STUDIES ON THE RELEASE, SURVIVAL AND GERMINATION OF CONIDIA OF PHYLLACHINIA CORYLEA (PERS.) KARST.

A thesis presented by

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ABSTRACT

Conidia of P. corylea germinated best at 25 - 28°C. and germinated well, uniformly and at the same rate at any humidity from zero to 100% R.H., but poorly in liquid water. Germ tubes of conidia germinating at lower humidities were short whilst those at high humidities were fairly long. Approximately 30 per cent of both germinated and ungerminated conidia shrivelled when incubated at 0% R.H. This value decreased with rise in humidity to show shrinkage in only one per cent of conidia held at 100% R.H.

The conidia had a brief latent period of germination of 2 - 3 hours. The germinating conidia usually produced a single germ tube and occasionally two. Branching of germ tubes was rare on glass surface but appressoria were freely formed. On the host (Carica papaya) leaf surface the germ tubes grew randomly over the epidermis and equally freely formed appressoria. The appressoria were either terminal, intercalary or lateral. Germ tubes produced at lower humidities collapsed and shrivelled within a few hours after emergence. All germ tubes produced and held at 0% R.H. shrivelled in 11 hours and those at 80.3% R.H. in 30 hours. Those at 92, 96.9 and 100% R.H. showed 94.5, 25.0 and 20.2% of shrivelled germ tubes respectively in 48 hours.

The Conidia germinated better in light and produced longer germ tubes but formed fewer appressoria than those incubated in darkness.

Conidia stored at 5°C. and at various relative humidities were preserved longest at the higher humidities and died quickest at the lower humidities. Longevity was however brief and did not exceed 20 days at any humidity.

Multicellular, non-branching conidiophores, commonly 300u long, arose perpendicularly from the superficial mycelium. Each was terminated by a generative cell which formed the conidia. Usually only one mature conidium was distinguishable at the tip of the conidiophore. Immediately after maturation of the conidium the generative cell bent approximately at the middle while the two arms of the cell stood at right angles. This movement loosened the attachment of the conidium to the conidiophore and the conidium fell at the slightest disturbance. The bent generative cell then abstricted the next conidium. In still air conidial chains were readily formed. A few conidia germinated whilst still attached to the conidiophore. The conidia matured predominantly in the dark and heaviest crop of spores were obtained in the morning.

The rate of bending of the generative cell was the same at 20 and 28°C., in light and in dark and at 76 and 100% R.H. The curvature in the generative cell remained unaltered in sucrose solutions and in water. The generative cell was not phototropic whilst the entire conidiophore

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I. INTRODUCTION

The powdery mildews (Erysiphaceae) are obligate parasites which generally occur as ectoparasites and grow exclusively on the surface of the host epidermis. The species, Leveillula taurica and Phyllactinia corylea are the only exceptions. Leveillula taurica is of particular interest because its mycelium is wholly endophytic and in like manner a portion of the mycelium of Phyllactinia corylea occurs between the mesophyll of its numerous hosts.

Both L. taurica and P. corylea are very familiar and occur extensively in tropical and sub-tropical regions. In Ghana, and in West Africa in general, L. taurica occurs with amazing frequency on leaves of pepper (Capsicum annuum L.) and no pepper plant ever seems to escape the disease. P. corylea, on the other hand, has just been recently found for the first time in Ghana by Clerk (1966) on pawpaw (Carica papaya L.) leaves. The disease appears as discrete patches of greyish-white, cobwebby growth on the abaxial surface of the leaf (Plates 1 and 2). Under very favourable conditions, the number of disease lesions is so great that the diseased spots tend to cover the entire leaf surface. Von Tubeuf (1897) and Yarwood (1956) found that there is occasional retention of chlorophyll at mildewed spots when the entire leaf became chlorotic. In the late stages of the disease the pawpaw leaves similarly became yellow except the sites supporting P. corylea which retained their chlorophyll and appeared as distinct

PLATE 1: Photograph of abaxial surface of leaves of pawpaw (C. papaya) showing white patches of mycelium of P. corylea.

A. Healthy leaf.

B. Mildewed leaf. Note the presence of chlorophyll at early stages of disease.

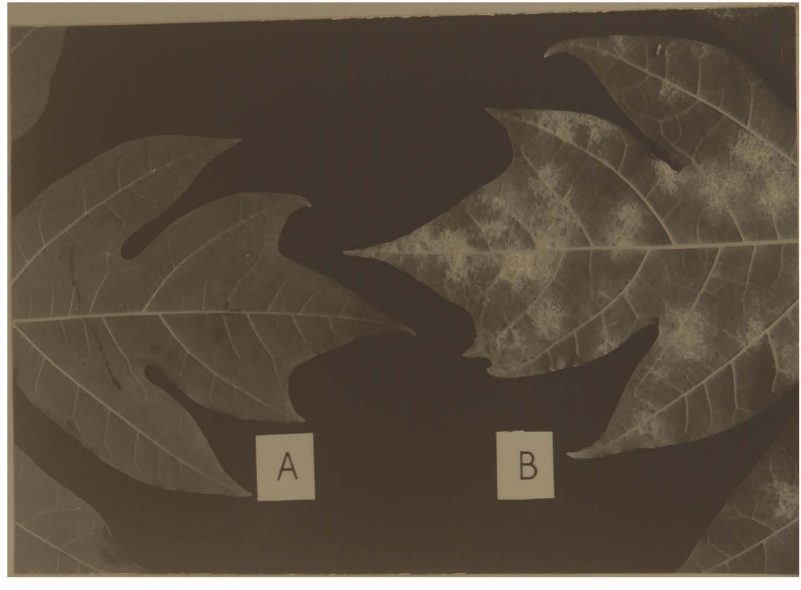


PLATE 2: Enlarged photographs of apices of healthy and mildewed pawpaw (C. papaya) leaves.

A. Healthy leaf.

B. Mildewed leaf.

"green islands" on a yellow background (Plates 3 and 4).

The host range of P. corylea is very wide and the fungus is an almost exclusive parasite of dicotyledonous plants. For example, Cardoso (1940), Hector (1927), Ramarishnan and Sundaran (1954), Rayss (1953), Ressencourt (1927), Rhind (1924), Wallace (1930) and Zaprometoff and Mikhailoff (1937) found P. corylea on mulberry. The fungus occurred on Palius australis (Foëx, 1926), jasmine (Sydow and Mitter, 1933), medlar (Voglino, 1932), Lagerstroemia (West, 1933), Corylus rostrata (Miller, 1934), pear (Blumer, 1936), lilac (Viennot-Bourgin, 1944) and on Dalbergia sissoo and teak (Bagchee, 1952). Although P. corylea has, at present, been found in Ghana on pawpaw alone, it is not unlikely that, in view of its reported wide host range, this fungus may be parasitic on more host species in this country than hitherto reported. P. corylea is certainly potentially of economic importance. Its significance and importance in Ghana becomes more profound with the current introduction of mulberry plants into this country in connection with the silk industry.

In the tropics and sub-tropics, the powdery mildews rarely produce cleistothecia and many species perpetuate themselves solely by means of conidia (Alexopoulos, 1952) and the conidia therefore remain the main agents of infection. Conditions which therefore

PLATE 3: Photograph of abaxial surface of chlorotic, mildewed leaf of pawpaw (*C. papaya*) at very advanced stage of disease.

Note green "islands" of chlorophyll at sites supporting *P. corylea* on a background of yellow leaf.

PLATE 4: Photograph of adaxial surface of chlorotic, mildewed leaf of pawpaw (C. papaya) at very advanced stage of disease. Note green "islands" of chlorophyll at sites supporting P. corylea on a background of yellow leaf.

affect germination of the conidia of P. corylea must be thoroughly studied and well defined in view of the epidemiological implications. It is surprising that a search through the literature revealed no pertinent information whatsoever.

This work was also carried out to provide information on another important aspect of the biology of P. corylea. Ressencourt (1927) and Viennot-Bourgin (1944) reported that the conidiophores of P. corylea were few in number, elongated, rigid and with only a single distinguishable conidium at the apex of each. Although other workers have made similar observations and had indicated that the mature conidium fell off before the succeeding one appeared, the reason why only a single conidium occurs on a conidiophore at any time has never been investigated.

The present studies were undertaken to provide some information on the development, release and germination of the conidia of P. corylea.

II. LITERATURE REVIEW

Very surprisingly there are very few references to the biology of P. corylea in the literature.

Most of the information available is in relation to host range and geographical distribution.

There is no information on the effect of different factors on the germination of the conidia of this fungus or on the pattern of conidial germination. The only information to be found in connection with temperature and the fungus is provided by Ressencourt (1927) who was infact describing a species of Phyllactinia which he thought to be at least closely related to P. corylea. He reported that the active development of this species was favoured by temperatures of 20 to 25°C. and retarded at 30°C. or over, as well as by intense damp or heavy rain.

Foex (1926) gave a very extensive account of the development of the conidiophore and formation of the conidia of P. corylea. He wrote, "The first indication of the conidiophore was the formation of a slender cylindrical cell which rapidly elongated perpendicularly to the hyphae on which it arose. Following nuclear division this cell separated into two, of which the basal cell was much longer and thinner than the apical; the protoplasm in the latter was much more denser than in the former. New elements were formed by continued

division of the apical cell. Finally the conidiophore was composed of a thread-like basal cell, in which the protoplasm was concentrated at the top. The terminal cell which was the only true conidium, was of a characteristic ovoid-conical shape." Hammarlund (1925), Viennot-Bourgin (1944) and Roger (1953) reported that the conidiophore of P. corylea bears only a single conidium at a time. Viennot-Bourgin, however, found that occasionally some conidiophores bore two conidia, whilst Hammarlund (1925) observed that P. corylea which frequently only bear a single conidium at the tip of the conidiophore, developed numerous chains in damp, still air. There is however no information on the mechanism of release of the mature conidium.

III. MATERIALS AND GENERAL METHODS

i. MATERIALS:

The culture of *P. corylea* used was a local isolate obtained from a naturally infected pawpaw (*Carica papaya* L.) plant. The conidia of *P. corylea* used in these investigations were obtained from stock culture maintained on glass house-potted pawpaw plants. Fresh seedlings were raised every month and were inoculated at the 6-leaf stage. The healthy seedlings were inoculated by gently adpressing diseased lesions of infected leaves unto the abaxial surface of the leaves. The temperature of the glass house remained constant at $27 \pm 1^{\circ}C$.

It was necessary to obtain conidia of uniform age throughout the work and the following procedure was adopted during these investigations. The leaves of pawpaw plants showing diseased lesions, which normally appear as white cobwebby patches on the abaxial surface of the leaves, were carefully tapped as a rule at 7.00 a.m. with a ruler, and a strong current of air from a bellow was blown over the surfaces of the leaves to remove any mature conidia present. The plants were then left to allow fresh crop of conidia to develop. Conidia were always, except where otherwise stated, removed from the leaves 48 hours after treatment, that is at 7.00 a.m. on the day of experimentation.

ii. GENERAL METHODS:

(a) HUMIDITY CHAMBERS

i) Plastic Boxes

Two types of plastic boxes were used as humidity chambers. They were either rectangular plastic boxes (21.5 cm long, 10.5 cm wide and 7.5 cm deep) (Fig. 1) or square plastic boxes (10 cm x 10 cm and 7 cm deep) with tightly fitting lids sealed airtight with cellotape. These were mostly used in germination tests requiring relative humidities of zero to 100%. The plastic boxes were found very convenient as very little condensation occurred when the atmospheric humidity was maintained at 100% R.H.

Glass slides holding conidia for germination and survival tests were supported at the bottom of the humidity chambers on V-piece glass rods. Water and solutions used for controlling the relative humidity of the atmosphere within the chambers were enough to cover the bottom of the boxes and rose just to midway of the thickness of the V-piece glass rod (Fig.1).

ii) Van Tieghem Cells

Solid watch glasses (3.7 x 3.7 x 1.6 cms) with a well 3 cms. in diameter and 1.0 cm deep were used to serve as Van Tieghem Cells (Fig. 2) for some of the conidium germination experiments. They were also used as humidity chambers. The well of each watch glass held 2 ml. of the appropriate solution

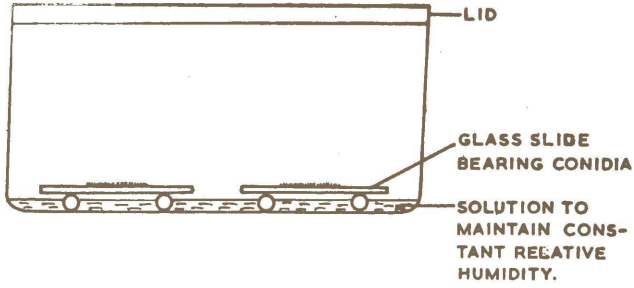


FIG. 1. RECTANGULAR PLASTIC BOX — HUMIDITY CHAMBER EMPLOYED FOR GERMINATION OF CONIDIA OF P. CORYLEA.

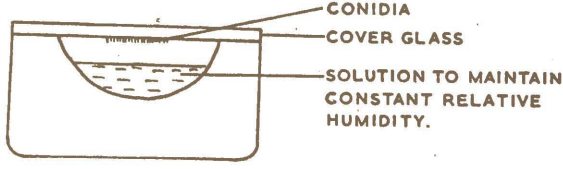


FIG. 2. SOLID WATCH GLASS USED TO SERVE AS VAN TIEGHEM CELL — HUMIDITY CHAMBER EMPLOYED FOR GERMINATION OF CONIDIA OF P. CORYLEA.

to give the desired relative humidity. The top edge of the watch glass was luted with vaseline in order to form an air-tight seal when the lid is placed on it. Conidia of P. corylea were dusted onto the centre of the glass lid, within a circumscribed area of 1.0 cm in diameter. The glass lid was then gently placed, with the conidium bearing face downwards, onto the watch glass. (Fig. 2).

(b) MAINTENANCE OF CONSTANT HUMIDITY

Various relative humidities were provided and maintained by either saturated aqueous salt solutions, which were prepared according to the data provided by Wexler and Hasegawa (1954) (See Table 1), or sulphuric acid solutions based on the data of Solomon (1952) (See Table 2).

Throughout the investigations, norminal 0% R.H. was maintained with anhydrous calcium chloride and water provided 100% R.H.

(c) METHODS OF STERILIZATION

Glass slides, glass covers, pippetes, flasks were all soaked in potassium dichromate for 48 hours, washed with detergents, rinsed under running tap and further thoroughly rinsed with distilled water. The slides and glass covers were stored in 90% ethyl alcohol and flame-sterilized just before use.

McCartney tubes which were used in some germination tests were sterilised together with the germination liquid medium by auto-claving for 20 minutes at 15 pounds per square inch steam pressure.

Table 1

Relative Humidity (Percentage) provided by Saturated Aqueous Salt Solutions.

(Data of Wexler, A. and S. Hasegawa, 1954)

Temperature in °C.	$LiCl\ H_2O$	$MgCl_2\ 6H_2O$	$Mg(NO_3)_2\ 6H_2O$	NaCl	$(NH_4)_2\ SO_4$	KNO_3	K_2SO_4
0	14.7	35.0	60.6	74.9	83.7	97.6	99.1
5	14.0	34.6	59.2	75.1	82.6	96.6	98.4
10	13.3	34.2	57.8	75.2	81.7	95.5	97.9
15	12.8	33.9	56.3	75.3	81.1	94.4	97.5
20	12.4	33.6	54.9	75.5	80.6	93.2	97.2
25	12.0	33.2	53.4	75.8	80.3	92.0	96.9
30	11.8	32.8	52.0	75.6	80.0	90.7	96.6
35	11.7	32.5	50.6	75.5	79.8	89.3	96.4

Table 2

H_2SO_4 Solutions for maintaining constant relative humidities

(Data of Solomon, 1952)

% Relative Humidity	Weight in gm. of H_2SO_4 per 100 gm. ofssolution	Weight of water in gm. per 100 gm. solution.
100	0.0	100.00
90	17.91	82.09
80	26.79	73.21
70	33.09	66.91
60	38.35	61.65
50	43.10	56.90
40	47.71	52.29
30	52.45	47.55
20	57.76	42.24
10	64.45	35.55
0	100.00	0.00

(d) METHODS OF PREPARING SPORE PRINTS FOR GERMINATION TESTS

Evenly scattered conidia for germination tests were obtained on glass slides and Van Tieghem glass covers by placing them about 1 cm below a diseased leaf held in a horizontal position. The leaf was then gently tapped with a pencil to dislodge the conidia.

(e) SPORE GERMINATION TESTS

i) The Slide Method

Glass slides holding conidia were placed on V-shaped glass rods in the humidity chambers with the spore-coated side facing upwards.

ii) The Van Tieghem Cell Method

This has been mentioned at page 15 under humidity chambers.

iii) Germination on Host Leaf

Leaf discs removed with No. 10 cork borer were used for germination tests on host leaf. The discs holding the conidia were placed with the spore-coated side facing upwards on moist filter paper in plastic chambers. The conidia were germinated on leaf discs at 100% R.H. only.

(f) INCUBATION

Germinating spores were incubated in electrically-controlled incubators. Slides on the development of the conidiophores, formation of the conidia and release of the conidia were carried out at

room temperature - 26 ± 2°C.

Spore survival tests were carried out in a refrigerator set at 5°C.

(g) ASSESSMENT OF CONIDIUM GERMINATION AND GERM TUBE LENGTH

At the end of the desired incubation period, observations were made immediately and counts taken. If observations could not be made at once, the slides or leaf discs were put into labelled petri dishes and stored in the refrigerator (2°C.) to stop growth and observations made later.

A conidium was considered to have germinated if a germ tube was discernable.

Assessment of conidium germination for each treatment was based on the percentage of conidia germinated and the mean length of 30 measured germ tubes.

The percentage germination was calculated from 600 observed conidia randomly selected. The "percentage germination" was calculated from the formula:-

$$\frac{100 \times \text{No. of germinated conidia}}{600}$$

The lengths of the germ tubes were measured, with a micrometer eye-piece. Where germ tubes branched or where a conidium produced more than one germ tube, germ tube length was taken as the sum of the lengths of the main germ tube and those of the branches or the sum of the lengths of the individual germ tubes of a conidium.

In the germination tests where observations were made on the formation of appressoria, percentage of germ tubes forming appresoria was calculated from the formula:-

$$\frac{100 \times \text{No. of conidia forming appresoria}}{\text{Total No. of germinated conidia}}$$

In the germination tests where observations were made on the rate of shrivelling of germ tubes, percentage of germinated conidia with shrivelled germ tubes was calculated from the formula:-

$$\frac{100 \times \text{No. of germinated conidia with shrivelled germ tubes}}{\text{Total No. of germinated conidia}}$$

During spore longevity tests conidia able to germinate after desired storage periods were considered viable.

(h) EXPERIMENTAL PRECAUTIONS

1. Corner (1935) and Nour (1958), among others, have noted that the germination of conidia of powdery mildews is very irregular. In some tests the conidia showed a high percentage germination whereas in other tests there was very little germination. To ensure reliable results and to cut down the irregularity to minimum the following precautions were taken:-

a) The age of the conidia used in the studies were standardized as indicated at page 23.

b) All germination tests were carried out three times on different occasions.

2. Yarwood (1957) noted that the degree of conidial germination in Erysiphaceae is more or less dependent on the time of the day of collection of the spores. For standardisation, in these studies conidia for all germination tests were strictly removed from the leaves at 7.00 a.m. except where the type of experiment necessitated commencement of investigation at a particular time. The conidia were incubated within 15 minutes of collection.

3. Care was always taken to prevent the sulphuric acid solutions or the saturated aqueous salt solutions coming into contact with the conidia especially when moving the germination chambers to the incubators.

4. When saturated aqueous salt solutions were used, it was ensured that there was always a solid phase of the salt.

5. Care was taken to obtain uniform distribution of the conidia on the slides, glass covers or the host leaf discs and to have the same spore density (about 30 conidia per microscope field) under low power magnification.

6. Conidia were never removed by pressing the slides or glass plates against the diseased lesions to avoid crushing the delicate conidia.

7. Glassware was kept scrupulously clean with detergents, washed well with tap water, rinsed three times with distilled water and was allowed to drain dry before use.

IV. RESULTS

A. EFFECT OF TEMPERATURE ON GERMINATION OF CONIDIA OF PHYLLACTINIA CORYLEA

One of the most important external factors which influence the germination of fungal spores is temperature. Temperature affects both percentage germination and the length of time required for germination, (Cochrane, 1958; Hawker, 1950; Lilly and Barnett, 1951). The temperature may even determine the type of germination. For example, the conidiosporangium of Phytophthora infestans may germinate by the formation of either a germ tube or zoospores depending on the environmental temperature. At 23°C. the conidiosporangia germinate best by means of germ tubes and 13°C. is optimum for zoospore production (Crozier, 1933; Melhus, 1915). Generally, the rate of germination is greatest at a temperature called the optimum temperature and decreases as the temperature is moved either way from the optimum. This optimum temperature differs from species to species. For example, the optimum temperature for Phyllosticta solitaria is 23°C. (Burgert, 1934) and 43 to 45°C. for Rhizopus chinensis (Weimer and Harter, 1923).

Conidia of many powdery mildews germinate best at a moderately low temperature about 21°C. (Cochrane, 1958). The optimum temperature of the tropical species will however, be slightly higher than this value. The effect of temperature on germination of conidia of

P. corylea was investigated.

Although Graf-Marin (1934), Yarwood (1936), Clayton (1942) and Grainger (1947), found that the germination of conidia of some members of Erysiphaceae was poor at 100% R.H. and that the optimum relative humidity lay at a level slightly below saturation, the results of Nour (1958) on Leveillula taurica and those of Manners and Hossain (1963) working on Erysiphe graminis, on the other hand, indicated that 100% R.H. is the most favourable level for germination of these conidia if care was taken to exclude condensation on the slide. Preliminary investigations during the present studies indicated that the conidia of P. corylea behave similarly.

Germination of the conidia of P. corylea held in atmospheres with a relative humidity of 100 per cent was studied at temperature levels of 15, 20, 25, 30 and 35°C. This range was adequate to cover the possible range of temperature the conidia will be subjected to in the tropics.

The conidia were incubated at the five different temperatures and at 100% R.H. for the various periods as indicated in the tables of results (Tables 3 to 7). Twelve slides with conidia were held at each temperature. Three slides were removed after 12, 18, 24 and 36 hours respectively and the percentage germination was estimated and the length of the germ tubes measured.

The results of these experiments are tabulated in Tables 3 to 7, and expressed graphically in Fig. 3.

The conidia germinated at all levels of temperature used, but germination at 15 and 30°C. was very poor. Less than 11 per cent of the conidia germinated at 15°C. in 36 hours and still fewer (4.6 per cent) germinated at 35°C. within the same period. Conidial germination at 20°C. was also low and there was only 22.0 per cent germination. At these temperatures (15, 20 and 35°C.) percentage germination and germ tube growth showed closely parallel temperature response.

The optimum temperature was found to be 25°C. supporting the highest mean percentage germination of 60.1 in 36 hours. Although 30°C. was evidently sub-optimal (45.4 per cent germination in 36 hours), the germ tubes were slightly longer at this temperature than at 25°C. The better growth of germ tubes at 30°C. suggests that probably the optimum temperature for P. corylea lies between 25 and 30°C.

At all levels of temperature, except 15°C., germination was such that almost all the conidia capable of germinating had germinated within 12 hours, the shortest observation time used. There was no increase in germination after 12 hours. It, however, took conidia at 15°C. 24 hours to attain the maximum percentage germination.

TABLE 3: Germination of Conidia of Phyllactinia corylea incubated at 15°C. and at 100% R.H.

Time in Hours	Experiment Number	% Germination	Mean Germ Tube Length in μ
12	1	3.6	20.1
	2	3.1	19.8
	3	3.9	21.4
	Mean	3.5	20.4
18	1	2.7	22.0
	2	4.8	20.1
	3	2.5	21.1
	Mean	3.0	21.1
24	1	16.2	28.0
	2	9.0	25.3
	3	5.1	21.0
	Mean	10.1	24.8
36	1	11.5	26.8
	2	8.5	26.3
	3	12.5	25.4
	Mean	10.8	26.2

TABLE 4: Germination of Conidia of Phyllactinia corylea incubated at 20°C. and at 100% R.H.

Time in Hours	Experiment Number	% Germination	Mean Germ Tube Length in μ
12	1	19.5	50.4
	2	12.2	37.8
	3	13.6	35.4
	Mean	15.1	41.2
18	1	17.6	44.1
	2	22.4	31.1
	3	12.9	36.4
	Mean	17.6	37.2
24	1	21.7	44.8
	2	19.9	44.8
	3	18.6	60.9
	Mean	20.1	50.2
36	1	21.8	79.2
	2	24.0	49.0
	3	20.6	50.1
	Mean	22.1	59.4

TABLE 5: Germination of Conidia of Phyllactinia corylea incubated at 25°C. and at 100% R.H.

Time in Hours	Experiment Number	% Germination	Mean Germ Tube Length in μ
12	1	59.5	90.4
	2	63.6	80.6
	3	58.7	57.8
	Mean	60.6	76.3
18	1	58.4	90.0
	2	56.4	86.2
	3	56.1	56.8
	Mean	57.0	77.7
24	1	60.0	111.0
	2	56.7	78.8
	3	60.0	89.0
	Mean	58.9	92.9
36	1	59.8	86.9
	2	59.1	91.5
	3	61.5	93.3
	Mean	60.1	90.6

TABLE 6: Germination of Conidia of Phyllactinia coryliea incubated at 30°C. and at 100% R.H.

Time in Hours	Experiment Number	% Germination	Mean Germ Tube Length in μ
12	1	49.8	83.7
	2	42.9	69.0
	3	50.5	61.6
	Mean	47.7	71.4
18	1	40.3	98.4
	2	45.3	63.7
	3	40.9	68.6
	Mean	42.2	76.9
24	1	39.4	107.5
	2	48.5	72.5
	3	44.9	111.3
	Mean	44.3	97.1
36	1	46.4	108.2
	2	42.9	128.8
	3	46.9	119.4
	Mean	45.4	118.8

TABLE 7: Germination of Conidia of Phyllactinia corylea incubated at 35°C. and at 100% R.H.

Time in Hours	Experiment Number	% Germination	Mean Germ Tube Length in μ
12	1	4.1	16.1
	2	3.7	30.0
	3	4.3	26.3
	Mean	4.0	24.1
18	1	3.1	20.3
	2	6.9	20.2
	3	4.6	20.2
	Mean	4.9	20.2
24	1	3.7	24.8
	2	3.2	24.5
	3	5.0	23.5
	Mean	4.0	24.3
36	1	4.4	18.2
	2	3.5	19.3
	3	5.8	19.2
	Mean	4.6	18.9

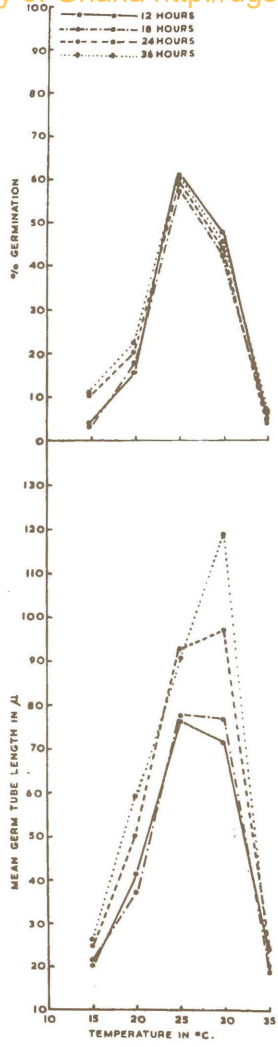


FIG. 3 EFFECT OF TEMPERATURE ON THE GERMINATION [illegible] INCUBATED AT 100% R.H.

