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Leaf Blight Disease of Coconut

2 Studies on Curvularia sp

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ABSTRACT

The cultural behaviour of *Curvularia lunata* Wakker, which caused lesions in leaves of coconut seedlings, was studied on different agar media. Temperature - growth studies indicated that the colony growth and sporulation were best at 30° C. The optimum temperature for germination of conidia on Water Agar was 30° C. Germination was reduced with the increase of concentration of conidia, and was less on leaf surface than on water agar.

When coconut leaves were inoculated with *C. lunata*, characteristic brown lesions appeared in three days. *C. eragrostidis* which was occasionally isolated from lesions, failed to produce any symptoms on inoculation.

INTRODUCTION

Leaf blight and Leaf spot diseases of coconut, *Cocos nucifera*, L., are common in many coconut growing countries. Several fungi such as *Pestalozzia palmarum* (Anon., 1961), *Drechslera incurvata* (Quillec & Renard, 1975), *Periconia saraswatipurensis* (Gupta & Ram, 1971) and *Curvularia* sp (Chan, 1974) have been found to be associated with these diseases.

In Sri Lanka, fungi associated with the leaf blight complex are *Drechslera incurvata* and *Pestalozzia palmarum*. However, it is doubtful whether the latter fungus is the primary cause of leaf blight (Fernando & Mahindapala, 1977). During routine laboratory investigations on leaf blight disease, a species of *Curvularia* was consistently isolated from lesions (Mahindapala, 1979).

Curvularia eragrostidis (=*C.maculans*) causes extensive leaf spots in coconut seedlings in nurseries during the wet months in West Malaysia (Chan, 1974). During the wet season of October-November, 1972, a serious outbreak of this disease occurred on *var.* Malayan Yellow dwarf with nearly all the nursery seedlings infected and over 50% of the frond area covered with spots (Chan, 1974).

The present study is the second in a series of studies on leaf blight disease and describes experiments on the effect of temperature on vegetative growth of the fungus, germination of conidia, sporulation and on disease development.

MATERIALS AND METHODS

Inoculum

Coconut leaves showing symptoms of leaf blight were collected from Tall X Dwarf (*var* CRIC 65) palms at the Coconut Research Institute, Lunuwila. To prepare a culture of the fungus leaf pieces bearing lesions were surface sterilized by immersing for 2 min in 0.1% mercuric chloride solution. These were then thoroughly washed in three changes of sterile distilled water, plated on Czapek-Dox Agar (CDA) medium and incubated at room temperature.

A pure culture of the fungus was prepared from hyphal tips. The fungus was identified as *Curvularia* sp. In the laboratory, the culture was maintained by regular transfer every seven days to Potato Dextrose Agar (PDA).

Inoculation techniques

Using a sterile 4 mm cork borer, an agar plug was cut from the leading edge of a 6—day old culture grown at 25° C, and inoculated centrally on fresh medium (20 cm^3). The inoculated plates were incubated at the test temperature $\pm$ 0.1 °C.

A conidial suspension was made by pouring five cm^3 of sterile distilled water on to a freshly spoulating culture medium and the spores dislodged using a sterile glassrod. The spore suspension was sprayed on to test plants using a laboratory atomizer.

Assessment of growth rate and germination of conidia

In experiments on assessment of growth rate, treatments were replicated four times at each temperature tested. The colony diameter was measured at 24 h intervals, starting 24 h after inoculation, using a pair of calipers and a centimeter scale. In each plate, two measurements were made at right angles to each other. Germination of conidia was assessed by spraying one cm^3 of conidial suspension on to a Petri-dish containing 10 cm^3 of 2.5% distilled water agar to give a uniform deposition of about 2×10^3 spores cm^{-2} which was found to be the ideal spore concentration on the test surface.

After incubation, discs 4 mm in diameter were removed randomly using a cork borer, and mounted in 0.1% cotton blue in lactophenol. These were examined under the microscope to assess germination.

To assess germination on the leaf, 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 leaf pieces were incubated in Petri-dishes lined with moist filter paper. After incubation, the leaf pieces were taken out of the Petri-dish and allowed to dry for a few minutes. Afterwards, a piece of sellotape was fixed, then removed and mounted in 0.1% cotton blue in lactophenol.

In germination assessment on Agar and on leaves, about 250 spores were examined from each treatment. A spore was scored as germinated if the length of the germ tube exceeded the breadth of the germinating cell of the conidium.

