STUDIES ON SURVIVAL OF *TRACHYSPHAERA FRUCTIGENA* Tabor & Bunting CAUSING MEALYPOD DISEASE OF COCOA *THEOBROMA CACAO* L.

A Thesis presented by

PHINEAS MARAMBA, B.Sc. (Hons.)

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University of Ghana,
LEGON.

I, the undersigned, PHINEAS MARAMBA, author of this thesis, do hereby declare that the work presented in this thesis

"STUDIES ON SURVIVAL OF
TRACHYSPHAERA FRUCTIGENA Tabor & Bunting CAUSING
MEALYPOD DISEASE OF COCOA THEOBROMA CACAO L"

was done entirely by me in the Department of Botany, University of Ghana, Legon, from July, 1972 to August, 1973. This work has never been presented either in whole or in part for any other degree of this University or elsewhere.

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Phineas Maramba, B.Sc. (Hons.) Botany (Legon)

University of Ghana,

LEGON.

ABSTRACT

Conidia of T. fructigena did not germinate at any relative humidity from zero to 100% R.H. Germination in distilled water was very poor, less than 2.0 percent at the optimum temperature, 25°C. PDA encouraged 96.8 percentage germination at 25°C. The conidia germinated very poorly on PDA at 35°C and a temperature of 40°C killed the conidia in approximately 12 hours.

T. fructigena conidia would only survive in atmospheres of extremely high humidity. Best survival occurred at 100% R.H. and 90.0, 29.7 and 0.6 percent of the conidia were viable after 10, 20 and 40 days' storage, respectively. In comparison more than 50 percent of the spores perished in 20 minutes at 90% R.H. The conidia were well conserved in water and they survived longer than at 100% R.H.

Conidia at humidities from zero to 100% R.H. eventually collapsed. Conidia exposed to atmospheres of humidities below 75% R.H. shrank instantly. There was increasing delay of shrinkage with rise in relative humidity above 75% R.H. Total shrinkage was observed after 1 hour, 1⅔ hours, 24 hours, 15 days and 30 days, respectively in conidia stored at 80, 85, 90, 95 and 100% R.H. The conidia were preserved longer at 20° than 28°C. Light up to 467.5 lux intensity had no effect on stored conidia. Removal of water from the spore by plasmolysis in 0.7M potassium nitrate, sodium chloride and sucrose solutions was not as damaging as loss of water during desiccation, and between 79.5 and 96.0 percent of the conidia endured an hour's plasmolysis. Damage only set in after prolonged immersion in the plasmolysing fluid beyond 12 - 24 hours.

No germination occurred in either non-sterile or autoclaved extracts of soil and partially decomposed leaves from cocoa farm. The fungistatic principle was seemingly non-volatile and non-fungicidal Highest survival of more than 50 percent was obtained in extracts of pH 8.0.

Conidia, both live and dead, buried in soil on slides were attacked by other microorganisms. Conidia killed before introduction into the soil, however, suffered considerably greater attack and were lysed very quickly.

T. fructigena showed limited competitive saprophytic ability. Reasonable percentage of baits was colonized only when large quantities of inoculum were used or when T. fructigena had earlier access to the bait than the other soil microorganisms. Sodium nitrate alone or a combination of sodium nitrate and dextrose encouraged greater saprophytic survival than ammonium tartrate, peptone or glucose applied separately.

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I. INTRODUCTION

Cacao (*Theobroma cacao* L.) is attacked by various fungi (Dade, 1932; Urquhart, 1961). The Phycomycetes, *Phytophthora palmivora* (Butl.) Butl. and *Trachysphaera fructigena* Tabor and Bunting, both belonging to the Order Peronosporales, are readily separated from the rest as fruit (pod) parasites. The two fungi have, however, not received equal attention because of three main reasons. First, *P. palmivora* causes a far greater loss, about 10 percent annually, (Gregory, 1969), than *T. fructigena* which destroys only one percent of the annual crop. Dade (1927) placed losses due to *T. fructigena* at as low as 0.1 percent. Secondly, while *T. fructigena*, as present knowledge indicates, is restricted to the cacao pod, *P. palmivora* affects every organ of the plant including the roots and thirdly, *T. fructigena* is restricted only to West Africa in contrast to the extensive occurrence of *P. palmivora* in all cacao growing countries (CMI 1952. Map 249; Gregory, 1969).

The scant studies which have been made on *T. fructigena* do not, however, seem reasonable, since it is generally known that *T. fructigena* attacks other valuable crops; and control measures would have been more rewarding if they were based on adequate knowledge of the biology of the fungus. *T. fructigena* is a parasite of banana (*Musa* spp.) (Brun, 1954; Brun and Merny, 1947; Meredith, 1960; Thorold, 1956), coffee (*Coffea* spp.) (Bunting, 1923; Morstatt, 1936; Resplandy *et al.*, 1954; Roger *et al.*, 1937; Tabor and Bunting, 1923) and Avocado pear (Persea gratissima Gaertn.) (Bunting, 1924). Indeed, the disease on coffee was considered serious enough to be controlled by quarantine regulations. The 1935 Regulation Ordinance applicable to the then Colony and Protectorate of Nigeria, including the Cameroons under British Mandate and the Importation of Plants Regulation Ordinance (1936) for the then Gold Coast prohibited the importation of coffee cherries unless certified to be free from T. fructigena.

T. fructigena causes a soft rot of banana fruit and a second fruit disease referred to as Cigar-end, sometimes called Finger-tip. In the Cigar-end disease, infection commences from the perianth. Germ tubes of conidia of T. fructigena germinating on the stigma grow down through the tissues of the style into the blossom-end of the fruit (Leach, 1953, 1954). Infection then slowly spreads along the finger to about 2 cm from the tip, causing the infected portion to change to dark-brown and finally to black. The pulp tissue seems to undergo what may be described as a dry rot and the affected region is sharply delimited from the uninvaded healthy tissue. The disease, in the field, makes no further progress. The rotted end closely simulating a burnt cigar end (Bouriquet, 1959; Ann. Report of the Cameroons Dev. Corp., 1956, 1957, 1958; Brun, 1955). The disease is very prevalent under conditions of high relative humidity (Brun and Champion, 1955).

The other disease, fruit rot, is mostly a storage malady that results in a general wet rot of the fruit (Brun, 1955; Meredith, 1960).

The damage caused by T. fructigena on bananas in West Africa may be extensive. In the Cameroons (Ann. Report - Dev. Corp., 1958) where banana is a staple food, cigar-end disease of banana caused in 1958 the loss of entire bunches of 10,485 plants. In the preceding three years, 1955 - 1957, cigar-end disease caused together by T. fructigena and the fungus, Stachylidium (Verticillium) theobromae, affected the entire crop of 65,000, 9,000 and 9,000 plants, respectively. In an Annual Report of the Cameroon Development Corporation (1957), it was stated that control of the cigar-end disease in banana should be sustained for about 9 months of the year.

T. fructigena attacks coffee cherries causing blackening and mummification (Resplandy et al., 1954). The disease is referred to as mealypod and was first reported on Liberian coffee (Coffea liberica), in Ghana by Tabor and Bunting (1923). On attack the berries, especially the young ones, turn dark purplish-brown and eventually shrivel and harden. The rotted fruit finally becomes covered with a white or pinkish-brown mealy incrustation of T. fructigena conidia. Entire crop had often been destroyed by the fungus under wet conditions (Mallamaire, 1934).

The disease is not confined to the fruits of coffee, for, in 1923 Bunting (1923) found *T. fructigena* on young shoots of *Coffea liberica* in Ghana.

T. fructigena was found as a wound parasite on Avocado pear in Ghana by Bunting (1923). The extent of damage to this crop has never been estimated.

Infected cacao pods become brown in colour, quite similar to early symptoms of *P. palmivora* infection. *T. fructigena* attack is soon distinguishable thereafter by the heavy sporulation of the fungus forming a thick pinkish encrusted mealy conidial mass over the surface of the pod. The disease is, therefore, termed mealypod. (See Plates 1, and 2). Infection is commonly through wounds, but the Report on Agricultural Development of the Government of the then Gold Coast for the year 1921, indicated that the organism was sometimes capable of infecting unwounded pods.

T. fructigena is undoubtedly economically important. Its significance and importance in West Africa becomes more profound with the usual agricultural practice of mixed farming that naturally encourages spread of the fungus among the various host species.

T. fructigena is largely a fruit parasite and, therefore, faces the problem of survival between successive fruit-bearing seasons. It is not unlikely that this facultative parasite could exist saprophytically in the soil in the absence of the fruits of the host plants. The conidia produced in large quantities would be especially important as the major agents of dispersal and infection. The literature, however, contains no information both on the survival of the mycelium and on the longevity of the conidia which are likely to be fragile, in common with the asexual propagules of other members of the Peronosporales.

In the investigation described below saprophytic survival of T. fructigena in soil and the factors affecting the survival of the conidia were extensively studied to provide valuable and indispensable information for the design of successful control measures.

Plate 1. Photograph showing early stages of development of mealypod on ripened and unripened cacao pods; wound-inoculated with conidial suspension of <u>T. fructigena</u> and incubated at 25°C in humid atmosphere for 3 days. (X $^9/_{26}$)

Plate 2. Photograph showing advanced stage of development of mealypod on ripened and unripened cacao pods; wound-inoculated with conidial suspension of <u>T. fructigena</u> and incubated at 25°C in humid atmosphere for 4 days. Note the encrusted mass of conidia and absence of external mycelium. (X $^1/_3$)

II. LITERATURE REVIEW

There are very few references to the biology of T. fructigena in the literature and the little interest so far shown in it might be due to its very limited geographical distribution. The physiology of the fungus can only be inferred from the results of the few inoculation experiments that have been carried out.

T. fructigena penetrates better non-fortified host tissues. Tabor and Bunting (1923) observed that mealypod was more serious among young cacao pods because the protective sclerotic tissues had not yet been formed. When Meredith (1960) placed conidia of T. fructigena in wounds made in the skin at the blossom-end and at the mid-region of the fruits of Jamaican bananas, only three of 25 fruits inoculated at the blossom-end were infected while 15 of the 25 fruits inoculated at the mid-region were rotted. In this instance resistance to infection might be due to another cause, since skin tissues at the blossom-end and mid-region of the fruit were apparently of the same thickness and contained identical cell types.

Temperature of 15°C was inferior to 21° - 27°C for growth of T. fructigena. Rate of rotting of infected banana fruits was almost doubled at 21° - 27°C (Buxton et al., 1962).

Both conidial formation and sexual reproduction were affected by the growing medium. Tabor and Bunting (1923) detected sexual organs in infected cacao pod husk but could not find any in either the pericarp of diseased coffee or on media containing extract of coffee pericarp.

When Meredith (1960) inoculated healthy skin of Jamaican bananas by placing conidial suspension on the intact skin surface, none of the fruits were infected although the conidia germinated very well. The germ tubes were seemingly unable to penetrate the outermost cells of the fruit. His observation, however, contrasted with an earlier observation of Brun and Merny (1947), who obtained infection, by similar inoculation procedure, of Gros Michel banana fruits. Were the conflicting results due to host varietal susceptibility or to varying potency of different isolates of <u>T. fructigena</u>?

There is absolutely no information on the survival of the fungus although its seasonal occurrence on banana had been observed in Cameroon. Leach (1953, 1954) reported that at certain times, for example, December, there was abundant evidence of <u>Trachysphaera</u> Cigar-end but little or no <u>Stachylidium</u> Cigar-end; but at other seasons, especially the dry season, <u>Stachylidium</u> was the evident superficial organism. Brun (1955) also observed that the rotting of bananas on certain plantings in Penja and Loum-Chontiers regions of Cameroon was very severe between the end of October and December, the disease disappearing almost completely from April to September. The sources of new infections in the wet season in both cases were not investigated.

III. MATERIAL AND GENERAL METHODS

(i) Material

T. fructigena used was isolated from a naturally infected cacao pod collected from a cacao farm at Aburi.

(ii) General Methods

(a) Spore Germination Tests

1. Slide Method

In many of the spore germination tests, the "slide" method was used. Spore suspensions of *T. fructigena* were prepared by transferring conidia removed from surface of culture on petri plates with a flamed inoculating needle into sterile water in McCartney tubes. The conidia dispersed readily and the suspension needed no shaking. The suspension was filtered through 4-layers of sterile muslin cloth to remove most of the hyphal fragments. The conidia were then washed free of any nutrients taken up from the petri plates by allowing them to sediment for half an hour in a test tube and discarding the supernatant fluid. The conidia were then re-suspended in sterile distilled water or other germinating medium. On other occasions washed conidia were recovered by centrifuging the suspension at 300 r.p.m. for 5 minutes.

The number of spores in suspension for every germination test was strictly standardised to 400,000 per millilitre of solution with the aid of a haemacytometer. Sterile petri dishes, each containing a sterile slide (7.5 x 2.5 cm) supported on a glass v-piece, over a small quantity of distilled water had, in the mean time, been equilibrated with the incubation temperature. This technique obviated evaporation of the germination test droplet at the initial stages of incubation.

Using an inoculating loop, two individual drops of spore suspension (about 0.1 ml in volume) were placed on each thermally equilibrated slide contained in its damp chamber, and the slide immediately returned to the incubation temperature. Each germination count was based on eight drops of suspension (i.e. two separate drops on each of four slides for each treatment).

2. Agar Plate Method

Spore germination was also observed on agar media in petri dishes (20 ml of agar medium per dish) in a considerable number of experiments in this investigation. Each agar plate was inoculated with 1 ml of spore suspension divided between 10 evenly spaced areas. The concentration and spatial spread of the spores corresponded closely to what obtained in the "slide" method.

(b) Assessment of Conidium Germination

At the end of the desired incubation period, the tested conidia were promptly stained with cotton blue (0.1%) in lactophenol. If observations could not be made at once drops of $N/40$ formaldehyde were added, in addition to the stain, to make sure that development of the spores was arrested.

The Percentage Germination was estimated from a total of not less than 300 conidia from randomly selected fields of the microscope. Any conidium with a discernible germ tube was counted as having germinated. This definition of germination was thus based on morphology. The numbers of conidia examined in each experiment are shown in the tables of data, to show the reliability of the percentage germination claimed for each treatment.

The germ tubes commonly branched so profusely that in most cases it was impossible to make accurate measurements. Plate 3 demonstrates extensive germ tubes produced by conidia of T. fructigena after only 3 hours incubation on PDA. The results, therefore, contain no quantitative information on amount of growth of germ tubes obtained and the effect of any treatment was assessed by percentage germination only.

(c) Humidity Chambers

Transparent plastic boxes, 21.5 cm long, 10.5 cm wide, and 7.5 cm deep with tightly fitting lids, served as Relative Humidity Chambers. The lids were usually held firmly in position with cellotape. These boxes proved to be very convenient in that very little condensation occurred at 100% relative humidity. The humidity chambers were used in experiments on conidial longevity.

Microscope slides bearing spore prints of conidia were placed on solid watch glasses standing in the humidity chambers, raising them well above the humidity-controlling solution at the bottom of the plastic chamber. Each humidity chamber held two microscope slides. Water and sulphuric acid solutions used for maintaining constant relative humidities within the chamber were 5 mm deep.

(d) Maintenance of Constant Relative Humidity

Atmospheres of different relative humidities were obtained and maintained with aqueous sulphuric acid solutions (Solomon, 1952) (see Table 1). Nominal zero percent relative

TABLE 1

Aqueous sulphuric acid solutions for maintaining constant relative humidities at 25°C (Extracted from data of Solomon, M.E., 1952).

% Relative Humidity at 25°C	Weight in g of sulphuric acid per 100 g of solution	Density at 15°C	Density at 20°C
100	0.0	0.999	0.998
95	11.02	1.075	1.073
90	17.91	1.126	1.123
85	22.88	1.165	1.162
80	26.79	1.196	1.193
75	30.14	1.223	1.219
50	43.10	1.334	1.330
25	55.01	1.499	1.445

humidity was maintained with anhydrous calcium chloride, while water provided 100 per cent relative humidity.

(e) Spore Prints for longevity tests

Preliminary studies have shown that the conidia are extremely sensitive to humidities lower than 100% R.H. Pertinent information has been presented at appropriate places in the text. The following method was therefore devised to obtain spores for longevity tests.

A rectangular piece of the culture, about 2 cm x 3 cm, was cut out of the petri plate from a 5 day-old pure culture with a flamed inoculating needle and lifted off the culture with the needle. The culture on the agar block was gently adpressed onto the slide to obtain a spore print. The slide was immediately transferred into the humidity chamber.

Such spore prints were also used in "buried slide" experiments investigating the fate of conidia in the soil. Further details of the "buried slide method" are presented in Chapter Q.

(f) Maintenance of Stock Culture

A stock culture was maintained on slopes of Potato Dextrose Agar Medium (200g. potato; 20g. dextrose; 20g. agar; 1000 ml de-ionised water) in McCartney Tubes at 25°C and subcultured fortnightly.

(g) Production of Conidia

Potato Dextrose Agar supported sufficiently heavy sporulation and was used for that purpose in this work.

Spores for use in experiments were consistently obtained from 6 day-old cultures raised in petri plates.

(h) Incubation

Incubation conditions varied with the experiment and are described at the appropriate places in the text.

(i) Aqueous Extract of Soil

Extract of soil needed during this investigation was made from soil collected from cocoa farms at Aburi where the disease is prevalent. The profile of the soil showed two distinct layers. There was an upper carpet of partially decomposed leaves, 3-4 inches thick, overlying a layer of black loam soil.

The soil extract was prepared as follows: the soils were moistened for two days (samples from the 2 zones were kept apart and used separately), and aliquots were packed into glass tubings, 300 mm long and 20 mm in diameter, with both ends open, though one was plugged with glass wool, before introducing the soil. A tiny glass tubing, 2 mm in diameter, was attached to the bottom of the soil tube (end plugged with glass wool) by a rubber bung, which led the draining fluid into a clean collecting flask. The soil in the glass tube formed a column of 200 mm. Sterile distilled water was then gradually poured by a pipette into the column of soil until it started to drain from the exit tube, and then an equal volume of the water so far introduced was added to the column. The extract collected at intervals was poured onto the soil column to drain through again. The process was continued for 3 hours.

All extracts were used immediately after preparation.

(j) Methods of Sterilization

All glass-ware and plastic containers were soaked in solutions of teepol, thoroughly washed, rinsed under running tap water and thoroughly rinsed in distilled water.

All media, medicinal flats, McCartney tubes, conical flasks, pint milk bottles, capped test-tubes and muslin filters, were sterilized by autoclaving for 15 minutes at 15 lbs per square inch steam pressure. Non-absorbent cotton wool plugs of culture vessels were temporarily covered with ~~grease~~ Kraft paper to prevent the penetration of any condensed water during autoclaving.

Petri-dishes and pipettes were sterilized by heating at 160°C for 6 hours in an electrically heated oven.

Inoculation needles, spatulae and microscope slides were flame sterilized. The microscope slides were stored in 90% ethyl alcohol, and flame sterilized just before use.

Surface of cacao pods were sterilized with 95% alcohol.

The inoculation room was sterilized by spraying with 5% dettol solution and kept closed for 10 minutes just before use.

(k) Experimental Precautions

1. Sulphuric acid solutions were prepared with caution. Care was taken to avoid generation of heat too quickly.

2. Soil for the preparation of extracts was not allowed to dry after collection from Aburi. They were

kept in plastic bags and regularly watered.

3. Spore print preparation was carried out with petri plates and humidity chambers under a hood of plastic sheet to prevent exposure of the conidia to dry air.

4. Spores of the same age were used in all experiments to prevent erratic results.

(1) Statistical Methods

Correlation and Regression

1. In the assessment of correlation between percentage of collapse and percentage of germination of conidia, the basic observations were n pairs of associated observations represented by (x, y) for % collapse and % germination, respectively. The assumption was that x and y followed, at least approximately, a bivariate normal distribution. The

correlation coefficient r was given by the equation:

$$r = \frac{\text{Covariance } x\ y}{\sqrt{\text{Variance } x.\ \ \text{Variance } y}}$$

which is the same as

$$r = \frac{\Sigma(x - \bar{x})\ (y - \bar{y})}{\sqrt{\Sigma(x - \bar{x})^2 .\ \Sigma(y - \bar{y})^2}}$$

To facilitate the use of calculating machines, the equivalent equation shown below was used:

$$r = \frac{\Sigma xy - \frac{\Sigma x\ .\ \Sigma y}{n}}{\sqrt{(\Sigma x^2 - \Sigma^2 x/n)\ .\ (\Sigma y^2 - \Sigma^2 y/n)}}$$

When it was found necessary, the r values were converted to the student's t-test values by the following equation:

$$t = \frac{r\sqrt{n - 2}}{\sqrt{1 - r^2}}$$

2. For the Regression, the true regression line for the regression of y on x is known to be: $y = a + \beta x$.
The true regression coefficient β was first estimated by the equation:

$$b = \frac{\Sigma(x - \bar{x})(y - \bar{y})}{\Sigma(x - \bar{x})^2}$$

which is the same as its equivalent and convenient equation below:

$$b = \frac{\Sigma xy - \frac{\Sigma x . \Sigma y}{n}}{\Sigma x^2 - \Sigma^2 x/n}$$

The constant a was calculated by the equation:

$$a = \bar{y} - b\,\bar{x}.$$

The fitted regression line was then as follows:

$$y = a + bx.$$

Coefficient b was only an estimate of the true regression coefficient β and so, it was necessary to prove that it was a good estimate of β. Since n was $\geqslant 30$, the normal test (where if b is more than 1.96 standard errors away from zero, then it is significant at the 5% level) was used. The standard error (s.e.) of b was calculated from the equation:

$$b \pm \text{s.e.} = b \pm \sqrt{\frac{\text{Variance } S^2}{\Sigma(x - \bar{x})^2}}$$

where variance $S^2 = \frac{1}{n - 2}\left\{\Sigma(y - \bar{y})^2 - \frac{[\Sigma(x - \bar{x})(y - \bar{y})]^2}{\Sigma(x - \bar{x})^2}\right\}$

Using the calculated constant a and regression coefficient b, two values of x were calculated, one from a given maximum value of y and another from a given minimum value of y. The two points were connected with a straight line to form a regression line on a graph, where the x axis shows percentage collapse and the y axis shows percentage germination.