B. THE GERMINATION OF CONIDIA OF P. CORYLEA AT VARIOUS RELATIVE HUMIDITIES

The powdery mildews have attracted considerable attention by their ability to germinate at low humidities and extensive pertinent studies are reported in the literature (Cherewick, 1944; Nour, 1958; Clerk and Ayesu-Offei, 1967; Yarwood, 1936; etc.). With the exception of very few species germination obtained at the lower humidities is rather low and the biological advantage of this habit is therefore limited. With species in which lower humidities permit sufficient conidial germination, considerable infection units could be mobilised under a wide range of atmospheric humidity conditions. The germination of conidia of P. corylea at various relative humidities and at 25°C., the optimum temperature obtained in the previous experiment (See Table 5, page 29) was investigated. This temperature was used in all subsequent experiments as temperature of incubation, except where otherwise stated.

The conidia were incubated at various relative humidities maintained with sulphuric acid solutions (see page 35) as indicated in the table of results (Table 8) for 24 hours, after which (a) percentage germination, (b) percentage of germinated conidia which had shrivelled, (c) percentage of ungerminated conidia which had shrivelled (d) percentage of germ tubes bearing appressoria, were estimated and the lengths of germ tubes measured.

It was observed that to study germination of the conidia in water on slides, some of the conidia floated whilst the rest remained submerged. To avoid creating two different physiological conditions, the conidia were incubated in sterile distilled water in McCartney bottles (20 mls capacity) and the bottles were continuously shaken for 24 hours on Griffin Flask Shaker at room temperature of 27°C. Drops of water with the conidia were removed at the end of the incubation period unto slides and percentage germination estimated.

The results of these experiments are tabulated in Table 8 and expressed graphically in Fig. 4 (Page 42) and also illustrated in Plates 5 - 13.

The data in Table 8 showed that conidia of P. corylea germinated at all the humidity levels used. Germination was surprisingly very uniform and very high at any humidity from zero to 100% R.H. The mean percentage germination recorded for 100% R.H. was 41.0 and that for 0% R.H. was 52.3. Germination was superior at any humidity level to that in liquid water, where a mean percentage germination of only 10.0 occurred.

Unlike the very uniform percentage germination obtained, the effect of different humidities was distinctly reflected in germ tube growth. The germ tube length increased with rise in relative humidity. Conidia germinating at 0% R.H. produced the shortest germ tubes (mean

TABLE 8: Effect of Relative Humidity on the germination of Conidia of P. corylea incubated at 25°C. for 24 hours. (Relative Humidity maintained with Sulphuric acid solutions).

% Relative Humidity	Experiment Number	% Germination	% Shrivelled germinated conidia	% Shrivelled ungerminated conidia	% of germ tubes with appressoria	Mean germ tube length in μ
Water	1	21.6	0	0	28.5	64.8
	2	4.5	0	0	28.6	41.7
	3	3.8	0	0	15.6	43.0
	Mean	10.0	0	0	24.2	49.8
100	1	35.5	1.8	2.5	14.4	88.6
	2	46.3	0.8	0.7	35.5	108.2
	3	41.3	0.8	1.3	17.9	100.5
	Mean	41.0	1.1	1.5	22.6	99.1
90	1	46.0	4.2	2.9	66.0	61.6
	2	49.5	3.7	4.0	59.3	53.9
	3	36.9	2.4	1.7	51.6	54.3
	Mean	44.1	3.4	2.9	59.0	56.6

Table 8 continued

Effect of Relative Humidity on the germination of conidia of P. corylea incubated at 25°C. for 24 hours. (Relative Humidity maintained with Sulphuric acid solutions).

% Relative Humidity	Experiment Number	% Germination	% Shrivelled germinated conidia	% Shrivelled ungerminated conidia	% of germ tubes with appressoria	Mean germ tube length in μ
80	1	38.8	22.3	12.1	51.2	48.6
	2	54.2	22.2	9.6	61.7	35.0
	3	45.4	13.3	7.5	65.4	53.6
	Mean	46.1	19.2	9.7	59.4	45.7
70	1	41.8	15.0	10.7	62.6	43.8
	2	53.8	28.9	19.2	53.8	56.0
	3	41.6	8.5	2.4	53.2	50.1
	Mean	45.7	17.5	10.8	56.5	50.0
60	1	43.6	19.5	18.0	66.8	40.2
	2	48.3	20.3	23.1	49.6	43.4
	3	46.1	10.3	4.8	57.5	42.7
	Mean	46.0	16.7	15.3	58.0	42.1

Table 8 continued

Effect of Relative Humidity on the germination of conidia of P. corylea incubated at 25°C. for 24 hours. (Relative Humidity maintained with Sulphuric acid solutions).

% Relative Humidity	Experiment Number	% Germination	% Shrivelled germinated conidia	% Shrivelled ungerminated conidia	% of germ tubes with appressoria	Mean germ tube length in μ
50	1	44.6	13.0	4.6	61.0	32.5
	2	54.4	7.7	3.8	58.7	39.9
	3	49.5	4.4	2.2	52.0	35.7
	Mean	49.5	8.4	3.5	57.2	36.0
40	1	48.2	12.2	9.7	64.5	32.5
	2	50.5	14.5	2.8	47.9	43.4
	3	53.0	23.0	19.3	54.5	28.3
	Mean	50.6	16.6	10.6	55.6	34.7
30	1	51.3	25.3	20.0	63.3	24.1
	2	52.2	26.3	23.5	54.3	29.7
	3	47.7	20.3	20.4	54.0	31.8
	Mean	50.4	24.0	21.3	57.2	28.5

Table 8 continued

Effect of Relative Humidity on the germination of conidia of P. corylea incubated at 25°C. for 24 hours. (Relative Humidity maintained with Sulphuric acid solutions).

% Relative Humidity	Experiment Number	% Germination	% Shrivelled germinated conidia	% Shrivelled ungerminated conidia	% of germ tubes with appressoria	Mean germ tube length in μ
20	1	48.1	38.1	22.8	72.3	19.9
	2	48.0	16.6	12.9	23.9	40.9
	3	48.4	31.7	16.1	57.9	28.4
	Mean	48.2	28.8	17.3	51.4	29.7
10	1	43.1	39.3	25.8	65.5	31.5
	2	45.0	9.5	2.1	37.0	52.8
	3	45.5	14.7	6.5	52.4	34.3
	Mean	44.5	21.2	11.5	51.6	39.5
0	1	56.1	47.7	45.6	39.5	24.9
	2	60.6	17.0	20.6	59.3	26.3
	3	40.2	22.5	27.1	44.0	30.4
	Mean	52.3	29.1	31.1	47.6	27.2

germ tube length of 27.2 μ) whilst the longest germ tubes (mean germ tube length of 99.1 μ) were found in conidia incubated at 100% R.H.

Manners and Hossain (1963) working on E. graminis stated that at 98% R.H. and 100% R.H. good growth occurred at suitable temperatures and appressoria were produced abundantly. The present work did not portray any relationship between humidity level and degree of formation of the appressoria. It was however found that the number of appressoria formed was consistently lower in water and at 100% R.H. than the lower humidities of 0 - 90% R.H. The poor development of appressoria in water and at 100% R.H. (where condensed water often appeared) may be due to the liquid water which seemed to prevent an establishment of a firm attachment by the germ tube unto the glass surface. The development of appressoria in germ tubes of conidia incubated and shaken in water indicate that contact with a solid substratum is not a sine qua non for appressorial formation.

Low humidity always caused considerable shrivelling of both germinated and ungerminated conidia of powdery mildews and this desiccation action is more pronounced at higher temperatures (Yarwood, 1936). The results of tests of the relation of temperature to desiccation of clover mildew and mustard mildew showed that the percentage of shrivelled conidia in the mounts over sulphuric acid was progressively greater as the temperature increased. Yarwood's

observation that volume changes of the non-germinated conidia was about the same as that of the germinated conidia makes it difficult to infer whether he associated the decrease in volume with the germination process or as an effect of low humidity.

In 1952, Yarwood working again on E. graminis came to some positive conclusions. He stated that germinating conidia lost more water than non-germinating conidia and that while both germinating and non-germinating conidia shrank in a dry atmosphere, the germinating spores shrank at a faster rate. Thus, the germinating spores decreased in volume at about three times the rate of non-germinating spores.

Brodie and Neufeld (1942) working with E. polygoni failed to observe any changes in volume of the conidia germinating under laboratory conditions at a relative humidity of from 65- - 80%. They positively concluded that the process of germination of conidia of E. polygoni under the conditions of their studies was not accompanied by any changes in volume.

Ayesu-Offei (1966) observed that both germinated and ungerminated conidia of L. taurica shrivelled when incubated at various relative humidities. He however noticed that, unlike observations of Yarwood (1952) germinated and ungerminated conidia of Leveillula taurica shrivelled to the same extent and did so at the same rate.

In the present studies germinated conidia and ungerminated ones shrivelled to the same degree. The percentage of conidia which shrivelled increased with decrease in humidity until a level of 30% R.H. A further fall in humidity failed to affect any further rise in quantity of shrunken conidia.

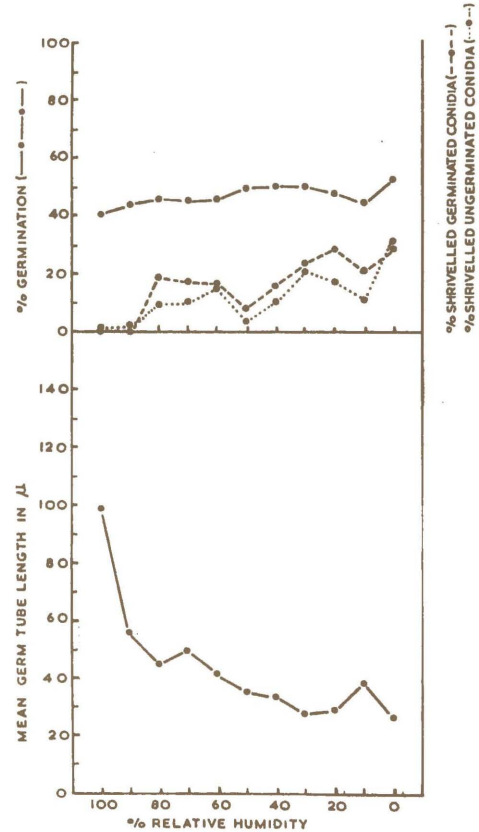


FIG. 4. EFFECT OF RELATIVE HUMIDITY ON GERMINATION OF CONIDIA OF P. CORYLEA INCUBATED AT 25°C. FOR 24 HOURS.

PLATE 5: Photomicrograph of conidia of P. corylea germinating at 0.0% R.H. and at 25°C. after 24 hours incubation.

Note: Shrivelled germ tubes and collapsed conidia.

X 110

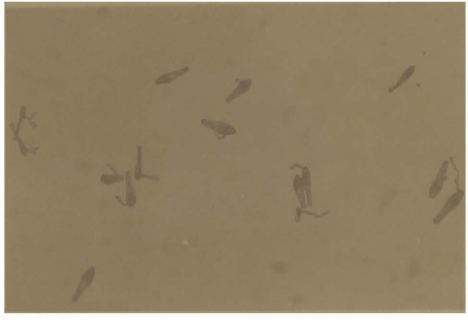


PLATE 6: Photomicrograph of conidia of P. corylea germinating at 12.0% R.H. and at 25°C. after 24 hours incubation.

Note: Most germ tubes have shrivelled.

X 110

PLATE 7: Photomicrograph of conidia of P. corylea germinating at 33.2% R.H. and at 25°C. after 24 hours incubation.

Note: Most germ tubes have shrivelled.

X 110

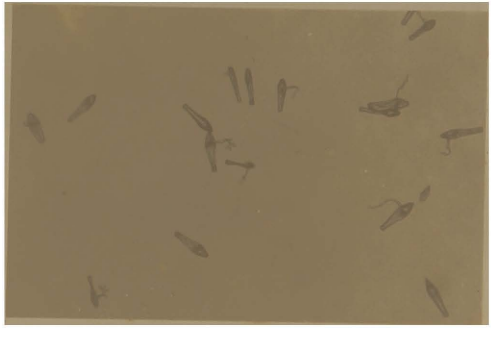


PLATE 8: Photomicrograph of conidia of P. corylea germinating at 53.8% R.H. and at 25°C. after 24 hours incubation.

Note: Shrivelling of just a few germ tubes.

X 110

PLATE 9: Photomicrograph of conidia of P. corylea germinating at 75.8% R.H. and at 25°C. after 24 hours incubation.

Note: Most germ tubes were turgid.

X 110

PLATE 10: Photomicrograph of conidia of P. corylea germinating at 80.3% R.H. and at 25°C. after 24 hours incubation.

Note: Most germ tubes were turgid.

X 110

PLATE 11: Photomicrograph of conidia of P. corylea germinating at 92.0% R.H. and at 25°C. after 24 hours incubation.

Note: Turgid and long germ tubes.

X 110

PLATE 12: Photomicrograph of conidia of P. corylea germinating at 96.9% R.H. and at 25°C. after 24 hours incubation.

Note: Turgid and long germ tubes.

X 110

PLATE 13: Photomicrograph of conidia of P. corylea germinating at 100% R.H. and at 25°C. after 24 hours incubation.

Note: Long and turgid germ tubes.

X 110

C. FURTHER EXPERIMENTS ON THE GERMINATION OF CONIDIA OF P. CORYLEA AT VARIOUS RELATIVE HUMIDITIES

(a) GERMINATION OF THE CONIDIA AT VARIOUS RELATIVE HUMIDITIES WITH SATURATED AQUEOUS SALT SOLUTIONS

It was observed in the previous experiment that germination was very uniform over the range of humidity used (0 - 100% R.H.) (Table 8). It was necessary to find whether this was the true behaviour of conidia of P. corylea. Germination over the same range of humidity was therefore examined again, this time using saturated aqueous salt solutions to maintain the relative humidities according to the data of Hexler and Hasegawa (1954) (See page 18). The specific levels of humidity employed are shown in the tables of results (Tables 9 and 10) and the conidia incubated for 24 hours. As a further confirmation two levels of temperature, 20 and 25°C. were used. Percentage germination was estimated, germ tube length measured and percentage of germ tubes showing appressoria assessed. These are presented in Tables 9 and 10, and in Fig. 5.

The data in Tables 9 and 10 showed that conidia of P. corylea germinated well and very uniformly at all the humidities (0 - 100% R.H.) maintained with saturated aqueous salt solutions incubated at both temperatures of 20 and 25°C. Germination at 100% R.H. at 25°C. was 56.9 per cent and that at 0% R.H. at the same temperature was

50.5 per cent. At 20°C., 41.2 per cent of the conidia germinated at 100% R.H. whilst 39.9 per cent did so at 0% R.H. Germination was lower at 20°C. than at 25°C. at each corresponding relative humidity.

The data showed that the higher the humidity the longer the germ tube length. Thus, at 25°C. 100% R.H. showed mean germ tube length of 112.6 μ whilst at 0% R.H. the mean germ tube length was only 17.3 μ. At 20°C. the mean germ tube length at 100% R.H. was 46.7 μ and that at 0% R.H. was 19.4 μ.

Appressorium formation again followed no definite pattern, indicating that relative humidity has no influence on appressorium development.

The data in these experiments conformed to the behaviour of the conidia of P. corylea found in the previous experiment.

TABLE 9: Effect of Relative Humidity on the Germination of Conidia of P. corylea incubated at 20°C. for 24 hours. (Relative Humidity maintained with saturated aqueous salt solutions).

% Relative Humidity	Saturated aqueous salt solution used	Experiment Number	% Germination	% Germ tube with appressoria	Mean germ tube length in μ
100	WATER	1	27.4	38.1	29.1
		2	41.4	8.4	44.1
		3	54.9	21.1	66.9
		Mean	41.2	22.5	46.7
97.2	K_2SO_4	1	30.5	48.8	24.2
		2	47.2	17.7	42.3
		3	53.9	23.4	49.0
		Mean	43.9	30.0	38.5
93.2	KNO_3	1	29.6	37.6	39.2
		2	42.1	23.4	44.1
		3	53.9	26.1	52.2
		Mean	41.9	29.0	45.2

Table 9 continued

Effect of Relative Humidity on the Germination of Conidia of P. corylea incubated at 20°C. for 24 hours.

(Relative Humidity maintained with saturated aqueous salt solutions).

% Relative Humidity	Saturated aqueous salt solution used	Experiment Number	% Germination	% Germ tube with appressoria	Mean germ tube length in μ
80.6	$(NH_4)_2SO_4$	1	23.9	38.6	20.6
		2	49.5	7.0	41.7
		3	52.3	23.4	51.5
		Mean	41.9	23.0	37.9
75.5	NaCl	1	26.0	34.2	23.4
		2	55.5	13.4	34.3
		3	47.7	24.0	48.3
		Mean	43.1	23.9	35.3
55.2	$Mg(NO_3)_2 6H_2O$	1	32.1	41.3	24.1
		2	47.0	19.0	38.1
		3	51.5	18.1	52.5
		Mean	43.5	26.1	38.2

Table 9 continued

Effect of Relative Humidity on the Germination of Conidia of P. corylea incubated at 20°C. for 24 hours. (Relative Humidity maintained with saturated aqueous salt solutions).

% Relative Humidity	Saturated aqueous salt solution used	Experiment Number	% Germination	% Germ tube with appressoria	Mean germ tube length in μ
33.6	$MgCl_2.6H_2O$	1	29.0	30.0	29.7
		2	39.1	6.3	35.7
		3	47.1	15.9	41.7
		Mean	38.4	17.4	35.7
12.4	$LiCl.H_2O$	1	30.5	32.3	24.8
		2	42.7	6.4	34.6
		3	45.5	8.1	39.6
		Mean	39.6	15.6	33.0
0	$CaCl_2$ (Anhydrous)	1	34.3	48.3	20.0
		2	31.6	66.7	22.4
		3	41.7	65.6	15.7
		Mean	35.9	60.2	19.4

TABLE 10: Effect of Relative Humidity on the Germination of Conidia of *P. corylea* incubated at 25°C. for 24 hours. (Relative Humidity maintained with saturated aqueous salt solutions).

% Relative Humidity	Saturated aqueous salt solution used	Experiment Number	% Germination	% Germ tube with appressoria.	Mean germ tube length in μ
100	WATER	1	48.0	68.1	52.2
		2	63.5	19.7	134.1
		3	59.2	40.9	151.6
		Mean	56.9	42.9	112.6
96.9	K_2SO_4	1	46.6	40.1	79.1
		2	58.0	25.1	92.5
		3	57.9	20.3	118.0
		Mean	54.2	28.5	96.5
92.0	KNO_3	1	42.8	37.1	98.0
		2	58.6	16.2	104.6
		3	58.8	23.1	119.4
		Mean	53.4	25.5	107.3

Table 10 continued

Effect of Relative Humidity on the Germination of Conidia of P. corylea incubated at 25°C. for 24 hours. (Relative Humidity maintained with saturated aqueous salt solutions).

% Relative Humidity	Saturated aqueous salt solution used	Experiment Number	% Germination	% Germ tube with appressoria	Mean germ tube length in μ
80.3	$(NH_4)_2SO_4$	1	42.5	35.7	48.3
		2	44.5	15.2	87.9
		3	58.4	24.7	77.7
		Mean	48.5	25.2	71.3
75.8	NaCl	1	31.5	30.2	45.8
		2	49.3	17.3	42.0
		3	56.7	24.2	49.3
		Mean	45.8	23.9	45.7
53.8	$Mg(NO_3)_2 \cdot 6H_2O$	1	49.0	24.2	54.3
		2	55.1	14.0	66.9
		3	53.7	1.3	67.9
		Mean	52.6	13.2	63.0

Table 10 continued

Effect of Relative Humidity on the Germination of Conidia of P. corylea incubated at 25°C. for 24 hours. (Relative Humidity maintained with saturated aqueous salt solutions).

% Relative Humidity	Saturated aqueous salt solution used	Experiment Number	% Germination	% Germ tube with appressoria	Mean germ tube length in μ
33.2	$MgCl_2.6H_2O$	1	50.5	29.1	48.0
		2	57.6	17.1	50.4
		3	56.0	8.5	41.3
		Mean	54.7	18.2	46.6
12.0	$LiCl.H_2O$	1	49.0	25.0	32.9
		2	51.9	13.7	37.5
		3	53.5	12.9	31.9
		Mean	51.5	17.2	34.1
0	$CaCl_2$ (Anhydrous)	1	56.7	64.9	17.8
		2	50.8	70.7	13.0
		3	44.1	31.1	21.0
		Mean	50.5	55.6	17.3

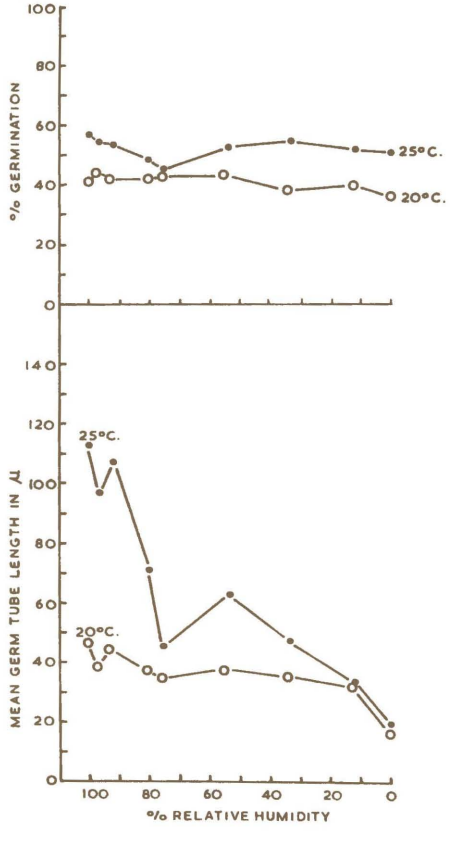


FIG. 5. EFFECT OF RELATIVE HUMIDITY ON THE GERMINATION OF CONIDIA OF P. CORYLEA INCUBATED AT 20 AND 25°C. FOR 24 HOURS.

(b) GERMINATION OF THE CONIDIA UNDER CURRENT OF AIR WITH ADJUSTED RELATIVE HUMIDITIES

Conidia of P. corylea germinate well and uniformly over humidity range of 0 - 100% R.H. maintained with either sulphuric acid or saturated aqueous salt solutions. These experiments were carried out with still air in chambers.

Brodie and Neufeld (1942), working with Erysiphe polygoni considered a possible source of error in the experiments with still air in humidity chambers in the study of germination of conidia. It was thought that under such circumstances some time might elapse before desired conditions especially at low humidities would really be established in the humidity chamber. Because conidia of P. corylea as will be shown later (page 83) have a very brief latent period of germination, it was possible that conidia placed in the closed chamber of lower humidities would begin their germination processes at higher humidity than desired and might be subjected to the desired low humidity only after germination was completed.

In order to leave no possibility of doubt of the ability of the conidia to germinate so well in dry air the conidia of P. corylea were germinated under a current of air with adjusted humidity as opposed to those under still air condition in the germination chamber.

Slides bearing conidia of P. corylea were placed with the spore-coated face uppermost on supporting V-bent glass rods on a glass plate and covered with a large bell jar. The rubber stopper of the jar carried two glass tubes. The bell jar was made airtight by luting the edge with vaseline. One of the glass tubes was connected to a series of four flat bottomed 500 ml. Erlenmeyer flasks which held appropriate sulphuric acid solution to provide a specific relative humidity. The second glass tube was connected to a vacuum pump (Fig. 6). Air was slowly drawn through the whole apparatus one hour before the slides bearing conidia were placed under the bell jar. The air was kept bubbling slowly through the solution and drawn over the conidia under the bell jar for 24 hours at laboratory temperature of 27 ± 1°C.

Three germination tests were made for each humidity level and percentage germination was estimated, germ tube length measured and percentage of germ tubes bearing appressoria assessed. These results are presented in Table 11 and expressed graphically in Fig. 7.

The data in Table 11 showed that conidia of P. corylea germinated very uniformly at all the humidities. Percentage germination here was surprisingly very high at all the humidities. The average percentage germination at 0% R.H. was 72.3 per cent. whilst that at 0% R.H. maintained with concentrated sulphuric acid in still air in chamber at 25°C. was 52.3 per cent and that at 0% R.H. maintained

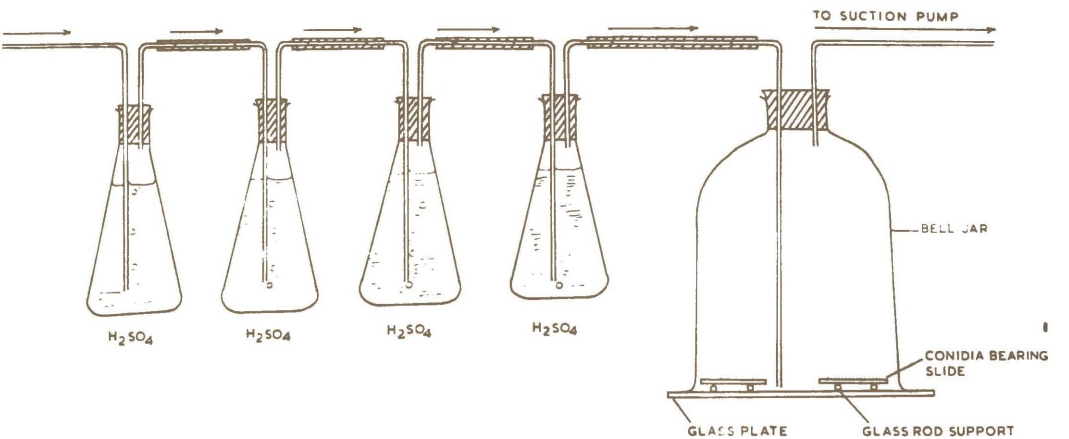


FIG. 6. APPARATUS FOR STUDING GERMINATION OF CONIDIA OF P. CORYLEA UNDER A CURRENT OF AIR OF ADJUSTED RELATIVE HUMIDITY.

with unhydrous calcium chloride at 25°C. was 50.5 per cent. At 100% R.H., 81.1 per cent of the conidia germinated.

Germ tube length followed the same pattern as in the previous experiments. The higher the humidity the longer the germ tube length Thus the mean germ tube length at 100% R.H. was 138.4 μ and that at 0% R.H. was 23.9 μ.

The higher percentage germination of conidia of P. corylea in this experiment might probably be due to two main factors. It might probably be due to slightly higher temperature than the 25°C. used in the previous experiments, the laboratory temperature being 27 ± 1°C. which was probably the true optimum (see page 26). It might also be due to the renewal of oxygen supply in the bell jar (the germination chamber) and the removal of carbon dioxide as the stream of air passed over the germinating conidia. The conditions here were evidently different from the conditions in the still air in the germination chambers where germination activity led to a decrease in oxygen level with time and carbon dioxide level on the other hand rose. The second proposition is however unlikely to be the cause of the higher germination since the plastic boxes used as humidity chambers were fairly large and the volume of air too large for its composition to be appreciably altered by the conidia.

TABLE 11: Effect of Relative Humidity on the Germination of Conidia of *P. corylea* incubated at 27 ± 1°C. for 24 hours. (Relative Humidity maintained with sulphuric acid solutions).

% Relative Humidity	Experiment Number	% Germination	% Germ tube with appressoria	Mean germ tube length in μ
100	1	76.1	27.0	141.6
	2	89.4	29.5	150.1
	3	77.7	19.6	123.5
	Mean	81.1	25.4	138.4
90	1	92.4	34.7	163.8
	2	96.4	29.6	150.9
	3	90.0	30.7	110.2
	Mean	92.9	31.7	141.6
80	1	87.8	20.1	110.2
	2	78.7	16.2	117.3
	3	80.9	25.2	100.1
	Mean	82.5	20.5	109.2

Table 11 continued

Effect of Relative Humidity on the Germination of Conidia of P. corylea incubated at 27 ± 1°C. for 24 hours.