Identity of the fungus

Using a camera lucida and a calibrated stage micrometer, the length and the breadth of about 100 spores together with other relevant morphological features were noted to determine the species using a key provided by Ellis (1971).

EXPERIMENTAL

Effect of temperature on growth of the fungus

Sterile Petri-dishes containing 15 cm^3 of Czapek-Dox agar were inoculated and incubated at 5°, 10°, 15°, 20°, 25°, 30°, 35° and 40°C. At each temperature the mean growth was assessed daily from four replicates. The fungus did not grow outside the range 10° - 40°C and the optimum temperature for growth is about 30°C (Fig. 1).

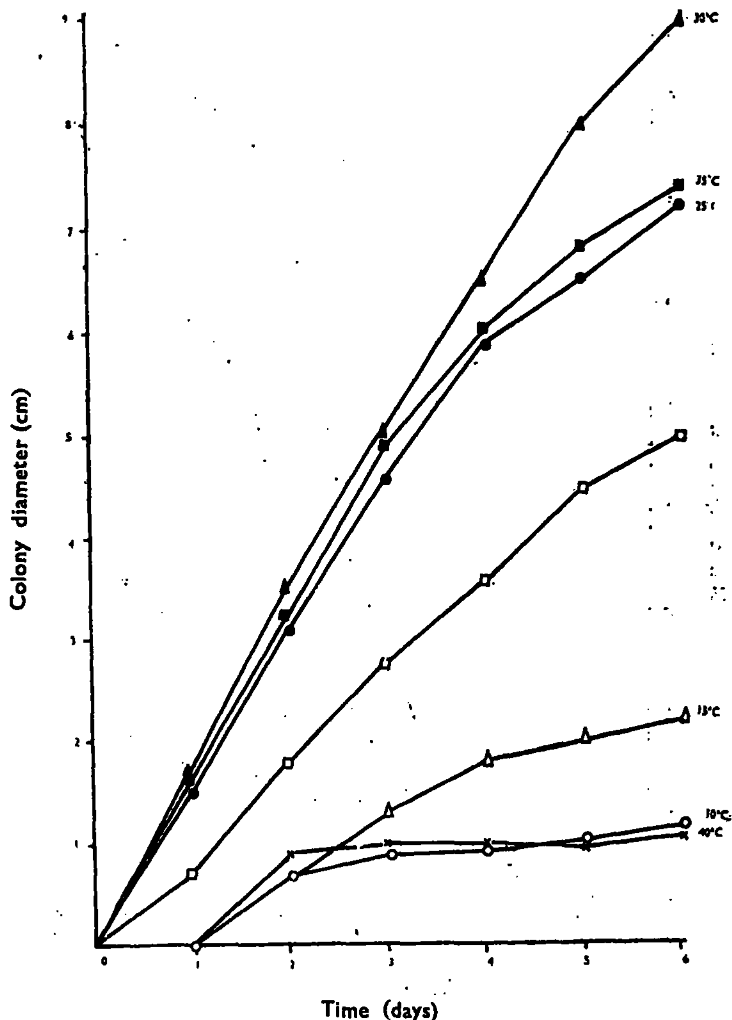

Fig. 1. Growth of *C. lunata* on Czapek-Dox agar at different temperatures.

Effect of temperature on Sporulation

Petri-dishes containing PDA were inoculated as described earlier and incubated at 10°, 15°, 20°, 30°, 35°, and 40°C. Ten plates were incubated at each temperature and the concentration of conidia was assessed using a conidial suspension made as described earlier at two day intervals from two replicates.

To determine the concentration, the number of conidia was counted in several droplets (each of volume 0.01 cm^3) of spore suspension using a haemocytometer. Mean values were obtained from five random samples.

Temperature considerably affected the sporulation time and the number of spores produced (Fig. 2). The optimum for sporulation (*i.e.* the shortest time for sporulation and the highest production of spores) appears to be 30°C.

