STUDIES ON THE SURVIVAL AND GERMINATION OF CONIDIA OF THREE ENTOMOGENOUS FUNGI.

A thesis presented by

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ABSTRACT

Conidia of Beauveria bassiana, Isaria farinosa and Metarrhizium anisopliae germinated best at respective temperatures of 18, 20 and 25°C., and at respective pH of pH 5.0, 4.0 and 5.0 - 7.6. Germ tubes of B.bassiana, I.farinosa and M.anisopliae growing in water attained respective maximum lengths of 1300, 1121 and 1326 µ. Light had no effect on germination of the conidia. Humidity ranges of 93.8 - 100, 97.2 - 100 and 96.9 - 100 % R.H. supported conidium germination in B.bassiana, I.farinosa and M.anisopliae respectively.

Yeast-extract, Casein hydrolysate and Dextrose enhanced conidium germination in all three fungi. All mineral salts tested, except $MgSO_4.7H_2O$, depressed percentage germination of M.anisopliae conidia, whilst they all stimulated germination in the other two, except $FeCl_3.6H_2O$ and ZnCl which inhibited germination in B.bassiana conidia, and $CuSO_4.5H_2O$ and NaCl which retarded germination in I.farinosa conidia.

Aqueous extracts of exoskeleton of silkworm highly stimulated germination of the conidia. The stimulatory property of silk extract was attributed to constituent amino acids which were separated by paper chromatography.

Germination of conidia of B.bassiana and I.farinosa was inhibited by aqueous extracts of their respective native soils.

Duration of viability of conidia, stored under various conditions, decreased with rise in temperature. The conidia lived longer when stored in darkness than in light. The longest demonstrated survival of conidia was for I.farinosa, 545 days and for B.bassiana, more than 635 days. In both, higher humidities led to earlier death of conidia than did low. M.anisopliae conidia, on the other hand lived longest at 0 - 25 and 75 - 95 % R.H., and died soonest at 40 - 55 % R.H. Conidia of M.anisopliae lived at best for more than 545 days. Early death of M.anisopliae conidia at the mid-humidities was prevented by either elevating CO_2 concentration in the humidity chambers or excluding O_2 from them.

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I. INTRODUCTION.

Fungal parasites of insects have received particular attention with regard to their possible use as a means of biological control of insect pests. The early observations of fungi parasitic upon insects are fascinatingly described by Steinhaus (1956) in his historical account of the development of the principles of biological control of insects.

Apparently the first significant experiments with use of fungi for control of insect pests were made in the 19th. century with Metarrhizium anisopliae (Metsch.) Sorokin, and results of these early tests and subsequent ones made at the beginning of this century were reviewed by Stevenson (1918). While many experiments were negative some workers reported success. For example, Rorer (1910, 1913) reported that a population of 184,000 of Tomaspis varia per acre, described as substantial, was destroyed by Metarrhizium anisopliae artificially applied. Similar successes were reported in later attempts to control insect pests with other entomogenous fungi (Picard, 1914 ; Berger, 1921, 1932 ; Watson and Berger, 1937). Despite this early emphasis on fungi as possible agents of microbial control, there has been rather limited progress along this line. The successes reported represented but a fraction of the many attempts made, and doubts on the feasibility of destroying insect pests by such means on commercial scales

have been expressed by several workers.

In recent years there has been a greater effort to under - stand the fundamental principles in microbial control and the epizootiology of mycoses. Biological control of insects appears dependent to a large extent on the favourability of environmen - tal factors ; these factors need close study. Petch (1921) pointed out that the problem which has yet to be solved by those who wish to control insects by means of fungi is to create an epidemic at a time when such an epidemic would not occur naturally.

Madelin (1960) pointed out that success in trials of biological control by fungi has been limited because very little is known of the behaviour or fate of the spores of most entomo - genous fungi broadcast in the field or introduced into the soil, and of the different climatic and edaphic factors determining their survival. It is necessary to know the role of the spores in the natural infection chain. Fawcett (1944) indicated that there is a complex of fluctuating factors that need to be unscrambled by experiments with controlled conditions to under - stand the numerous factors involved in successful establishment of fungus - insect relationship.

Since spore germination in the infection court of the insect is a pre - requisite of infection, the factors affecting spore survival and germination are of prime importance. Such information is not only essential to an understanding of the

role of spores in the natural infection - chains, but is also needed to show how best spores can be employed in the biological control of insects.

In the investigation described below, the factors affecting survival and germination of conidia of three entomogenous fungi, Beauveria bassiana (Bals.) Vuill., Isaria farinosa Fries and Metarrhizium anisopliae (Metsch.) Sorok. were extensively studied. These were chosen for this investigation because of their frequent and widespread association with several different insect species, which suggests that they may be important in the natural control of many insect pests.

II. REVIEW OF LITERATURE.

Beauveria bassiana : B.bassiana first came into prominence when it was identified (Balsamo, 1835) as the agent responsible for a disease of silkworms, Bombyx mori, that was well known in the 18th century. It has become evident, however, that while B.bassiana has often proved to be a costly pest to the sericultural industry it is nevertheless of some importance in the control of many noxious insect pests.

The vast host range and extensive geographical distribution of this fungus is reported in detail by Charles (1941), Gösswald (1939), MacLeod (1954), Petch (1925), Rockwood (1951), Siemaszko (1937) and Steinhaus (1949). It has also proved possible to infect in the laboratory many insect species, for which natural occurrence of infection have as yet not been noted (Dresner, 1949 ; Gösswald, 1939 ; Rex, 1941).

There have been many attempts to employ B.bassiana in the biological control of insects. Field-scale attempts include those of Dieuzeide (1925), Schaerffenberg (1957), Telenga, Dyadechko and Sikura (1959) and Samšiňáková and Čermáková (1960) to control Leptinotarsa decemlineata. Metalnikov and Toumanoff (1928) and Steyaert (1935) used B.bassiana in attempts to control respectively, Pyrausta nubilalis and Stephanoderes hampei. Broadcast spore suspensions of B.bassiana are reported to have caused high mortality in populations of

hibernating Aradus cinnamomeus (Smirnov, 1954). One of the few bio - preparations which have recently been described as useful in the control of insect pests is that reported by Tolenga (1959). He applied conidia of B.bassiana in combination with either hexachlorane or D.D.T. to the soil and reported effective control of Bothynoderes punctiventris and Cydia pomonella.

Although B.bassiana has so often been used in biological control trials, there is very little information from the literature of deliberate and special study of how the various environal conditions influence the longevity and germination of the conidia. Generally, information available on the spore behaviour derives from incidental observations made during pathogenicity and infection studies.

Arnaud (1927), Hart and MacLeod (1955), Muller - Kogler (1942) and Steyaert (1935) observed that the conidia would only germinate at very high relative humidities, between 90 and 100 % R.H. Gabriel (1959) however reported that conidia of B.bassiana germinated very tardily in water, but germinated well in NaCl solution.

Arnaud (1927) reported that conidia of B.bassiana germinated between 8 and 40° C. with an optimum at 22° C. Schaerffenberg (1957) reported germination from 0 to 40°C. with an optimum between 20 and 30°C. Hart and MacLeod (1955) obtained best germination in humid air at 28°C.

Rozsypal (1930) found that conidia of B.bassiana germinated best in a medium of neutral reaction.

Toumanoff (1933) stated that the conidia became incapable of infection after three hours' exposure to sunlight, and Gosswald (1939) also reported that the viability of the conidia was well conserved when protected from the sun.

Lombardi (1952) found that the conidia remained viable for not more than 12 months, and in inoculation experiments old conidia proved as virulent as younger ones. Steinhaus (1960) found that conidia of B.bassiana lost their viability most rapidly at 38°C. At 24 - 30°C. they died within approximately 7 weeks. At 23°C. they survived slightly longer and at 4°C. they lived up to 128 weeks.

Huber (1958) observed that conidia of B.bassiana were unable to germinate in fresh garden soil and concluded that it was presumably due to the presence of antibiotics in the soil.

Isaria farinosa : I.farinosa parasitises Lepidoptera, Hymenoptera, Coleoptera, Diptera, Aphididae and Arachnida (Petch, 1932). Although it is among the few better known examples of the entomogenous - Fungi Imperfecti, it has received less study as a biological control agent than has B.bassiana. A few attempts have been made to control insects with it. (eg., Metalnikov and Toumanoff, 1928), and Korner (1959) succeeded

in infecting insect species from which the fungus had not been previously isolated.

Information on factors which affect spore survival and germination appears to be meagre. Müller - Kögler (1942) found that the optimum relative humidity (R.H.) for infection lies between 90 and 100 % R.H. Kornbluth (1961) reported that the lowest R.H. for the germination of the conidia was 92 % R.H., although with the technique used, in the vicinity of the conidia the R.H. might have been higher than this.

The reported minimum temperature for germination of conidia of I.farinosa is 0.5°C. (Müller - Kögler, 1942). Brown and Smith (1957) stated that germination was very tardy at 8°C., slow at 10 - 14°C., moderate at 24°C. and nil at 37°C.

Kornbluth (1961) found that in carbon dioxide - free air, conidia of I.farinosa did not germinate until the 7th day as compared with 2 days in normal air.

Metarrhizium anisopliae : In 1879, Metschnikoff in Russia made the first significant experiments on the destruction of injurious insects by the use of micro-organisms when he was able to infect larvae of the beetle, Anisoplia austriaca with the fungus M.anisopliae. Since then many insect species have been reported as hosts of this fungus, e.g., approximately 70 species in North America (Charles, 1941) with an equal or

greater number from other parts of the world (Petch, 1924, etc.).

Many attempts to use M.anisopliae for biological control have been made. Some trials, for example, those made by Rorer (1910, 1913), Briton - Jones (1927) and Friederichs (1913) were successful, whilst many more failed, for example, the experiments of Groenewege (1916), Stevenson (1918), Toumanoff and Grison (1954). etc. Tolenga (1959) used M.anisopliae conidia together with hexachlorane in a bio - preparation which effectively con - trolled Bothynoderes punctiventris and Cydia pomonella.

Vouk and Klas (1932) found the optimum temperature for conidium germination to be 24 - 26°C.

Masera (1957) found the conidia germinable at any pH within the range of pH 2 to 10. Vouk and Klas (1932) earlier observed a similarly wide range of pH tolerance (pH 4.7 to above pH 10).

Glaser (1926) stated that the conidia of M.anisopliae could survive in a dried state for 3 years, whilst Masera (1957) reported that the conidia lose their virulence and viability, under laboratory conditions and temperature (25 - 29°C.), within 4 months.

Manifestly, the factors that affect the germination of fungal spores are of two types : internal and environmental or external. The ability to germinate primarily depends on the

internal factors, aspects of which include, the maturity, longevity, dormancy and vitality of the spore ; the permeability of its wall and the amount and nature of the available food reserves. Even when a spore possesses the maximum innate ability to germinate, it may yet fail to do so under certain environal conditions, eg. of temperature, aeration, light, pH and moisture ; and the presence or absence of external nutrients, or of stimulatory or inhibitory substances. The importance of these factors has been reviewed by Gottlieb (1950), Hawker (1950) and Cochrane (1958) etc., and further reference will be made to these factors in appropriate places in the text.

Until recently studies on fungal spore germination have been concerned chiefly with environmental influences, but the recent advent of many new research techniques has facilitated investigation into the metabolism of spore germination. For example, Gottlieb and Caltrider (1963) reported that the activity of particular enzymes changed during the germination of spores of Ustilago zeae.

III. MATERIALS AND GENERAL METHODS.

(i) Materials.

The isolates of B.bassiana (MFM.HERB.1082), I.farinosa and M.anisopliae (MFM.HERB.1118) used were obtained from the Stock Culture Collection of the Department of Mycology and Plant Pathology, Imperial College of Science and Technology, London,S.W.7. B.bassiana was originally isolated from an adult of Phytodecta olivacea, I.farinosa from a Lepidopterous larva and M.anisopliae from Tomaspis portica, collected in Trinidad.

(ii) Methods.

(a) Spore Germination Tests.

I. Slide Method.

In most of the spore germination tests, the "slide" method was used. Spore suspensions of M.anisopliae and B.bassiana were prepared by flooding tube cultures with a few drops of sterile water or liquid medium and shaken gently. These suspensions were then decanted into sterile McCartney tubes. Spores were thereby obtained in suspension without hyphal fragments or pieces of agar medium. Spores of I.farinosa were gathered solely from coremia, using a sterile camel hair brush, and similarly transferred into liquid medium or water in McCartney tubes. The spore suspensions were shaken by hand

for 10 minutes to give uniform dispersion. The number of spores in suspension for every germination test was strictly standardised to 500,000 per millilitre of solution with the aid of a haemacytometer. Sterile petri dishes each containing a sterile slide (7.5 x 2.5cm.) supported on a glass V - piece, over a small quantity of the same medium as that in which the germination of the spore was to be tested (i.e. sterile water or dilute nutrient solution) had, in the mean time, been equilibrated with the incubation temperature. This technique obviated both evaporation of the germination test droplet and condensation of moisture. Using a sterile dropping pipette, three individual drops of spore suspension (about 0.1ml. in volume) were placed on each thermally equilibrated slide contained in its damp chamber, and the slide immediately returned to the incubator. Each germination count was based on nine drops of suspension (i.e. three separate drops on each of three slides for each treatment). Petri dishes were always ramdomised in the incubators to nullify positional effect.

2. Agar Plate Method.

Spore germination was also observed on agar media in petri dishes (20ml. of agar per dish) in a considerable number of experiments in this investigation. Each agar plate was inoculated with 1 ml. of spore suspension (standardised at

500,000 spores per ml.) divided between 10 evenly spaced pre-marked areas. Two dishes (20 test areas) were used for each germination test. The concentration and spatial spread of the spores corresponded closely to what obtained in the "slide" method.

(b) Assessment of Conidium Germination.

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

The "Percentage Germination" was calculated by the formula :

$$\frac{100 \times \text{Number of Spores which germinated}}{\text{Total Number of Spores (germinated and ungerminated) examined}}$$

The lengths of a hundred germ tubes were also measured, using a micrometer eye - piece, and the mean evaluated. Any spore whose germ tube was as long as the shortest diameter of a spore was counted as having germinated. This definition of germination was thus based on morphology. Gottlieb and Caltrider (1963) have recently shown that external evidences of spore germination are long preceded by enzymatic changes within the spore, and that a definition of germination should

include this state of physiological change in the spore. Methods have yet to be devised for quick determination of such enzymatic changes to suit germination tests of this kind. The Percentage Germination was calculated from counts made in 45 random fields of the microscope (with high power, x 6, objective), distributed among the nine drops, or 20 areas containing spores (see above). The numbers of spores examined are shown in the tables of data, to show the reliability of the percentage germination claimed for each treatment. Where germ tubes branched, or where a spore produced more than one, "Germ Tube Length" was taken as the sum of the lengths of the main axis and the branches or the individual germ tubes.

(c) Experimental Precautions.

Several workers have commented on the frequently erratic nature of spore germination (e.g., Ferguson, 1902 ; Child, 1929 ; Webb, 1921 and Wilkins, 1938). Ferguson (1902) reported that with spores of Agaricus campestris one culture out of four gave 90 per cent germination, while the other three gave none ; no explanation could be given of these variations.

To reduce erratic results, the following methods, based on the recommendations of Wilkins (1938), were strictly adhered to in these investigations.

1. Spores of the same age were used in all experiments.

2. In the "slide" method, 5 fields were counted at random in each of the nine drops (giving the 45 counts), and at least two fields were observed from each seeded area on the agar plates.

3. Results were based on tests, each with three slides or two agar plates, as the case may be. Each "Percentage Germination" represents a count of approximately 2000 spores.

4. Glassware was kept scrupulously clean. Glassware which had already been cleaned with water and detergents, was rinsed three times with warm tap water and three times with distilled water and allowed to drain before use.

(d) Methods of Sterilisation.

All media, conical flasks, McCartney tubes, camel hair brushes and pipettes were sterilised by autoclaving for 20 minutes at 15 pounds per square inch steam pressure. Cotton wool plugs were temporarily covered with aluminium foil caps to prevent the penetration of any condensed water during autoclaving. Pipettes and camel hair brushes were wrapped in brown paper.

Slides and cover slips were first soaked in cleansing solution of potassium dichromate ($K_2Cr_2O_7$, 100 g. ; conc. H_2SO_4, 1000 ml. ; and water, 1000 ml.) for 24 hours, followed by immersion in a dilute solution of NaOH and were finally rinsed several times in distilled water. They were stored in 90 % ethyl

alcohol and flamed sterilised just before use.

Petri dishes were sterilised by heating at 165°C. for 6 hours.

The inoculation room was sterilised with ultra-violet light for 20 minutes just before use.

(e) Maintenance of Stock Cultures.

Stock cultures of B.bassiana and M.anisopliae were grown on slopes of V-8 and Yeast-extract agar medium respectively, in McCartney tubes. They were incubated at 25°C., and sub-cultured every 14 days. The cultures of I.farinosa were maintained on autoclaved short-grain (pudding) rice in conical flasks (5 g. polished rice, 25 ml. water) and incubated in the laboratory (temperature of 21°C.). They were sub-cultured at monthly intervals.

(f) Production of Conidia.

Because large quantities of spores were needed in certain investigations, several media were tested to find the one giving most profuse sporulation of each fungus, to be used for that purpose in this work. The best media for sporulation of B.bassiana, I.farinosa and M.anisopliae were, respectively, V-8 agar, boiled short-grain (pudding) rice and yeast-extract agar.

Spores for use in experiments were consistently obtained from 8 days' old cultures of B.bassiana, 21 days' old cultures of I.farinosa and 5 days' old culture of M.anisopliae

(g) Monospore Culture.

Where monospore isolates were required, the individual conidia were selected after microscopic examination from plates inoculated with very dilute suspension of conidia.

(h) Incubation.

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

(i) Constant Humidity Solutions.

To obtain and maintain the different relative humidities, aqueous saturated salt solutions were prepared according to the data provided by Wexler and Hasegawa (1954). (see Table 1.). Relative humidities were also maintained in some experiments with aqueous glycerol (Johnson, 1940), H_2SO_4 and KOH solutions (Solomon, 1952). (see Tables 2 and 3). Whenever solutions for control of humidity were prepared, the densities were carefully determined by weighing before being used.

TABLE 1

Relative Humidity (Percentage) of Saturated Salt Solutions.

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

Temperature in °C.	$LiCl.H_2O$	$MgCl_2 6H_2O$	$Na_2CrO_7 2H_2O$	$Mg(NO_3)_2 6H_2O$	NaCl	$(NH_4)_2SO_4$	KNO_3	K_2SO_4
0	14.7	35.0	60.6	60.6	74.9	83.7	97.6	99.1
5	14.0	34.6	59.3	59.2	75.1	82.6	96.6	98.4
10	13.3	34.2	57.9	57.8	75.2	81.7	95.5	97.9
15	12.8	33.9	56.6	56.3	75.3	81.1	94.4	97.5
20	12.4	33.6	55.2	54.9	75.5	80.6	93.2	97.2
25	12.0	33.2	53.8	53.4	75.8	80.3	92.0	96.9
30	11.8	32.8	52.5	52.0	75.6	80.0	90.7	96.6
35	11.7	32.5	51.2	50.6	75.5	79.8	89.3	96.4

TABLE 2

Maintenance of Atmospheric Humidities with Glycerol-water mixtures.

(Data of Johnson,C.G., 1940)

% R.H. at 20°C.	% Weight Glycerol.	Specific Gravity.	Water V.P. mm.
0.0	100.0	1.261	-
1.7	98.0	1.256	0.4
16.9	92.0	1.240	4.0
33.8	83.0	1.215	8.0
44.3	75.0	1.194	10.5
62.4	60.0	1.153	14.8
73.4	50.0	1.126	17.4
85.2	35.0	1.085	20.2
92.8	25.0	1.059	22.0
97.0	15.5	1.036	23.0
100.0	0.0	0.998	23.7

TABLE 3

H_2SO_4 and KOH Solutions for the control of Atmospheric Humidity.

(Data of Solomon,M.E., 1952).

SULPHURIC ACID SOLUTIONS.				POTASSIUM HYDROXIDE SOLUTIONS.		
% Relative Humidity at 25°C.	%(Wt./Wt.) (Gm.Conc. H_2SO_4/100 Gm.soln.)	Density at 15°C.	Density at 20°C.	% R. H. at 20°C.	%(Wt./Wt.) (Gm.KOH / 100 Gm. soln.)	Density at 15°C.
100	0.0	0.999	0.998	100	0.00	1.000
95	11.02	1.075	1.073	95	7.00	1.064
90	17.91	1.126	1.123	90	11.75	1.105
85	22.8	1.165	1.162	85	15.80	1.147
80	26.79	1.196	1.193	80	19.25	1.181
75	30.14	1.223	1.219	75	22.25	1.211
70	33.09	1.248	1.245	70	25.00	1.239
65	35.80	1.271	1.267	65	27.30	1.262
60	38.35	1.292	1.289	60	29.50	1.285
55	40.75	1.313	1.309	55	31.60	1.307
50	43.10	1.334	1.330	50	33.70	1.330
45	45.41	1.355	1.351	45	35.80	1.353
40	47.71	1.377	1.373	40	37.95	1.378
35	50.04	1.400	1.396	35	40.10	1.400
30	52.45	1.423	1.419	30	42.30	1.425
25	55.01	1.499	1.445	25	44.50	1.450
20	57.76	1.478	1.474	20	47.00	1.479
15	60.80	1.511	1.507	15	49.80	1.512
10	64.45	1.551	1.546	-	-	-
5	69.44	1.608	1.604	-	-	-

(j) Buffer Solutions.

Adjustment of the pH of media, when required, was obtained by adding N/10 HCl, N/10 NaOH or Buffer solutions. Buffer solutions were prepared according to the data provided by Clark (1925). All adjusted media were checked with a "Cambridge" pH meter.

(k) Analysis of Amino Acids by Paper Chromatography.

Standard paper chromatographic techniques were employed. (Smith, 1958). Descending chromatograms on Whatman No.1 paper, size, 24" x 9" were developed with n-butanol, glacial acetic acid and distilled water (4 : 1 : 5 by volume) at 21°C. for 16 hours, dried and sprayed with ninhydrin (Triketo-hydrindene hydrate - 2 % solution in 95 % butanol and 5 % 2N Acetic Acid). Known amino acids were used as markers. Amino acids were identified both by their calculated R_f and by comparison with the markers. After decisive identifications, approximate quantitative determinations were made by visually comparing colour intensities and sizes of the developed spots with dilution series of respective markers.

(l) Respiration studies with Warburg Apparatus.

Intake of oxygen of the conidia was measured by

manometric methods using Warburg apparatus. (Umbreit, et.al., 1959)

When the respiration rate of dry conidia was to be measured in atmospheres of different relative humidities, a modified procedure was followed. The flasks were kept stationary in the water bath as there was no need to facilitate gas exchange in a respiratory fluid medium, and also to prevent the spores from scattering into the alkali solution. KOH solutions were used to provide the desired humidities in the respiratory flask (see above) and also to absorb CO_2. 1 ml. of KOH was put into the side arm and 0.4 ml. into the central well, and the spores spread out in a thin layer in the main compartment of the Warburg flask. The open flask was then incubated for 12 hours, at 25°C. in a desiccator, holding 40 ml. of similar strength of KOH solution to maintain the same humidity. This allowed the spores to become adequately equilibrated with the ambient humidity before respiratory tests were made. Manometric readings were taken at intervals in the usual way and the volume of oxygen used up calculated. Flasks acting as thermo-barometers contained KOH solutions, instead of water, to provide similar vapour pressures to that in the respiratory flasks.

(n) Culture Media.

All chemicals used in the preparation of the media

were of the "Analar" grade. 20 ml. of agar medium was used per petri dish, and 15 ml. per McCartney tube or 40 ml. per conical flask. The compositions of the media used were as follows :

1. Bean - Extract Agar : Bean extract, 64 g. ; Agar, 20 g. ; Water, 1000 ml.
2. Boiled Rice : 5 g. of short - grain (pudding) rice in 25 ml. of water in each vessel, directly autoclaved without any previous boiling.
3. Brown's Agar Medium : Dextrose, 2 g. ; Asparagine, 2 g. ; K_3PO_4, 1.25 g. ; $MgSO_4.7H_2O$, 0.75 g. ; Agar, 20 g. ; Water, 1000 ml.
4. Czapek - Dox Agar Medium : Sucrose, 15 g. ; $NaNO_3$, 2 g. ; KH_2PO_4, 1 g. ; $MgSO_4.7H_2O$, 0.5 g. ; KCl, 0.5 g. ; $FeSO_4.7H_2O$, 0.01 g. ; Agar, 20 g. ; Water, 1000 ml.
5. Flour and Water Mixture : A flour and water mixture was made just thin enough to pour. McDougall's Self-Raising Flour was used. 10 ml. of the mixture was put into each culture vessel and autoclaved.
6. Glucose - Peptone Agar.: Glucose, 10 g. ; Peptone, 2.5 g. ; KH_2PO_4, 1 g. ; $MgSO4.7H_2O$, 0.5 g. ; Agar, 20 g. ; Water, 1000ml.
7. Glycerinated Potato : Potato wedges were cut and soaked in 25 % solution of glycerol for 20 minutes, and inserted into test tubes. Cotton wool paddings placed at the bottom of the tubes were also moistened with 25 % solution of glycerol. When conical flasks and petri dishes were used, broad thin potato

discs to fit the bottoms of these were prepared instead of wedges.

8. Ground Bean : Dry broad bean seeds were soaked in water for 4 days and ground to a paste.

9. Kauffman's Agar Medium : Maltose, 5 g. ; $Ca(NO_3)_2$, 0.5 g. ; KH_2PO_4, 0.25 g. ; $MgSO_4.7H_2O$, 0.1 g. ; Agar, 20 g. ; Water, 1000 ml.

10. Leonian's Agar Medium : Fructose, 30 g. ; KNO_3, 2 g. ; KH_2PO_4, 1 g. ; $MgSO_4.7H_2O$, 1 g. ; Agar, 20 g. ; Water, 1000 ml.

11. Malt - Extract Agar Medium : Malt - extract powder, 20 g. ; Agar, 20 g. ; Water, 1000 ml.

12. Oat - Meal Agar Medium : Oats (ground), 20 g. ; Agar, 20 g. ; Water, 1000 ml.

13. Potato - Dextrose Agar Medium : 200 g. of peeled potato were boiled in 500 ml. of water, and strained and made up to 1000 ml. ; 20 g., glucose ; 20 g. Agar.

14. Potato - Extract Agar Medium : As in (13) but without glucose.

15. Richard's Agar Medium : Sucrose, 10 g. ; KNO_3, 10 g. ; KH_2PO_4, 1 g. ; $MgSO_4.7H_2O$, 2.5 g. ; $FeCl_3$, a trace ; Agar, 20 g. ; Water, 1000 ml.

16. V - 8 Agar Medium : 200 ml. of V-8 juice ; 800 ml., water ; 20 g. agar. Adjusted with NaOH to pH 6.

17. Weindling's Agar Medium : Glucose, 25 g. ; NH_4 - tartrate, 2 g. KH_2PO_4, 1 g. ; $MgSO_4.7H_2O$, 1 g. ; Agar, 20 g. ; Water, 1000 ml.

18. Yeast - Extract Agar Medium : Glucose, 10 g. ; Peptone, 10 g. ; Yeast - extract, 3 g. ; Agar, 20 g. ; Water, 1000 ml.

(n) Pyridine - washed Agar.

Whenever nutrient - free agar was needed, it was leached with pyridine according to the method of Robbins (1939) to remove most of the physiologically active compounds.

IV. RESULTS.

A. PRELIMINARY EXPERIMENTS.

SPORULATION ON DIFFERENT MEDIA.

B.bassiana, I.farinosa and M.anisopliae are known to grow and sporulate well on several media. MacLeod (1954) observed that the heaviest spore production among the B.bassiana isolates he studied occurred on Sabouraud maltose agar, followed by Molisch medium, Blood agar base, Raulin-Thom and Czapek-Dox media, Potato dextrose and finally Corn meal agar. Kornbluth (1961) showed that I.farinosa could utilise a number of monosaccharides, polysaccharides and fatty acid radicals as sources of carbon ; and nitrate- , ammonium- and organic-sources of nitrogen for coremium production. Early workers, for example, Metchnikoff (1879), Krassilstchik (1886, 1888) and Rorer (1910) raised large quantities of conidia of M.anisopliae on relatively simple media. Notini et al. (1944) found that for M.anisopliae, asparagine was a superior source of nitrogen to ammonium nitrate in the production of spores, and that although M.anisopliae was vitamin-autotrophic on purely synthetic nutrient solution, the addition of a small amount (0.2 %) of yeast extract resulted in increase in conidial production.

An experiment was conducted to determine for each fungus the medium supporting the heaviest sporulation. Twenty two media were tested (see Tables 4 - 6). Cultures of B.bassiana and M.anisopliae were grown in petri dishes and of I.farinosa in 150 ml.

conical flasks. Each medium had four replicates. The media for B.bassiana and M.anisopliae were inoculated with discs, 3 mm. in diameter, taken from the growing periphery of 10 days' old cultures on Potato dextrose agar, and incubated in light at 25°C. for 10 days. The diameters of the growing colonies of both fungi were measured along diameters at right angles to each other. After 10 days' incubation, the degree of sporulation on the various media was determined. 4 discs, 11 mm. in diameter, were removed from each culture plate with a No. 7 cork borer from 4 symmetrical positions equidistant from the centre of the culture (Plate 1). Each disc was placed in a McCartney tube holding 10 ml. of distilled water, and shaken for 10 minutes to disperse the spores. The number of spores per ml. of suspension prepared from each disc was determined with a haemacytometer. Each result is the mean of counts from 16 discs. Results are presented in Tables 4 and 6.

For I.farinosa, the media in the conical flasks were inoculated with one drop (0.1 ml.) of spore suspension, prepared with conidia from coremia of a month old culture on rice. The cultures were placed on shelves in the laboratory for 28 days (Temperature 21°C.). Such conditions of light and temperature favour coremium production (Kornbluth, 1961). Degree of sporulation (Plate 2) was estimated after 28 days by taking a direct count of the number of coremia produced and by measuring their lengths. The actual number of spores were not counted. The results are presented in Table 5.

TABLE 4

Degree of Sporulation of B.bassiana on Different Media.

MEDIUM	Mean Diameter of Colony in mm. on		Number of Spores per ml. of suspen - sion on 10th Day.
	5th Day.	10th Day.	
Bean extract agar	37.2	65.0	51.68×10^6
Boiled rice	24.4	37.2	23.16×10^6
Brown's agar	26.0	48.8	2.99×10^6
Czapek-Dox agar	22.5	45.8	7.08×10^6
CDA. with Glycerol[1]	22.0	48.8	4.03×10^6
CDA.with Starch[1]	28.5	44.4	7.62×10^6
CDA. with Arginine[2]	21.8	39.5	0.12×10^6
CDA. with Tyrosine[2]	22.5	46.0	0.23×10^6
Flour and water	22.4	44.0	13.40×10^6
Glucose-peptone agar	16.6	27.5	1.72×10^6
Glycerinated potato	22.4	39.2	15.66×10^6
Ground bean	19.8	32.2	19.18×10^6
Kauffman's agar	24.5	45.8	2.09×10^6
Leonian's agar	20.4	31.5	0.62×10^6
Malt-extract agar	20.8	29.3	1.56×10^6
Oat-meal agar	24.5	40.8	0.90×10^6
Potato dextrose agar	21.2	29.0	17.32×10^6
Potato-extract agar	28.0	65.2	12.72×10^6
Richard's agar	22.2	49.0	5.87×10^6
V - 8 agar	28.4	41.6	52.60×10^6
Weindling's agar	16.2	25.0	3.54×10^6
Yeast-extract agar	22.6	37.0	1.66×10^6

[1,2] Czapek-Dox with a substituted carbon[1] or nitrogen[2] source.

TABLE 5

Degree of Sporulation of I.farinosa on Different Media.

MEDIUM	Degree of production of coremia[1]	Mean length of coremia in mm.	Non-coremial (Paecilomyces) Sporulation.
Bean extract agar	∓ ∓ ∓	11.3	Very sparse
Boiled rice	∓ ∓ ∓ ∓	23.5	Fairly heavy.
Brown's agar	∓ ∓	4.8	Heavy
Czapek-Dox agar	∓ ∓	16.2	Fairly heavy
CDA. with Glycerol[2]	∓ ∓ ∓ ∓	14.0	Heavy
CDA. with Starch[2]	∓	10.3	Heavy
CDA. with Arginine[2]	∓	22.8	Very sparse
CDA. with Tyrosine[2]		-	Very sparse
Flour and water	∓ ∓	4.0	Very sparse
Glucose-peptone agar	∓ ∓ ∓	18.5	Sparse
Glycerinated potato	-	-	-
Ground bean	∓	5.2	Sparse
Kauffman's agar	∓	12.4	Heavy
Leonian's agar	∓ ∓ ∓	17.9	Heavy
Malt-extract agar	∓ ∓ ∓ ∓	18.0	Fair
Oat-meal agar	∓	25.0	Fairly heavy
Potato dextrose agar	∓ ∓ ∓	15.8	Fairly heavy
Potato-extract agar	∓ ∓	12.3	Fairly heavy
Richard's agar	∓ ∓	12.0	Fairly heavy
V - 8 agar	∓ ∓	12.5	Heavy
Weindling's agar	∓ ∓ ∓ ∓	15.7	Fairly heavy
Yeast-extract agar	∓ ∓ ∓ ∓	12.9	Very sparse

[1] See foot - note of Table 4.

[2] ∓ : 1-10 coremia; ∓ ∓ : 11-20 coremia; ∓ ∓ ∓ : 21-30 coremia; ∓ ∓ ∓ ∓ : over 30 coremia.

TABLE 6

Degree of Sporulation of M. anisopliae on Different Media.

MEDIUM	Mean Diameter of Colony in mm. on		Number of Spores per ml. of suspen - sion on 10th Day.
	5th Day.	10th Day	
Bean extract agar	22.6	44.0	32.24×10^6
Boiled rice	18.5	31.0	38.32×10^6
Brown's agar	18.0	31.5	5.48×10^6
Czapek-Dox agar	21.0	36.2	3.82×10^6
CDA. with Glycerol[1]	17.0	26.6	1.61×10^6
CDA. with Starch[1]	19.6	33.2	0.67×10^6
CDA. with Arginine[1]	17.0	29.8	41.92×10^6
CDA. with Tyrosine[1]	20.0	35.6	7.52×10^6
Flour and water	16.6	33.0	53.44×10^6
Glucose-peptone agar	25.3	48.2	40.24×10^6
Glycerinated potato	16.7	32.3	30.02×10^6
Ground bean	18.4	34.2	35.36×10^6
Kauffman's agar	18.0	31.5	15.02×10^6
Leonian's agar	20.2	33.5	0.13×10^6
Malt-extract agar	25.8	51.8	7.00×10^6
Oat meal agar	25.5	46.8	4.90×10^6
Potato dextrose agar	29.5	57.5	31.60×10^6
Potato-extract agar	27.0	44.5	40.20×10^6
Richard's agar	19.8	37.5	8.16×10^6
V - 8 agar	28.0	53.5	10.88×10^6
Weindling's agar	16.5	27.0	7.20×10^6
Yeast-extract agar	27.8	52.0	68.52×10^6

[1] See foot - note of Table 4.

B.bassiana sporulated best on V - 8 agar medium.

(52.6 x 10^6 spores per ml. of spore suspension).

Boiled rice was best for coremium formation in I.farinosa. The number of coremia produced within 28 days exceeded 30, and the mean coremial length was 23.5 mm.

M.anisopliae sporulated best on Yeast - extract agar medium

(60.52 x 10^6 spores per ml. of spore suspension).

These three were adopted for all subsequent production of spores.