(Relative Humidity maintained with sulphuric acid solutions)

% Relative Humidity	Experiment Number	% Germination	% Germ tube with appressoria	Mean germ tube length in μ
70	1	64.4	16.0	116.6
	2	65.7	30.2	96.8
	3	83.5	21.2	99.1
	Mean	71.2	22.5	104.2
60	1	87.5	33.7	92.4
	2	74.9	22.1	89.5
	3	65.9	19.2	79.0
	Mean	76.1	25.0	87.0
50	1	69.2	9.8	90.6
	2	64.1	7.2	88.9
	3	79.6	29.5	77.0
	Mean	71.0	15.5	85.5
40	1	83.3	34.1	79.8
	2	76.3	32.5	76.8
	3	78.5	20.1	69.5
	Mean	79.4	28.9	75.4

Table 11 continued

Effect of Relative Humidity on the Germination of Conidia of P. corylea incubated at 27 ± 1°C. for 24 hours. (Relative Humidity maintained with sulphuric acid solutions).

% Relative Humidity	Experiment Number	% Germination	% Germ tube with appressoria	Mean germ tube length in μ
30	1	63.2	14.1	71.4
	2	56.4	18.8	76.5
	3	85.1	18.1	50.9
	Mean	68.2	17.0	66.3
20	1	53.2	21.8	35.0
	2	77.6	19.5	39.0
	3	80.4	30.4	38.2
	Mean	70.4	23.9	37.4
10	1	75.5	35.5	37.8
	2	79.6	21.5	40.5
	3	69.9	36.5	32.4
	Mean	75.0	31.2	36.9
0	1	67.6	11.2	21.4
	2	79.4	13.5	30.1
	3	69.9	14.6	20.2
	Mean	72.3	13.1	23.9

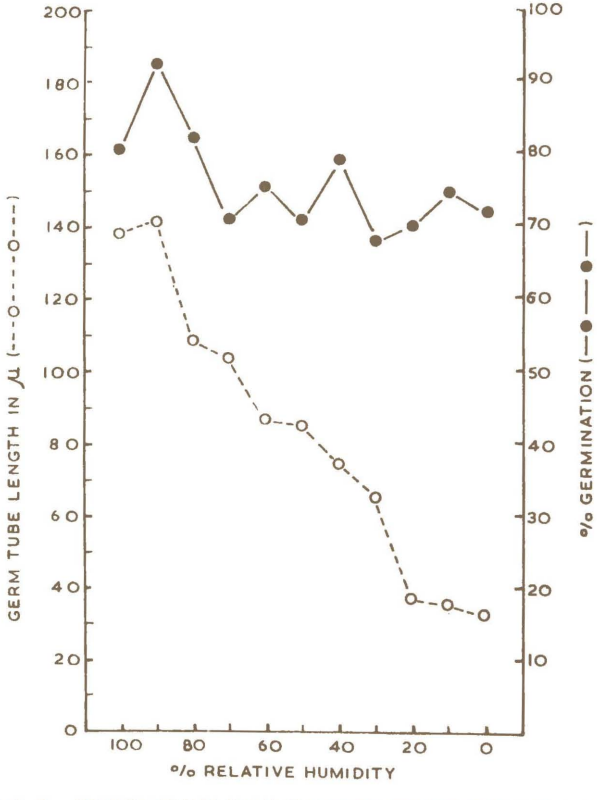


FIG. 7. EFFECT OF RELATIVE HUMIDITY ON THE GERMINATION OF CONIDIA OF P. CORYLEA INCUBATED AT 27 ± 1°C. FOR 24 HOURS.

D. EFFECT OF FLUCTUATING ATMOSPHERIC HUMIDITIES ON THE GERMINATION OF CONIDIA OF P. CORYLEA AT THE TEMPERATURE OF THE AIR

The previous experiments on germination of the conidia have been carried out under constant humidity conditions. In nature, the spores are invariably subjected to fluctuating humidities. It is probable that where the changes in humidity are abrupt, stresses may be imposed on the membranes of the spores especially at the period of onset of germination when the spore has lost protection of the quiescent state, which may lead to disfunction of the conidia. The germination of conidia of P. corylea exposed to atmospheric humidity on glass slides was examined.

Dry slides bearing conidia on the upper surface were exposed to the atmospheric humidity on an open verandah for 24 hours. The conidia were collected and incubated at 3 p.m. of the day of the experiment and the percentage germination estimated and germ tube length measured at 3 p.m. on the following day. The data for the daily and hourly atmospheric humidity of Legon, (Fig. 8) were compiled from the weather charts of Department of Geography, University of Ghana, Legon. This department is situated south-east and 200 yards away from the Botany Department.

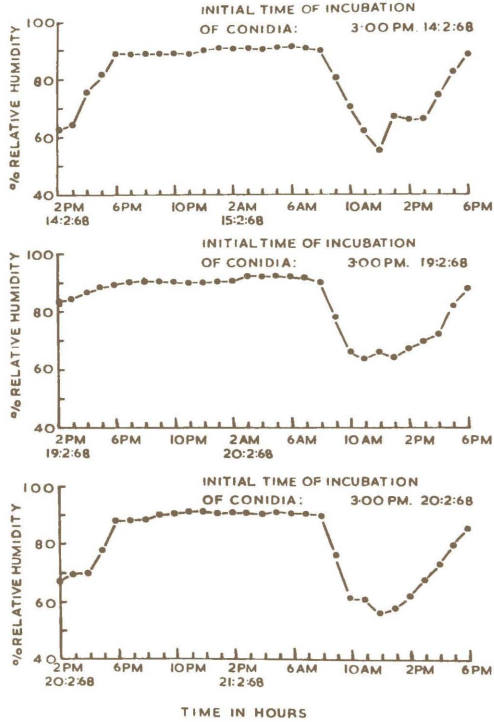


FIG. 8. RELATIVE HUMIDITY OF THE ATMOSPHERE AS RECORDED BY A THERMOHYGROGRAPH DURING GERMINATION OF CONIDIA OF P.COPYLEA UNDER ATMOSPHERIC HUMIDITIES.

From the data of the results (Table 12) (page 73) it would be seen that germination was high and uniform for the three days. On the 14th February 1968, germination was 63.9 per cent though the humidity rose from 64.0% R.H. at 3.00 p.m. to 86.8% R.H. at 6.00 p.m. the critical period for germination. On the 19th February 1968, germination was 80.4 per cent when the atmospheric humidity shifted from 84% R.H. at 3.00 p.m. to 89% R.H. at 6.00 p.m., and on the 20th February 1968, germination was 74.9 per cent. This happened under atmospheric humidity which changed from 69.9% at 3.00 p.m. to 88.1% at 6.00 p.m.

It was interesting to note that where the change in humidity was slight in the latent period, (84.0 to 89.0% R.H.), germination was better (80.4%) than when there was a considerable change. (For example, 63.9 per cent of the conidia germinated when the humidity shifted from 64.0 to 86.8% R.H. The latent period of germination of P. corylea as will be shown later (see page 83) was between 2 - 3 hours. The conidia of P. corylea therefore germinated very well within this period when the atmospheric humidity changed rather sharply between 3.00 p.m. and 6.00 p.m. on the days of the experiment.

Extensive studies have been carried out to determine the ability of conidia of various members of Erysiphaceae to germinate under natural atmospheric humidities. A comprehensive list of such record

on Erysiphe graminis extracted from a similar compilation by Brodie (1945) on some powdery mildews is presented in Table 13. By comparison, conidia of P. corylea belong to the category of those powdery mildews capable of germinating very well under atmospheric humidities.

TABLE 12: Germination of conidia of P. corylea on dry slides exposed to natural Atmospheric Humidities at atmospheric temperature for 24 hours.

Experiment Number	Initial time of Incubation	Time of Observation	Average Atmospheric Temperature	% Germination	Mean germ tube length in μ
1	3.00 p.m. 14/2/68	3.00 p.m. 15/2/68	29.0°C.	63.9	87.2
2	3.00 p.m. 19/2/68	3.00 p.m. 20/2/68	28.3°C.	80.4	97.0
3	3.00 p.m. 20/2/68	3.00 p.m. 21/2/68	28.8°C.	74.9	90.1

TABLE 13: Reported data on germination of conidia of *Erysiphe graminis* exposed to atmospheric humidities.

Host species	Temperature °C.	Atmospheric Humidity	% Germination	Author	Date
Hordeum vulgare	23	-	20	Brodie	1945
" "	23	-	22	Brodie	1945
" "	23	-	19	Brodie	1945
" "	21	40	12	Brodie	1945
" "	21	40	33	Brodie	1945
" "	20	50	14	Brodie	1945
" "	20	50	22	Brodie	1945
" "	20	46	29	Brodie	1945
" "	18	-	9	Cherewick	1944
Triticum aestivum	22	58	35	Brodie	1945
" "	22	43	30	Brodie	1945
" "	22	41	40	Brodie	1945
" "	18	-	40	Cherewick	1944

Table 13 continued

Reported data on germination of conidia of Erysiphe graminis exposed to atmospheric humidities.

H Host species	Temperature °C.	Atmospheric Humidity	% Germination	Author	Date
Avena sativa	18	-	16	Cherewick	1944
Poa pratensis	22	-	15	Brodie and Neufeld	1942
Agropyron repens	25	-	18	Brodie	1945
" "	23	-	30	Brodie	1945
Agropyron sp.	18	-	20	Cherewick	1944
Delphinium spp.	22	-	30	Brodie and Neufeld	1942
Phaseolus vulgaris	-	50	25	Brodie	1945
Brassica oleracea	-	-	35	Clayton	1942
Brassica sp.	24	61	5	Brodie	1945
Aster sp.	22	65	16	Brodie	1945
Oenothera	-	-	15	Clayton	1942

E. EFFECT OF VARIOUS RELATIVE HUMIDITIES ON THE RATE OF GERMINATION OF CONODIA OF P. CORYLEA

It is now convincingly established that germination is equally good over a very wide humidity range (0 - 100% R.H.). The length of germ tubes of the conidia was however not uniform and using this value as a criterion it could be suggested that the more favourable humidities (higher humidities) would support longer germ tubes than the less favourable ones (lower humidities). Another useful criterion which can be used is to examine the rate of germination of the conidia at the various relative humidities. More favourable humidities will encourage faster rate of germination and *vice versa*. The following account reports of findings of this investigation.

The conidia were incubated at different humidity levels as shown in Table 14, maintained with saturated aqueous salt solutions at 25°C. for only 12 hours, since experiment in chapter 1 indicated that the conidia gave maximum germination for each humidity level within that period. Four slides bearing spores were placed in each humidity chamber. Two were withdrawn after 6 hours and the remaining two after 12 hours and (a) percentage germination estimated, (b) percentage of germ tubes with appressoria assessed, and (c) germ tube length measured, for each period of incubation (6 hours and 12 hours).

The results are shown in Table 14 and illustrated graphically in Fig. 9.

The results showed that at 6 hours, the earliest time of observation, germination was approximately uniform at all relative humidities, and similar to values obtained after 12 hours incubation. It seems that for a critical estimation of rate of germination of these conidia, shorter intervals are necessary.

TABLE 14: Effect of Relative Humidity on the rate of germination of conidia of P. corylea incubated at 25°C for 12 hours.

% Relative Humidity	Experiment Number	INCUBATION PERIOD IN HOURS					
		6			12		
		% Germination	Germ tube with appressoria	Mean germ tube length in μ	% Germination	Germ tube with appressoria	Mean germ length in μ
100	1	59.0	12.5	85.4	57.2	23.1	150.9
	2	68.2	15.4	90.7	71.6	11.3	144.6
	3	80.2	9.4	86.8	79.4	9.2	147.4
	Mean	69.1	12.4	87.6	69.4	14.5	147.6
96.9	1	55.9	10.9	94.9	65.7	19.1	120.0
	2	70.2	13.5	85.7	75.2	7.7	168.0
	3	91.1	21.7	77.4	90.5	29.0	109.6
	Mean	72.4	15.4	86.0	77.1	18.6	132.6
92.0	1	58.5	8.5	106.4	53.8	21.0	113.1
	2	73.2	16.2	85.0	67.9	7.6	116.6
	3	86.6	20.7	75.9	88.5	22.0	117.2
	Mean	72.8	15.1	89.1	70.1	16.9	115.6

Table 14 continued

Effect of Relative Humidity on the rate of germination of conidia of P. corylea incubated at 25°C. for 12 hours.

% Relative Humidity	Experiment Number	INCUBATION PERIOD IN HOURS					
		6			12		
		% Germination	Germ tube with appressoria	Mean germ tube length in μ	% Germination	Germ tube with appressoria	Mean germ length in μ
80.3	1	54.2	11.0	73.5	62.2	18.1	93.5
	2	75.2	3.6	92.7	76.3	17.4	120.0
	3	85.6	21.4	51.8	91.5	18.4	96.6
	Mean	71.7	12.0	72.7	76.7	18.0	103.4
75.8	1	56.6	14.5	67.2	53.3	15.7	85.7
	2	63.9	7.6	72.8	66.0	13.8	79.4
	3	84.8	15.3	74.6	85.1	8.8	91.0
	Mean	68.4	12.5	71.5	68.1	12.8	85.4
53.8	1	58.4	10.3	68.6	63.3	12.6	81.2
	2	72.7	16.0	63.3	72.7	13.0	70.3
	3	85.3	13.5	61.3	85.6	19.0	75.6
	Mean	72.1	13.3	64.4	73.9	14.9	75.7

Table 14 continued

Effect of Relative Humidity on the rate of germination of conidia of P. corylea incubated at 25°C. for 12 hours.

% Relative Humidity	Experiment Number	INCUBATION PERIOD IN HOURS					
		6			12		
		% Germination	Germ tube with appressoria	Mean germ tube length in μ	% Germination	Germ tube with appressoria	Mean germ length in μ
33.2	1	58.8	12.6	62.6	57.4	13.6	63.4
	2	63.7	8.1	71.1	72.0	9.0	75.6
	3	83.3	9.2	60.9	87.3	10.7	67.6
	Mean	68.6	10.0	64.9	72.2	11.1	68.9
12.0	1	57.3	5.7	54.3	54.2	4.3	56.7
	2	67.1	9.8	50.8	65.8	2.5	59.8
	3	79.2	6.7	51.5	83.5	6.5	52.8
	Mean	67.9	7.4	52.2	67.8	4.4	56.4
0	1	54.8	8.2	52.9	53.3	9.3	53.2
	2	56.6	14.2	47.2	65.2	12.2	53.6
	3	86.7	7.0	52.2	86.1	14.7	43.8
	Mean	66.0	9.8	50.8	63.2	12.1	50.2

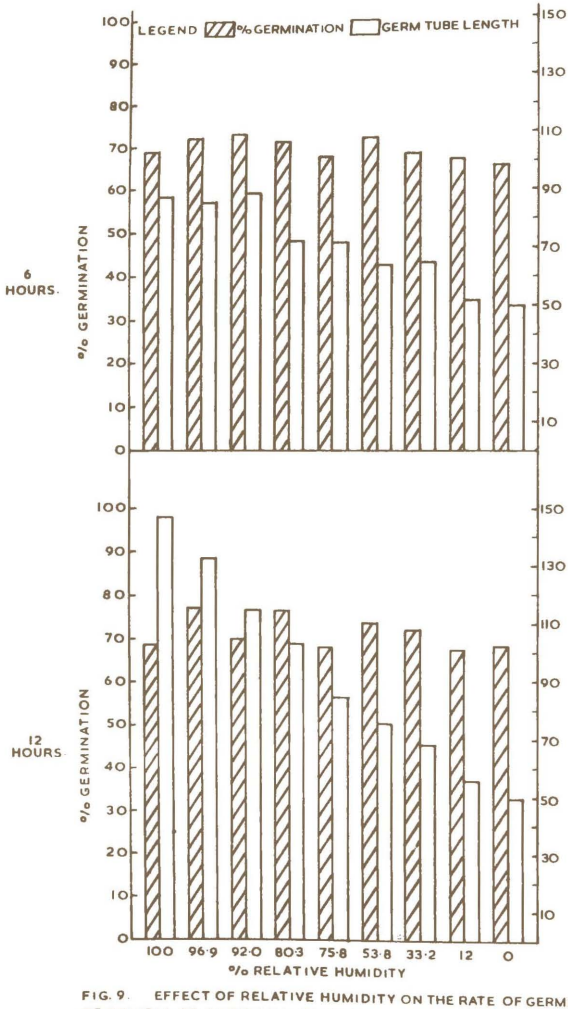


FIG. 9. EFFECT OF RELATIVE HUMIDITY ON THE RATE OF GERMINATION OF CONIDIA OF P. CORYLEA AT 25°C.

F. FURTHER EXPERIMENTS ON THE EFFECT OF VARIOUS HUMIDITIES ON THE RATE OF GERMINATION OF CONIDIA OF P. CORYLEA

Most studies on spore germination employ as criterion the final germination of a population to determine the effect of the environmental factors on germination. Another major criterion often used is the latent period of germination defined as the time required for either germination to begin or the percentage germination to reach some specified low value (Bonner, 1948; Groom and Panisset, 1933; Tomkins, 1932).

In the previous experiment (page 77) the conidia at every humidity level tested showed the possible maximum germination after 6 hours. Percentage germination was besides uniform over the entire humidity range. The search for the optimum relative humidity for germination of conidia of P. corylea could be extended further to either examining the rate of germination within the 0 - 6 hours interval after incubation or finding the latent period of germination of the conidia. The experiment below was designed to investigate both.

The conidia were incubated in the usual manner at the different humidity levels shown in Table 15 at 25°C. for 6 hours. Humidities were maintained with saturated aqueous salt solutions. Twelve slides bearing spores were placed in each humidity chamber. Two were withdrawn at hourly intervals for a total period of 6 hours and percentage

germination estimated. The results are presented in Table 15 and Fig. 10. The latent period of germination defined as "the period or time required by 50% of total germinable conidia to produce germ tubes" was then calculated from the graph in Fig. 10.

The effects of the various levels of humidity on the germination of the conidia were closely similar. The latent period of germination of the conidia of P. corylea at each humidity level was between 2 and 3 hours after incubation. Besides, the conidia showed identical rates of germination over the entire humidity range.

TABLE 15: Percentage of conidia of *P. corylea* able to germinate at various relative humidities at 25°C.

% Relative Humidity	Experiment Number	INCUBATION PERIOD IN HOURS					
		1	2	3	4	5	6
100	1	0.7	20.9	56.1	65.1	66.8	68.0
	2	0.4	21.0	49.3	50.3	56.9	57.9
	3	0.0	19.1	50.0	58.9	66.8	69.8
	Mean	0.4	20.3	51.8	58.1	63.5	65.2
96.9	1	0.9	13.2	23.2	43.8	50.8	51.0
	2	0.2	20.3	45.5	47.9	55.5	49.2
	3	0.0	21.1	53.6	61.0	68.0	70.3
	Mean	0.4	18.2	40.8	50.9	58.1	56.8
92.0	1	0.0	20.6	56.8	74.5	80.8	80.9
	2	0.0	18.9	54.7	65.7	60.7	62.2
	3	0.0	19.2	50.4	59.9	65.9	64.5
	Mean	0.0	19.6	54.0	66.7	69.1	69.2

Table 15 continued

Percentage of conidia of P. corylea able to germinate at various relative humidities at 25°C.

% Relative Humidity	Experiment Number	INCUBATION PERIOD IN HOURS					
		1	2	3	4	5	6
80.3	1	0.6	24.2	54.4	59.9	47.4	59.5
	2	0.0	20.0	43.7	50.3	56.4	66.6
	3	0.0	26.0	40.1	55.6	60.1	61.0
	Mean	0.2	23.4	46.1	55.3	54.6	62.4
75.8	1	0.0	26.8	30.2	52.7	68.0	76.9
	2	0.6	20.9	34.8	49.3	52.9	50.1
	3	0.0	17.3	59.1	71.9	72.5	75.2
	Mean	0.2	21.7	41.4	58.0	64.5	67.4
53.8	1	0.4	19.0	49.3	55.6	62.3	63.3
	2	0.0	18.6	30.1	39.5	48.2	50.2
	3	0.0	20.2	44.3	56.4	61.9	61.0
	Mean	0.1	19.3	41.2	50.5	57.5	58.2

Table 15 continued

Percentage of conidia of P. corylea able to germinate at various relative humidities at 25°C.

% Relative Humidity	Experiment Number	INCUBATION PERIOD IN HOURS					
		1	2	3	4	5	6
33.2	1	0.9	20.4	42.2	50.0	50.1	52.5
	2	0.0	12.7	39.9	40.5	45.5	46.1
	3	0.0	19.9	51.9	58.9	60.4	60.2
	Mean	0.3	17.7	44.7	49.8	52.0	52.9
12.0	1	0.6	24.3	57.3	65.9	69.0	69.5
	2	0.0	6.1	24.9	43.1	54.7	56.9
	3	0.0	26.5	49.1	65.0	65.5	64.0
	Mean	0.2	19.0	43.8	58.0	63.1	63.5
0.0	1	0.3	18.5	31.8	45.7	51.3	51.5
	2	0.1	27.6	57.2	66.5	70.1	69.5
	3	0.0	8.3	39.8	53.1	60.2	59.3
	Mean	0.1	18.1	42.9	55.1	60.5	60.1

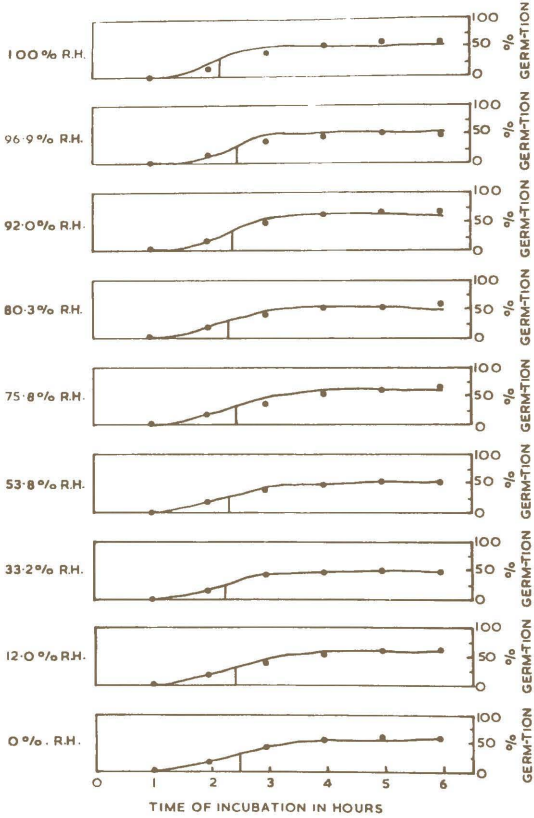


FIG.10. THE EFFECT OF VARIOUS RELATIVE HUMIDITIES ON THE RATE OF GERMINATION OF CONIDIA OF P.CORYLEA INCUBATED AT 25°C.

G. EFFECT OF HUMIDITY ON THE RATE OF GERM TUBE GROWTH

Most fungi require a high humidity usually 95% or more for growth, but a few, such as some grain deteriorating fungi, can tolerate humidities as low as 85% to 90% R.H. (Hawker, 1950; Lilly and Barnett, 1951; Cochrane, 1958).

The previous experiments have shown that:
(a) the conidia of P. corylea germinate uniformly over the humidity range of 0 - 100% R.H., (b) that the rate of germination is uniform for this humidity range and (c) that the latent period of germination is approximately similar. It was impossible therefore to assess the comparative importance of these humidities in germination by these criteria. The only feature where variation has consistently been observed is the length of the germ tubes (Tables 8, 9, 10, 11 and 14). It seems therefore that the rate of germ tube growth could be used to assess the more favourable humidity for the growth of P. corylea. It will however be pointed out that this criterion is being used to embrace both germination of the conidia and growth of the germ tubes more particularly. In an experiment set up to establish the influence of the humidities on growth of the germ tubes, percentage of germination was again uniform and occurred at the same time, and even germ tubes were quite similar in length at 6 hours (Table 15 and Fig. 10). It was after this stage did marked differences appear in the lengths

of germ tubes, obviously due to different rates of extensional growth. This experiment was again carried out by incubating eight glass slides bearing conidia of P. coryloa at 25°C. at each humidity (Table 16). Two slides were removed at 6 hour-intervals and percentage germination was estimated and measurements made of the lengths of 50 germ tubes for each treatment. The entire experiment was extended over 24 hours.

The mean lengths of the germ tubes are shown in Table 16 and are illustrated graphically in Fig. 11. The percentage of germination was practically identical to those already recorded and so omitted from the table of results.

The results (Table 16) indicate that germ tubes of conidia germinating at 0.0, 12.0 and 33.2% R.H. stopped growth after 6 hours. Those at 53.8 and 75.8% R.H. grew for 12 hours after which no further increase in length could be observed. Growth of the germ tubes at 100% R.H., however, showed a uniform growth rate, practically linear, over the entire period of 24 hours and it was evidently the most favourable humidity for germ tube growth. Germ tubes growing at 80.3, 92.0 and 96.9% R.H. although showed extension throughout the period of incubation, growth rate was variable. Two main periods were observed; in a first period of the 0 - 12 hour interval where growth rate was high and a period of very slow growth in the 12 - 24 hour interval, where the rate of growth was approximately a sixth

of the former.

The germ tubes at the lower humidities did not only stop growth quite early during incubation but quickly became dry and shrivelled and probably dead. This raises an important question about the biological value of the ability of P. corylea conidia to germinate so profusely at the lower humidities. If these conidia are not brought under the influence of more favourable humidities after germination they are almost wasted instantly.

TABLE 16: Effect of various Relative Humidities on the rate of growth of germ tubes of P. corylea at 25°C.
(Relative Humidity maintained with saturated aqueous salt solutions).

% Relative Humidity	Experiment Number	Germ tube length in μ at indicated hours			
		6	12	18	24
100	1	86.9	151.2	160.0	190.1
	2	91.7	144.8	188.0	204.8
	3	88.6	148.4	180.2	199.0
	Mean	89.1	148.1	176.1	198.0
96.9	1	95.6	119.1	136.1	140.2
	2	86.0	167.2	163.4	152.2
	3	76.6	108.9	147.4	154.0
	Mean	86.1	131.7	149.0	148.8
92.0	1	106.3	112.3	105.3	111.9
	2	84.9	116.8	112.1	123.2
	3	75.8	116.2	126.0	145.2
	Mean	89.0	115.1	114.5	126.8

Table 16 continued

Effect of various Relative Humidities on the rate of growth of germ tubes of P. corylea at 25°C. (Relative Humidity maintained with saturated aqueous salt solutions).

% Relative Humidity	Experiment Number	Germ tube length in μ at indicated hours			
		6	12	18	24
80.3	1	73.4	93.4	106.0	116.5
	2	91.9	119.9	121.4	122.7
	3	52.6	95.6	99.4	97.0
	Mean	72.6	103.0	108.9	112.1
75.8	1	66.9	85.7	79.5	84.4
	2	72.8	79.3	76.3	81.2
	3	75.6	90.9	92.8	94.2
	Mean	71.8	85.3	82.9	86.6
53.8	1	68.6	81.3	79.1	80.1
	2	64.3	70.2	76.3	63.4
	3	60.3	76.2	86.4	87.8
	Mean	64.4	75.9	80.6	77.1

Table 16 continued

Effect of various Relative Humidities on the rate of growth of germ tubes of P. corylea at 25°C. (Relative Humidity maintained with saturated aqueous salt solutions).