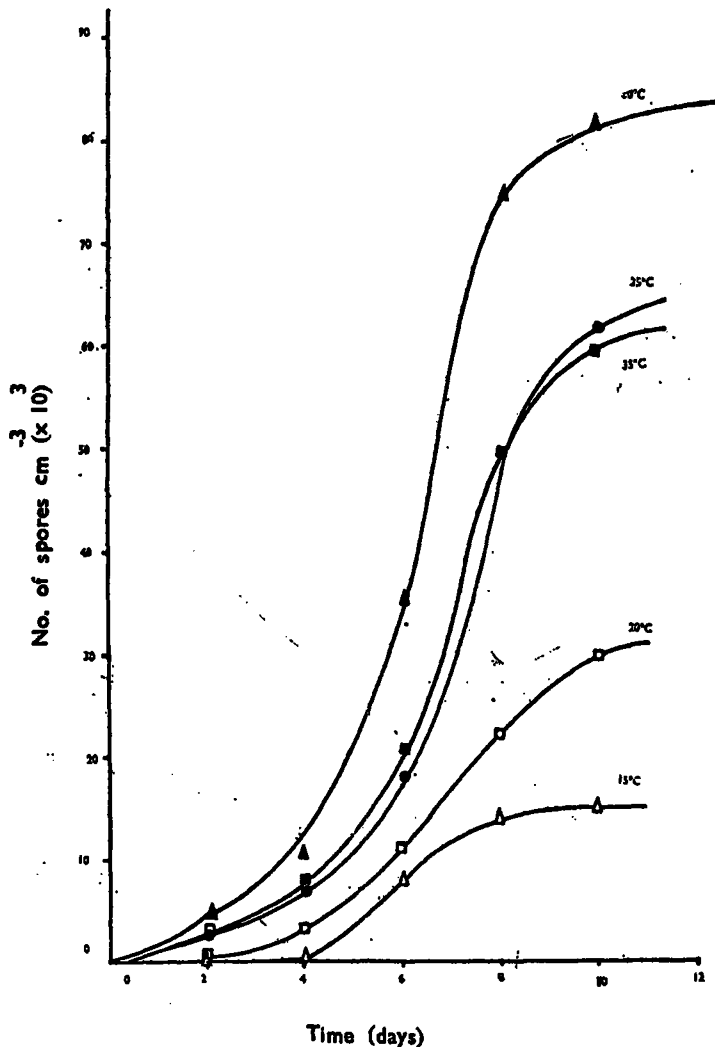

Fig. 2. Effect of sporulation of *C. lunata* at different temperatures.

Effect of temperature on germination of conidia

Distilled water Agar plates (2.5%) were pre-incubated for 2 h at the test temperature in order to equilibrate the temperature of the Agar medium with that of the environment. The agar plates were then inoculated with freshly made suspension of conidia and incubated for 6h-at 5°, 10°, 15°, 20°, 30°, 40° and 45°C. Germination was assessed hourly after inoculation.

The results of this experiment, which are presented in Figure 3, show that germination occurred at all temperatures tested except 5° and 45°C. The optimum temperature for germination appears to be 30°C. At this temperature, germination was observed within an hour and reached the maximum (98%) within 6 h.

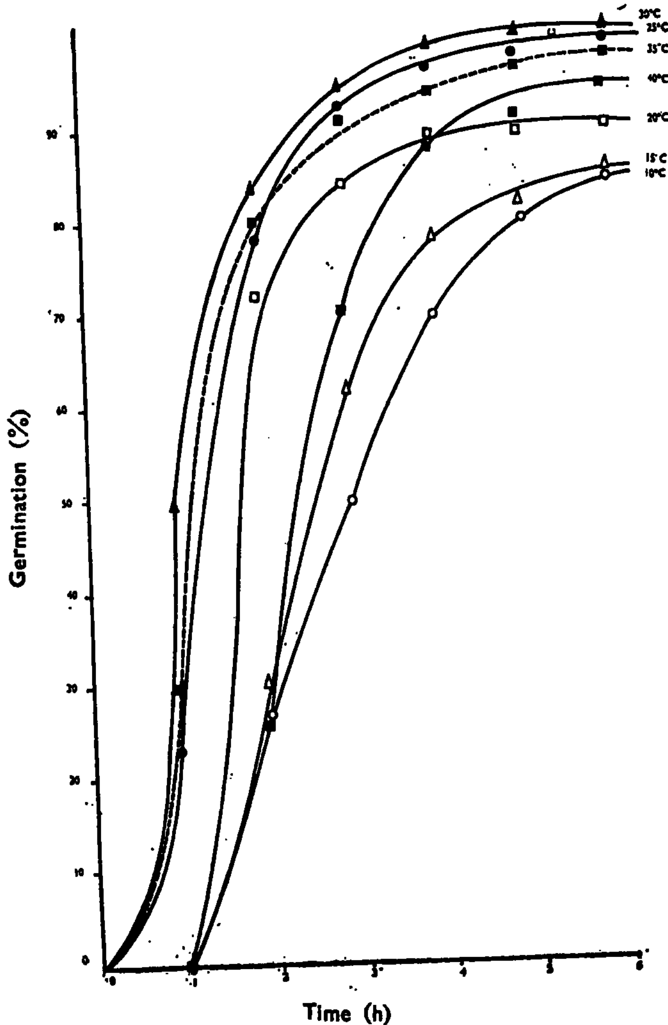

Fig. 3. Germination of *C. lunata* conidia with time.