The information was obtained from Bailey's Statistical Methods in Biology (Bailey, 1959).

Plate 3. Photomicrograph of germinating conidia of *T. fructigena* incubated on Potato Dextrose Agar at 25°C for 3 hours. (X 120)

IV. RESULTS

A. Effect of Temperature on Germination of Conidia of *T. fructigena*

The major portion of this investigation examined the longevity of the conidia. It was considered important to determine the germination capacity of the conidia after various storage treatments at the optimum temperature, where germination activity would be maximum.

The germination of conidiosporangia of several members of the Peronosporales has been studied. In most of these species the phenomenon of germination differs from that of *T. fructigena*. The conidiosporangia germinate either directly by producing germ tubes or indirectly by means of zoospores. Germination of *T. fructigena* conidia is comparable with direct germination of these species. The conidiosporangia, in most instances, produce zoospores at considerably low temperatures with an optimum temperature below average tropical atmospheric temperatures. Germ tubes, on the other hand, are formed at higher temperatures. To illustrate, Clerk (1972) reported that indirect germination of sporangia of *Phytophthora palmivora* in distilled water was optimal at 22°C over the range 10° to 34°C while direct germination occurred only at 30° and 34°C. Wolf and Wolf (1947) reported an optimum temperature of 12° - 13°C for indirect germination of sporangia of

Phytophthora infestans, and best germination, by means of germ tubes, at 24°C. Aragaki *et al*. (1967), who did not obtain any direct germination in water in *Phytophthora parasitica* sporangia, found a comparatively higher optimum temperature of 24° - 28°C for indirect germination.

The behaviour of the conidiosporangia could, however, be influenced by other factors, particularly nutrients. Thus Clerk (1972) found that sporangia of *P. palmivora* germinated well by means of germ tubes at 22°C in peptone and yeast extract solutions and in the extract of cocoa pod husk. Similarly, Aragaki *et al*. (1967) reported that papaya extract supported the best direct germination of 51 percent at 28°C.

In finding the optimum temperature for germination of the conidia of *T. fructigena* to be used in subsequent experiments, the results would also indicate, although, uniformity is not expected, ~~not expecting uniformity,~~ whether its optimum temperature is close to that for direct germination of other species of the Peronosporales.

The slide method was used. Aqueous conidial suspension drops were incubated at 15°, 20°, 25°, 30°, 35° and 40°C. There were four slides at each temperature and observations were made on the same suspension drops over the entire period. The slides were withdrawn at 6, 12, 24, 36 and 48 hours; for counts of germinated conidia to be quickly made and returned to the incubators. The results are presented in Table 2.

The results show that germination was poor in distilled water. There was no germination at 40°C, even after 48 hours of incubation. Germination was clearly very low at 35°C. There was only 0.5 percent germination at 35°C, compared to 1.4, 1.4, 1.2 and 1.1 percent respectively, at 15°, 20°, 25° and 30°C after 48 hours.

Although percentage germination was practically the same at 15° - 30°C, the germ tubes were far shorter at 30°C than at 15° - 25°C.

TABLE 2

Germination of conidia of *T. fructigena* incubated in distilled water at different temperatures for 48 hours.

Temperature of incubation °C	Period of incubation in Hours	Total number of conidia observed	Percentage Germination
15	6	393	0.3
	12	410	1.7
	24	413	1.2
	36	406	1.2
	48	414	1.4
20	6	410	0.5
	12	445	0.5
	24	452	1.1
	36	468	1.3
	48	442	1.4
25	6	385	0.8
	12	406	1.2
	24	423	1.2
	36	434	1.2
	48	416	1.2

TABLE 2 (cont'd.)

Germination of conidia of _T. fructigena_ incubated in distilled water at different temperatures for 48 hours.

Temperature of incubation °C	Period of incubation in Hours	Total number of conidia observed	Percentage Germination
30	6	385	1.3
	12	392	1.3
	24	504	1.0
	36	453	1.1
	48	457	1.1
35	6	459	0.2
	12	423	0.2
	24	452	0.4
	36	392	0.5
	48	412	0.5
40	6	354	0.0
	12	398	0.0
	24	405	0.0
	36	409	0.0
	48	371	0.0

B. Further experiments on the effect of Temperature on the Germination of Conidia of *T. fructigena*

It was observed in the previous experiment that germination of the conidia of *T. fructigena* was best favoured at 15°, 20° and 25°C. The amount of germination in water was, however, so low that it was not possible to make any reliable comparison. The conidia were, therefore, germinated again, using in this instance PDA medium. The same range of temperature of 15° to 40°C, was again used in this experiment. The temperature levels of 35° and 40°C which were clearly unsuitable in the distilled water experiment were included to find out whether germination would be improved in the presence of extraneous nutrients.

The 'Agar Plate Method' was used and for each temperature, assessment of percentage germination was again made after 6, 12, 24, 36 and 48 hours. There were four petri plates for each temperature. The results are presented in Table 3.

There was again no germination at 40°C. Germination at some of the temperature levels was, however, highly stimulated. At 12 hours, the last recording time for 20° and 25°C, percentage germination was 94.0 and 96.5 percent at 20° and 25°C, respectively and 33.6 and 30.2 percent at 15° and 30°C, respectively, for the same period. Although almost the same amount of germination was

TABLE 3

Germination of conidia of *T. fructigena* on Potato Dextrose Agar at different temperatures for 48 hours.

Temperature of incubation °C	Period of incubation in Hours	Total number of conidia observed	Percentage Germination
15	6	421	13.4
	12	440	33.6
	24	404	35.1
	36	417	45.8
	48	408	52.7
20	6	359	87.2
	12	400	94.0
	24	——*	——
	36	——	——
	48	——	——
25	6	373	96.8
	12	428	96.5
	24	——	——
	36	——	——
	48	——	——

*Observations were discontinued because the germ tubes developed so profusely that their origin could not be traced while some hyphae began to produce conidia after 36 hours.

TABLE 3 (cont'd.)

Germination of conidia of *T. fructigena* on Potato Dextrose Agar at different temperatures for 48 hours.

Temperature of incubation °C	Period of incubation in Hours	Total number of conidia observed	Percentage Germination
30	6	305	34.4
	12	334	30.2
	24	429	26.1
	36	——+	——
	48	——	——
35	6	429	0.0
	12	418	0.0
	24	418	0.2
	36	397	0.3
	48	——	——
40	6	382	0.0
	12	441	0,0
	24	390	0.0
	36	395	0.0
	48	420	0.0

+Although germ tubes were discernible, new conidia were produced and so observations were discontinued.

eventually attained at 12 hours, at 20° and 25°C, rate of germination was slightly faster at 25°C. At 6 hours, there was 96.8 percent germination at 25°C and 87.2 percent at 20°C. The optimum temperature was apparently 25°C and was adopted for subsequent experiments.

The poor germination at 35°C, 0.3 percent germination at 36 hours, indicated that the conidia might be rather sensitive to higher temperatures. It was not possible to extend reading beyond this period for further observation of the conidia because of the production of conidia by the germ tubes (Fig. 1).

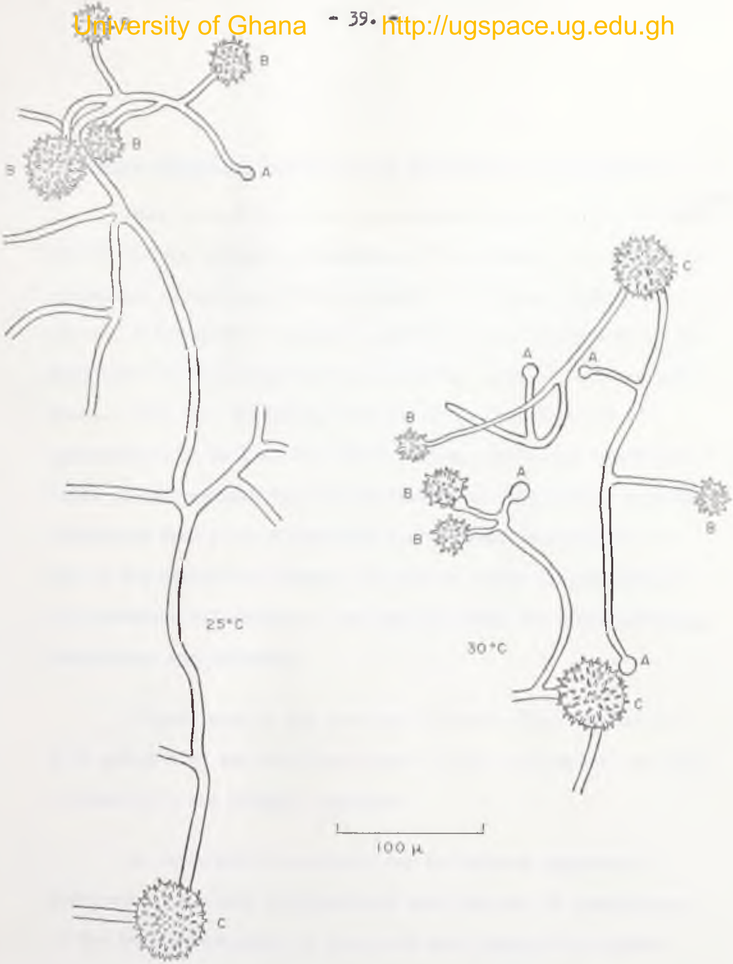


FIG. I DEVELOPMENT OF CONIDIA BY GERMINATING <u>T. FRUCTIGENA</u> CONIDIA INCUBATED ON PDA AT 25°C AND 30°C FOR 36 HOURS.

A, PRIMODIAL CONIDIA B, NEW DEVELOPED CONIDIA

C, OLD CONIDIA

C. Latent Period of Germination of the Conidia at 20° and $25^\circ C$

Many criteria are used in assessing the effect of environmental factors on spore germination. These mainly are, estimating percentage germination after a specific time lapse; noting time for the first spore to produce a germ tube and, measurement of the time taken by 50 percent of the germinable spores to produce germ tubes. This last criterion, referred to as latent period of germination, as well as the second method, enables us to differentiate clearly between two treatments with closely similar effects, especially when percent germination are almost identical at the end of the incubation period. The latent period of germination also provides information on the rate at which the final percentage germination was achieved.

It was found in the previous experiment (see Chapter B) that germination was very high after 12 hours at both 20° and $25^\circ C$, although $25^\circ C$ was slightly superior.

An experiment was carried out to estimate percentage germination at hourly intervals and thus the rate of germination at the two temperatures, to bring out more clearly the effects of these two temperatures on the germination of the conidia.

The conidia were germinated on PDA, using the 'Agar Plate Method' over 8 hours. There were four petri plates at each temperature and hourly observations were made on the same plates throughout the experiment. The results, percentage germination for each hour, are recorded in Table 4 and graphically represented in Fig.2.

The results (Table 4) show that the rate of germination of conidia of T. fructigena was faster at 25°C. The latent period of germination at 25°C was, as calculated from the graph, 96 minutes while that at 20°C was 150 minutes. The earliest germinating spores at 20° and 25°C were observed after 56 and 32 minutes, respectively. Germination was almost completed after 3 hours at 25°C and after 4 hours at 20°C. Again, the final percentage germination was slightly higher at 25°C.

TABLE 4

Germination of conidia of *T. fructigena* incubated on PDA at 20° and 25°C for 8 hours.

Temperature of incubation °C	Period of incubation in Hours	Total number of conidia observed	Percentage Germination
20	1	352	1.4
	2	375	22.7
	3	380	66.1
	4	386	80.6
	5	402	83.4
	6	369	86.4
	7	359	83.0
	8	360	84.7
25	1	363	16.8
	2	369	65.0
	3	374	86.7
	4	415	90.2
	5	337	87.7
	6	385	90.8
	7	379	91.0
	8	338	88.4

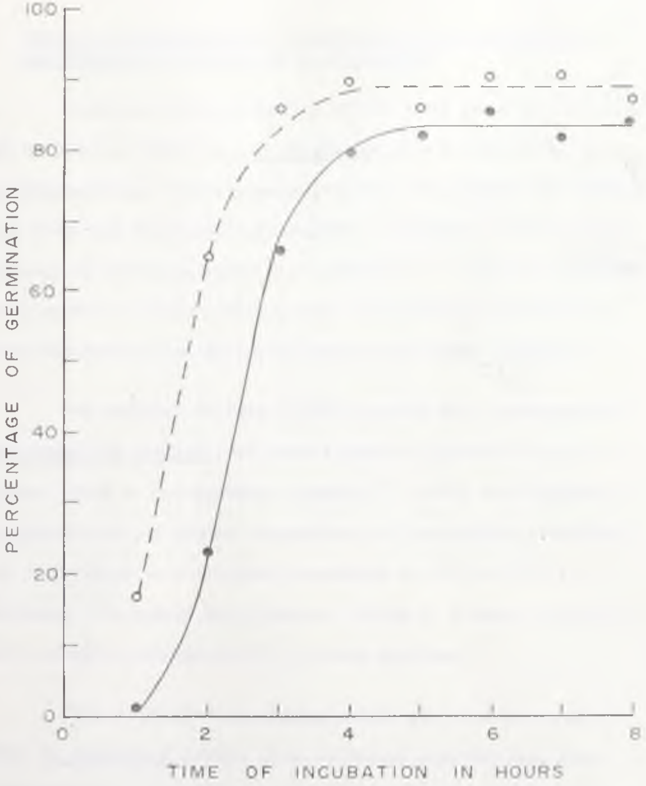


FIG. 2 GERMINATION OF CONIDIA OF <u>T. FRUCTIGENA</u> INCUBATED AT 20° AND 25° C.

●———● 20° C ○- - - - -○ 25° C

D. Effect of Moderately High Temperatures on the Germination and Viability of Conidia of *T. fructigena*

No germination occurred at 40°C in both water (see Table 2) and on PDA (see Table 3). *T. fructigena* clearly appears to be non-thermophilic. It is necessary to find out whether the conidia were killed by the relatively moderate temperature of 40°C within 12 hours or merely prevented from germination. There are instances where spores of other fungal species that have not germinated at such temperature have done so on transfer to lower temperatures.

For example, Frossard (1962) observed that, ascospores of *Mycosphaerella musicola*, the causal agent of Sigatoka disease of banana, which do not germinate beyond 38° - 40°C, were capable of germination at the optimum temperature for germination after they had been placed in a saturated atmosphere at 40° and 42°C for 24 hours. The spores were, however, killed by 6 hours exposure to 44°C and after storage at 50°C for only one hour.

This experiment was carried out to find out the extent to which *T. fructigena* conidia could withstand slightly more than normal tropical temperatures, which are not unlikely to occur in a vigorously respiring cocoa pod or in decomposing leaf litter on the ground.

Sixteen petri plates of PDA with spore suspension drops were incubated at 40° and another similar set at 45°C. Four plates were withdrawn from each temperature at 3, 6, 12 and 16 hours and placed at 25°C for 12 hours. The percentage germination of the conidia was then assessed at the end of this period. The results are presented in Table 5.

The results show that there was no germination after incubation at 45°C, even for as short a period as 3 hours. Some germination was obtained in conidia kept for 12 hours at 40°C. Survival was, however, very low, 0.5 percent, and most of the conidia would be killed by this temperature in less than 12 hours. Only 28.5 percent of the conidia were viable after 6 hours' exposure to 40°C.

TABLE 5

Germination of conidia of *T. fructigena* stored on PDA at 40° and 45°C for varying periods (HOURS) and then transferred to the optimum temperature (25°C) for 12 hours.

Temperature of storage °C	Period of storage in Hours	Total number of conidia observed	Percentage Germination
	3	401	52.1
	6	432	28.5
40	12	397	0.5
	18	399	0.0
	3	348	0.0
	6	334	0.0
45	12	342	0.0
	18	336	0.0

E. Survival of Conidia of *T. fructigena*

The length of life of fungal spores, as measured by their ability to germinate after various periods, is affected, in the main, by environmental conditions of humidity, temperature and light. At any given relative humidity, increasing the temperature generally decreased the viability of fungal spores, and lower temperatures, above freezing, favour longevity (Cochrane, 1958; Ainsworth and Sussman, 1968).

Fergus and Schein (1960) found that light had a deleterious effect on viability of stored ascospores of *Vinula craterium*, and spores exposed to light died quicker than those kept in darkness. Clerk and Madelin (1965) also observed that *Metarrhizium anisopliae* conidia lived longer in darkness particularly when the spores were stored at 8°C.

The relationship between relative humidity and viability is not simple, and four main sorts of relationship have been reported, and have been recently fully reviewed by Ainsworth and Sussman (1968).

(1) Several investigators have found that lower relative humidities favour retention of viability of specific fungus spores, (deBruyn, 1926; Harrison, 1942; Maneval, 1924; McCrea, 1923; McKay, 1935).

(2) Hart (1926) reported that uredospores of *Melampsora lini* retained their viability for a longer time at mid-humidities (40% and 60% R.H.) than at higher and lower humidities, while Rosen and Weetman (1940), and Naqvi and Good (1957), respectively, found similar behaviour in uredospores of crown rust of oats and conidia of *Monilinia fructicola*.

(3) Merek and Fergus (1954) found that at 12° - 24°C, the endoconidia of *Endoconidiophora fagacearum* remained viable longer at 95% than at 75% R.H., and Goos and Tschirsch(1962) reported that spores of *Gloeosporium musarum* survived longest at higher humidities (60% - 80% R.H.) than at lower humidities (0% - 20% R.H.). Hyre and Cox (1953) found that at 20° and 30°C sporangia of *Phytophthora phaseoli* did not germinate in any saturated atmospheres, unless the sporangia were in free water. At 20°C, nearly 40 percent sporangia were viable after 27 hours exposure to 95% or 100% R.H. and 25 percent were viable after 50 hours. They found that longevity decreased rapidly at lower humidities - less than 10 percent was viable after 4 hours, 1 hour and 15 minuties at 90%, 86% and 81% R.H., respectively. Fewer sporangia were viable after exposure at corresponding humidities and periods of time during storage at 30°C. Less than 10 percent were viable at 93%, 97% and 100% R.H. after 2, 10 and 27 hours, respectively.

(4) Teitell (1958) reported that viability of conidia of <u>Aspergillus flavus</u> was preserved longest at 0% and 85% R.H., and lost quickest at 75% R.H. The only other observation of this response was that of Clerk and Madelin (1965) who found that conidia of <u>Metarrhizium anisopliae</u> survived longest at the extreme relative humidities, 5% - 35% R.H. and 85% - 95% R.H. and lived shortest at the median humidities, 55% - 65% R.H.

Teyegaga and Clerk (1972) found that the response of conidia of <u>Cercospora canescence</u> to storage humidity was different in light and in dark. In the dark, the conidia lived longest at the lower humidities, 0% - 20% R.H. and longevity shortened with increasing relative humidity. In light, however, humidities of 20% to 40% R.H. were the most favourable for survival, with the conidia dying quickest at 0% R.H. and 60% - 80% R.H.

An experiment was carried out to find the effect of relative humidity - which would be the most important climatic factor on cacao farms - on the survival of <u>T. fructigena</u> conidia. Atmospheric temperatures vary very little (Boateng, 1960), the range being on the average, 26.1° to 28.9°C annually for Ghana.

Information from three experiments - the results of which have not been quoted in full since no germination occurred in any - preceded this major survival experiment. The experiments were briefly as follows:-

1. The conidia did not germinate at 80% - 100% R.H.
2. The conidia did not survive 24 hours storage at 0% - 80% R.H.
3. In a second experiment the conidia did not survive 6 hours storage at 0% - 80% R.H.
4. In a third experiment no conidia survived one hour storage at 0% - 80% R.H.

Every experiment was repeated three times.

T. fructigena conidia were apparently very sensitive to drying and a major experiment was set up using 75% - 100% R.H. as the storage humidity and observations of their viability commencing after only 20 minutes of storage.

Spore prints for longevity tests were prepared and immediately transferred into the humidity chambers, as outlined in sections (c), (d) and (e) of General Methods. The spores were placed in plastic chambers with constant internal humidities of 75%, 80%, 85%, 90%, 95% and 100% R.H. and incubated at 25°C in incubators with no internal lighting.

Samples from each humidity level were withdrawn at 20 minutes intervals for the first two hours of incubation and then, at hourly intervals until the 6th hour of incubation, followed by germination tests at 9, 12, 24 hours and, thereafter, after the number of days of storage indicated in Tables 10 and 11, which concerned those stored at 95% and 100% R.H. only.

On withdrawal after storage, the spores on the slides were washed off with sterile distilled water to make a spore suspension which was used in seeding petri plates of PDA. The inoculated petri plates were then incubated at 25°C for 12 hours and the percentage germination, and hence percentage viability, assessed. Whenever zero percent germination was recorded, germination tests were made on the two succeeding sampling times to confirm total death earlier recorded.