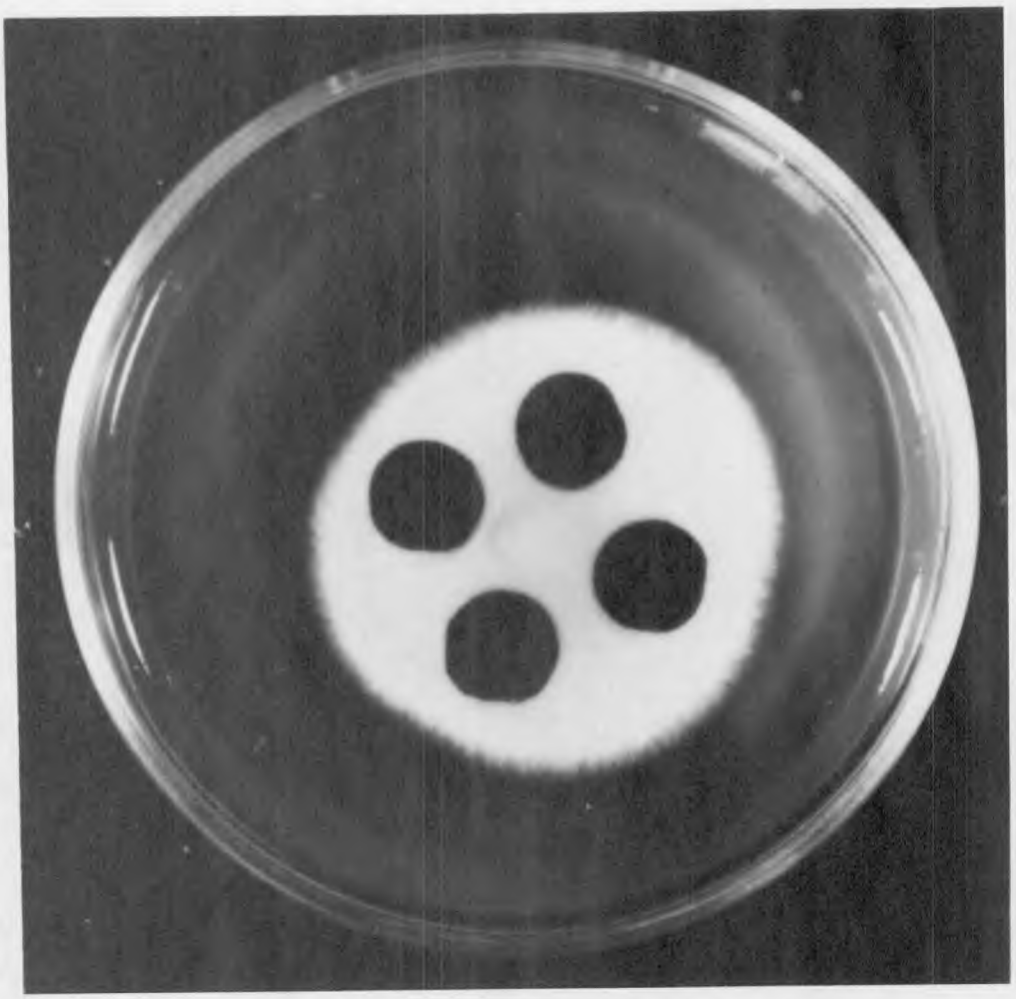
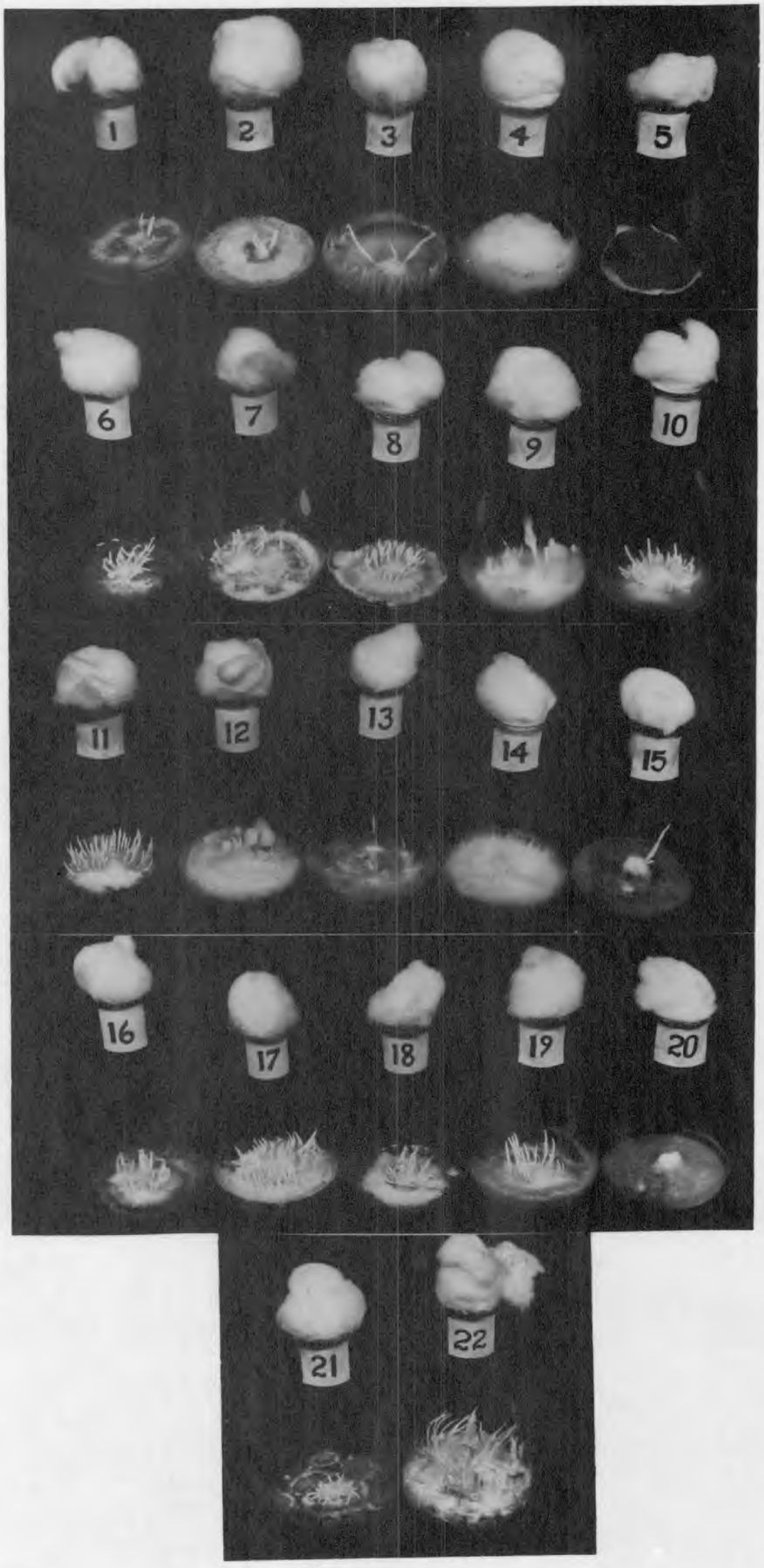


PLATE 1. 10-DAY OLD CULTURE OF B.BASSIANA SHOWING EXCISION OF 4 DISCS FOR SPORE DENSITY TEST. (x $1\frac{3}{8}$).

PLATE 2. - DEGREE OF SPORULATION OF I.FARINOSA ON DIFFERENT MEDIA.

1. Kauffman's Agar Medium.
2. Czapek-Dox Agar Medium with Starch as Carbon source.
3. Bean-Extract Agar.
4. Ground Bean.
5. Glycerinated Potato.
6. Potato-Extract Agar.
7. V-8 (Vegetable) Agar.
8. Yeast-Extract Agar.
9. Czapek-Dox Agar.
10. Weindling's Agar Medium.
11. Malt-Extract Agar.
12. Richard's Agar Medium.
13. Oatmeal Agar.
14. Flour and Water Mixture.
15. Czapek-Dox Agar Medium with Arginine as Nitrogen source.
16. Glucose-Peptone Agar.
17. Czapek-Dox Agar Medium with Glycerol as Carbon source.
18. Leonian's Agar Medium.
19. Potato-Dextrose Agar.
20. Czapek-Dox Agar Medium with Tyrosine as Nitrogen source.
21. Brown's Agar Medium.
22. Boiled Rice.


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B. EFFECT OF TEMPERATURE ON GERMINATION.

Temperature is one of the most important external factors which influence germination. It not only affects the percentage germination, but also the length of time required for germination, and often, determines the method of germination. The effect of temperature on germination of conidia of B.bassiana and I.farinosa during an incubation period of 48 hours, and of conidia of M.anisopliae during an incubation period of 24 hours was determined. The "slide" method was used. The conidial suspensions prepared with sterile distilled water were incubated in the dark at selected temperatures (see Tables 7 - 10). The percentage germination and germ tube lengths were finally recorded.

The results in Table 7 and Fig. 1 show that B.bassiana conidia were capable of germinating at low temperatures. Within 24 hours of incubation, 4.79 per cent of the conidia germinated at 4^{o}C., and the germination increased to 23.26 per cent in a further period of 24 hours. The minimum temperature (4^{o}C.) obtained in this experiment was lower than those reported by Rozsypal (1930) and Müller - Kögler (1948). They both reported a minimum temperature of 8^{o}C. Since 4^{o}C. was the lowest temperature available, Schaerffenberg's (1957) reported minimum temperature of 0^{o}C. could not be verified. The optimum was found to be 18^{o}C, supporting the highest percentage germination and the greatest germ tube growth. Schaerffenberg op cit.

and Hart and MacLeod (1955) reported higher optimum temperatures of, between 20 and 30°C. and 28°C. respectively. No germination was observed at 33°C. and above. Percentage germination and the germ tube growth showed closely parallel temperature characteristics.

TABLE 7

Effect of Temperature on Germination of conidia of B.bassiana incubated in water in darkness.

Temp. of Incubation in °C.	Observations after 24 HRS.			Observations after 48 HRS.		
	No. of conidia examined	% Germi - nation.	Mean Germ tube length in μ	No. of conidia examined	% Germi - nation.	Mean Germ tube length in μ
4	3552	4.79	1.8	3712	23.26	5.2
10	3490	8.17	8.1	3556	42.79	48.6
15	3036	59.16	27.1	3473	62.88	241.0
18	3725	61.27	39.2	4092	65.41	443.7
20	3114	59.81	24.0	3211	60.92	333.2
25	3455	52.59	17.4	3875	57.60	88.0
33	3222	0.00	-	3616	0.00	-
35	3696	0.00	-	3505	0.00	-
38	3372	0.00	-	3344	0.00	-

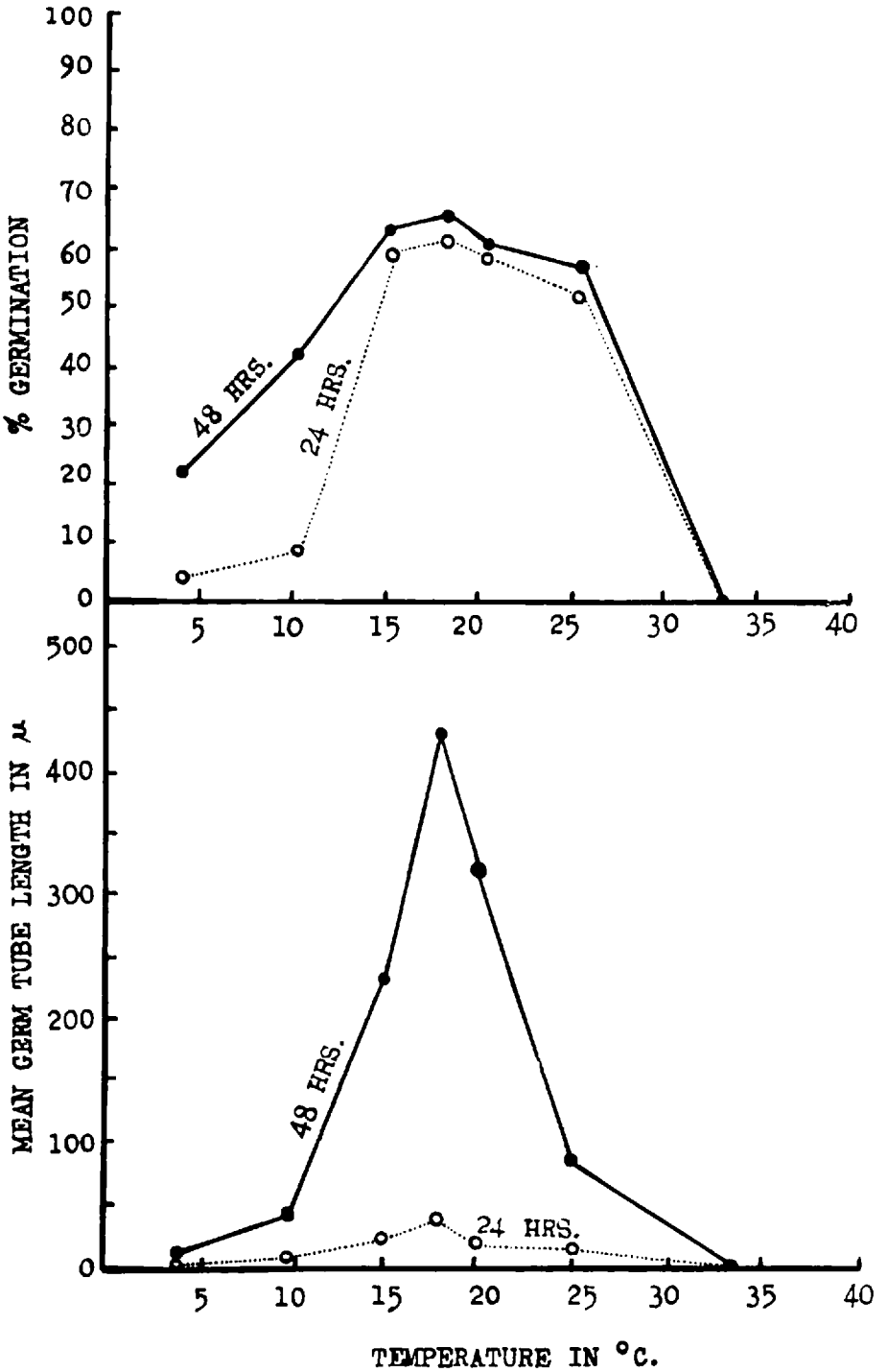


FIG.1. EFFECT OF TEMPERATURE ON GERMINATION OF CONIDIA OF B.BASSIANA SOWN IN WATER.

TABLE 8

Effect of Temperature on Germination of conidia of I.farinosa incubated in water in darkness.

Temp. of Incubation in °C.	Observations after 24 HRS.			Observations after 48 HRS.		
	No. of conidia examined	% Germination.	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
4	6243	0.00	-	7316	0.00	-
10	4915	0.00	-	9655	2.13	16.0
15	4424	0.72	6.8	4869	3.30	45.3
18	7376	2.56	11.4	5160	7.05	56.5
20	5736	13.48	32.5	9280	16.98	180.9
22	5113	13.23	31.0	6844	16.42	177.5
25	8730	2.71	3.2	6967	3.15	14.9
33	8548	0.00	-	9156	0.00	-
35	4900	0.00	-	7833	0.00	-
38	5756	0.00	-	6004	0.00	-

Conidia of I.farinosa from the coremia germinated rather poorly in water (Table 8). Maximum germination within 48 hours was only 16.98 per cent. No conidia germinated within 48 hours at 4°C. and at 33°C. and above. The optimum temperature lay around 20 - 22°C. Germination at 10°C. was considerably delayed ;

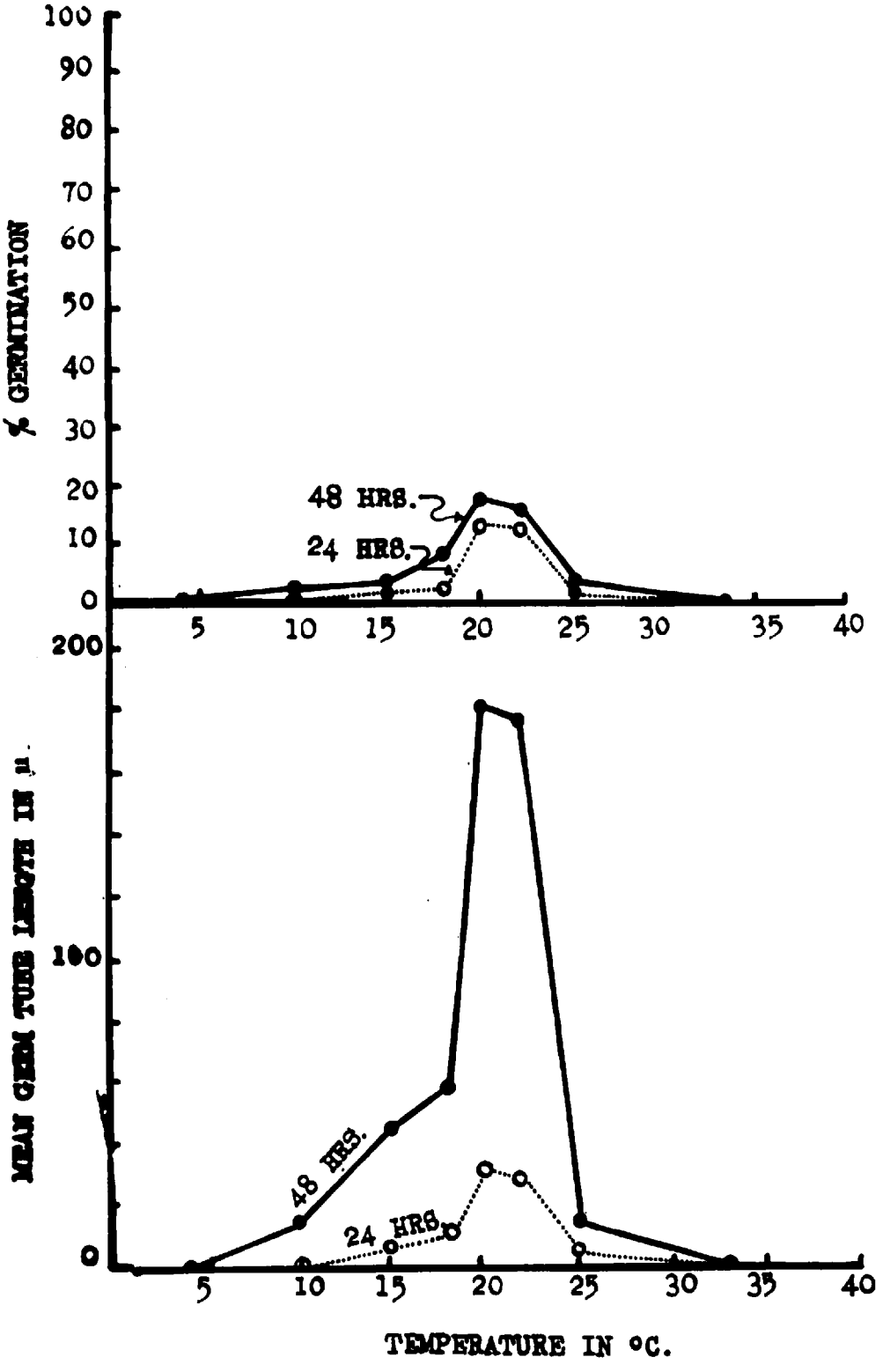


FIG. 2. EFFECT OF TEMPERATURE ON GERMINATION OF CONIDIA OF I.FARINOSA FROM COREMIA SOWN IN WATER.

none had germinated in 24 hours. The trend of temperature response by the conidia observed here, was closely similar to the data of Brown and Smith (1957). They stated that, germination was very tardy at 8°C., slow at 10 - 14°C., moderate at 24°C. and nil at 37°C. The minimum temperature found, however, differed from that reported by Müller - Kögler (1948), viz. 0.5°C.

As in B.bassiana, growth of germ tubes again responded similarly to percentage germination. The temperature which supported the highest percentage germination, promoted the greatest germ tube growth.

Effect of Temperature on Germination of conidia of Paecilomyces - state of I.farinosa.

I.farinosa produces two types of conidia - those borne on the erect coremia, and conidia produced by phialides borne directly on the undifferentiated vegetative hyphae, referred to as the Paecilomyces - state. In the above experiment, conidia from the coremia germinated poorly in water. It was considered desirable to investigate the germination of conidia of the Paecilomyces - state in water under similar temperature conditions. The results are presented in Table 9 and in Fig. 3.

The results revealed that even fewer of the conidia of Paecilomyces - state of I.farinosa germinated in water than

those from the coremia. The conidia moreover failed to germinate at 10°C. within the period of 48 hours. The optimal and maximal temperatures were again about 20°C. and 33°C. respectively. Germ tube elongation was generally slower.

TABLE 9

Effect of Temperature on Germination of conidia of Paecilomyces-state of I.farinosa incubated in water in darkness.

Temp. of Incubation in °C.	Observations after 24 HRS.			Observations after 48 HRS.		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
4	9974	0.00	-	8064	0.00	-
10	5144	0.00	-	9266	0.00	-
15	6130	0.17	4.3	9328	0.55	12.7
18	6192	0.93	7.2	8262	4.20	71.4
20	8926	5.18	18.3	9018	10.12	104.3
22	6797	2.93	5.9	6593	7.92	49.9
25	5560	2.11	5.5	7111	2.21	17.1
33	9355	0.00	-	8717	0.00	-
35	5202	0.00	-	7499	0.00	-
38	6569	0.00	-	5433	0.00	-

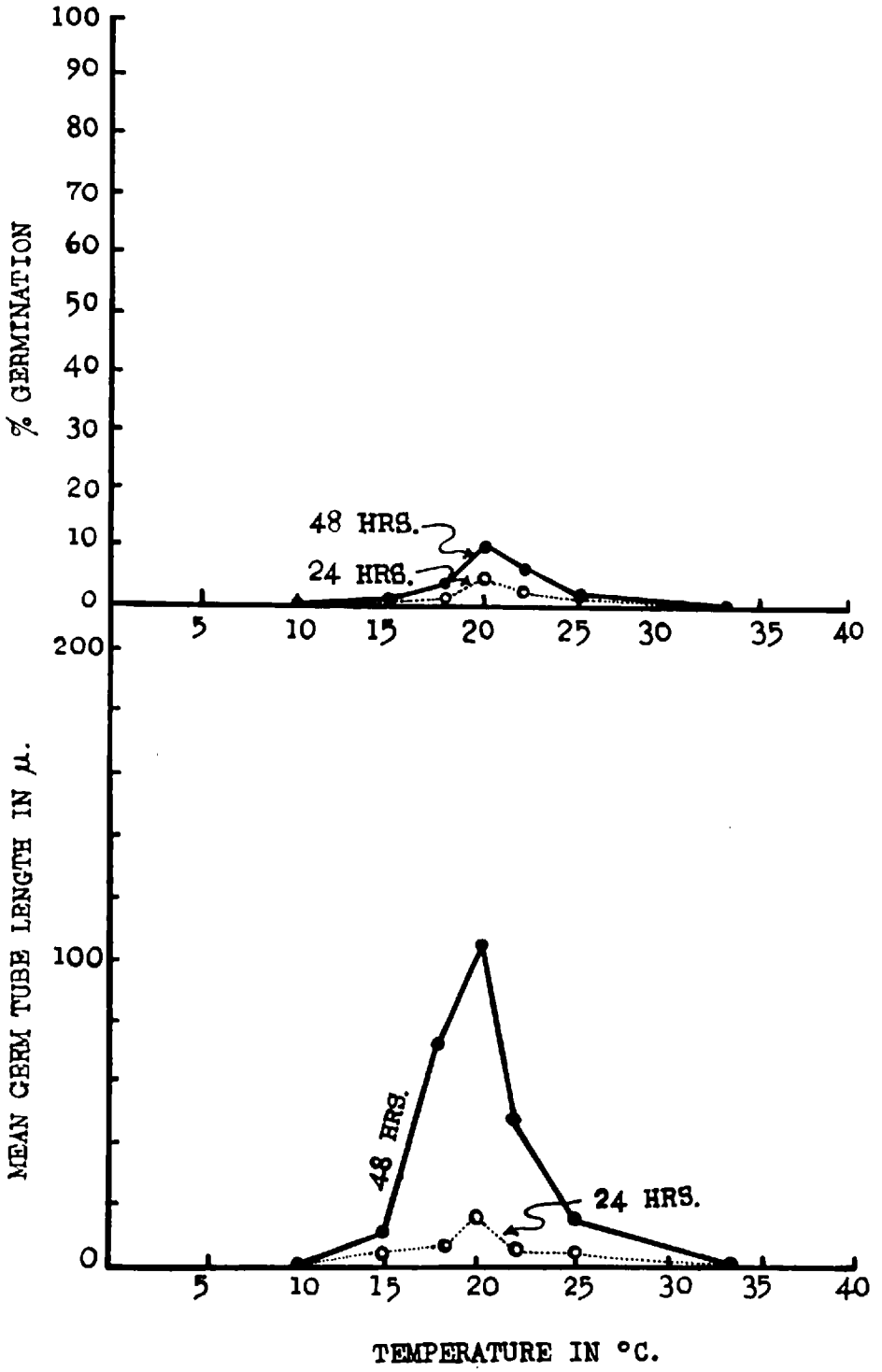


FIG. 3. EFFECT OF TEMPERATURE ON GERMINATION OF CONIDIA OF I.FARINOSA OF PAECILOMYCES STATE SOWN IN WATER.

TABLE 10

Effect of Temperature on Germination of conidia of M.anisopliae incubated in water in darkness.

Temp. of Incubation in °C.	Observations after 12 HRS.			Observations after 24 HRS.		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
4	4270	0.00	-	4255	0.00	-
10	4175	0.00	-	4182	0.00	-
15	3946	2.16	9.6	4050	32.54	11.7
18	3885	18.86	14.1	4169	75.70	18.0
20	4240	31.39	21.7	4079	83.03	29.9
25	4100	87.52	38.0	4422	99.78	82.0
33	4375	23.34	26.8	4335	40.74	45.3
35	4202	0.59	21.5	3995	0.70	26.0
38	4413	0.00	-	3890	0.00	-

Conidia of M.anisopliae (of which the isolate was of tropical origin) germinated over a higher range of temperature than did conidia of B.bassiana and I.farinosa. The conidia germinated within 24 hours over the temperature range of 15 - 35°C. (Table 10), showing a very distinct optimum at 25°C., where

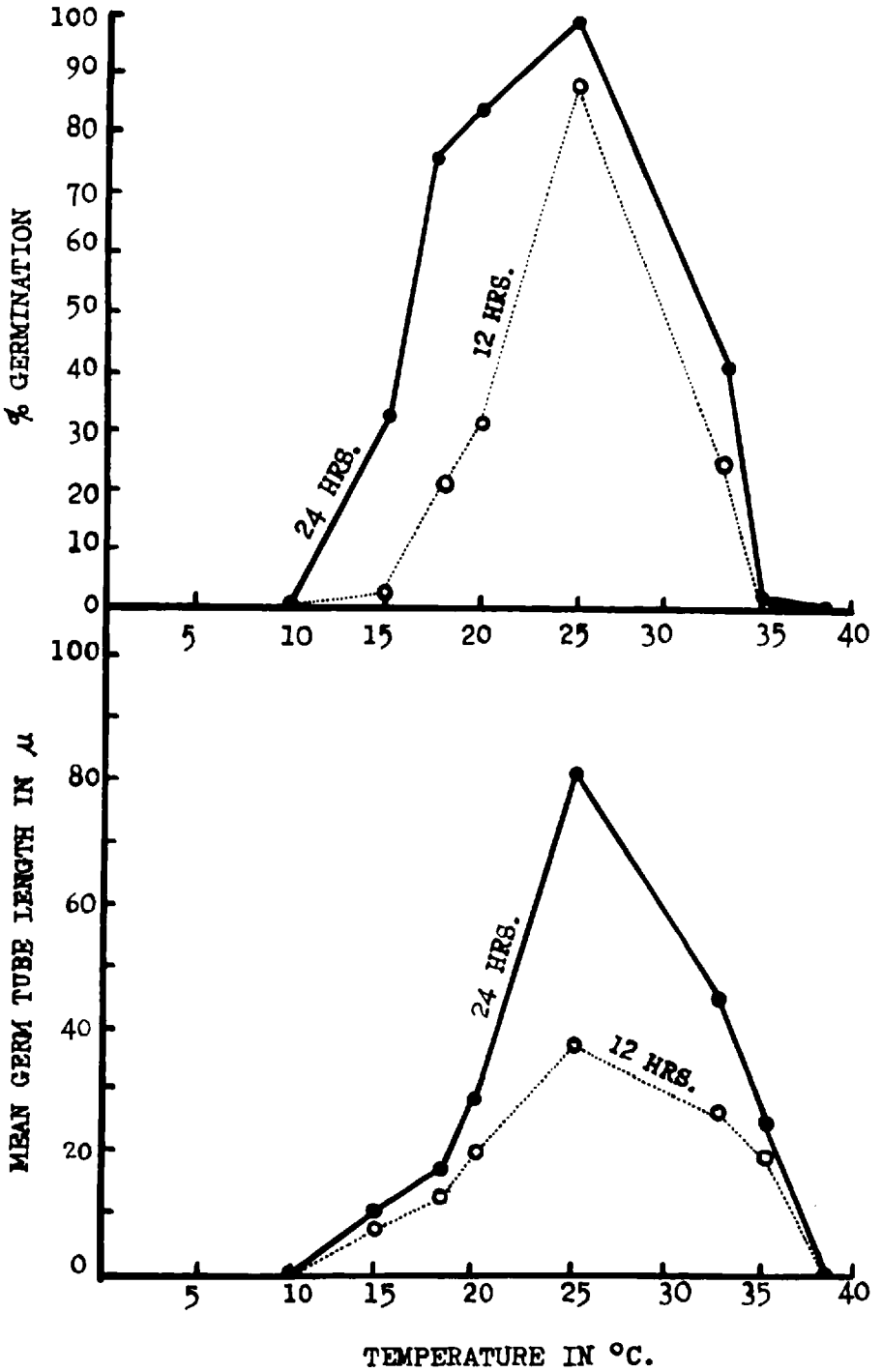


FIG. 4 EFFECT OF TEMPERATURE ON GERMINATION OF CONIDIA OF M.ANISOPLIAE SOWN IN WATER.

87.57 per cent of the conidia germinated after only 12 hours. Germination was also high at 18 and 20°C. Conidia at 35°C. produced peculiarly swollen and abnormal germ tubes, which were never observed under any other conditions in this work. The structural details of these abnormal germ tubes are described below on page 73 . Minimal, optimal and maximal temperatures for percentage germination and germ tube growth were the same (Fig. 4).

The optimum temperature for germination (25°C.) agrees with that reported by Vouk and Klas (1932) (24 - 26°C.).



x 160



x 160



x 160

CONIDIA GERMINATING AFTER 24 HOURS IN DISTILLED WATER AT TEMPERATURES OPTIMAL FOR THE SPECIES. PLATE 3.- CONIDIA OF B.BASSIANA AT 18°C. PLATE 4.- CONIDIA OF I.FARINOSA AT 20°C. PLATE 5.- CONIDIA OF M.ANISOPLIAE AT 25°C.

C. Influence of Temperature on Germination during Prolonged Periods.

In the previous experiments the incubation period for conidia of B.bassiana and I.farinosa was restricted to 48 hours and that for conidia of M.anisopliae to 24 hours. The effect of temperature on conidial germination during a longer incubation period was also investigated. Conidia in sterile distilled water, were incubated in moist chambers in darkness for 144 hours, and percentage germination recorded at regular 24 hour - intervals. The germ tube lengths were not measured, as they grew and branched extensively with time creating technical difficulty. The results are presented in Tables 11 - 13 and in Figs. 5 - 7.

Germination of B.bassiana conidia (Table 11 and Fig. 5) at the optimum temperature (18°C.) was so rapid that almost all the conidia capable of germinating in water, had germinated within the first 24 hours. Conidia held at temperatures near the optimum, i.e., 15, 20 and 25°C. showed more or less similar behaviour, viz., a fairly vigorous germination rate within the first 24 hours, succeeded by a period of about 24 - 48 hours in which maximum conidial germination for the respective temperatures was achieved. There was no increase after the 72nd hour. At 4 and 10°C., the initially vigorous germination was lacking. Germination at these temperatures was slow to start, but progressed gradually with time to achieve the rather high figures of 44.39 and 51.86 per cent germination at 4 and 10°C. respectively. There was no germination within 144 hours at 33, 35 and 38°C.

TABLE 11

Effect of Temperature during Prolonged Periods on Germination of conidia of B.bassiana incubated in water in darkness.

Temp. of Incubation in °C.	Details of Conidia	PERIOD OF INCUBATION IN HOURS.					
		24	48	72	96	120	144
4	No. examined	2709	3129	2584	2896	2879	2799
	% Germination	1.00	5.21	9.44	26.43	32.37	44.39
10	No. examined	3665	3998	4981	2797	2742	2667
	% Germination	2.21	10.15	22.18	48.66	48.46	51.86
15	No. examined	3114	3562	3355	3367	3036	3473
	% Germination	62.36	66.92	72.54	71.64	72.36	72.58
18	No. examined	2461	2493	3590	3399	3246	3259
	% Germination	71.48	71.83	72.22	72.18	71.98	72.87
20	No. examined	2844	2735	2798	2934	2998	2964
	% Germination	54.92	66.44	66.00	66.47	66.61	66.89
25	No. examined	2713	3328	3538	3592	3281	3658
	% Germination	45.85	60.18	62.37	64.23	63.94	64.35
33	No. examined	2947	3175	2425	3042	3114	3450
	% Germination	0.00	0.00	0.00	0.00	0.00	0.00
35	No. examined	3562	3136	3229	3628	3344	3575
	% Germination	0.00	0.00	0.00	0.00	0.00	0.00
38	No. examined	3643	3387	3156	3584	3281	3655
	% Germination	0.00	0.00	0.00	0.00	0.00	0.00

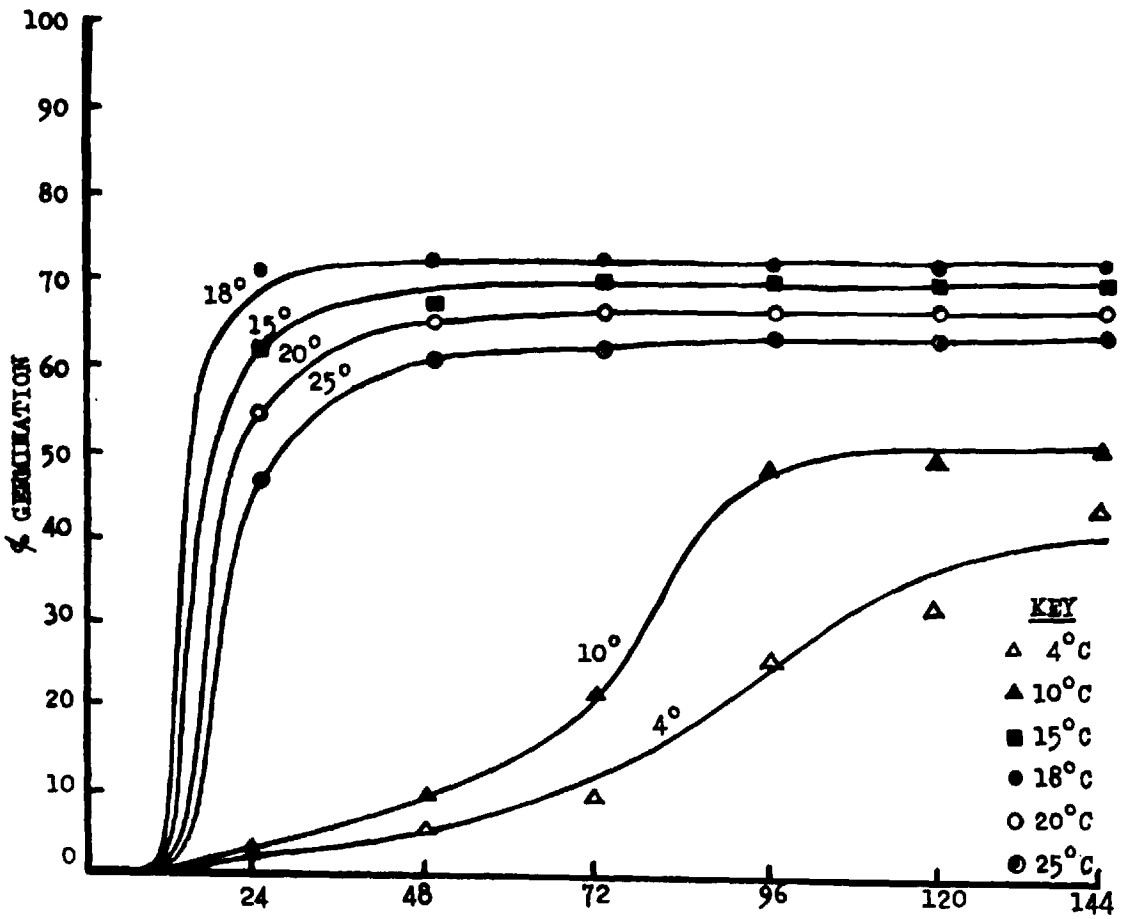


FIG. 5. INFLUENCE OF TEMPERATURE ON GERMINATION OF CONIDIA OF B.BASSIANA DURING PROLONGED PERIODS.

TABLE 12

Effect of Temperature during Prolonged Periods on Germination of conidia of I.farinosa incubated in water in darkness.