Effect of the concentration of conidial suspension on germination

(*a*) Distilled water agar (2.5%) plates were inoculated with spore suspension of different concentrations made from freshly sporulated plates and incubated for 8 h at 30°C. Germination was assessed hourly.

(*b*) Four coconut leaves from 3 month old seedlings were excised and inoculated with different concentrations of freshly made suspension of conidia. Different concentrations of conidia were made by serially diluting a spore suspension of concentration 10^5 spores cm^{-3}.

While spraying, care was taken to prevent run-off and the inoculated leaflets were incubated at 30°C in a saturated atmosphere. Germination was assessed 2-hourly using the Sellotape method.

Results of these experiments are presented in Fig. 4. Although the rate of germination on coconut leaves was less than that on DWA, a higher percentage germination at low spore concentrations has been noted.

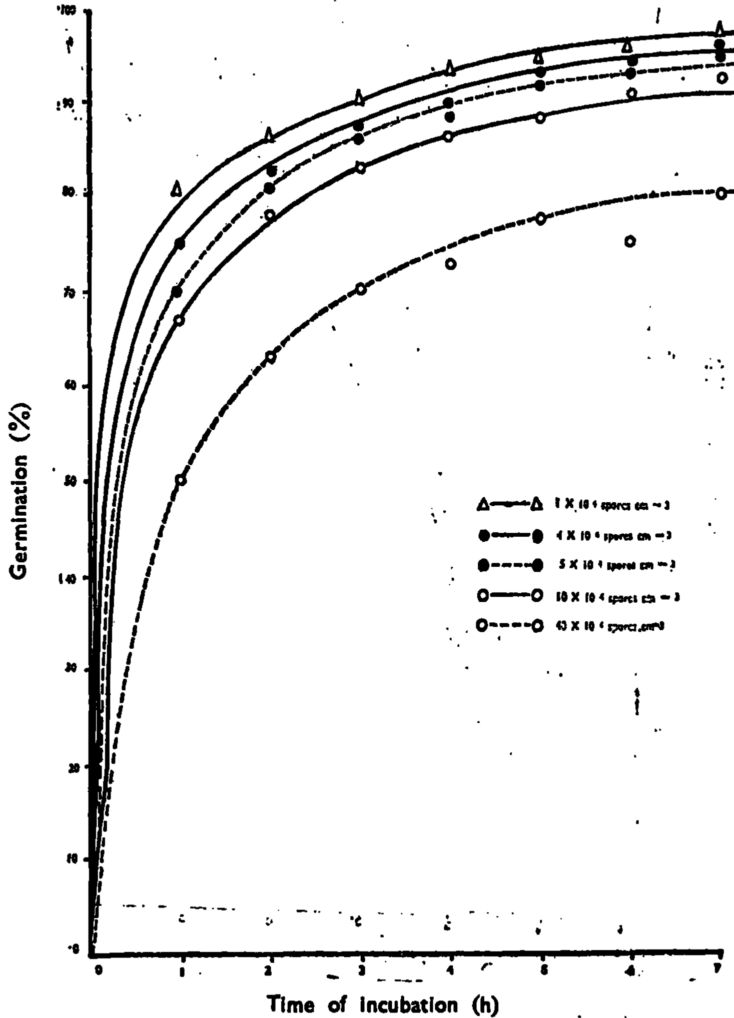

Fig. 4. (a) Effect of spore density of *C. lunata* on germination on Water Agar surface.

Disease development and the identity of the fungus

Coconut leaves were inoculated and incubated at the ambient temperature. The mode of infection was studied by examining epidermal peels mounted on 0.1% cotton blue in lactophenol under the microscope.