% Relative Humidity	Experiment Number	Germ tube length in μ at indicated hours			
		6	12	18	24
33.2	1	61.9	62.5	61.2	64.1
	2	72.0	75.9	67.9	69.0
	3	60.6	68.5	67.2	68.6
	Mean	64.8	69.0	68.8	67.2
12.0	1	50.8	55.6	48.0	47.1
	2	54.3	59.9	64.0	58.1
	3	50.5	53.8	63.4	57.4
	Mean	51.9	56.4	58.5	54.2
0.0	1	51.9	53.3	49.0	37.1
	2	48.3	52.6	54.9	55.7
	3	52.1	44.8	50.0	51.4
	Mean	50.8	50.2	51.3	48.1

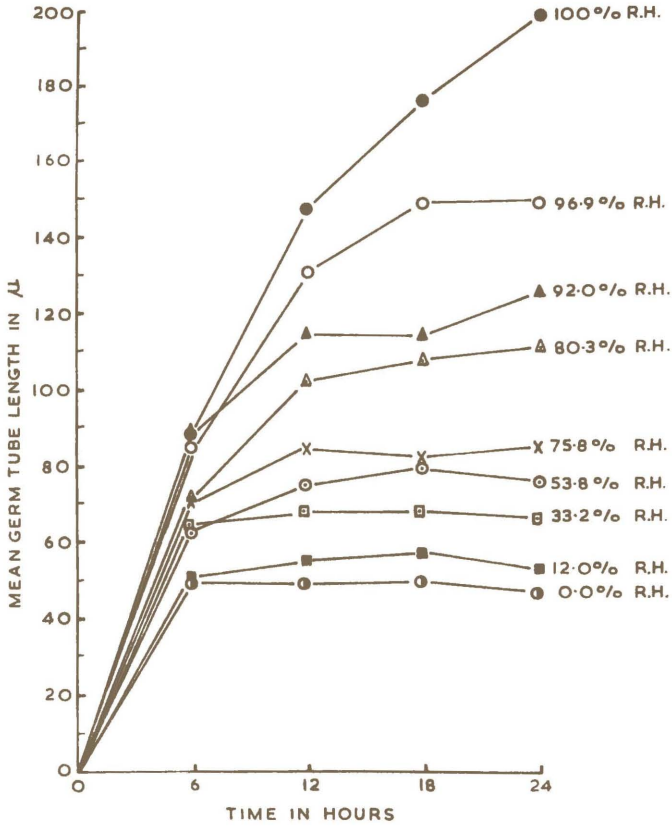


FIG.11. EFFECT OF VARIOUS RELATIVE HUMIDIES ON THE RATE OF GROWTH OF GERM TUBE OF P. CORYLEA AT 25°C.

H. GERMINATION OF CONIDIA OF P. CORYLEA STILL ATTACHED TO CONIDIOPHORE

Brodie and Neufeld (1942) and Clerk and Ayesu-Offei (1967) after exhaustive examination failed to find any spores of the respective powdery mildews, E. polygoni and L. taurica germinating while still attached to the conidiophores. Cherewick (1944) on the other hand found that conidia of E. graminis freely germinated in situ.

During the present studies it was observed that conidia populations obtained on slides occasionally included germinated ones. The proportion of these, however, is extremely low (about one in a thousand). Such conidia might have two possible origins; either they were conidia which might have germinated in situ or were detached conidia which had germinated on the host epidermis. The former is more probable since the diseased lesions occur only on the abaxial surfaces with the conidiophores hanging down. Detached conidia would therefore more naturally drop downward into the air.

Several diseased leaves were therefore examined under the microscope for germinating conidia.

Indeed, some conidia, but rather scantily were observed to have germinated whilst still attached to the conidiophore. (Plates 14, 15 and 16). It is highly probable that these conidia might have been stimulated under unknown peculiar conditions, which

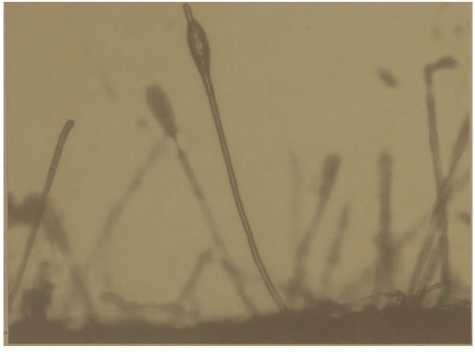


PLATE 14: Photomicrograph of conidium of P. corylea at early stages of germination whilst still attached to the conidiophore.

X 190

PLATE 15: Photomicrograph of conidium of P. coryleа at advanced stage of germination whilst still attached to the conidiophore.

X 190

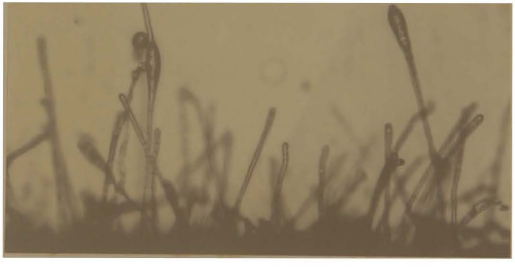


PLATE 16: Photomicrograph of conidium of P. corylea germinating whilst still attached to the conidiophore. Note the absence of constriction between conidium and conidiophore tip.

X 190

occurred even before the spore was fully prepared for detachment (see pages 159 - 162).

On the other hand, some of the detached conidia lying either directly on the epidermis or trapped in the ectotrophic mycelium were found to have germinated. In this case, again, the number of conidia doing so was low, involving about 1 per cent of the total number of detached conidia.

I. SURVIVAL OF THE GERMINATED CONIDIA OF P. CORYLEA

It has been reported that germinated spores of some fungi survive drying for considerable periods (Calpouzos, 1955; Grindle and Good, 1961; Goos and Tschirch, 1962). This does not appear to be so in other species (Doran, 1962; Yarwood, 1936). It seems that the role of the spore dried after it has germinated, but before it has penetrated the host tissue is important from the standpoint of infection. If only a small proportion of such spore survives a dry period, this may have considerable effect on the epidemiology of the disease.

With P. corylea a large percentage of conidia (more than 50%) germinate at even 0% R.H. The germ tubes produced at the lower humidities were observed in the preceding experiments to dry up quite readily. Spores under such conditions which do not permit long survival of the emerged germ tubes, may therefore have very limited value as infection units. This experiment therefore attempts to establish the longevity of the germ tubes under the various humidities.

Conidia on glass plates (42 x 42 mm) inverted over Van Tieghem cells (see general method, page 16) holding saturated aqueous salt solutions to provide various relative humidities (see Tables 17 to 19)

were incubated at laboratory temperature (27 ± 1°C.) for a total period of 48 hours. At 6 hours when all the germinable conidia had germinated, the respective percentage of germ tubes which have shrivelled at the various humidity levels were observed. These observations were continued at the indicated intervals in Tables 17 to 19. In each case percentage of germinated conidia with shrivelled and twisted germ tubes were assessed. From this value was also calculated the percentage of the germinated conidia with unshrivelled germ tubes, a datum used for the graph in Fig. 12. No further reading for a particular sample was taken after the percentage of germinated conidia with shrivelled twisted germ tubes showed 100% shrinkage for three consecutive observations. The results are presented in Tables 17, 18, 19 and 20 and in Fig. 12.

Camera lucida drawings of representative germinated conidia at intervals of 3, 6 and 12 hours held at some of the relative humidities (0.0, 33.2, 53.8, 75.8, 96.9% R.H.) were made and illustrated in Figs 13 to 17.

The results showed that the germ tubes shrivelled quickest at 0% R.H., where all were dry after 12 hours. As the humidity increased, however, the rate of shrivelling of the germ tubes decreased. The germ tubes therefore remained turgid longest at 100% R.H. At this humidity about 80 per cent of the germ tubes were still fully turgid after 48 hours.

TABLE 17: The rate of shrivelling of germ tubes of conidia of *P. corylea* incubated at 0.0, 12.0 and 33.2% R.H. and at 25°C.

Time of Incubation in Hours	Percentage of germinated conidia with shrivelled germ tubes at indicated relative humidities.		
	0.0%	12.0%	33.2%
6	15.5	10.9	1.5
7	20.5	14.0	2.5
8	39.2	26.6	5.9
9	48.4	52.5	6.8
10	68.8	62.5	23.5
11	100	80.7	34.9
12	100	87.4	40.3
13	100	94.4	46.5
14	-*	100	55.2
15	-	100	69.6
16	-	100	83.2
17	-	-	93.2
18	-	-	100
19	-	-	100
20	-	-	100
21	-	-	-

* No further reading taken.

TABLE 18: The rate of shrivelling of germ tubes of conidia of P. corylea incubated at 53.8, 75.8 and 80.3% R.H. and at 25°C.

Time of Incubation in Hours	Percentage of germinated conidia with shrivelled germ tubes at indicated relative humidities.		
	53.8%	75.8%	80.3%
6	1.0	0.0	1.0
7	4.4	0.0	2.5
8	6.0	3.2	8.3
9	18.0	8.9	15.7
10	27.8	11.0	14.8
11	31.0	12.6	15.0
12	33.4	17.7	19.3
13	47.2	32.8	26.3
14	49.2	39.6	29.7
15	52.7	47.1	29.2
16	70.0	53.0	31.8
17	86.4	62.0	39.4
18	91.6	65.7	40.2
19	94.1	71.3	56.0

Table 18 continued

The rate of shrivelling of germ tubes of conidia of *P. corylea* incubated at 53.8, 75.8 and 80.3% R.H and at 25°C.

Time of Incubation in Hours	Percentage of germinated conidia with shrivelled germ tubes at indicated relative humidities.		
	53.8%	75.8%	80.3%
20	100	72.3	70.3
21	100	81.2	79.1
22	100	83.2	80.9
23	-*	85.0	81.1
24	-	95.0	85.6
25	-	98.0	90.0
26	-	100	92.5
27	-	100	97.7
28	-	100	98.1
29	-	-	99.0
30	-	-	100
31	-	-	100
32	-	-	100

* No further reading taken.

TABLE 19: The rate of shrivelling of germ tubes of conidia of P. corylea incubated at 92.0, 96.9 and 100% R.H. and at 25°C.

Time of Incubation in Hours	Percentage of germinated conidia with shrivelled germ tubes at indicated relative humidities.		
	92.0%	96.9%	100%
6	0.0	0.0	0.0
7	3.0	0.0	0.0
8	4.9	0.0	0.0
9	9.1	1.5	0.0
10	9.5	2.5	0.0
11	10.0	3.9	1.0
12	12.0	5.0	2.5
13	20.0	5.5	2.5
14	23.2	5.7	2.7
15	25.5	6.0	4.0
16	27.9	7.5	4.0
17	37.4	10.0	4.9
18	42.7	14.0	5.0
19	44.2	16.0	5.0
20	50.2	16.9	7.5
21	61.8	17.0	9.5

Table 19 continued

The rate of shrivelling of germ tubes of conidia of *P. corylea* incubated at 92.0, 96.9 and 100% R.H. and at 25°C.

Time of Incubation in Hours	Percentage of germinated conidia with shrivelled germ tubes at indicated relative humidities.		
	92.0%	96.9%	100%
22	66.1	17.1	9.5
23	67.6	19.0	10.0
24	68.7	20.0	11.0
25	70.2	21.0	11.0
26	72.8	21.0	14.0
27	79.2	22.5	16.0
28	80.0	22.5	18.0
29	81.7	22.9	18.0
30	82.5	23.0	18.5
31	90.5	23.5	18.5
32	91.6	23.5	19.0
33	93.8	24.2	20.0
34	93.8	24.2	20.0
35	93.9	24.6	20.0
36	94.0	24.9	20.2
48	94.5	25.0	20.2

TABLE 20: Percentage of germinated conidia with unshrivelled germ tubes at various relative humidities and at 25°C. (Relative Humidities maintained with saturated aqueous salt solutions).

% Relative Humidity	Time of incubation in Hours					
	6	12	18	24	30	36
100	100	97.5	95.0	89.0	81.5	79.8
96.9	100	95.0	86.0	80.0	77.0	75.0
92.0	100	88.0	57.3	31.3	17.5	6.0
80.3	99.0	80.7	59.8	14.4	0.0	0.0
75.8	100	82.3	34.3	5.0	0.0	0.0
53.8	99.0	66.6	8.4	0.0	0.0	0.0
33.2	98.5	59.7	0.0	0.0	0.0	0.0
12.0	89.1	12.6	0.0	0.0	0.0	0.0
0.0	84.5	0.0	0.0	0.0	0.0	0.0

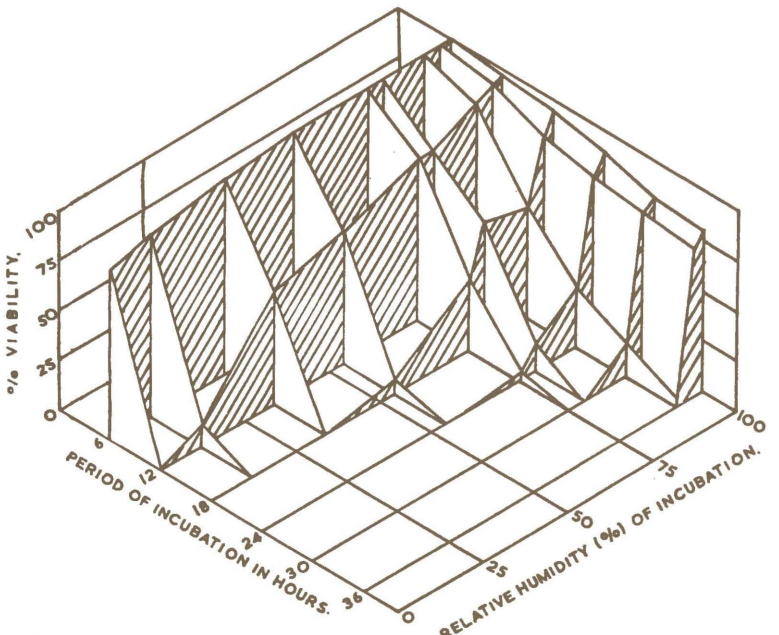


FIG.12.
PERCENTAGE OF GERM TUBES OF P.CORYLEA CONIDIA VIABLE AFTER STORAGE AT DIFFERENT RELATIVE HUMIDITIES FOR VARYING PERIODS AT 27 ± 1°C.

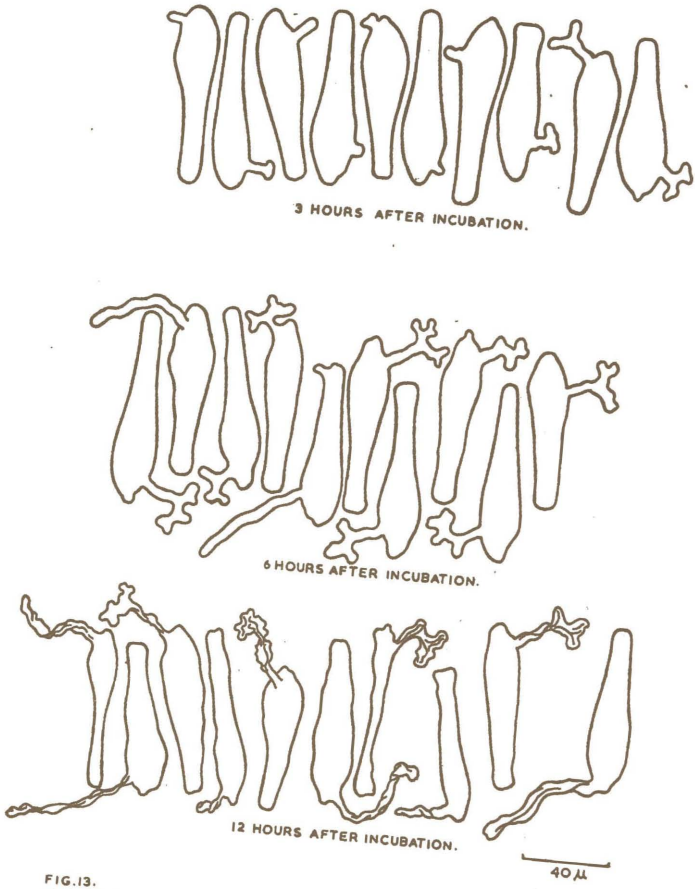
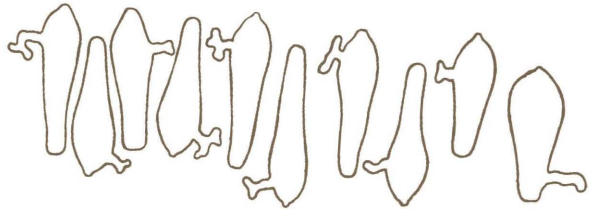
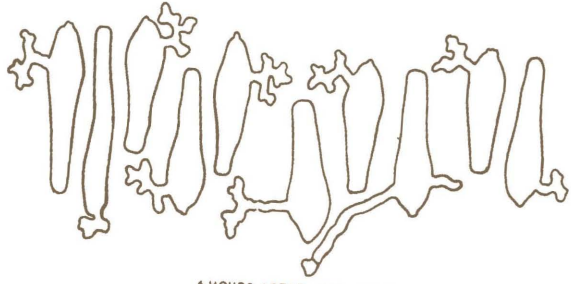


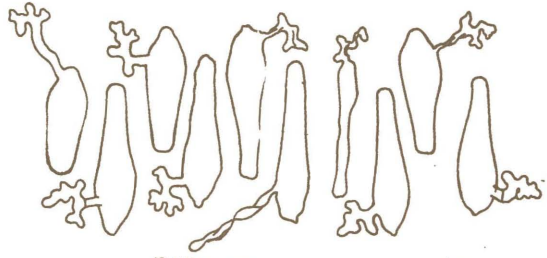
FIG.13.
CAMERA LUCIDA DRAWINGS OF GERMINATED CONIDIA OF P.CORYLEA INCUBATED AT 0·0% R.H. AND AT 25°C. SHOWING RATE OF DRYING OF GERM TUBES.



3 HOURS AFTER INCUBATION.



6 HOURS AFTER INCUBATION.



12 HOURS AFTER INCUBATION.

40μ

FIG.14.
CAMERA LUCIDA DRAWINGS OF GERMINATED CONIDIA OF P.CORYLEA INCUBATED AT 33·2% R.H. AND AT 25°C. SHOWING RATE OF DRYING OF THE GERM TUBES.

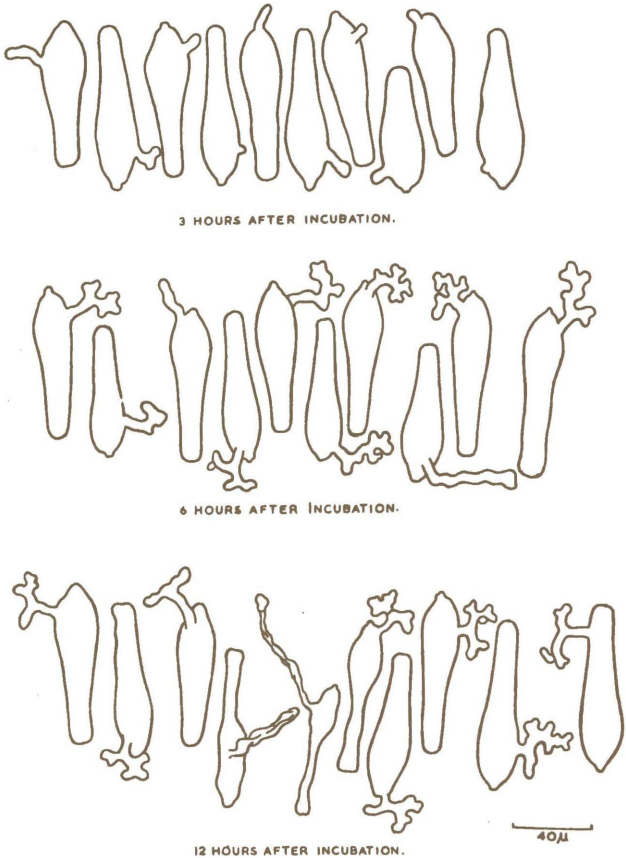


FIG. 15.
CAMERA LUCIDA DRAWINGS OF GERMINATED CONIDIA OF P. CORYLEA INCUBATED AT 53·8% R.H. AND AT 25°C. SHOWING RATE OF DRYING OF THE GERM TUBES.

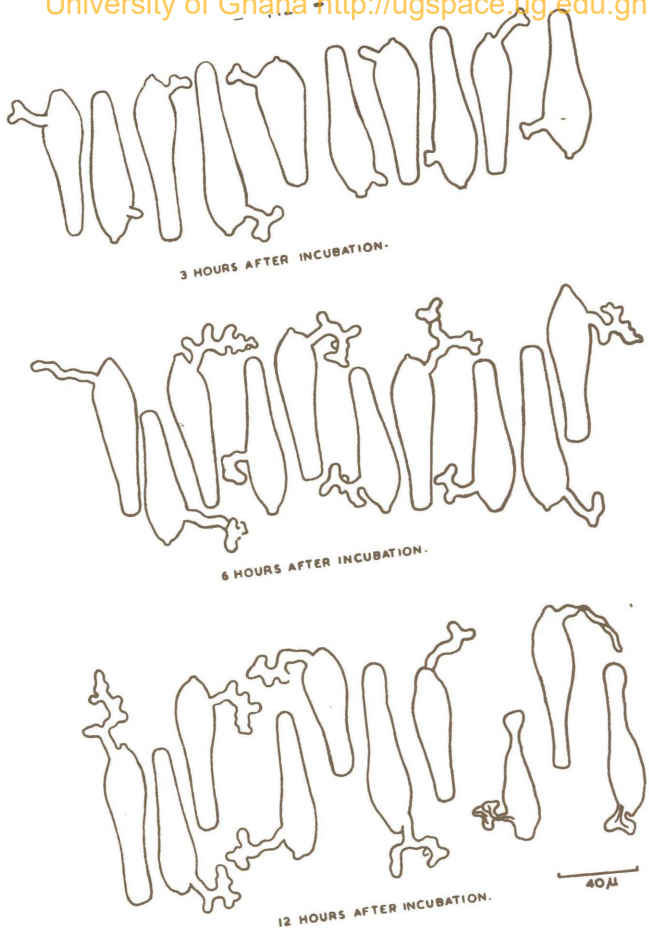


FIG.16.
CAMERA LUCIDA DRAWINGS OF GERMINATED CONIDIA OF P. CORYLEA INCUBATED AT 75·8% R.H. AND AT 25°C. SHOWING RATE OF DRYING OF THE GERM TUBES.

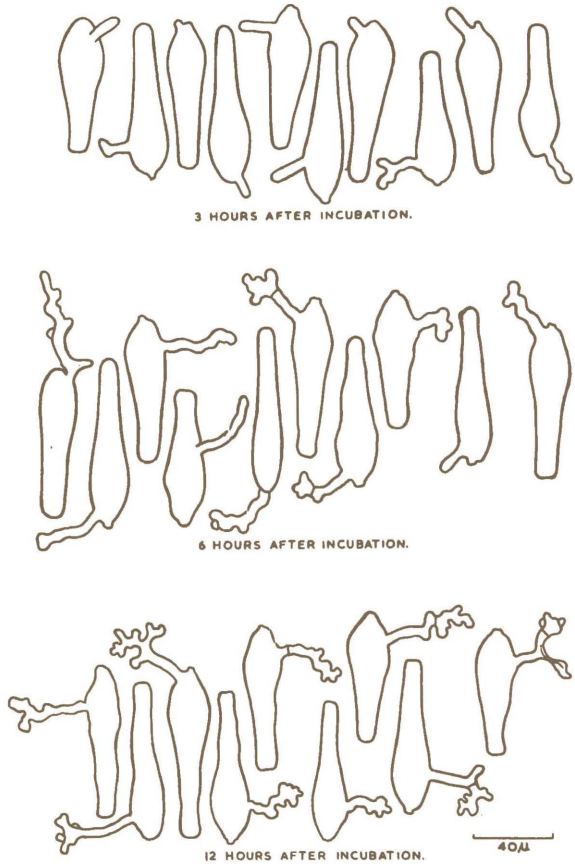


FIG.17.
CAMERA LUCIDA DRAWINGS OF GERMINATED CONIDIA OF P.CORYLEA INCUBATED AT 96·9% R.H. AND AT 25°C. SHOWING RATE OF DRYING OF THE GERM TUBES.

It was also observed that slight wrinkles appeared on some of the germ tubes and the conidia but these did not show clear visible shrinkage and these were therefore excluded from those judged shrivelled. If these in reality were showing a stage or another form of shrivelling, then the values of percentage of shrivelled germ tubes might be slightly higher by about 4 - 6% of the expressed figures in each case. The results also showed that shrivelled twisted germ tubes were observed even at 100% R.H. as early as 11 hours after incubation. This shrinkage of germinated conidia at 100% R.H. probably showed death of the germinated conidia through another cause and not by actual loss of water. On the whole it was noticed that the longer germ tubes collapsed earlier than the shorter germ tubes, perhaps due to exposure of greater surface area to the influence of the humidity.

Throughout this discourse it has been implied that shrinkage is synonymous to death. Since certain conidia are known to recover when returned to favourable conditions after they had been desiccated for some time (Grindle and Good, 1961; Goos and Tschirch, 1963) the shrivelled conidia of P. corylea could only be described as dead if they failed to regain their turgidity if placed at higher humidities. In each case, therefore, where 100 per cent shrinkage was achieved during these studies, viz., conidia at 0.0, 12.0, 33.2, 53.8, 75.8

and 80.3% R.H., the conidia were transferred to humidity chambers of 100% R.H., and observed after 12 and 24 hours. In none could any recovered germ tube be found.

J. PATTERN OF GERMINATION OF CONIDIA OF P. CORYLEA

The pattern of germination of the conidia of some powdery mildews has been studied by many workers and observations are quite varied. Brodie and Neufeld (1942) showed that in germinating conidia of E. polygoni the germ tubes emerged excentrically from one end of the conidium and never directly from the end or from the sides. Similar behaviour was noted in germinating conidia of Leveillula taurica by Nour (1958) and Clerk and Ayesu-Offei (1967). Yarwood (1957) stated that this mode of germination was common to the Erysiphaceae in general. The latent period of germination also seems to be quite uniform among the powdery mildews. Brodie and Neufeld (1942) indicated that germination starts within 2 hours. Germ tubes produced on glass surface generally showed remarkably poor branching.

Certain features of the germinating conidium are considered to be of taxonomic importance, and attempts have been made to identify the powdery mildews by conidial characters. Thus Neger (1902) and Hirata (1942, 1955) found for various powdery mildews that the shape of the germ tubes was characteristic for each species; Hirata found that Sphaerotheca fuliginea was unique in producing forked germ tubes. De Bary (in Blumer, 1933) observed that Erysiphe galeopsidis was easily distinguished by its lobate appressoria.

Zaracovitis (1964a, b) divided a number of powdery mildews into two groups on the basis of certain germination habits. Conidia of Group I produced on germination, short germ tubes terminating in conspicuous lobate appressoria. Some members of the group are E. polygoni, E. betae, Microsphaera berberidis and Oidium begoniae. Group 2 included those which formed thick-walled club-shaped appressoria. This group included E. cichoracearum, Sphaerotheca epiboli and Oidium spp. L. taurica is unique in that variable forms of appressoria are formed, among these are knob-like, branching and net-like types.

Finally, the conidia of P. corylea, like those of other powdery mildews when germinating at 100% R.H. more often retained their original dimensions. Under reduced humidity conditions the conidium visibly shrank, when in both germinated and ungerminated state. This has been reported in this thesis at pages 33 to 42.

The germinating conidia of P. corylea on both glass surface and on the host leaf surface were critically observed and the other attendant features of germination studied.

Sixteen glass slides bearing conidia were incubated at 100% R.H. and at 25°C. Thirty-two host (C. papaya) leaf discs 15 mm. in diameter removed by means of a cork borer held conidia for germination

studies. Sixteen of the leaf discs held conidia on the adaxial surface and the rest on the abaxial surface. These were placed on moist filter paper with the respective spore coated surfaces facing upwards and incubated at 100% R.H. Two of the glass slides, two of the leaf discs bearing conidia on the adaxial surface and two bearing conidia on the abaxial surface were removed at 6-hour intervals for a total period of 72 hours and the following observations made:

(a) point of emergence of germ tube

(b) habit of germ tube

(c) position, architecture and number of appressoria, and,

(d) maximum length of germ tubes sustained solely by endogenous substrate of the spore.

Observations of germinated conidia on the leaf were made after the leaf has been cleared and stained. This was made by immersing discs of host leaf (C. papaya) with germinating conidia in chloral hydrate solution for 24 hours to clear the leaf of chlorophyll. It was then stained with cotton blue in lactophenol. The course of germ tube growth on the leaf, that is directional growth as well as position of appressoria in relation to the configuration of the epidermal surface and distribution pf stomata was carefully investigated.

Camera lucida drawings of these observations and photomicrographs are presented in Figs 18 to 23 and Plates 17 to 18.