Temp. of Incubation in °C.	Details of Conidia	PERIOD OF INCUBATION IN HOURS					
		24	48	72	96	120	144
4	No. examined	4485	3798	3005	3549	3585	3443
	% Germination	0.00	0.00	0.00	0.00	0.00	0.00
10	No. examined	2232	2715	2372	2220	2247	2200
	% Germination	0.00	5.76	8.55	9.66	12.42	13.14
15	No. examined	4748	3290	3582	3578	3568	3558
	% Germination	0.44	8.81	8.26	10.78	11.09	12.19
18	No. examined	3698	2434	2510	2540	2512	2854
	% Germination	3.59	12.98	13.07	16.38	17.52	22.42
20	No. examined	2786	2299	2245	1878	1922	2161
	% Germination	6.68	14.18	16.44	21.40	21.96	30.40
22	No. examined	3680	1918	2027	2008	1995	1968
	% Germination	4.62	12.14	12.40	14.95	16.29	17.02
25	No. examined	2228	1961	1949	1847	1908	1902
	% Germination	2.65	6.32	6.64	7.36	7.44	8.20
33	No. examined	2516	2607	2382	2469	2411	2384
	% Germination	0.00	0.00	0.00	0.00	0.00	0.00
35	No. examined	2645	2152	2427	2777	2284	2423
	% Germination	0.00	0.00	0.00	0.00	0.00	0.00

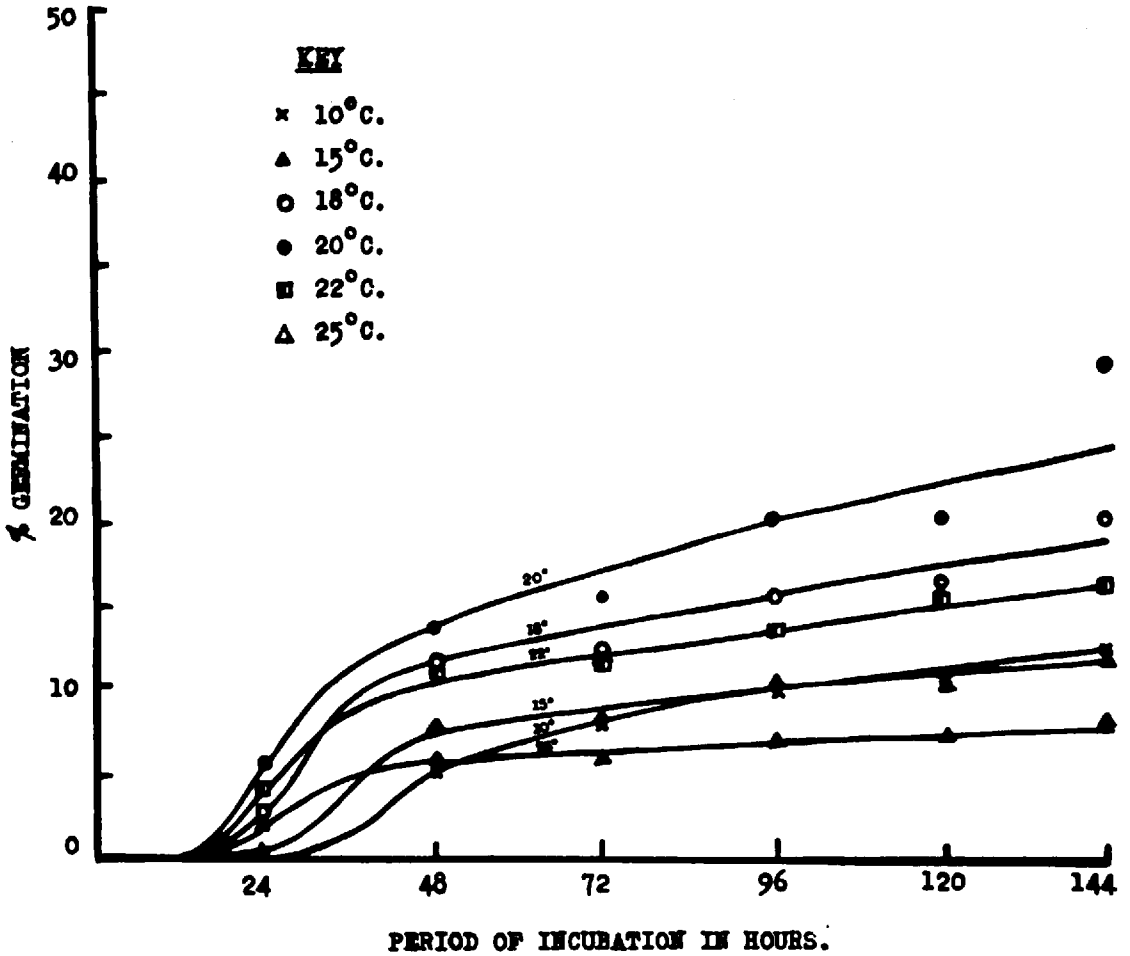


FIG. 6. INFLUENCE OF TEMPERATURE ON GERMINATION OF CONIDIA OF I. FARINOSA DURING PROLONGED PERIODS.

With I.farinosa the poor germination of the conidia in water was again evident (Table 12 and Fig. 6). Even at the optimum (20°C.) only 30.40 per cent germinated within 144 hours. This being attained by a gradual increase in germination over the entire incubation period. This was the pattern of response at all temperatures. There was again no germination at 4, 33 and 35°C. The behaviour of the conidia held at 10°C. observed in the previous experiment (see Table 8) was confirmed here, namely, that there was no germination in the first 24 hours of incubation. Later, the conidia began to germinate, to show 5.96 per cent germination after 48 hours.

Extension of the incubation period beyond 48 hours, revealed a well defined optimum at 20°C. (cf., Table 8 and Fig. 2).

With M.anisopliae, it was apparent that a period of 48 hours, at 18, 20 and 25°C. (Table 13 and Fig. 7) was long enough to ensure the maximum germination possible. At 15°C. it took 72 hours to attain maximum germination (ca., 100 %). At the optimum temperature (25°C.), 96.52 per cent of the conidia germinated in 24 hours and practically all did so within 48 hours. At 18 and 20°C., about 75 per cent germinated within 24 hours, increasing to approximately 98 per cent within the next 24 hours. Although the initial germination rate at 15°C. was less rapid, almost all the conidia germinated in 72 hours. 15°C. was the lowest sub-optimal temperature allowing germination within

TABLE 13

Effect of Temperature during Prolonged Periods on Germination of conidia of M.anisopliae incubated in water in darkness.

Temp. of Incubation in °C.	Details of Conidia	PERIOD OF INCUBATION IN HOURS					
		24	48	72	96	120	144
4	No. examined	3528	3114	2974	3165	3046	2981
	% Germination	0.00	0.00	0.00	0.00	0.00	0.00
10	No. examined	4225	3172	3595	2765	3660	3592
	% Germination	0.00	0.00	0.00	0.00	0.00	0.00
15	No. examined	5153	5357	4049	3377	3340	3027
	% Germination	30.64	97.13	99.28	99.76	99.91	100.0
18	No. examined	6699	4642	3963	3994	3561	3775
	% Germination	74.58	97.39	99.67	99.97	100.0	100.0
20	No. examined	5700	4096	3111	3915	3034	3201
	% Germination	75.22	98.12	99.45	99.85	100.0	100.0
25	No. examined	3746	4628	3199	3625	3614	3401
	% Germination	96.52	98.74	99.50	100.0	100.0	100.0
33	No. examined	5610	8068	6846	3984	3107	3070
	% Germination	33.63	34.54	81.71	85.54	87.77	87.90
35	No. examined	7566	5714	5165	4990	4451	4430
	% Germination	3.36	10.39	17.17	46.51	60.84	60.84
38	No. examined	3038	4799	3228	3316	3656	5121
	% Germination	0.00	0.00	0.00	0.00	0.00	0.00

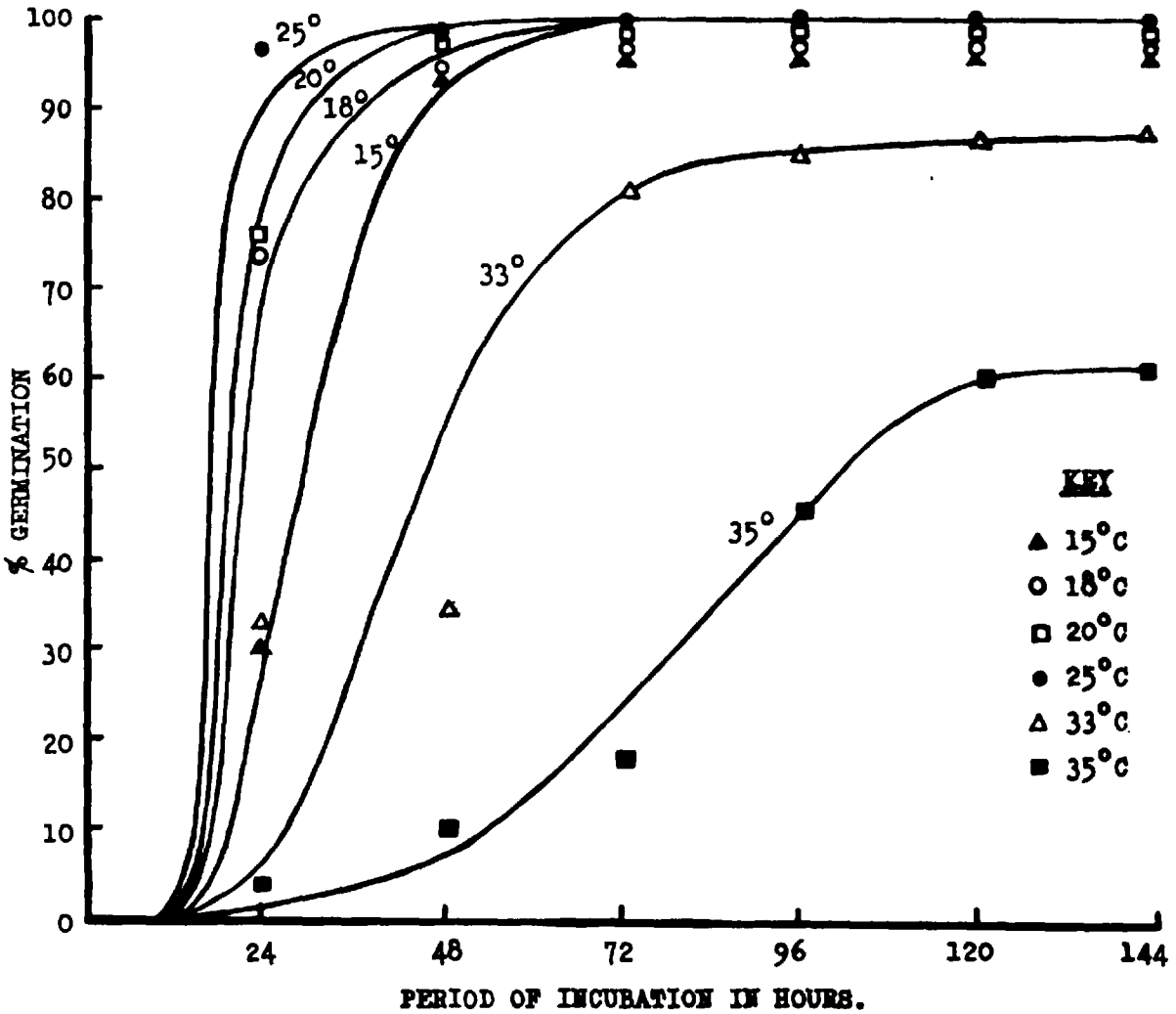


FIG. 7. INFLUENCE OF TEMPERATURE ON GERMINATION OF CONIDIA OF M.ANISOPLIAE DURING PROLONGED PERIODS.

144 hours. At 33 and 35°C., the initial germination rate was slow, but after about 120 hours, percentage germinations of 87.77 and 60.84 per cent respectively, were attained. However no conidia germinated at 38°C.

D. GERMINATION OF CONIDIA OF B.BASSIANA AND M.ANISOPLIAE IN WATER FROM DIFFERENT SOURCES.

Gabriel (1959) reported that conidia of M.anisopliae did not germinate in water, and that B.bassiana conidia germinated to only a very small degree. Since high percentage conidium germination was consistently attained in water with M.anisopliae and B.bassiana in the experiments described above, further studies were carried out.

Conidial suspensions were prepared with water from different sources. The distilled water used in the previous experiments was obtained from a metal still. It was considered necessary to find out whether this water acquired some traces of minerals from the apparatus which might have stimulated conidial germination. Distilled water, obtained from a glass still, and rain water were used in the present investigation in addition to the metal-distilled water. The rain water was collected on the roof of the Botany Department building of Imperial College of Science and Technology, London, in a clean pyrex glass bowl. Water from the three sources was autoclaved and used in the preparation of conidial suspensions. The slide method was employed and drops of conidial suspensions of B.bassiana and M.anisopliae were incubated at 18 and 25°C. respectively for 24 hours. The results are presented in Table 14.

TABLE 14

Germination of conidia of B.bassiana and M.anisopliae in water from different sources. (Observations after 24 hours' incubation)

SOURCE OF WATER.	B.bassiana (Incubated at 18°C.)			M.anisopliae (Incubated at 25°C.)		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
Metal still	2543	74.44	38.1	3950	93.95	86.0
Glass still	2557	73.18	38.4	4061	94.82	85.0
Rain	2773	77.31	37.3	3765	95.13	89.4

The results in Table 14 showed that conidia of B.bassiana and M.anisopliae germinated equally in water from the three sources. There was thus no evidence that germination in the previous experiments (see Tables 7, 10, 11 and 13) had been encouraged by traces of minerals in water from the metal still.

E. COMPARISON OF GERMINATION OF CONIDIA OF B.BASSIANA AND M.ANISOPLIAE COLLECTED BY FLOODING OF CULTURE SLANTS AND IN DRY STATE.

Conidia of B.bassiana and M.anisopliae used in the earlier experiments, had been collected from the culture slants by flooding with water. The possibility of the water acquiring traces of nutrients from the agar medium during flooding, and that these nutrients had enhanced germination could not be ignored. In the following experiment, conidia for germination tests were removed from culture surfaces by two methods :

a) flooding with distilled water, and

b) removing the conidia in the dry state with a camel hair brush.

The conidia of B.bassiana and M.anisopliae were incubated in glass distilled water at 18 and 25°C., respectively, for 24 hours, using the slide method. The results are presented in Table 15.

The data reveal that conidia of B.bassiana and M.anisopliae removed in the dry state with a camel hair brush germinated as well as those removed from the culture slants by flooding.

The results confirmed that conidia of B.bassiana and M.anisopliae germinate in pure water, and that any contaminant nutrients acquired when flooding the cultures, had apparently exercised no influence on germination in the previous experiments.

TABLE 15

Comparison of Germination of conidia of B.bassiana and M.anisopliae collected by Flooding culture slants and in Dry state. (Observations after 24 hours' incubation.)

METHOD OF COLLECTION OF CONIDIA	B.bassiana (Incubated at 18°C.)			M.anisopliae (Incubated at 25°C.)		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
Flooding	3812	68.80	32.6	3472	97.23	92.9
Dry state	3620	69.55	33.2	3596	97.55	94.8

F. EFFECT OF CONIDIAL DENSITY ON GERMINATION OF CONIDIA OF B.BASSIANA AND M.ANISOPLIAE SOWN IN WATER.

Previous experiments showed that conidia of B.bassiana and M.anisopliae germinated extremely well in just water. Because the conditions under which the germination tests of Gabriel (1959) were conducted were not specified, it was not possible to conclude why he obtained such low germination with B.bassiana and none with M.anisopliae in water. The rate of fungal spore germination is often determined by the age of the spores, and also by the concentration of the conidia in the germination drop. The ages of B.bassiana and M.anisopliae conidia used in the above experiments were only 8 and 5 days respectively. The manner in which age of the conidia might have influenced the results of Gabriel's op. cit. work is discussed below in connection with investigations on longevity of the conidia. Experiments were conducted to test the second possibility, viz., that conidial density affected germination. Standard conidial suspensions in water were prepared and used in germination tests. The slide method was used, and the conidia were incubated at their respective optimal temperatures for 24 hours, after which the percentage germination was determined and germ tube lengths measured. The results are presented in Tables 16 and 17.

TABLE 16

Effect of conidial density on Germination of conidia of B.bassiana sown in water. (Observations after 24 hours' Incubation at 18°C.)

Conidia per ml. of water	No. of conidia examined	% Germination	Mean Germ tube length in μ
105	48	95.83	28.1
105 x 10	86	97.67	32.5
105 x 10^2	373	87.68	29.3
105 x 10^3	3642	67.55	26.6
105 x 10^4	40095	40.81	18.8
105 x 10^5	78647	3.06	9.3
105 x 10^6	174545	0.14	4.3

TABLE 17

Effect of conidial density on Germination of conidia of M.anisopliae sown in water. (Observations after 24 hours' Incubation at 25°C.)

Conidia per ml. of water	No. of conidia examined	% Germination	Mean Germ tube length in μ
146	95	100.0	85.5
146 x 10	363	100.0	84.7
146 x 10^2	970	97.94	88.9
146 x 10^3	3108	95.88	87.5
146 x 10^4	13576	49.14	60.3
146 x 10^5	24899	5.12	33.5
146 x 10^6	193825	3.84	13.6

The conidia of B.bassiana (Table 16) and M.anisopliae (Table 17) germinated at all concentrations tested, but in different proportions. Conidia in suspensions of lower concentrations germinated very well, but as the concentration increased, percentage germination fell. The percentage germination of B.bassiana conidia in suspensions of concentrations of less than 105 x 10^2 conidia per ml. of water are much higher than those hitherto recorded (e.g., 71.48 % - Table 11). This increase might be an apparent one, attributable to the difficulty of locating all ungerminated conidia (because of the sparseness of their distribution), rather than a real effect of extreme dilution.

Within the limits of spore concentration likely to be employed in germination tests, conidia of B.bassiana and M.anisopliae germinate very well in water. The reason for the failure of the conidia to germinate in Gabriel's (1959) experiments therefore appears not to be connected with spore density.

G. DETAILS OF STRUCTURE AND EARLY STAGES OF GERMINATION OF CONIDIA OF B.BASSIANA, I.FARINOSA AND M.ANISOPLIAE.

Having established the respective optimum temperatures for germination of the conidia, it became possible to study in detail the stages in germination of the conidia under the most congenial temperature conditions. Distilled water was used as the germination medium to reduce the number of external influences.

Conidia germinated by the "slide" method were incubated at their respective optimum temperatures and examined at regular half-hour intervals. Pertinent drawings of stages of germination were made with the aid of a camera lucida.

Details of Structure of Conidia of B.bassiana.

Primary Conidia : The isolate of B.bassiana employed in this investigation was found to possess a mixture of globose and oval primary conidia (Text-Fig. 1, Fig. 8). Of 1,000 conidia examined and measured (with a micrometer eye-piece and an apochromatic oil-immersion objective), 68 per cent were globose and the rest oval. Both globose and oval conidia were hyaline and smooth-walled.

The observations here made that primary conidia of B.bassiana were a mixture of globose and oval ones, agreed with the findings of Lefebvre (1931), MacLeod (1954) and Petch (1926, 1931). The diameter of 2.1 - 2.8 μ of the globose conidia of the

isolate studied in this investigation fell into the intermediate range of the reported dimensions cited by MacLeod (1954). Oval conidia measured 2.1 - 2.4 x 2.8 - 4.7 µ. These were bigger than those studied by MacLeod (1954) and Petch (1926, 1931) viz., 2 x 1.5 µ and 1.5 - 2 x 1.2 - 2 µ respectively, but very close in dimensions to those studied by Lefebvre (1931).

Secondary Conidia. De Bary (1869) found that when primary conidia from cultures of Beauveria species were germinated in a thin film of water, the germ tubes, after reaching a length of 6 to 10 times the diameter of the conidia, produced oblong-cylindric secondary conidia. He named these " cylinder conidia ".

Secondary conidia were also reported among Beauveria species by Pettit (1875), Siemaszko (1937) and MacLeod (1954).

During this investigation, similar secondary conidia were produced by germ tubes of primary conidia sown in distilled water on glass slides (Text - Fig. 2). These were again observed when primary conidia were germinated at 100 % relative humidity. Secondary conidia were borne directly on germ tubes of primary conidia of varying lengths, over the range of 5 to 36 µ. The secondary conidia measured 1.5 - 2.5 x 3.5 - 4.6 µ.

The class distributions of the primary and secondary conidia according to sizes are presented in Tables 18a and 18b.

TABLE 18 (a)

Distribution of Primary Conidia of B.bassiana in classes according to size.[1]

	LENGTH						WIDTH		
OVAL CONIDIA (32.0%)	% Conidia in 0.4μ classes.						% Conidia in 0.2μ classes.		
	2.8μ	3.2μ	3.6μ	4.0μ	4.4μ	4.8μ	2.0μ	2.2μ	2.4μ
	2.0%	12.9%	42.9%	37.5%	4.7%		8.7%	91.3%	

	DIAMETER					
GLOBOSE CONIDIA (68.0%)	% Conidia in 0.2μ classes					
	2.0 μ	2.2 μ	2.4 μ	2.6 μ	2.8 μ	3.0 μ
	0.6%	2.1%	57.6%	39.4%	0.3%	

TABLE 18 (b)

Distribution of Secondary Conidia of B.bassiana in classes according to size.[2]

LENGTH						
% Conidia in 0.2 μ classes						
3.4μ	3.6μ	3.8μ	4.0μ	4.2μ	4.4μ	4.6μ
0.2%	1.3%	3.1%	17.3%	31.9%	46.2%	

WIDTH						
% Conidia in 0.2 μ classes						
1.4μ	1.6μ	1.8μ	2.0μ	2.2μ	2.4μ	2.6μ
0.1%	0.3%	2.2%	42.3%	51.3%	3.8%	

[1] Determinations based on 1,000 conidia observed.

[2] Determinations based on 200 conidia observed.

Early Stages of Germination of Conidia of B.bassiana.

Conidia of B.bassiana always swell prior to germination. Germ tubes appeared to arise from any point on the wall of the conidium. The germ tubes were non - branching, thin - walled, hyaline and continuous. Germinating conidia usually produced one (Text-Fig. 1, Fig. 9) or two germ tubes (Text - Fig. 1, Fig. 10), but sometimes three (Text - Fig. 1, Fig. 11). Three were the maximum observed. When there were two, the angle separating them might range from a very acute one to 180° (Text - Fig. 1, Fig. 10)

The first germinating conidia were observed at 5 hours. Subsequent examinations showed germ tube lengths ranging from a few microns to as much as 12.8, 15.3 and 16.4 μ at 7, 8 and 9 hours respectively (Text - Fig. 1, Fig. 9). One or two cross - walls were present in some of the longer germ tubes at 12 hours (Text - Fig. 1, Fig. 9h). Secondary branches started to form at 30 hours. The period before germ tubes emerged was shorter than that recorded by MacLeod (1954), who noticed the first germ tubes at 8 hours, in conidia sown on Sabouraud - maltose agar.

Details of Structure of Conidia of I.farinosa.

Conidia of I.farinosa were oval, hyaline and smooth - walled (Text - Fig. 3, Fig. 12): the lengths ranged from 2.3 to 2.6 μ, mostly 2.5 μ, and widths were 1.4 μ. The observed size and shape agreed with the reports of Petch (1926) and, Brown and Smith

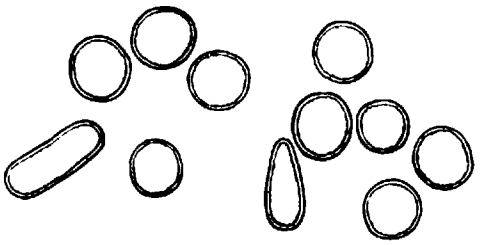


FIG. 8 SPORES OF BEAUVERIA BASSIANA (BALS) VUILL. SHOWING VARIATION IN SIZE AND SHAPE.

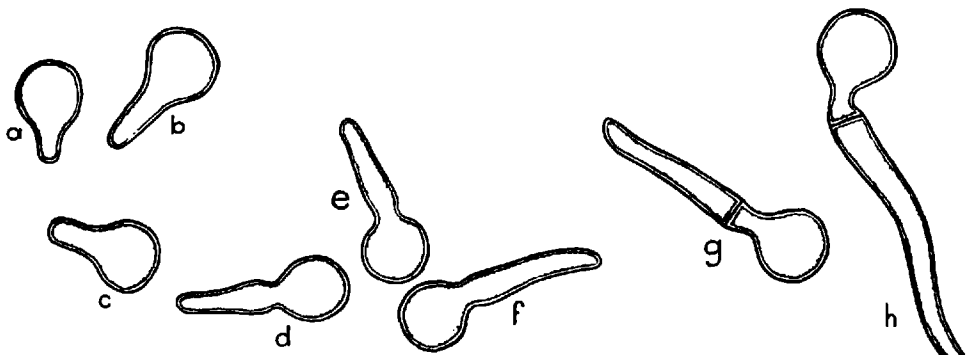


FIG. 9 GERMINATING SPORES.

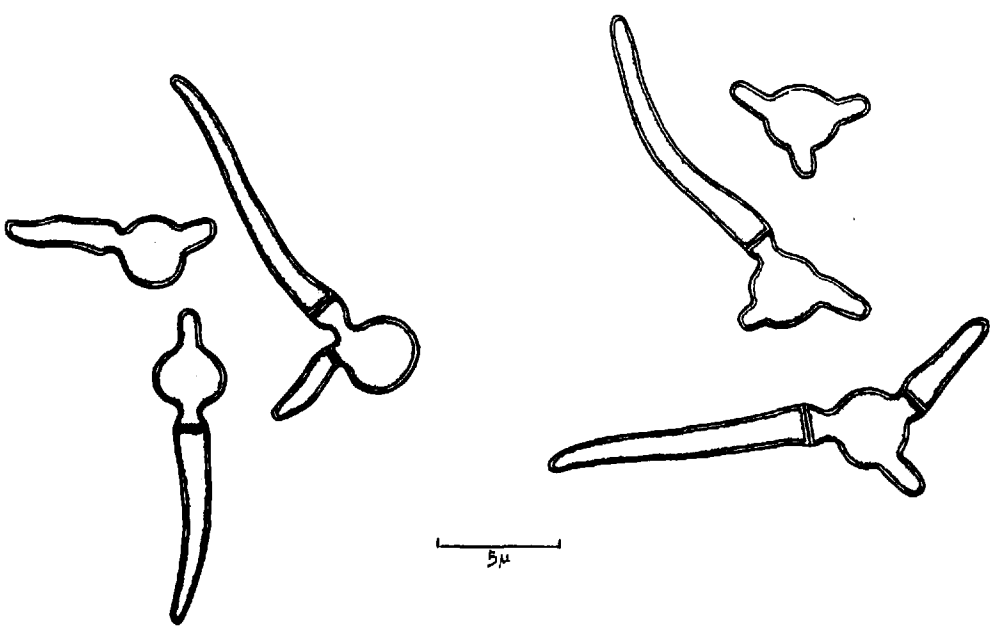
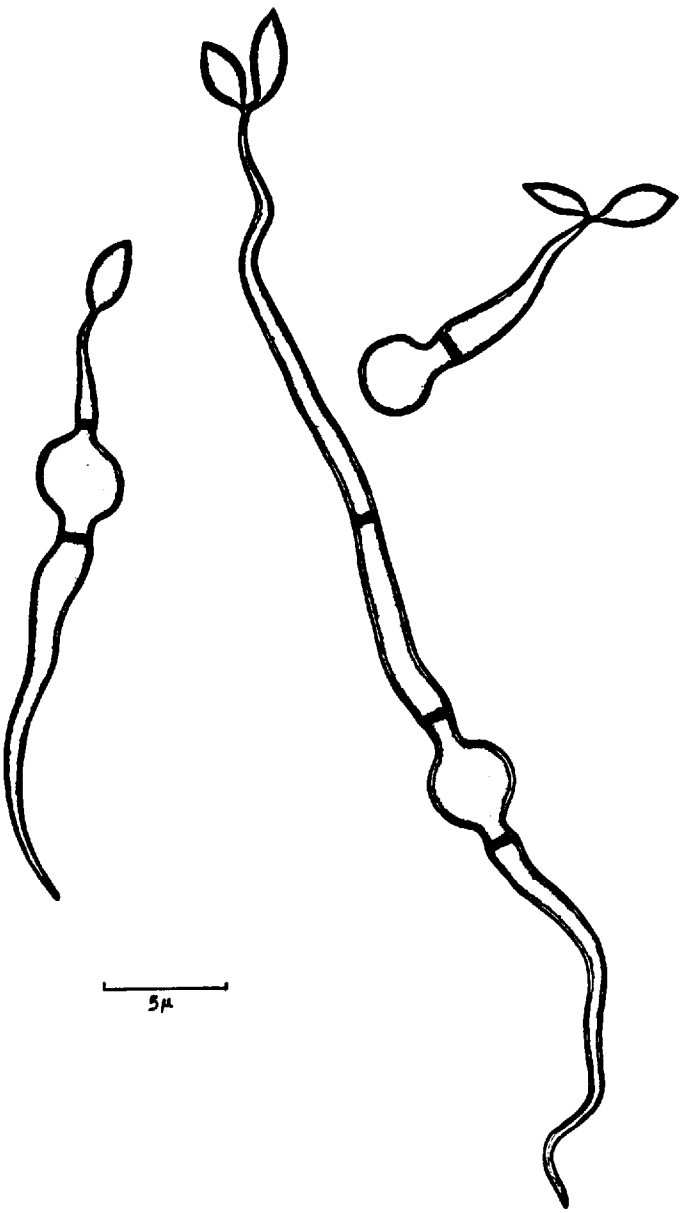


FIG.10.SPORES WITH TWO GERM TUBES DEVELOPING AT VARIOUS PLACES ON THE SPORE.

FIG. 11. SPORES WITH THREE GERM TUBES DEVELOPING AT VARIOUS PLACES ON THE SPORE.

TEXT-FIG. 1. SPORE GERMINATION IN BEAUVERIA BASSIANA (BALS) VUILL.



TEXT-FIG. 2. SECONDARY SPORES BORNE AT THE TIP OF GERM TUBES OF PRIMARY SPORES OF BEAUVERIA BASSIANA (BALS) VUILL. GERMINATING IN WATER AT THE END OF A 36 HOUR PERIOD

(1957). In Saccardo's Sylloge Fungorum (IV, 1886) the conidia were described as globose.

Early Stages of Germination of Conidia of I.farinosa.

The conidia were observed always to swell prior to germination. The swelling, usually apparent after 10 hours of incubation in the fastest germinating conidia, practically doubled their linear dimensions. The first germ tubes were observed at 12 hours. Subsequent examinations showed germ tubes 4.0 μ in length at 16 hours and 12 μ at 20 hours (Text - Fig. 3, Fig. 13). Cross - walls appeared within 6 hours of germ - tube emergence. Secondary branches were initiated at quite early stages of germination, even as early as the sixth hour of the germ tube's existence. (Text - Fig. 3, Fig. 15).

The conidia successively produced one or two hyaline germ tubes. They emerged solely from the ends of the conidia. When two developed, they always emerged from opposite ends. (Text - Fig. 3, Fig, 14). Germ tubes rarely maintained a uniform width. Usually it varied between about 1.2 and 1.8 μ.

Brown and Smith (1957) observed production of germ tubes in as little as 10 hours. The germ tubes sometimes branched after only two hours.

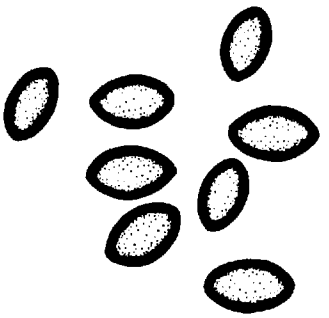


FIG.12 SPORES OF ISARIA FARINOSA.

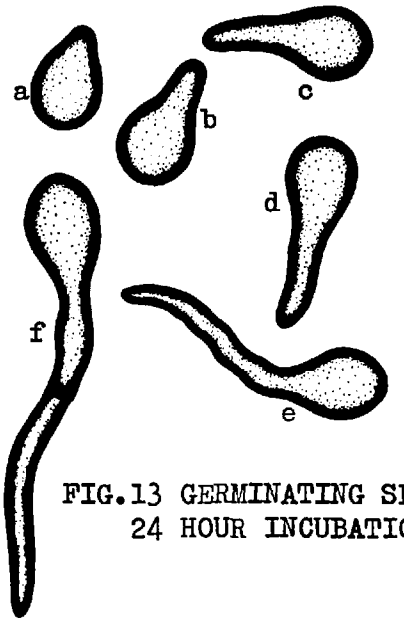


FIG.13 GERMINATING SPORES - AFTER 24 HOUR INCUBATION PERIOD.

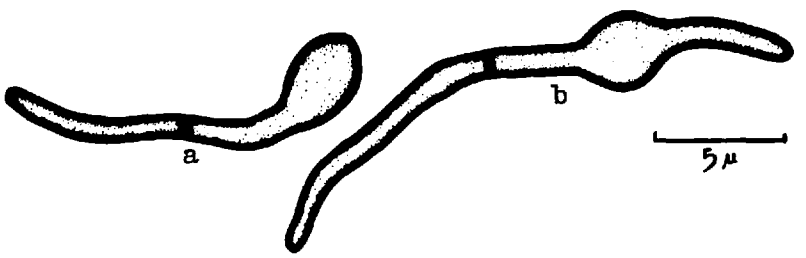


FIG.14 SPORES WITH ONE OR TWO GERM TUBES SHOWING POINTS OF EMERGENCE.

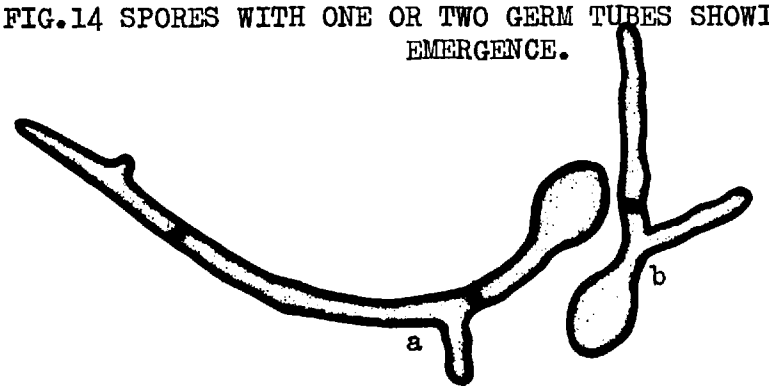


FIG.15 GERM TUBES BRANCHING AFTER 6 HOURS OF EXISTENCE.

TEXT-FIG. 3 SPORE GERMINATION IN ISARIA FARINOSA FRIES.

Details of Structure of Conidia of M.anisopliae.

The conidia of M.anisopliae (the "Green Muscardine") when first formed were white , but gradually became olive green. The conidia were smooth - walled, uniformly cylindrical, and rounded at the ends (Text - Fig. 4, Fig. 16). Their lengths ranged from 4.7 to 9.4 μ, and widths from 2.2 to 2.6 μ, being mostly 2.5 μ. (See Table 19).

TABLE 19

Distribution of Conidia of M.anisopliae in classes according to length[1]

% Conidia in 0.8 μ classes						
4.7μ	5.5μ	6.3μ	7.1μ	7.9μ	8.7μ	9.5μ
2.8%	23.0%	71.7%	1.5%	0.65%	0.34%	

[1] Determinations based on 1,000 conidia observed.

Johnston (1915) described two forms of M.anisopliae, major and minor, occurring on different hosts, and Friederichs (1920) similarly recognised long- and short-spored forms. Radha et. al. (1956) are inclined to accept Johnston's division of the species, with 10.6 to 12 μ and 3.5 to 8.2 μ as the two characteristic ranges of the spore length. According to Johnston's classification, the isolate of M.anisopliae studied in this investigation thus appear to be of the minor form.

Early Stages of Germination of Conidia of M.anisopliae.

Conidia swelled prior to germination. The germ tubes were

first observed at 6 - 7 hours, an hour after the swelling of the conidium had occurred. At the 8th hour, the germ tubes ranged from a few microns to as long as 14.1 μ. The fastest growing germ tubes measured 22 μ at 9 hours, and most of them had a cross-wall (Text - Fig. 4, Fig. 17d). At 24 hours, they ranged from 70 μ to as much as 120 μ in length. Secondary branches started to appear at 30 hours.

The germ tubes always emerged eccentrically from the ends of the cylindrical body of the conidium (Text - Fig. 4, Fig. 17a). The maximum number of germ tubes produced by a conidium was four, of which no more than two developed at one end of the spore (Text - Fig. 4, Fig. 18e). Germ tubes produced in water were characteristicly hyaline, continuous, non - branching and of uniform width of about 1.0 μ.

Abnormal Germ Tube Development by Conidia of M. anisopliae at 35°C.