In the earlier experiments outlined above, the conidia were found to collapse or shrink at all the humidities between 0% and 80% R.H. Normal conidia and shrunken conidia are shown in Plates 4 and 5, respectively. Perhaps shrinkage was related to death of the spore and in all the present tests, slides selected at random from each humidity level were examined under the microscope and the number of spores which had collapsed was quickly estimated. These data are included in the results presented in Tables 6 to 11 and graphically represented in Figs. 3, 4 and 5.

The results in Tables 6 and 7 show that the conidia died very quickly at 75% and at 80% R.H., and the conidia survived for

TABLE 6

Survival of Conidia of *T. fructigena* stored at 75% R.H. at 25°C

Period of storage in hours	Estimation of Percentage of conidia that collapsed in storage		Estimation of Percentage germination of viable conidia on PDA after 12 hours	
	Total number of conidia observed	Percentage collapse	Total number of conidia observed	Percentage Germination
$\frac{1}{3}$	296	97.6	382	0.5
$\frac{2}{3}$	309	99.0	422	0.0
1	298	99.3	419	0.0
$1\frac{1}{3}$	284	99.3	381	0.0

TABLE 7

Survival of Conidia of *T. fructigena* stored at 80% R.H. at 25°C

Period of storage in hours	Estimation of Percentage of conidia that collapsed in storage		Estimation of Percentage germination of viable conidia on PDA after 12 hours	
	Total number of conidia observed	Percentage collapse	Total number of conidia observed	Percentage Germination
1/3	285	95.1	385	1.6
2/3	284	95.8	395	0.5
1	289	98.6	386	0.0
1 1/3	286	98.3	365	0.0
1 2/3	286	100.0	397	0.0

TABLE 8

Survival of Conidia of *T. fructigena* stored at 85% R.H. at 25°C

Period of storage in hours	Estimation of Percentage of conidia that collapsed in storage		Estimation of Percentage germination of viable conidia on PDA after 12 hours	
	Total number of conidia observed	Percentage collapse	Total number of conidia observed	Percentage Germination
⅓	296	86.8	397	4.3
⅔	285	94.4	387	0.8
1	278	97.1	392	1.0
1⅓	284	93.3	365	1.1
1⅔	285	99.3	393	0.5
2	265	99.2	384	0.0
3	269	99.6	381	0.8
4	261	99.2	384	0.8
5	248	96.8	377	0.3
6	279	97.5	366	0.6
9	280	100.0	382	0.3
12	271	95.2	383	0.0
24	268	99.3	382	0.0
48	301	100.0	354	0.0

TABLE 9

Survival of Conidia of *T. fructigena* stored at 90% R.H. at 25°C

Period of storage in hours	Estimation of Percentage of conidia that collapsed in storage		Estimation of Percentage germination of viable conidia on PDA after 12 hours	
	Total number of conidia observed	Percentage collapse	Total number of conidia observed	Percentage germination
$\frac{1}{3}$	281	23.8	343	43.1
$\frac{2}{3}$	277	48.0	385	25.5
1	282	81.6	408	6.1
$1\frac{1}{3}$	272	93.4	369	10.3
$1\frac{2}{3}$	258	97.3	387	1.6
2	265	97.0	390	2.6
3	275	80.0	434	10.1
4	273	86.1	399	12.8
5	261	82.0	379	9.0
6	261	92.7	369	4.6
9	258	93.0	378	2.4
12	281	90.7	374	0.3
24	274	98.9	373	0.0

TABLE 9 (cont'd.)

Survival of Conidia of _T. fructigena_ stored at 90% R.H. at 25°C

Period of storage in Days	Estimation of Percentage of conidia that collapsed in storage		Estimation of Percentage Germination of viable conidia on PDA after 12 hours	
	Total number of conidia observed	Percentage collapse	Total number of conidia observed	Percentage Germination
2	275	94.2	376	0.3
3	270	98.2	390	0.0
4	250	95.6	391	0.0
5	282	93.3	383	0.0

TABLE 10

Survival of Conidia of *T. fructigena* stored at 95% R.H. at 25°C

Period of storage in hours	Estimation of Percentage of conidia that collapsed in storage		Estimation of Percentage Germination of viable conidia on PDA after 12 hours	
	Total number of conidia observed	Percentage collapse	Total number of conidia observed	Percentage Germination
$\frac{1}{3}$	279	3.2	396	92.2
$\frac{2}{3}$	300	5.0	359	83.0
1	258	22.1	359	75.8
$1\frac{1}{3}$	270	19.3	379	81.0
$1\frac{2}{3}$	264	24.2	381	51.2
2	303	34.3	422	41.7
3	252	46.0	422	71.1
4	274	25.2	378	61.9
5	276	21.7	386	81.6
6	316	26.7	358	71.2
9	294	23.8	337	77.7
12	260	22.3	413	65.4
24	259	49.8	434	33.2

TABLE 10 (cont'd.)

Survival of Conidia of <u>T. fructigena</u> stored at 95% R.H. at 25°C

Period of storage in Days	Estimation of Percentage of conidia that collapsed in storage		Estimation of Percentage Germination of viable conidia on PDA after 12 hours	
	Total number of conidia observed	Percentage collapse	Total number of conidia observed	Percentage Germination
2	291	68.7	369	10.3
3	291	90.7	379	5.3
4	308	91.1	396	20.7
5	257	88.9	375	11.7
10	272	90.4	365	0.0
15	298	99.3	387	0.0
20	319	100.0	358	0.0

TABLE 11

Survival of Conidia of *T. fructigena* stored at 100% R.H. at 25°C

Period of storage in hours	Estimation of Percentage of conidia that collapsed in storage		Estimation of Percentage Germination of viable conidia on PDA after 12 hours	
	Total number of conidia observed	Percentage collapse	Total number of conidia observed	Percentage Germination
$\frac{1}{3}$	245	1.6	396	94.7
$\frac{2}{3}$	304	2.3	382	97.1
1	297	2.7	392	95.9
$1\frac{1}{3}$	273	1.8	369	95.9
$1\frac{2}{3}$	269	4.5	381	93.4
2	282	11.7	395	89.9
3	269	3.7	397	91.9
4	267	2.3	365	87.1
5	270	11.9	373	95.7
6	273	15.4	371	91.9
9	303	5.0	389	97.4
12	266	2.6	409	94.1
24	277	15.5	366	90.2

TABLE 11 (cont'd.)

Survival of Conidia of *T. fructigena* stored at 100% R.H. at 25°C

Period of storage in Days	Estimation of Percentage of conidia that collapsed in storage		Estimation of Percentage Germination of viable conidia on PDA after 12 hours	
	Total number of conidia observed	Percentage collapse	Total number of conidia observed	Percentage Germination
2	267	14.6	364	91.5
3	259	20.8	411	82.7
4	297	33.0	424	88.4
5	280	6.8	403	93.5
10	279	11.8	389	90.0
15	261	16.5	409	66.7
20	293	49.8	330	29.7
30	283	99.3	336	14.9
40	261	100.0	352	0.6
50	272	98.2	341	0.0
60	285	99.3	346	0.0
70	269	100.0	331	0.0

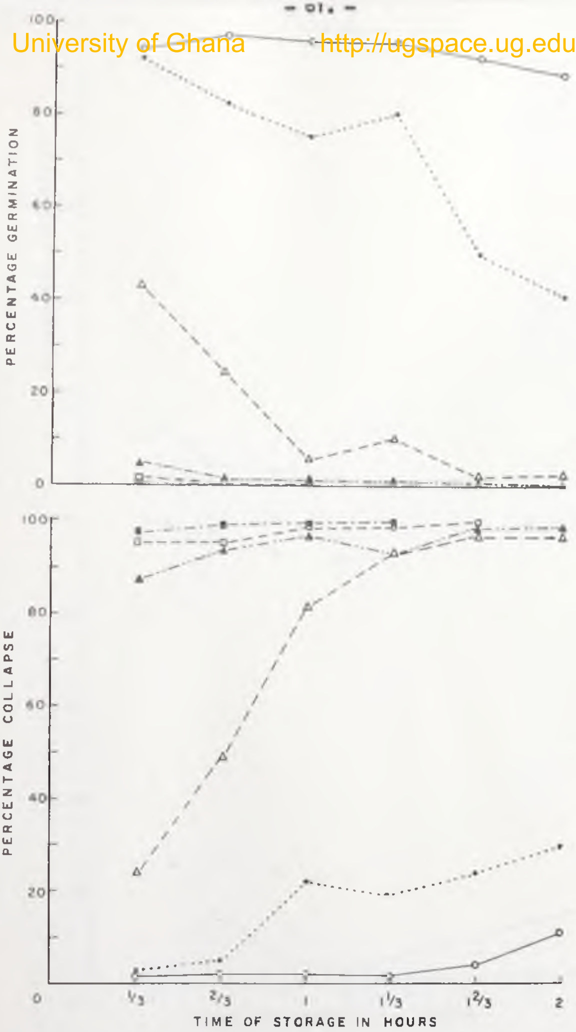


FIG. 3 EFFECT OF RELATIVE HUMIDITY ON LONGEVITY OF CONIDIA OF <u>T. FRUCTIGENA</u> STORED IN LIGHT AT 25°C

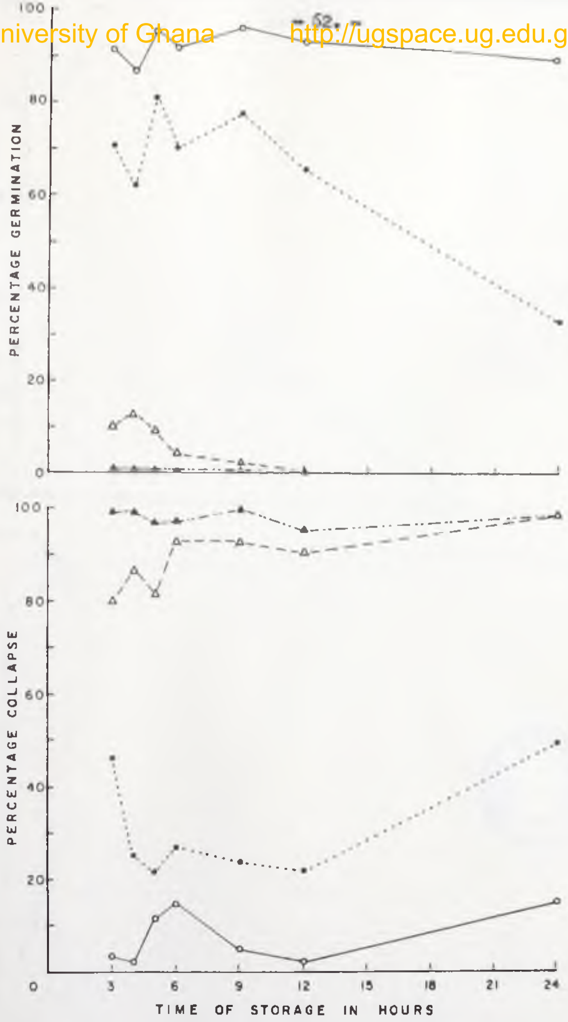
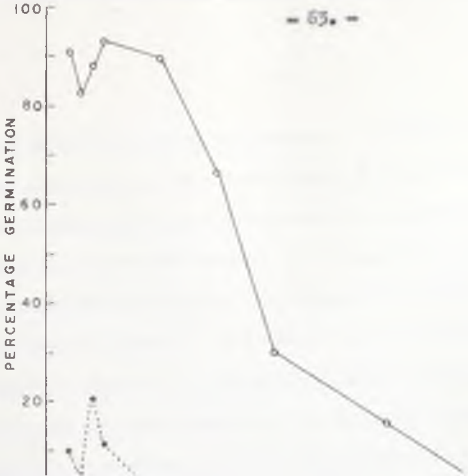


FIG. 4 EFFECT OF RELATIVE HUMIDITY ON LONGEVITY OF CONIDIA OF T. FRUCTIGENA STORED IN LIGHT AT 25°C


PERCENTAGE COLLAPSE
100
80
60
40
20
0


PERCENTAGE GERMINATION
100
80
60
40
20

only 20 and 40 minutes, respectively. At 85% R.H. (Table 8), some conidia (0.3%) were viable after 9 hours but none after 12 hours. This humidity level was unfavourable for conidial survival was below one percent after 80 minutes. The conidia of T. fructigena were clearly extremely sensitive to drying, for at 90% R.H. (Table 9) more than 50 percent of the spores died in 20 minutes and all lost viability between 5 and 10 days at 95% R.H. (Table 10). They were only well preserved under very high humidity conditions (Table 11). Survival at 100% R.H. was 90.0 percent after 10 days and 29.7 percent after 20 days while a few (0.6%) were still viable after 40 days.

There seemed to be a strong relationship between death and shrinkage. This was clearly visible in Tables 10 and 11, where the conidia lived sufficiently long. It was, therefore, decided to test the relationship by means of a correlation coefficient r and to graphically represent the data by means of a regression line. Since one of the most noteworthy assumptions in calculating correlation coefficient is that the distribution of scores must display at least a rough approximation to the normal curve or linear plot, a scatter diagram was plotted on a graph to determine whether there was an apparent degree of association or correlation between conidial collapse and viability (see Fig. 6). When it was clear that the association was apparent, the correlation coefficient r was calculated according to the statistical procedure outlined in the General Methods, section (1) 1, using the data of Tables 8 to 11, excluding the figures which had zero percentage germination.

Experimental r was $= -0.989$.

The theoretical 5% level of significance when $n = 62$, was $r = \pm 0.250$ and at the 0.1% level r was $= \pm 0.408$.

Since $0.989 > 0.408 > 0.250$, the experimental r was found significant even at the 0.1% level of significance.

Probability P. was < 0.0001.

Converted to the t- test, experimental t was $= 51.764$, and the 5% level of significance $t = 1.95$, while 0.1% level $t = 3.48$.

Since $51.764 > 3.48 > 1.95$, then correlation coefficient r was significant, indicating a high degree of negative correlation between collapse and viability.

A regression line was drawn calculated according to the method in section (1) 2, (General Methods). The Standard Error of b when $n = 62$ was found to be:

$b \pm s.e. = -0.9885 \pm 0.0334$, showing b to be 29.6 Standard Errors away from zero. Since $29.6 > 1.96$, then it was significant and so, b was regarded as a good estimate of the true regression coefficient β and was used to calculate and draw the regression line $y = 95.71 - 0.9885x$ which is graphically represented in Fig.6.

It is clear from the result in Fig.6 that an increase in percentage collapse was associated with a decrease in percentage viability.

A few remarkable features of the shrunken conidium are worthy of note. The conidium collapsed in a characteristic way; one hemisphere of the spore sank into the other hemisphere simulating a deflated ball with one side pushed in (Plate 5).

When a shrunken conidium was immersed in water, the dead conidium assumed again its spherical shape. Such a conidium could, however, be readily distinguished from a living conidium by its dense rounded cytoplasmic mass well withdrawn from the spore wall (Plates 6 and 7).

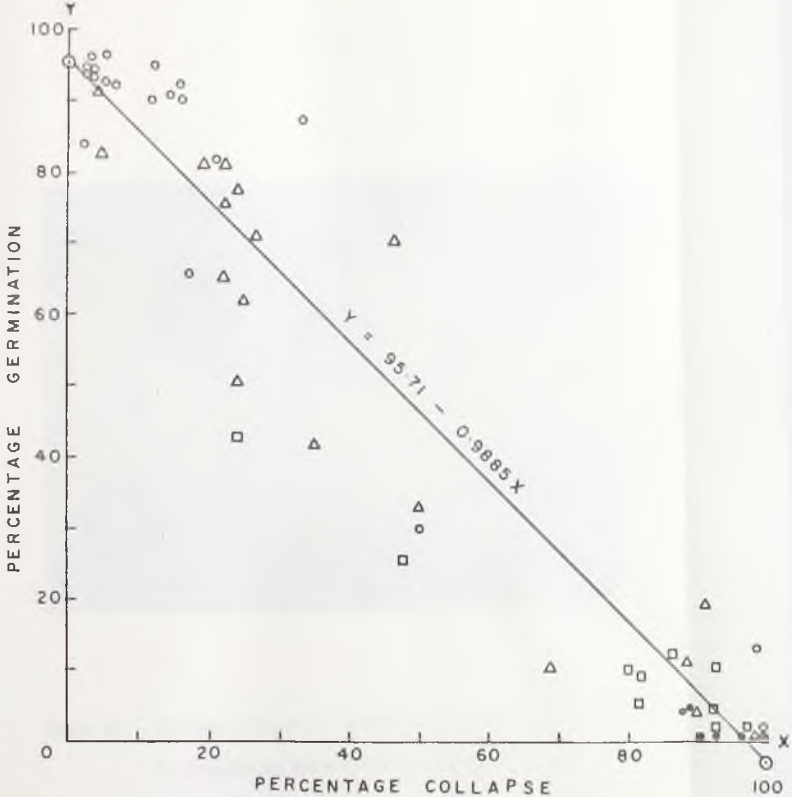


FIG. 6 CORRELATION BETWEEN PERCENTAGE COLLAPSE AND PERCENTAGE SURVIVAL OF CONIDIA OF <u>T. FRUCTIGENA</u> STORED AT VARIOUS RELATIVE HUMIDITIES AT 25°C

(DATA FROM TABLE 8 TO 11)

●, 85 % R.H. □, 90% R.H. △, 95 % R.H ○, 100% R.H.

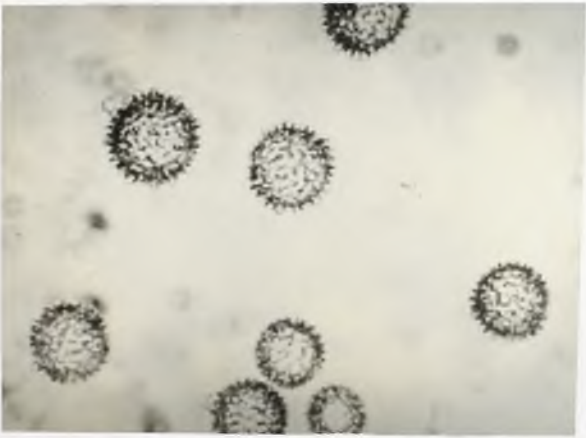


Plate 4. Photomicrograph of unstained conidia of *T. fructigena* maintained in water. (X 400)

Plate 5. Photomicrograph of unstained conidia of *T. fructigena* which have shrunken on exposure to the atmosphere on a dry slide. (X 400)

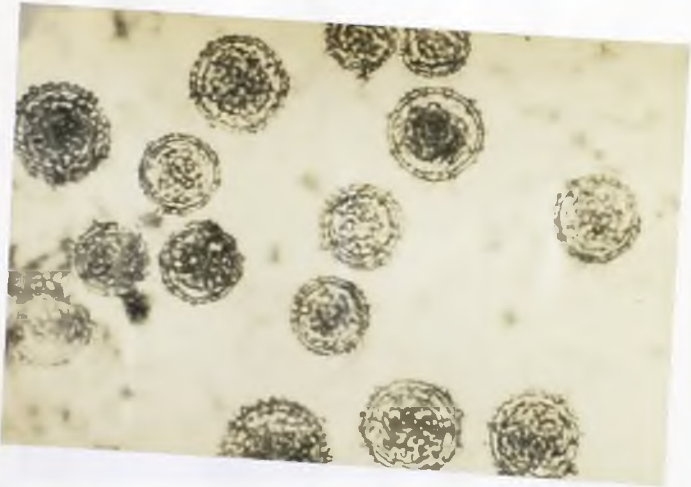


Plate 6. Photomicrograph of unstained dead shrunken conidia of <u>T. fructigena</u> which have rounded up again on immersion in water. Note the central rounded cytoplasmic mass. (X 400)

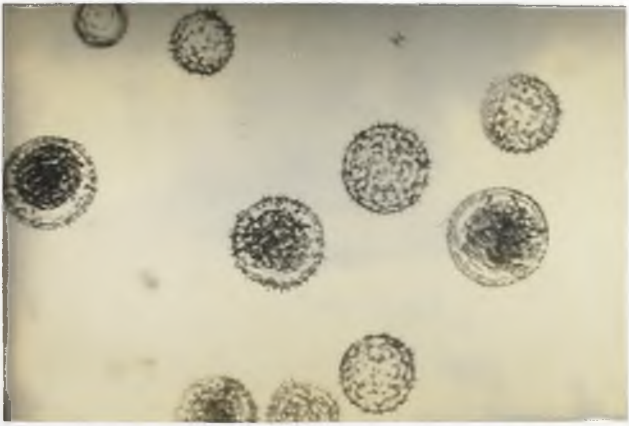


Plate 7. Photomicrograph of five unstained living conidia of *T. fructigena* in water and five dead shrunken conidia of *T. fructigena* which have rounded up again on immersion in water, identified by the central rounded cytoplasmic mass. (X 400)

F. Longevity of Conidia of T. fructigena stored at 100% R.H. in Light and Dark

The experiments on survival had been carried out at a single temperature of 25°C and in incubators which did not have internal lighting. It is reasonable to presume that the spore viability would follow the common trend in relation to temperature. That is, lower temperature would allow a slow rate of metabolism and so prolong the life of the spore, while higher temperatures would shorten it by hastening metabolism, with attendant accumulation - within the spore - of deleterious compounds. The important aspect of the temperature relationship, therefore, for each individual species, is the extent of decrease of longevity with temperature rise. The conidia were incubated at 100% R.H., at 20°C (air-conditioned room) and 28°C (laboratory), where light and dark treatments could be given, over 20 days. Light was provided by combined day-light and fluorescent light to give a light intensity of 275.0 and 467.5 lux at 20° and 28°C, respectively.