The conidia of P. corylea were observed to have a very short latent period of germination, usually within 2 hours of incubation, and all germinable conidia germinated before 6 hours. Some of the germinating conidia commonly produced a single germ tube. Occasionally, more than one germ tube emerged from a conidium. This occurred in about 3 per cent of the total number of conidia observed. No conidium was found with more than two germ tubes (Fig.18). The germ tubes more frequently emerged from variable spots at the club-end of the conidium (Fig. 18). Germ tubes were less frequently produced from other sites either on the "stem" of the spore or directly from the basal end of the conidium (Fig. 18: B, D, H, I, M, S, T). When two germ tubes occurred, their distribution was strictly random (Fig. 18: L to T).

The germ tubes freely produced appressoria on glass surface. Commonly the conidia on germination produced short germ tubes about 4.2 to 70.0 μ which terminated in conspicuous appressoria (Fig. 19: B, D, J). At times these appressoria appeared on fairly long germ tubes, (as long as 200 to 224 μ) (Fig. 19: F, H). The appressoria may be knob-like in shape (Fig. 19: B, C, D, F), repeatedly branching organ (Fig. 19: E, D, K) or

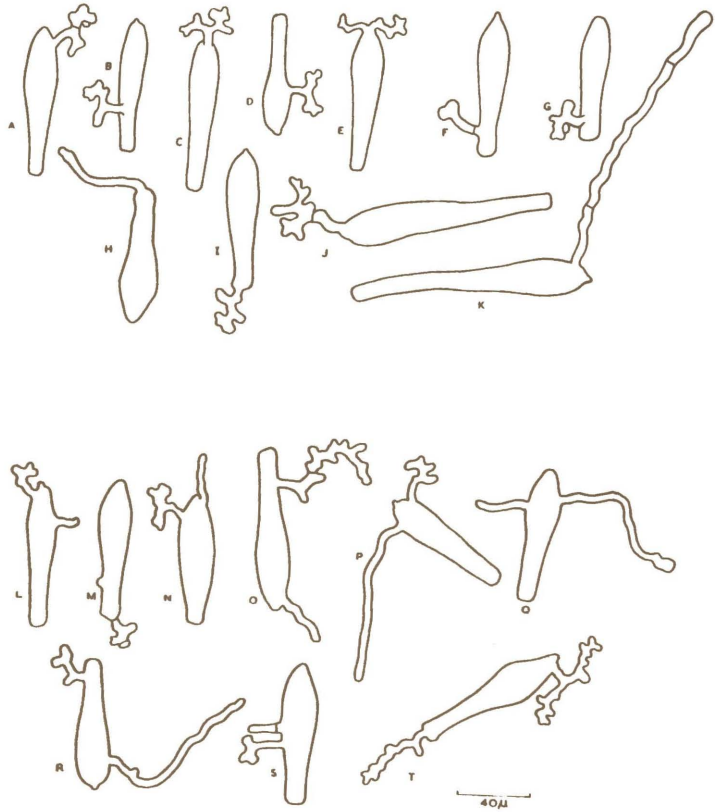


FIG.18.
CONIDIA OF P CORYLEA SHOWING GERMINATION PATTERNS AFTER 24 HOURS INCUBATION AT 25°C ON GLASS SURFACE IN AN ATMOSPHERE OF 100% R.H.

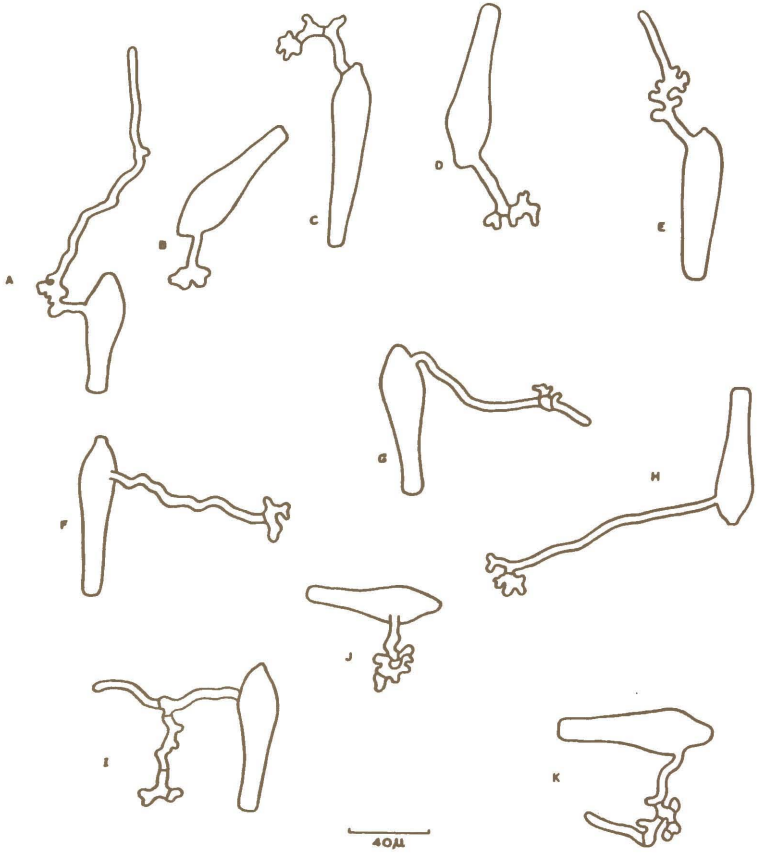


FIG.19.
VARIOUS TYPES AND POSITION OF APPRESSORIA OF GERM TUBES OF P.CORYLEA CONIDIA GERMINATING ON GLASS SURFACE AT 25°C. AND AT 100% R.H.

repeatedly branching short hyphae which fuse to form loops (Fig 19: J,). On glass slides some of the germ tubes did not grow further after forming the appressoria, but on host leaf surface and some of those on glass surface, growth ultimately resumed and the appressoria became 'intercalary' in position (Fig. 19: A, E, G, K; Fig. 20: A, B). In some cases the germinating conidia initially formed long germ tubes (100 to 198 u) long before initials of appressoria arose along its length (Fig. 19: G). These initials later developed into appressoria which always appeared as distinct outgrowths (Fig. 19: H, K). When this happened the appressoria looked either as simple knobs (Fig. 19: G, H) or branching hyphae-like outgrowths (Fig. 19: K). Appressoria could be formed on both sides of the germ tube at a locus (Fig. 19: A, E) or on only one side (Fig. 19: G, H; Fig. 21: B). Appressoria were not necessarily formed by all the germ tubes, and some might grow very long for many hours without producing any (Fig.18: K; Fig. 21: C, D; Fig. 20: C; Fig. 22: A, B).

The conidia germinated well on both surfaces of the host leaf. Observations of conidia germinating on discs of host leaf surface as shown by camera lucida drawings showed germ tubes running randomly on host leaf surface either across the

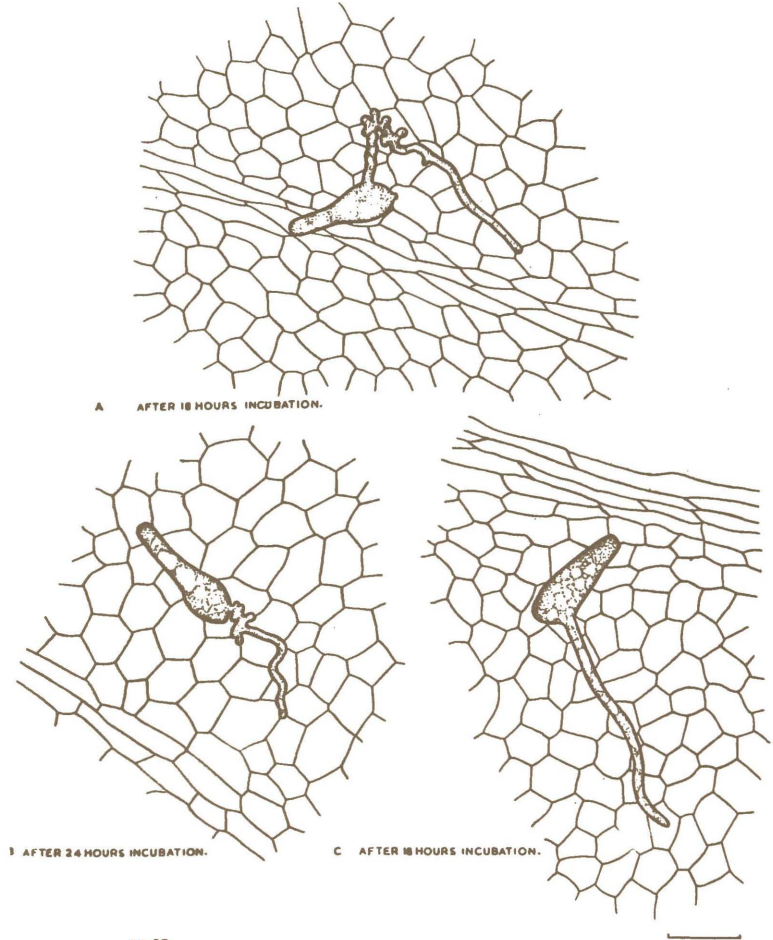


FIG.20.
GERMINATION OF CONIDIA OF P.CORYLEA ON ADAXIAL SURFACE OF LEAF OF C.PAPAYA, INCUBATED AT 100% R.H. AND AT 25°C., SHOWING GERM TUBES RUNNING RANDOMLY OVER HOST LEAF SURFACE.

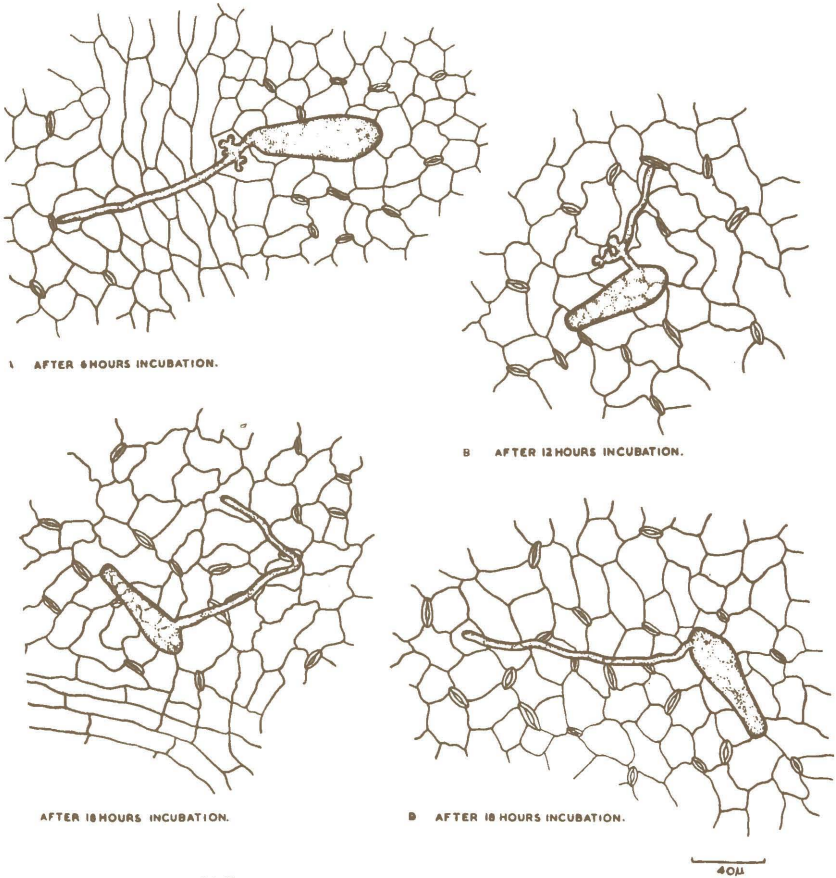


FIG. 21.
GERMINATION OF CONIDIA OF P. CORYLEA ON ABAXIAL SURFACE OF LEAF OF C. PAPAYA INCUBATED AT 100% R.H. AND AT 25°C, SHOWING GERM TUBES RUNNING RANDOMLY OVER HOST LEAF SURFACE.

veins (Fig. 21: A; Fig. 22: A, B; Plate 17) or along them (Fig. 22: C; Plate 18) or irregularly over the epidermal cells (Figs 20: A, B, C and 21: B, C, D).

There was some branching of germ tubes on glass surface but this was rather not extensive (Fig. 23). The germ tubes on slides attained a maximum length of 196.0 - 201.6 μ, in 36 hours, when growth ceased whilst those on host leaf surface continued to grow thereafter to achieve a lenght of over 450.0 μ, no doubt supported in part by extraneous nutrients from the host (Table 21).

The germ tube is septate. The cross walls often appeared closely associated with the appressoria. Septa also appeared in non-appressorium bearing germ tubes. Observations on growth of germ tubes on host leaf surface was confined to the initial stages, and development of these into ectotrophic mycelium lay beyond the scope of these studies.

During germination the conidia remained still attached to the mycelium. The contents of the conidium were never seen emptied into the developing germ tube and the spore retained its protoplasmic contents.

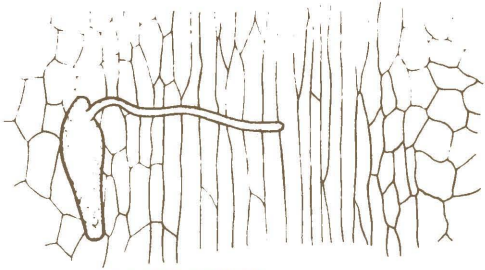


FIG.22 A. AFTER 18 HOURS INCUBATION.

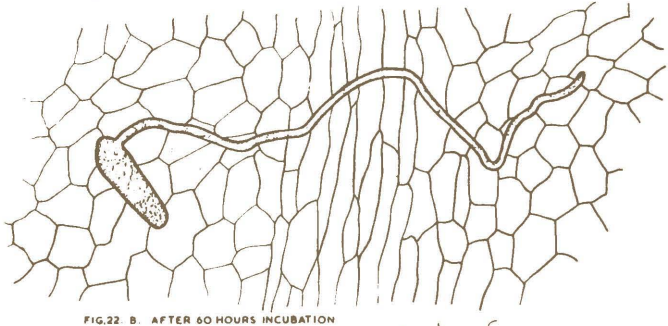


FIG.22. B. AFTER 60 HOURS INCUBATION

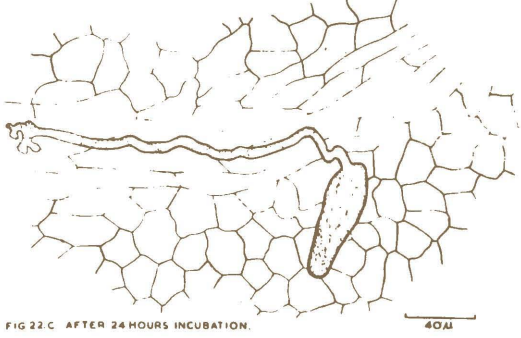


FIG 22.C AFTER 24 HOURS INCUBATION.

FIG. 22
GERMINATION OF CONIDIA OF P. CORYLEA ON ADAXIAL SURFACE OF LEAF OF C. PAPAYA INCUBATED AT 100% R.H. AND AT 25°C SHOWING GERM TUBES RUNNING RANDOMLY ON HOST LEAF SURFACE EITHER ACROSS (FIGS A AND B) OR ALONG (FIG C).

PLATE 17: Photomicrograph of germinating conidium of *P. corylea* on pawpaw (*C. papaya*) leaf surface. Note germ tube growing across leaf vein.

X 175

PLATE 18: Photomicrograph of germinating conidium of *P. corylea* on pawpaw (*C. papaya*) leaf surface. Note germ tube growing along leaf vein.

X 175

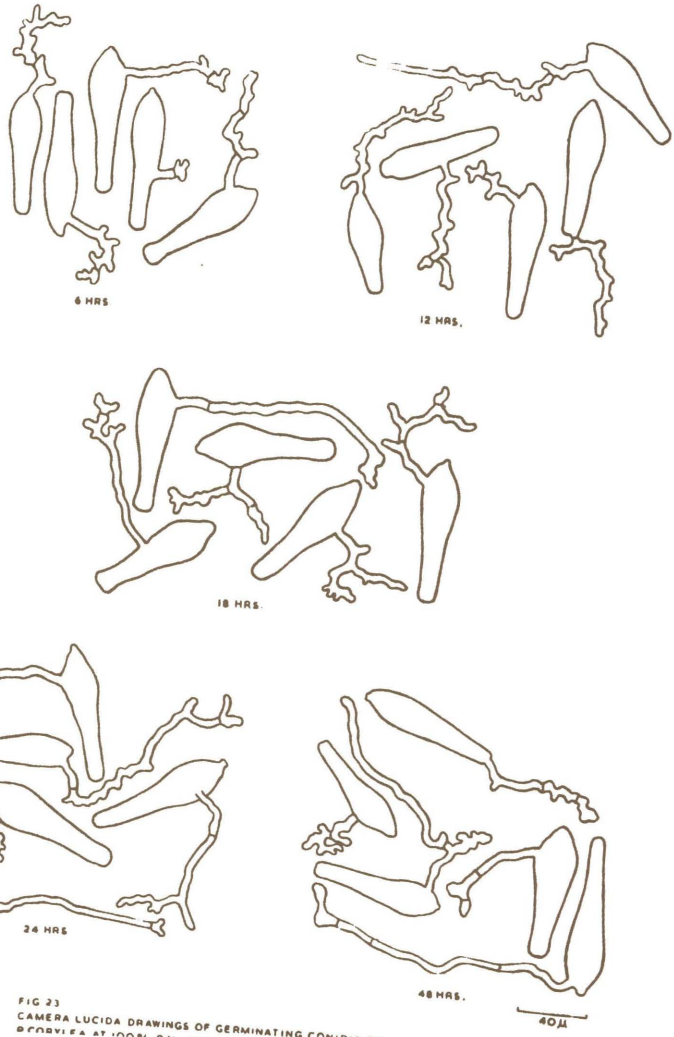


FIG 23
CAMERA LUCIDA DRAWINGS OF GERMINATING CONIDIA OF
OTE THE RATHER

TABLE 21: Comparative growth of Germ Tubes of *P. corylea* on glass and on host leaf surfaces at 100% R.H. and 25°C. (Measurement in each case is a mean of germ tubes).

Time of Incubation in Hours	MEAN GERM TUBE LENGTH IN μ		
	Glass Slides	Leaf	
		Adaxial Surface	Abaxial Surface
6	61.6	24.5	29.4
12	142.8	59.7	47.6
18	156.2	100.8	110.5
24	173.6	191.8	164.5
36	196.0	270.2	225.4
48	196.0	361.2	387.8
60	197.8	439.1	409.9
72	201.6	455.6	458.4

K. EFFECT OF LIGHT ON CONIDIAL GERMINATION

The pertinent literature reveals that different fungi differ in their light requirements for the germination of their spores. Several reports indicate that most spores germinate equally well in light and in darkness (Cochrane, 1958; Gottlieb, 1950; Marsh, Taylor and Bassler, 1959; Hawker, 1950). Doran (1922) noted that conidia of Sclerotina fructigena germinate equally well in direct light or diffuse light or darkness.

Light, however, has been found to increase spore germination in some species (Neergaard, 1941; Hebert and Kelman, 1958). For example, Neergaard (1941) found that the germination of conidia of Alternaria oleracea is stimulated by light. The depressing effect of strong light, on the other hand, on germination has been noticed by many workers. Cochrane (1945a) observed that the germination of uredospores of Phragmidium mucronatum is retarded by light of 1,250 foot candles in intensity. This inhibitory effect increased with rise in intensity of illumination.

Light effect in certain cases may be affected by other environmental conditions. Thus Chorewick (1944) working with Erysiphe graminis found that at high temperatures (30, 35°C.)

spore germination was depressed by light whilst at low temperatures (10 - 25°C.) it was stimulated.

In this experiment, the effect of artificial light and complete darkness on the germination of the conidia of P. corylea was studied at 100% R.H. and at 21°C.

Two plastic boxes (10 x 10 x 7 cm.) were used as humidity chambers. One of the boxes was completely covered with black paper. Two glass slides bearing conidia were put on V-shaped glass rods in each of the humidity chamber, and the two boxes were placed side by side on a bench in an air conditioned room to ensure that the temperature remained constant throughout the period of growth. They were exposed to a 25-Watt electric lamp held 15 cm. above the germination chambers for 24 hours. The intensity of illumination could not unfortunatoly, be measured since no light metre was available in the University. The temperature of the incubation room was 21°C.

The percentage germination, percentage of germ tubes with appressoria and the mean germ tube length of 30 randomly selected germinated conidia were estimated. These are presented in Table 22.

The results showed considerable differences in percentage germination and percentage of germ tubes forming appressoria,

between conidia kept in continuous light and those stored in continuous darkness. Germination was appreciably higher in continuous light whereas much as twice the number of conidia which germinated in continuous dark did so. The number of germ tubes forming appressoria on the other hand, was higher in the darkness than in the light. This might be associated with light effect on direction of germ tube growth and has been commended fully on at page 215. Maximum lengths of the germ tubes also showed a difference, with those in light growing longer than those in dark. Evidently, appressorium formation might have in a way interfered with full development of the germ tubes. It was thus clear that light had an effect on the germination of conidia of P. corylea.

TABLE 22: Germination of conidia of P. corylea incubated in continucus light and continuous darkness at 100% R.H. and at 21°C. for 24 hours.

Experiment Number	% Germination		% Germ Tubes producing appressoria		Mean germ tube length in µ	
	Light	Dark	Light	Dark	Light	Dark
1	54.4	26.8	8.8	15.0	136.8	108.2
2	54.5	21.4	1.7	29.9	135.4	72.4
3	43.4	22.3	2.3	21.7	138.6	94.2
4	58.0	39.4	1.8	31.0	120.8	112.4
Mean	52.6	27.5	3.7	24.4	132.9	96.8

L. LONGEVITY OF CONIDIA OF P. CORYLEA STORED AT DIFFERENT RELATIVE HUMIDITIES

The viability of fungal spores measured by their ability to germinate after various periods is affected mainly by environmental conditions of humidity, temperature and light. Humidity and temperature usually interact. However, one factor or the other may exert so strong an influence that it can be said to be isolated.

Various investigators found different kinds of relationships between relative humidities and longevity of fungal spores. At the moment four ways in which longevity of fungus spores is related to atmospheric humidity are recognised. Several investigators (Anderson et al., 1948; Groom and Panniseet, 1933; McLaughlin and True, 1952, Page et al., 1947 etc.) have found that lower relative humidities favour retention of viability of specific fungal spores.

Hart (1926) found that uredospores of Melampsora lini retained their viability for a longer time at intermediate humidities (40 and 60% R.H.) than at higher and lower humidities. Rosen and Weetman (1940) and Nagvi and Good (1957) respectively found similar behaviour in uredospores of crown

rust of oats and conidia of Monilinia fructicola. Nagvi and Good (1957) reported that the conidia of Monilinia fructicola survived longest at 75% R.H.

Other investigators for example, Merrick 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.). A fourth type of relationship was reported by Teitell (1958) who found that within the range of atmospheric humidities that was too dry to permit of germination, survival of Aspergillus flavus conidia was longest at low (0% R.H.) and high (85% R.H.) humidities and briefest at a narrow intermediate range close to 75% R.H. He found a similar relationship also for an isolate of A. terreus. A possible third example of this sort of relationship is provided by the ascospores of Endoconidiophora fagacearum. Although Merek and Fergus (1954) interpreted their results on longevity of ascospores of this species as indicating longest survival at the lowest humidity and least at high, they did in fact show that between 12 and 24°C. all the

ascospores died in less time at 75% R.H. than as 50, 25 and 10% R.H. Conidia of Metarrhizium anisopliae, appears to be what is so far only the fourth known example of a fungus with spores surviving for the least time at a median humidity (c. 45% R.H.), and longest above and below this level (Clerk and Madelin, 1965).

The effects of light and temperature on spore longevity shows less variation. Almost without exception, survival of fungus spores is greatest at low temperatures (Cochrane, 1958) and in spores sensitive to light, their viability tends to be shortened by light; this effect being more marked in hyaline than in coloured spores,

With P. corylea conidia which readily germinate at any humidity level at any favourable temperature, longevity studies could only be carried out within a limited range of temperature, that is, between freezing and the minimum temperature permitting germination. This was the condition, therefore, under which the longevity of the conidia was examined.

Conidia were stored on glass slides over respective saturated aqueous salt solutions (which maintained constant relative humidities) in humidity chambers sealed with cellotape, in darkness in a refrigerator set at 5°C. Anhydrous calcium

chloride and water were used for constant humidities of 0% and 100% R.H. respectively. Viability was determined after desired intervals of storage by transferring and germinating the conidia at 27°C. and at 100% R.H. for 24 hours. Percentage germination was then determined and the length of germ tubes measured. When in the longevity test there was no germination in a sample, further samples were taken from the same vessel at the next two sampling dates and tested for germination. If still negative, the population was presumed wholly to have lost the ability to germinate and no further samples were taken.

The data of the results (Table 23 and Fig. 24) show clearly that at even such a low temperature of 5°C., the conidia of P. corylea were poorly preserved. In no sample did longevity exceed 20 days. Conidia of P. corylea were very short-lived at low humidities and loss of ability to germinate was extremely rapid in conidia stored at 0% R.H., so that by the fifth day there were no viable spores. Rising humidity increased longevity and the longest survival was at 82.6 to 100% R.H.; between 0.2 and 0.4% conidia were still able to germinate after 15 days.

TABLE 23: Percentage of conidia of P. corylea able to germinate after storage in darkness in atmospheres of various humidities at 5°C.

(Percentage germination at 100% R.H. after 24 hours at 27°C.)

% Relative Humidity	Period of Storage in Days					
	1		2		3	
	% Germination	Mean germ tube length in μ	% Germination	Mean germ tube length in μ	% Germination	Mean germ tube length in μ
0.0	13.6	114.1	4.1	72.5	0.9	45.7
14.0	14.8	140.0	5.6	37.7	1.4	47.6
34.6	10.9	109.0	9.6	98.8	1.8	21.5
59.2	11.8	92.1	11.2	103.0	3.5	70.9
75.1	12.4	117.3	9.2	87.7	3.3	56.3
82.6	15.1	119.8	5.3	90.0	4.0	90.4
96.6	11.5	121.2	4.8	105.5	6.0	89.8
100.0	9.6	115.9	4.9	94.7	1.6	86.4

Table 23 continued

Percentage of conidia of P. corylea able to germinate after storage in darkness in atmospheres of various relative humidities at 5°C.

(Percentage germination at 100% R.H. after 24 hours at 27°C.)

% Relative Humidity	Period of Storage in Days					
	4		5		6	
	% Germination	Mean germ tube length in μ	% Germination	Mean germ tube length in μ	% Germination	Mean germ tube length in μ
0.0	0.6	19.6	0.0	-	0.0	-
14.0	0.3	98.0	0.4	24.0	0.5	24.5
34.6	0.7	56.0	0.5	18.2	0.5	28.0
59.2	4.2	106.8	0.1	24.5	0.1	20.2
75.1	5.9	88.7	2.4	82.6	0.9	49.0
82.6	4.0	90.4	4.5	74.1	1.0	35.0
96.6	6.6	110.6	2.6	61.8	1.2	35.0
100.0	4.5	100.9	3.5	68.2	1.0	38.5

Table 23 continued

Percentage of conidia of P. corylea able to germinate after storage in darkness in atmospheres of various relative humidities at 5°C.

(Percentage germination at 100% R.H. after 24 hours at 27°C.)

% Relative Humidity	Period of Storage in Days					
	7		8		9	
	% Germination	Mean germ tube length in μ	% Germination	Mean germ tube length in μ	% Germination	Mean germ tube length in μ
0.0	0.0	-	-	-	-	-
14.0	0.4	24.0	0.2	17.5	0.0	-
34.6	0.6	20.0	0.4	11.6	0.2	11.8
59.2	0.1	18.0	0.0	-	0.0	-
75.1	0.4	41.9	0.6	12.3	0.4	50.4
82.6	1.2	36.1	1.4	32.6	0.4	63.0
96.6	1.3	36.1	1.4	35.0	1.2	54.0
100.0	2.0	60.0	3.7	50.1	1.5	58.8

Table 23 continued

Percentage of conidia of *P. corylea* able to germinate after storage in darkness in atmospheres of various relative humidities at 5°C.

