterilized by immersing for 2 min in 0.1% mercuric chloride solution. These were then thoroughly washed in two changes of sterile distilled water, plated on Czapek-Dox Agar(CDA) medium and incubated at 28°C.

In all instances, fungal growth was observed, and a pure culture was prepared by hyphal tips. The fungus was identified as *Pestalozzia palmarum* Cooke. In the laboratory, the culture was maintained by regular transfer to CDA.

Inoculation techniques

Using a sterile 4 mm cork borer, an agar plug was cut from the leading edge of a 5-day old culture grown at 28°C, and inoculated centrally on fresh medium (20 cm^3). The inoculated plates were then incubated at the desired temperature $\pm$0.1°C.

When necessary, conidial suspensions were made by pouring 10 cm^3 of sterile distilled water on to freshly sporulating cultures and the spores dislodged using a sterile glass rod. The suspensions thus made were sprayed on to test surfaces using a laboratory atomizer.

Assessment of growth rate and of germination of conidia

In experiments involving assessment of growth rate, treatments were carried out in four replicates where inoculation was done as described earlier. The colony diameter was measured at 24 h intervals, starting 24 h after inoculation, using a pair of calipers and a millimetre scale rule, two measurements being made at right angles on each colony at each time.

Germination of conidia was assessed by spraying 1 cm^3 of conidial suspension containing 3 x 10^3 conidia per cm^3 with a laboratory atomizer, on to a Petri dish containing 10 cm^3 of 2.5% distilled water agar (pH 5.4). After incubation, the agar plate was sprayed with 0.1% cotton blue in lactophenol and discs 4 mm in diameter were removed randomly using a cork borer. These were examined under the microscope to assess germination.

To assess the germination on leaf surface, leaves from 3-month old seedlings were sprayed with a freshly made suspension of conidia. Care was taken to prevent run-off and the inoculated seedlings were incubated in a moist chamber at the required temperature. After incubation, a piece of sellotape was placed on the conidia-bearing surface and lightly pressed before being removed and mounted in 0.1% cotton blue in lactophenol. These were then examined under the microscope to assess germination.

In the assessment of germination, about 400 conidia were examined from each treatment. A conidium was scored as germinated if the length of the germ tube exceeded the breadth of the germinating cell of the conidium.

EXPERIMENTAL

Effect of temperature on growth of the fungus

Sterile Petri dishes containing 15 cm^3 of Czapek-Dox agar were incoulated and incubated at 5, 10, 15, 20, 25, 30 and 35°C. The mean growth rate was assessed daily.

In artificial media, the fungus *P. palmarum* did not grow outside the range 5—35°C and the optimum temperature for growth is about 25°C. The growth rate at 10 and 15°C was much slower than at 25°C, where the Petri dish was completely covered in about 7 days (Fig. 1).

Growth of *P. palmarum* in different culture media

This experiment was carried out using Czapek-Dox agar, Potato Sucrose agar, Potato Dextrose agar, Potato Carrot agar and Malt Extract agar, which were prepared as indicated elsewhere (Anon., 1974).

Each dish was inoculated centrally and incubated at 25°C and measurements were taken daily. The fungus grew best in Potato Dextrose agar (Fig. 2).