Two sets of spore prints were prepared for each temperature, one set to be kept in light and the other in dark. Those for dark treatment were contained in plastic chambers covered with black photographic paper. Samples were withdrawn after 5, 10, 15 and 20 days and their viability assessed by germinating them on PDA for 12 hours. The results are presented in Table 12 and in Fig.7.

TABLE 12

Longevity of Conidia of T. fructigena stored at 100% R.H. in light and dark and at different temperatures over 20 days.

Temperature (°C)	Light Treatment	Period of storage in Days	Total number of conidia observed	Percentage Germination
20	Light (275.0 lux)	5	379	79.9
		10	360	84.7
		15	359	39.0
		20	355	25.9
	Dark	5	370	74.9
		10	331	81.9
		15	385	56.9
		20	364	20.6
28	Light (467.5 lux)	5	364	71.4
		10	329	21.9
		15	350	21.4
		20	367	17.7
	Dark	5	358	73.2
		10	328	31.7
		15	327	21.0
		20	353	15.9

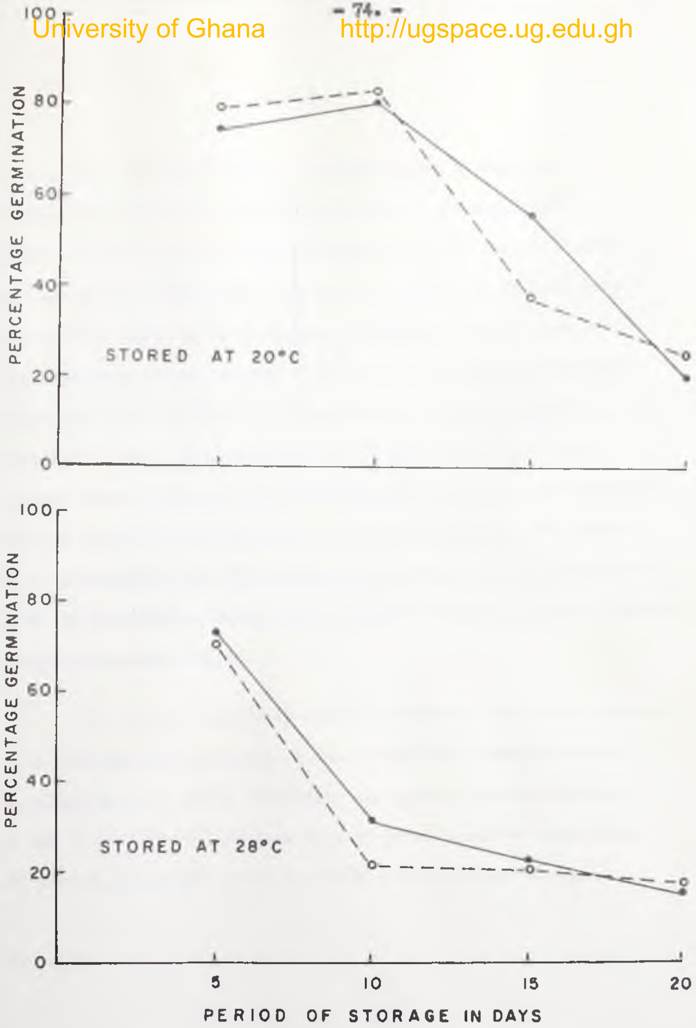


FIG. 7 LONGEVITY OF CONIDIA OF <u>T. FRUCTIGENA</u> STORED AT 100% R.H. IN LIGHT AND DARK AND AT 20°C, 28°C

o– – –o STORED IN LIGHT ●——● STORED IN DARK

The results (Table 12) show that some conidia were still viable after 20 days at both temperatures. Although conidia in dark and light showed closely similar amounts of viable conidia at the end of the experiment, thus at 20°C, there was 25.9 percent survival in light and 20.6 percent in dark while the respective corresponding viable conidia at 28°C were 17.7 and 15.9 percent; there was an indication that these levels of survival were reached faster in light. For example, at 20°C while viability of 84.7 percent after 10 days fell to 39.0 percent in 5 days, it fell from 81.9 to 56.9 percent in dark within the same period. The great drop in viability at 28°C occurred earlier and the implication of both the temperature and the higher light intensity in this instance would be discussed later.

The conidia evidently survived better at the lower temperature and greater proportion of the conidia was conserved at all sampling times at 20°C. Viability was greatly reduced between 5 and 10 days at 28°C to less than 32 percent, whilst more than 80 percent of conidia stored at 20°C survived after 10 days.

G. Longevity of Conidia of *T. fructigena* stored in water

Most observations of longer survival at high humidities recorded in the literature do not contain any information on survival of the spores in liquid water. Physical conditions imposed by liquid water are very different from those of humid air. The longevity of *T. fructigena* conidia in water could be conveniently tested for in these studies since rather very low germination takes place in water and large quantities of ungerminated conidia would be available for subsequent germination tests. Aqueous spore suspensions in petri dishes were stored in the dark at 25°C. Four dishes were withdrawn after specific intervals as indicated in Table 13 and the ungerminated conidia sown on petri plates containing PDA, after germination percentage in the distilled water had been assessed.

The few germinated conidia usually floated on the water surface, while the ungerminated conidia sedimented at the bottom of the dish and were easily sucked up with a pipette. This spore suspension was then added to an equal volume of double strength of Potato Dextrose Broth (400g. potato, 40g. dextrose, 1000 ml. distilled water) and incubated at 25°C, for 12 hours. On mixture the Potato Dextrose Broth was diluted to normal concentration.

The percentage germination obtained in the nutrient medium at each sampling time is shown in Table 13.

The results indicate that *T. fructigena* conidia were well conserved in water. Longevity in water was better than at 100% R.H. (Table 11). Percentage survival after 20 days was 80.9 and 29.7 percent in water and at 100% R.H., respectively, (Tables 11 and 13).

TABLE 13

Longevity of Conidia of *T. fructigena* stored in Distilled Water at 25°C for 20 Days

Period of storage in Days	Germination in Distilled water during storage		Germination in Potato Dextrose Broth after storage	
	Total number of conidia observed	Percentage Germination	Total number of conidia observed	Percentage Germination
1	1472	0.2	1492	97.1
2	1477	0.5	1493	96.1
3	1483	0.3	1518	92.3
6	1564	0.1	1561	96.0
9	1578	0.2	1576	95.1
12	1513	0.2	1584	90.9
15	1576	0.1	1547	82.7
20	1582	0.0	1609	80.9

H. Effect of loss of water, by Plasmolysis, on Germination Capacity of Conidia of *T. fructigena*

The proportion of spores that collapsed at any relative humidity was closely related to the percentage of conidia that died (Chapter E). The percentage of spores which had collapsed could thus be used as a criterion of the percentage viability of any batch of spores. Was death due to mere loss of water from the cytoplasm or to an effect on the spore membrane or to both?

To find whether egress of water from the spore - under conditions that did not permit wall collapse - would kill the conidia at the same rate as those exposed to the atmosphere, the conidia were placed in solutions of commonly used plasmolysing agents, Potassium nitrate, sodium chloride and sucrose for 60 minutes and then sown on petri plates of PDA for 12 hours, to determine their germination capacity. The spore suspensions of plasmolysing fluids were kept in test tubes and the spores were heavily deposited at the bottom of the tubes at the end of one hour. The supernatant fluid was poured off and the spore deposits, with very little of the plasmolysing fluid seeded onto the plates. The plasmolysing fluid was not washed off as it was not known how damaging the sudden movement of water into the plasmolysed spores might be.

The results are shown in Tables 14 to 16. No germination occurred in any of the plasmolysing solutions and none of the spores collapsed. Percentage survival in all solutions remained above 77 percent even after storage for 60 minutes at osmotic pressure of 29.9 atmospheres (Tables 14, 15 and 16).

The high germination obtained indicated that the method followed was reasonable. The little quantity of plasmolysing fluid that was taken up with the unwashed conidia did not obviate the effects of the nutrients of the PDA.

TABLE 14

Germination of Conidia of *T. fructigena* plasmolysed in Potassium Nitrate Solution prior to Incubation on PDA at 25°C for 12 hours

Time kept in Plasmolysing solution (Minutes)	Molarity (M)*	Shrinkage of conidia in Plasmolysing Fluid		Germination on PDA after Plasmolysis	
		Total number of conidia observed	Percentage Collapse	Total number of conidia observed	Percentage Germination
20	0.1	342	0.0	376	93.6
	0.2	387	0.0	368	77.4
	0.3	356	0.0	398	81.2
	0.7	370	0.0	413	79.0
40	0.1	390	0.0	404	92.6
	0.2	410	0.0	362	91.7
	0.3	375	0.0	382	94.0
	0.7	386	0.0	356	94.1
60	0.1	350	0.0	367	93.7
	0.2	392	0.0	359	95.0
	0.3	381	0.0	352	92.3
	0.7	372	0.0	393	89.1

* 0.1M = 4.22 atm.; 0.2M = 8.04 atm.,

0.3M = 11.2 atm.; 0.7M = 24.6 atm.

TABLE 15

Germination of Conidia of *T. fructigena* plasmolysed in Sodium chloride solution prior to Incubation on PDA at 25°C for 12 Hours.

Time kept in Plasmolysing solution (Minutes)	Molarity (M)*	Shrinkage of Conidia in Plasmolysing fluid		Germination on PDA after Plasmolysis	
		Total number of conidia observed	Percentage Collapse	Total number of conidia observed	Percentage Germination
20	0.1	384	0.0	357	96.1
	0.2	402	0.0	349	90.5
	0.3	390	0.0	375	84.8
	0.7	373	0.0	372	77.5
40	0.1	370	0.0	367	96.5
	0.2	364	0.0	409	88.8
	0.3	386	0.0	388	77.3
	0.7	399	0.0	376	85.1
60	0.1	382	0.0	368	93.8
	0.2	357	0.0	349	96.3
	0.3	377	0.0	350	97.7
	0.7	396	0.0	396	82.6

* 0.1M = 4.45 atm[illegible].; 0.2M = 8.73 atm[illegible].;

0.3M = 13.0 atm[illegible].; 0.7M = 29.9 atm[illegible].

TABLE 16

Germination of Conidia of T. fructigena plasmolysed in Sucrose Solution prior to Incubation on PDA at 25°C for 12 Hours.

Time kept in Plasmo-lysing solution (Minutes)	Molarity (M)*	Shrinkage of Conidia in Plasmolysing fluid		Germination on PDA after Plasmolysis	
		Total number of conidia observed	Percentage Collapse	Total number of conidia observed	Percentage Germination
20	0.2	375	0.0	344	94.8
	0.3	384	0.0	348	96.0
	0.5	349	0.0	332	95.5
	1.0	390	0.0	339	94.7
40	0.2	347	0.0	354	98.6
	0.3	388	0.0	361	96.1
	0.5	365	0.0	358	88.0
	1.0	372	0.0	371	96.0
60	0.2	394	0.0	374	94.1
	0.3	403	0.0	358	98.9
	0.5	386	0.0	354	98.0
	1.0	354	0.0	378	93.9

* 0.2M = 4.96 atm[illegible].; 0.3M = 7.49 atm[illegible].;
0.5M = 12.4 atm[illegible].; 1.0M = 25.0 atm[illegible].

I. Further Experiments on the Effect of Loss of Water by Plasmolysis on Germination Capacity of Conidia of *T. fructigena*

The previous experiment showed that *T. fructigena* conidia plasmolysed in 0.7 M solutions of Potassium nitrate and Sodium chloride and in 1.0 M solution of Sucrose, were hardly affected and conidia of all treatments subsequently germinated very well on PDA. The maximum time of immersion was 60 minutes which was long enough to induce plasmolysis. It is possible that the spores have sufficiently high osmotic pressure to prevent excessive plasmolysis. Instances of high osmotic pressures have been recorded in the literature. Osmotic pressures of 63 atmospheres for conidia of *Erysiphe graminis* and 68 atmospheres for *Erysiphe polygoni* had been reported by Brodie (1945). The experiment below was designed to investigate the effect of higher concentrations of sucrose solution only, on the viability of the conidia.

The procedure of the previous experiment (Chapter H) was followed, using this time 1.5 and 2.0 M. sucrose solutions. The conidia were transferred to PDA plates after 60 minutes immersion in the plasmolysing solutions and incubated at 25°C for 12 hours, after which percentage germination was assessed. The results are presented in Table 17.

TABLE 17

Germination of Conidia of *T. fructigena* Plasmolysed in Sucrose Solutions prior to Incubation on PDA at 25°C for 12 Hours.

Molarity (M)*	Time kept in Plasmolysing Solution (Minutes)	Shrinkage of Conidia in Plasmolysing Fluid		Germination on PDA after Plasmolysis	
		Total number of conidia observed	Percentage Collapse	Total number of conidia observed	Percentage Germination
1.5	20	354	0.0	363	93.4
	40	339	0.0	378	89.9
	60	382	0.0	411	90.5
2.0	20	397	0.0	388	90.7
	40	375	0.0	409	87.0
	60	407	0.0	419	85.9

* 1.5M = 37.5 atmos.; 2.0M = 50.0 atmos.

The results show that 60 minutes plasmolysis at the higher concentrations of sucrose was not more damaging than the lower concentrations of the previous experiment. As high as 90.5 and 85.9 percent viability were recorded for spores plasmolysed for 60 minutes in 1.5 and 2.0 M sucrose solutions.

In the preceding experiment and in the present one, the conidia were found to have been plasmolysed to varying degrees. This was ~~could be~~ re-examined in the next experiment.

J. Longevity of Conidia of T. fructigena stored in Sucrose solutions

Cursory examination of conidia in the previous experiment showed that they were plasmolysed in the sucrose solutions. Immersion for only one hour was, evidently, too brief to effect serious disruption in the spores judging by the high percentage survival of all the treatments (Tables 14 to 17). Although plasmolysed spores survived far better than conidia that lost water at such high relative humidity as 90% R.H. (Table 9), it was presumed that they would live - because of their apparent sensitivity to loss of water - for far shorter periods than conidia stored in water, in the unplasmolysed state. This hypothesis was tested by keeping the conidia in both 1.5 M and 2.0 M sucrose solutions for 72 hours. Conidia in distilled water served as control. Table 18 indicates the proportion of conidia that survived plasmolysis in the sucrose solutions after 6, 12, 24, 48 and 72 hours.

Results in Table 18 show that again no conidia collapsed. Lengthening the period of spores in plasmolysing solutions of 1.5 M and 2.0 M sucrose shortened their longevity. Survival after 72 hours in 1.5 M and 2.0 M sucrose solutions was, respectively, 34.1 and 32.7 percent, whilst a corresponding storage in distilled water (control after 72 hours) showed 94.7 percent viability.

TABLE 18

Longevity of Conidia of *T. fructigena* stored in 1.5 and 2.0M Sucrose Solutions for 72 Hours.

Molarity (M)	Time kept in Plasmolysing solution (Hours)	State of Conidia in Sucrose Solution			Percentage on PDA after Plasmolysis	
		Total number of conidia observed	Percentage Plasmolysed	Percentage Collapse	Total number of conidia observed	Percentage Germination
1.5	6	391	100.0	0.0	420	88.8
	12	398	100.0	0.0	400	75.8
	24	388	100.0	0.0	397	69.5
	48	378	100.0	0.0	385	56.6
	72	395	100.0	0.0	414	34.1
2.0	6	381	100.0	0.0	419	89.7
	12	378	100.0	0.0	426	80.6
	24	377	100.0	0.0	392	87.5
	48	371	100.0	0.0	391	63.4
	72	400	100.0	0.0	410	32.7
Distilled H_2O	72	-	-	-	454	94.7

Every conidium observed after storage in the sucrose solutions was found to have plasmolysed. The spores were, however, not plasmolysed to the same extent as demonstrated by Plates 8 and 9. It is not possible from observations here to relate degree of plasmolysis to viability. It could be conjectured that death would occur when a certain stage of plasmolysis had been exceeded.

Plate 8. Photomicrograph of plasmolysed unstained conidia of T. fructigena after 12 hours incubation in 1.5M Sucrose solution. Note the varying degrees of plasmolysis. (X 200)

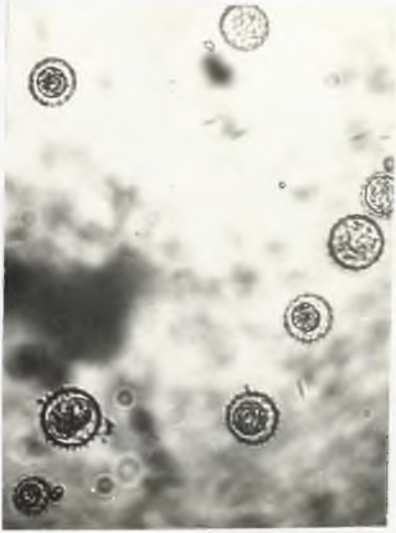


Plate 9. Photomicrograph of plasmolysed unstained conidia of T. fructigena after 12 hours incubation in 2.0M Sucrose solution. Note the varying degrees of plasmolysis. (X 200)

K. Possible Effects of Storage in Water on Metabolism of T. fructigena conidia

When conidia of T. fructigena were stored at 100% R.H. and in water, a high percentage of the conidia survived for more than 10 days and germinated very well on PDA (Tables 11 and 13). It has generally been presumed that metabolic activities of fungal spores would proceed at a high rate under such conditions and the effects would be shown in two ways. Either metabolic activities under high humidity conditions would better 'prepare' the spore for germination and so hasten germination when favourable conditions are provided or the attendant accumulation of metabolites within the spore would depress metabolic activity and delay germination. These hypotheses could be examined in various ways. Often the length of the germ tubes is an indication of time of their production. The extensively branching nature of T. fructigena germ tubes did not permit the use of this criterion. One other method would be an estimation of the rate of germination of the conidia stored for varying periods, by measuring percentage germination at closely-spaced intervals. Such studies were carried out on conidia stored at 100% R.H. and in water and at 25°C. The maximum storage time was 10 days when more than 90 per cent of the conidia would be alive (Tables 11 and 13). The conidia were, as usual, germinated on PDA for 12 hours at 25°C after storage. The results are presented in Tables 19 and 20 and graphically represented in Fig.8.

TABLE 19

Germination of *T. fructigena* conidia on PDA in 12 hours after storage in dark at 100% R.H. and at 25°C.

Period of storage at 100% R.H. in Days	Period of incubation on PDA in Hours	Total number of conidia observed	Percentage Germination
2	2	381	7.3
	4	396	16.2
	6	393	30.3
	8	348	55.5
	10	385	73.0
	12	390	89.7
4	2	356	8.4
	4	384	22.1
	6	392	38.3
	8	343	62.1
	10	386	72.3
	12	386	90.2

TABLE 19 (cont'd.)

Germination of *T. fructigena* conidia on PDA in 12 hours after storage in dark at 100% R.H. and at 25°C.

Period of storage at 100% R.H. in Days	Period of Incubation on PDA in Hours	Total number of conidia observed	Percentage Germination
6	2	381	23.6
	4	350	41.1
	6	379	58.0
	8	405	70.6
	10	387	78.6
	12	372	86.8
8	2	385	9.9
	4	354	20.3
	6	356	29.8
	8	367	43.9
	10	410	65.4
	12	400	75.8

TABLE 19 (cont'd.)

Germination of <u>T. fructigena</u> conidia on PDA in 12 hours after storage in dark at 100% R.H. and at 25°C.

Period of storage at 100% R.H in Days	Period of Incubation on PDA in Hours	Total number of conidia observed	Percentage Germination
10	2	360	12.5
	4	353	24.1
	6	388	30.7
	8	370	45.7
	10	348	59.6
	12	391	73.4

TABLE 20

Germination of *T. fructigena* conidia on PDA in 12 hours after storage in dark and in water at 25°C.

Period of storage in Water in Days	Period of Incubation on PDA in Hours	Total number of conidia observed	Percentage Germination
2	2	336	43.5
	4	358	91.1
	6	349	95.7
	8	406	96.8
	10	371	94.9
	12	390	95.4
4	2	357	19.0
	4	373	94.4
	6	379	97.4
	8	372	96.0
	10	393	95.7
	12	370	96.5

TABLE 20 (cont'd.)

Germination of <u>T. fructigena</u> conidia on PDA in 12 hours after storage in dark and in water at 25°C.

Period of storage in water in Days	Period of Incubation on PDA in Hours	Total number of conidia observed	Percentage Germination
	2	364	23.4
	4	416	83.9
	6	364	95.3
6	8	394	94.7
	10	385	96.6
	12	377	93.9
	2	382	21.7
	4	340	72.1
	6	369	91.1
8	8	366	94.0
	10	384	92.4
	12	382	93.5

TABLE 20 (cont'd.)

Germination of *T. fructigena* conidia on PDA in 12 hours after storage in dark and in water at 25°C.