Notini and Mathlein (1944) observed that at 34°C. growth of the germ tubes of M. anisopliae soon ceased and they became swollen and abnormal in appearance. The hyphae resumed growth after transfer to 20°C. Abnormalities were also noticed among conidia germinating at 35°C. in this work (Text - Fig. 4, Fig. 19). These germ tubes however continued to elongate, even when held at that temperature over prolonged periods. Swollen sections were entirely confined to the base of the germ tubes, extending over lengths ranging from 10 to 20 μ. The conidia initially produced

a normal sized germ tube, until a length of about 1.0 μ had been attained, when further elongation of the germ tube was then accompanied by exaggerated increase in hyphal width to about 2.5 - 3.5 μ. This increase was sustained over a length of 10 - 20 μ, when the growing germ tube suddenly assumed the normal hyphal width of about 1.0 μ again (Text - Fig. 4, Fig. 19d). The germ tube thereafter continued to grow maintaining a normal hyphal width. Some conidia at 35°C. differed in the course of development of the germ tubes. In these, the production of the germ tube involved almost the entire end of the conidium. The enlarged base of the germ tube was thus directly confluent with the conidium (Text - Fig. 4, Fig. 19c). The initial width of about 2.5 - 3.0 μ was maintained over a length of 15 to 18 μ, before reverting to the normal hyphal width of 1.0 μ.

FIG. 16. SPORES OF METARRHIZIUM ANISOPLIAE (METCH) SOROKIN.

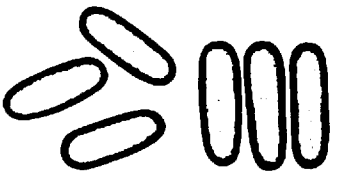


FIG. 17. GERMINATING SPORES AT THE END OF A 9 HOUR PERIOD.

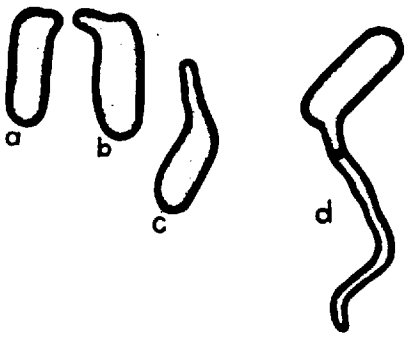


FIG.18. SPORES WITH TWO, THREE OR FOUR GERM TUBES DEVELOPING AT VARIOUS PLACES, EXCENTRICALLY, AT THE ENDS OF THE SPORE.

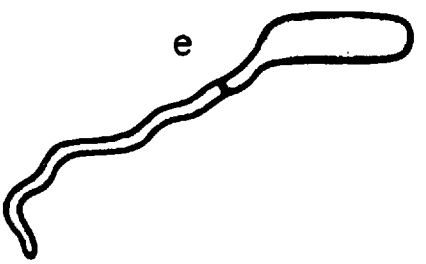
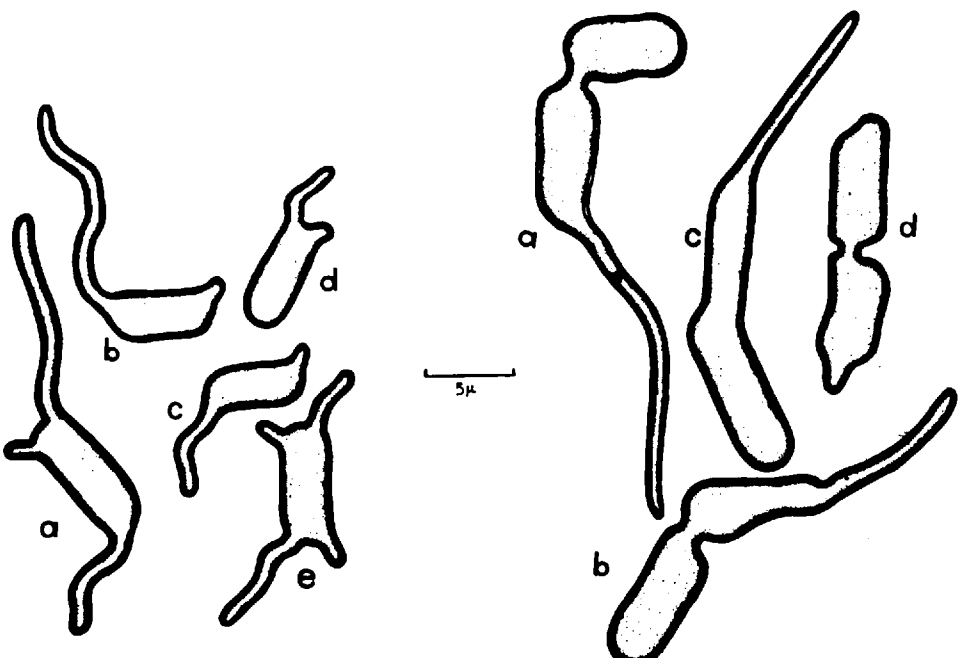


FIG. 19. SPORES GERMINATING AT 35° C. SHOWING PRODUCTION OF PECULIAR DEVELOPMENTAL STRUCTURES.



TEXT-FIG. 4. SPORE GERMINATION IN METARRHIZIUM ANISOPLIAE (METCH) SOROKIN.

H. MAXIMUM LENGTH OF GERM TUBES.

Germination may be considered as a growth process in which some or all the necessary nutrients are present in the spore ab initio. This is necessarily so when germination occurs in water. Since the spore stores only a limited quantity of nutrients, there must be a definite limit to which the germ tubes can grow sustained entirely by these endogenous nutrients, if exogenous substrates are not available. The amount and duration of growth which the endogenous nutrients can support in germinating spores of pathogenic fungi may be very important in the epidemiology of disease.

To determine the maximum length of the germ tubes supported by endogenous nutrients alone, conidia of B.bassiana, I.farinosa and M.anisopliae were germinated in water at their respective optimum temperatures and the germ tube lengths measured at regular intervals until there was no further increase. By setting up one set of experiments in the morning of the first day of the investigation and another set at night on the same day, "day - time" readings of the second set were substituted for what might have been the night observations of the first set. These results were then fitted together to obtain repre - sentative measurements of the germ tubes over an uninterrupted period of 120 hours. Germ tube lengths of 20 conidia were measured in each case.

It was necessary to measure all the ramifications of the germ tubes, and to conduct the measurements on the same conidia throughout the investigation. A very dilute spore suspension was used (approximately 60 conidia per ml. of water). The branches of each germ tube were completely free from interference from hyphal branches of other conidia. To ensure identification of individual conidia, a grid of 25 squares (each square, 1 mm. x 1 mm.) was etched in the centre of glass slides, with a glass cutter, and the conidial drops placed on these grids. On the first signs of germination, the position of the conidium on the grid was carefully noted, and only those labelled conidia were measured on subsequent examinations.

The results are presented in Fig. 20. The two sets of tests, differing in age by 12 hours, gave results which linked together with barely perceptible irregularity. The results showed that the growth curves of the germ tubes were sigmoid. After a latent period of germination, which averaged 7 hours in conidia of B.bassiana, 12 hours in I.farinosa and 6 hours in M.anisopliae, there were respective initial growth periods of about 18, 36 and 18 hours when growth of the germ tubes was slow. This was followed by a period of almost linear, fast growth lasting approximately 40 hours for germ tubes of B.bassiana, 38 for I.farinosa, and 60 for M.anisopliae. The

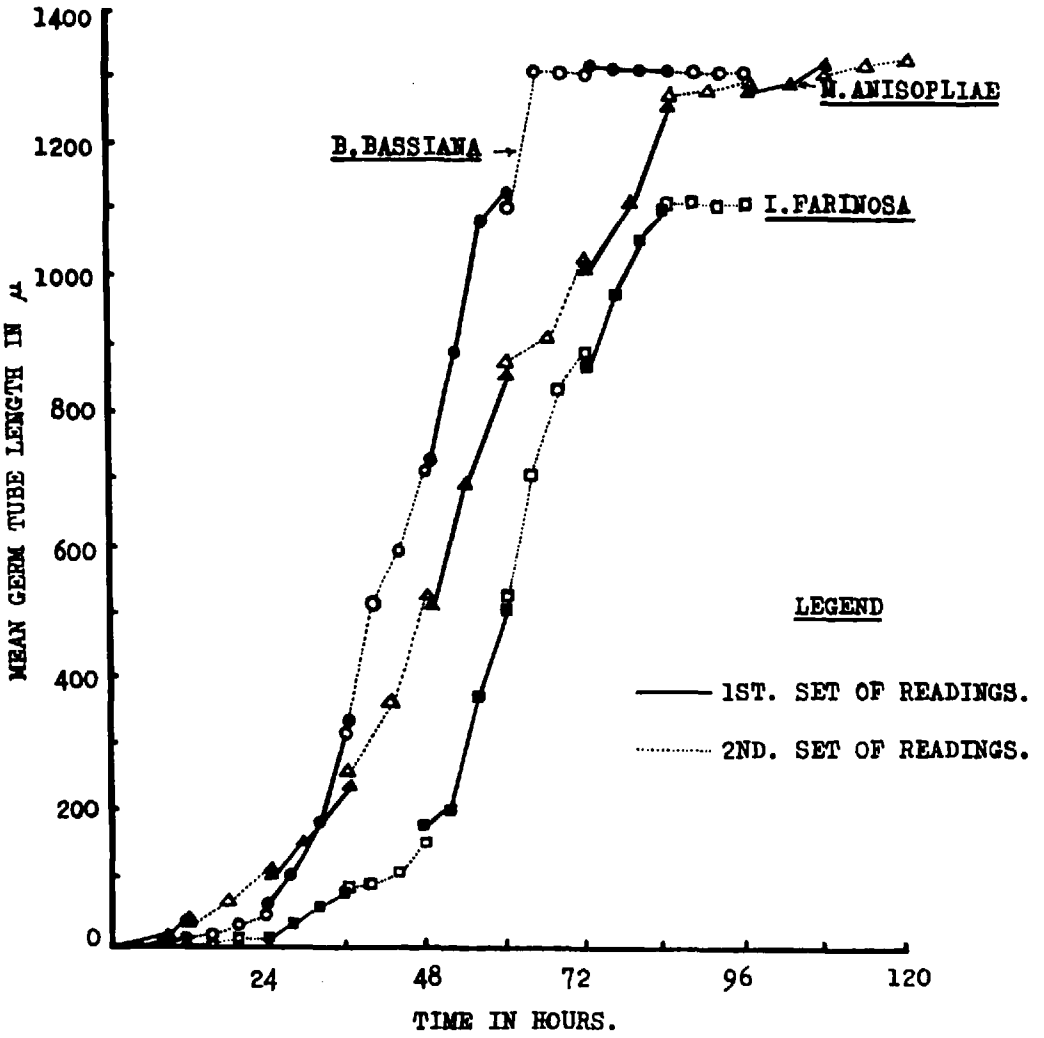


FIG. 20. MAXIMUM LENGTH OF GERM TUBES OF B.BASSIANA, I.FARINOSA AND M.ANISOPLIAE GROWING IN WATER AT 18, 20 AND 25°C. RESPECTIVELY.

third and last period was the slowing down and cessation of growth, undoubtedly caused by depletion of endogenous substrates.

A maximum length of 1300 μ was attained by germ tubes of B.bassiana in approximately 60 hours of germ tube growth. Germ tubes of I.farinosa needed about 80 hours to reach a maximum length of 1121 μ, and those of M.anisopliae attained a maximum length of 1326 μ in 100 hours.

I. EFFECT OF LIGHT ON GERMINATION.

Most reports show that light has no influence on spore germination (Cochrane, 1958 ; Gottlieb, 1950 ; Hawker, 1950 ; Marsh, Taylor and Bassler, 1959). However, in some, light has been reported to inhibit spore germination, and in others to be necessary for germination.

An experiment was set up to investigate the effect of light on germination of conidia of B.bassiana, I.farinosa and M.anisopliae. The conidia were germinated in sterile distilled water using the slide method in two lighted incubators set at 25 and 18°C. and of respective light intensities of 30 and 78 foot-candles. Since 18°C. was quite close to the optimum temperature for conidium germination in I.farinosa, this temperature was used for tests with both B.bassiana and I.farinosa. Conidia of M.anisopliae were incubated at 25°C. Petri dishes holding conidia for tests in darkness were wrapped in black paper and placed in plastic boxes (17.5 x 11.5 x 6.0 cm.). These boxes were in turn wrapped in black paper before putting them into the incubators.

Conidia of B.bassiana and I.farinosa in light and darkness were sampled and inspected at 24 hour - intervals over a period of 96 hours. Conidia of M.anisopliae were examined at 12 hour - intervals over a period of 48 hours. Percentage Germination was determined and germ tube lengths measured. The results are presented in Tables 20 - 22 and in Fig 21.

There were negligible differences (see Tables 20 - 22), in both percentage germination and average germ tube lengths, between conidia kept in light and in darkness in all three fungi. It was evident that light had no effect on the germination of conidia of B.bassiana, I.farinosa and M.anisopliae.

TABLE 20

Germination of Conidia of B.bassiana in LIGHT of 78 foot - candles and in DARKNESS, sown in water at 18°C.

INCUBATION PERIOD IN HOURS.	LIGHT			DARKNESS		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
24	4320	48.07	41.2	4106	50.57	39.9
48	4066	55.00	500.8	3734	53.88	487.6
72	3555	58.22	-[1]	3073	61.18	-
96	3308	59.53	-	3872	61.33	-

[1] Because germ tubes had grown extensively measurements were discontinued.

TABLE 21

Germination of Conidia of I.farinosa in LIGHT of 78 foot - candles and in DARKNESS, sown in water at 18°C.

INCUBATION PERIOD IN HOURS.	LIGHT			DARKNESS		
	No. of conidia examined.	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
24	6012	8.91	13.8	7681	8.79	15.4
48	6738	10.62	125.2	5933	10.81	130.9
72	5170	17.41	- [1]	5517	18.07	-
96	5234	17.84	-	5500	18.10	-

[1]See foot - note of Table 20.

TABLE 22

Germination of Conidia of M.anisopliae in LIGHT of 30 foot - candles and in DARKNESS, sown in water at 25°C.

INCUBATION PERIOD IN HOURS.	LIGHT			DARKNESS		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
12	3600	94.71	36.8	4112	94.68	42.9
24	3575	98.86	91.9	3900	99.40	95.0
36	3892	99.90	202.5	4215	99.58	210.0
48	3723	100.00	-[1]	4004	100.00	-

[1]See foot - note of Table 20.

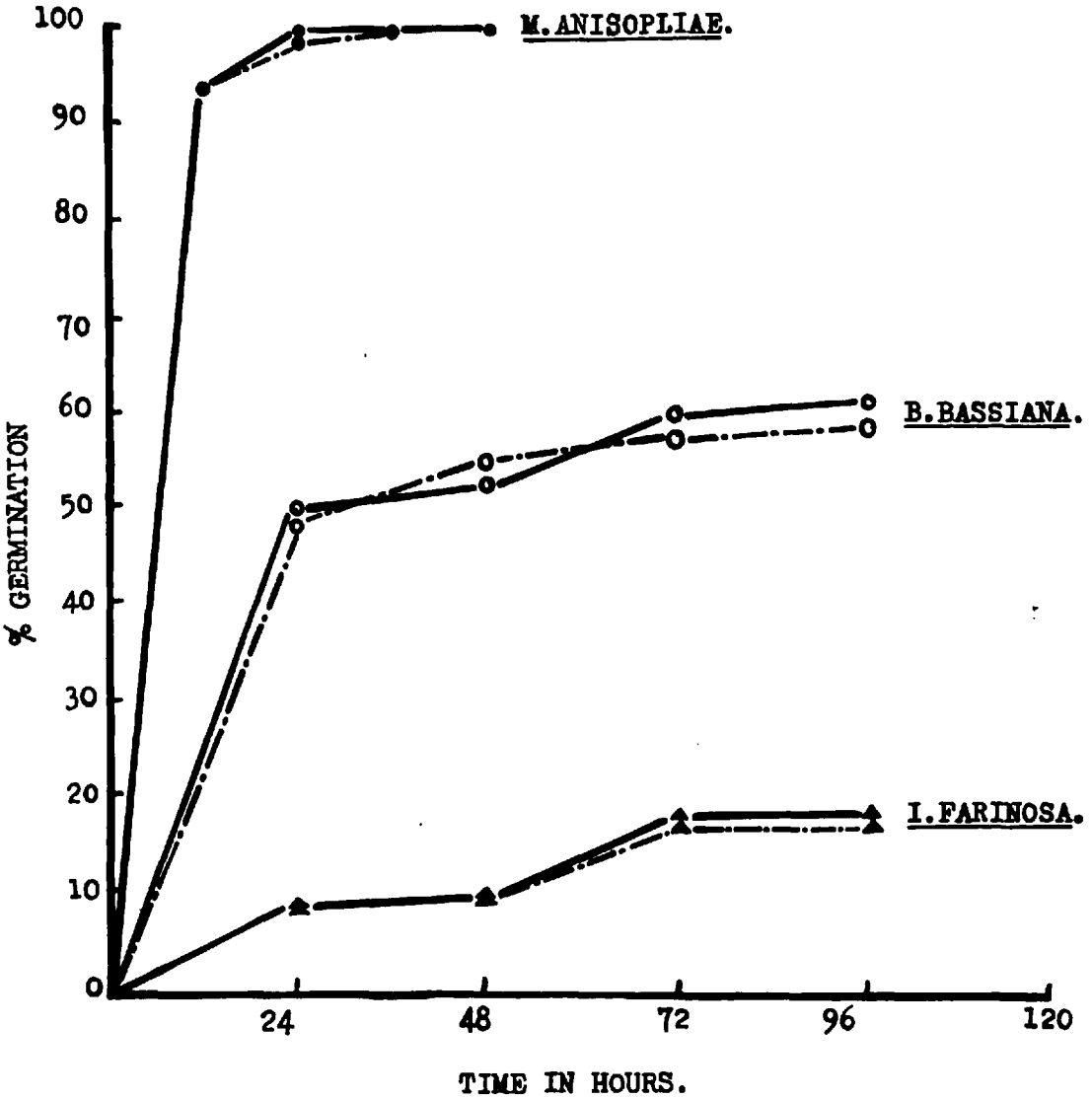


FIG.21 GERMINATION OF CONIDIA OF B.BASSIANA, I.FARINOSA AND M.ANISOPLIAE AT 18, 20 AND 25°C. RESPECTIVELY, IN LIGHT (—·—·—) AND IN DARKNESS (———)

J. EFFECT OF HUMIDITY ON GERMINATION.

The importance of high humidities in germination of conidia of B.bassiana, I.farinosa and M.anisopliae has often been stressed in the literature. (Arnaud, 1927 ; Hart and MacLeod, 1955 ; Steyaert, 1935 etc. etc.). Germination of the conidia at different relative humidities was therefore studied. Constant relative humidities were maintained with saturated aqueous salt solutions, prepared according to the data of Wexler and Hasegawa (1954) (see page 21).

Saturated aqueous salt solutions were prepared in screw - capped jars, 5 cm. high and 7 cm. in diameter. Open - ended glass cylinders 1.5 cm. high and 1.5 cm. in diameter were placed upright in the bottom of the jars. One drop of suspension of conidia of the particular fungus to be studied was placed on each clean, sterile coverslip 5/8 inch in diameter. The drops on the coverslips were left exposed 1 to 2 hours at 23°C. to dry, after which the coverslips were inverted and laid on top of the cylinders in the screw - capped jars. The jars were then sealed and placed in the incubator for a period of 24 hours. The conidia were incubated at their respective optimum temperatures and the humidity range used are shown in the tables below. (see Tables 23 - 25).

The results are presented in Tables 23 - 25 and in Fig. 22. These as usual were based on percentage germination and average germ tube length.

TABLE 23

Effect of Humidity on Germination of Conidia of B.bassiana at 18°C.

(Observations after 24 hours' incubation)

% Relative Humidity	Saturated aqueous salt solution used	No. of conidia examined.	% Germination	Mean Germ tube length in μ
100.0	Distilled water	2744	81.05	50.0
97.3	K_2SO_4	3920	61.53	32.2
93.8	KNO_3	3520	6.30	5.9
80.6	$(NH_4)_2SO_4$	3136	0.00	-

TABLE 24

Effect of Humidity on Germination of Conidia of I.farinosa at 20°C.

(Observations after 24 hours' incubation)

% Relative Humidity	Saturated aqueous salt solution used	No. of conidia examined.	% Germination	Mean Germ tube length in μ
100.0	Distilled water	3293	19.64	13.5
97.2	K_2SO_4	3019	1.37	2.2
93.2	KNO_3	3225	0.00	-

TABLE 25

Effect of Humidity on Germination of Conidia of M.anisopliae at 25°C.

(Observations after 24 hours' incubation)

% Relative Humidity	Saturated aqueous salt solution used	No. of conidia examined.	% Germination	Mean Germ tube length in μ
100.0	Distilled water	3630	76.47	62.7
96.9	K_2SO_4	3933	12.89	24.2
92.0	KNO_3	3578	0.00	-

The data in Table 23 showed that conidia of B.bassiana germinated best at 100 % R.H., and fairly well at 97.3 % R.H. 93.8 % R.H. was the lowest humidity which supported germination. Germ tubes were longest at 100.0 % R.H., and diminished in length with decrease in humidity level. The range of humidity which supported germination found here agreed with that reported by Arnaud (1927), Hart and MacLeod (1955), Müller - Kögler (1942) and Steyaert (1935).

97.2 % R.H. was the lowest humidity which supported germination of conidia of I.farinosa, and germination increased as the humidity level rose (Table 24).

Conidia of M.anisopliae germinated substantially at 100.0 % R.H. (Table 25). 96.9 % R.H. was the lowest relative humidity where conidium germination occurred. The humidity level which supported the highest percentage germination, promoted the greatest germ tube growth.

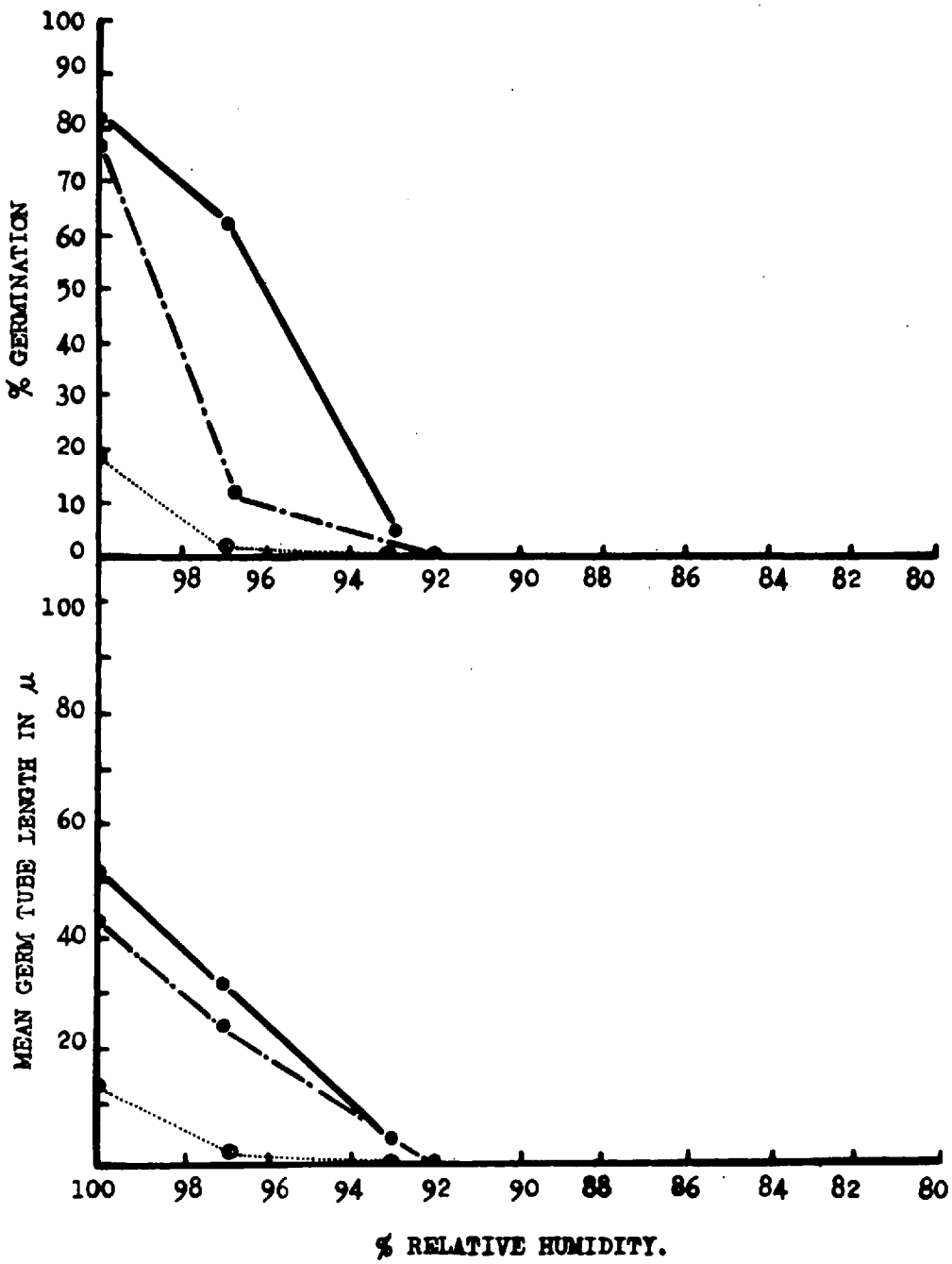


FIG. 22 EFFECT OF RELATIVE HUMIDITY ON GERMINATION OF CONIDIA OF B.BASSIANA(——•——), I.FARINOSA(·····•·····) AND M.ANISOPLIAE(—·—•—·—) INCUBATED, RESPECTIVELY, AT 18, 20 AND 25°C.

K. THE INFLUENCE OF HUMIDITY ON GROWTH RATE OF GERM TUBES.

The experiment above had shown the exact ranges of humidity within which the conidia of B.bassiana, I.farinosa and M.anisopliae would germinate. Within these ranges, germ tube length in a given time increased with the relative humidity. The rate of growth of the germ tubes at the different relative humidities was therefore studied to elucidate the influence of humidity in the early stages of growth.

The procedure outlined in the previous experiment was followed. Germ tubes of conidia which had been labelled by their position on a grid of squares (each square measured 1 mm. x 1 mm.) etched on the coverslip, were measured throughout this investigation. Very dilute conidial suspensions (approximately 75 conidia per ml. of water) were used to disperse the conidia as much as possible. 20 labelled germ tubes were measured in each case at 24 hour - intervals over a period of 120 hours. Comparative treatments with conidia in free water were run. The conidia were incubated at their respective optimum temperatures and the results are presented in Tables 26 - 28 and in Figs. 23 - 25. Through the later part of each curve (see Figs. 23 - 25) was drawn a straight line to show the mean rate of growth, and from the slopes of these lines were derived the approximate mean growth rates of the germ tubes presented in the last columns of the tables.

TABLE 26

Growth rates of Germ tubes of Conidia of B.bassiana at Relative Humidities of 100.0, 97.2 and 92.3 % at 18°C.

Readings of Germ tube length in μ.

% Relative Humidity	INCUBATION PERIOD IN HOURS.					Growth rate in μ / Hour.
	24	48	72	96	120	
Free water	46.5	623.9	-[1]	-	-	24.06
100.0	53.9	211.9	-	-	-	6.58
97.2	29.2	51.1	74.2	230.3	-	3.90
93.2	2.1	8.5	20.7	29.4	47.6	0.37

[1]Measurements were discontinued (see Text)

The growth rate of the germ tubes of B.bassiana (Table 26, Fig. 23) was greatest in free water. Though the germ tubes were slightly longer at 100.0 % R.H. than in water in the first 24 hours, a far greater growth occurred in water within the next 24 hours to give a length of 623.9 μ compared to the length of 211.9 μ attained at 100.0 % R.H. As the humidity decreased growth rate of the germ tubes correspondingly fell. Measurements of germ tubes in water and at 100.0 % R.H. were discontinued after 48 hours, because of the extensive growth of the germ tubes which required too long a time for measurement, but by then the significance of the particular treatments had been established.

TABLE 27

Growth rates of Germ tubes of Conidia of I.farinosa at Relative Humidities of 100.0 and 97.2 % at 20°C.

Readings of Germ tube length in μ.

% Relative Humidity	INCUBATION PERIOD IN HOURS.					Growth rate in μ / Hour.
	24	48	72	96	120	
Free water	22.3	144.9	-[1]	-	-	5.11
100.0	8.0	16.0	55.5	104.3	126.9	1.49
97.2	0.3	3.9	16.8	57.6	73.2	0.96

[1] See foot - note of Table 26.

Growth rate of the germ tubes of I.farinosa (Table 27 , Fig. 24) was greatest in free water, followed by that at 100.0 % relative humidity. A decrease in humidity reduced the growth rate of the germ tubes.

The superiority of water to support greater germ tube growth rate in conidia of M.anisopliae to 100.0 and 96.9 % R.H. was shown by the results in Table 28 and in Fig. 25. Growth rate of the germ tubes, again, gradually fell with decrease in relative humidity.

TABLE 28

Growth rates of Germ tubes of Conidia of M.anisopliae at Relative Humidities of 100.0 and 96.9 % at 25°C.

Readings of Germ tube length in μ.

% Relative Humidity	INCUBATION PERIOD IN HOURS.					Growth rate in μ / Hour.
	24	48	72	96	120	
Free water	100.6	498.2	- 1	-	-	16.56
100.0	32.6	75.9	119.7	138.7	315.5	3.33
96.9	20.6	29.5	82.4	89.9	178.9	2.01

[1] See foot - note of Table 26.

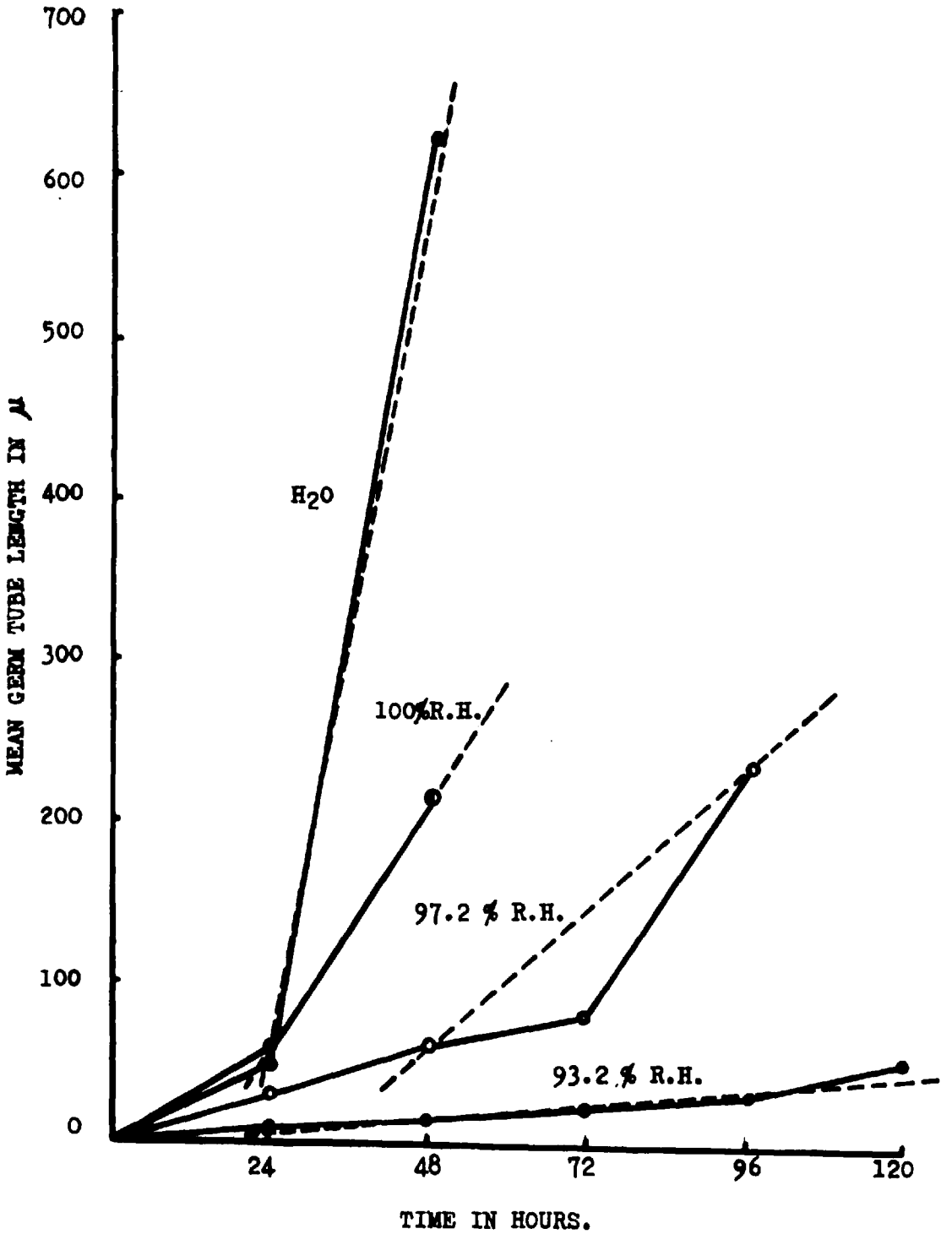


FIG.23. GROWTH OF GERM TUBES OF B.BASSIANA IN ATMOSPHERES OF DIFFERENT RELATIVE HUMIDITIES AT 18°C.

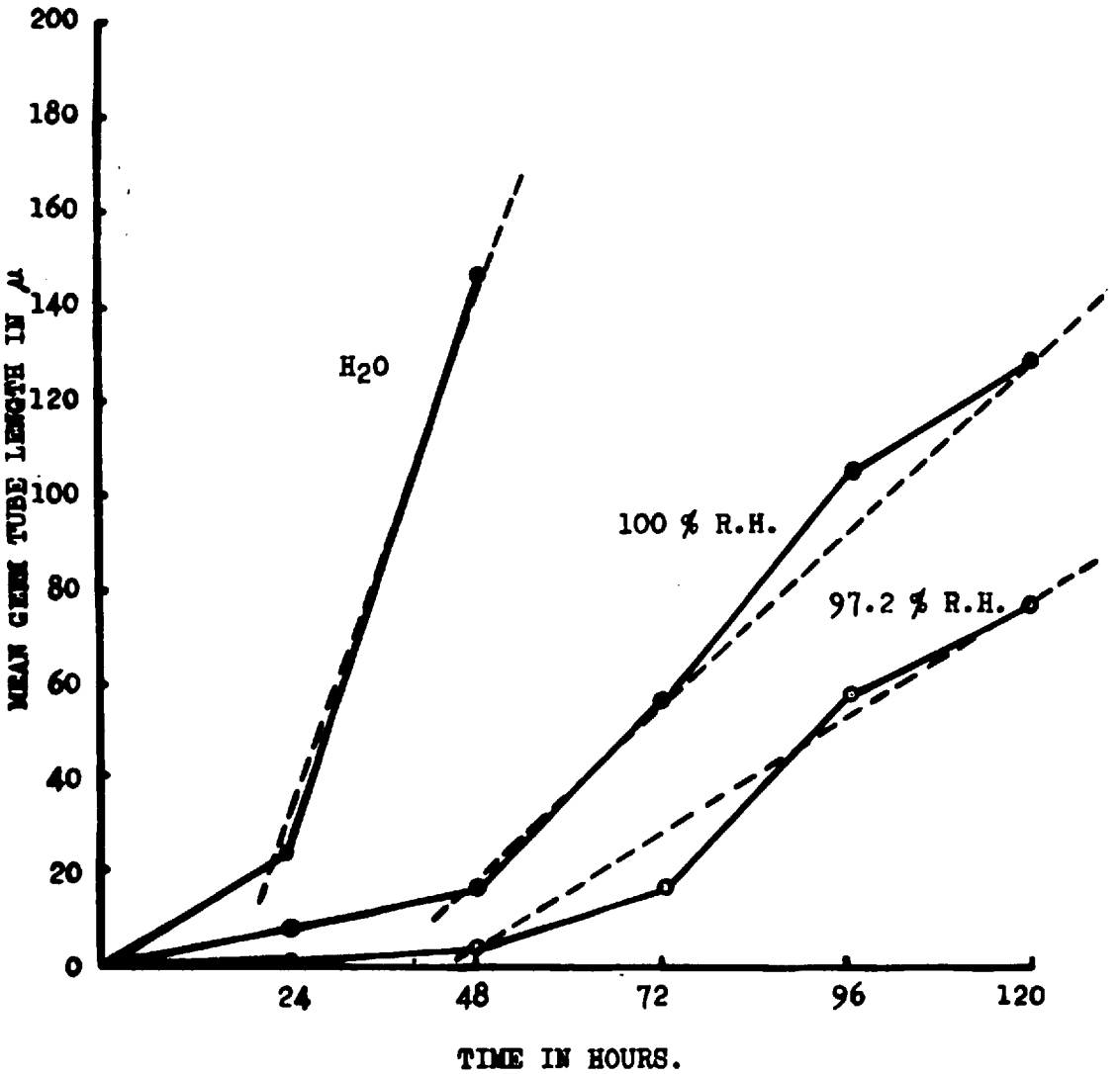


FIG. 24 GROWTH OF GERM TUBES OF I.FARINOSA IN ATMOSPHERES OF DIFFERENT RELATIVE HUMIDITIES AT 20°C.