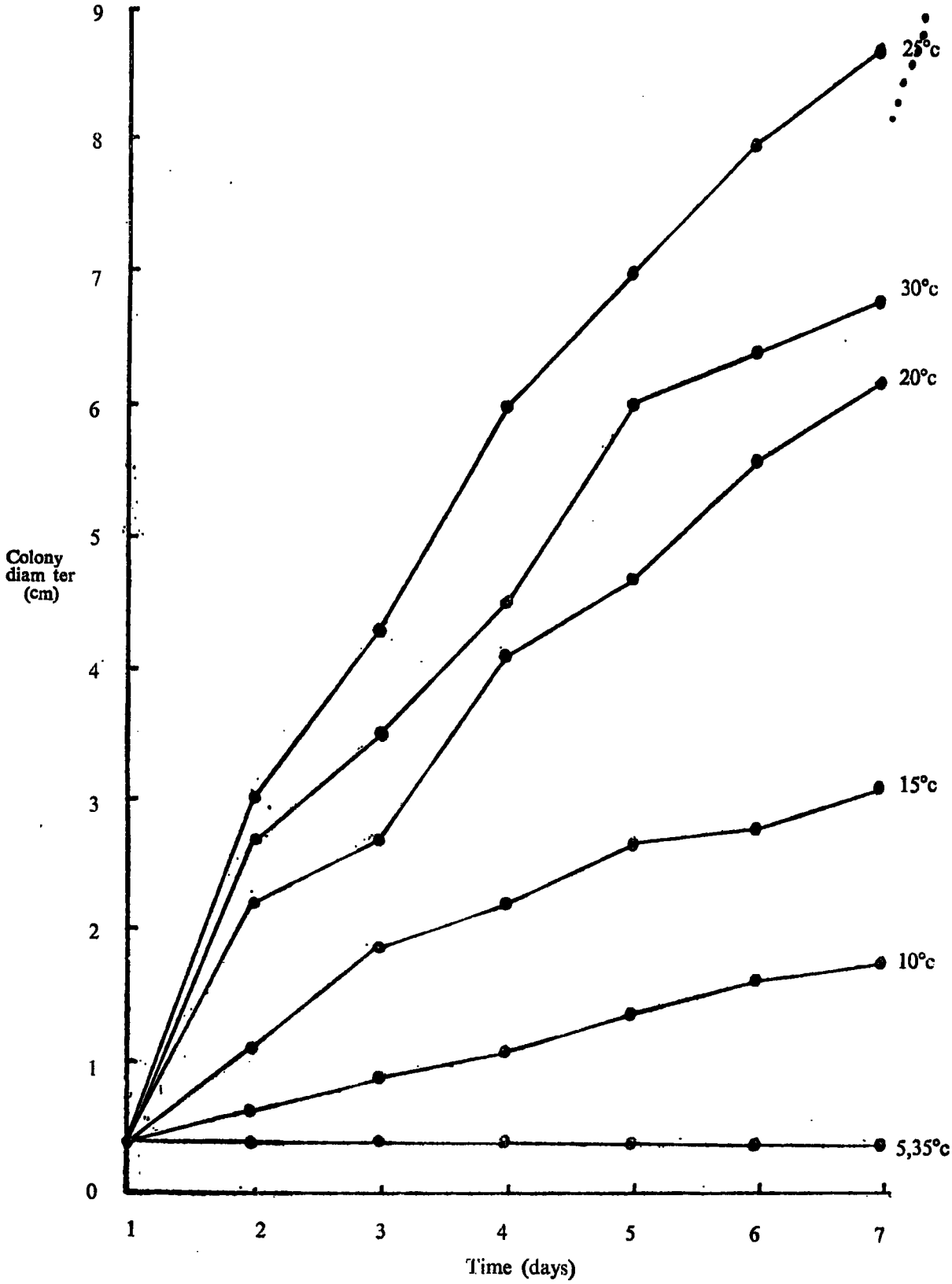

Fig. 1. Growth of *P. palmarum* on Czapek-Dox agar at different temperatures.

