data at soil temperatures of 70°F and 40 to 55°F, respectively. Observations on yam plots adjacent to the experimental plot indicated that soil temperature ranged between 89 and 95°F during the experimental period. This temperature range was much higher than those under which Goring (1962b) and Turner *et al* (1962) conducted their experiments. The soils on the farm were rather porous (80 — 92 percent sand, Table 1). In addition to the high temperature, the porosity of the soils would be a factor accelerating the loss of N-serve through volatilization.

The concentration of N-serve (120 ppm. N-serve) which assisted in partially conserving ammonium nitrogen in this study is several times greater than that observed to be toxic to many crops (Goring 1962a: Mckell and Whalley, 1964). It may be concluded that under the conditions of this experiment the use of N-serve at concentrations that are not toxic to plants will not prevent a rapid loss of ammonium fertilizers in the soil. This conclusion may also hold true for other tropical rain forest areas where high soil temperatures generally prevail.

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Preliminary Studies on the Epidemiology of *Phytophthora palmivora* Butler. II. Effects of Relative Humidity on Growth, Sporulation and Viability

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INTRODUCTION

TOWARDS the end of 1963, casual inspections carried out in some cocoa plots in Ibadan showed that sporangia of *Phytophthora palmivora* obtained from cocoa pods attacked by the pathogen appeared empty and dead. During the 1964 cocoa season similar observations were also made and it was found that the amount of aerial hyphae and sporangia produced on *Phythophthora*-infected cocoa pods determined by subjective visual estimation of the whitish mycelial tufts on black-pods, varied with the time of the year.

As a result of these observations, it was suggested that the production of sporangia by *P. palmivora* might be affected by environmental factors such as temperature and humidity, and that the dry atmospheric conditions which prevail during the 'harmattan' months from November to January might play an important role in the survival of the fungus on cocoa pods. Consequently, the following experiments were carried out to investigate the effects of humidity on *P. palmivora* under laboratory conditions.

MATERIALS AND METHODS

Different relative humidities were obtained by using saturated solutions of various salts viz:- Magnesium chloride (33% relative humidity); potassium carbonate (43%); magnesium nitrate (52%); sodium nitrite (63%); ammonium sulphate (80%); potassium nitrate (91%); potassium sulphate (96%); and distilled water (approximately 100%).

Growth and sporulation of *P. palmivora* at different relative humidities.

Small amounts of the above solutions (in contact with excess salt in each case) were poured into 25 mm. diameter boiling tubes. Strips, measuring 10 x ½ x ½ cm; were cut from cocoa pods and suspended in the tubes by means of adapted fishing hooks, and aluminium caps were placed over the mouth of the tubes. The latter were autoclave-sterilised and then sealed in 35 mm diameter polythene bags for two weeks to bring the water content of each strip into equilibrium with that of the atmosphere within the tube.

At the end of this period each polythene bag was carefully opened, the aluminium cap removed from the top of the tube, and an agar disc of cultured *P. palmivora* was placed on the proximal end of the cocoa strip under asceptic conditions. The aluminium cap was then replaced and the bag carefully re-sealed. Each treatment was replicated ten times.

After incubation at room temperature for one week, sporulation and growth of the fungus in each strip were assessed.

By surface-scraping with a sterile needle, small amounts of materials were removed from the strips of cocoa, mounted on slides with lactophenol, and examined under the microscope for presence of sporangia of *P. palmivora.*

The linear growth of the fungus was determined by cutting the cocoa into segments at intervals of 1 cm. wound-inoculating materials from them into green healthy cocoa pods, the wounds on the latter being made by means of a 7 mm. diameter cork borer. Care was taken to prevent the transfer of inoculum from one treatment to another. The inoculated green pods were incubated in humid chambers for 3 to 4 days, and presence of *P. palmivora* was shown by the development of typical black pod lesions from which the fungus was re-isolated.

Effect of humidity and secondary invading micro-organisms on viability of *P. palmivora* in cocoa pods

Secondary invading fungi, bacteria and other micro-organisms are often present in lesions caused by *P. palmivora* on cocoa pods. The probable effect of these microorganisms and humidity on the viability of *P. palmivora* was investigated as follows:

7 mm. cocca discs were autoclave-sterilised and placed on one week old colonies of *P. palmivora* for four days. The discs were then removed and strung into bead-like chains with nylon threads, each chain being kept at known humidity in a boiling tube using the saturated solutions described above. The tubes were capped with aluminium foil and sealed in 35 mm diameter polythene bags. Controls with sterile discs only were also set up. Each treatment was replicated ten times.

At weekly intervals the polythene bags were carefully opened and a disc from each tube was wound-inoculated into healthy green pods, the bags being sealed again as quickly as possible.