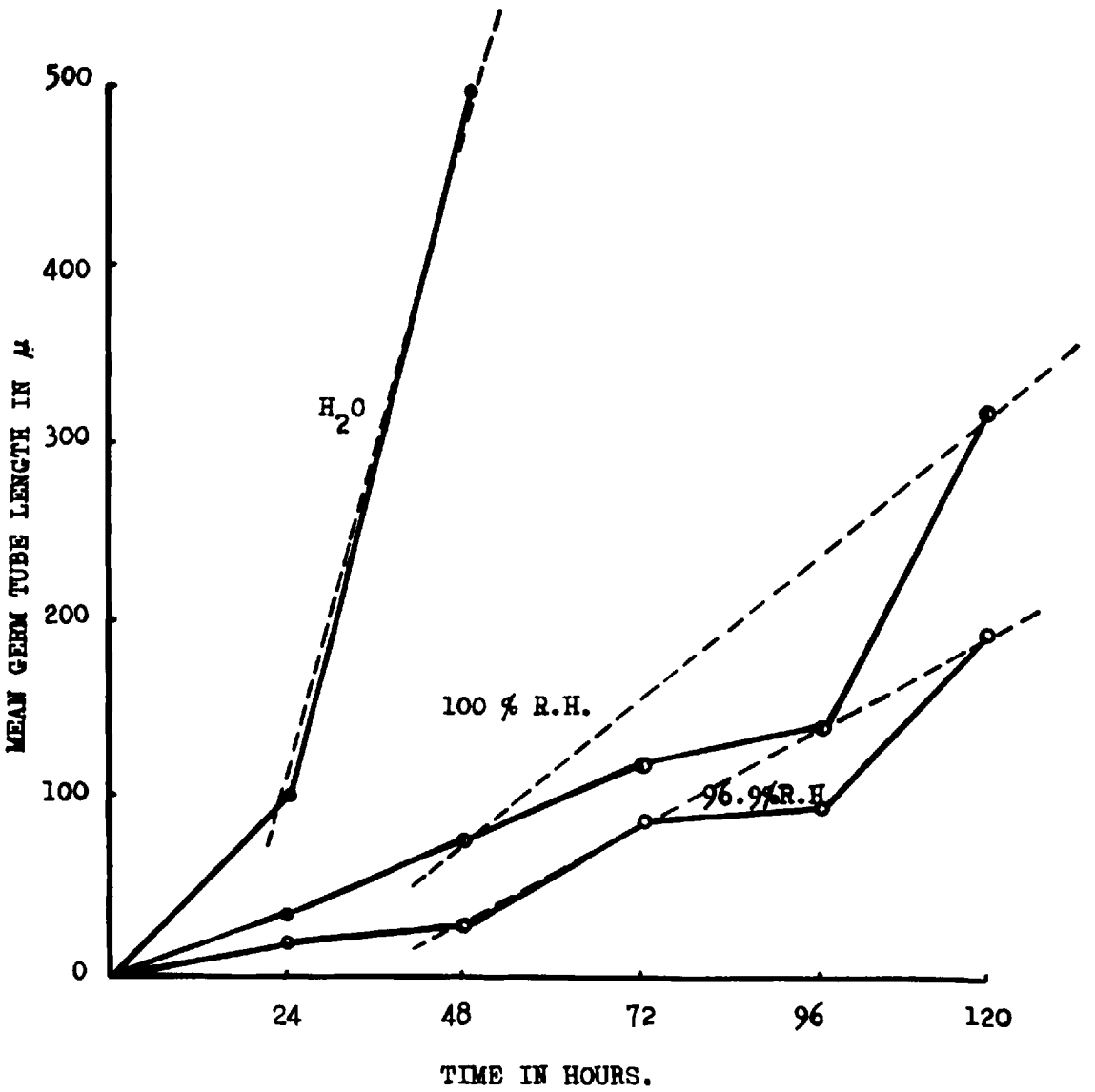


FIG. 25 GROWTH OF GERM TUBES OF M.ANISOPLIAE IN ATMOSPHERES OF DIFFERENT RELATIVE HUMIDITIES AT 25°C.

L. JOINT INFLUENCE OF TEMPERATURE AND HUMIDITY ON GERMINATION.

As an extension of studies on the importance of relative humidity in germination of the conidia, the joint effect of humidity and temperature was investigated. The humidity jars with their loads of conidia on coverslips were incubated at selected temperatures (see Tables 29 - 31) for a period of 24 hours. Because incubation temperatures were different, the relative humidities provided by the saturated salt solutions (K_2SO_4, KNO_3 and $(NH_4)_2SO_4$) are not all the same at the different temperatures. At the end of the incubation period, percentage germination was determined and germ tube lengths measured. The results are presented in Tables 29 - 31 and in Figs. 26 - 28.

The results in Table 29 and in Fig. 26 showed clearly the relation of germination of conidia of B.bassiana to humidity and temperature. At all relative humidities tested, 18°C. was optimal. While germination was possible at about 97 % R.H. at all tempera - tures from 10 to 25°C., it failed to occur at 4°C. There was some indication that in the range 4 to 25°C., the higher the temperature, the lower was the minimum relative humidity allowing germination.

TABLE 29

Joint Effect of Humidity and Temperature on Germination of Conidia of B.bassiana

(Observations after 24 hours' incubation)

Temperature in °C.	% Relative Humidity	No. of conidia examined	% Germination	Mean Germ tube length in μ
4	100.0	4473	0.42	4.7
	98.5	4242	0.09	1.3
	96.8	4670	0.00	-
10	100.0	3731	35.03	13.3
	97.9	2443	15.88	9.8
	96.6	3681	0.41	3.0
15	100.0	3567	73.31	45.0
	97.5	2444	35.90	22.7
	94.4	3837	3.15	2.8
18	100.0	3044	79.05	53.0
	97.4	3280	64.11	30.4
	93.8	3580	8.82	5.0
20	100.0	4716	34.62	23.3
	97.2	3380	28.31	14.9
	93.2	4441	2.27	3.7
25	100.0	3865	22.50	14.2
	96.9	3447	18.14	5.3
	92.0	3971	0.00	-
33	100.0	3225	0.00	-
	96.5	3367	0.00	-
	90.5	3052	0.00	-

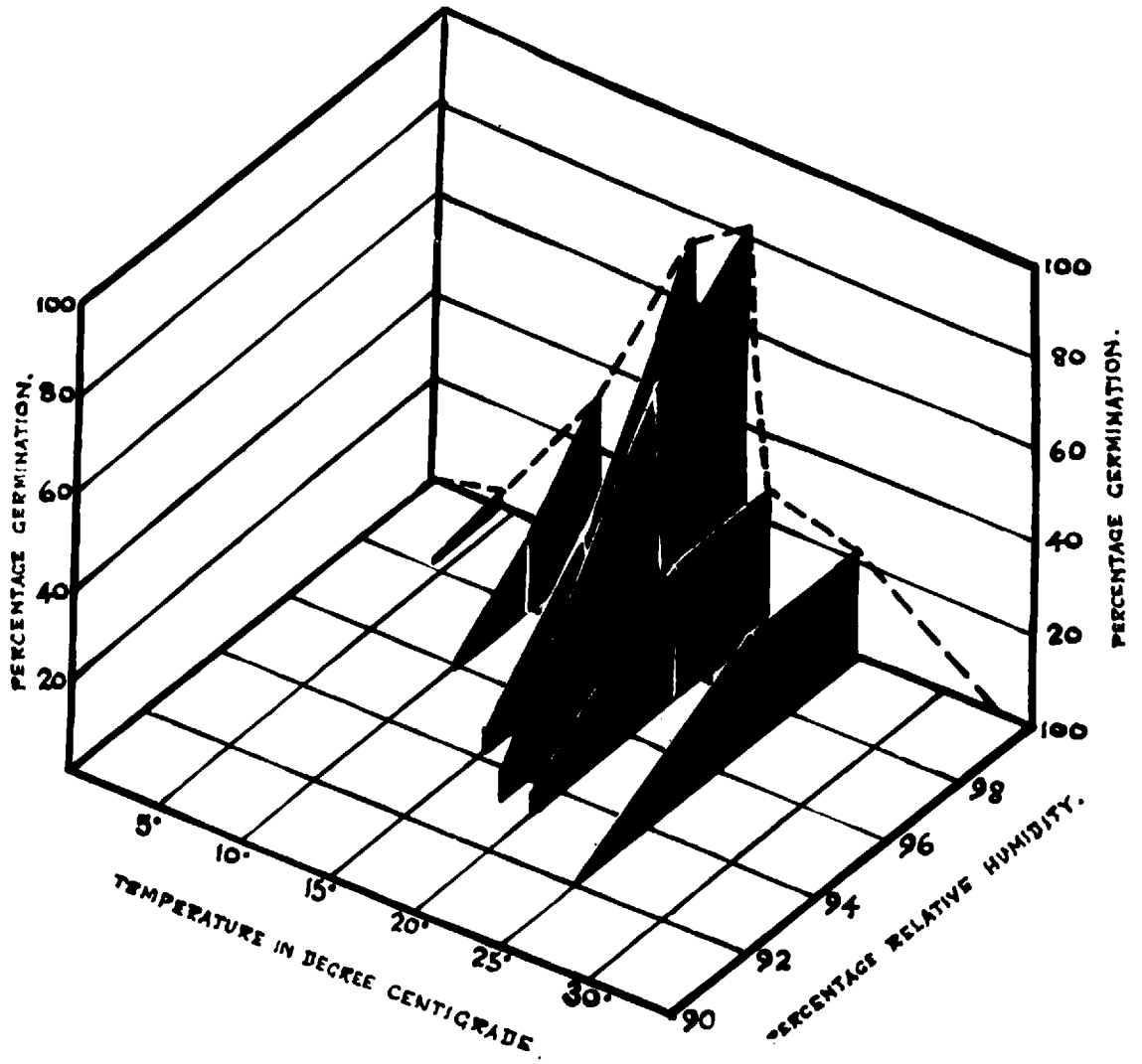


FIG. 26. GERMINATION OF CONIDIA OF B.BASSIANA, WITHIN 24 HOURS, IN ATMOSPHERES OF DIFFERENT RELATIVE HUMIDITIES AND AT DIFFERENT TEMPERATURES.

TABLE 30

Joint Effect of Humidity and Temperature on Germination of Conidia of I.farinosa.

(Observations after 24 hours' incubation)

Temperature in °C.	% Relative Humidity	No. of conidia examined	% Germination	Mean Germ tube length in μ
10	100.0	3688	10.31	11.1
	97.9	4750	0.00	-
	95.5	4302	0.00	-
15	100.0	4176	17.34	11.3
	97.5	7642	0.10	1.9
	94.4	6200	0.00	-
18	100.0	4162	18.84	11.6
	97.4	4553	0.14	2.5
	93.8	5291	0.00	-
20	100.0	6586	18.95	16.8
	97.2	6431	0.42	5.8
	93.2	6125	0.00	-
22	100.0	4264	17.15	13.1
	97.1	7932	0.42	5.2
	92.7	6514	0.00	-
25	100.0	6238	8.50	8.3
	96.9	7899	0.21	4.7
	92.0	7672	0.00	-
33	100.0	5270	0.00	-
	96.5	4844	0.00	-
	90.5	4536	0.00	-

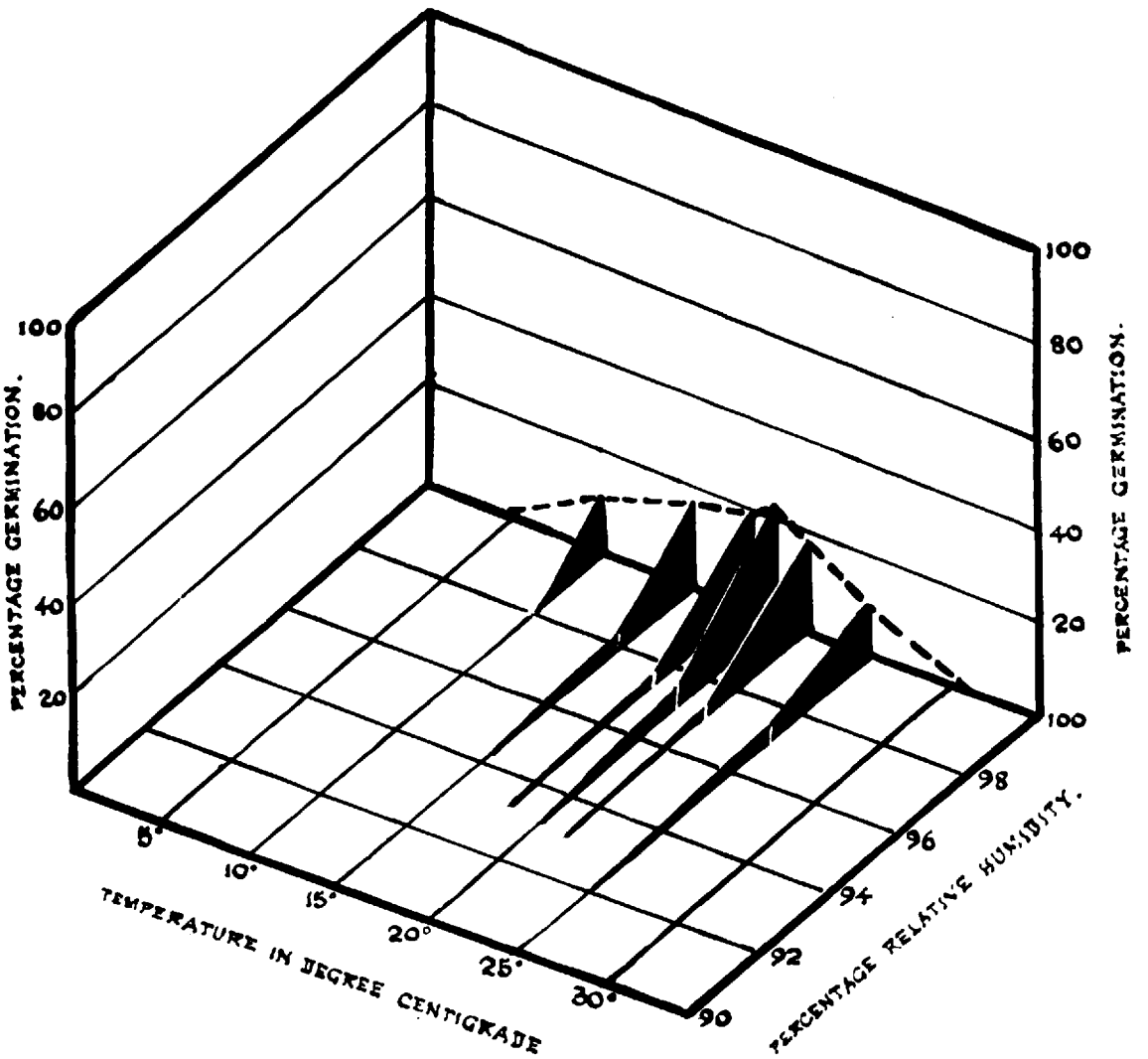


FIG. 27. GERMINATION OF CONIDIA OF I.FARINOSA, WITHIN 24 HOURS IN ATMOSPHERES OF DIFFERENT RELATIVE HUMIDITIES AND AT DIFFERENT TEMPERATURES.

The best conidium germination of I.farinosa at all relative humidities (Table 30, Fig. 27) occurred at 20°C. A temperature range of 10 to 25°C. supported conidium germination at 100.0 % R.H. At low temperatures (eg. 10°C.) the minimum relative humidity for spore germination was higher than at temperatures above 15°C. This is similar to the behaviour of B.bassiana conidia.

The results for M.anisopliae in Table 31 and in Fig. 28 showed that the effect of temperature and humidity on conidium germination was generally the same as found in the investigations with conidia of B.bassiana and I.farinosa. At relatively low temperatures (18°C. and below) there was no germination at about 97 % R.H., but germination was possible at this relative humidity at temperatures from 20 to 30°C. 25°C. was optimal at all relative humidities.

The requirement for atmospheric humidity is greater in all three species at temperatures approaching the minimum for these species, than at more nearly optimal temperatures.

TABLE 31

Joint Effect of Humidity and Temperature on Germination of Conidia of M. anisopliae.

(Observations after 24 hours' incubation)

Temperature in °C.	% Relative Humidity	No. of conidia examined	% Germination	Mean Germ tube length in μ
15	100.0	6911	0.00	-
	97.5	4272	0.00	-
	94.4	4839	0.00	-
18	100.0	6767	28.42	4.0
	97.4	4328	0.00	-
	93.8	5007	0.00	-
20	100.0	5496	28.98	6.9
	97.2	6541	0.31	2.7
	93.2	5992	0.00	-
22	100.0	4549	35.42	30.6
	97.0	7819	0.87	14.2
	93.0	5272	0.00	-
25	100.0	3630	74.50	45.6
	96.9	2622	15.00	20.6
	92.0	3025	0.00	-
30	100.0	3624	69.48	16.2
	96.9	3560	12.29	14.2
	90.7	4118	0.00	-
33	100.0	4222	11.00	3.5
	96.5	3756	0.00	-
	90.5	3688	0.00	-
35	100.0	4172	0.00	-
	96.4	3155	0.00	-
	89.3	3602	0.00	-

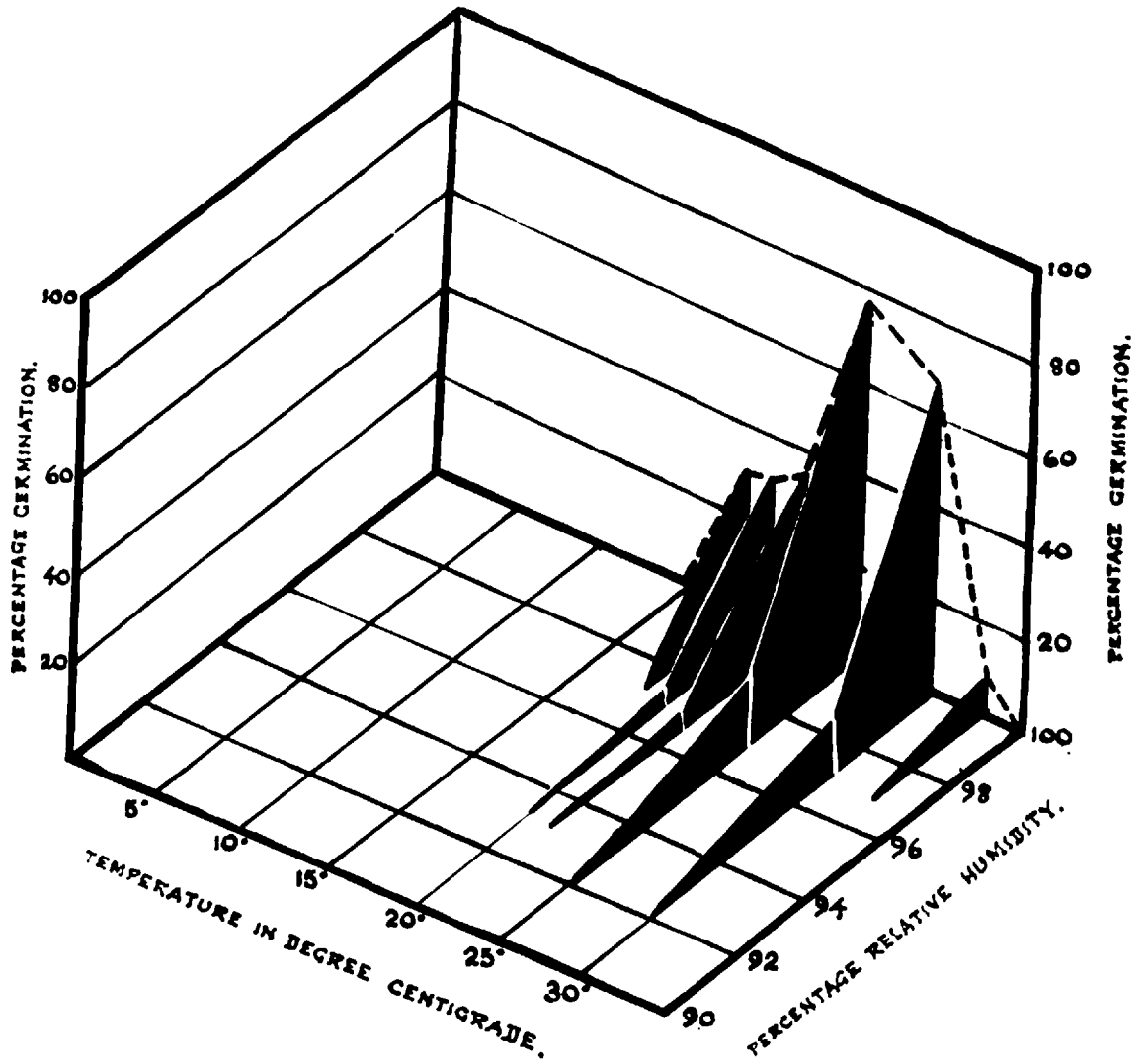


FIG. 28. GERMINATION OF CONIDIA OF M.ANISOPLIAE, WITHIN 24 HOURS, IN ATMOSPHERES OF DIFFERENT RELATIVE HUMIDITIES AND AT DIFFERENT TEMPERATURES.

M. EFFECT OF pH ON GERMINATION.

Generally the spores of fungi germinate best in acid media (Hawker, 1950 ; Wolf and Wolf, 1947). The hydrogen - ion concentration in an 'infection drop' is undoubtedly a factor of considerable importance affecting the behaviour of spores of a pathogen. This section of the work was designed to investigate the nature of this influence by determining the optimum pH for conidium germination and the range of pH over which conidia of B.bassiana, I.farinosa and M.anisopliae could germinate.

The conidia were germinated on buffered 'pyridine - washed' plain agar. This was made by dissolving 2% (weight / volume) washed agar in buffer solutions of the desired pH levels. A pH range of pH 2.2 to 12.0 was used. This range was obtained by using different buffer mixtures prepared according to data by Clark (1925), for different sections of the pH range. Combining the results for different buffer systems produced reasonably continuous germination curves. However, the separate sections of the graphs, representing the different buffer mixtures, are indicated in the figures showing the results. (see Figs. 29 - 31).

Buffered agar plates were seeded with conidial suspensions prepared with buffer solutions of similar pH, and incubated at the respective optimum temperatures of the conidia for a period

of 24 hours. Percentage germination was calculated and germ tube lengths measured at the end of that period. The results are presented in Tables 32 - 35 and in Figs. 29 - 31.

TABLE 32

Effect of pH on Germination of Conidia of B.bassiana sown on Buffered Agar at 18°C.

(Observations after 24 hours' incubation)

pH and Buffer Solution used	No. of conidia examined	% Germination	Mean Germ tube length in μ
McIlvaine's Standards[1]			
2.2	2967	0.51	5.1
2.6	3130	6.93	6.2
3.0	3772	11.66	10.9
3.6	3403	89.62	25.6
4.0	2788	89.99	30.6
5.0	2451	98.69	44.9
6.0	2813	78.88	30.0
7.0	2643	78.60	21.8
8.0	2915	44.66	14.0
Ringer's Mixtures[2]			
10.9	2559	0.31	4.4
11.7	2610	0.14	3.5
12.0	2879	0.00	-

[1] A mixture of 0.2 M. Na_2HPO_4 and 0.1 M. Citric acid.

[2] A mixture of 0.15 M. Na_2HPO_4 and 0.1 M. NaOH.

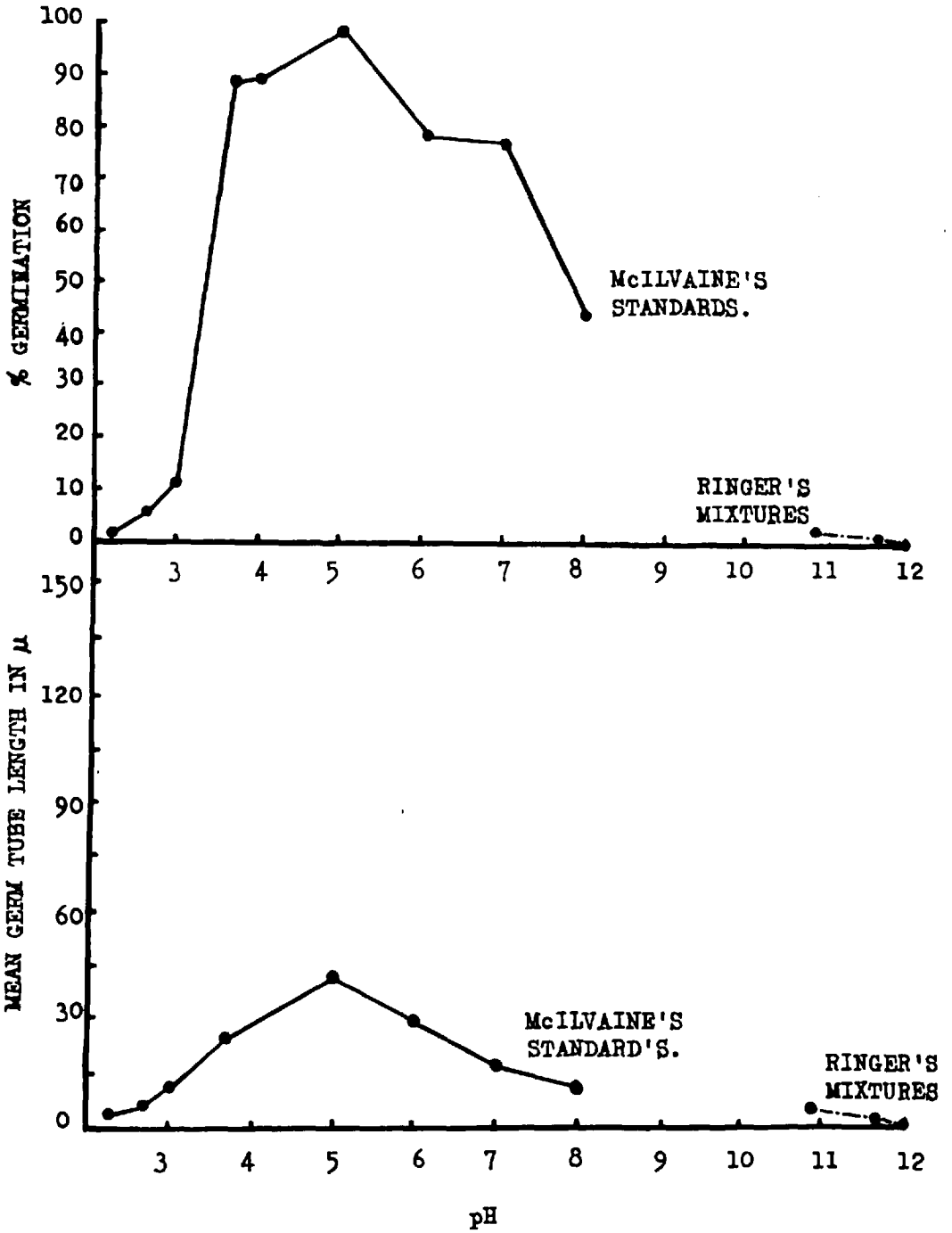


FIG. 29. EFFECT OF pH ON GERMINATION OF CONIDIA OF B.BASSIANA SOWN ON BUFFERED-WASHED PLAIN AGAR, AND INCUBATED AT 18°C. FOR 24 HOURS.

The results in Table 32 and Fig. 29 show that the optimum for germination of conidia of B.bassiana was about pH 5. The range of tolerance was rather wide, conidia germinating from pH 2.2 to 11.7. The upper limit lay between pH 11.7 and 12.0. The limit on the acid side was not determined with the pH range used.

The optimum for both percentage germination and germ tube growth (Table 33 and Fig. 30) for conidia of I.farinosa was about pH 4.0. Conidia germinated over the range of pH 2.6 to 7.0. The alkaline limit for conidium germination was between pH 7.0 and 8.0, and the acid limit between pH 2.2 and 2.6. The graph of the results shows the presence of a subsidiary peak at pH 6, besides the greater one at pH 4. At pH 5, germination and germ tube growth were less than at either pH 4 or 6. This "double pH optima" is discussed further below (page 332).

The percentage germination figures obtained in this experiment are far higher than those recorded in earlier tests with spores in pure water, on glass surfaces, where, with optimal conditions of temperature, only 10 – 16 % of the spores produced germ tubes within 24 hours. In the current experiment, on agar with McIlvaine's buffer (Na_2HPO_4 and Citric acid), the optimum pH gave no less than 86 % germination. This high germination may be in part due to specific effects of the components of the buffer solutions. (see below, pages 123-130 – salts, etc., affecting germination of conidia of I.farinosa.).

TABLE 33

Effect of pH on Germination of Conidia of I.farinosa sown on Buffered Agar at 20°C.

(Observations after 24 hours' incubation)

pH and Buffer Solution used	No. of conidia examined	% Germination	Mean Germ tube length in μ
McIlvaine's Standards[1]			
2.2	3822	0.00	-
2.6	3332	11.28	9.5
3.0	3048	22.85	10.3
3.6	3126	41.97	10.6
4.0	3175	86.75	26.1
5.0	3660	60.48	6.4
6.0	3238	65.41	14.5
7.0	3895	12.17	6.4
8.0	3304	0.00	-
Ringer's Mixtures			
10.9	3000	0.00	-
11.7	3412	0.00	-
12.0	3708	0.00	-

[1], [2] See foot - note of Table 32.

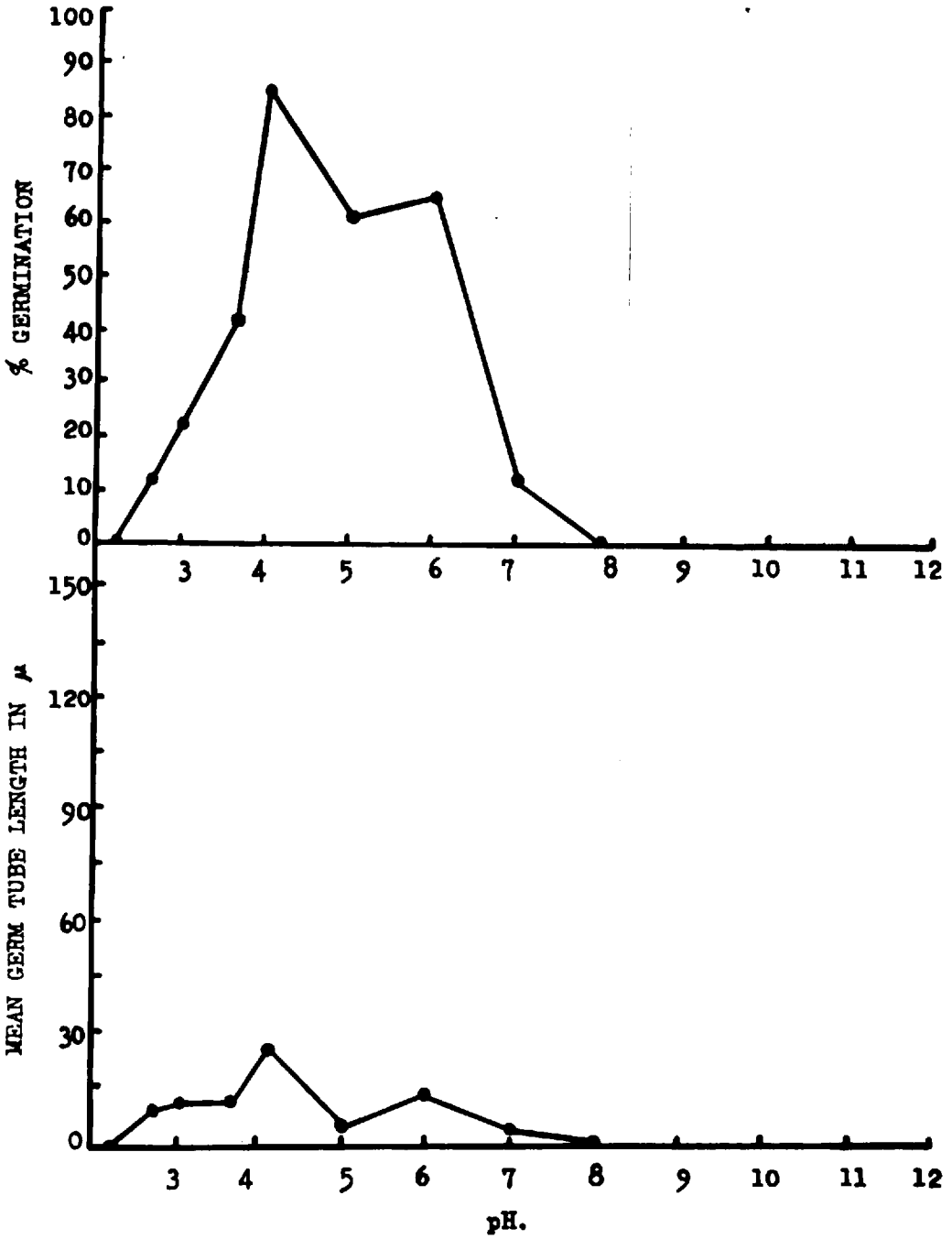


FIG. 30. EFFECT OF pH ON GERMINATION OF CONIDIA OF I.FARINOSA SOWN ON BUFFERED (McILVAINE'S STANDARDS) WASHED PLAIN AGAR, AND INCUBATED AT 20°C. FOR 24 HRS.

TABLE 34

Effect of pH on Germination of Conidia of M.anisopliae sown on Buffered Agar at 25°C.

(Observations after 24 hours' incubation)

pH and Buffer Solution used	No. of conidia examined	% Germination	Mean Germ tube length in μ
McIlvaine's Standards[1]			
2.2	2528	0.00	-
2.6	2464	2.49	4.3
3.0	3052	30.08	25.1
3.6	2628	40.36	29.0
4.0	2073	85.70	61.2
5.0	3075	99.54	98.7
6.0	2638	99.53	97.0
7.0	2672	99.32	96.2
7.6	2548	98.97	85.1
8.0	3150	89.78	42.7
Ringer's Mixtures[2]			
10.9	2640	41.10	14.4
11.7	3944	8.11	5.2
12.0	2435	2.06	4.4

[1], [2] See foot - note of Table 32.

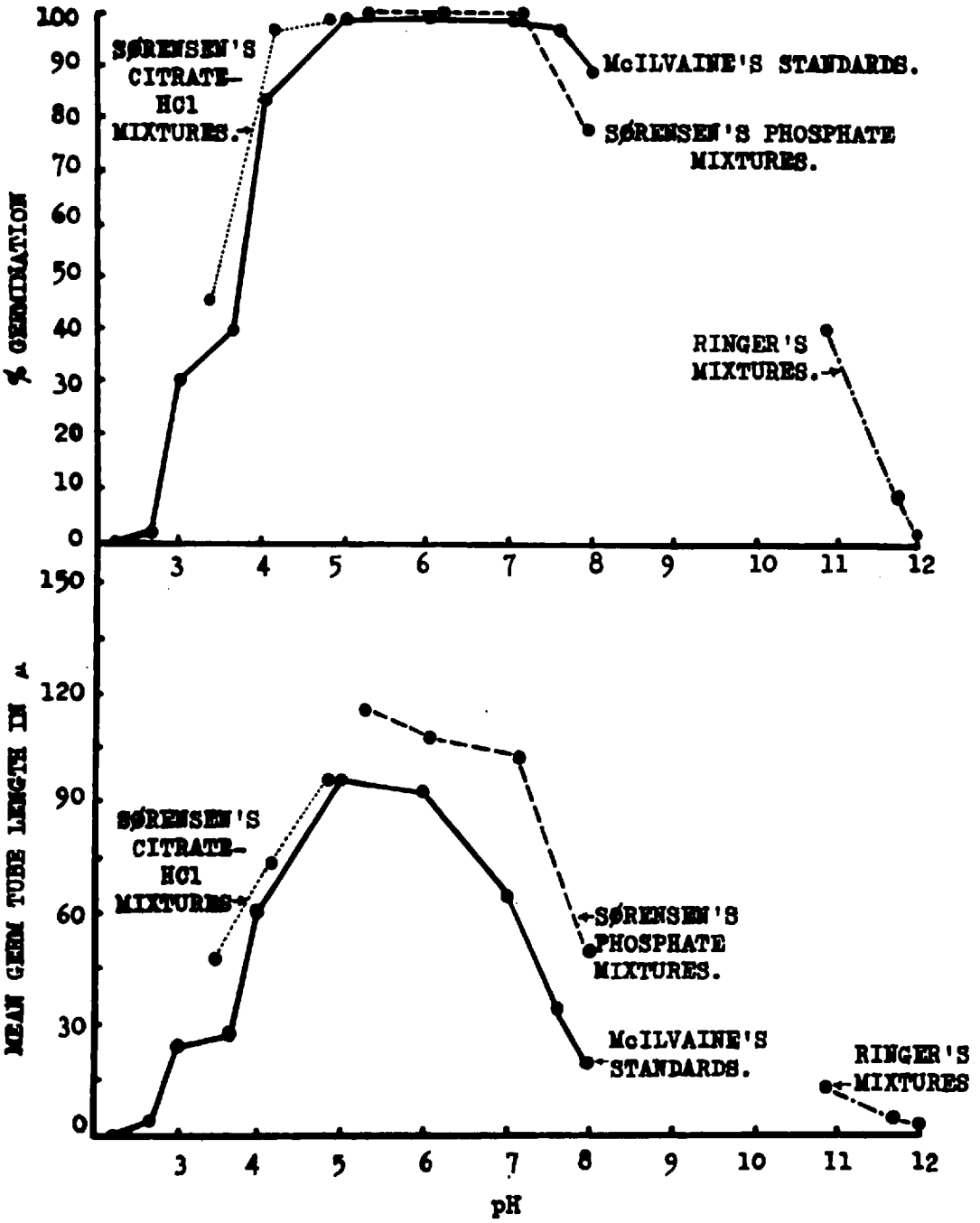


FIG. 31. EFFECT OF pH ON GERMINATION OF CONIDIA OF M.ANISOPLIAE SOWN ON BUFFERED-WASHED PLAIN AGAR, AND INCUBATED AT 25°C. FOR 24 HOURS.