Period of storage in Water in Days	Period of Incubation on PDA in Hours	Total number of conidia observed	Percentage Germination
10	2	361	36.8
	4	407	75.4
	6	386	87.3
	8	383	92.7
	10	380	93.4
	12	374	92.0

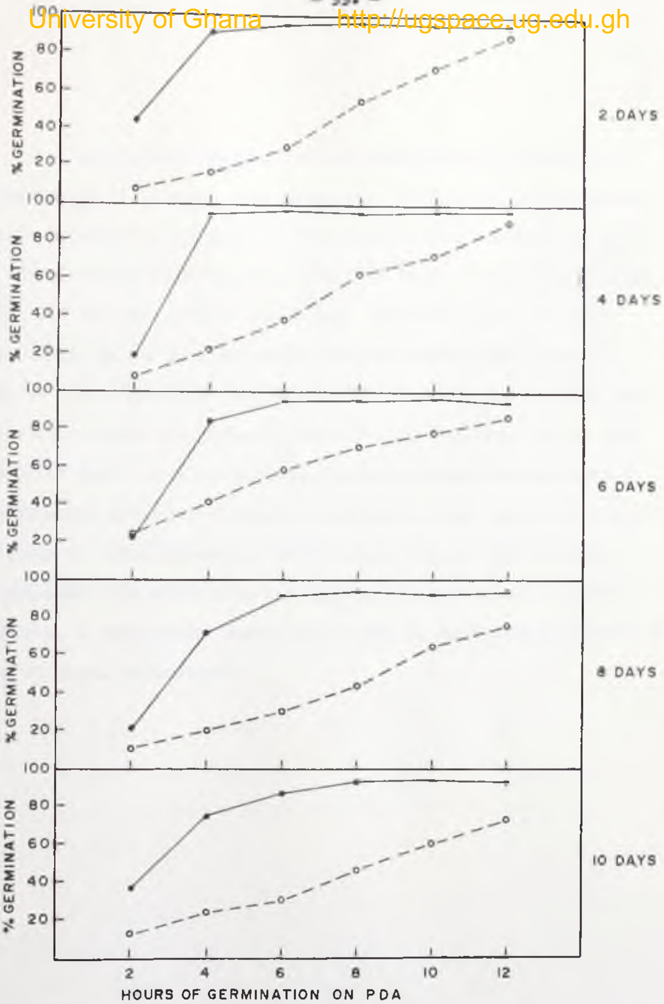


FIG. 8 RATE OF GERMINATION OF T. FRUCTIGENA CONIDIA AFTER STORAGE AT 100% R.H. AND IN LIQUID WATER FOR 2, 4, 6, 8 AND 10 DAYS

●——● STORED IN LIQUID WATER ○– – –○ STORED AT 100% R.H.

The results show that rate of germination was faster in conidia stored in water than those kept at 100% R.H. For example, maximum percentage germination (91.1 percent) of conidia stored for 2 days in water was attained on PDA in 4 hours (Table 20). Conidia kept for the same period at 100% R.H. needed as long as 12 hours incubation on PDA to achieve maximum germination (89.7 percent) (Table 19). Conidia stored at 100% R.H. at different sampling days similarly showed the highest germination at 12 hours. Spores kept in water for 4 days, in contrast, germinated well showing maximum germination after only 4 hours incubation on PDA. Six to ten days' storage in water, however, affected spore vigour. The conidia germinated at a slower rate and maximum germination was observed after 6, 6 and 8 hours' incubation on PDA in conidia stored for 6, 8 and 10 days, respectively.

L. Germination of Conidia of *T. fructigena* in aqueous extract of native soil

Husks of cocoa pods from which the beans have been extracted are left in heaps on the ground of cocoa farms for very prolonged periods. These heaps naturally form a very good food base for *T. fructigena* and, furthermore, form a springboard for soil infection. The fate of conidia, either washed off from mycelium growing on the pod heaps into the soil, or those formed by mycelium in the soil is of extreme importance in relation to the establishment of the fungus on new crop of pods. If they remain alive in soil, they could be splashed by rain drops onto pods at the lower regions of the trunk, or carried from soil up the trunk by ants.

The subject of spore germination has more recently been reviewed by many (Cochrane, 1966; Gottlieb, 1966; Sussman, 1966). Dormancy as defined by Gottlieb (1950) has been qualified by Sussman (1966) as *constitutive dormancy*, to distinguish it from *exogenous dormancy*, imposed upon the spore by environmental conditions that inhibit germination.

If *exogenous dormancy* were imposed by soils of cocoa farms on *T. fructigena* conidia, they could play the role of infection units much better if they do not germinate in soil at least indiscriminately unless in the presence of a food base that would ensure support for the resultant mycelium or until the dormant spore is dispersed onto pods.

Inhibition of fungal spores by soils has been described by several investigators (e.g. Chinn, 1953; Dobbs and Hinson, 1953; Jackson, 1958; Stover, 1958, etc.) and the concept of a general widespread fungistasis in soils has been developed.

The germination of T. fructigena in extracts of soil of a cocoa farm at Aburi, with high mealypod incidence, was examined to find out whether the conidia would be prevented from germination.

Conidial suspensions were separately prepared with non-sterile extracts of (a) soil (pH 9.9) and (b) partially decomposed leaves (pH 8.2) of the cocoa farm. Mycelial fragments in the suspensions were removed by straining the suspension with sterile muslin cloth. Aliquots of 20 ml of each spore suspension were pipetted into sterile petri-dishes and incubated at 25°C. Percentage germination was assessed after 12 hours. Conidia in distilled water served as control.

There was no germination in all three treatments. The minimum number of conidia examined ~~observed~~ during observation of the incubated conidia at the end of the incubation period was more than 740 for each treatment.

N. Cause of Exogenous Dormancy in Conidia of *T. fructigena* in Soil extract

One of the major causes of fungistasis in soil is considered to be the inhibitory action of products of microbial metabolism. Inhibition could, therefore, be removed through elimination of the microorganisms by sterilization (Dobbs and Gash, 1965; Dobbs and Hinson, 1953).

Conidia of *T. fructigena* were, as a sequel to the experiment in the previous chapter, germinated in autoclaved extracts of the partially decomposed leaves and soil for 12 hours at 25°C.

There was again no germination in the autoclaved extract of partially decomposed leaves (out of 736 observed conidia) and autoclaved soil extract (out of 755 observed conidia).

N. Examination of the Presence of Fungistasis in native soil

From the results of the experiment of Chapter L, it could be inferred that fungistasis was present in non-sterile extracts and permitted absolutely no germination. Since there was also no germination in the sterile extracts (Chapter M), either there was a residual fungistasis which was strong and effective, or nutrients in the extracts were too low to stimulate germination after removal of the fungistatic principle. The results so far do not, however, establish conclusively the presence of fungistasis since the conidia did not also germinate in water. The presence of fungistasis in the extracts could be demonstrated by supplementing extracts of the soils with different concentrations of nutrients. Very high concentrations of nutrients should overshadow the fungistatic effect. On the other hand, at very low nutrient concentrations, fungistasis should exert its influence and allow lower germination in comparison to germination in unamended nutrient of identical concentration. This experiment verified this hypothesis - by using Potato Dextrose Broth of the concentrations N, N/2, N/4, N/8 and N/16, N denoting the normal concentration of Potato Dextrose Broth medium, where N = (200g. potato; 20g. dextrose; 1000 ml de-ionised water). Double concentrations of both the media and extracts were prepared in order to achieve the desired concentrations on mixture of equal volumes of the two media.

Spore suspensions prepared with the 'mixture' solution were incubated at 25°C for 12 hours. Percentage germination obtained for the various treatments are presented in Table 21 and in Fig.9.

The results clearly indicate fungistatic effect in extract of partially decomposed leaves. Fungistasis eclipsed, partially, the nutrients of low concentrations, N/8 and N/16, of the PDB. For example, whilst PDB alone at N/16 supported 98.6 percent germination, only 17.0 percent of the conidia germinated when the extract was added. The germ tubes were in addition very short.

TABLE 21

Germination of conidia of *T. fructigena* in non-sterile Extracts of Soil and Partially Decomposed Leaves supplemented with Potato Dextrose Broth, at 25°C in 12 hours.

PDB amended with	Concentration of PDB	Total number of conidia observed	Percentage Germination
Soil Extract	N	1481	98.1
	N/2	1436	97.8
	N/4	1437	98.2
	N/8	1430	98.3
	N/16	1422	97.9
Extract of Partially Decomposed Leaves	N	1453	97.3
	N/2	1437	97.6
	N/4	1422	96.8
	N/8	1468	70.5
	N/16	1512	17.0
Distilled Water	N	1456	99.1
	N/2	1461	98.7
	N/4	1434	99.0
	N/8	1468	99.1
	N/16	1438	98.6

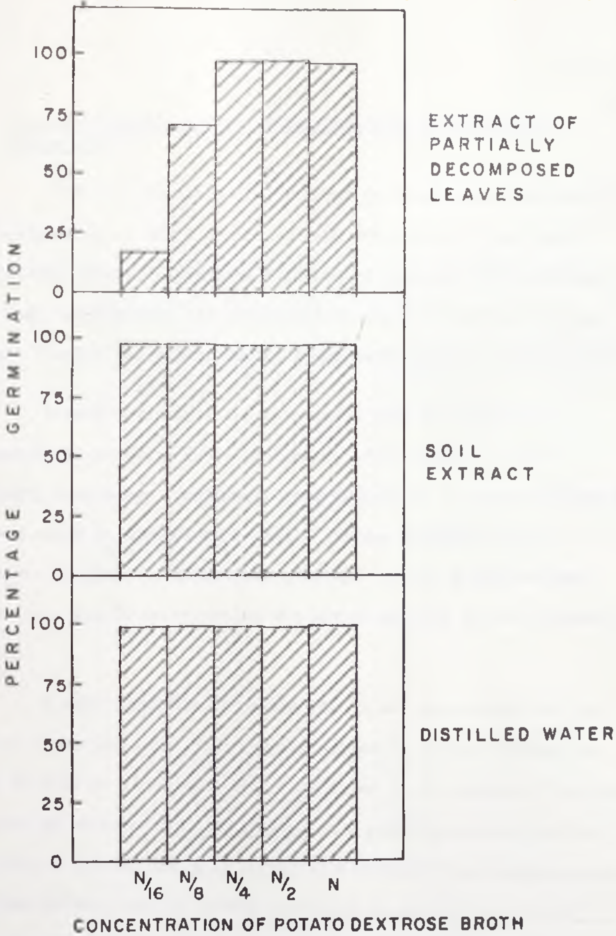


FIG. 9 EFFECT OF FUNGISTASIS IN POTATO-DEXTROSE ENRICHED EXTRACTS OF SOIL AND PARTIALLY DECOMPOSED LEAVES ON GERMINATION OF CONIDIA OF *T. FRUCTIGENA* AT 25° C

O. Possible Volatile Nature of the Fungistatic Principle of native soil

With the observation of reduced germination at N/8 and N/16 concentrations of the PDB amended with the extract of partially decomposed leaves, a positive evidence of presence of fungistasis had been established. An investigation was next carried out to define further the nature of the fungistatic principle of this soil.

Recent investigations have shown that the fungistatic principle is volatile (Hora and Baker, 1970; 1972). Such a property thus makes fungistasis more effective, as spores distantly placed could be influenced as well. It was thought desirable to find out whether the inhibitory principle of the Aburi soil was volatile. Fig.10 demonstrates the method adopted in this investigation.

A small quantity of molten PDA (5 ml) was poured into the lid of a sterile petri dish and was spread by gently tilting the lid, so that it set into a thin flat layer in the centre of the lid. A piece of culture of T. fructigena in a petri plate was then cut out with a sterile scalpel, lifted with a flamed inoculating needle, and the Culture Surface gently adpressed to the surface of the agar in the lid. The lid was then placed

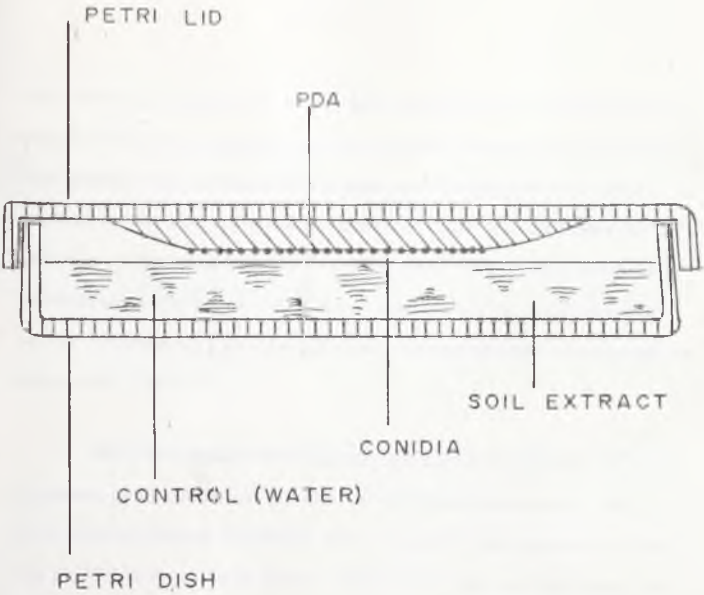


FIG. 10 SECTIONAL DIAGRAM ILLUSTRATING CONIDIA OF T. FRUCTIGENA SOWN ON PDA ON PETRI LIDS EXPOSED TO SOIL EXTRACT.

back onto the bottom half of the petri dish which had been almost fully filled with non-sterile soil extract, leaving a space of only 2 mm between the surfaces of the agar and the extract. In this condition, the conidia would be directly under the influence of any volatile matter emanating from the extract. PDA of N/8 and N/16 concentrations only were used since fungistasis was only detected in the presence of these low nutrient concentrations in the previous experiment (Table 21).

When the conidia were examined after incubation at 25°C for 12 hours, germination was similar in all three treatments, viz, petri dishes holding distilled water (control) and extracts of soil and partially decomposed leaves (Table 22). Even on N/16 PDA, the respective percentage germination was 93.5, 92.9 and 93.6 percent in the presence of water, and extracts of soil and partially decomposed leaves. The fungistatic principle was seemingly non-volatile.

TABLE 22

Germination of *T. fructigena* conidia sown on PDA and exposed to non-sterile extracts of soil and partially decomposed leaves for 12 hours at 25°C.

Extract	Concentration of PDA	Total number of conidia observed	Percentage Germination
Loamy Soil	N/8	374	93.6
	N/16	396	92.9
Partially Decomposed Leaves	N/8	387	97.4
	N/16	391	93.6
Distilled Water	N/8	396	86.9
	N/16	387	93.5

P. Survival of Conidia of *T. fructigena* in Extracts of native soil

Extracts of soil of the cocoa farm at Aburi were fungistatic and they imposed exogenous dormancy on conidia of *T. fructigena*. An information of practical importance is the period for which viability of the conidia would be sustained under that condition. There are various possible factors that would prevent long survival. Excessive mineral salts would impose plasmolysis problems. The spores may form substrates for soil bacteria. Lingappa and Lockwood (1961) have shown that spore-surface bacteria subsist on spore-exudate nutrients. The rate at which bacteria would flourish would be closely related to the pH of the soil, and they would most likely operate best at alkaline pH, following the observations of Jackson (1958) which showed that fungistatic effect decreased with increasing acidity-conditions that prevent bacterial action and growth. Some of these problems were considered in the following investigation.

Conidial suspensions were prepared with extracts of soil and, partially decomposed leaves adjusted to pH 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0, measured with capillators, with either dilute hydrochloric acid or 0.1N sodium hydroxide and stored at 25°C for 15 days. After storage, the conidial suspensions were separately seeded onto PDA and incubated at 25°C for 12 hours before assessing percentage germination. The results are presented in Table 23 and in Fig.11.

More than 50 percent of the spores was still viable after 15 days at some pH levels. The results show that the best pH for survival was pH 8.0, where 69.6 percent of the conidia in the soil extract survived 10 days' storage and 51.0 percent was alive after 15 days. Longevity in the extract of partially decomposed leaves was closely similar; 60.6 and 56.8 percent survival, respectively, after 10 and 15 days. Survival then declined as the pH moved to both the acidic and the alkaline ends. In both extracts pH 4.0, which was farthest removed from the optimum, was most unsuitable for storage (Fig.11).

TABLE 23

Survival of _T. fructigena_ conidia stored for 15 days at 25°C at various pH levels of extracts of Partially decomposed leaves and soil, (Conidia germinated on PDA for 12 hours at 25°C after storage).

Period of storage in Days	pH of Extract	Survival in Extracts of:			
		Partially Decomposed leaves		Loam Soil	
		Total number of conidia observed	Percentage Germination	Total number of conidia observed	Percentage Germination
10	4.0	381	10.0	412	14.1
	5.0	389	42.4	415	14.7
	6.0	398	43.0	423	40.9
	7.0	412	53.9	395	44.8
	8.0	393	60.6	424	69.6
	9.0	433	47.3	411	66.6
	10.0	412	50.5	455	67.9
15	4.0	458	23.4	385	3.1
	5.0	414	29.2	419	25.5
	6.0	446	48.7	427	39.1
	7.0	444	54.7	412	49.8
	8.0	414	56.8	414	51.0
	9.0	440	32.3	453	37.7
	10.0	454	30.2	435	42.0

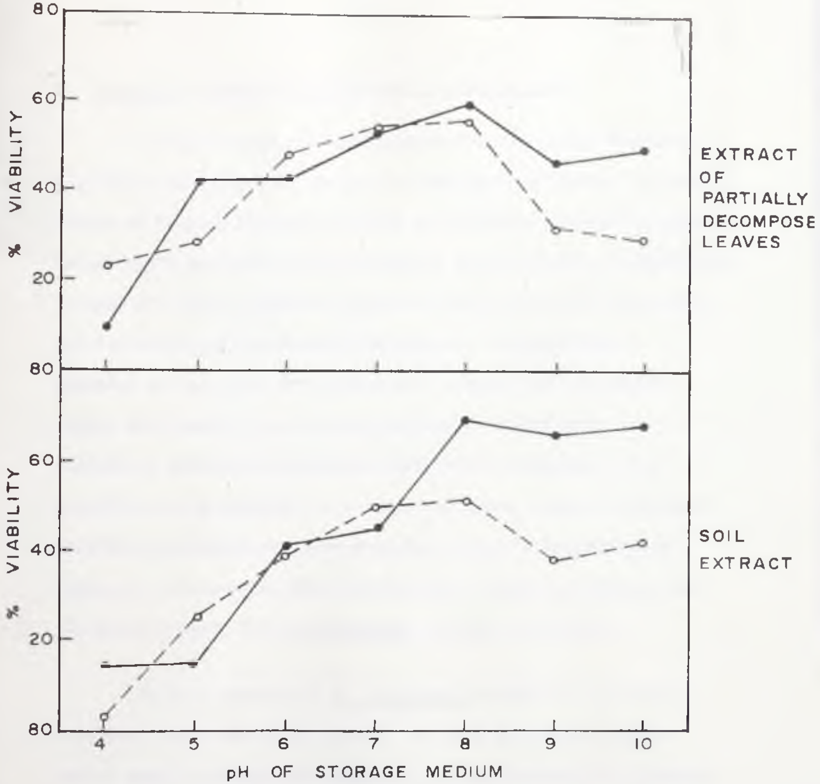


FIG. II SURVIVAL OF T. FRUCTIGENA CONIDIA INCUBATED FOR 10 AND 15 DAYS IN EXTRACTS OF PARTIALLY DECOMPOSED LEAVES AND SOIL ADJUSTED TO VARIOUS pH LEVELS. (CONIDIA GERMINATED ON P D A FOR 12 HOURS AFTER STORAGE)

●——● 10 DAYS OF INCUBATION ○– – –○ 15 DAYS OF INCUBATION

Q. Lysis of Conidia of T. fructigena in native soil

In most studies on fungistatic effect of soils, attention has mostly been given to the fate of the spore or hyphae. In his review on factors limiting survival and activity of fungi in soil, Brian (1960) explained that heterolysis could be due to oxygen-lack because of a large microbial population and due also to some antibiotics acting as respiratory inhibitors. He cited further examples of bacterial and actinomycete enzymes that can disrupt fungal cell walls or protoplast membranes. If the spore is capable of sufficient metabolism under soil conditions, it is possible that metabolites of a spore and hypha could in turn exert inhibitory influence on micro-organisms close to them and they could, at least, delay their destruction. There is evidence from the present study that *T. fructigena* conidia could do so.

In this experiment, *T. fructigena* conidia in two states were used, dead and viable spores. To kill the conidia, spore prints were incubated over anhydrous calcium chloride, to desiccate the spores, for 6 hours. The slides bearing the conidia were then buried in loam soil from the cocoa farm at Aburi, and adjusted to 20% moisture content.

Four slides bearing the spore prints were then placed vertically, standing on the longer edge, in a glass bowl (14 cm. diameter and 6 cm. deep) filled with the soil. In order to avoid wiping off the conidia during insertion of the slides, four parallel equidistantly spaced slits were made with a scalpel into which the slides were put. The soil was then pressed firmly against the slide. There were four bowls of four slides each for each ~~type~~ state of spore. The bowls were finally covered with polythene sheets held in position with rubber bands to keep the moisture content constant and incubated at 25°C.

Two bowls, one containing slides bearing initially dead conidia and the other living conidia, were withdrawn at 24-hour intervals. A drop of distilled water was quickly placed on the viable spore print on withdrawal of the slide to prevent desiccation and the conidia examined under the microscope for presence of micro-organisms and signs of degradation. The conidia were then, as spore suspension, germinated on PDA in petri plates at 25°C for 12 hours after which assessment of percentage germination (percentage survival) was made.

The results presented in Table 24 and 25 clearly show a difference in the extent of visible attack by micro-organisms between originally viable and dead conidia. On the 4th day only 0.9 percent of the 1690 initially viable conidia observed carried a microflora visible under high power of the microscope. Survival after 4 days in the soil was 54.5 percent. Dead conidia, on the other hand, showed heavy incidence of saprophytes. Out of 1814 conidia observed after 4 days' burial in the soil, 85.9 percent had been visibly attacked by saprophytes (Plate 10) and 22.1 percent was completely lysed and they could be only identified by their faint outline (Plate 11). Such lysis had seemingly been delayed in spore prints of viable conidia as no lysis was detected after 4 days' burial in soil. This impression was confirmed by a supplementary experiment in which the conidia of initially viable spore print were examined under the microscope after withdrawal from the soil without adding the drop of distilled water. This kept the conidia in position and would allow detection of any lysed spores. These spores were not germinated on PDA, as they might have perished after exposure during examination under the microscope.