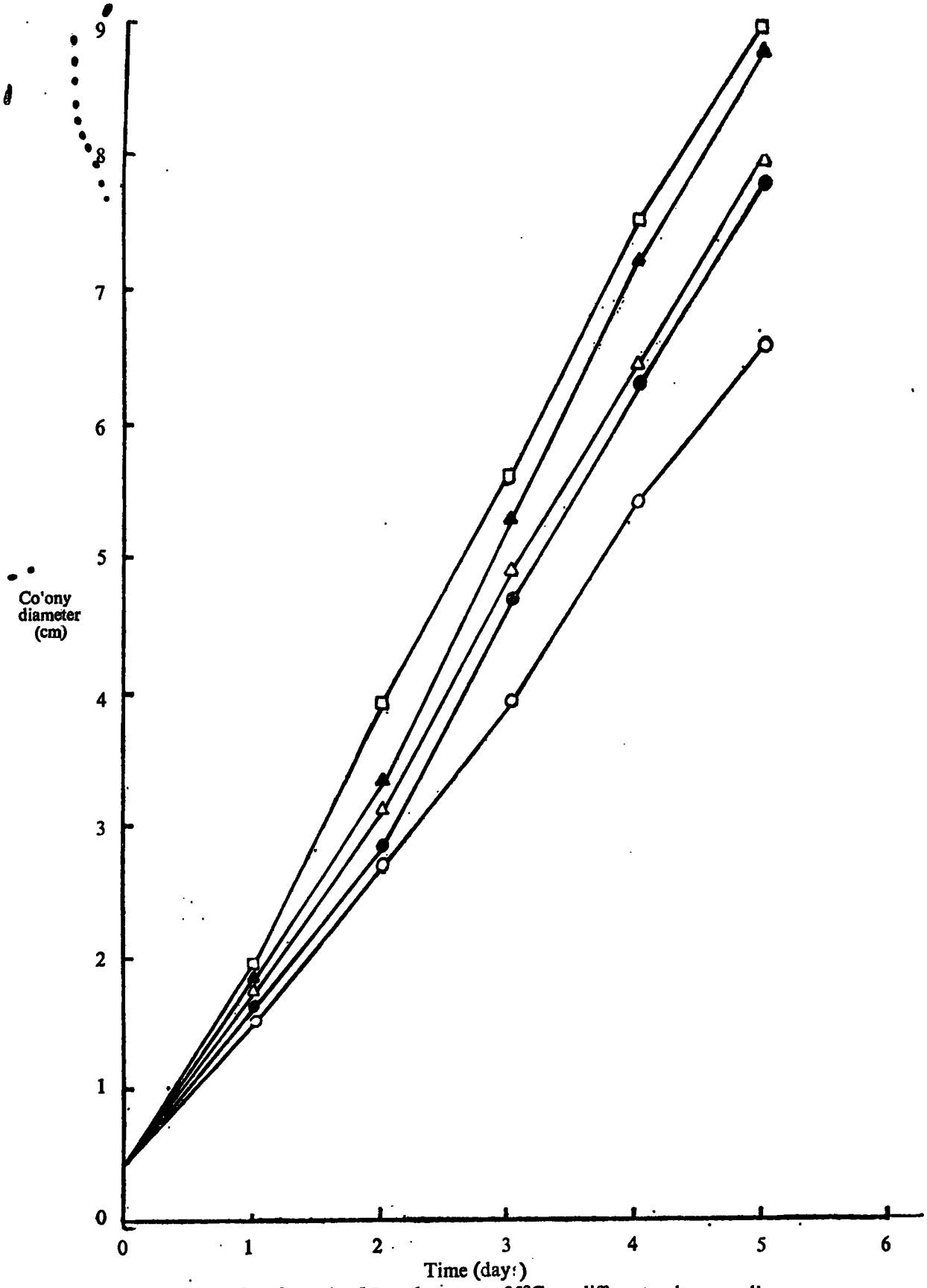

Fig. 2. Growth of *P. palmarium* at 25°C on different culture media.

(○) Malt Extract agar
(●) Potato Carrot agar
(△) Czapek-Dox agar
(▲) Potato Sucrose agar
(□) Potato Dextrose agar

Effect of temperature on sporulation of the fungus

Petri dishes containing Potato Dextrose agar were inoculated and incubated at 5, 10, 15, 20, 25, 30 and 35°C respectively. At each temperature, the mean sporulation time was assessed from four replicates.