The results in Table 34 and Fig. 31 show that conidia of M. anisopliae were capable of germinating over a wide pH range, from pH 2.6 to 12.0. The acid limit for conidium germination was between pH 2.2 and 2.6. It was not possible to determine the alkaline limit for germination. This however, could be inferred from the steep slope of the growth curve at the alkaline end, and was presumably just beyond pH 12. This toleration of high alkalinity is not exceptional. Child (1929) working with spores of Daldinia vernicosa, stated that pH 9.6 stimulated the spores to almost maximum germination. Other similar instances of spore germination in extremely alkaline media have been reported by other workers (eg. Wilkins, 1938 ; Goos and Tschirsch, 1962).

A striking feature of the results was the broad optimum pH for germination, viz., from pH 5.0 to 7.6.

This broad optimum pH for germination occurred on washed agar buffered with McIlvaine's Standard buffer solutions. To confirm the validity of this broad optimum pH, the experiment was partially repeated, using pyridine-washed agar buffered with Sørensen's mixtures (Citrate - HCl and $Na_2HPO_4.2H_2O$ - KH_2PO_4). The range of pH used (pH 3.5 - 8.0) was just enough to include the optimum pH observed in the previous experiment. The experiment was run over a period of 24 hours. The results are presented in Table 35 and in Fig. 31.

TABLE 35

Effect of pH on Germination of Conidia of M.anisopliae sown on Buffered Agar at 25°C.

(Observations after 24 hours' incubation)

pH and Buffer Solution used	No. of conidia examined	% Germination	Mean Germ tube length in μ
Sørensen's Citrate - HCl Mixtures[1]			
3.5	2622	45.35	47.6
4.2	3186	97.86	73.3
4.9	3454	99.55	99.7
Sørensen's Phosphate Mixtures[2]			
5.3	3198	99.74	112.9
6.2	3042	99.57	108.5
7.2	3278	99.56	106.6
8.0	3796	77.95	49.5

[1] A mixture of 0.1 M. Citrate and N/10 HCl.

[2] A mixture of M/15 $Na_2HPO_4 \cdot 2H_2O$ and M/15 KH_2PO_4

The optimum pH for germination of conidia of M.anisopliae was again observed to be a broad one from pH 4.9 to 7.2.

N. EFFECT OF NUTRIENTS AND MINERAL SALTS ON GERMINATION

That water alone is often insufficient for the germination of many fungal spores has been recognised by several workers. Tomkins (1932) recommended the use of nutrient solutions rather than water, on the grounds that the germination rate was higher and the results more consistent. Various reports indicate the stimulatory effect of nutrients and mineral salts on spore germination (Gottlieb, 1950 ; Hawker, 1950). On the other hand, there are incidents where spore germination had been depressed by the addition of nutrients. For example, Wilkins (1938) found that glucose, maltose and sodium nitrate actually reduced the amount of germination of spores of Ustulina vulgaris below that in water.

From the standpoint of germination of conidia of entomogenous fungi on the insect host, nutrient media as such are of less significance ; water, soil extracts and aqueous extracts of insect cuticle will give conditions more closely approaching the natural conditions of germination. The following considerations however, prompted an investigation into the effects of selected nutrients and mineral salts on germination of conidia of B.bassiana, I.farinosa and M.anisopliae.Firstly, experiments (described above) indicated that conidia of I.farinosa germinated rather poorly in pure water but well on buffered agar, especially at and around the optimum pH. It was desirable to find

to what extent pH of the medium was responsible for the good germination obtained. Wolf and Wolf (1947) remark that though there is specificity of pH requirements for various activities in fungi, the effects of the concentration of the hydrogen ions could not be isolated completely from those induced by other ions.

Secondly, Gabriel (1959) had found that B.bassiana and M.anisopliae conidia germinated very well in NaCl solution, but not in water. The conidia had however germinated well in water in this investigation. It would be useful to investigate their response to such mineral salts as NaCl for purposes of comparison.

Lastly, when entomogenous fungal spores are broadcast in the field, they invariably come in contact with soluble organic and inorganic materials in the soil, which might influence their ability to germinate.

The substrates used (see Tables 36 - 44) are mostly the constituents of nutrient media in common use, and were used individually over specific ranges of concentrations. (Organic nutrients measured in % - weight/volume and mineral salts in Molal concentration). The conidia were germinated in aqueous solutions using the 'slide' method ; control conidia were germinated in water. The pH's of the media were determined with a Cambridge pH meter immediately before germination tests were run, and though the difference in the pH introduced a second variable, the media were not standardised as that would have necessitated the introduction of additional elements.

The conidia were incubated for 24 hours at their respective optimum temperatures and results were estimated on the basis of percentage germination and average germ tube length. The results are presented in Tables 36 - 44 and in Figs. 32 - 37.

TABLE 36

Effect of Yeast - extract and Casein hydrolysate on the Germination of Conidia of B.bassiana at 18°C.

(Observations after 24 hours' incubation)

% Concentration (Wt. / Vol.)	YEAST - EXTRACT (pH 6.4)			CASEIN HYDROLYSATE. (pH 6.2)		
	No. of conidia examined	% Germi - nation.	Mean Germ tube length in μ	No. of conidia examined	% Germi - nation.	Mean Germ tube length in μ
Water	2561	60.15	41.4	2396	61.22	40.0
2×10^{-5}	3240	62.10	38.9	3268	61.61	39.2
2×10^{-4}	3800	60.31	40.6	3205	62.69	39.9
2×10^{-3}	2074	69.71	36.0	3002	60.30	45.9
2×10^{-2}	3268	87.51	56.9	2176	66.67	95.1
0.2	2058	98.93	110.2	2888	84.63	99.6
2.0	2134	98.23	113.2	2920	92.05	112.7
4.0	3392	96.36	80.0	2705	87.47	90.3

TABLE 37

Effect of Mineral Salts on Germination of Conidia of *B.bassiana* at 18°C.

(Observations after 24 hours' incubation)

MINERAL SALT		CONCENTRATION IN MOLAL.					
		H_2O	$1x10^{-7}$	$1x10^{-5}$	$1x10^{-3}$	0.1	0.5
CaCl (pH 6.3)	No. of conidia examined	2464	2200	2036	3292	2145	2592
	% Germination	63.42	81.64	77.33	68.40	45.68	43.29
	Mean Germ tube length in μ	42.1	37.6	38.5	28.1	22.3	11.8
$CuSO_4.5H_2O$ (pH 5.3)	No. of conidia examined	2134	2528	2226	2348	2418	2005
	% Germination	58.76	91.46	68.59	0.00	0.00	0.00
	Mean Germ tube length in μ	38.4	48.1	32.5	-	-	-
$FeCl_3.6H_2O$ (pH 3.6)	No. of conidia examined	2275	3292	2150	2724	2430	2986
	% Germination	64.25	16.89	4.62	2.05	0.00	0.00
	Mean Germ tube length in μ	39.64	7.64	3.06	2.68	-	-
K_2HPO_4 (pH 7.3)	No. of conidia examined	2917	2360	2050	2364	2738	2416
	% Germination	62.45	93.56	94.33	96.92	68.29	5.13
	Mean Germ tube length in μ	39.5	52.8	53.1	52.0	43.5	6.1
$MgSO_4.7H_2O$ (pH 6.1)	No. of conidia examined	2504	2196	2345	2944	2673	2828
	% Germination	60.20	96.17	96.82	97.69	97.00	95.47
	Mean Germ tube length in μ	41.3	114.6	112.5	129.4	130.3	63.3

TABLE 37 (cont'd)

Effect of Mineral Salts on Germination of Conidia of *B.bassiana* at 18°C.

(Observations after 24 hours' incubation)

MINERAL SALT		CONCENTRATION IN MOLAL.					
		H_2O	$1x10^{-7}$	$1x10^{-5}$	$1x10^{-3}$	0.1	0.5
$MnSO_4.4H_2O$ (pH 6.2)	No. of conidia examined	2255	1928	2000	2314	2105	1884
	% Germination	59.25	58.82	62.08	75.19	68.29	52.69
	Mean Germ tube length in μ	42.0	25.5	24.6	27.9	20.4	10.4
NaCl (pH 6.5)	No. of conidia examined	2142	2856	2876	2754	2086	2482
	% Germination	59.80	69.11	67.22	89.55	68.26	52.21
	Mean Germ tube length in μ	48.1	66.8	102.1	87.3	61.4	60.5
$Na_2MoO_4.2H_2O$ (pH 6.5)	No. of conidia examined	2042	2728	2433	2616	1955	1828
	% Germination	64.25	81.82	82.00	81.93	26.28	0.11
	Mean Germ tube length in μ	38.4	42.8	40.5	40.7	10.4	2.8
$(NH_4)_2.HPO_4$ (pH 7.5)	No. of conidia examined	1832	2132	2061	2456	1964	2712
	% Germination	58.78	89.49	96.58	96.42	66.36	60.02
	Mean Germ tube length in μ	42.4	56.4	78.0	75.3	29.5	17.7
ZnCl (pH 6.2)	No. of conidia examined	2060	3048	2453	3064	2237	2882
	% Germination	64.39	51.99	56.36	53.19	7.26	0.00
	Mean Germ tube length in μ	42.1	15.1	27.25	23.4	4.4	-

TABLE 38

Effect of Dextrose on Germination of Conidia of B.bassiana at 18°C.

(Observations after 24 hours' incubation)

% Concentration (Wt. / Vol.)	No. of conidia examined	% Germination	Mean Germ tube length in μ
Water	2775	60.65	38.7
0.125	2376	97.47	111.2
0.25	3208	100.00	166.9
0.5	2829	100.00	178.8
1.0	2966	99.80	146.3
2.0	3046	99.93	126.2
4.0	2970	98.25	125.0
8.0	3220	91.18	38.7

Results in Tables 36 to 38 showed that percentage germination of B.bassiana conidia in water (control) was between 58 and 65 per cent and the only substrates associated with reduced germination at all concentrations used were $FeCl_3$ and ZnCl. Increased germination to varying degrees, occurred in all the rest. Increased germination, was however, not always accompanied by corresponding greater growth of the germ tubes .

In brief the influence of these substrates were :

Dextrose : There was a very high percentage germination (above 91 %) at all concentrations of Dextrose used.

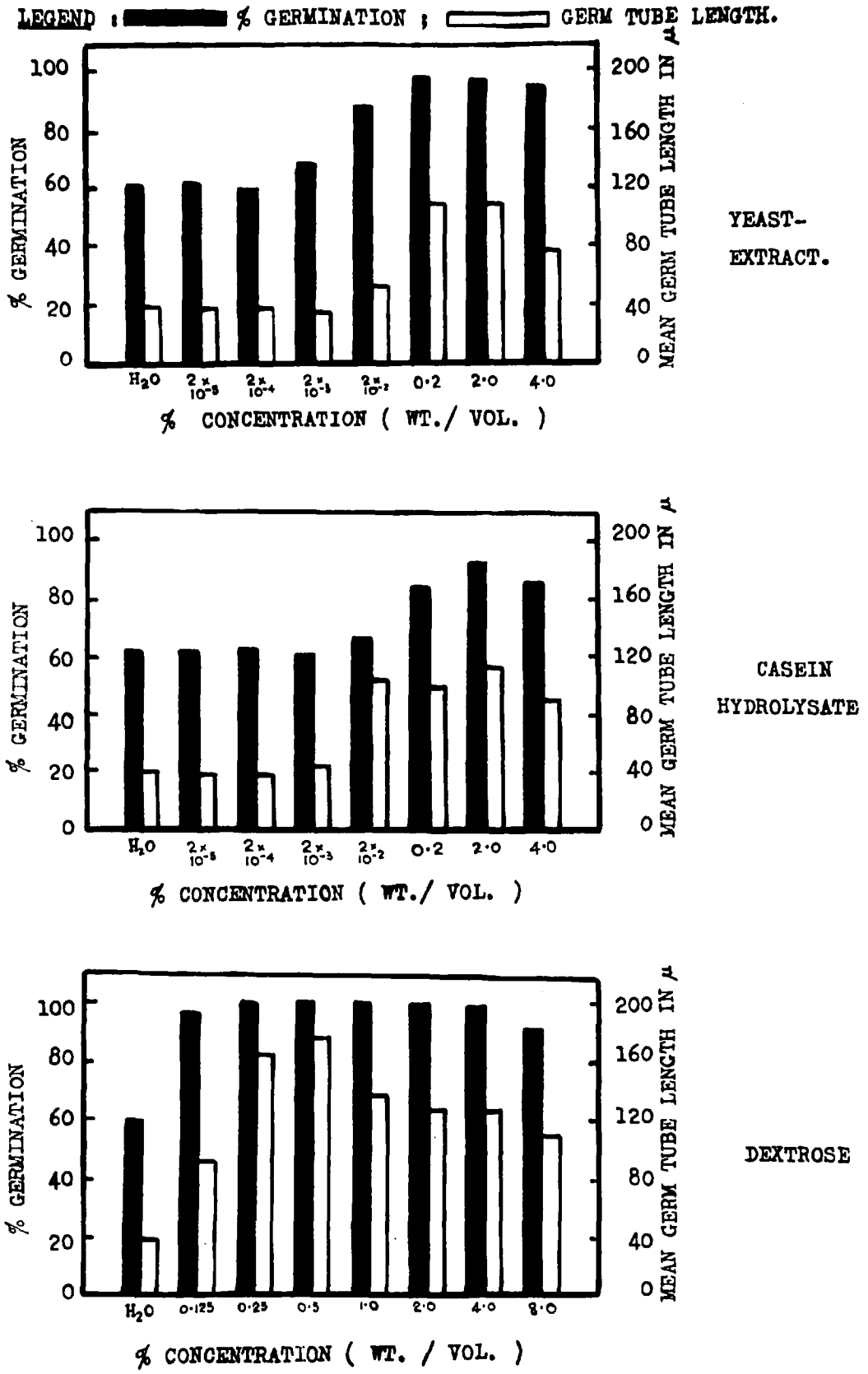


FIG.32. EFFECT OF ORGANIC NUTRIENTS ON GERMINATION OF CONIDIA OF B.BASSIANA

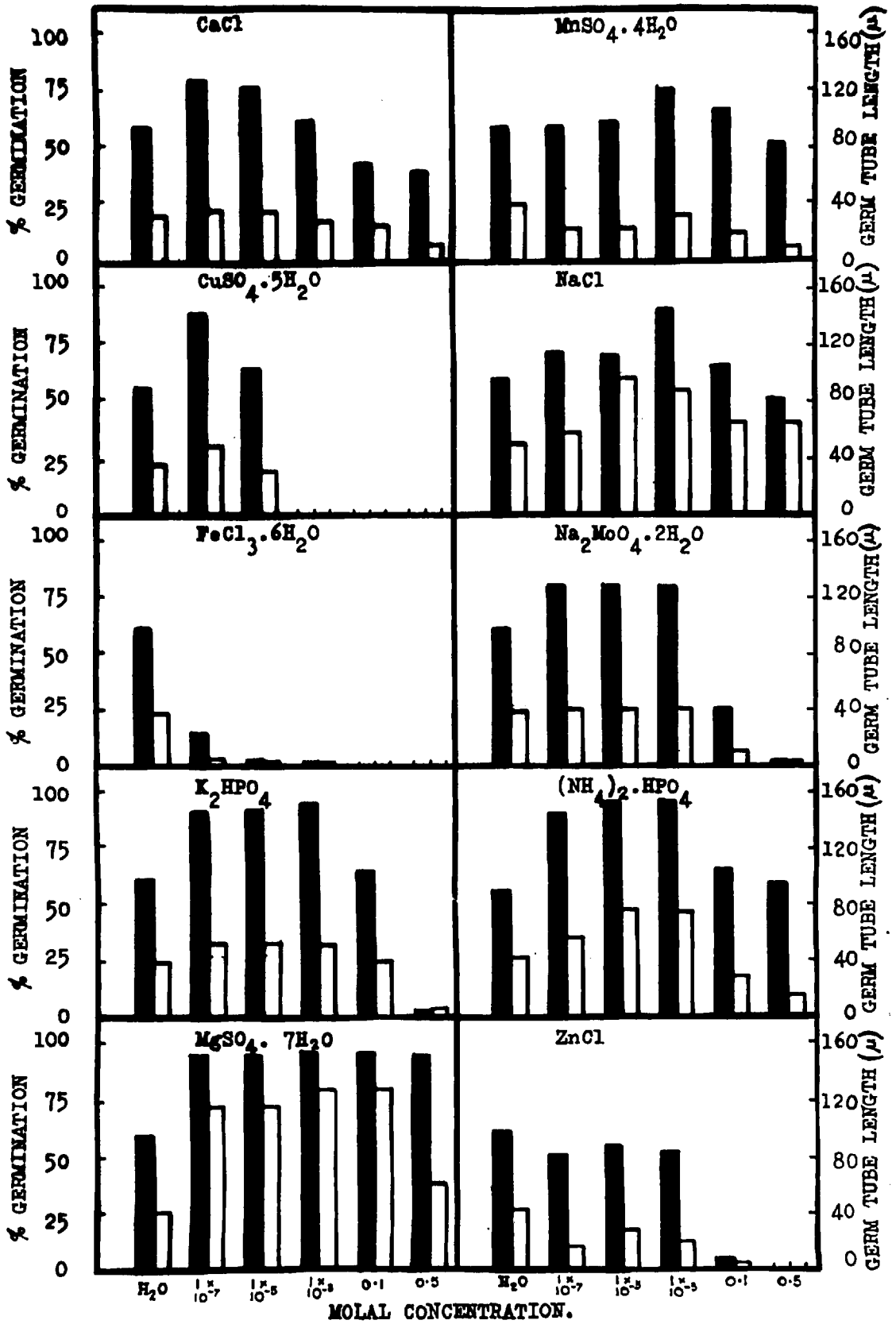


FIG. 33. EFFECT OF MINERAL SALTS ON GERMINATION OF CONIDIA OF B. BASSIANA.

Concentrations of 0.25 and 0.5 % gave 100.0 % germination. Dextrose supported extensive growth of the germ tubes as well (Table 38, Fig. 32).

Casein hydrolysate : Germination at 2 x 10^{-4} to 2 x 10^{-2} % was approximately the same as that of the control (Table 36, Fig. 32). Concentrations of 0.2 to 4.0 % tremendously increased conidium germination and encouraged extensive germ tube growth. There is a suggestion that concentration above 2.0 % is supraoptimal.

Yeast - extract : The pattern of response of the conidia in Yeast - extract (Table 36, Fig. 32) was similar to that observed in casein hydrolysate. However with 0.2 - 2.0 % Yeast - extract, germination closely approached 100.0 per cent.

Calcium chloride : Calcium chloride was stimulatory at very low concentrations, and the percentage germination dropped with increase in concentration. The percentage germination thus fell below that of the control at concentrations at 0.1 Molal and above (see Table 37 and Fig. 33 for particulars of all salts tested).

Copper sulphate : The conidia germinated at concentrations of 1 x 10^{-7} to 1 x 10^{-5} Molal. At 1 x 10^{-7} Molal, germination was highly stimulated (to 91 %). Increase in concentration above 1 x 10^{-5} Molal became highly inhibitory.

Ferric chloride : Ferric chloride depressed germination at all concentrations used, its effect increasing with increase

in concentration.

Di-potassium hydrogen phosphate : Stimulated conidium germination (about 98 %) occurred at concentrations of 1 x 10^{-7} to 1 x 10^{-3} Molal, accompanied by slightly greater germ tube growth. At 0.1 Molal, the percentage germination was identical to that of the control (about 62 %), and any further increase in concentration became inhibitory.

Magnesium sulphate : There was uniformly high stimulation of germination (above 95 %) at all concentrations used. Germ tube growth was similarly stimulated.

Manganese sulphate : There was a slight increase in germination at 1 x 10^{-3} to 0.1 Molal, but otherwise, manganese sulphate had little effect. Germ tube growth was reduced at all concentrations.

Sodium chloride : There was an increase in germination (to 90 %) at 1 x 10^{-3} Molal. Germ tubes grew slightly better at all concentrations than in the controls.

Sodium molybdenate : This was inhibitory (germination less than 26 %) at concentrations of 0.1 Molal and above, but germination was considerably stimulated (about 82 %) at the lower concentrations of 1 x 10^{-7} to 1 x 10^{-3} Molal.

Di-ammonium hydrogen orthophosphate : This stimulated germination and germ tube growth to a great extent at the lower concentrations of 1 x 10^{-7} to 1 x 10^{-3} Molal, with a peak (96 %) between 1 x 10^{-5} and 1 x 10^{-3} Molal. Above this, percentage

germination fell as the concentration rose, but did not drop below that of the control (59 %).

Zinc chloride : ZnCl decreased germination and germ tube growth at all concentrations.

TABLE 39

Effect of Yeast - extract and Casein hydrolysate on Germination of Conidia of I.farinosa at 20°C.

(Observations after 24 hours' incubation)

% Concentration (Wt. / Vol.)	YEAST - EXTRACT (pH 6.4)			CASEIN HYDROLYSATE (pH 6.2)		
	No. of conidia examined	% Germi - nation.	Mean Germ tube length in μ	No. of conidia examined	% Germi - nation.	Mean Germ tube length in μ
Water	2012	9.00	29.4	2114	9.52	28.4
2×10^{-5}	3220	8.41	28.5	1950	10.50	29.2
2×10^{-4}	2770	7.50	29.5	1880	10.66	28.0
2×10^{-3}	2449	11.31	35.0	2640	18.48	58.5
2×10^{-2}	2504	62.30	39.2	2542	19.91	59.0
0.2	2553	100.00	64.8	1846	84.52	72.6
2.0	1879	100.00	151.9	1702	100.00	77.0
4.0	2633	100.00	68.8	2686	100.00	63.1

TABLE 40

Effect of Mineral Salts on Germination of Conidia of *I.farinosa* at 20°C.

(Observations after 24 hours' incubation)

MINERAL SALT		CONCENTRATION IN MOLAL.					
		H_2O	$1x10^{-7}$	$1x10^{-5}$	$1x10^{-3}$	0.1	0.5
CaCl (pH 6.3)	No. of conidia examined	1962	2589	2075	2510	2133	2244
	% Germination	10.48	13.32	18.40	21.35	3.26	0.00
	Mean Germ tube length in μ	31.2	36.9	42.3	58.0	7.4	-
$CuSO_4.5H_2O$ (pH 5.3)	No. of conidia examined	1958	2118	2003	2510	1962	1898
	% Germination	12.42	4.22	4.50	3.74	0.00	0.00
	Mean Germ tube length in μ	31.6	6.9	6.7	3.5	-	-
$FeCl_3.6H_2O$ (pH 3.6)	No. of conidia examined	2043	2090	2112	2054	2160	2858
	% Germination	10.67	11.25	16.50	17.04	4.06	0.00
	Mean Germ tube length in μ	29.6	40.7	36.2	47.9	3.8	-
K_2HPO_4 (pH 7.3)	No. of conidia examined	1892	2164	2008	2078	1995	2488
	% Germination	9.64	27.35	28.00	20.21	0.21	0.00
	Mean Germ tube length in μ	27.5	50.9	47.3	35.9	2.4	-
$MgSO_4.7H_2O$ (pH 6.1)	No. of conidia examined	2246	1898	2000	1801	1922	1817
	% Germination	10.57	11.69	12.30	12.45	15.26	16.12
	Mean Germ tube length in μ	28.0	30.0	28.1	26.8	27.0	28.0

TABLE 40 (cont'd)

Effect of Mineral Salts on Germination of Conidia of *I.farinosa* at 20°C.

(Observations after 24 hours' incubation)

MINERAL SALT		CONCENTRATION IN MOLAL.					
		H_2O	$1x10^{-7}$	$1x10^{-5}$	$1x10^{-3}$	0.1	0.5
$MnSO_4.4H_2O$ (pH 6.2)	No. of conidia examined	2736	1769	2005	2682	2177	3184
	% Germination	12.29	13.06	18.72	23.93	23.04	25.19
	Mean Germ tube length in μ	33.3	30.7	26.4	40.6	39.0	17.5
NaCl (pH 6.5)	No. of conidia examined	2111	2236	2524	2264	1948	3357
	% Germination	13.74	6.19	5.62	6.61	2.00	1.16
	Mean Germ tube length in μ	30.6	31.7	32.3	29.8	8.3	4.4
$Na_2MoO_4.2H_2O$ (pH 6.5)	No. of conidia examined	1877	2534	2001	2562	2180	2390
	% Germination	12.49	37.20	10.40	4.06	0.00	0.00
	Mean Germ tube length in μ	28.0	50.9	26.4	16.3	-	-
$(NH_4)_2.HPO_4$ (pH 7.5)	No. of conidia examined	2445	1809	2020	2818	1980	2372
	% Germination	11.92	10.17	18.22	23.84	0.00	0.00
	Mean Germ tube length in μ	29.0	29.1	28.6	30.4	-	-
ZnCl (pH 6.2)	No. of conidia examined	1926	2132	2566	2392	2114	1900
	% Germination	9.74	13.41	3.68	0.25	0.00	0.00
	Mean Germ tube length in μ	33.7	43.6	22.4	14.0	-	-

TABLE 41

Effect of Dextrose on Germination of Conidia of I.farinosa at 20°C.

(Observations after 24 hours' incubation)

% Concentration (Wt. / Vol.)	No. of conidia examined	% Germination	Mean Germ tube length in μ
Water	2343	11.62	30.5
0.125	2579	25.78	61.0
0.25	2019	24.26	61.5
0.5	2661	22.77	59.9
1.0	3742	44.52	94.8
2.0	1971	47.29	62.5
4.0	2932	43.69	36.6
8.0	1928	43.11	32.8

In I.farinosa, with the exception of conidia at relatively high concentrations of casein hydrolysate and yeast - extract (Table 39, Fig. 34) germination never exceeded 50 % with any of the substrates. (Tables 40 and 41, Figs. 34 and 35). The conidia were highly sensitive to the higher concentrations of the salts (eg. 0.1 Molal). Several salts at these concentrations completely inhibited germination even though substantially increasing it at the lower concentrations. The percentage germination of conidia in distilled water (control) ranged from 9.00 to 13.74 per cent.

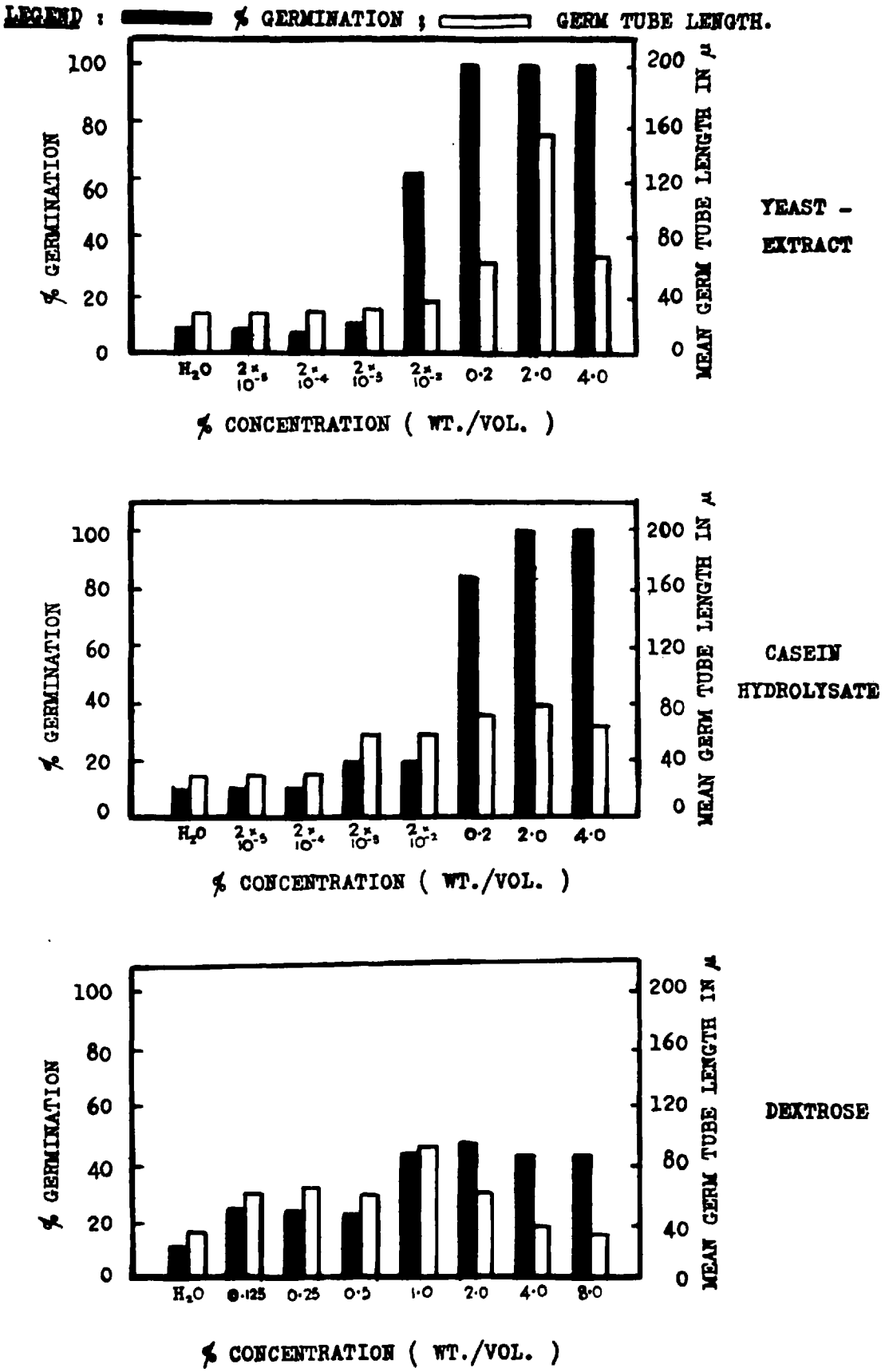


FIG. 34. EFFECT OF ORGANIC NUTRIENTS ON GERMINATION OF CONIDIA OF I. FARINOSA

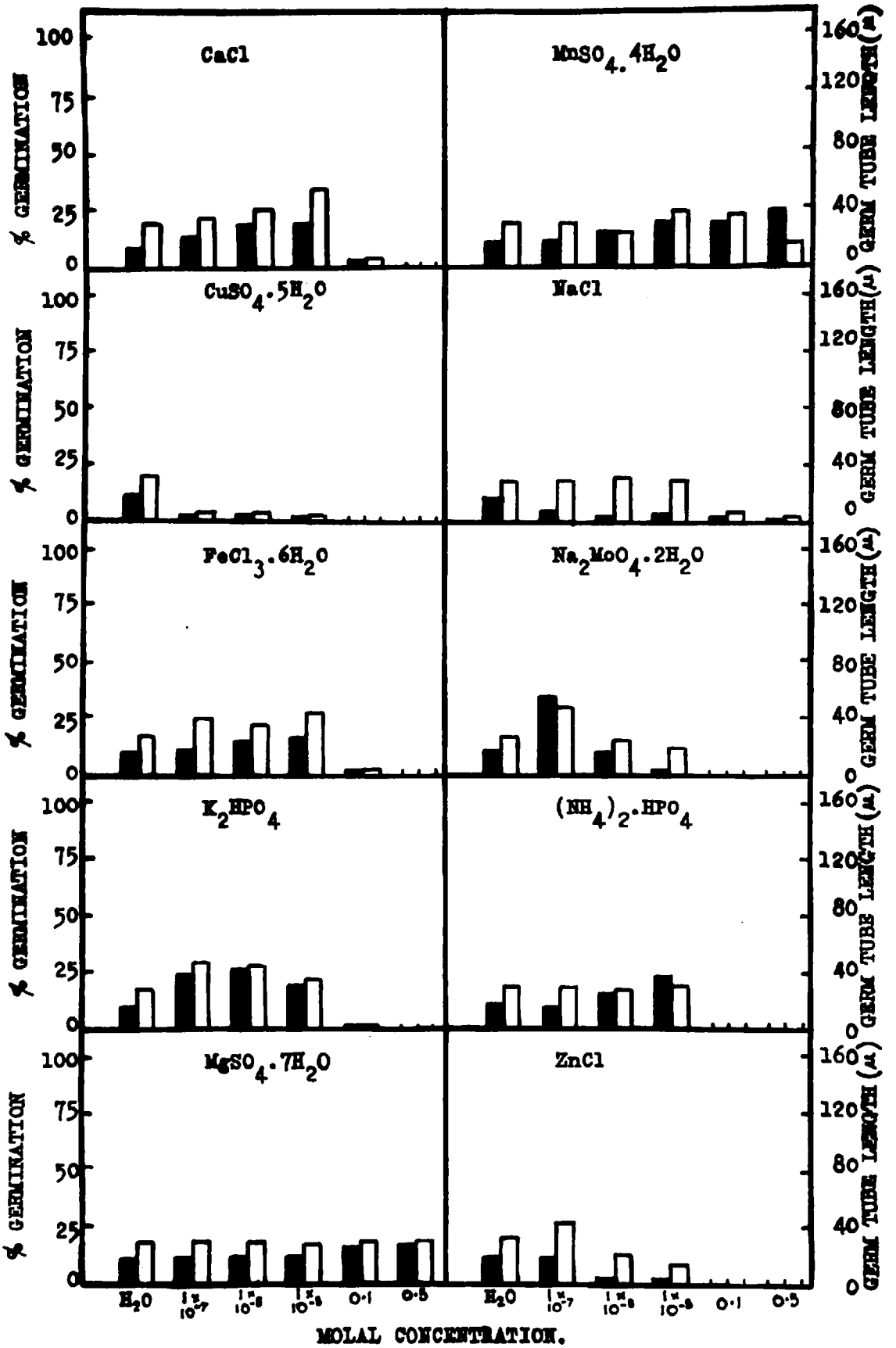


FIG. 35. EFFECT OF MINERAL SALTS ON GERMINATION OF CONIDIA OF I. FARINOSA.

Dextrose : All concentrations stimulated conidium germination, with a peak at about 1.0 to 2.0 %.

Casein hydrolysate : Percentage germination increased with concentration to attain 100.0 per cent germination at 2.0 and 4.0 %. Germ tube growth was similarly stimulated.

Yeast - extract : The response was similar to that of conidia germinating in casein hydrolysate solutions, though 100 per cent germinated with as little as 0.2 % yeast - extract.

Calcium chloride : Percentage germination was greatest (21 %) at a concentration of 0.001 Molal. Germination then fell with increase in concentration, becoming inhibited at 0.5 Molal.

Copper sulphate : Depressed germination at all concentrations.

Ferric chloride : Concentrattions of 1×10^{-5} to 1×10^{-3} Molal weakly increased germination, whilst concentrations of 0.1 Molal and above were inhibitory.

Di - potassium hydrogen phosphate : Germination was stimulated at concentrations of 1×10^{-7} to 1×10^{-5} Molal, but depressed at 0.1 Molal and above.

Magnesium sulphate : Conidial germination was stimulated, rising with increased concentration in the range tested.