TABLE 24

Survival of viable conidia of T. fructigena buried in native soil (20% W.H.C.) on slide for 4 days at 25°C.

Period in soil (Days)	Conidia with no visible signs of microbial attack		Conidia with visible signs of microbial attack	
	Total number of conidia observed	Percentage Germination	Total number of conidia observed	Number of conidia Germinating
1	1455	60.1	17	13
2	1847	61.4	20	14
3	1569	54.7	7	4
4	1690	54.5	16	8

TABLE 25

Degradation of dead conidia of *T. fructigena* buried in native soil (20% W.H.C.) on slide for 4 days at 25°C.

Period in soil (days)	Total number of conidia observed	Percentage of conidia with visible signs of microbial attack	Percentage of conidia lysed
1	2210	29.4	0.1
2	2113	57.6	6.5
3	1636	76.5	16.8
4	1814	85.9	22.1

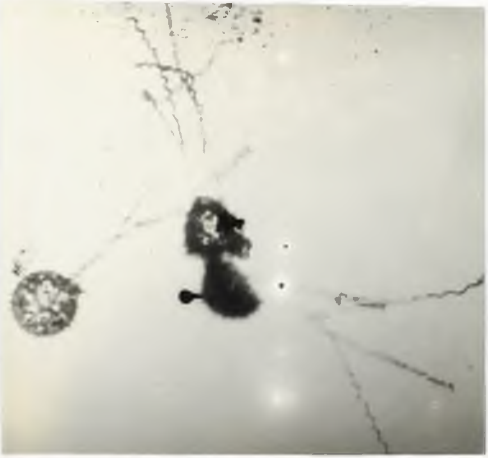


Plate 10. Photomicrograph of Actinomycetes growing on 'dead' conidia of <u>T. fructigena</u> buried in soil for 4 days. (X 200)

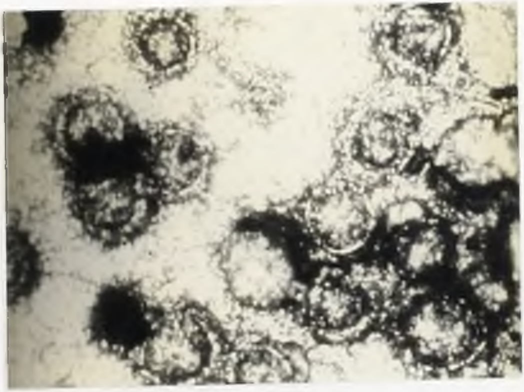


Plate 11. Photomicrograph showing outline of lysed 'dead' conidia of <u>T. fructigena</u> buried in soil for 4 days. (X 400)

R. Saprophytic Survival of *T. fructigena* in soil

There are no reports on the occurrence of *T. fructigena* in soil. The most important reason is that it has not yet been sought. It has been suggested earlier (Chapter L) that the practices of cocoa farmers in West Africa are likely to encourage the invasion of the soil by this fungus. As a sequel to the present findings on the fate of spores of *T. fructigena* in soil, the ability of the mycelium to live as a saprophyte in soil was studied.

For a long time interest in the behaviour of pathogenic fungi in soil has been confined to root-infecting fungi. Garrett (1950) reviewed the inter-relationships between root-disease fungi and the associated soil microflora and he distinguished between specialised root-inhabiting parasites and unspecialised soil-inhabiting parasites. This classification did not include shoot pathogens. Garrett (1970) has recently enumerated all possible modes of survival of root-disease fungi that have been discovered during the numerous pertinent investigations:

(1) as competitive saprophytes on dead organic substrates;

(2) saprophytic survival on dead tissues of a host crop or weeds, infected during the parasitic phase;

(3) dormant survival as 'resting' propagules, e.g. sexually produced oospores and other spores, asexually produced chlamydospores, and multicellular sclerotia;

(4) parasitic survival on living roots and other underground parts of used hosts and 'volunteer' susceptible crop plants;

(5) parasitic survival on living root systems of plants that show no disease symptoms above ground.

At least <u>T. fructigena</u>, a facultative parasite, should be able to use the first two modes of survival. The present work investigated this.

The soil used was loam soil from the cocoa farm at Aburi. It was passed through a 2 mm sieve and adjusted to a moisture content of 45% saturation capacity.

A maize meal-sand medium of 100 parts sand, 3 parts ground maizemeal and 13 parts water (by weight) was in the meantime inoculated with <u>T. fructigena</u> and the culture allowed to grow over a month. The culture was shaken occasionally to ensure thorough growth of the fungus in the entire medium. At the end of the month, the culture was also adjusted to a moisture content of 45% saturation capacity and then mixed with the unsterilised loam soil in the following proportions, the culture, hereafter referred to as inoculum, coming first in the ratios: 100:0 (inoculum control); 95:5; 90:10; 75:25; 50:50; 25:75; 10:90; 5:95 and 0:100 (soil control). Immediately after the preparation of the mixture, 50 pieces of dried unsterilized cocoa pod husk and coffee pericarp used as baits were stirred into 400 g portions of the inoculum/soil mixtures in Kilner jars and the jars with their contents weighed. The jars were covered with perforated aluminium foil sheets which prevented ~~avoided~~ rapid drying of the inoculum/soil mixture but ensured sufficient aeration. ~~The jars were~~ Throughout the incubation period of 4 weeks, the jars were weighed on each other day and the amount of water lost was made up with sterile distilled water. The jars were stacked on a wooden platform in the laboratory (26 ± 1°C) with legs standing in bowls of oil to avoid invasion by non-soil crawling insects and flying insects were kept off by a cover of nylon mosquito netting.

The baits were prepared in the following way: the husk of cacao pods and the pericarp of coffee berries were cut into small uniform pieces, approximately 1 cm by 2 cm. The cocoa pods were first peeled before the pieces were cut. The pieces were 5 mm thick.

The husk and pericarp pieces were separately dried in the sun until they had completely dried to uniform weight. They were not sterilized and were suspended in sterile distilled water for 1 hour immediately before they were used.

At the end of the 4-week incubation period, the baits were recovered, washed free of adhering soil and inoculum and surface sterilized by immersing for 1 minute in mercuric chloride of dilution 1:1000. They were then rinsed twice with sterile distilled water containing streptomycin. Each bait was cut into two but kept together and plated out - five (10 half pieces) per petri dish - on PDA containing a drop of 1% solution of streptomycin. The plated baits were incubated at 25°C for five days and the number of baits yielding T. fructigena was recorded. Despite the immense growth of saprophytes on the plates it was possible to identify T. fructigena by its distinctly recognisable echinulated conidia and their unique mode of production on the conidiophores, by careful microscopic examination. This was an extremely time consuming but rewarding exercise that required 3 days for a complete examination of each experiment. The results are presented in Table 26.

TABLE 26

Colonization of unsterilized Cocoa pod husk and Coffee berries in soil by *T. fructigena*.

(Percentage colonized: based on number of baits (out of a total of 50) bearing mycelium of *T. fructigena*, identified by the presence of the conidia, after incubation on PDA for 5 days at 25°C).

Inoculum (%)	Percentage Coffee fruits colonized	Percentage Cocoa pod husk colonized
100	58	70
95	14	30
90	10	12
75	6	0
50	6	2
25	4	0
10	0	0
5	0	0
0	0	0

The results indicate that T. fructigena has limited competitive saprophytic ability. In cocoa pod husk, saprophytic survival of T. fructigena fell rapidly from 70 percent survival in 100% inoculum to 2 percent survival in 50% inoculum. The decline in percentage survival conversely followed an increase in unsterilized loam soil. Similar trend - from 58 percent survival in 100% inoculum to 6 percent survival in 50% inoculum, for the pericarp of coffee was observed. Curiously, T. fructigena survived better in cocoa husk at higher percentage inoculum than in coffee pericarp, but this was reversed with the increase in unsterilized soil.

A second method of assessment was attempted using only cocoa pod husk pieces, anticipating a less exhaustive method of assessment than the 'Agar plate method'. This was the 'cocoa pod infection method'. Infection through baits containing the fungus would be expected to produce encrusted mass of conidia of T. fructigena on the pod surface (see Plate 1).

The baits from the inoculum of soil mixtures at the end of the 4th week, were placed in wounds made in the pericarp of mature green cocoa pods, after they had been surface sterilized with mercuric chloride. The cocoa pods were washed and surface sterilized with 5%

sodium hypochlorite. Triangular wounds, each side measuring 2 cm, and 5 mm deep were made with a flamed scalpel. The piece of husk removed was placed back after the bait had been inserted. The thickness of the bait, however, lifted the 'lid' and extensively exposed the cut surface. The inoculated pods were finally placed on moist cotton wool inside cellophane bags on the laboratory bench.

There was no infection of any of the cocoa pods. It is probable that nutrients from the husk did not diffuse sufficiently well into the bait to encourage the growth of mycelium of T. fructigena present in the bait.

Besides this set-back, the large number of pods needed for this method of assessment would be a deterrent. In this experiment 450 pods, kindly provided by the Agronomist of the Cocoa Research Institute, Tafo, were used.

S. Further Experiments on the Saprophytic Colonization of Cocoa pod husk buried in soil by T. fructigena

The considerable difference between the number of buried cocoa pod husk pieces colonised in the medium of 100 percent inoculum and that of 95 percent inoculum in the previous chapter (Table 26) showed that T. fructigena was not very successful in the colonization of cocoa pod husk in competition with microflora of the soil. Colonization by a test fungus can be improved by various means. Naturally by invading the tissues first, the fungus would establish itself well before the entry of other microflora. Butler (1953) demonstrated this in a very simple way. He coated wheat straws with the inoculum of Ophiobolus graminis before burying them in a culture/soil mixture and compared colonization with straws coated with soil and those not treated at all before burial. There was increased colonization by O. graminis of the straws coated initially with inoculum. There was low colonization of straws coated with soil, which may conversely be attributed to the comparable advantage thereby given to other soil micro-organisms. Butler's experiment was repeated in this investigation.

The soil was prepared and maize meal-sand culture of T. fructigena raised according to the procedure in Chapter R. Cocoa pod husk pieces were used as baits and buried in inoculum/soil mixtures prepared to provide inoculum concentrations of 95%, 90% 75% and 25%. Three sets of the experiment were set up. Maize meal-sand culture was pasted onto the baits of one set, while baits of the second set were plastered with moist soil. The third set was a repetition of the previous experiment. The results are presented in Table 27 and Fig.12.

The results clearly indicate that the position of T. fructigena in relation to its competitors is an important factor. In the 95:5 inoculum/soil mixture, 40 percent colonization was recorded for husk pieces coated initially with inoculum while only 24 percent colonization was recorded for husk pieces coated initially with unsterilized soil. This advantage still persisted even with increased soil content in the mixture with 75% inoculum. Cocoa pod husk pieces buried without any treatment showed better colonization than the soil-coated husk pieces and inferior colonization to inoculum-coated husk pieces.

TABLE 27

Colonization of unsterilized Cocoa pod husk in soil by <u>T. fructigena</u> placed at different positions from the baits in relation to soil microflora.

(Percentage colonized: based on number of baits (out of a total of 50) bearing mycelium of <u>T. fructigena</u>, identified by the presence of the conidia, after incubation on PDA for 5 days at 25°C).

	Percentage of husk colonized		
Inoculum (%)	Husk coated with inoculum	Husk coated with unsterilized soil	Husk placed in inoculum/soil mixture
95	40	24	30
90	24	10	16
75	10	0	6
25	0	0	0

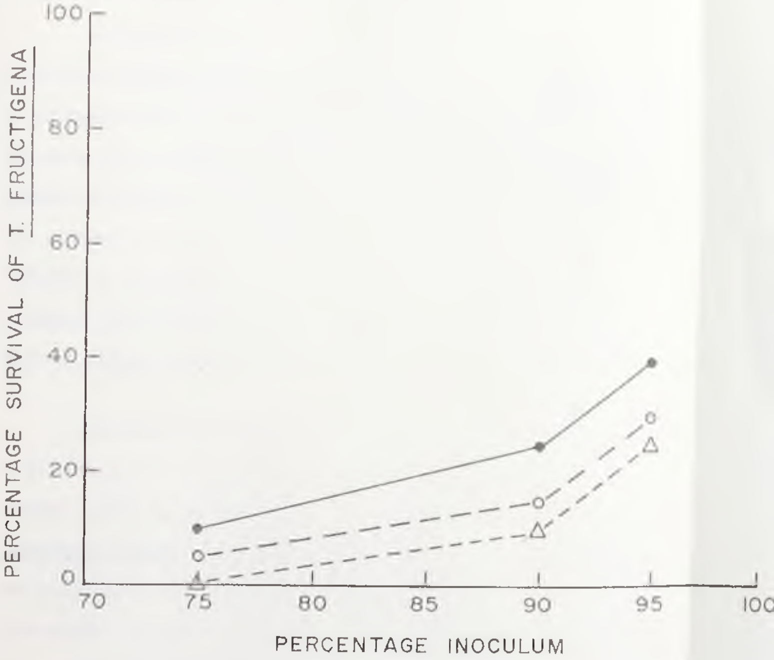


FIG. 12 COLONISATION OF BAIT (CACAO POD HUSK) BY T. FRUCTIGENA PLACED AT VARIOUS POSITIONS FROM THE BAITS

●——● BAITS COATED WITH INOCULUM
Δ– – –Δ BAITS COATED WITH SOIL
o- - - -o NON-COATED BAITS

T. Saprophytic Colonization of Cacao pod husk of increased nutrient content in soil by *T. fructigena*

It is generally true that the population level of a particular fungus in natural soil, and also its inoculum dosage in an experiment, is one of the most important factors determining the share of a substrate that will be obtained by that fungal species in competition with others. But it is not the only factor. The success of a fungus in competitive colonization will also be affected by such important factors as nutrients available in the substrate and environmental conditions. These will affect its rate of growth on the substrate, and similarly that of its competitors.

Nutrients have been shown to be one of the most important soil factors that would influence saprophytic colonization. Garrett (1938) provided the earliest evidence in his work on *Ophiobolus graminis*. *O. graminis* was found to die out early from the rigid and little-decomposed wheat straws buried in nitrogen-poor soils, whereas it was found to survive in almost 100 percent of straws in nitrogen-rich soils. This finding has been confirmed by Butler (1953; 1959), Chambers and Flentje (1969) and Macer (1961). *O. graminis* dies out pre-maturely from carbon starvation in wheat straws buried in nitrogen-poor soil, because it cannot obtain the nitrogen needed for continued mycelial growth and hydrolysis of fresh areas of cellulose within the straw tissue.

The effect of nutrients, particularly nitrogen, has not been found to be uniform. Butler (1959) discovered that excess soil nitrogen actually reduced longevity of Cochliobolus sativus in wheat straw, while Curvularia ramosa appeared to be indifferent to the level of soil fertility and nitrogen content. These contrasting effects of nutrients have been variously interpreted and the various views have been mentioned in the discussion of this thesis. The response of T. fructigena to added nutrients has been investigated here, as an indication of how it might be affected in soils with different nutrient content.

This was an extensive investigation that covered a period of 3 months, consisting of three experiments, set up in series. These were:

(a) April 1973 - May 1973: the effects of three nitrogen compounds, ammonium tartrate, peptone and sodium nitrate were examined and the compound encouraging best survival - sodium nitrate - was used in subsequent experiments.

(b) May 1973 - June 1973: the effects of various concentrations of Dextrose and sodium nitrate were examined and the concentrations for best survival selected for the third experiment.

(c) June 1973 - July 1973: the effects of a combination of dextrose and sodium nitrate was compered with those of the compounds when present separately.

Cocoa pod husk cut out into pieces and dried were soaked in the various solutions for 2 hours and then stirred into the various maize-sand culture/soil mixtures shown in Tables 28 to 31 and incubated at room temperature, 26°C, for 4 weeks, in each case. The husk pieces were then plated on PDA in petri plates for 5 days at 25°C, after they had been surface sterilized with mercuric chloride. The results are presented in Tables 28 to 31 and Figs. 13 to 16.

Of the three nitrogen compounds, sodium nitrate encouraged best survival (Table 28). Approximately 50 percent of the buried cocoa pod husk soaked in 0.5% (50 percent colonization) and 1.5% (56 percent colonization) sodium nitrate solutions contained

TABLE 28

Colonization of unsterilized Cocoa pod husk, pre-treated with various Nitrogen Compounds, in soil by *T. fructigena*.

(Percentage colonization: based on number of baits (out of a total of 50) bearing mycelium of *T. fructigena*, identified by the presence of the conidia, after incubation on PDA for 5 days at 25°C).

Nitrogen Compound	Concentration %(W/V)	Percentage colonization
Ammonium tartrate	0.5	30
	1.0	44
	2.0	20
Peptone	0.5	20
	1.0	6
	2.0	16
Sodium nitrate	0.5	50
	1.0	40
	1.5	56

TABLE 29

Colonization of unsterilized Cocoa pod husk, pre-treated with various concentrations of sodium nitrate, in soil by *T. fructigena*.

(Percentage colonized: based on number of baits (out of a total of 50) bearing mycelium of *T. fructigena*, identified by the presence of the conidia, after incubation on PDA for 5 days at 25°C).

Concentration %(W/V)	Percentage Inoculum	Percentage Colonized
0.5	100	50
	95	50
	90	30
	75	40
1.0	100	40
	95	36
	90	40
	75	30
1.5	100	56
	95	60
	90	56
	75	44

TABLE 30

Colonization of unsterilized Cocoa pod husk, pre-treated with various concentrations of Dextrose, in soil by *T. fructigena*.

(Percentage colonized: based on number of baits (out of a total of 50) bearing mycelium of *T. fructigena*, identified by the presence of the conidia, after incubation on PDA for 5 days at 25°C).

Concentration %(W/V)	Percentage Inoculum	Percentage Colonized
0.5	100	16
	95	16
	90	20
	75	4
1.0	100	20
	95	10
	90	20
	75	●
2.0	100	10
	95	10
	90	14
	75	●

TABLE 31

Colonization of unsterilized Cocoa pod husk, pre-treated with Dextrose, sodium nitrate and Dextrose-Sodium nitrate mixture, in soil by *T. fructigena*.

(Percentage colonized: based on number of baits (out of a total of 50) bearing mycelium of *T. fructigena*, identified by the presence of the conidia, after incubation on PDA for 5 days at 25°C).