(Percentage germination at 100% R.H. after 24 hours at 27°C.)

% Relative Humidity	Period of Storage in Days					
	10		15		20	
	% Germination	Mean germ tube length in μ	% Germination	Mean germ tube length in μ	% Germination	Mean germ tube length in μ
0.0	-	-	-	-	-	-
14.0	0.0	-	0.0	-	-	-
34.6	0.0	-	0.0	-	0.0	-
59.2	0.0	-	-	-	-	-
75.1	0.4	14.0	0.0	-	0.0	-
82.6	0.4	54.5	0.4	17.5	0.0	-
96.6	1.2	68.7	0.4	13.0	0.0	-
100.0	0.4	67.7	0.2	17.0	0.0	-

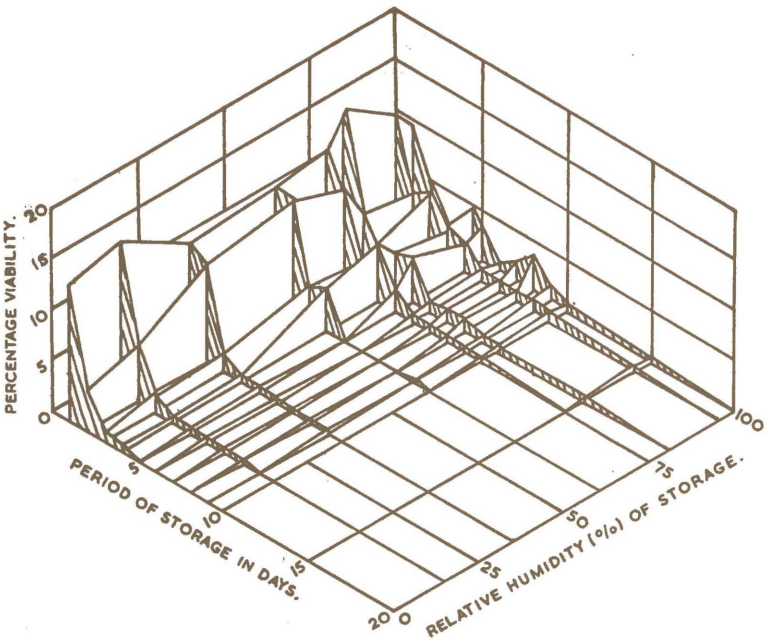


FIG.24.
EFFECT OF RELATIVE HUMIDITY ON LONGEVITY OF CONIDIA OF R CORYLEA INCUBATED AT 5°C.

M. DEVELOPMENT AND RELEASE OF CONIDIA OF P. CORYLEA

The following experiments were conducted to examine two aspects of the biology of P. corylea; these are, the morphology of the conidiophore and formation of the conidium and the manner of release of the mature conidium.

The structure of powdery mildew conidiophores is sometimes confused by the amazing variation in the same species on the same host as observed by different investigators. For example, the illustrations of conidiophores of Uncinula nector from grape by Viala (1893) and Cooke (1906) in Europe and by Galloway (1895), Longyear (1904) and Duggar (1909) in the United States are clearly different from each other yet all are generally regarded as of the same species. Whether these differences represent truly different fungi or whether they are differences due to environment, host variety or differences in the illustrating ability of the observers cannot be determined at this time. Further examples with the same species, as well as with others, could be given. On the other hand, uniformity in reported structure is quite common. Thus, Yarwood (1957) found that the conidiophores of Uncinula nector as well as of Erysiphe polygoni, E. graminis and E. cichoracearum are reasonably constant in the same and different collections

if actively growing specimens are examined. The morphology of the conidiophore, formation and development of the conidia in P. corylea were studied to find out to what extent observations here agree with earlier descriptions.

There is an often-reported fascinating aspect of the fate of the mature conidium of P. corylea which has remained unanswered. It is generally reported that the conidiophore of P. corylea bears at any time only a single conidium at its tip which immediately falls off before the next one appears (Bouwens, 1924; Ressencourt, 1927; Roger, 1953; Viennot-Bourgin, 1944). It might be implied that an active mechanism in the conidiophore is responsible for the release of the mature conidium. This has never been investigated. Hammarlund (1925), on the contrary, observed that in still and damp air P. corylea which frequently only bore a single conidium at the tip of the conidiophore developed numerous chains. It is most unlikely as suggested by this information, that wind alone prevents the formation of conidial chains by P. corylea, since similarly delicately poised dry conidia of other fungal species for example, Erysiphe, Aspergillus and Penicillium, freely form conidial chains. The inability of P. corylea to form conidial chains under normal field conditions was therefore critically examined.

Direct microscopic observations, under low power magnification, on conidium development and release were made on pawpaw (Carica papaya) leaves still attached to potted plants and held in position on the microscope stage with cellotape. Infected leaves were folded so that the conidiophores on the abaxial surface, under observation, projected beyond the folded edge of the leaf. Except where otherwise stated the observations were made in the laboratory with an average temperature of 28°C. and with free air circulation.

Observations were recorded at intervals both by photomicrography and by camera lucida drawings.

a. DEVELOPMENT AND MORPHOLOGY OF THE CONIDIOPHORE

The conidiophores were typically unbranched, multicellular, about 10.5 μ in diameter, up to 300 μ long and occurred randomly along the ectophytic hyphae at right angles to the host surface. Typically there was a stipe of two or three cells, terminated by a generative cell which is responsible for the formation of the conidia (Fig. 25 B, C, D and E) Plate 19. At the tip of the generative cell would usually be seen a single maturing conidium. Conidiophores stained with lactophenol cotton blue showed that the protoplasm was mostly concentrated in the generative cell and the developing conidium (Plate 20: A). The first indication of the conidiophore was the formation of a slender cylindrical cell which rapidly elongated perpendicularly to the hypha on which it arose. This cell eventually divided transversely into two (Fig. 25, A). The upper cell next divided again into two, the apical cell of which would again divide. The conidiophore when fully built consisted of usually two short basal cells, on elongated middle cell terminated by a short generative cell. The generative cell of the newly formed conidiophore was strictly erect (Figs 25 and 26: A), (Plates 22 and 23).

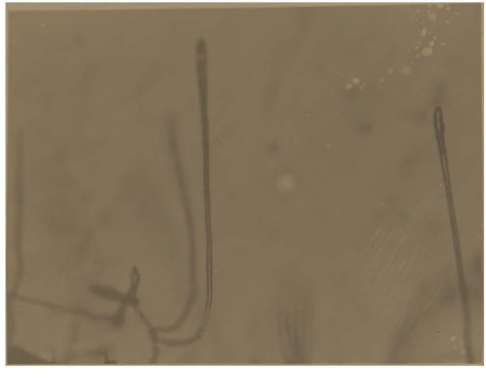


PLATE 19: Photomicrograph of conidiophores of *P. corylea* showing their origin from superficial hypha. X 120

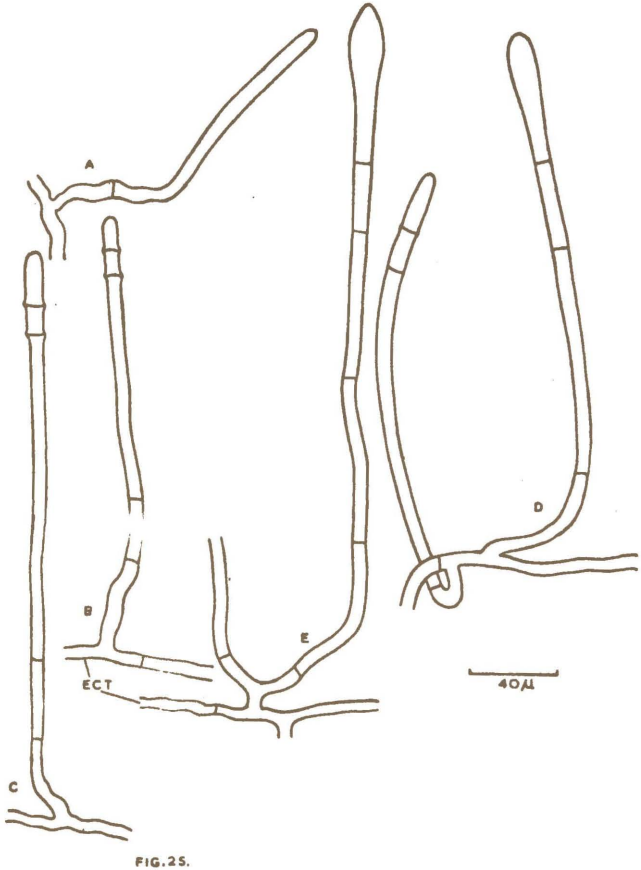


FIG. 25.
DEVELOPMENT OF CONIDIOPHORE OF P. CORYLEA FROM ECTOPHYTIC HYPHA (ECT.) AND FORMATION OF CONIDIUM.
A: YOUNG CONIDIOPHORE
B, C : CONIDIOPHORE WITH NEWLY FORMED CONIDIUM.
D, E : CONIDIOPHORE WITH DEVELOPING CONIDIUM.

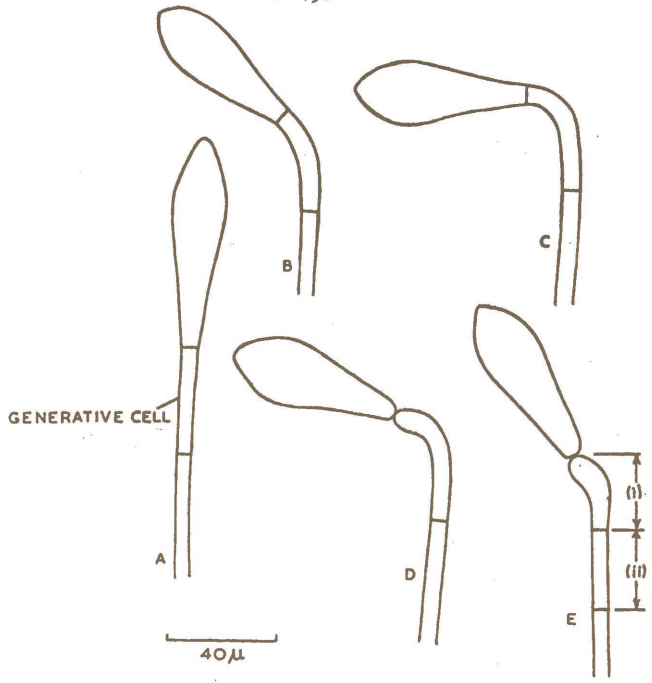


FIG. 26.
CAMERA LUCIDA DRAWINGS OF THE DISTAL ENDS OF DIFFERENT CONIDIOPHORES OF P. CORYLEA SHOWING VARIOUS ACTIVITIES OF THE GENERATIVE CELL:
(A) ERECT GENERATIVE CELL OF NEWLY-FORMED CONIDIUM.
(B) BENDING GENERATIVE CELL.
(C) FULLY BENT GENERATIVE CELL.
(D) ROUNDING-OFF OF BASAL WALL OF CONIDIUM AND APEX OF FULLY BENT GENERATIVE CELL.
(E) GENERATIVE CELL DIVIDED INTO TWO FORMS AN UPPER CONIDIUM-INITIAL(i) AND A LOWER NEW GENERATIVE CELL (ii).

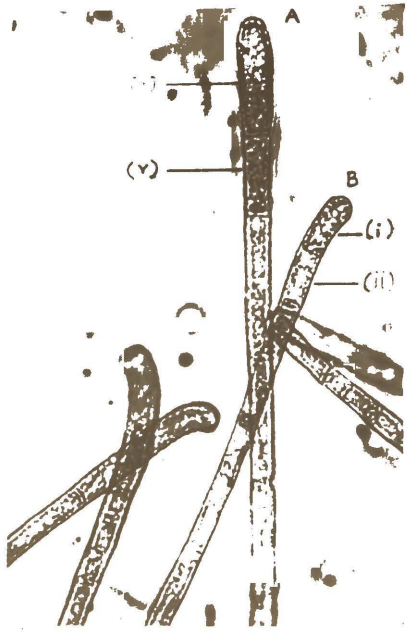


PLATE 20: Photomicrograph of stained apical regions of conidiophores of P. corylea showing B. Transversely dividing bent generative cell to give rise to second generation of spore (i) and new generative cell (ii). A. Young conidiophore with initial conidium portraying heavy concentration of protoplast in the generative cell and developing conidium.

X 400

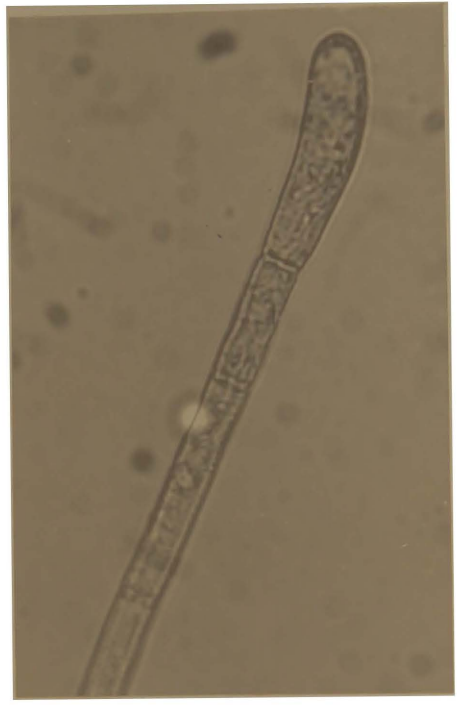


PLATE 21: Photomicrograph of stained apical region of conidiophore of P. corylea showing development of conidium from the bent generative cell. Terminal curved cell is the new conidium just abstricted from the generative cell.

X 800

b. FORMATION AND DEVELOPMENT OF THE CONIDIUM

The generative cell divided transversely into two, the lower cell persisting as the generative cell and the upper cell developing into the characteristic club-shaped conidiur, on the whole, with a diamond-shaped look (Fig. 25, E). The conidia measured 58.7 - 132.0 µ x 18.0 - 28.7 µ and on the average 92.0 x 24.0 µ (Fig. 27). A conidium took 24 hours under optimal conditions to develop fully after it had been delimited by the generative cell. Full maturity of the conidium was indicated by the simultaneous rounding up of the distal wall of the generative cell and the basal wall of the conidium (Plate 26).

Features of the conidiophore and pattern of its development and conidium formation observed in these studies are similar to those found by other workers (Ressencourt, 1927; Foex, 1926).

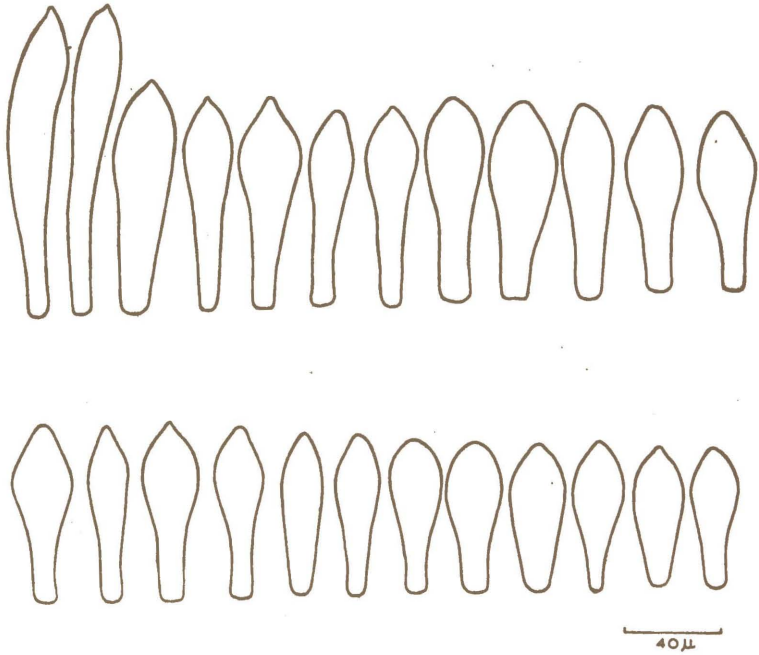


FIG.27. RANGE OF CONIDIAL SIZE AND SHAPE IN P. CORYLEA.

c. RELEASE OF MATURE CONIDIUM

Under normal laboratory conditions the mature conidium was invariably released from the conidiophore. Investigations here have shown that conidium release was due to the combined effect of wind action and fascinating structural changes in the conidiophore.

Immediately after the conidium had fully matured the generative cell began to bend. This was accompanied by the rounding up of the basal wall of the conidium and upper end-wall of the generative cell. The plane of bending was along either the midrif or more commonly slightly above it. Bending continued until the two arms of the generative cell stood at right angles to each other (Plates 23, 24, 25, 26 and 27 and Fig. 26, A - D). The conidium was in the event swung from the vertical to a horizontal position. The force behind this, however, was not sufficiently great to completely dislodge the spore but enough to loosen the attachment and render the conidium liable to detachment on the slightest disturbance.

It was found necessary to confirm that bending of the generative cell alone was insufficient to cause spore detachment. Further observations were made on diseased leaves in a laboratory with all windows shut to create a condition of still

air. The peculiar behaviour of the conidiophore described above was once again observed but unlike those in the previous experiment in laboratory with open windows the conidia remained perched, though precariously, at the curved conidiophore tips (Fig. 28 A - G).

The effect of wind was next tested by suddenly opening the windows. The conidia were readily blown off the conidiophores leaving a crop of conidiophores with the characteristic bent generative cells (Plate 28).

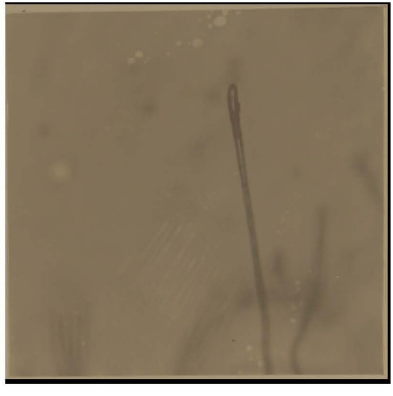


PLATE 22: Photomicrograph of young conidiophore of P. corylea showing developing first conidium subtended by erect generative cell.

X 190

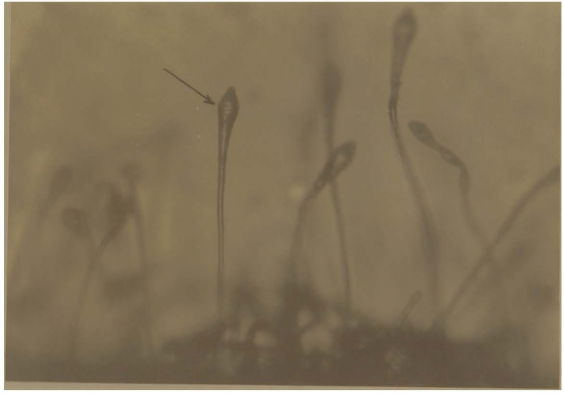


PLATE 23: Photomicrograph of conidiophore of P. corylea bearing fully developed conidium subtended by erect generative cell (arrowed).

X 190

PLATE 24: Photomicrograph of conidiophore of P. corylea showing initial stages of bending of the generative cell after conidium has fully developed. Note the initial stages of constriction between conidium and generative cell.

X 190

PLATE 25: Photomicrograph of conidiophore of P. corylea in the process of bending. Note the appearance of constriction between conidium and bent generative cell.

X 190

PLATE 26: Photomicrograph of conidiophore of P. corylea with fully bent generative cell and with complete rounding up of basal wall of conidium and apical wall of generative cell. Note plane of curvature about midrif of generative cell.

X 190

PLATE 27: Photomicrograph of conidiophore of P. corylea with fully bent generative cell. Note that plane of curvature lay above the midrif of generative cell.

X 190

PLATE 28: Photomicrograph of conidiophores of P. corylea immediately after shedding the conidia. Note the characteristic curved tips of the conidiophores - portraying the bent generative cells.

X 75

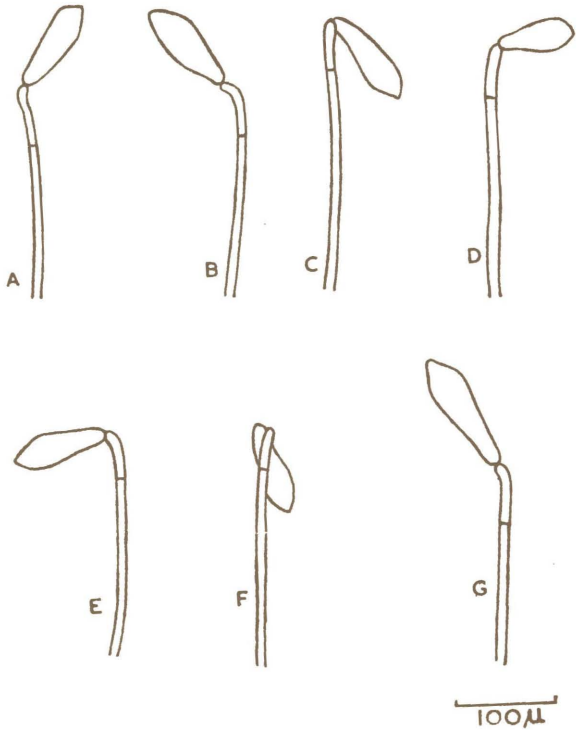


FIG.28.
CONIDIOPHORES OF P.CORYLEA SHOWING VARIOUS PRECARIOUSLY-PERCHED MATURE CONIDIA ON BENT CONIDIOPHORES.

d. FORMATION OF SUBSEQUENT CONIDIA

It was observed under low power magnification that a succeeding conidium was formed immediately after the mature conidium had fallen off. Details of development were more distinctly observed in stained specimens under high power magnification. Epidermal strips of diseased leaves were removed and stained with either lactophenol cotton-blue or 5 per cent iodine in potassium iodide. Observations were once again recorded by means of photomicrographs and camera lucida drawings.

Conidiophores examined for prolonged periods under the microscope during developmental studies were in reality hanging in air. Those therefore obtained in perfect focus at the beginning of an observation were found to have varyingly moved out of focus during growth, invariably attaining different planes. These could never be brought into focus together. When several conidiophores therefore portrayed perfect features deserving recording, it was found necessary to photograph the same view a number of times moving the microscope objective to bring the different conidiophores into focus in turn.

The bent generative cell divided transversely (Plate 20: B and Fig. 26: E) in a way to include the curved section in the

upper sector, the conidium initial. The bent upper cell then gradually developed into a normal-shaped conidium (Plate 21 and Fig. 29: A - F). The generative cell immediately bent again on maturity of the conidium. The sequence of events from the development of a new conidiophore, through the formation of the first conidium, bending of the generative cell, release of the conidium and development of the succeeding conidium is fascinatingly portrayed in the time series photomicrographs of Plates 29 - 31 and camera lucida drawings in Fig. 30: A and B.

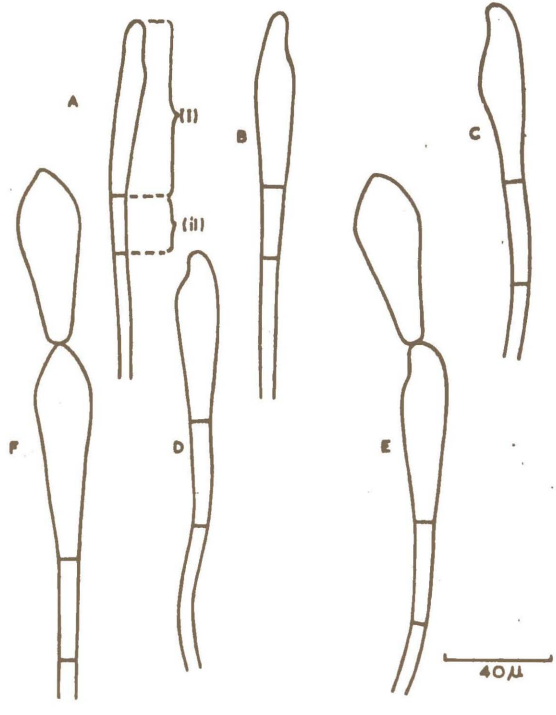


FIG. 29.
CAMERA LUCIDA DRAWINGS OF DISTAL ENDS OF DIFFERENT CONIDIOPHORES OF P. CORYLEA SHOWING STAGES OF DEVELOPMENT OF CONIDIUM FROM BENT GENERATIVE CELL AFTER DIVIDING INTO (I) CONIDIUM INITIAL AND (II) GENERATIVE CELL (A)- (E) PROGRESSIVE SWELLING OF BENT CONIDIUM INITIAL. (F) FULLY-FORMED CONIDIUM.

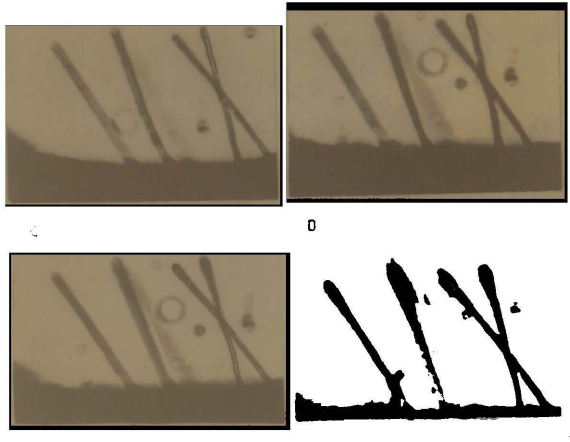


PLATE 29: Photomicrographs of conidiophores of P. corylea showing development of conidia with time.

A: Taken 20TH MARCH 1968, 6.00 A.M. Conidiophores with initial conidia.

B: Taken 20TH MARCH 1968, NOON. Developing conidia.

C: Taken 20TH MARCH 1968, 6.00 P.M. Developing conidia.

D: Taken 21ST MARCH 1968, MIDNIGHT. Developing conidia.

X 150

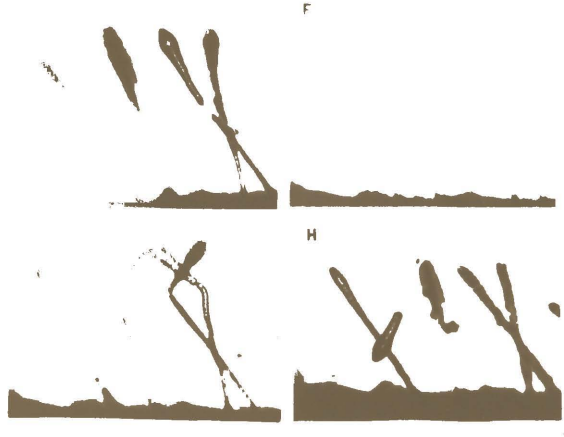


PLATE 30: Photomicrographs of conidiophores of P. corylea showing bending of the generative cells after formation of the conidium and later release of the conidia.

E: Taken 21ST MARCH 1968, 6.00 A.M. First generation conidia fully developed.

F: Taken 21ST MARCH 1968, NOON. Bent generative cells.

G: Taken 21ST MARCH 1968, NOON. Bent generative cells. Same as F but taken to bring other conidiophores into focus.

H: Taken 21ST MARCH 1968, 6.00 P.M. Release of conidia and development of succeeding conidia. Note one detached conidium lying over conidiophore.