Manganese sulphate : Conidial germination was enhanced, and rose with increase in concentration.

Sodium chloride : This depressed germination at all

concentrations used.

Sodium molybdenate : This stimulated conidium germination at 1×10^{-7} Molal but depressed it as the concentration rose above 1×10^{-5} Molal.

Di-ammonium hydrogen orthophosphate : Germination was completely inhibited at 0.1 Molal. It was about double that of the control at 1×10^{-5} to 1×10^{-3} Molal.

Zinc chloride : ZnCl became inhibitory at a concentration of 1×10^{-5} Molal. At lower concentration of 1×10^{-7} Molal germination was practically the same as in water.

TABLE 42

Effect of Yeast - extract and Casein hydrolysate on Germination of Conidia of M.anisopliae at 25°C.

(Observations after 24 hours' incubation)

% Concentration (Wt. / Vol.)	YEAST - EXTRACT (pH 6.4)			CASEIN HYDROLYSATE (pH 6.2)		
	No. of conidia examined	% Germi - nation.	Mean Germ tube length in μ	No. of conidia examined	% Germi - nation	Mean Germ tube length in μ
Water	2181	97.85	88.1	1949	96.02	94.0
2×10^{-5}	2122	98.71	88.9	1850	98.52	97.4
2×10^{-4}	2058	99.22	88.4	1662	98.98	98.1
2×10^{-3}	1972	99.96	89.7	2640	99.50	101.2
2×10^{-2}	2144	99.90	90.4	1777	99.96	218.0
0.2	2018	100.00	458.1	2055	100.00	306.4
2.0	2152	100.00	472.8	1821	100.00	307.3
4.0	1995	100.00	474.4	2067	100.00	245.5

TABLE 43

Effect of Mineral Salts on Germination of Conidia of M. anisopliae at 25°C.

(Observations after 24 hours' incubation)

MINERAL SALT		CONCENTRATION IN MOLAL					
		H_2O	$1x10^{-7}$	$1x10^{-5}$	$1x10^{-3}$	0.1	0.5
CaCl (pH 6.3)	No. of conidia examined	2062	2024	2161	2512	2244	2839
	% Germination	98.22	89.84	85.00	80.73	36.44	25.36
	Mean Germ tube length in μ	91.1	62.8	62.1	59.6	23.0	8.3
$CuSO_4.5H_2O$ (pH 5.3)	No. of conidia examined	2995	2534	2270	2325	2614	2310
	% Germination	97.80	75.61	21.26	2.58	0.00	0.00
	Mean Germ tube length in μ	92.4	34.3	8.7	1.6	-	-
$FeCl_3.6H_2O$ (pH 3.6)	No. of conidia examined	2171	2136	1888	1772	1965	2000
	% Germination	98.82	47.09	30.25	29.74	0.32	0.00
	Mean Germ tube length in μ	94.6	54.2	36.5	30.4	7.4	-
K_2HPO_4 (pH 7.3)	No. of conidia examined	2411	2552	2640	2372	2252	3058
	% Germination	99.00	98.59	84.00	77.90	6.39	0.13
	Mean Germ tube length in μ	92.4	52.6	42.5	40.1	12.6	2.4
$MgSO_4.7H_2O$ (pH 6.1)	No. of conidia examined	2005	2046	2171	2122	1900	2204
	% Germination	98.00	97.26	95.00	95.09	94.21	93.64
	Mean Germ tube length in μ	90.7	82.4	84.9	77.1	75.3	67.6

TABLE 43 (cont'd)

Effect of Mineral Salts on Germination of Conidia of *M.anisopliae* at 25°C.

(Observations after 24 hours' incubation)

MINERAL SALT		CONCENTRATION IN MOLAL					
		H_2O	$1x10^{-7}$	$1x10^{-5}$	$1x10^{-3}$	0.1	0.5
$MnSO_4.4H_2O$ (pH 6.2)	No. of conidia examined	2400	2524	2200	2354	2180	2311
	% Germination	97.29	97.54	93.60	77.06	73.42	67.80
	Mean Germ tube length in μ	88.7	84.4	79.8	62.1	60.0	42.9
NaCl (pH 6.5)	No. of conidia examined	2558	2866	2846	2137	2848	2047
	% Germination	98.41	97.98	86.01	64.36	2.31	0.00
	Mean Germ tube length in μ	94.3	89.5	88.8	59.5	34.6	-
$Na_2MoO_4.2H_2O$ (pH 6.5)	No. of conidia examined	2985	2164	2462	2202	2005	2474
	% Germination	98.02	98.89	90.04	89.91	7.22	0.00
	Mean Germ tube length in μ	95.4	89.6	76.5	45.1	26.5	-
$(NH_4)_2.HPO_4$ (pH 7.5)	No. of conidia examined	2080	2678	2200	2234	2171	2448
	% Germination	98.42	98.38	88.55	87.10	13.62	4.11
	Mean Germ tube length in μ	91.1	73.8	70.1	63.8	21.4	7.1
ZnCl (pH 6.2)	No. of conidia examined	2212	3332	3490	3523	2507	2648
	% Germination	96.02	83.49	84.00	82.27	8.72	0.00
	Mean Germ tube length in μ	91.4	79.7	75.6	61.1	21.4	-

TABLE 44

Effect of Dextrose on Germination of Conidia of M.anisopliae at 25°C.

(Observations after 24 hours' incubation)

% Concentration (Wt. / Vol.)	No. of conidia examined	% Germination	Mean Germ tube length in μ
Water	2256	98.78	94.6
0.125	2074	100.00	170.0
0.25	2838	100.00	168.3
0.5	2896	100.00	163.5
1.0	3252	100.00	160.7
2.0	3154	100.00	168.6
4.0	3134	100.00	156.8
8.0	2700	100.00	154.7

Results in Tables 42 - 44 and in Figs. 36 and 37 revealed a unique response of conidia of M.anisopliae to the substrates tested. In water, usually about 97 per cent of the conidia germinated. While dextrose (Table 44), casein hydrolysate and yeast - extract (Table 42) enhanced germination, all the remaining substrates (Table 43) depressed it to varying degrees at almost all the concentrations used. At the lowest salt concentrations used, germination was at most equal to that in

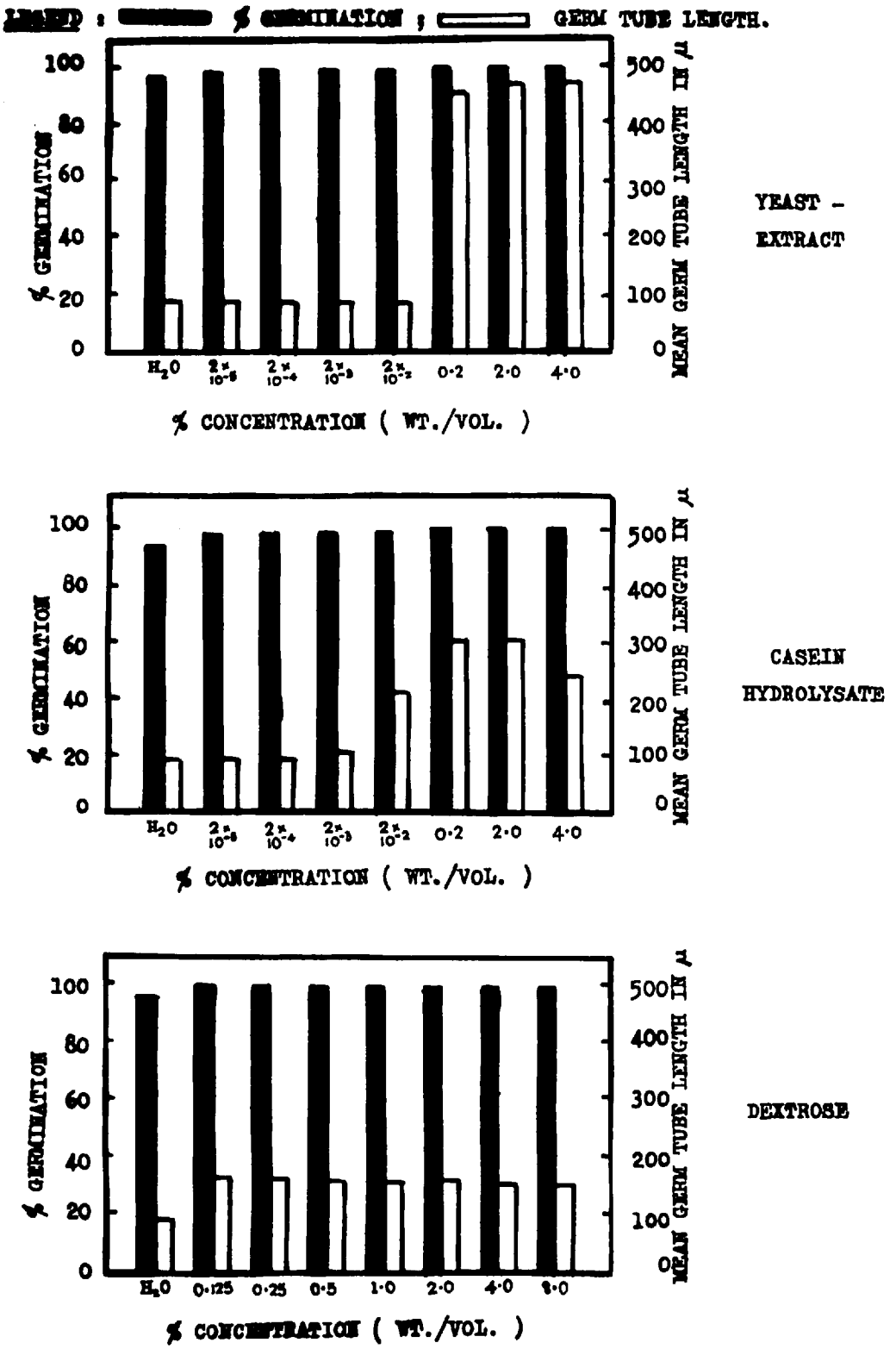


FIG. 36. EFFECT OF ORGANIC NUTRIENTS ON GERMINATION OF CONIDIA OF M. ANISOPLIAE

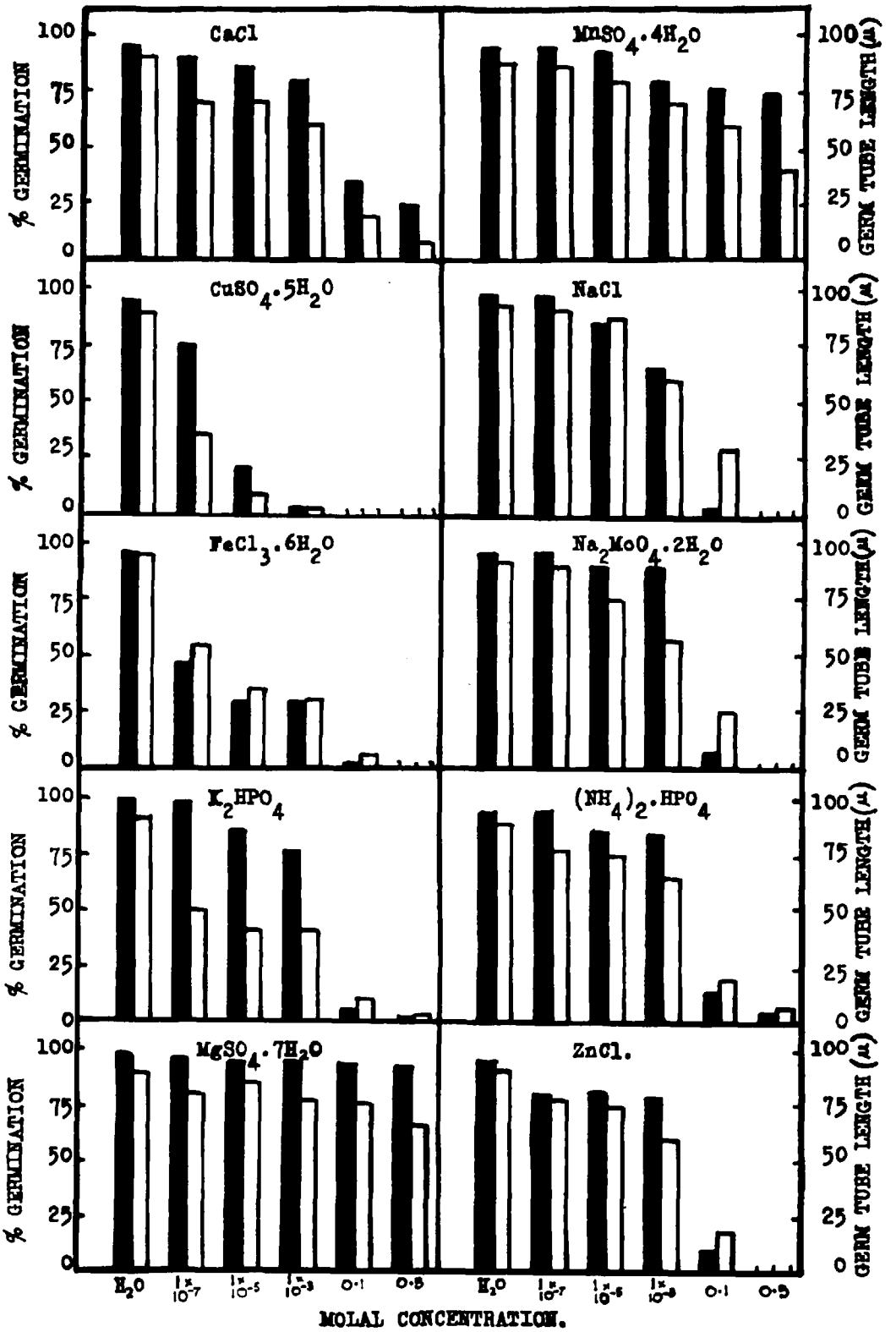


FIG.37. EFFECT OF MINERAL SALTS ON GERMINATION OF M.ANISOPLIAE CONIDIA.

water, and sometimes less. Germination thereafter diminished with increase in concentration.

Dextrose : 100 per cent germination occurred at all concentrations and the germ tubes grew exceedingly well.

Casein hydrolysate : This was highly stimulatory, particularly at the higher concentrations.

Yeast - extract : Yeast - extract was stimulatory, especially at 0.2 to 4.0 %.

Magnesium sulphate : This had little or no depressant effect over the range of concentrations tested.

Of the remaining salts, two categories could be differentiated. Copper sulphate and Ferric chloride strongly inhibited germination at all concentrations ; the rest were inhibitory only at the higher concentrations, and at their lower concentrations reduced germination but slightly.

These experiments thus reveal that the three species show wide differences in percentage germination in water (approximately, 60, 10 and 97 per cent for B.bassiana, I.farinosa and M.anisopliae respectively). For B.bassiana, this proportion can be increased by organic supplements, especially of dextrose and, curiously enough, by several unrelated mineral salts at appropriate levels (especially $CuSO_4 \cdot 5H_2O$, K_2HPO_4, $MgSO_4 \cdot 7H_2O$ and $(NH_4)_2HPO_4$, all of which can lead to over 90 % germination.)

I.farinosa could be induced to germinate 100 per cent by

organic supplements such as yeast - extract and vitamin - free casein hydrolysate, but not by dextrose. This suggests that amino acids may be critical factors. Some mineral salts at certain levels were slightly stimulatory. The best stimulation was by $Na_2MoO_4 \cdot 2H_2O$ at 1×10^{-7} Molal, giving 37 per cent germination. (A connection with N - metabolism might be inferred from the known role of Molybdenum in such processes).

M.anisopliae conidia germinate well in water (eg. 97 %). However complete germination can be induced by organic supplements. No salt was able to do this. On the contrary, most exercised a depressant effect, especially at about 0.1 Molal and above.

O. THE EFFECT OF AQUEOUS EXTRACTS OF EXTERNAL PARTS OF INSECTS ON GERMINATION

i. Effect of Aqueous Extracts from Bombyx mori on Germination.

Several investigators have shown that host stimulation in the germination of the spores of parasitic fungi is a comparatively frequent phenomenon. The pertinent literature on plant parasites has been reviewed by Brown (1946). Throughout these instances, the basis of the stimulation was undoubtedly the same : it involved the production, by the active tissue of the host, of a chemical substance which, though not a sine qua non, appeared to have hastened and encouraged germination of the spore of the parasite.

It has often been reported that entry of entomogenous fungi into the insect directly through the integument is a very frequent form of attack on the insect body (Lefèbvre, 1934 ; Toumanoff, 1933 ; Wallengren, 1929). The degree to which the germination capacity of the conidia on the cuticle of the insect is influenced by the substrate was therefore investigated. Bombyx mori (silkworm), which is a well known host to B.bassiana (MacLeod, 1954) and also susceptible to I.farinosa (Aoki, 1939) and M.anisopliae (Glaser, 1926), was used. Conidial germination tests were made with aqueous extract of the egg - shell, pupal cuticle, silk of pupal cocoon and cuticle of the adult insect.

Silkworm eggs, pupae, pupal cocoons and the adult insects

were obtained from Worldwide Butterflies Ltd., Seafields House, Charmouth, Dorset. Pupae and adult insects were procured alive, and killed with ether vapour immediately before use. Excised cuticular pieces of the pupa and adult were scrupulously cleaned by scrapping the inner surfaces with a sharp micro-spatula to remove any attached fragments of muscles. The items were used without any previous washing.

Two sets of experiments were carried out with each item. The eggs or skeletal fragments were placed in conidial drops on slides (slide method). Each germination drop held either one egg or a mm. square of cuticle or silk of pupal cocoon. In the second set, the skeletal structures were embedded in pyridine-washed plain agar, and the plates later seeded with the conidia. Each agar plate held either 20, 0.1 inch square pieces of the exoskeleton, or 30 eggs. The conidia were incubated at their respective optimum temperatures for 24 hours, after which percentage germination was calculated and germ tube lengths measured. Control germination tests were made in either distilled water or on plain agar. The results are presented in Tables 45 - 47.

TABLE 45

Effect of Aqueous extract of External parts of Bombyx mori (silkworm) on Germination of Conidia of B.bassiana at 18°C.

(Observations after 24 hours' incubation)

EXOSKELETON	TREATMENT	No. of conidia examined	% Germination	Mean Germ tube length in μ
EGG SHELL	Suspension Drop	2109	100.00	120.4
	Control - Water	1905	53.20	29.3
	Agar	2425	100.00	155.0
	Control - Agar	1725	62.41	37.2
CUTICLE OF PUPA	Suspension Drop	2088	100.00	126.4
	Control - Water	2268	60.81	34.3
	Agar	3041	100.00	161.0
	Control - Agar	2172	64.25	35.7
SILK OF PUPAL COCOON	Suspension Drop	2619	100.00	186.0
	Control - Water	1817	50.39	29.7
	Agar	1997	100.00	202.3
	Control - Agar	2468	65.96	34.1
CUTICLE OF IMAGO	Suspension Drop	2206	99.55	100.9
	Control - Water	4278	60.07	32.0
	Agar	2077	100.00	126.6
	Control - Agar	1992	52.66	35.0

TABLE 46

Effect of Aqueous extract of External parts of Bombyx mori (silkworm) on Germination of Conidia of I.farinosa at 20°C.

(Observations after 24 hours' incubation)

EXOSKELETON	TREATMENT	No. of conidia examined	% Germination	Mean Germ tube length in μ
EGG SHELL	Suspension Drop	2306	81.87	34.6
	Control - Water	2692	7.50	18.4
	Agar	3150	68.89	52.7
	Control - Agar	2450	10.12	27.1
CUTICLE OF PUPA	Suspension Drop	1964	100.00	62.9
	Control - Water	2063	8.69	19.2
	Agar	1734	100.00	72.5
	Control - Agar	1949	14.52	21.8
SILK OF PUPAL COCOON	Suspension Drop	2399	100.00	121.9
	Control - Water	2124	12.99	20.5
	Agar	2479	100.00	130.9
	Control - Agar	1966	16.60	26.3
CUTICLE OF IMAGO	Suspension Drop	2485	100.00	73.2
	Control - Water	1886	13.22	20.7
	Agar	2108	100.00	67.3
	Control - Agar	2030	14.64	20.0

TABLE 47

Effect of Aqueous extract of External parts of Bombyx mori (silkworm) on Germination of Conidia of M.anisopliae at 25°C.

(Observations after 24 hours' incubation)

EXOSKELETON	TREATMENT	No. of conidia examined	% Germination	Mean Germ tube length in μ
EGG SHELL	Suspension Drop	1803	100.00	400.6
	Control - Water	2060	94.52	82.2
	Agar	2705	100.00	401.2
	Control - Agar	2200	96.92	107.4
CUTICLE OF PUPA	Suspension Drop	2789	100.00	377.2
	Control - Water	2548	95.97	102.9
	Agar	3020	100.00	424.7
	Control - Agar	2609	96.74	102.7
SILK OF PUPAL COCOON	Suspension Drop	2062	100.00	451.4
	Control - Water	2516	94.20	92.3
	Agar	2498	100.00	469.1
	Control - Agar	2900	96.69	107.9
CUTICLE OF IMAGO	Suspension Drop	1909	100.00	400.0
	Control - Water	2020	97.12	102.3
	Agar	2151	100.00	405.3
	Control - Agar	2860	96.99	99.7

Results in Table 45 showed that germination of conidia of B.bassiana in the different extracts of the exoskeleton of Bombyx mori was consistently high - about 100 per cent in all cases, whether in suspension drops or on the agar plates. Germ-tube growth was similarly highly stimulated. On the agar plates in the presence of the exoskeleton, they showed slightly superior growth to those in the suspension drops under the influence of identical exoskeletal structures. This presumably was due to better aeration on the agar plates.

The extracts of exoskeleton of Bombyx mori greatly stimulated germination of conidia of I.farinosa. Extracts of pupal cuticle, silk of pupal cocoon and cuticle of the adult insect led to 100 per cent germination in 24 hours. Extract of egg shell was not as stimulatory as the other extracts (Table 46). In all cases, germ tube growth was also increased, especially by silk extract.

In the four different extracts, the percentage germination of conidia of M.anisopliae (Table 47) was consistently 100 per cent. Because it's conidia germinated well in water any way, the stimulatory effects of the extracts are more clearly displayed by the data for growth of the germ tubes. These, in the suspension drops or on agar, in the presence of the exoskeletal materials were 4 times the length of those in the controls.



CONIDIA OF B.BASSIANA AND I.FARINOSA GERMINATING IN WATER EXTRACT OF SILK OF PUPAL COCOON OF BOMBYX MORI AFTER 24 HOURS' INCUBATION.

PLATE 6. - CONIDIA OF B.BASSIANA AT 18°C. (x 160).

PLATE 7. - CONIDIA OF I.FARINOSA AT 20°C. (x 160).

(cf. GERMINATION OF THE CONIDIA IN WATER - PAGE 47 - PLATES 3 & 4).

ii. CHEMOTROPIC EFFECT OF EXTRACTS ON GERM TUBES.

The problems of the nature of the stimulus leading to penetration of parasitic fungi into the host tissue have long interested plant pathologists. Germ tube tropisms towards water and nutrients have been reported, but the evidence is conflicting and inconclusive. The earlier workers considered that the stimulus was a chemical one. Miyoshi (1895) reported that germ tubes were attracted by extracts of plants at suitable concentrations. Fulton (1906) however, stated that the chemotropism of germ tubes was a negative one, away from the products of their own metabolism. Graves (1916) concluded that both types of chemotropism occurred, but that Fulton's type was the more usual.

Further investigation was therefore carried out to determine whether the active exoskeletal extracts possessed any chemotropic stimulus in addition to their general stimulatory properties.

For the determination of chemotropic effect of extracts of the exoskeleton on the germ tubes, a modified version of Graves' (1916) method was adopted. Each preparation consisted of a perforated impermeable 'polyglaze' plate bearing a thin film of pyridine-washed plain agar on each side of it's surfaces. (Fig. 38) The agar film on one side contained the conidia, while the other contained eggs or fragments of the exoskeleton. Stimulatory

substances from the agar film containing exoskeletal fragments on one side of the polyglaze plate would diffuse through the agar plugs in the perforations to the agar film holding the conidia on the other side. If there was any positive chemotropic stimulus the germ tubes in the immediate vicinity of the diffusing stimulus would bend towards the source of the stimulus.

When washed agar, previously melted, had cooled to exactly 40°C., a suspension of conidia was added and shaken to give an even dispersion, and 2 mls. of this are quickly poured into each of several sterile petri dishes. The gelled agar was cut into 19 x 19 mm. squares, which were then transferred singly onto sterile glass slides. In the meantime, the centre of rectangular (25 x 40 mm.) 'polyglaze' plates was punched with a sharp needle. The area perforated measured 20 x 20 mm., and held 9 holes, 5 mm. apart, in 3 rows of 3. These perforated polyglaze plates were placed upon the agar squares on the glass slides so that the perforated area coincided with the agar film. The polyglaze plate was gently pressed down until agar was forced up through the holes. Next 0.2 ml. of cool molten agar containing exoskeletal fragments, was dropped and spread on the upper surface of the polyglaze plate, up to the boundaries of the perforated area (Fig. 38).

The preparation with its supporting glass slide was now

rested on V - shaped glass rods in a petri dish lined with water - soaked filter paper, and incubated at the optimum temperature of the fungus for 24 hours.

Method of estimating chemotropic reaction : The procedure of estimating the reaction of the germ tubes adopted by Graves (1916) was followed. Camera lucida drawings were made of the germ tubes within the immediate vicinity of the hole, which were apparently under the general stimulatory influence of the extract, judging by their rates of growth. The area to be considered, for the purposes of standardisation, was in each case delimited by a circle, 2 inches in radius, on the drawing sheet, the centre of that area coinciding with the centre of the hole.

The next step was to determine from the drawings the direction taken by the germ tube. Straight lines were drawn from the centre of the hole to the conidium itself, in cases where the hypha was fairly straight (Fig. 39 a). That line was supposed to make angles of 45° with two other lines intersecting one another at right angles at the conidium. The 4 regions, of 90° each, thus marked out by these last two lines, were then called A, B, C and D with orientation as shown in Fig. 39. The hyphae were then classified as A, B, C and D, according to the quarter in which they grew. If the hyphae curved markedly, only its tip was considered, since the ultimate effect of the reaction was



FIG. 38. APPARATUS FOR DETERMINING CHEMOTROPIC STIMULUS OF STIMULATORY SUBSTANCES OF EXTRACTS OF EXTERNAL PARTS OF BOMBYX MORI. 1. IMPERMEABLE POLYGLAZE PLATE. 2. GLASS SLIDE. 3. PYRIDINE-WASHED PLAIN AGAR HOLDING FRAGMENTS OF EXTERNAL PARTS OF BOMBYX MORI. 4. PYRIDINE-WASHED PLAIN AGAR HOLDING CONIDIA.

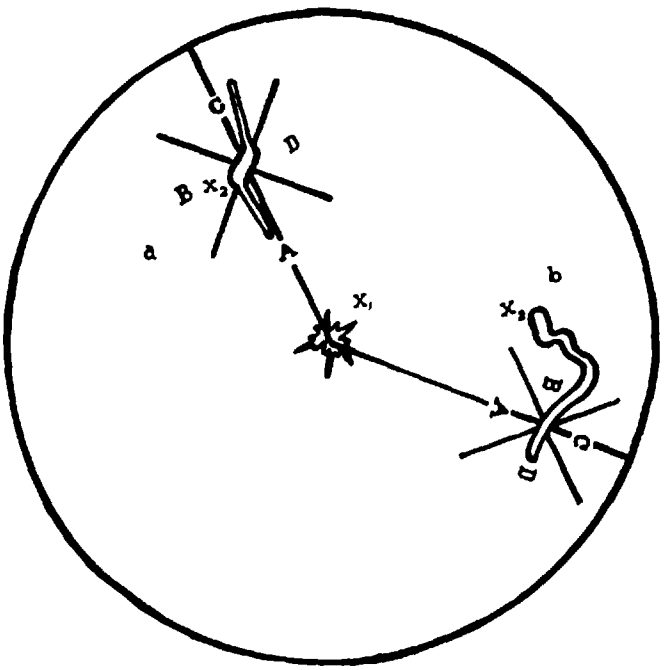


FIG. 39. METHOD OF ESTIMATION OF DIRECTION OF GROWTH OF GERM TUBE. X_1 - PERFORATION. X_2 - GERMINATING SPORE.

expressed in this portion (Fig. 39b), and the hypha was then classified according to the quarter into which the tip pointed.

The basis of the interpretation of the figures thus obtained lay in the assumption that the average number of hyphae in each of the 4 classes, considering a large number of holes, and supposing that no attractive or repellent force existed, should on average be approximately the same. Any considerable deviation from that would indicate a reaction to a disturbing force. Since the B and D regions were of equal volume and equally subjected to repellent or attractive forces, they might be left out of consideration, except that the number occurring in them went to make up the total for each hole, and influenced the final percentage of reaction. Further, if there were no external influences, apart from those related to the perforation, the numbers of hyphae in classes B and D should be virtually the same. One was concerned therefore first of all with the A and C classes. If it was assumed in the first instance that an attractive force existed which was causing the hyphae to grow towards the hole 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 increment had been derived from those which would normally point B or D. 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 hyphae originally in the C class had turned into B or D direction, and the remaining C's would be fewer than one-fourth of the total. The difference between the remaining C's and the normal number would then represent those which had turned from the C direction into B or D. 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 3/4 of the total number of hyphae counted in the whole area, since only 3/4 of the hyphae were dealt with, the 1/4 represented by the normal A's being unaffected.

Let " n " (1/4 of the total) denote the normal number to be expected in each region, the number actually in each region by it's corresponding letter, and the total number of hyphae in the whole area " t " ; the formula as derived by Graves (1916) thus reads as follows

$$\frac{(A - n) \mp (n - C)}{3/4t}$$

$$= \frac{A - C}{3/4t} = \text{fraction of hyphae reacting.}$$

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

Three separate tests (numbered a, b and c in Tables of results) were made with each exoskeletal material. Each test comprised 12 slides. The results are presented in Tables 48 - 50.

TABLE 48

Chemotropic effect of aqueous extract of egg shell, pupal cuticle, silk of pupal cocoon and cuticle of adult of Bombyx mori on germ tubes of B.bassiana incubated at 18°C.

(Observations after 24 hours' incubation)

EXOSKELETON		[1]Mean Germ tube length within 0.5 mm of hole	[1]Mean Germ tube length 2-3 mm from hole	Total No. of Germ tubes plotted	Direction of hyphae around hole in circumscribed area				% Reaction
					A	B	C	D	
EGG SHELL	a	119.2	22.6	519	159	95	167	98	- 2.12
	b	120.2	23.1	548	164	103	175	106	- 2.51
	c	118.9	26.4	550	172	107	160	111	∓ 2.90
CUTICLE OF PUPA	a	146.4	25.4	510	164	90	159	97	∓ 1.30
	b	140.2	30.4	913	263	189	257	204	∓ 0.87
	c	132.7	28.5	923	269	185	277	192	- 1.15
SILK OF PUPAL COCOON	a	131.6	22.7	529	161	102	162	104	- 0.25
	b	126.2	25.8	968	301	152	338	177	- 3.37
	c	121.5	23.2	928	309	171	288	160	∓ 3.01
CUTICLE OF IMAGO	a	148.0	24.2	672	180	149	187	156	- 1.38
	b	142.6	25.4	688	192	154	187	155	∓ 0.96
	c	139.8	22.0	704	200	157	200	147	0.00
Sum of hyphae in each class :					2534	1654	2557	1606	

[1]Length of Germ tube in μ.

TABLE 49

Chemotropic effect of aqueous extract of egg shell, pupal cuticle, silk of pupal cocoon and cuticle of adult of Bombyx mori on germ tubes of I.farinosa incubated at 20°C.

(Observations after 24 hours' incubation)

EXOSKELETON		[1]Mean Germ tube length within 0.5 mm of hole	[1]Mean Germ tube length 2-3 mm from hole	Total No. of Germ tubes plotted	Direction of hyphae around hole in circumscribed area				% Reaction.
					A	B	C	D	
EGG SHELL	a	71.3	21.2	462	135	93	139	95	- 1.15
	b	70.0	22.2	389	110	80	116	83	- 2.05
	c	65.4	23.6	422	126	92	115	89	± 3.55
CUTICLE OF PUPA	a	67.3	19.0	363	102	82	98	81	± 1.46
	b	71.6	20.2	355	92	79	99	85	- 2.62
	c	72.1	20.7	356	89	83	97	87	- 2.99
SILK OF PUPAL COCOON	a	85.9	24.1	386	132	65	126	63	± 2.07
	b	71.6	20.2	405	125	70	130	80	- 1.60
	c	70.1	20.7	428	119	94	117	98	± 0.63
CUTICLE OF IMAGO	a	70.8	22.3	520	152	109	153	106	- 0.25
	b	73.0	21.1	529	147	110	150	122	- 0.75
	c	71.4	22.1	499	155	143	144	157	± 2.27
Sum of hyphae in each class :					1484	1100	1484	1146	

[1] Length of Germ tube in μ.

TABLE 50

Chemotropic effect of aqueous extract of egg shell, pupal cuticle, silk of pupal cocoon and cuticle of adult of Bombyx mori on germ tubes of M. anisopliae incubated at 25°C.

(Observations after 24 hours' incubation)

EXOSKELETON		[1] Mean Germ tube length within 0.5 mm of hole	[1] Mean Germ tube length 2-3 mm from hole	Total No. of Germ tubes plotted	Direction of hyphae around hole in circumscribed area				% Reaction
					A	B	C	D	
EGG SHELL	a	127.0	32.2	754	224	164	214	152	∓ 1.76
	b	128.5	29.8	750	240	136	232	142	∓ 1.41
	c	117.6	31.3	620	200	116	204	100	- 0.85
CUTICLE OF PUPA	a	116.7	34.1	596	166	130	168	132	- 0.44
	b	159.9	51.1	460	158	76	158	68	0.00
	c	138.0	51.7	468	140	96	134	98	∓ 1.58
SILK OF PUPAL COCCON	a	129.8	37.1	460	140	96	136	88	∓ 1.15
	b	135.6	36.8	548	160	98	158	132	∓ 0.48
	c	142.1	31.5	674	202	128	210	134	- 1.58
CUTICLE OF IMAGO	a	138.3	42.3	528	136	122	140	130	- 1.01
	b	129.7	50.8	469	128	100	135	106	- 1.98
	c	130.0	49.1	552	158	130	147	117	∓ 2.65
Sum of hyphae in each class :					2052	1392	2036	1399	

[1] Length of Germ tube in μ.

The results in Tables 48 to 50 show that there was negligible difference between the number of " A " and " C " hyphae, whatever the treatment. Evidently the extracts of the exoskeleton of Bombyx mori had no general chemotropic properties, either attractive or repellent. The small and inconsistent values of percentage reaction obtained were clearly of no significance. In no treatment was there a consistent + or - percentage reaction.

One interesting feature was the regular occurrence of greater number of germ tubes in classes A and C than in B and D. Such an effect might result from the existence of tropic responses, in which the sign of the response depends on either the concentration of the excitant, or the characteristic of the individual spore, which vary within a population, or on both. Unfortunately, the available data do not permit this possibility to be evaluated.

The general stimulatory powers of the extracts were reflected in the greater germ tube lengths in conidia immediately around the holes.

It is evident that germ tubes of conidia of M. anisopliae, both under and away from the influence of the stimulatory substance, were shorter in this experiment (Table 50) than in the previous experiment (Table 47). Germ tube growth in conidia of M. anisopliae was presumably reduced in this experiment by submergence in the agar.

iii. DETERMINATION OF NATURE OF STIMULANT IN AQUEOUS EXTRACT OF SILK FROM BOMBYX MORI.

a. Constitutive or induced nature of stimulant.

Aqueous extracts of the exoskeleton of Bombyx mori were found in the previous experiments above to enhance germination of conidia of B.bassiana, I.farinosa and M.anisopliae. The nature of the stimulant in the extracts was examined in the following experiments. This investigation was confined to studies on extract of the silk of pupal cocoon, and I.farinosa conidia alone were used, where necessary, as the test organism.

Firstly, an investigation was conducted to find out whether stimulatory substances were passively released by the exoskeletal material, or whether the conidia acted on the exoskeleton, most probably enzymatically, to release substances which enhanced germination. Pyridine-washed plain agar was poured into petri dishes holding several 0.2 inch square pieces of silk cut from the pupal cocoon. These plates were stored at 4°C. for 24 hours. At the end of that period, the silk fragments in half the number of the dishes were removed with a cork borer. Silk fragments in the remaining plates were left intact. Both types of plates were then seeded with conidia of I.farinosa and incubated at 20°C. for 24 hours.

If the stimulatory substances were passively released from

the silk, percentage germination in plates of both treatments should be the same, since sufficient time was allowed for diffusion of stimulants from the silk fragments into the agar. If however, germinating conidia must act on the silk to release the stimulant, only those conidia seeded over the silk pieces left in the agar would show enhanced germination. Control germination tests were made on unamended washed agar.