Inoculum (%)	Percentage colonization of husk treated with			
	Water	1.0%(W/V) Dextrose	1.5%(W/V) Sodium nitrate	1.0%(W/V) Dextrose + 1.5%(W/V) Sodium nitrate
100	66	20	60	90
95	40	20	60	100
90	16	20	50	86
75	4	4	50	50

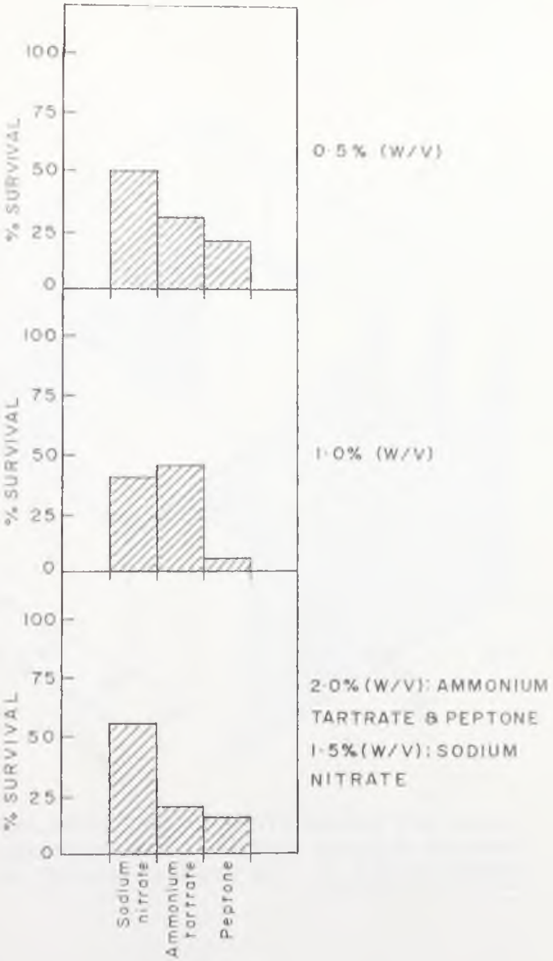


FIG. 13 COLONISATION OF BAITS (CACAO POD HUSK) AMENDED WITH SOLUTIONS OF VARIOUS NITROGEN COMPOUNDS

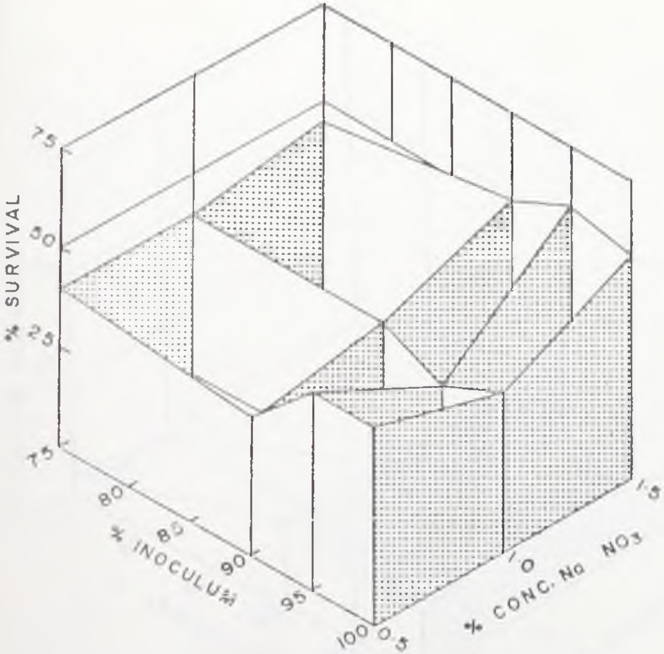


FIG. 14 COLONISATION OF BAITS (CACAO POD HUSK) AMENDED WITH VARIOUS CONCENTRATIONS OF SODIUM NITRATE BY T. FRUCTIGENA

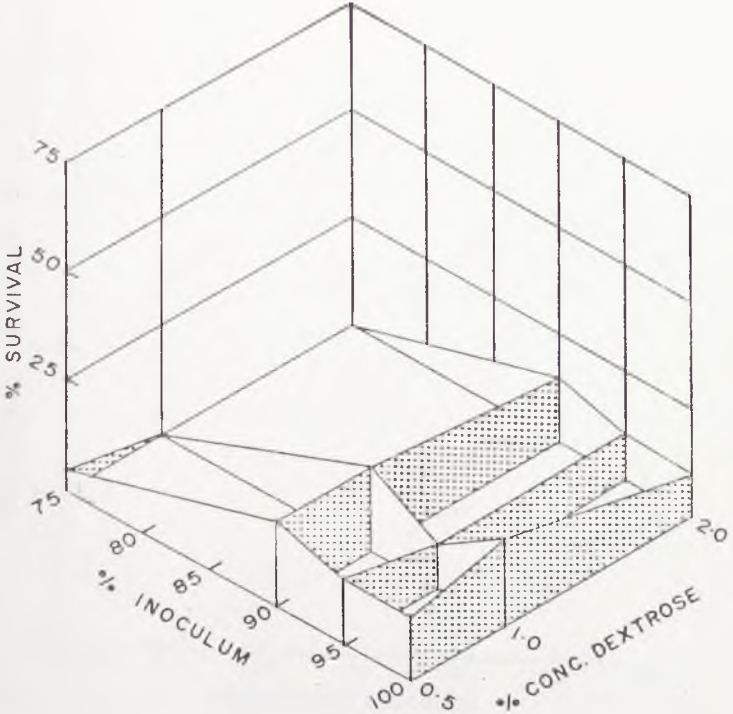


FIG. 15 COLONISATION OF BAITS (CACAO POD HUSK) AMENDED WITH VARIOUS CONCENTRATIONS OF DEXTROSE BY <u>T. FRUCTIGENA</u>

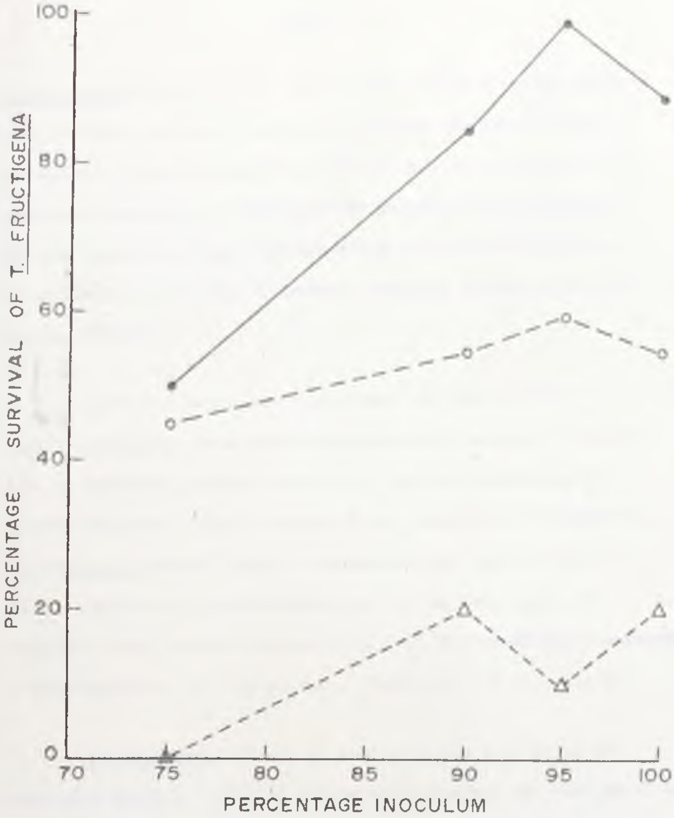


FIG. 16 COLONISATION OF BAITS (CACAO POD HUSK) AMENDED WITH DEXTROSE AND SODIUM NITRATE BY T. FRUCTIGENA

(Data from Tables 29 , 30 and 31)

●———● 1·0% DEXTROSE + 1·5% SODIUM NITRATE
o— — —o 1·5% SODIUM NITRATE, △- - - -△ 1·0% DEXTROSE

T. fructigena after 4 weeks. Saprophytic survival was slightly less in buried pod husk containing ammonium tartrate, ranging between 20 percent colonization at 2.0% ammonium tartrate to 44 percent colonization at 1.0% ammonium tartrate. T. fructigena did not persist as long in buried cocoa pod husk with peptone. The highest survival was 20 percent, recorded in husk with 0.5% peptone (Fig.13).

Untreated buried cocoa pod husk had earlier shown rapidly declining percentage colonization with decreasing quantities of inoculum (Tables 26 and 31). In soil containing 90 percent inoculum, only 12 percent of the buried husk contained T. fructigena after 4 weeks. Colonization was vastly improved by the addition of sodium nitrate and for the same level of inoculum dosage, sodium nitrate of 0.5 - 1.5% concentration sustained a colonization of 30 - 56 percent. (Tables 29 and 31; Fig.14)

T. fructigena did not persist extensively in cocoa pod husk with dextrose. Even in 100 percent inoculum, the best survival after 4 weeks was 20 percent at 1.0% dextrose (Table 30; Fig.15).

Table 31 clearly demonstrates that a combination of dextrose (1.0% W/V) and sodium nitrate (1.5% W/V) highly increased percentage colonization of buried cacao pod husk by T. fructigena. The fungus persisted in 50 percent of the cacao pod husk buried in soil containing only 75 percent inoculum, while approximately maximum colonization occurred at 95 and 100 percent inoculum (Table 31; Fig.16).

GENERAL DISCUSSION

The most important crop in Ghana is cocoa and any malady of this crop is of immense concern to us. T. fructigena is a pod pathogen of cocoa causing mealypod disease (see Plates 1 and 2). The extent of damage caused by the fungus is at present considered insignificant. Judging from the ease with which infection was obtained during the present studies by artificial inoculation and the frequent appearance of the disease on a particular farm at Aburi, it is likely that the disease may reach economic levels in cocoa heavily infested with rodents which will both create wounds for the entry of the pathogen and carry the fungus from pod to pod.

The object of the present investigation was to define factors that influence survival of conidia of T. fructigena and examine other aspects of survival of the fungus as a contribution to our knowledge on this fungus of which very little is known. There are other reasons for undertaking this investigation. Its importance in Ghana, at least, is misjudged, and it is supposed to be of uneconomical importance because it causes a very small annual loss of cocoa of only one percent. It is, however, not a pathogen of cocoa alone. It attacks fruits of the important crops, banana, (Brun, 1955; Meredith, 1960; Simmonds, 1960), Coffee (Bunting, 1924; Morstatt, 1937; Roger, *et al.*, 1937) and Avocado pear (Bunting, 1924). Spread from one crop to the other is very readily encouraged under the common agricultural practice of mixed farming in West Africa, as isolate of one crop infects the other host plants. Tabor and Bunting (1923) reported that experimental cross inoculations from coffee to cocoa and vice versa gave typical symptoms of the disease in each case.

T. fructigena has, besides, been found as a parasite of wild forest trees which could act as disease reservoir. Turner and Lovi (1962) found *T. fructigena* on fruits of *Mimusops elengi* and *Mimusops commersonii* in Ghana.

Finally it has become a serious parasite elsewhere. It is considered serious enough as a pathogen of banana to merit control measures. The disease becomes very severe from June to December in Cameroons necessitating control spraying on a 2-day cycle (Ann. Rept. of the Cameroons Development Corporation, 1958). A thorough knowledge of the biology of the fungus would be invaluable in the design of any successful control measure, especially if the attack is to be directed at the most vulnerable phase of the pathogen.

The conidia produced in such large masses on the infected pod would unquestionably be of infinite importance in the epidemiology of mealypod disease of cocoa.

Very great differences have been found in the survival potential of different kinds of fungal spores. The viability of all spores decreases with time, and rate of loss of vigour is dependent on the inherent characteristics of the spore and upon environmental conditions, especially, temperature, humidity and light (Ainsworth and Sussman, 1968; Cochrane, 1958). Pertinent information from the literature has already been mentioned in Chapter E.

High temperature shortens viability while ~~long survival is correlated with~~ low temperature permits long survival. It is probable that moderately high temperatures increase the respiration rate of the quiescent spore resulting in the accumulation of deleterious metabolites and early death. Very high temperatures would obviously kill the spore by denaturing its proteins.

The period of viability may be longer in dark than in light in some fungus spores (Clerk and Madelin, 1965; Dillon-Weston and Halnan, 1931; Markett, 1953; Tinline *et al.*, 1960; Wolf, 1934).

Dillon-Weston and Halnan (1932) showed that the most active portion of the visible spectrum was blue light. It is generally believed that spore pigment protects the spores against radiation. Dillon-Weston and Halnan (1931, 1932) showed that white and orange spores of *Puccinia graminis tritici* are more easily killed by ultraviolet light than are grey and red spores. The survival value of pigmentation is generally recognised. Ingold (1953) on coprophilous fungi remarked: "There is evidence that light has an injurious effect on fungal spores, and it is, therefore, of interest to notice in these three fungi that in the spore-masses discharged on to the grass the protoplasm is shaded by the pigment of the spore-wall in *Dasyobolus* and *Sordaria* and by the black part of the sporangial wall in *Pilobolus* which effectively covers up the discharged mass of almost colourless spores cemented to a blade of grass". He continued further on, "It is, however, interesting to notice that coprophilous agarics have black (*Coprinus*. *Anelloria*, *Panaeolus*) or dark-brown (*Stropharia semiglobata*) spores, so that again the protoplasm is protected from light during the sojourn of the spores on the grass before it is eaten".

Humidity also greatly influences the length of time during which fungal spores remain viable. Quiescent fungal spores show four different types of responses to humidity (see Chapter E). Naturally humidity has different effects on the conidia of the various survival patterns.

In common with all observed fungal spores in storage, conidia of T. fructigena survived longer at the lower temperature of 20°C than at 28°C (see Table 12). The influence of temperature is reflected especially by the rate at which the levels of viability on the 20th day of storage were achieved. The higher temperature destroyed the spores quicker as demonstrated by a viability of 31.7 percent in dark at 28°C in contrast to 81.9 percent viability at 20°C after 10 days. In light, the difference was more accentuated. It is likely that the higher light intensity of 467.5 lux might have contributed in addition to the temperature effect.

It is unlikely that death under these circumstances would be due within periods as short as 10 days to either accumulation of toxic products of increased respiration or to loss of essential reserves. Denaturing of the spores' proteins was more likely to supersede the effect of higher respiration rate, for the conidia seemed to be sensitive to even moderately high temperatures. Supporting evidence would be found in the germinating experiments of Chapters B and D. In contrast to many mesophilic tropical fungi, the conidia barely germinated at 35°C. Only 0.3 percent of the conidia was able to germinate on PDA at this temperature. Also the conidia which do not germinate at 40°C were not capable of germination at the optimum temperature after they had been placed on PDA at 40°C for more than 12 hours (see Table 5). The ascospores of Mycosphaerella musicola causing leaf spot of banana occurring in the same environment with T. fructigena endured longer a similar treatment (Frossard, 1962). The ascospores germinated after 24 hours incubation at 40°C.

The good germination at 20°C (94.0 percent after 12 hours), fairly close to that at the optimum temperature, 25°C (96.5 percent after 12 hours) (see Table 13) and the high germination of 52.7 percent at 15°C is another evidence that T. fructigena conidia require lower temperature than most tropical fungi for maximum activity. This behaviour of the conidia might be of some significance in nature. The conidia on the fruits, even in the shade, could occasionally be subjected to moderately high temperatures over 30°C, that would lower their germination rate considerably. The data of shade Temperature of a cocoa farm at Tafo, Ghana, tabulated by Urquhart illustrate this (see Table 32, below).

TABLE 32

Shade Temperature of Cocoa Farm at Tafo, Ghana (15-year period, 1938-1952)

Data from Urquhart (1955).

Month	Mean Max. (°F)	Mean Minimum (°F)
January	91.4	59.1
February	93.7	61.2
March	93.5	65.8
April	93.7	66.1
May	92.1	66.6
June	89.3	66.1
July	86.8	66.2
August	86.4	65.0
September	88.0	66.6
October	89.8	66.3
November	90.5	65.0
December	90.6	62.7

Several months, it would be observed (June - October) have sufficiently low temperatures favourable to T. fructigena.

In the open, the temperature could rise considerably above 30°C and this would be important for T. fructigena formed on coffee berries and banana fruits which usually grow in the open. Lawson et al. (1970) recorded maximum air temperatures in the open at Kade, Ghana, a moist semi-deciduous forest, of 38°C, 37.6°C and 34.8°C, respectively, at 10cm, 35cm and 150cm above ground level.

If the fungus grows in soil as well, bush fires set by hunting gangs are likely to raise the temperature of the ground sufficiently high to destroy the conidia or prevent germination. Lock and Milburn (1971) have shown that the recorded maximum soil temperature after burning was 59.0°, 37.7° and 32.4°C at the surfaces of the soil and 1.0cm and 5.00cm deep, respectively.

Light did not have any significant effect. It is even likely that differences observed would be due to normal heterogeneity of fungal spore population. Percentage viability was slightly higher in light (275.0 lux) than in dark after 5, 10 and 20 days' storage at 20°C. Higher percentage survival was also recorded on some occasions in light (467.5 lux) at 28°C (see Table 12). Conidia of *T. fructigena* were obviously not affected by light intensity of 467.5 lux. It remains to be found whether higher light intensities would have some deleterious effect.

Cocoa pods are borne on the trunks and branches of the trees and are always heavily shaded by the closed canopy of the trees. Very little light, therefore, ever reaches them, an intensity of less than 467.5 lux.

Some fungi are sensitive to dehydration. Merek and Fergus (1954) found that at 12° - 24°C, the endoconidia of *Endoconidiophora fagacearum* remained viable longer at 95% than at 75% R.H., and Goos and Tschirsch (1962) reported that spores of *Gloeosporium musarum* survived longest at higher humidities (60 - 80% R.H.) than at lower humidities (0 - 20% R.H.). Moreover, the viability of several powdery mildews is best preserved in wet conditions as in the case of conidia of *Erysiphe graminis* (Metzger, 1942), *Erysiphe polygoni* (Yarwood *et al*., 1954), *Oidium heveae* (Corner, 1935) and *Phyllaotinia corylea* (Ankora, 1968). *T. fructigena* conidia belong to this category.

Humidity was found to be a factor powerfully influencing survival. Conidia of T. fructigena survived longest at 100% R.H. and lost viability very rapidly at any humidity below 95% R.H. It was possible to examine survival over the entire humidity range because no germination occurred even at optimum temperature (25°C). No conidium survived an hour's storage at 0 - 80% R.H. In fact, only 1.6 percent of the conidia stored at 80% R.H. was viable after only 20 minutes (see Table 7). Reasonable longevity was found at storage humidities above 90% R.H. Approximately 33 percent conidia survived 24 hours' storage at 95% R.H. and storage for 5 - 10 days destroyed all the conidia (see Table 10). Survival was considerably better at 100% R.H., and survival after 5 days was 93.5 percent and, 66.7 percent after 15 days. Viability was lost rapidly thereafter to give 29.7 percent survival after 20 days and less than one percent survived 40 days' storage at 100% R.H. (see Table 11).

Quick death at lower humidities was accompanied by other events. The conidia apparently possess thin walls that collapsed as water was lost in air (see Plate 5). The rate of shrinkage depended upon the surrounding humidity. Thus all the conidia collapsed in 100 minutes when exposed to an atmosphere of 80% R.H. (see Table 7). The time taken to shrink then lengthened with increasing relative humidity (see Figs. 3, 4 and 5). Maximum shrinkage was therefore observed after 9 hours, 24 hours, 15 days and 30 days during storage at 85, 90, 95 and 100% R.H., respectively (see Tables 8 - 11). Evidently, humidities at 95% R.H. and below would encourage loss of water from the spore. As water came out of the vacuoles, the protoplast withdrew from the wall causing a collapse of the wall. It is difficult, however, to imagine how water could be lost by the spore into a humid air of 100% R.H. and an alternative mechanism might have brought changes in vacuole volume and that of protoplast. The cause of shrinkage of the conidia at 100% R.H. needs further investigation.

There are many reports (e.g. Ankora, 1968; Brodie, 1945; Manners and Hossain, 1963; Nour, 1958; Yarwood, 1936) that conidia of powdery mildews collapse when exposed to humidities less than 100% R.H. It has been suggested that shrinkage may be due both to loss of water by the spore to the air, especially in non-germinated conidia, and its use by the conidium for germination activities. Yarwood (1952) did indeed observe that germinating conidia of *Erysiphe polygoni* showed greater decrease in volume than shrunken non-germinated conidia. The conidia of powdery mildews do not shrink at 100% R.H. and conidia of *T. fructigena* might therefore have mechanisms not present in the powdery mildew spore.

The conidia retained a more stable constitution and survived longer in water than at 100% R.H. as shown by the contrasting 80.9 percent survival in water after 20 days (see Table 13) and 29.7 percent survival at 100% R.H. (see Table 11). Humid air and liquid water apparently exert completely different influences on the spore. The extremely low germination of the conidia in water, less than 2.0 percent at the optimum temperature (see Table 2) permitted the extension of the survival experiments to examination of the fate of the conidia in water, which has not been possible for spores that survive best at higher humidities as they germinate well in water.

The manner in which water is lost from the conidia affected the vigour of the spore. Conidia plasmolysed for 1 hour in 0.7M Potassium nitrate solution (24.6 atmospheres), 0.7M sodium chloride solution (29.9 atmospheres) and 1M sucrose solution (25.0 atmospheres) were barely affected showing 89.1, 82.6 and 93.9 percent germination on PDA, respectively (see Tables 14 - 16). When a 2.0M sucrose solution was used, germination was still very high, 85.9 percent, on PDA (see Table 17). Conidia from which water had been withdrswn (see Plate 9) would, however, eventually lose vigour. Table 18 shows that an effect was noticeable for immersion, in sucrose solution, exceeding 24 hours. The data may be intepreted as showing that death may be due to two causes operating together in air. These are loss of water and a disruption in the functioning of the membranes. The latter does not seem to occur when submerged in fluids and onset of death is delayed, depending on injury caused by loss of water alone.

The plasmolysis experiments throw light on the mode of shrinkage of the conidia. Diminishing size of the protoplast as it lost water at humidities below 100% R.H., or through as yet unknown changes in the protoplasm at 100% R.H., caused the fragile spore wall to lose its support and to collapse. When the conidium was made to lose water by plasmolysis and indeed, decreased the volume of the protoplast (see Plates 8 and 9) the wall was held back by the plasmolysing fluid that occupied the space between the plasma membrane and the spore wall. The changes in the protoplast were, however, irreversible. Shrunken conidia floated in water showed distinctly rounded up protoplast and the resumption of the spherical form was due to water that had passed through the spore wall pushing back the infolded wall (see Plates 6 and 7). Shrinkage at humidities below 100% R.H. is, therefore, a sequel to actual events of death. It, however, affords a most convenient means of assessing viability of the conidia.

An outstanding feature of most fungal species is the enormous spore production to compensate for the unavoidable waste of reproductive units of all organisms which seems to reach its extreme in the fungi. Vast quantities of T. fructigena are likely to die immediately on production, since the atmospheric humidity of the cocoa farm, even under the canopy never reaches 90% R.H., as again other pertinent data of Urquhart (1955) show in Table 33. It is, however, possible that some of the accumulated conidia on the infected tissues would be protected by overlying conidia and may be found in an atmosphere of considerably higher moisture content. Greater proportion of the conidia might therefore remain viable than would have been expected.

The mode of dispersal of the conidia would be critical to their survival. Prolonged exposure would be hazardous. The efficiency of cocoa farm ants, reported to carry the conidia of T. fructigena by Dade (1927) is doubtful. The movement of ants is erratic and their coming across a pod is a matter of chance. Rodents which, on the other hand, seek the pods would be more effective dispersal agents. They move directly from pod to pod, ensuring not only quick transfer but also inflicting the wound, which ants cannot do, needed for infection by the fungus.

TABLE 33

Atmospheric Relative Humidity of Cocoa Farm at Tafo, Ghana (15-year period, 1938 - 1952) Data from Urquhart (1955).

Month	Mean % R.H. 9.00 a.m.	Mean % R.H. 3.00 p.m.
January	85.9	54.1
February	83.7	51.3
March	81.6	54.7
April	80.5	57.9
May	81.7	62.3
June	84.7	67.9
July	86.0	68.9
August	85.9	61.3
September	85.1	70.6
October	83.1	69.8
November	81.8	65.4
December	84.2	61.4

Substances in water extract of partially decomposed leaves of soils of farms in which T. fructigena had been found depressed germination (see Table 21). That stimulatory and inhibitory substances exist at the same time in soil, with the latter often predominating, is generally recognised. The failure of the conidia to germinate in the soil extracts (see Chapter M) in this investigation could be due to either very high levels of inhibitory substances or to low nutrient levels. It is possible that both were operating together. Unlike observations of Hora and Baker (1970; 1972), the inhibitory substances here were not volatile as was clearly shown by the experiment in Chapter O and actual contact with the spore would be necessary for its operation.