X 150

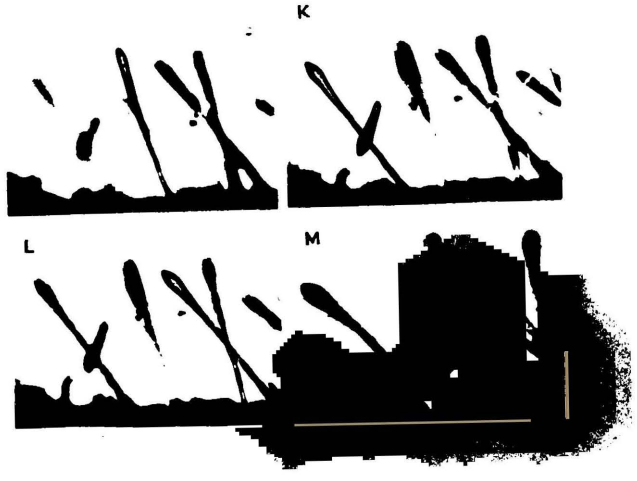


PLATE 31: Photomicrographs of conidiophores of P. corylea showing development of succeeding conidia.

J: Taken 22ND MARCH 1968, MIDNIGHT. Developing conidia.

K: Taken 22ND MARCH 1968, 6.00 A.M. Developing conidia.

L: Taken 22ND MARCH 1968, 6.00 A.M. Developing conidia. Same as K but taken to bring other conidiophores into focus.

M: Taken 22ND MARCH 1968, NOON. Second generation conidia fully developed.

X 150

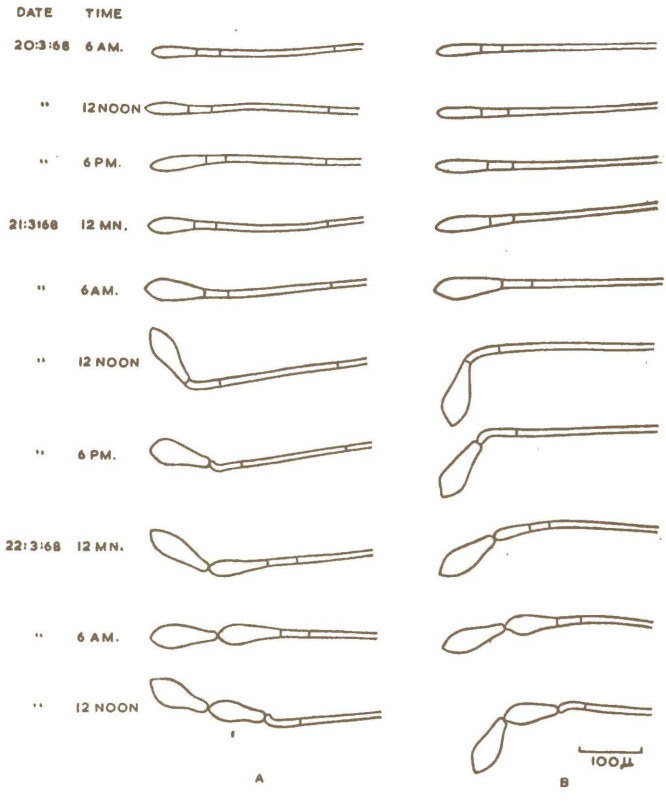


FIG. 30.
CAMERA LUCIDA DRAWINGS OF DEVELOPING CONIDIUM OF P. CORYLEA AND ASSOCIATED BENDING OF THE GENERATIVE CELL AT 28°C.

e. FORMATION OF CONIDIAL CHAINS

If wind or physical disturbance was essential in spore detachment in P. corylea it would be possible then to prevent spore release and obtain conidial chains in still air. Diseased leaves were prepared again in the usual manner in a room with all windows shut and the conidiophores were again observed under low power magnification. Indeed, under still air, P. corylea freely formed conidial chains (Plates 32, 33 and 34 and Fig. 31: A - H). It was found out that the mature conidium though precariously perched on the bent generative cell might remain undetached until a succeeding conidium had fully developed, resulting in a conidial chain. Under such conditions, however, the alternating swinging of the conidia from the upright position to the horizontal and back to the upright as succeeding conidia were abstricted, was sufficient to break up the rather dangling chain, when it attained a certain minimum length. Conidial chains, therefore, formed in still air during extensive studies, most commonly contained only two spores (Plate 35) and at most four.

Failure of the conidiophores of P. corylea to form conidial chains in the field might not be as absolute as reported by earlier workers (Ressencourt, 1927; Viennot-Bourgin, 1944). In fact, conidial chains could be formed in the field on conidiophores occurring

in sheltered spots. Thus those shielded from the wind by other conidiophores or growing on the leeward side of a prominent vein usually produced conidial chains. Indeed, during these studies conidial chains were a common occurrence and with each spore sample obtained on glass slides for germination studies conidia held in chains made up between 20 and 25 per cent of the entire spore population.

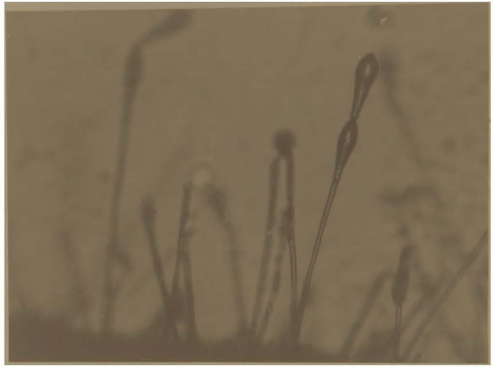


PLATE 32: Photomicrograph of conidiophores of P. corylea kept in sealed room showing conidial chains (2 spores).

X 190

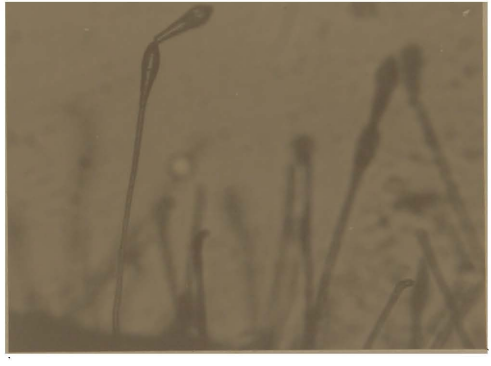


PLATE 33: Photomicrograph of conidiophores of P. corylea kept in sealed room showing conidial chains. Photograph of same conidiophores in Plate 32 taken to bring other conidiophores into focus.

X 190

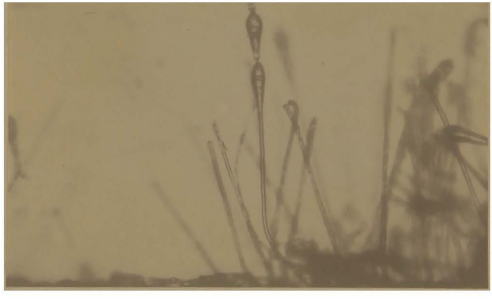


PLATE 34: Photomicrograph of conidiophore of P. corylea kept in sealed room showing conidial chains (3 spores).

X 150

PLATE 35: Photomicrograph of spore print of conidia of P. corylea kept in sealed room. Note the numerous conidial chains which commonly contain two spores.

X 100

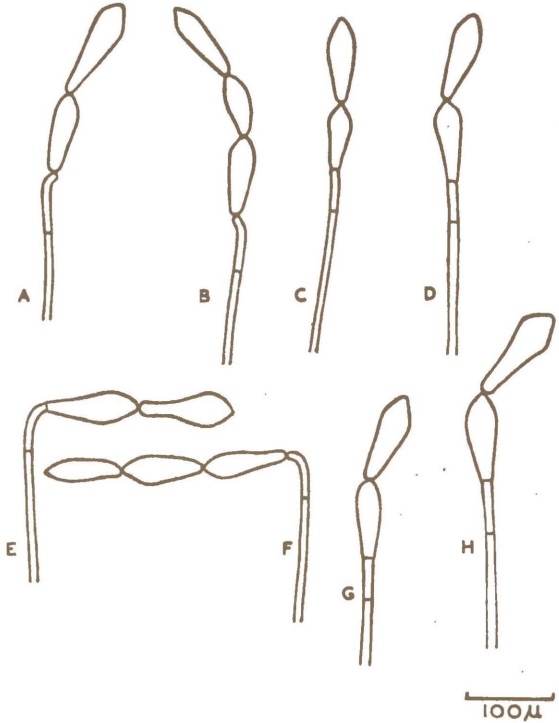


FIG. 31.

CONIDIOPHORES OF P. CORYLEA FORMED ON LEAF OF C. PAPAYA INCUBATED IN SHELTERED CONDITIONS SHOWING VARIOUS PRECARIOUSLY PERCHED CONIDIAL CHAINS. NOTE DIFFERENT ANGLES OF INCLINATIONS OF CHAINS DUE TO DIFFERENT STAGES OF BENDING OF SUB-TENDING GENERATIVE CELL.

f. PERIODICITY OF CONIDIUM DEVELOPMENT

During preliminary studies which preceded the major investigations reported here, it was observed that scanty conidia were obtained at any time of the day except in the mornings. Spores for germination tests were therefore, as mentioned earlier (see page 23) removed from the diseased leaves in the mornings only. It was possible that greater proportion of the conidia matured in the night to provide this morning heavy crop of spores. Any periodicity in maturation of the conidia could best be established counting trapped P. corylea conidia using a spore trap, preferably Hirst spore trap over a complete period of 24 hours for several days. Since this trap was not available in our laboratories, maturation of the conidia was examined directly under the microscope.

Three diseased leaves, again still attached to the stem, were individually held in the usual manner onto the microscope stage and proliferating conidiophores were observed at 6- hourly intervals. Three categories of conidiophores were studied. Conidiophores with newly delimited conidia were selected at 6.00 a.m., noon and 6.00 p.m. and the rates of development of the respective groups of conidia were examined.

Generally, conidia delimited at 6.00 p.m. developed rapidly in the night to become fully mature within 12 hours, whilst development

was slower in the remaining groups where conidia delimited at 6.00 a.m. and noon showed little development in the day followed by more rapid rate of development in the night. The conidia therefore took approximately 24 and 18 hours respectively to mature. Fig. 32: A, B and C are typical of numerous observations made.

Apparently, the conidia of P. corylea developed quicker in dark than in light imposing a periodicity on conidium maturation.

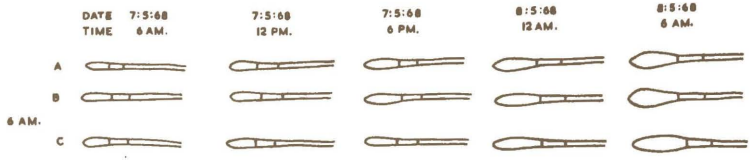


FIG.32. A. CONIDIOPHORES INCUBATED INITIALLY AT 6-00 AM.

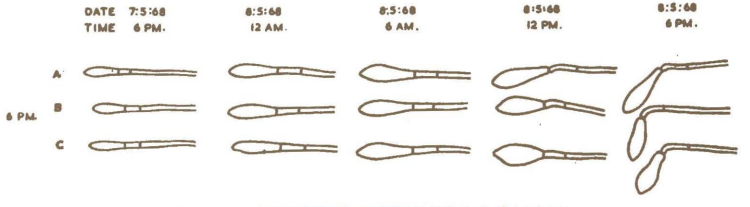


FIG.32. B. CONIDIOPHORES INCUBATED INITIALLY AT 6-00 PM.

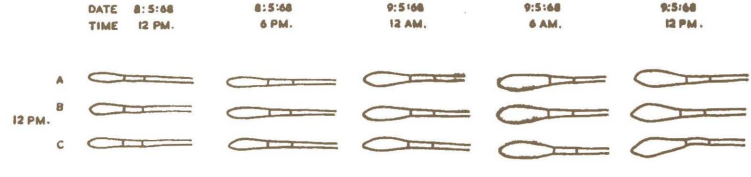


FIG.32. C. CONIDIOPHORES INCUBATED INITIALLY AT 12-00 PM.

FIG.32. CAMERA LUCIDA DRAWINGS OF DEVELOPING CONIDIA OF P.CORYLEA AT DIFFERENT TIMES OF DAY AT 27±1°C.

N. FURTHER OBSERVATIONS ON CONIDIOPHORE BEHAVIOUR

There was overwhelming evidence that bending of the generative cell of the conidiophore of P. corylea was a developmental phenomenon. It is likely that some environmental factors may exert some influence on the rate and degree of bending of the generative cell which will give a clue to the mechanism involved. The relationships of temperature, moisture and unilateral light to bending of the generative cell were investigated.

a. EFFECT OF TEMPERATURE ON BENDING OF THE GENERATIVE CELL

Infected pawpaw (C. papaya) leaves still attached to the plants were again held in position on the microscope stage in the usual manner for observations on the generative cell. Two similar experiments were set up, one in the laboratory with a temperature of 28°C. and the other in an air-conditioned room with a temperature of 20°C.

A number of newly formed conidiophores under view in each case was selected for observation. Camera lucida drawings were made of these as soon as the first conidia were fully formed and at hourly intervals thereafter until a fully bent generative cell was obtained. From the numerous observations made of which camera lucida drawings in Figs 33: A, B; 34: A, B are typical, it seemed that the generative cells bent at the same rate and to the same degree at 20 and 28°C.

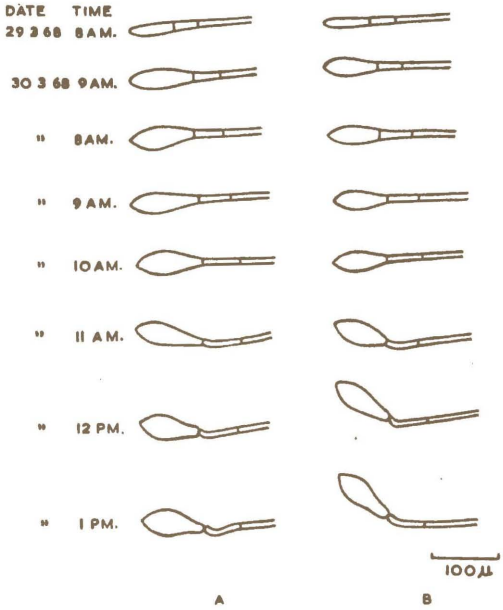


FIG. 33.
CAMERA LUCIDA DRAWINGS OF DEVELOPING CONIDIUM OF P. CORYLEA AND ASSOCIATED BENDING OF THE GENETIVE CELL AT 20° C.

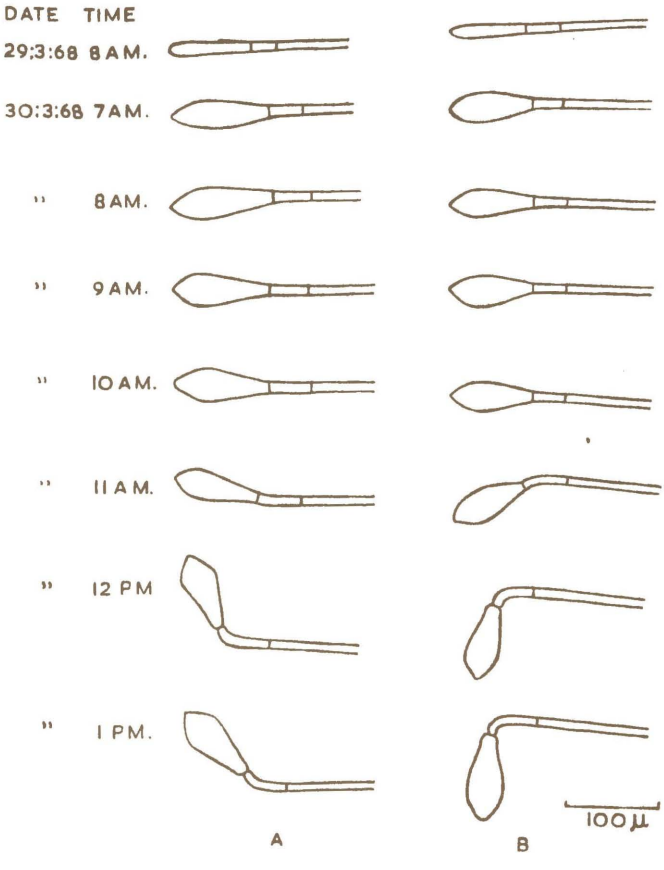


FIG. 34.
CAMERA LUCIDA DRAWINGS OF DEVELOPING CONIDIUM OF P.CORYLEA AND ASSOCIATED BENDING OF THE GENERATIVE CELL AT 28°C.

b. EFFECT OF MOISTURE ON BENDING OF THE GENERATIVE CELL

If moisture plays a role in the bending mechanism, that is, the generative cell either loses or takes in water during bending this may be accordingly affected by different moisture levels. Conidiophores on diseased leaves were again kept under investigation in the usual manner in the laboratory (temperature 28°C.). Two experiments were set up. One experiment was under a plastic hood which had previously been sprinkled with water to provide an atmosphere of 100% R.H. The other remained uncovered and was placed near a table fan to keep the humidity slightly lower than atmospheric humidity (atmospheric humidity at time of experimentation 76% R.H.). The conidiophores were again observed at hourly intervals after the conidia had developed and the bending was recorded by means of camera lucida drawings. The hood over the microscope was lifted for drawings to be made and quickly put back. Generative cells under the two moisture regimes bent at the same rate and attained the same degree of curvature.

If curvature was due to influx of water into the generative cell, it could possibly might have been transferred from the mycelium through the conidiophore into the generative cell. Under such conditions atmospheric moisture might not be directly involved. An experiment was therefore next designed to investi-

gate further the effect of moisture.

Pieces of mildewed pawpaw leaves bearing conidiophores with fully bent generative cells were individually placed in sucrose solutions of 0.5, 1.0 and 2.0 M and in distilled water. If bending involved water intake, the generative cells would be straightened in the stronger sucrose solutions. If, on the other hand, loss of water is involved in the bending mechanism, generative cells in distilled water would absorb sufficient water to straighten out or at least considerably alter the extent of curvature.

Several observations, an example of which is presented in Table 24 indicated that distilled water did not alter the curvature of generative cell.

TABLE 24: Degree of curvature of bent generative cell of conidiophore of P. corylea placed in distilled water at 28°C.

Time of observation in Hours (GMT)	Curvature of Generative Cell in degrees		
	A	B	C
06.00	130	145	120
08.00	127	150	125
10.00	125	145	120
12.00	130	150	118
14.00	127	145	119
16.00	133	145	120
18.00	130	144	120

c. EFFECT OF LIGHT ON THE BENDING OF THE GENERATIVE CELL

Although fungi lack chlorophyll, it is known that radiant energy influences rate of growth and that species in which growth or elongation is localised might be affected by light. The problems of the nature of the stimulus leading to the curvature of some organs of fungi have long interested many mycologists.

Phototropic responses are common in fungi, examples include the orientation of basidiomycete sporophores (Borris, 1934; Streeter, 1909; Walker, 1927), of asci (Backus, 1937; Brefeld, 1877; Buller, 1934), of sporangiophores (Castle, 1931; Flint, 1942; Galston, 1950), and of conidiophores of powdery mildew (Domsch, 1953).

The following experiments were set up to find out whether (a) light is necessary for the bending of the generative cell and (b) light affects the direction of bending.

1. EFFECT OF LIGHT AND DARK ON BENDING OF THE GENERATIVE CELL

Two experiments were set up. Conidiophores of P. corylea on mildewed pawpaw leaves were kept under observation under two microscopes. As soon as the conidia matured one microscope was completely covered with black cloth whilst the other was kept under continuous light provided by a fluorescent light. No observations were made during the course of bending as it was likely intermittent exposure may affect the dark treatment. The conidiophores were examined after 1 hour, when it was found that the generative cells of all conidiophores bearing mature conidia in both light and dark had fully bent. It seems that the generative cell is capable of bending in both light and dark.

ii. EFFECT OF LIGHT ON DIRECTION OF BENDING OF THE GENERATIVE CELL

Phototropic response of the bending generative cell of P. corylea was examined by admitting white light into a box containing the diseased leaf through a hole in the side of the box. A rectangular plastic, transparent, 8.5 inches long, 5.8 inches broad and 1.6 inches deep, with a replaceable lid (Figs 35 and 36) was painted all over with black paint except a rectangular area of 0.6 by 0.4 inches on one vertical side which acted as a "window" to admit unilateral light into the box. A diseased leaf of a potted pawpaw plant still attached to the plant was carefully placed in the box with abaxial side facing upwards and held in position unto the floor of the box with cellotape. The petiole of the leaf passed through a special hole in the box prepared for this purpose.

Diseased lesions lying directly in the path of the unilateral light were noted and used in all observations. The lid of the box was then replaced and screwed down. Any space found between the wall of the hole admitting the petiole and the petiole was sealed with dark cotton wool.

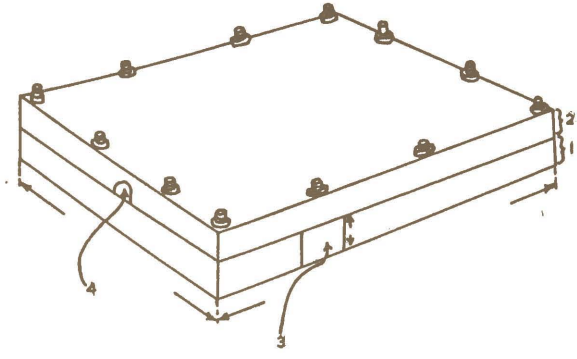


FIG. 35. APPARATUS FOR DETERMINING THE EFFECT OF LIGHT ON THE DIRECTION OF BENDING GENERATIVE CELL OF P.CORYLEA.

1. THE BOX IN WHICH LEAF WAS INSERTED.
2. LID OF THE BOX.
3. UNPAINTED PART OF BOX TO ADMIT UNILATERAL LIGHT-"WINDOW".
4. HOLE THROUGH WHICH PETIOLE OF LEAF WAS INSERTED.

FIG. 36. DIAGRAM OF SECTION OF LEAF CHAMBER ALONG PETIOLE-MIDRIB AXIS TO SHOW POSITION OF LEAF

1. PETIOLE OF LEAF.
2. ABAXIAL SURFACE OF LEAF.
3. CONIDIOPHORES.
4. LEAF CHAMBER.

A lighted Radium electric bulb, 25W. was then placed 7 inches away in front of the "window" to provide a unilateral light. The experiment was left for 24 hours. After which the lid was removed and the leaf carefully transferred to a microscope stage and conidiophores of the marked diseased lesions which lay in the direct track of the light were examined under low power magnification to find out the direction of curvature of the conidiophores. Observations were made against a dark field in which the conidiophores appeared distinctly as pearly white structures. It was observed that the generative cells bent randomly in any direction and were never affected by the direction of light. The generative cells were non-phototropic.

It was however observed that the conidiophores were strongly phototropic and there was a distinct bending of the conidiophores towards the source of light. Several subsequent experiments were carried out to make a quantitative estimation of the phototropic response of the conidiophores.

Method of Estimating Phototropic Reaction

The method used by Graves (1916) during studies on chemotropism of germ tubes of Rhizopus nigricans was adopted and modified. During observations each microscopic view under low power was in each case delimited by a circle, 2 inches in radius

on a drawing sheet using a camera lucida, with the centre of that area coinciding with the centre of the microscopic view.

The direction of the conidiophores within this area was then recorded by drawing each observable conidiophore on the drawing sheet, and their direction of growth assessed from these drawings. The field on the drawing sheet was first divided into 4 quarters by two diameters running at right angles to each other with one diameter lying along the direction of the unilateral light. These four quarters were next bisected by appropriate radii, to divide the field into 8 equal sectors. Two adjacent sectors lying along each of the original radii were combined to form a unit - thus creating four equal areas or quarters as follows: (i) facing source of light - A (ii) directly away from source of light - D (iii) remaining two lying at right angles to path of light - B and C (Fig. 37).

Each conidiophore on the drawing sheet was then classified as A, B, C and D according to its direction of orientation.

The basis of the interpretation of the figures thus obtained lay in the assumption that the average number of conidiophores in each of the 4 classes under uniform illumination and supposing that no attractive or repellent force existed, should on the average be approximately the same. Any considerable deviation

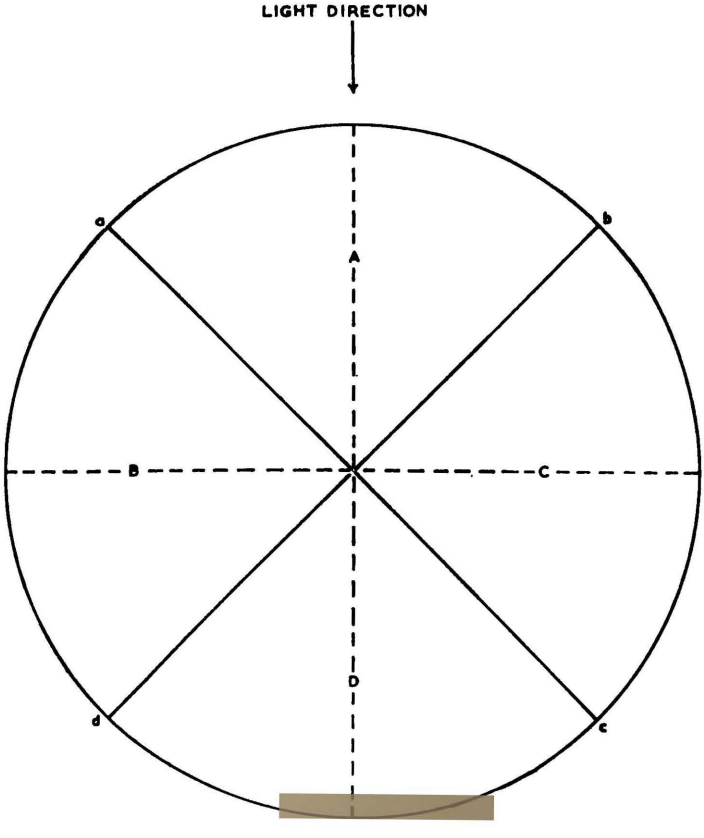


FIG. 37. METHOD OF ESTIMATION OF DIRECTION OF CONIDIOPHORES
A. TOWARDS LIGHT B. TOWARDS LEFT OF LIGHT.
C. TOWARDS RIGHT OF LIGHT. D. AWAY FROM LIGHT.

from that would indicate a reaction to a disturbing force. Since B and C regions were almost of equal number and equally subjected to repellent or attractive force, they might be left out of consideration or to be considered as the effect of the diffused light on both sides of the path of light.

One was concerned therefore with A and D classes. If it was assumed in the first instance that an attractive force existed which was causing the conidiophores to grow or bend towards the source of light making the number of the A class greater than the normal number, it could be concluded that those in excess of one-fourth had been attracted into that class. If the attractive force was not a very strong one, one might be certain that the increase had been derived from those which would normally point to B or C. The number of A's in excess of the normal, however, did not give a complete measure of the attraction, for in all probability some conidiophores originally in the D class had turned into B or C direction, and the remaining D's would be fewer than one-fourth of the total. The difference between the remaining D's and the normal number would then represent those which had turned from the D direction into B or C. By adding that number to those in excess of the normal in A, the total number affected could be arrived at. In order to get

the total percentage of reaction, that number was divided by $\frac{3}{4}$ of the total number of conidiophores counted in the whole area, since only $\frac{3}{4}$ of the conidiophores were dealt with, the $\frac{1}{4}$ represented by the normal A's being unaffected.

Let "n" ($\frac{1}{4}$ of the total) denote the normal number to be expected in each region, the number actually obtained in each region by its corresponding letter, and the total number of conidiophores in the whole area "t", the formula as derived by Graves (1916) thus reads as follows:

$$\frac{(A - n) \pm (n - D)}{\frac{3}{4}t}$$

$$= \frac{A - D}{\frac{3}{4}t} = \text{fraction of conidiophores reacting.}$$

In case a repellent force existed, raising the number of D's above the normal, the final percentage would then be a minus quantity.

The results in Table 25 show that there was difference between the number of "A" and "D" conidiophores. Evidently the conidiophores responded to unilateral light, and the conidiophores of P. corylea are positively phototropic. The consistently high values of percentage reaction obtained were clearly significant. Every treatment showed a consistent positive percentage reaction.

TABLE 25: Phototropic effect of unilateral light on conidiophores of **P. corylea**.