The inoculated pods were incubated in humid chambers, and surface scrapings from the resulting lesions were examined for the presence of *P. palmivora.*

A similar experiment was conducted with discs taken from black-pod disease lesions on cocoa pods which were naturally infected for about one week in the field. This presumably ensured the presence of secondary fungi and other microorganisms which invade black pod lesions under natural conditions. Controls with sterile discs were also set up. Viability of *P. palmivora* was tested at weekly intervals as in the previous experiment.

Table 1. Growth and Sporulation of *P. palmivora* at different relative humidities after one week's incubation (Mean of 10 Replicates)

Relative Humidity	*Distance of growth in cm.*	*Sporangia Production*
100	10	Sporangia produced
96	8	,,
91	6	,,
80	3	No sporangia
63	0	,,
52	0	,,
43	0	,,
33	0	,,

RESULTS

The results of the experiment are shown in Tables 1 &2. In both groups no infection of experiments resulted from the controls.

These data suggest that although growth could take place in a relative humidity range of 80 — 100%, a saturated atmosphere (R.H. 100%) was the most suitable for rapid growth of *P. palmivora.* Sporangial production took place at high humidity, the minimum requirement being about 90%. Although infection and growth occurred at 80%, no sporangia were produced at this humidity, thus suggesting that infection and growth on one hand, and the production of sporangia on the other, have different humidity requirements.

Table 2. Effect of Humidity and Secondary Invading Microorganisms on viability of *P. palmivora* in Cocoa Pods. (Mean of 10 Replicates).

Percentage	*Viability in Weeks*	
Relative Humidity	*P. palmivora alone*	*P. palmivora Plus Secondary Invaders*
100	9	7
96	9	6
91	7	5
80	5	4
63	4	3
52	3	3
43	3	2
33	2	2

In the second group of experiments (Table 2), the presence of secondary invading microorganisms reduced the period of viability of *P. palmivora* by 1 — 3 weeks at relative humidities between 63 and 100%. At lower humidities the effect of these was insignificant.

DISCUSSION

The data obtained from these experiments suggest that relative humidity greatly influences infection, growth, sporulation and viability of *P. palmivora*. In Table 1 extensive growth of the fungus through sterilised cocoa pods occurred only above 90% relative humidity, and none at less than 80%. These results also suggest in nature that the rate of spread of the pathogen through infected pods would increase with increasing atmospheric humidity, resulting in a higher infection rate of rotting pods, and seed destruction in more humid farms than in drier ones. Thus, whereas a farmer in a less humid area may be able to retrieve some good quality cocoa beans from diseased pods, a farmer in a wet area may not be able to do so.

Since sporangia are important means of disease spread, information about the influence of atmospheric humidity and other environmental factors on their formation will be of value in devising methods of control.

That there is a positive correlation between rainfall and the incidence of black pod disease is an accepted fact in many cocoa growing countries, but Orellana and Som (1957), in their statistical study of relationship between weather factors and black pod of cocoa said, "Contrary to general expectations, rainfall has very little effect on the disease incidence." Thus instead of the current practice of increasing fungicidal spraying when rains are more frequent, a better control may possibly be achieved by spraying when meteorological data indicate that atmospheric humidity of over 80 per cent is likely to prevail for a certain period. As our knowledge of the influence of climatic factors on *P. palmivora* increases, it may be possible to devise a system for the successful forecasting of outbreak and spread of black pod disease, thus leading to more efficient spraying programmes.

Table 2 suggests that in addition to adverse effects of low humidity the presence of secondary microorganisms in black pod lesions hasten the death of *P. palmivora*. This may be due to the production of inhibitory compounds or straight forward competition for food by the secondarily invading microorganisms.

It is probable that at the lower humidities where the effect of these microorganism was negligible, the humidity was too low for their survival and establishment.

SUMMARY AND CONCLUSION

The effects of relative humidity on growth, sporulation and viability of *P. palmivora* were studied.

Under the conditions of these laboratory experiments, a minimum relative humidity of 80 per cent was necessary for infection. Growth and spread of the disease was quickest at higher humidities, and a minimum of 90 per cent was required for production of sporangia.

The viability of the pathogen was adversely affected by dry conditions and by the secondary invaders. These factors are probably significant in the disappearance of *P. palmivora* from diseased pods in the field at certain times of the year.

ACKNOWLEDGEMENT

I wish to express my deep gratitude to Dr. R. N. Smith of the University of Ibadan for his advice; and to Dr. L. K. Opeke, the Director, Cocoa Research Institute of Nigeria for his help in ensuring a regular supply of some of the materials used for this work and for permission to publish these results.

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