These inhibitory substances were fungistatic but not fungicidal. Conidia of T. fructigena which had remained dormant for 15 days under the influence of the inhibitory substances of the soil had later been stimulated into germination with nutrient medium (see Table 23). Similar observations had been made by other workers. Lingappa and Lockwood (1961), for example stimulated spores of Fusarium oxysporum f. lycopersici. Glomerella cingulata and Penicillium frequentans which had lain dormant under the influence of the inhibitory substances for 5 days, into germination with nutrients.

The quiescent conidium in the soil faces many hazards. Both organic and inorganic ions in the soil, when present at high levels are likely to impose plasmolytic problems which would shorten longevity. Two other important factors have been demonstrated in this work. The pH of the soil in which the spore is deposited would greatly influence its survival during its exogenously induced dormancy. Survival at the optimum pH of pH 8.0 was more than fifteen fold that at pH 4.0 (see Table 23). Besides, the conidia would face inevitable lytic action of other soil microflora. One of the most ecologically significant result of these experiments was the fact that viable and dead conidia were differently attacked by soil microflora. While approximately one percent of the originally viable conidia showed signs of attack on the 4th day, as much as 85.9 percent of originally dead conidia had been attacked and 22.1 percent had been completely broken down by lytic action (see Tables 24 and 25). The results suggest that the spores produced metabolites that might have affected the microflora around them affording protection. The possibility of substances passing out of spores has also been postulated elsewhere. Lingappa and Lockwood (1962) reported the observation of areas of stimulation of bacteria in the vicinity of fungal spores added to soil. These observations led them to the hypothesis that individual spores might release nutrients into the soil, stimulating the growth of antagonistic organisms in their vicinity. The hypothesis proposed here might be valid for a short period as metabolite production could not be expected to be sustained to a rate sufficient to maintain a defensive barrier for more than a few days. The diminishing longevity with increasing period of incubation (see Table 24) supports this view.

Death of the spore of a fungus would not necessarily lead to immediate attack by soil microorganisms as the result here might suggest. The nature of the wall had been found to be an important factor in spore lysis. Chu and Alexander (1972) found that spores of various fungi killed by ultraviolet light were not equally lysed in soil. Spores of Aspergillus niger and Aspergillus phoenicis were resistant to lysis in soil and only indications of discoloration of the spores were detected after 2 weeks. By contrast, Colletotrichum lagenarium spores were ruptured to a significant extent by the fifth day. Thielaviopsis basicola spores lost their long side walls as well as cytoplasm within 5 days and Penicillium atrovenetum spores exhibited wall breakage by the seventh day.

A relation between the melanin content of fungal walls and their resistance to enzymatic degradation was noted by Potgieter and Alexander (1966), and quantitative studies in melanin-containing and melanin-free strains of Aspergillus nidulans have confirmed this relation (Kuo and Alexander, 1967). It was found in these studies that synthetic melanin inhibited a gluconase, chitinase and proteinase in vitro and that such a melanin may be very resistant to microbial degradation in soil. The melanin-resistance mechanism is clearly independent of the health of the spore. There are most likely other modes of resistance to lysis.

Fungistasis of cocoa farm soil imposed exogenous dormancy on conidia of T. fructigena. Because this fungistasis is capable of modification or elimination by other factors in the environment, e.g. nutrients, it is possible to predict the behaviour of such conidia formed by mycelium in the soil.

The survival of such mycelium in soil has been the subject of study in this work. The concept of competitive saprophytic ability has been much used recently and many relevant investigations have been carried out. Generally, fungi with very low competitive saprophytic ability do not survive long as saprophytes. Garrett (1950) listed four general attributes that are likely to contribute to a high degree of competitive saprophytic ability. They are:

(1) rapid germination of spores and a high rate of hyphal growth, both favouring rapid colonization;

(2) good enzyme production, which favours rapid and extensive substrate utilization;

(3) production of substances toxic to other organisms, which may reduce competition for the available substrates; and

(4) tolerance of anti-biotic substances produced by other organisms.

There is no evidence from the present studies to show that _T. fructigena_ possesses the last two attributes. The greater colonization of the cocoa pod husk baits when the fungus had first access to the bait (see Table 27) indicates that the necessary enzymes were present. The conidia, in addition, germinated quickly when provided with nutrients (see Table 3) and they produced extensive hyphae (see Plate 3).

An attempt to increase the nutrient level of the cocoa pod husk baits did not bring uniform rate of colonization. _T. fructigena_ was clearly encouraged well by sodium nitrate only (see Table 28).

Sodium nitrate benefited T. fructigena because it might be one of the most suitable nitrogen-compounds for its growth while most probably the other nitrogen compounds favoured other soil micro-organisms. It remains to be established in further investigations on T. fructigena whether good growth is produced in sodium nitrate-medium.

Concentration effects cannot, however, be ruled out. Perhaps at other concentrations both T. fructigena and other soil microflora would behave differently.

There are no reasons why T. fructigena cannot colonize other decomposing plant materials, especially the rotting cocoa leaves which litter the farms and form a thick carpet over the ground. Under such circumstances, the survival rate of T. fructigena, although low, in the unamended bait, would be sufficient to ensure continual growth from one rotten leaf to a newly fallen one. Large inoculum without which colonization would be unsuccessful could be readily provided by rotted diseased pods with their extensive crop of conidia on the surface and mycelium in the tissues.

Studies on survival of a member of the Peronosporales cannot be complete without a consideration of the oospores. An insight of the immense constitutive dormancy of oospores of Peronosporales was provided by Blackwell's (1943a and b) intensive study of the oospores of *Phytophthora cactorum*. The cospore has an after-ripening period of 6-8 months at 15° - 20°C. Tabor and Bunting (1923) had shown that *T. fructigena* oospores are not readily come by. None of the innumerable plates that were cultured during the course of this work produced oospores. Survival of the oospores should be studied when a method to produce the oospores in large quantities has been devised.

VI. SUMMARY

1. Germination of conidia of T. fructigena in distilled water was extremely poor.

2. Germination rates in distilled water at 15° - 30°C were identical, ranging from 1.0 to 1.4 percent after 48 hours.

3. There was no germination in water at 40°C and only 0.5 percent conidia germinated at 35°C in 48 hours.

4. On PDA, there was no germination at 40°C in T. fructigena conidia.

5. PDA highly stimulated germination at 15° - 35°C but not at 35° and 40°C. It supported 33.6, 94.0, 96.5 and 30.2 percent germination at 15°, 20°, 25° and 30°C, respectively, after 12 hours.

6. The maximum possible germination was attained in a shorter time at 25°C, the optimum temperature than at 20°C where germination was also very good.

7. The latent period of germination at 25°C was 96 minutes while that at 20°C was 150 minutes.

8. Poor germination of conidia at 35°C, 0.5 percent germination on PDA after 36 hours, indicated that conidia were sensitive to higher temperatures.

9. There was no germination after exposure of *T. fructigena* conidia on PDA to 45°C, even for as short as 3 hours and conidia would not survive incubation at 40°C for more than 12 hours.

10. *T. fructigena* conidia did not germinate at 80% - 100% R.H.

11. Conidia of *T. fructigena* were extremely sensitive to drying.

12. Conidia of *T. fructigena* died within less than 20 minutes when exposed to atmospheres of 0% - 70% R.H.

13. The conidia lived for only 20 and 40 minutes, respectively at 75% and 80% R.H.

14. At 85% R.H. the conidia perished after 6 hours, and only one percent was viable after an hour's storage.

15. All the conidia lost viability between 5 and 10 days at 95% R.H. and between 2 and 3 hours at 90% R.H.

16. Best survival occurred at 100% R.H. There was 90.0 percent viability after 10 days and 29.7 percent after 20 days, while a few (0.6 percent) were still viable after 40 days.

17. Conidia exposed to humidities of 0% to 100% R.H. collapsed. Time for onset of shrinkage was related to storage humidity. Shrinkage was instant in conidia stored at humidities below 75% R.H. and all the conidia at 100% R.H. collapsed after 40 days.

18. A scatter diagram indicated an apparent degree of association between conidial collapse and viability.

19. A statistical test for correlation revealed a significant negative correlation between viability and shrinkage, even at 0.1 level of significance.

20. Probability P. was < 0.0001 for correlation between viability and shrinkage.

21. The regression line $y = 95.71 - 0.9885x$ showed that an increase in percentage collapse was associated with a decrease in percentage viability.

22. When a shrunken conidium was immersed in water, the dead conidium assumed again its spherical shape. Such a conidium could, however, be readily distinguished from a living one by its dense rounded cytoplasmic mass well withdrawn from the spore wall.

23. Light of intensities 275.0 and 467.5 lux had no adverse effect on stored conidia.

24. Viability was greatly reduced between 5 and 10 days at 28°C to less than 32 percent, whilst more than 80 percent of conidia stored at 20°C was still alive.

25. Conidia of *T. fructigena* were well conserved in water.

26. Longevity in water was better than at 100% R.H. After 20 days, percentage survival was 80.9 and 29.7 percent in water and at 100% R.H., respectively.

27. The rate of germination was faster in conidia stored in water than those kept at 100% R.H. for the same time.

28. Plasmolysed conidia showed distinctly rounded protoplast, but did not shrink.

29. A large percentage, above 77 percent, of the conidia remained alive after plasmolysis in 0.7M potassium nitrate, sodium chloride and sucrose solutions for 60 minutes.

30. Viability of T. fructigena conidia plasmolysed for 60 minutes by sucrose solutions of higher osmotic pressures of 1.5M and 2.0M was also high; 90.5 and 85.9 percent, respectively.

31. Viability was affected when the conidia were kept in 1.5M and 2.0M sucrose solutions for more than 24 hours.

32. Survival of conidia after 72 hours of storage in 1.5M and 2.0M sucrose solutions was, respectively, 34.1 and 32.7 percent, whilst conidia in distilled water showed 94.7 percent viability.

33. Every conidium observed after storage in sucrose solutions of 1.5M and 2.0M was plasmolysed, though to varying degrees.

34. No germination occurred in non-sterile extracts of soil (pH 9.9) and partially decomposed leaves (pH 8.2) of the cocoa farm.

35. No germination also occurred in autoclaved extracts of soil and partially decomposed leaves.

36. Fungistasis could be demonstrated in extract of partially decomposed leaves by its ability to eclipse partially the nutrients of low concentration, N/8 and N/16, of potato dextrose broth.

37. Whilst PDB alone at N/16 supported 98.6 percent germination, only 17.0 percent of the conidia germinated when the extract was added. The germ tubes were, in addition, very short.

38. The fungistatic principle was seemingly non-volatile.

39. Approximately 70 percent of the conidia stored in soil extract of pH 8.0 survived 10 days of storage and 51.0 percent was alive after 15 days. That was the highest percentage survival in the pH levels of 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and pH 10.0.

40. The fungistatic principle was not fungicidal and the conidia survived for several days in the soil extracts.

41. Longevity in the extracts of partially decomposed leaves was closely similar to that of soil, and the conidia were preserved best at pH 8.0 in both extracts.

42. In both extracts, pH 4.0 was most unsuitable for storage of _T. fructigena_ conidia.

43. There was considerable difference between the extracts of visible attack by microorganisms of originally viable and dead conidia buried in native soil of 20% moisture content.

44. On the 4th day, only 0.9 percent of the 1690 initially viable conidia observed carried a microflora visible under high power of the microscope.

45. Survival after 4 days in the soil was 54.5 percent.

46. Initially dead conidia showed heavy incidence of saprophytes:

    (a) Out of 1814 initially dead conidia observed after 4 days of burial in soil, 85.9 percent had been visibly attacked by saprophytes, and;

    (b) 22.1 percent was completely lysed and could only be identified by their faint outline of their disintegrated walls.

47. Lysis was seemingly prevented, at least for some time, by viable conidia. No lysis was detected after 4 days burial in soil.

48. T. fructigena demonstrated limited competitive saprophytic ability. Saprophytic colonization of pieces of cocoa pod husk fell rapidly from 70 percent survival in 100% inoculum to 2 percent survival in 50:50 inoculum/unsterilized soil mixture.

49. A similar trend occurred when pericarp of coffee was used as bait. There was 58 percent survival in 100% inoculum and 6 percent survival in 50:50 inoculum/soil mixture.

50. *T. fructigena* survived better in cocoa husk at higher percentage inoculum than in coffee pericarp, but this was reversed with increased proportion of unsterilized soil.

51. The position of *T. fructigena* in relation to its saprophytic competitors was clearly indicated to be an important factor. In the 95:5 inoculum/soil mixture 40 percent colonization was recorded for cocoa husk pieces initially coated with inoculum while only 24 percent colonization was recorded for husk pieces initially coated with unsterilized soil.

52. Cocoa pod husk pieces buried without any treatment showed better colonization than the soil coated husk pieces and inferior colonization to inoculum coated husk pieces.

53. Of the three nitrogen compounds viz; ammonium tartrate, peptone and sodium nitrate, sodium nitrate encouraged best survival.

54. Approximately 50 percent of the buried cocoa pod husk soaked in 0.5% (50 percent colonization) and 1.5% (56 percent colonization) sodium nitrate solution contained *T. fructigena* after 4 weeks.

55. Saprophytic survival was slightly less in buried pod husk pieces containing ammonium tartrate, ranging between 20 percent colonization at 2.0% ammonium tartrate to 44 percent colonization at 1.0% ammonium tartrate.

56. *T. fructigena* did not persist as long in buried cocoa pod husk pieces with peptone. The highest survival was 20 percent, recorded in husk pieces with 0.5% peptone.

57. *T. fructigena* did not persist extensively in cocoa pod husk pieces with dextrose. Even in 100% inoculum, the best survival after 4 weeks of burial at 1.0% dextrose was 20 percent.

58. A combination of dextrose (1.0% W/V) and sodium nitrate (1.5% W/V) highly increased percentage colonization of buried cocoa pod husk pieces by *T. fructigena*. The fungus persisted in 50 percent of the cocoa pod husk pieces buried in soil containing only 75% inoculum.

59. Approximately maximum colonization occurred at 90%, 95% and 100% inoculum.

60. The implications of the findings have been discussed.

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1. (1921) "Report on the Agricultural Department, Government of the Gold Coast, for the year 1921, p.77". REV. APP. MYCOL., II: p.203, (1923).

2. (1935) "Regulations made under the Importation of Plants Regulation Ordinance (applicable to the Colony and Protectorate of Nigeria, including the Cameroons under British mandate) 1935. No.4 of 1936 - 6pp.". REV. APP. MYCOL., 16: p.79, (1937).

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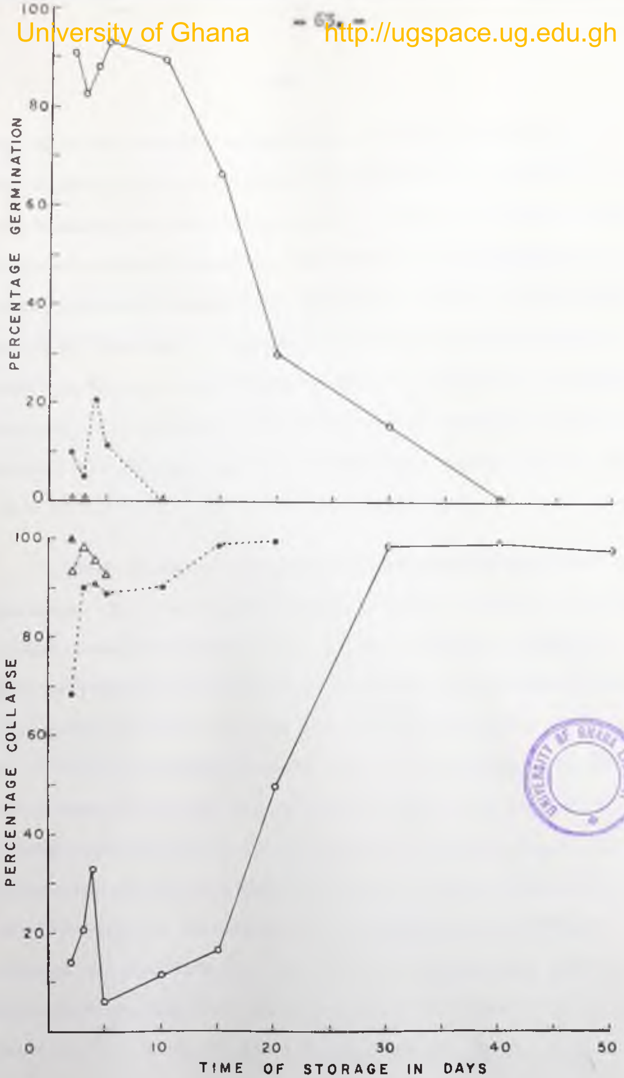

FIG. 5 EFFECT OF RELATIVE HUMIDITY ON LONGEVITY OF CONIDIA OF T. FRUCTIGENA STORED IN LIGHT AT 25° C

■–·–■ 75% R.H □– – –□ 80% R.H ▲–··–▲ 85% R.H

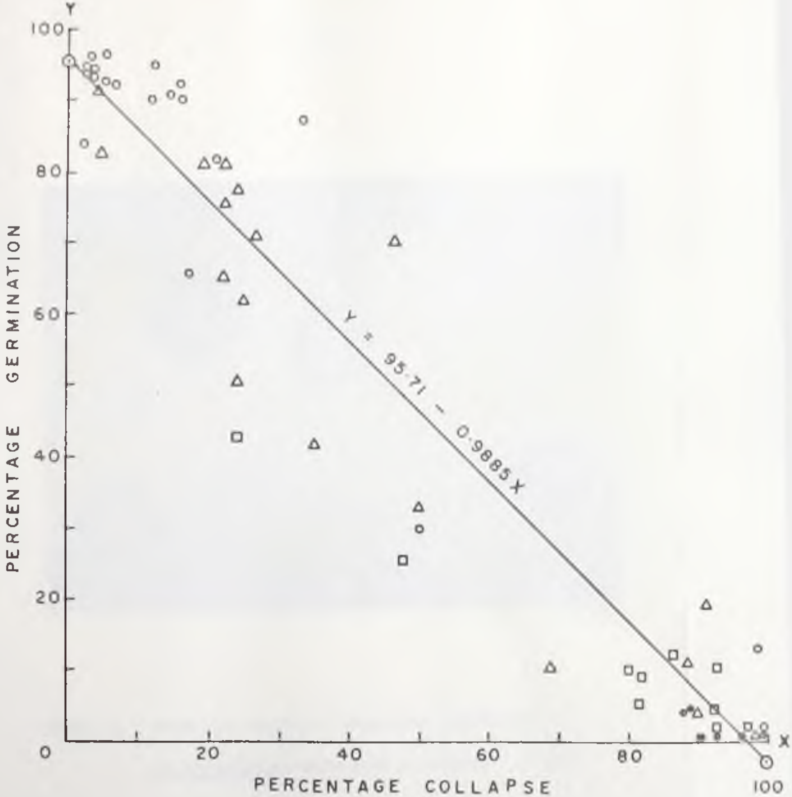

FIG. 6 CORRELATION BETWEEN PERCENTAGE COLLAPSE AND PERCENTAGE SURVIVAL OF CONIDIA OF T. FRUCTIGENA STORED AT VARIOUS RELATIVE HUMIDITIES AT 25°C

(DATA FROM TABLE 8 TO 11)

●, 85 % R.H. ☐, 90% R.H. △, 95 % R.H ○, 100% R.H.

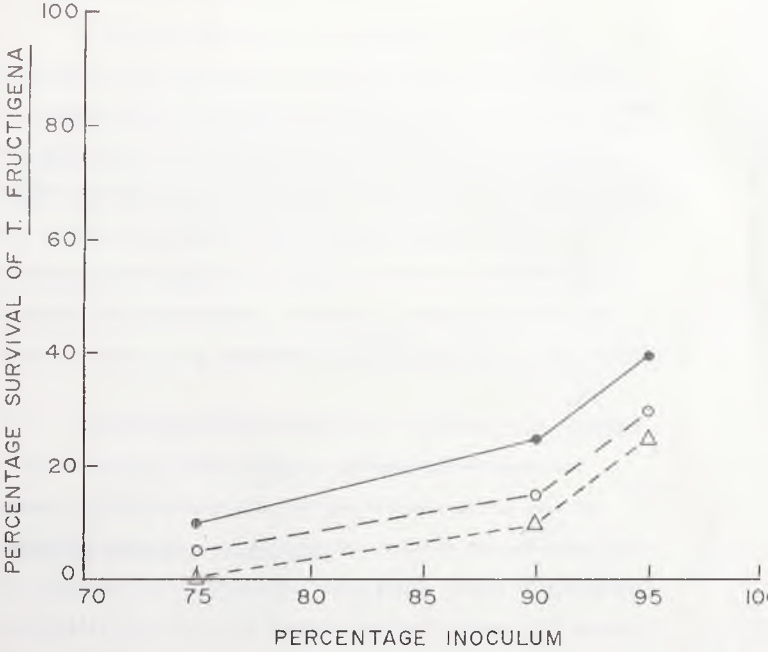

FIG. 12 COLONISATION OF BAIT (CACAO POD HUSK) BY T. FRUCTIGENA PLACED AT VARIOUS POSITIONS FROM THE BAITS

●——● BAITS COATED WITH INOCULUM

Δ— — —Δ BAITS COATED WITH SOIL

o- - - -o NON-COATED BAITS