Considerable variation of sporulation time was observed at different temperatures (Table 1). The sporulation was quickest at 25°C, which was also the optimum temperature for mycelial growth.

Table 1

Time taken for sporulation by P. palmarum at different temperatures.
(* indicates no sporulation after 18 days).

Temperature °C	*Time taken for sporulation (days)*
5	*
10	*
15	*
20	12
25	7
30	8
35	*

Effect of temperature on germination of conidia

Distilled water agar (2.5%) plates were pre-incubated for 2 h at the test temperature before inoculation. The agar plates were then inoculated with a freshly made suspension of conidia and incubated for 24 h at 5, 10, 15, 20, 25, 30 and 35°C. Germination was assessed every 2 h after inoculation.

The results of this experiment indicate that germination occurred at all temperatures tested except 5°C. The optimum temperature for germination appears to be about 25°C (Fig. 3). Twenty four hours after incubation, the highest percentage germination was observed at 25°C. At lower temperatures, initiation of germination was observed to take longer than 2 h (Fig. 3).

Effect of the concentration of conidial suspension on germination

Distilled water agar (2.5%) plates were inoculated with spore suspensions of varying concentrations, made from freshly sporulated agar plates, and incubated at 25°C for 24 h.

After incubation, germination was assessed as described elsewhere. Results of this experiment (Fig. 4) indicate that germination is significantly reduced at higher spore concentrations ($P=0.01$).

Inoculation of plants

Glasshouse-raised, three-month old potted coconut seedlings were sprayed with a conidial suspension and incubated for 72 h at about 25°C in a moist chamber. At the end of the incubation period plants were taken out and left outdoors. These plants were regularly examined for symptom development. In inoculated leaves mycelial strands were observed after about 7 days of inoculation but no further symptoms were observed until the 45th day, when a