Experiment Number	Direction of conidiophores				Total Number in each Area	% Reaction
	A	B	C	D		
	Towards	Left	Right	Away		
1	519	31	102	36	688	+ 93
2	701	113	100	25	939	+ 96
3	1,700	293	213	78	2,284	+ 94
4	650	103	124	24	901	+ 92
5	232	58	56	23	369	+ 76
6	335	106	65	19	525	+ 80
7	311	54	43	30	434	+ 86
8	119	16	29	20	184	+ 72
Sum of Conidia in each class	4,567	774	732	255	6,328	

One interesting feature was the regular occurrence of greater number of conidiophores in class "A" than in class B, C and D put together. Class B and C also showed in the majority of cases equal number of conidiophores.

V. GENERAL DISCUSSION

The powdery mildew Phyllactinia corylea occurs extensively in tropical and subtropical regions with a very wide host range. It has been found most commonly on the mulberry plant (see page 8). This fungus is certainly of economic importance. At present though it is only found on pawpaw (C. papaya) in Ghana its significance in this country becomes more profound with the current introduction of mulberry in connection with the silk industry. Some factors affecting germination of the conidia of P. corylea, the major infection units, have been studied in addition to some aspects of the biology of this fungus.

The climatic factors affecting the germination of the conidia studied here are temperature, humidity and light. Temperature relation of the powdery mildews has been reviewed by Yarwood, Sidky and Cohen (1954). In their review, it was reported that the optimum temperature for powdery mildews ranged from 11 to 28°C. depending on the species and the average was 22°C. Manners (1963) stated that the percentage germination of conidia of E. graminis was appreciable at all temperatures employed up to 25°C. Conidia of P. corylea were germinated at the temperatures 15, 20, 25, 30 and 35°C., a range which covers that found in Ghana. The conidia germinated best at 25°C. where

60.1 per cent germinated in 36 hours (see Tables 3 to 7). The germ tubes on the other hand grew best at 30°C. Frick (1943), Gaumann (1946) and Saccas (1951) pointed out that germ tube growth and conidial germination may respond alike to temperature, and this, indeed, is a common feature in fungi. Cochrane (1945) and Yarwood et al. (1945) have, however, also indicated that there may be pronounced differences in germ tube growth and percentage germination of conidia in relation to temperature. In this studies, percentage germination and germ tube growth showed parallel temperature characteristics except at 25 and 30°C. These results suggested that the optimum temperature most probably lay between 25 and 30°C., and it was possible that a temperature level between these two would support together the highest percentage germination and best germ tube growth. Indeed, this assumption was proved in subsequent experiments when the conidia were incubated at the laboratory temperature of 28°C. Conidia held at 100% R.H. and at 28°C. showed 81.1 per cent germination and a mean germ tube length of 138.4 μ in 24 hours (see Table 11) whilst at 25 and 30°C. and at 100% R.H., the respective percentage germination and mean germ tube lengths were 58.9 and 44.3 per cent and 92.9 and 97.1 μ within the same period (see Tables 5 and 6). The optimum temperature

for the germination of the conidia was therefore either 28°C. or very close to it. This is considerably higher than the average of 22°C. reported for powdery mildews which may be due to its tropical origin. Temperature would never be a limiting factor during disease establishment in this country where the atmospheric temperature is quite uniform and mostly 28°C. The development of P. corylea has indeed been found to have occurred best disease under such temperature conditions. Bagchee (1952) reported that the attack of teak by P. corylea became epidemic under conditions of high temperature and humidity. Further work using a wider range of temperature will be necessary to obtain the maximum and minimum temperatures for germination of the conidia.

Findings on the moisture requirements of germinating conidia of P. corylea reported here make the first recorded pertinent information. Extensive reports indicate that most powdery mildews have the ability to germinate at very low humidities, even over a desicant (Cherewick, 1944; Clerk and Ayesu-Offei, 1967; Nour, 1958; Yarwood, 1936, 1937). Very few species among these, however, show any substantial germination at the lower humidities (Yarwood, 1936) and the role this ability to germinate at low humidity in most species plays in epidemiology of disease

is doubtful. Results of the present investigations have shown that conidia of P. corylea germinate at all relative humidities from zero to 100% R.H. (see Tables 8 to 11). This is another evidence of the ability of the conidia of a powdery mildew to germinate at extremely low humidities. It is very fascinating to note here that, unlike most of the species of Erysiphaceae, germination of conidia of P. corylea was surprisingly uniform and very substantial at any humidity from zero to 100% R.H. in atmospheres whose humidities were maintained with either saturated aqueous salt solutions or sulphuric acid solutions. The percentage germination of conidia of P. corylea at 0% R.H. of 79.4 per cent observed in one of the experiments (see Table 11) seems to be the highest germination at this humidity recorded among the Erysiphaceae. No particular humidity level could therefore be considered as optimum with regards to conidial germination in P. corylea since germination was so uniform and even the rates of germination (see Table 15) were similarly identical. It was however possible to establish this by the amount of germ tube growth supported. Germ tubes were always longest at 100% R.H. (99.1 - 138.4 μ) and shortest at 0% R.H. (17.3 - 27.2 μ) (see Tables 8, 10 and 11).

With species such as P. corylea in which lower humidities permit sufficiently high conidial germination, considerable infection units could be mobilised under a wide range of atmospheric humidity conditions. Many workers claim that in general the powdery mildews spread rapidly under dry climatic conditions (Volk, 1931; Honecker, 1936; Cherewick, 1944; Last, 1955). This is however not always so, for the powdery mildew, L. taurica has been observed to occur abundantly in the wet season in Ghana and very sparsely in the dry season (Ayesu-Offei, 1966). The ability of the conidia of P. corylea to germinate very well at extremely low humidities probably explains the persistence of the disease on pawpaw plants throughout the year, and flourishes in the rainy as well as in the dry (harmattan) seasons in this country. Furthermore, the conidia of P. corylea could germinate very well under fluctuating atmospheric humidities, thus seemingly well suited for its role as infection units.

The virtually unique property of germination at zero relative humidity confers on the powdery mildew fungi considerable theoretical importance. This ability to germinate at very low relative humidities has received two interpretations. Comparative studies of the water content of most fungal spores is low. For example, Yarwood (1950) found that the water content

of spores of Penicillium digitatum and Aspergillus niger were only 6.0 and 13.0 per cent respectively. Yarwood, on the other hand, found that the water content of conidia of the powdery mildews E. polygoni, E. graminis and E. cichoracearum ranged from 52 to 75% of their fresh weight. Apparently, the low water content of most fungal spores demands presence of external water for germination. Yarwood (1950) suggested that the high water content of powdery mildew conidia, on the other hand, may explain their ability to germinate at low relative humidities. He argued that there is enough water in the conidium to initiate germination. His observations of shrinking conidia of the three species during germination at low humidities was put forward to support his claim. The shrinkage of the thin delicate walls of the conidia was thought to be due to fall in water level in the conidium. Yarwood (1952) working again on E. grminis substantiated his earlier propositions. He observed that germinating conidia lost more water than non-germinating conidia and that while both germinating and non-germinating conidia shrank in a dry atmosphere the germinating spore shrank at a faster rate.

Brodie and Neufeld (1942) had earlier presented an alternative interpretation. They contended that the mature conidia of E. polygoni and E. graminis contain very little water before commencement of

germination but water is released through changes in colloidal materials containing bound water during respiration. They claimed that no shrinkage was observable in conidia germinating at the lower humidities. Brodie (1945) further suggested that the high osmotic pressure of the conidia of E. polygoni and E. graminis could enable them to absorb water from relatively dry atmosphere, hence their ability to germinate at these humidities. It is however highly improbable that conidia will find sufficient moisture to absorb at extremely low humidities and this hypothesis fails completely to account for germination over desiccants (zero per cent relative humidity) where the atmosphere is completely devoid of moisture.

Observations made in the present work favour Yarwood's (1950) hypothesis that the source of water for germination of the conidium of the powdery mildew at low humidity is stored water in the conidium. P. corylea conidia at any humidity germinated to the same degree, probably depending entirely on endogenous water supply. On the other hand, those at the higher humidities showed longer germ tubes, may be sustained by external moisture whilst those produced at the lower humidities remained short because of lack of additional water supply from the atmosphere.

Besides, germinating and non-germinating conidia of P. corylea did not shrirk when incubated at 100% R.H., whilst varying percentages of the conidia shrank at relative humidities below 100% R.H. The percentage of conidia shrinking at the higher humidities was small and increased at the lower humidities (see Table 8). This shrinkage has been accepted as evidence of loss of water, a hypothesis consistent with observations here. Yarwood (1952) has ruled out the possibility that a volatile substance escaping from the spore might be the causative factor of this collapse. He argued that this then could have taken place at lower humidities as well as 100% R.H. Most probably the germinating conidia obtained abundant external supply of water at the higher humidities, and need not be sustained solely by the internal water. It can be concluded from results of this work that the shrinkage may be due both to loss of water by the spore to the air, especially in non-germinated conidia, and to use by the conidium for germination activities. Yarwood (1952) did indeed observe that germinating conidia of E. polygoni showed a greater decrease in volume than shrunken non-germinated conidia. The physiology of germination of the spores has not been studied, and until this is done, knowledge of metabolic activities involved in germination of powdery mildew

conidia will be a matter for conjecture.

Many workers (e.g. Graf-Marin, 1934; Yarwood, 1936; Clayton, 1942; Grainger, 1947) have stated that germination of conidia of the Erysiphaceae is poor at 100% R.H. and that the optimum relative humidity lies at a level slightly below saturation. The present observations (see Tables 10 and 15) and the results of Nour (1958) on L. taurica and those of Manners and Hossain (1963) working on E. graminis and of Ayesu-Offei (1966) also working on L. taurica indicate that 100% R.H. is the most favourable level for germination of these conidia if care is taken to exclude liquid water. It is only when condensation occurs copiously that poor germination obtains. A supporting evidence is provided by data on germination of the conidia of P. curylea sown in water (see Table 8). It will be useful to examine further this aspect of the physiology of the germinating spore. Corner (1935), Chorowick (1944) and Dickinson (1949) have observed the same behaviour in conidia of other species of Erysiphaceae when sown in liquid water. Lowered germination may be due to either lack of oxygen or disruption of osmotic balance of the spore due to entry of water. Evidences at hand are in favour of the latter. Jhooty (1967) was able to germinate conidia of E. polygoni at 6% O_2, which is much less than the 20% O_2 of

the air. Similarly, Allen (1955) has demonstrated that uredospores of Puccinia graminis var. tritici were able to attain maximum or near maximum germination at 30 - 38 mm of O_2. Conidia of Botrytis cinerea were able to germinate normally at 5% O_2 (Brown, 1922). Therefore it appears that reduced oxygen concentrations below atmospheric level are adequate for the germination of these conidia, and so it may be, for other conidia. Again high P_{CO_2} had an inhibitory effect on germination of conodia of E. polygoni (Jhooty, 1967) but it became pronounced only at very high levels of CO_2, when compared with natural CO_2 level of the atmosphere. The chances of O_2 and CO_2 levels reaching inhibitory levels when condensation occurred on slides around the conidia of P. corylea are practically nil, and besides conidia germinated in water did so on a shaker which ensured sufficient aeration. Decreased germination under these circumstances was most likely not caused by either accumulation of CO_2 or lack of O_2. Delp (1954) also provides another evidence of disruption of physiological balance of powdery mildew spore in water. Jhooty reported that conidia of grape powdery mildew burst in pure water rather soon after immersion.

No information was found in the pertinent literature on the pattern of germination of conidia of P. corylea. Various

observations on germinating conidia of powdery mildews have been recorded and this has been outlined at pages 24 - 41.
The latent period of germination for P. corylea conidia was 2 - 3 hours at 25°C. in atmospheres of zero to 100% R.H. They therefore behave similarly in this respect as many other powdery mildews. In P. corylea the germ tubes emerged more frequently from variable sites at the club-end of the conidium and germ tubes were less frequently produced, again randomly, on the "stem" of the conidia or directly from the basal end of the conidium. When two germ tubes appeared they emerged at no particular or specific spots (see Fig. 18). A few germ tubes branched on glass slides but branching was not extensive.
It was observed that germ tubes on glass slides stopped growth early and attained maximum length of 196.0 - 201.6 μ in 36 hours, whilst those on host leaf surface continued to grow thereafter to achieve a length of over 450.0 μ, no doubt, supported by extraneous nutrients from the host leaf (see Table 21).

Appressoria formation was a habitual feature of the germ tubes. Their appearance was not related to the relative humidity, and they occurred with the same frequency over the entire humidity range used in these experiments (see Tables 8, 11 and 14). There are, however, some few interesting observations. In some

experiments (see Tables 9 and 10) germ tubes forming appressoria at lower humidities were extremely high andformation of appressorium was slightly inferior at higher humidities. Surprisingly, considerable number of germ tubes of conidia germinating in liquid water formed appressoria (see Table 8). This indicates that contact with a firm surface is not the only condition necessary for appressorium formation. The stimulus for appressorium formation in P. corylea needs further examination.

The germ tubes of conidia of P. corylea produced various patterns of appressoria typical of the Erysiphaceae (see Fig. 19). Certain species of the powdery mildews form only specific types of appressoria. P. corylea resembles L. taurica in the production of variously-shaped appressoria. It however differs from L. taurica by having no specific sites for germ tubes emergence.

The conidia produced the same germination patterns on the host leaf surface and freely produced appressoria. It was surprising to note that branching was not extensive on the leaf surface, at least, within the period of observation, which was as long as 72 hours. The germ tubes ran randomly over the host leaf surface (see Figs 20 - 22 and Plates 17 and 18). It was interesting to note that the conidia showed similar intial rates of germination on both adaxial and abaxial surfaces of the leaf.

The failure of the fungus to develop and establish itself on the adaxial surface of the leaf is not at least due to inhibition of conidial germination and development might have been arrested further on in the growth of the hyphae. It will be of interest to extend observations to find out at what stage growth stops and the cause of it.

Germ tube growth responded differently to humidity. Higher relative humidities supported longer germ tubes, and at lower humidities the germ tubes were extremely short (see Tables 8, 9, 10, 11 and 14). Germ tubes produced at 0.0, 12.0 and 33.2% R.H. and at 25°C. stopped growth within 6 hours of emergence accompanied by rapid collapse and shrivelling of the germ tubes. This raises an important question. Does the fungus actually benefit from this ability of its conidia to germinate so profusely at the lower humidities? If these conidia are not brought under the influence of more favourable humidities after germination they may dry up and be lost as disease inoculum altogether. Attempts were made to establish the longevity of the germ tubes under the various humidities and hence fate of conidia germinating at the specific humidities. It was found that the germ tubes shrivelled very quickly at 0% R.H. where all dried up within 12 hours and the rate of shrivelling fell

with increase in humidity (see Tables 17 - 19 and Fig. 12).

Although certain conidia are known to recover when returned to humid conditions after they had been dried for some time (Grindle and Good, 1916; Goos and Tschirch, 1963) those of P. corylea failed to do so and none of the shrivelled germ tubes of the germinated conidia ever regained turgidity nor resumed growth after transfer to 100% R.H. and were judged dead. Since during these trials the transfer was made only after all the germ tubes had shrivelled (see page 114) it might be argued that the germ tubes might have been retained too long in the dried state before being transferred to the high humidity of 100% R.H. Since, however, the germ tubes did not shrink at the same time, the last germ tubes to do so before transfer should at least might have showed recovery. It is probable that biochemical changes internally might have long commenced before the shrivelling of the germ tubes became evident externally, thus death perhaps immediately followed or even occurred concurrently with shrinkage.

Behaviour of the conidia on glass slides however could indicate to a limited extent the fate of the germ tubes under natural conditions where the transpiring surface of the host leaf creates a microhabitat of a higher relative humidity than

the atmospheric one for the germ tube. Perhaps germ tubes are normally therefore never subjected to the extremely low humidities used in the laboratories and spore waste never attains the magnitude obtained on glass surface. Finding here could not be translated directly into conditions in the field.

A familiar effect of visible light on fungal spores is either acceleration or inhibition of germination. This is especially well documented in the rust fungi; the literature is reviewed briefly by Cochrane (1945). The striking aspect of this inhibition is that experiments with filters indicate that the longer wavelengths - red, orange and yellow light - are the effective wavelengths, and that the higher energy radiations in the blue regions are ineffective (Dillon Weston, 1932). This rather surprising conclusion is borne out by studies on the inhibition by light of germination of conidia of E. graminis tritici, which are of course not obviously pigmented (Pratt, 1944).

Gottlieb (1950) reviews the early literature on light acceleration of spore germination, most of which is suggestive only. However, the more recent work of Ziegler (1948) on spore germination in the Saprolegniales, and especially the data of Hebert and Kelman (1958) on Physoderma maydis definitely establish a role of light energy in spore germination. P. corylea

belongs to this category. Conidia of P. corylea incubated in light at 21°C. germinated far better than those held in darkness (see Table 22). Germ tubes produced in light were also longer (averagely, 132.9 μ) than those in darkness (averagely 96.8 μ), but produced fewer appressoria. This condition could arise in one of the two ways. If darkness promotes higher frequency of appressorium formation, this might have interfered with full development of the germ tubes and would account for the shorter germ tubes in the dark. On the other hand, similar results will occur if light inhibits appressorium formation or accelerates extensional growth of the germ tubes.

Very great differences have been found in the survival potential of different fungal spores. The viability of all spores decrease with time, and the rate of loss of vigour is dependent on the inherent characteristics of the spore and upon environmental conditions, especially temperature, humidity and light (Gottlieb, 1950; Cochrane, 1958). Pertinent information from the literature had already been outlined at pages

In this investigation the effect of humidity alone on longevity of P. corylea conidia was studied at one temperature only, since any temperature slightly above this will incite germination of the stored conidia. The conidia lived for a very

short period and in no sample did longevity exceed 20 days. The conidia survived shortest at 0% R.H. Rising humidity increased longevity and the longest survival was at 82.6 to 100% R.H. (see Table 23). It is apparent that the conidia were incapable of withstanding desiccation and the physiological activity quickly impaired by a drop in water level in the conidium. At the low temperature of 5°C. metabolic activities of the spores were considerably slowed down and periods of viability registered here could be described as prolonged. At higher temperatures, therefore, in the presence of raised physiological activities longevity will be even far shorter. This, therefore raises another question whether the proportion of conidia which failed to germinate in the fore-going germination tests were actually dead? This may be answered by growing the spores on nutrient media to find out whether an improved germination might occur.

The amount of growth made in 24 hours by the conidia after various storage periods seemed to have been affected by conditions of storage. The germ tubes were shorter the nearer the storage humidity approached zero per cent R.H. That a decline of vigour such as this should precede complete loss of the ability to germinate is not unexpected.

The formation of the conidiophores and the conidia and the subsequent release of the conidia have been critically studied. The conidiophores of this isolate are similar morphologically to those studied by others (Foex, 1926). Earlier workers who observed the presence of only a single conidium at the tip of the conidiophore (Bouwens, 1924; Hammarlund, 1924; Ressencourt, 1927) failed to elucidate the exact fate of the mature conidium and the mechanism involved in the release of the conidium, which is one of the most fascinating features of P. corylea recorded here for the first time (see Figs 26, 28, 29 and 31) and Plates 23 - 27, 29 - 31). The generative cell bent at the end of the development of the conidium and the attachment of the spore, in the event, became loose and was easily dislodged by wind or on shaking. Conidia of P. corylea were not wholly actively liberated by the conidiophores. On the other hand, discharge cannot be absolutely described as passive, since the swinging of the conidial chain brought about by the bending of the generative cell could succeed in breaking up the spore chain (see pages 155 to 173).

Since it was noted that the conidia matured mainly at night (see pages 179 to 181) most of the bending of the generative cells with attendant spore detachment would occur in the morning.

From the point of view of the spread of plant diseases, the time of day when the spores of a pathogen are set free may be of importance. For delicate, short-lived spores, such as those of P. corylea, it may be vital to the spread of the pathogen that liberation occur at a time of day when the air is damp and germination of the spores and growth of the germ tubes are most likely to occur. The conidia of P. corylea therefore stand a very good chance of being released and landing on the host leaf at a time when high atmospheric humidity of early morning will permit successful establishment of the fungus. The periodicity of spore production has been studied in a limited experiment in the laboratory and this needs to be confirmed in an examination of similar cycle of the spore content in the air in the neighbourhood of infected plants.

No external stimuli tested, light, moisture and temperature (20 and 28°C.) was observed to influence the bending of the generative cell. The temperature range used is not wide enough to permit a decisive conclusion. This factor needs further examination. From evidences here it seems that bending of the generative cell is a sequence in growth of the conidiophore. The most impressive feature is that the curvature is not permanent, and a bent cell could readily be moulded later into a normal-

shaped conidium (see Plate 21). Further studies are needed to unravel the underlying mechanism of the bending of the generative cell.

Responses by growth towards asymmetrical light stimuli are shown by representatives of all the major groups of filamentous fungi. Much of the literature on phototropism is included in the comprehensive and critical review by Banbury (1959) and the subject has been treated by Carlile (1965). In a few cases, phototropic responses of vegetative hyphae have been observed, but in most cases it is reproductive structures that respond to unilateral light and various attempts have been made to explain the underlying mechanism (Buller, 1934; Castle, 1933). In P. corylea bending occurs in the mature conidiophore at a point at the base of the stipe. No further observations have been made here to allow a guess at the possible mechanism involved.

Finally, the ability of the conidia of the powdery mildews togerminate at very low humidities presents a problem. They will germinate on the parent mycelium as soon as they mature unless prevented from doing so. That conidia of these species are always obtained ungerminated from the conidiophores for germination tests indicates that this is so. Careful examinations, however, for germinating conidia of powdery mildews in situ

are few. Brodie and Neufeld (1942) and Clerk and Ayesu-Offei (1967) failed to observe germinating conidia of E. polygoni and L. taurica respectively while they were still attached to the conidiophores. Cherewick (1944) on the other hand found that conidia of E. graminis germinated in situ. In the present studies it was observed that the majority of conidia of P. corylea never germinated whilst still attached to the conidiophore. A few, however, under some peculiar undetermined circumstances could germinate on the conidiophores (see Plates 14 to 16). In these instances the conidia always germinated when not fully mature. As soon as they become fully mature they failed to germinate in the presence of the parent mycelium.

There are many reports of the inhibition of spore germination brought about by the activity of the parent mycelium. Stadler (1952) found that the germination of spores of Rhizopus stolonifer was inhibited by a highly unstable product of the mycelium, a substance which also apparently occurred in the spores and was responsible for the negatively chemotropic response of their germ tubes with respect to other spores. Park (1961) showed that aging cultures of Fusarium oxysporum produce an unstable but non-volatile factor with profound effects on growth and morphogenesis and able to inhibit spore germination. He produced evidence for the occurrence of an identical or similar

factor in many, but not all, of the other fungus species he examined. Carlile and Sellin (1963) also found that colonies of Botrytis cinerea produced a factor with an inhibitory effect, which did not affect the spores permanently. They further showed that this factor is not produced equally by the entire culture of the fungus, and inhibitory effect of the mycelium increases with age.

On the other hand, fragments of fruit-body of many basidiomycetes have a beneficial effect on spore germination, and a volatile metabolic product in Agaricus campestris mycelium has been shown to stimulate germination (McTeague, Hutchinson and Reed, 1959), and Carlile and Sellin (1963) found that spores of Polyporus arcularis and Chaetomium jonesii germinated normally over the parent colonies.

The behaviour of conidia of P. carylea may be due to one of two causes or both. The conidiophores either produced different levels of an inhibitory factor with age; the factor being in low concentrations at the early stages of spore development permitting some germination and then increasing to inhibitory levels at last stages of spore development or produced both inhibitory and stimulatory factors together in different proportions at different stages of spore development. The spore may then germinate or remain quiescent depending on which factor is predominant.

VI. SUMMARY

1. Conidia of P. corylea germinated at all the five temperatures viz., 15, 20, 25, 30 and 35°C., used.

2. The respective mean percentage germination at 15, 20, 25, 30 and 35°C. was 10.8, 22.0, 60.6, 47.7 and 4.9 per cent. in 24 hours.

3. Although the conidia germinated best at 25°C. the germ tubes at 30°C. were slightly longer than those at 25°C.

4. The optimum temperature apparently lay between 25 and 30°C.

5. Only 10.0 per cent of P. corylea conidia germinated in liquid water at 25°C.

6. Conidia incubated at 25°C. showed the respective mean percentage germination of 41.0, 44.1, 46.1, 45.7, 46.0, 49.5, 50.6, 50.4, 48.2, 44.5 and 52.3 at humidities of 100, 90, 80, 70, 60, 50, 40, 30, 20, 10 and 0% R.H.

7. At 25°C. and at respective humidities of 100, 96.9, 92.0, 80.3, 75.8, 53.8, 33.2, 12.0 and 0% R.H., 56.9, 54.2, 53.4, 48.5, 45.8, 52.6, 54.7, 51.5 and 50.5 per cent of the conidia germinated.

8. Conidia incubated at 27 ± 1°C. attained the respective percentage germination of 81.1, 92.9, 82.5, 71.2, 76.1, 71.0, 79.4, 68.2, 70.4, 75.0 and 72.3 at humidities of 100, 90, 80, 70, 60, 50, 40, 30, 20, 10 and 0% R.H.

9. Conidia exposed to fluctuating atmospheric relative humidities of 64.0 to 89.0% R.H. during initial period of germination showed 63.9 to 80.4 per cent germination.

10. The rate of germination was uniform over humidity range of 0 to 100% R.H. and the latent period of germination was 2 - 3 hours for each of these humidities.

11. The germ tubes grew more rapidly and longer at 100% R.H. than at any other humidity. Lengths of germ tubes diminished with decrease in relative humidity.

12. Equal proportions of germinated and ungerminated conidia shrivelled at the various relative humidities.

13. Appressoria formation followed no definite pattern, indicating that relative humidity has no influence on appressoria development.

14. The germ tubes of P. corylea emerged more frequently but randomly from the club-shape end of the conidia.

15. Only a single germ tube usually emerged from each donidium and less frequently two.

16. Branching of germ tubes on glass surface was rare, and if present was not extensive.

17. The conidia germinated at both surfaces of the host (C. papaya) leaf and the germ tubes grew randomly over the host leaf surface.

18. Germ tubes shrivelled quickest at 0% R.H. and the rate of shrivelling of germ tubes decreased as the humidity rose.

19. Conidia germinated better and produced longer germ tubes though fewer appressoria in light than in dark.

20. Conidia of P. corylea lost viability most rapidly at the lower humidities and were preserved longest at the higher humidities. Conidium longevity was however very brief. The longest surviving conidia stored at 82.6, 96.6 and 100% R.H. lost viability in 15 - 20 days.

21. The conidiophores are averagely 300 μ long, septate, 2 - 3 celled, unbranched and arose from the ectophytic hyphae at right angle to the leaf surface.

22. The first conidium of each conidiophore was cut off from a terminal erect generative cell.

23. Immediately after the conidium had fully matured the generative cell began to bend across the middle and which continued until the two arms of the generative cell stood approximately at right angles to each other.

24. Full maturity of the conidium was indicated by the simultaneous rounding up of the distal wall of the generative cell and the basal wall of the conidium.

25. During bending of the generative cell, the attachment of the conidium to the conidiophore was weakened and readily fell off on slightest disturbance.

26. Some conidia germinated whilst still attached to the conidiophore and before the bending of the generative cell occurred.

27. The formation of the subsequent conidium evolved the transverse division of the bent generative cell. The upper cell was the conidium initial and the lower one persisted as the generative cell.

28. The generative cell bent again on maturity of each succeeding conidium.

29. Conidiophores frequently produced one conidium at the tip. Conidiophores in still air and those in sheltered spots on the leaf and hence undisturbed formed conidial chains.

30. Conidial chains in still air were however short, commonly composed of 2 conidia and at most four.

31. The conidia matured predominantly in the night and heaviest crop of spores were obtained in the morning.

32. Rate of bending of the generative cell was the same at 20 and 28°C.

33. Rate of bending of the generative cell was the same at 76 and 100% R.H.

34. The generative cell bent in both dark and light and was not phototropic.

35. The shape of the bent generative cell remained unaltered in water and sucrose solutions.

36. The entire conidiophore was positively phototropic.

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