By examining a large number of infections, it was noted that 72% of the germinated conidia entered leaf by direct penetration of the cuticle while the rest entered through stomata. It was also observed that a minimum period of between 2-4 h was required for the initiation

of infection. Two days after inoculation, infection appeared in the form of small roundish or oval shaped brown lesions each about 0.6 mm in diameter. After the 3rd day, the spots enlarged and appeared slightly darker. Within 7 days, about 1/3 of the leaf was subjected to extensive necrosis.

Fig.5 shows the structure of conidiophores. Conidia are formed at the tip of the conidiophore and are 19.9-25.5 × 8.6-11.3 μm in size. The fungus was identified as *C. lunata*.

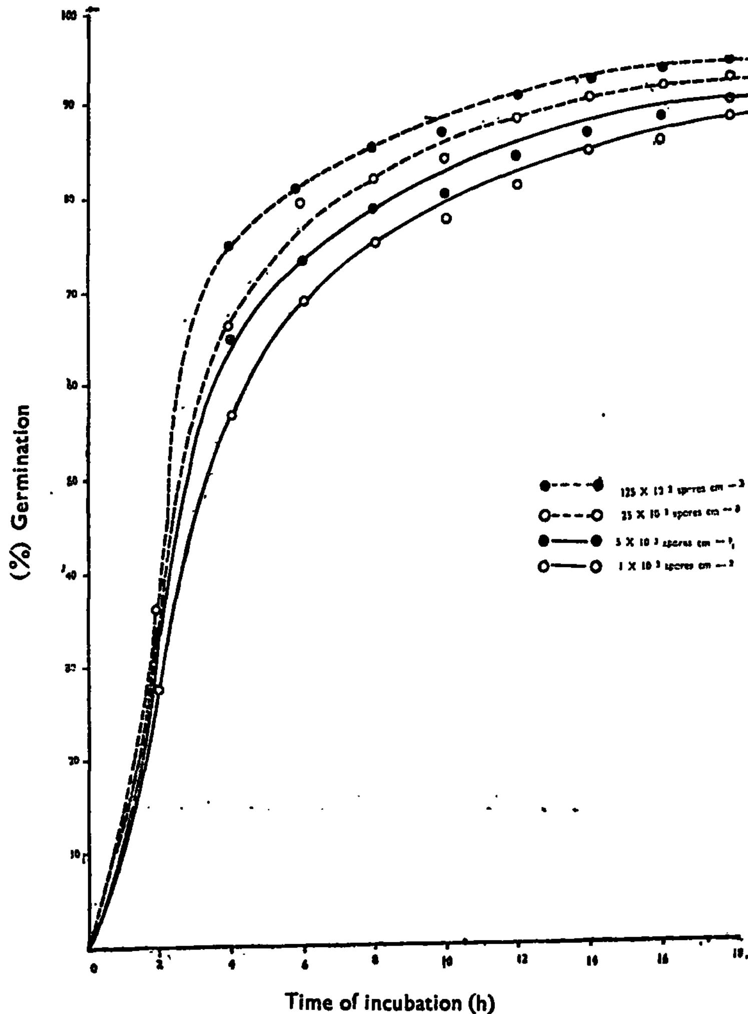

Fig. 4. (b) Effect of spore density of *C. lunata* on germination on coconut leaves.

In certain isolations, another species of *Curvularia*, distinct from *C. lunata* was observed. This fungus was identified as *C. eragrostidis* (= *C. maculans*) where the conidia are straight, symmetrical, four-celled, dark brown with the two mid cells showing a darker shade (Fig. 5) However, inoculation experiments with *C. eragrostidis* failed to produce any symptoms.