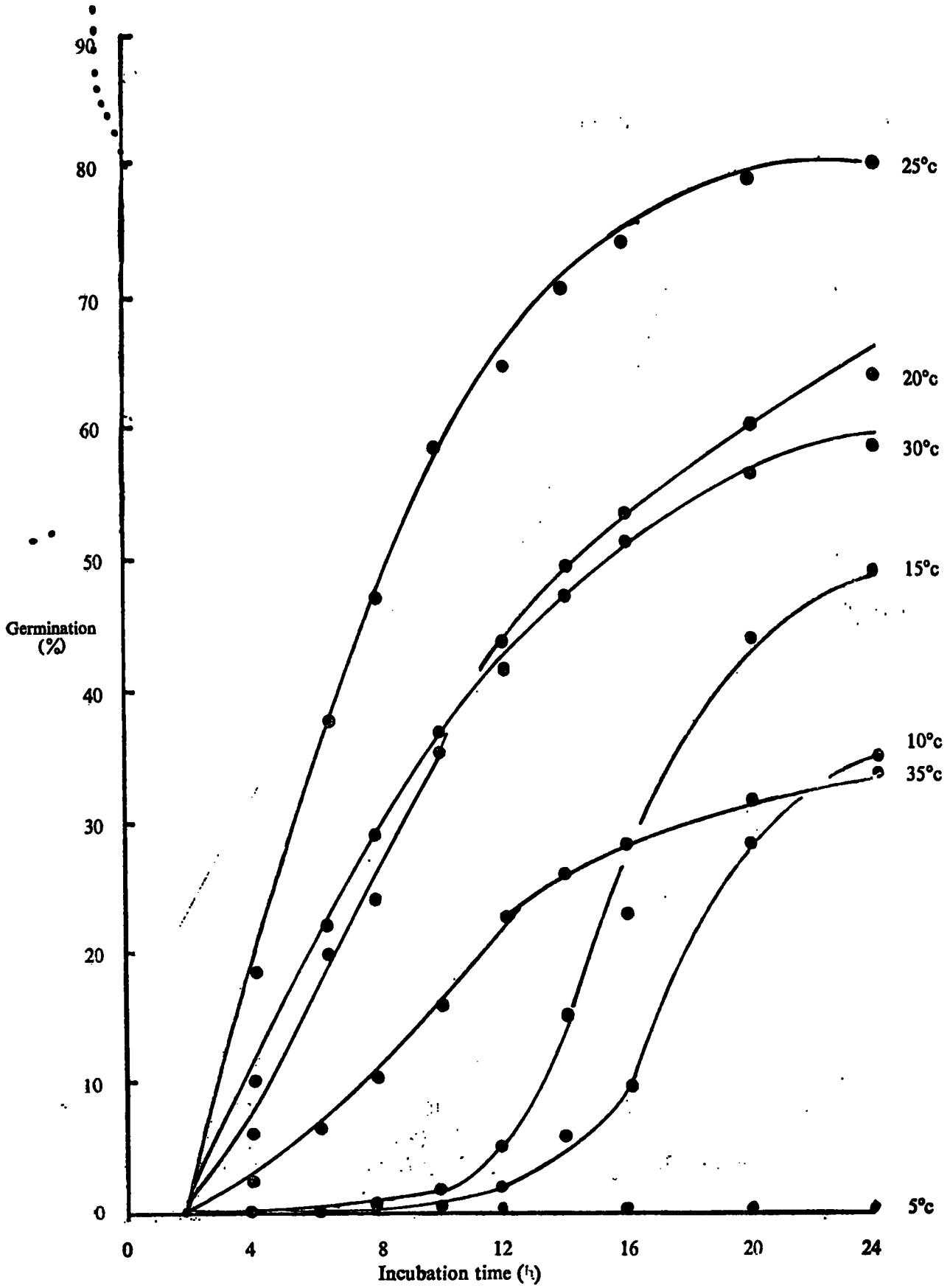

Fig. 3. Germination of *P. palmarum* conidia with time.