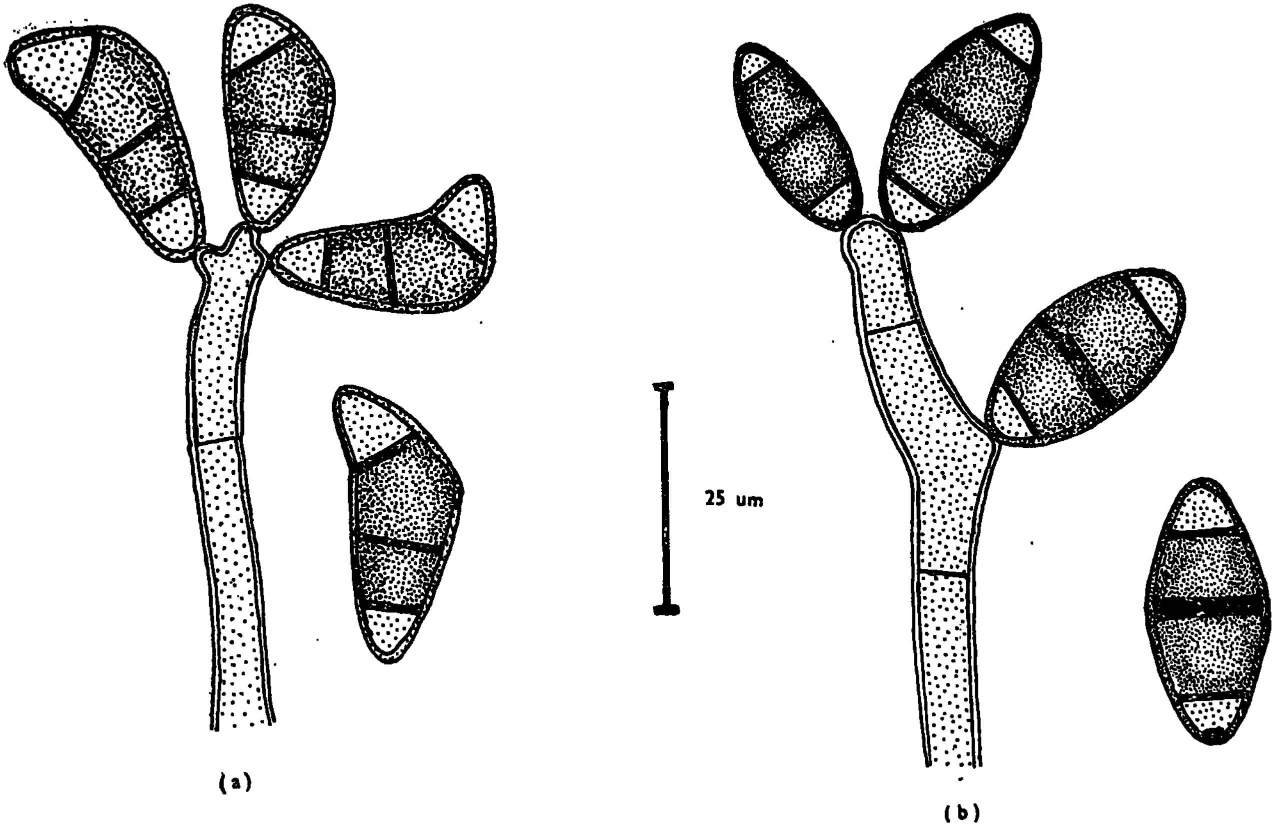

Fig. 5. (a) *Curvularia lunata* (b) *Curvularia eragrostidis*.

DISCUSSION

From the features examined, the fungus appears to be *Curvularia lunata* (Wakker), the conidial state of *Cochliobolus lunatus* Boedjin. Although *C. eragrostidis* (= *C. maculans*), which is considered to be a serious pathogen of coconut in Malaysia (Chan, 1974), was also isolated from some of the lesions, it does not seem to be a primary invader of tissue.

The temperature-growth curve for this fungus is similar to that of other fungi. The optimum temperature for growth (30°C) relates to the general outdoor temperature, and is likely that the fungus grows well under these conditions.

The sporulation was quickest at the optimum temperature for mycelial growth (30°C) and this indicates that under field conditions there could be profuse sporulation. The optimum temperature for spore germination is similar to that for mycelial growth and sporulation. However, the temperature range which allows germination is broader than that for growth.

Germination on coconut leaves took about 12 h to reach the maximum whereas on Agar medium it took only about 6 h at 30°C. This is perhaps due to the available moisture conditions. A noteworthy characteristic of the fungus is the considerable germination observed after one hour's incubation. This would help the fungus in spreading very quickly under wet conditions in nurseries, where the leaf surface humidity is high due to the very close planting of seedlings.

There is also a reduction of germination with the increase of spore concentration, a phenomenon also observed with *Pestalozzia palmarum* (Fernando & Mahindapala, 1977). This perhaps is due to competition for limiting nutrients or due to the release of inhibitory substances by the spores themselves (Sussman & Halvorson, 1966).

The mode of entry is both by direct penetration through the cuticle and through the stomata. The observation that about 72% of germinated spores enter by direct penetration gives an indication of the pathogenicity of the fungus. In the experiments, symptoms on inoculated plants appeared after three days. Under favourable conditions, a rapid development of the disease in the host has been found.

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