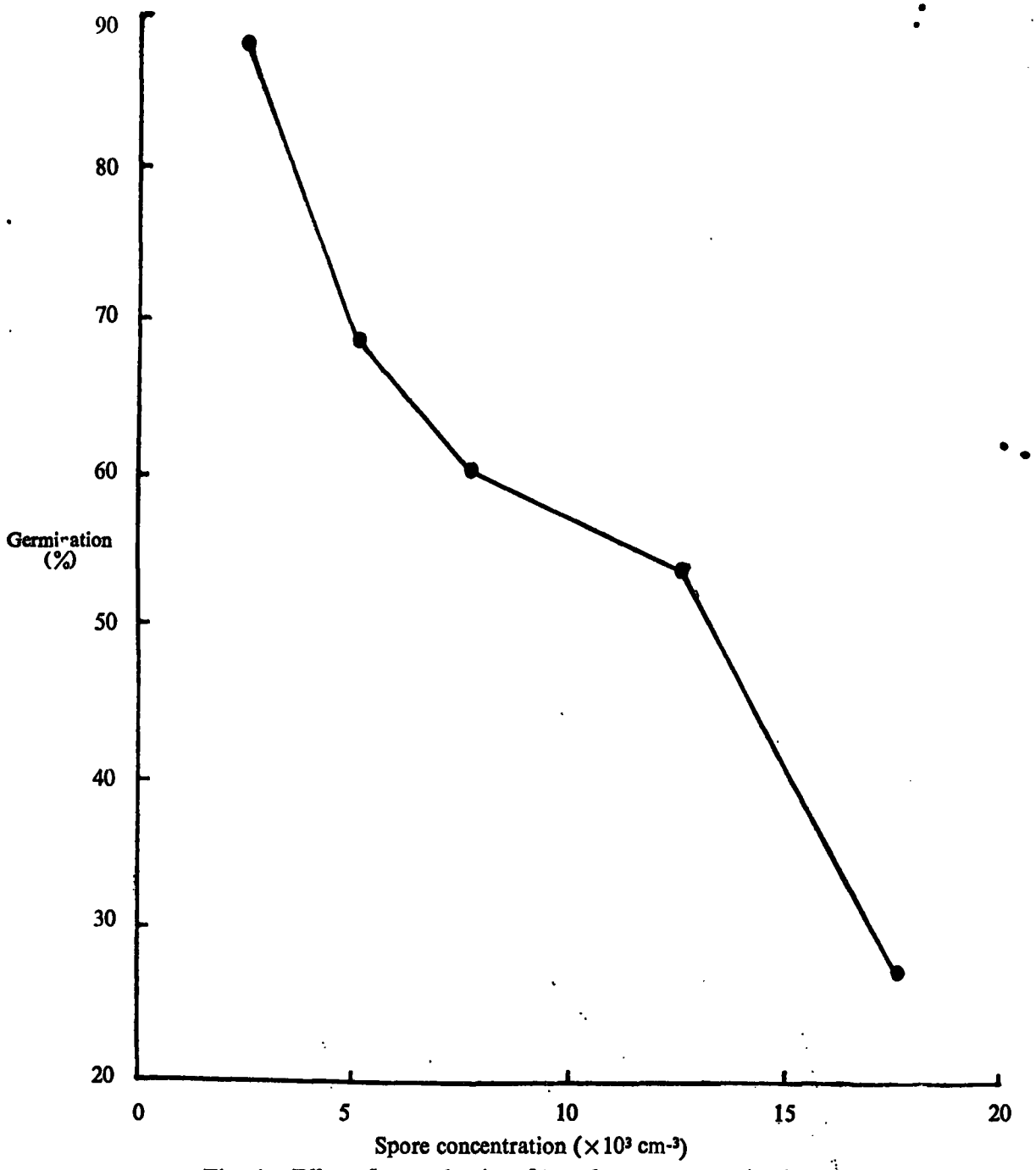

Fig. 4. Effect of spore density of *P. palmarum* on germination.

few small necrotic spots appeared. Uninoculated leaves too showed a few necrotic spots at this time. Attempts to reisolate from both inoculated and uninoculated leaves yielded several fungi, out of which *P. palmarum*, *Botryodiplodia theobromae*, *Nigrospora oryzae* and *Phoma cocoina* were consistently recovered.

DISCUSSION

The temperature-growth curve for this fungus is similar to that of other fungi. The optimum temperature for growth (25°C) is about the average outdoor temperature, and it is likely that the fungus grows profusely under these conditions. The fungus grew on all media tested but the best growth was observed in Potato Dextrose agar, which can be recommended for the laboratory culturing of the fungus. The sporulation was quickest at the optimum temperature for mycelial growth (25°C), and this indicates that under field conditions there is abundant sporulation. It is noteworthy that no sporulation occurred outside the range 20-30°C. The results of the present investigation show that the optimum temperature for spore germination is similar to that for mycelial growth and sporulation. This too would be of importance in the development of the fungus in the field, particularly because germination was observed within 2 h at 25°C. Results also show a reduction of germination with the increase of spore concentration. Competition for limiting nutrients or the release of inhibitory substances by the spores themselves could explain this phenomenon (Sussman and Halvorson, 1966).

Inoculation of coconut seedlings did not produce typical symptoms. This is one of the most important aspects of this study. Under the conditions of the experiments, small spots were seen 45 days after inoculation. Although it was possible to reisolate *P. palmarum* together with several other fungi such as *Botryodiplodia theobromae*, *Nigrospora oryzae* and *Phoma cocoina* from lesions, it is doubtful whether *P. palmarum* was the primary cause. Some of the control plants too developed small lesions which too yielded *P. palmarum* and some of the other fungi already mentioned. It is likely that *P. palmarum* can only infect already weakened tissue. These results indicate the need for a complete re-examination of the role of *P. palmarum*, hitherto considered to be the primary pathogen, in the leaf blight complex of coconut.

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