The results, presented as percentage germination and mean germ tube lengths, are shown in Table 51.

TABLE 51

Constitutive or induced nature of stimulant in extract of silk from Bombyx mori.

Conidia of I.farinosa germinated at 20°C. for 24 hours.

TREATMENT	No. of conidia examined	% Germination	Mean Germ tube length in μ
Conidia seeded over silk left intact in agar.	2363	100.00	53.1
Conidia seeded on agar with excised silk	2740	100.00	59.9
Control - Unamended agar	2879	18.22	21.5

Conidia of I.farinosa germinated extremely well in both treatments (Table 51). The results thus indicate that the stimulatory substances were released passively into the agar wherein they influenced the conidia.

b. Effect of Dilution of aqueous extract of silk.

The effect of dilution of the aqueous extract of silk on germination of conidia of I.farinosa was examined. In this experiment and subsequent ones, for the purposes of standardisation, aqueous extract of silk denoted as Normal (N) was made by shaking 1 gm. of silk of cocoon in 10 ml. of water in a McCartney tube on a reciprocating shaker for 4 hours.

The ' Normal ' extract was diluted to give a range of solutions (see Table 52) which were used to prepare conidial suspensions. The suspension drops were then incubated at 20°C. for 24 hours, using the 'slide' method. Percentage germination was calculated and germ tube lengths measured at the end of that period. The results are presented in Table 52.

The extract was found to be highly stimulatory over the wide range of concentrations used. (Table 52), for at the lowest concentration of N/64, percentage germination of conidia of I.farinosa was approximately 6 times that of the control. Germination increased with increase in concentration to attain 100 per cent at a concentration of N/2. Germ tube growth was similarly stimulated.

TABLE 52

Effect of dilution of aqueous extract of silk from Bombyx mori on germination of conidia of I.farinosa at 20°C.

(Observations after 24 hours' incubation)

Concentration of Extract	No. of conidia examined	% Germination	Mean Germ tube length in μ
N	1973	100.00	98.4
N/2	2266	100.00	95.2
N/4	1978	99.29	86.7
N/8	2652	90.31	68.5
N/16	2112	79.21	66.9
N/32	2284	62.96	27.9
N/64	2290	60.87	23.0
Control - Water	2111	10.42	19.8

c. Therno-stability of Extract.

The extract had hitherto been used without previous sterilisation. This experiment was designed to examine the effect of heating, by autoclaving for 20 minutes at 15 pounds per square inch steam pressure, on the stimulatory properties of the extract. An aqueous extract of silk of " N " concentration was autoclaved and used to prepare a conidial suspension. Germination tests were then made by the 'slide' method at 20°C. for 24 hours. Controls were run with non-autoclaved aqueous extract of silk of the same concentration (N). The percentage germination and germ tube lengths were then determined at the end of the period. The results are presented in Table 53.

TABLE 53

Germination of I.farinosa conidia in Autoclaved and Non-autoclaved aqueous extract of silk from Bombyx mori at 20°C., for a period of of 24 hours.

TREATMENT	No. of conidia examined	% Germination	Mean Germ tube length in μ
Autoclaved Extract	2780	100.00	118.0
NOn-autoclaved Extract	2563	100.00	115.7

The results in Table 53 show that conidia of I.farinosa germinated equally well in both autoclaved and non-autoclaved extracts.

d. Germination of Conidia of I.farinosa in aqueous extract of silk at different pH levels

This experiment was designed to find the response of conidia of I.farinosa to the aqueous extract of silk at different pH levels.

Silk extract of a concentration of 2N was prepared, as well as buffer solutions of selected pH levels according to McIlvaine's standards - mixture of 0.2 M. Na_2HPO_4 and 0.1 M. Citric acid. (Clark, 1925). To adjust the pH of the extract, equal volumes of silk extract (2N) and buffer solution of the desired pH were mixed. The adjusted extract was thus simultaneously diluted to " N " concentration. pH of the adjusted extracts was checked with a Cambridge pH meter. Control experiments were concurrently run with McIlvaine's buffer solutions mixed with equal volumes of pure water. Conidia of I.farinosa were then germinated in the adjusted silk extract and unamended buffer solutions by the 'slide' method at 20°C. for 24 hours. Percentage germination was calculated at the end of the incubation period, and lengths of the germ tubes measured. The results are tabulated in Table 54.

The results in Table 54 revealed that the extract greatly modified the response of I.farinosa conidia to pH. 100 per cent of the conidia germinated over the pH range of pH 3.0 to 7.0 in the buffered extracts. As shown previously in Table 33, pH 4.0

was optimal in unamended buffer solutions, giving much higher germination than in water alone.

TABLE 54

Germination of Conidia of I.farinosa in aqueous extract of silk from Bombyx mori at different pH levels at 20°C. for 24 hours.

(Extract buffered with McIlvaine's Buffer Solutions)

pH	BUFFERED SILK EXTRACT			UNAMENDED BUFFER SOLUTIONS (CONTROL)		
	No. of conidia examined	% Germination	Mean Germ tube length(μ)	No. of conidia examined	% Germination	Mean Germ tube length(μ)
3.0	2798	100.00	82.3	2484	10.96	6.7
4.0	2094	100.00	101.3	2077	70.58	18.3
5.0	1996	100.00	100.1	2272	52.37	9.9
6.0	1911	100.00	75.4	2648	52.42	10.8
7.0	1918	100.00	73.0	2079	10.96	5.8
8.0	1722	98.15	22.7	2021	0.00	-

e. Paper Chromatographic analysis of Extract.

A more direct attempt was made to analyse the aqueous extract of silk for the presence of fractions of nutritive value.

Silk of the pupal cocoon is spun by the insect in the form of a filament composed of two strands of fibroin cemented together by a water soluble silk gelatin known as serecin. (Frey-Wyssling, 1938 ; Ohara, 1933a, 1933b.). This water soluble sericin is characterised by extremely high content of hydroxy amino acids. (Alders, 1927 ; Ito and Komori, 1939 ; Nicolet and Saidel, 1941 ; Lang, 1948a, 1948b.) These workers have identified as components of sericin, the amino acids : glycine, serine, threonine, tyrosine, histidine, tryptophane, proline, arginine, lycine and cystine.

The aqueous extract of silk from Bombyx mori was therefore chromatographically analysed. Developed chromatograms were tested for carbohydrates and amino acids. Silk extract of " N " concentration was used. From a series of chromatographic tests (20 tests), the constitute amino acids were identified (Plate 8) and their respective quantities estimated. The results are presented in Table 55, while Plate 8 shows a representative chromatogram. Quantitative estimates were based on visual comparisons of the spots (of identified amino acids) with spots produced by known quantities of corresponding markers.

Results of
/ Several tests made for the presence of carbohydrates were negative. For these tests, the spots were developed with n-butanol-pyridine-water-benzene (5,3,3,1) and sprayed with benzidine-acetic acid,

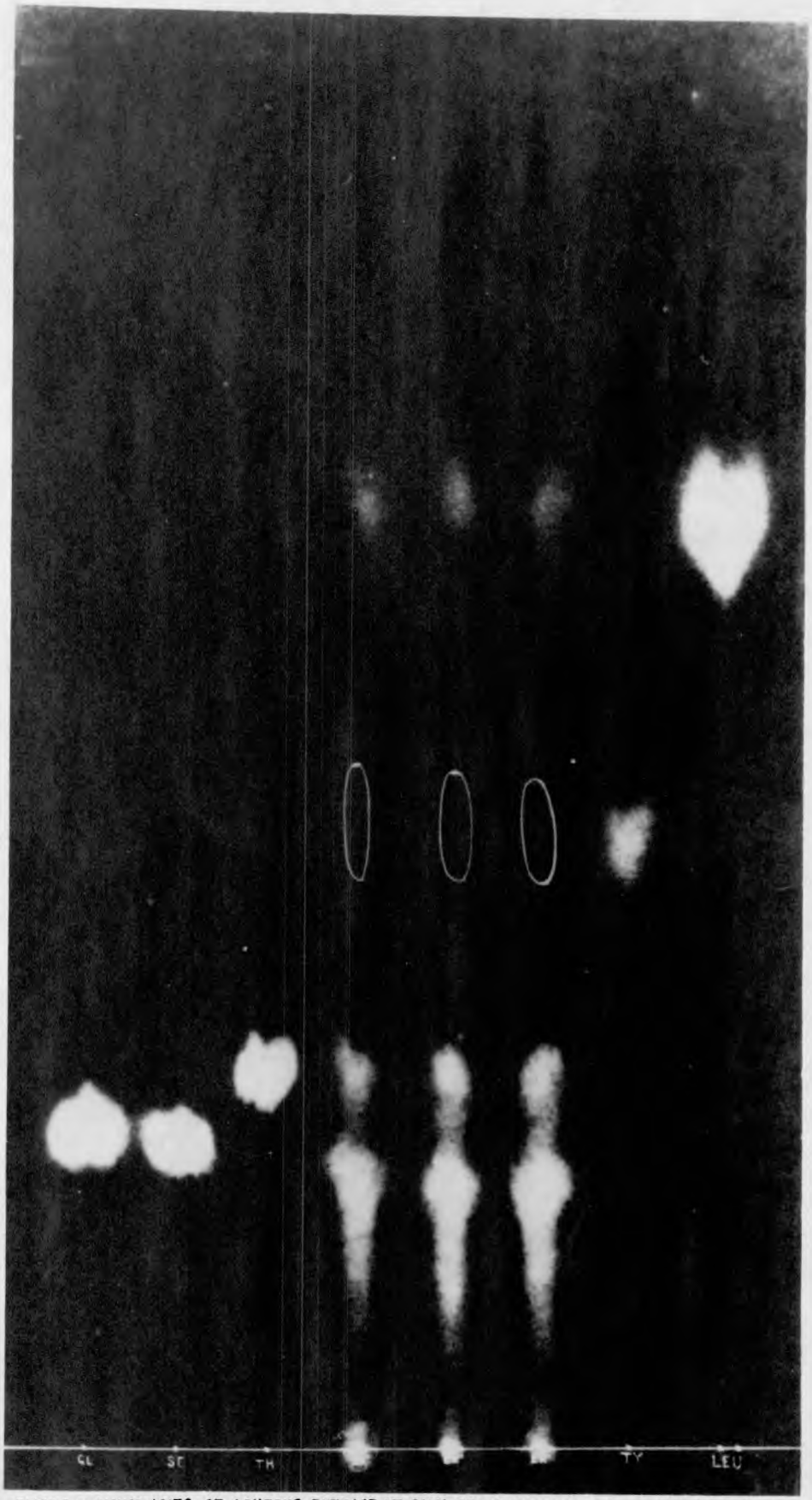


PLATE 8.- AMINO ACIDS OF AQUEOUS EXTRACT OF SILK FROM BOMBYX MORI (EX.) SEPARATED BY PAPER CHROMATOGRAPHY, WITH CORRESPONDING MARKERS (GL. - GLYCINE ; SE.- SERINE ; TH. - THREONINE ; TY.- TYROSINE ; LEU.- LEUCINE.).

TABLE 55

Amino acids in aqueous extract of silk from Bombyx mori identified by paper chromatography.

Amino acid	Estimated quantity in extract of "N" concentration in p.p.m.
Tyrosine	30
Leucine	45
Glycine	85
Serine	85
Threonine	125

Five amino acids were definitely identified by both R_f values and comparison with known markers (Table 55, Plate 8). Of the amino acids identified, four, tyrosine, glycine, serine and threonine, had also been identified by earlier workers (Lang, 1948a, 1948b ; Alders, 1927). Leucine, which was consistently obtained in this work, had not been previously recorded.

f. Effect of constituent amino acids of extract.

Glycine, leucine, serine, threonine and tyrosine have been positively identified as constituents of aqueous extract of silk from Bombyx mori. The effect of these constituent amino acids, individually, on germination of I.farinosa conidia was then investigated.

Several chromatographs of the aqueous extract of the silk were run simultaneously on broad strips of chromatograph paper. A narrow strip bearing one spot and it's separated fractions was then cut off from each dried but unsprayed sheet. This narrow strip was sprayed with ninhydrin, and from the positions of the developed spots (amino acids), the positions of those on the other unsprayed sheets were inferred. These " inferred " spots were cut out. Excised pieces from identical spots were embedded in pyridine-washed plain agar in petri dishes, and the agar plates were inoculated with conidia of I.farinosa, and incubated at 20°C. The amino acid in the embedded chromatograph paper was expected to diffuse into the agar and influence the seeded conidia. Two controls were set up - one agar plate held pieces of an unspotted chromatograph paper which had previously been wetted with the solvent (butanol-acetic acid-water) and later dried ; and, a second control comprised agar without pieces of chromatograph paper. Percentage germination was determined and

germ tube lengths measured after 24 hours' incubation. The results are presented in Table 56.

TABLE 56

Effect of constituent amino acids of aqueous extract of silk from Bombyx mori on germination of conidia of I.farinosa at 20°C.

(Observations after 24 hours' incubation)

Amino acid	No. of conidia examined	% Germination	Mean Germ tube length in μ
Glycine	1920	100.00	67.4
Leucine	2355	98.09	51.2
Serine	2124	100.00	63.9
Threonine	2280	98.94	51.9
Tyrosine	2148	85.25	36.3
Control - Unamended Agar	2088	20.27	19.7
Control - Agar with Unspotted paper.	2135	34.44	23.8

The data in Table 56 show that within 24 hours, each constituent amino acid highly stimulated conidium germination. It would however be difficult to know the degree of importance to be attached to the differences in percentage germination among conidia under the influence of the different amino acids, for it was clear that, the variation in percentage germination (Table 56) bore an apparent relationship to the differences in quantity of amino acid in the extract (Table 55). That difference in quantity

of the fractions might most likely be responsible for the variation in the enhanced germination attained. The stimulatory effect appeared therefore to be caused by unspecific amino acids.

g. Effect of extract on germination of spores of non-entomogenous fungi.

Several workers (e.g. Brown, 1922) have shown that the source of the activator, in the question of biological stimulation, for any particular spore is not specific. Different tissues from a large number of species might provide the stimulation. Further, the extract of a particular tissue can stimulate spores of different fungal species.

It was considered necessary to ascertain whether the response to these insect extracts was restricted to conidia of entomogenous fungi. Spores of non-entomogenous fungi (see Table 57) were therefore germinated, by the slide method, at 23°C., in aqueous extract of silk. The isolates of the fungi were obtained from the stock culture collection at the Department of Mycology and Plant Pathology, Imperial College of Science and Technology, London, S. W. 7. Control experiments were run with distilled water.

Percentage germination was calculated and germ tube lengths measured after an incubation period of 24 hours. The results are presented in Table 57.

The results in Table 57 show that the extract of silk enhanced germination to varying degrees in all the spores tested. Germ tubes also showed stimulated growth in the silk extract. (Plates 9 and 10). The stimulant was clearly not specific to

entomogenous fungi alone.

TABLE 57

Germination of spores of non-entomogenous fungi in aqueous extract of silk from Bombyx mori at 23°C.

(Observations after 24 hours' incubation)

FUNGUS	TREATMENT	No. of conidia examined	% Germination	Mean Germ tube length in μ
Aspergillus niger	Silk-extract	2452	99.83	188.3
	Control-H_2O	2388	0.00	-
Botrytis allii	Silk-extract	2432	100.00	200.6
	Control-H_2O	2536	93.48	43.0
Cladosporium herbarum	Silk-extract	1764	91.67	246.9
	Control-H_2O	2224	43.88	24.0
Fusarium culmorum	Silk-extract	2540	36.53	212.9
	Control-H_2O	2684	22.35	103.2
Penicillium chrysogenum	Silk-extract	2244	100.00	157.0
	Control-H_2O	1959	0.26	15.3
Penicillium digitatum	Silk-extract	2572	21.22	166.1
	Control-H_2O	2850	0.00	-
Rhizopus stolonifera	Silk-extract	2336	63.86	416.3
	Control-H_2O	2620	10.38	66.5



9a x 160



9b x 160



10a x 160



10b x 160

SPORES OF BOTRYTIS ALLII GERMINATING AFTER 24 HOURS AT 23°C. IN WATER (PLATE 9a) AND IN WATER EXTRACT OF SILK FROM BOMBYX MORI (PLATE 9b).

SPORES OF CLADOSPORIUM HERBARUM GERMINATING AFTER 24 HOURS AT 23°C. IN WATER (PLATE 10a) AND IN WATER EXTRACT OF SILK FROM BOMBYX MORI. (PLATE 10b).

P. GERMINATION OF CONIDIA OF B.BASSIANA AND I.FARINOSA IN AQUEOUS EXTRACT OF NATIVE SOIL.

i. Germination in unsterile aqueous soil extract.

Entomogenous fungi in biological control trials, have commonly been introduced into the soil and expected there to attack stages of the insect life cycle, especially larval and pupal stages, spent in the soil. The fate of the spore introduced into the soil therefore is of extreme importance in relation to the establishment of the fungus on the host.

Inimical effects of soils on fungal spores and mycelium have been described by several investigators (eg., Chinn, 1953 ; Dobbs and Hinson, 1953 ; Jackson, 1958 ; Stover, 1958 etc., etc.), and the concept of a general widespread fungistasis in soils has been developed. Lingappa and Lockwood (1961) also reported the possibility of individual fungus spores serving as nutrient microsubstrates in the soil for soil microbes.

On the other hand, the presence of stimulatory substances in soil, which enhance fungus spore germination, has been reported by James (1958) and Lingappa and Lockwood (1961).

Few observations have been made on the ability of conidia of entomogenous fungi to germinate in soil. Huber (1958) concluded that B.bassiana conidia were unable to germinate in fresh garden soil and thought that this was presumably due to

the presence of antibiotics produced by actinomycetes.

This experiment was set up to examine germination of B.bassiana and I.farinosa conidia in aqueous extracts of soils from which the relevant diseased insects had originally been collected. M.anisopliae is a tropical fungus and it's native soil was not available ; it was excluded from this investigation.

Insects infected by B.bassiana were collected at Silwood Park, Sunninghill, Berks., and soil from that site was used in experiments with B.bassiana conidia. A fresh strain of I.farinosa was isolated from lepidopterous larvae collected from St. Anne's Hill, near Chertsey in Surrey, and soil from that site was accordingly used in experiments with conidia of I.farinosa. (It was only for these experiments involving soil that this new isolate of I.farinosa was employed.) .

The soil profile of the two sites were similar. There was an uppermost carpet of partially decomposed leaves, overlying a layer of humus and a third layer of sandy-loam. These three strata were referred to as Zones A, B and C, respectively.

The soil extract was prepared as follows : the soils were moistened for three days (Samples from the 3 zones of each soil were kept apart and used separately), and aliquots were packed into glass tubes, 145 mm. long and 15 mm. in diameter, with both ends open, though one end was plugged with glass wool, before introducing the soil. A tiny glass tube, 2 mm. in diameter, was

attached to the bottom of the soil tube (end plugged with glass wool) by a rubber bung, which led the draining water into a clean collecting flask. Water was gradually poured by a pipette into the soil column, until it started to drain from the exit tube, and then an equal volume of the water so far introduced was added to the soil column. The water collected at intervals was poured unto the soil column to drain through again. This process was continued for 4 hours.

The conidia were then germinated in the unsterilised soil extracts at their respective optimum temperatures using the slide method, for a period of 36 hours. Percentage germination was calculated and germ tube lengths measured at the end of that period. The results are presented in Table 58.

The data in Table 58 were representative of extensive experiments made. The results show that non-sterile extracts of all three zones of the soil significantly suppressed conidium germination in B.bassiana (Plates 11, 14 and 17). Percentage germination of the conidia in non-sterile extract of Zone C (loam) was exactly half of germination in the control (distilled water). Germination was strongly depressed in extracts of Zones A and B. The germ tubes similarly grew tardily in the extracts.

Non-sterile extracts of Zones A and B of native soil of I.farinosa were very inhibitory to the conidia, and germ tubes also grew poorly in these extracts. Conidia of I.farinosa, however,

germinated to the same degree as in the control in non-sterile extract of Zone C (loam). The germ tubes however, did not grow as extensively as those in the controls.

TABLE 58

Germination of conidia of B.bassiana and I.farinosa in non-sterile aqueous extract of soil.

(Observations after 36 hours' incubation)

FUNGUS	SOURCE OF EXTRACT	No. of conidia examined	% Germination	Mean Germ tube length in μ
B.bassiana Incubated at 18°C.	Zone A	2340	4.27	12.2
	Zone B	3748	9.18	12.5
	Zone C	3776	20.72	39.5
	Control - Water	2900	55.86	84.3
I.farinosa Incubated at 20°C.	Zone A	3002	0.68	16.2
	Zone B	2777	0.51	14.1
	Zone C	4262	10.57	41.0
	Control - Water	2687	9.20	116.9

ii. Germination in Filtered, Heat sterilised and Amended Extracts.

Inhibition of germination of conidia of B.bassiana and I.farinosa in unsterilised soil extracts in the above experiment might be attributed to different reasons. This inhibition might be caused by high concentration of mineral salts, for, the release of minerals from the decomposition of leaf litter had been known to reach high proportions (Burges, 1956). It seemed more likely, however, that decreased germination was caused by a general fungistasis in the soil extracts ; the abundance of bacteria to be seen in the extracts suggested such a fungitoxin, if present, might well be of bacterial origin.

Fungistasis in the soil has been demonstrated to be nullified by various treatments, for example, air-drying of the soil, addition of nutrients, treatment of soil extract with activated charcoal, filtration of the extract, and sterilisation of the soil or the extract. (Dobb and Hinson, 1953 ; Jackson, 1958 ; Stover, 1958).

This investigation was therefore designed to examine germination of conidia of B.bassiana and I.farinosa in extracts which have received some of these treatments, to test the hypothesis that the conidia failed to germinate because of the presence of fungistasis in the extracts.

Aqueous extracts of the three zones of the soils were :

(a) filtered (using millipore filter) (b) heat-sterilised (autoclaved for 20 minutes at 15lbs. / in^2.) (c) amended with 1 % dextrose, and, (d) amended with "N" concentration extract of silk from Bombyx mori.

Soil extracts which had received the different treatments were used in germination tests, employing the 'slide' method. The conidia in the germination test drops were incubated at their respective optimum temperatures for 36 hours, after which, percentage germination was evaluated and germ tube lengths measured. The results are presented in Tables 59 - 62.

TABLE 59

Germination of conidia of B.bassiana and I.farinosa in FILTERED aqueous soil extract.

(Observations after 36 hours' incubation)

FUNGUS	EXTRACT.		No. of conidia examined	% Germination	Mean Germ tube length in μ
	TREATMENT	ORIGIN			
B.bassiana Incubated at 18°C.	FILTERED	Zone A	2364	82.57	76.1
		Zone B	2672	88.17	76.8
		Zone C	2133	92.50	99.0
	UNFILTERED	Zone A	2965	3.72	11.4
		Zone B	2280	8.44	12.5
		Zone C	2455	20.00	35.6
	Control - Water		2106	61.22	74.5
I.farinosa Incubated at 20°C.	FILTERED	Zone A	4168	17.87	20.5
		Zone B	3570	7.59	28.9
		Zone C	5577	40.02	42.7
	UNFILTERED	Zone A	3521	0.72	17.3
		Zone B	3228	0.50	14.0
		Zone C	3411	14.42	40.3
	Control - Water		3000	8.01	95.3

The results in Table 59 show that filtration eliminates the inhibitory effect of soil extracts on germination. The inhibitory effect must therefore have been associated with particulate matter in the extract. It seems most probable that the active particles were the bacteria which were largely, though not entirely, eliminated by filtration.

A second feature shown by the results was the way germination in filtered extracts far exceeded germination in water. Removal of the inhibitory agents evidently unmasked the presence of stimulatory substances in the extracts, which under normal conditions were eclipsed by that of inhibitory substances.

Filtered extracts of all zones highly stimulated conidium germination and germ tube growth in B.bassiana. Germination was stimulated to the same degree in all extracts.

There persisted however a difference in percentage germination in conidia of I.farinosa in filtered extracts of the different zones. Germination was considerably stimulated in filtered extract of the loamy soil (Zone C), whilst germination in the filtered extract of humus (Zone B) approximated to germination in water. This difference in germination might be a response to either different levels of residual fungistasis or different levels of stimulatory substances in the extracts.

TABLE 60

Germination of conidia of B.bassiana and I.farinosa in HEAT-STERILISED aqueous soil extract.

(Observations after 36 hours' incubation)

FUNGUS	EXTRACT		No. of conidia examined	% Germination	Mean Germ tube length in μ
	TREATMENT	ORIGIN			
B.bassiana Incubated at 18°C.	STERILISED	Zone A	2028	98.83	143.6
		Zone B	3104	89.40	76.6
		Zone C	2204	98.72	161.6
	NON-STERILISED	Zone A	2640	5.00	12.6
		Zone B	2377	11.27	13.1
		Zone C	2189	25.26	38.7
	Control - Water		2270	61.00	80.5
I.farinosa Incubated at 20°C.	STERILISED	Zone A	2314	32.41	108.0
		Zone B	2592	9.33	148.1
		Zone C	2135	64.96	130.2
	NON-STERILISED	Zone A	2255	0.54	17.3
		Zone B	2270	0.50	12.9
		Zone C	2438	10.62	45.0
	Control - Water		2320	9.27	96.0

The results (Table 60) on the effect of heat sterilisation of the soil extracts were essentially the same as in the previous experiment. Heat sterilisation did not only nullify the fungistatic

effect of the extracts but also unmasked the effects of the constituent stimulatory substances.

TABLE 61

Germination of conidia of B.bassiana in unsterilised aqueous extract of soil, amended with Dextrose and Extract of silk from Bombyx mori at 18°C.

(Observations after 36 hours' incubation)

AMENDMENT	SOURCE OF EXTRACT	No. of conidia examined	% Germination	Mean Germ tube length in μ
1 % DEXTROSE	Zone A	2428	97.85	176.7
	Zone B	2188	97.07	206.1
	Zone C	2136	98.48	147.4
"N"Concentration Extract of silk from Bombyx mori	Zone A	2372	96.62	181.1
	Zone B	2080	97.88	246.9
	Zone C	1788	99.32	278.6
UNAMENDED.	Zone A	2576	2.01	11.2
	Zone B	2424	10.22	10.6
	Zone C	2800	26.53	30.6
DISTILLED WATER	-	2422	66.57	83.6

TABLE 62

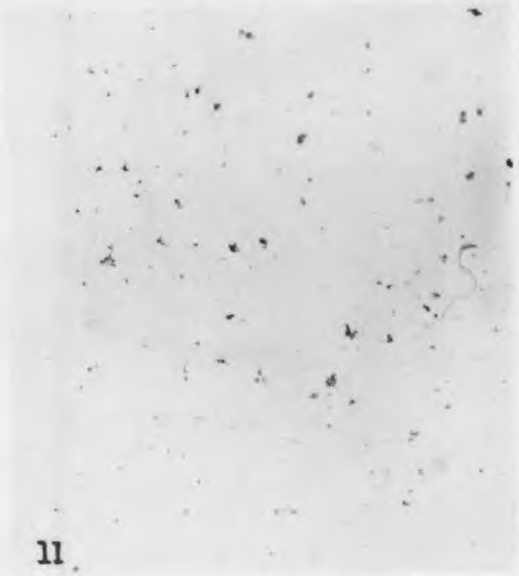
Germination of conidia of I.farinosa in unsterilised aqueous extract of soil, amended with Dextrose and Extract of silk from Bombyx mori at 20°C.

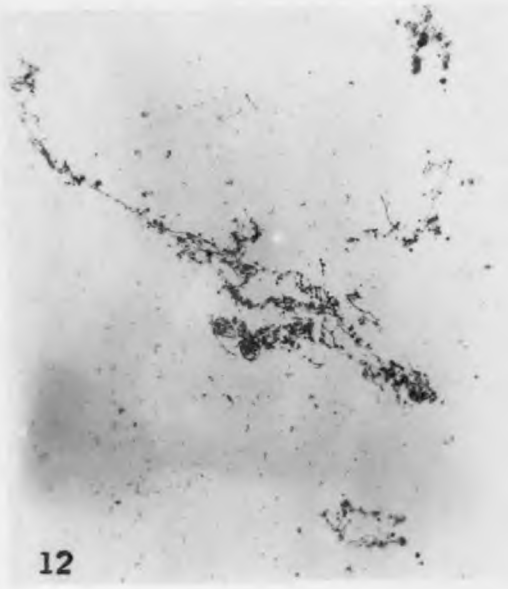
(Observations after 36 hours' incubation)

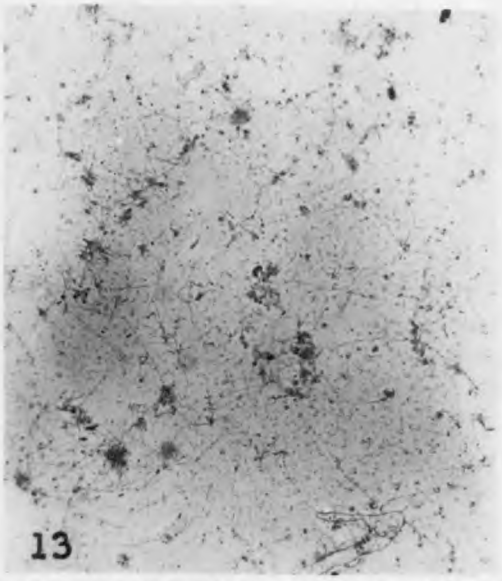
AMENDMENT	SOURCE OF EXTRACT	No. of conidia examined	% Germination	Mean Germ tube length in μ
1 % DEXTROSE	Zone A	2077	0.67	11.9
	Zone B	2689	0.70	14.7
	Zone C	4275	43.02	40.4
"N" Concentration Extract of silk from Bombyx mori	Zone A	2945	0.75	13.1
	Zone B	2250	0.89	13.7
	Zone C	4132	99.92	162.1
UNAMENDED	Zone A	2255	0.21	11.2
	Zone B	3310	0.52	10.3
	Zone C	4459	5.72	40.2
DISTILLED WATER	-	2169	9.28	101.5

Results of experiments with amended soil extracts and conidia of B.bassiana are shown in Table 61. Earlier experiments had indicated the presence of both stimulatory and inhibitory substances in the extracts. Filtration and heat-sterilisation eliminated the source of the inhibitory substances and exposed the effects of the constituent stimulatory substances. Addition of 1 % Dextrose and " N " extract of silk from Bombyx mori greatly enhanced germination in the non-sterile extracts (Plates 12, 13, 15, 16, 18 and 19), completely swamping the native inhibitory effect.

Though these amendments also increased germination of conidia of I.farinosa (Table 62) in the non-sterile extract of the loamy soil (Zone C), they failed to encourage higher germination in the non-sterile extracts of Zones A and B, where even in the presence of the amendments, fewer than 1 in a hundred spores germinated.

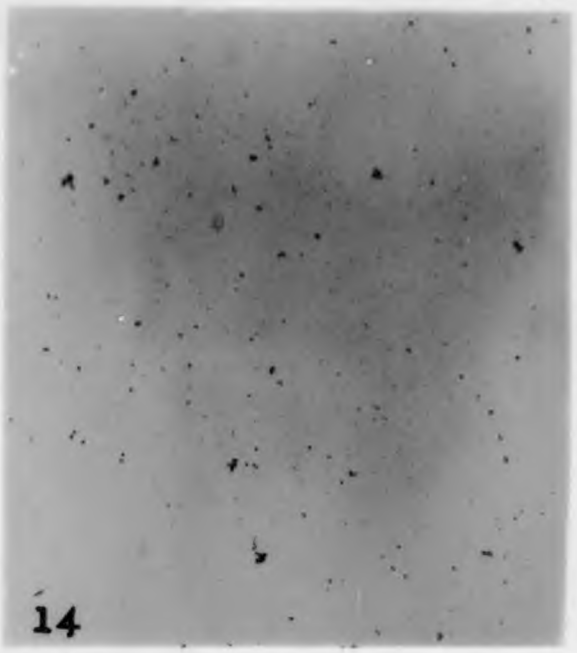


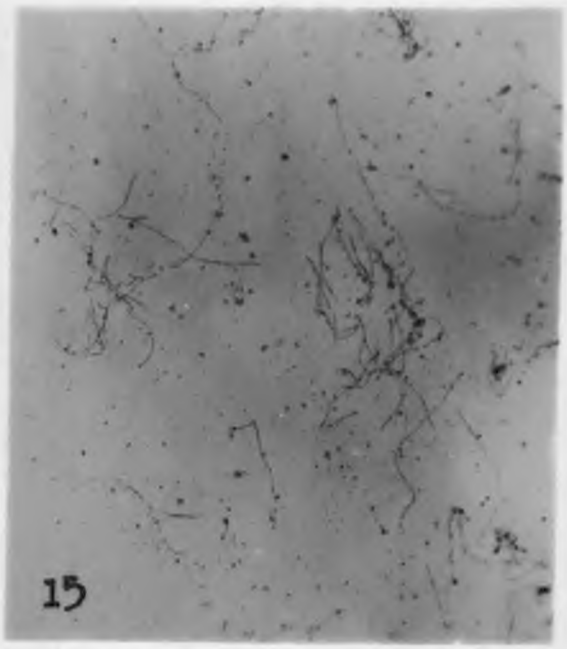


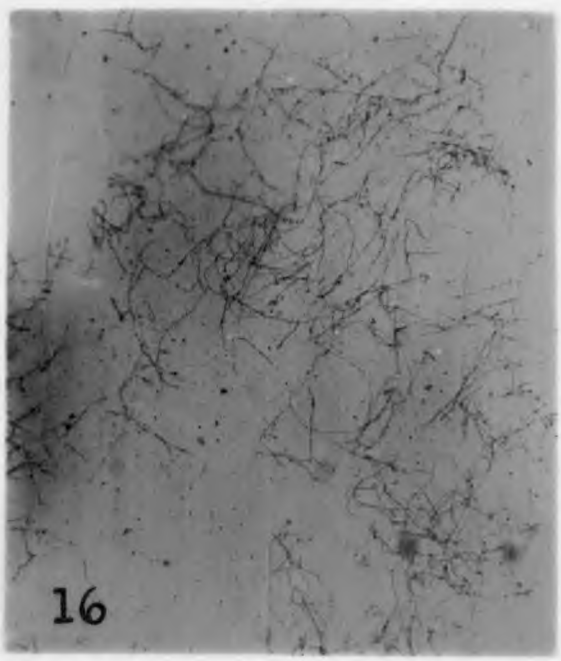


CONIDIA OF B.BASSIANA GERMINATING IN WATER EXTRACT OF PARTIALLY DECOMPOSED LEAVES AFTER 36 HOURS AT 18°C.

PLATE 11.- CONIDIA IN UNSTERILISED EXTRACT. (x 160).
PLATE 12.- CONIDIA IN UNSTERILISED EXTRACT AMENDED WITH 1% DEXTROSE (x160).
PLATE 13.- CONIDIA IN UNSTERILISED EXTRACT AMENDED WITH "N" CONCENTRATION OF EXTRACT OF SILK FROM BOMBYX MORI. (x 160).







CONIDIA OF B.BASSIANA GERMINATING IN WATER EXTRACT OF HUMUS AT 18°C.
PLATE 14.- CONIDIA IN UNSTERILISED EXTRACT. (x 160).
PLATE 15.- CONIDIA IN UNSTERILISED EXTRACT AMENDED WITH 1% DEXTROSE (x160).
PLATE 16.- CONIDIA IN UNSTERILISED EXTRACT AMENDED WITH "N" CONCENTRATION OF EXTRACT OF SILK FROM BOMBYX MORI. (x 160).

(GERMINATION AFTER 36 HOURS)



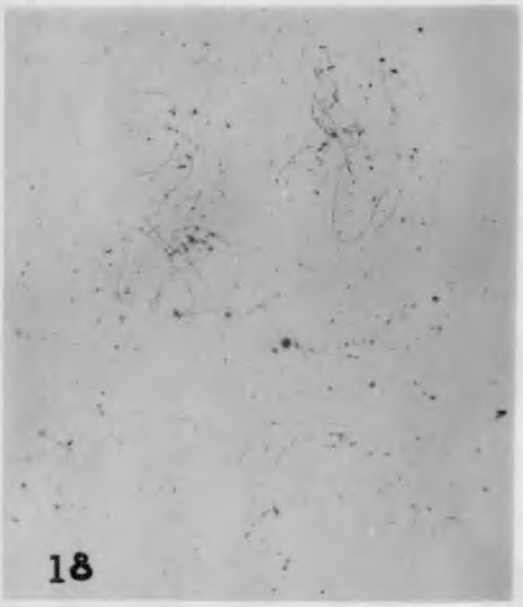




PLATE 17.- CONIDIA OF B.BASSIANA GERMINATING IN UNSTERILISED WATER EXTRACT OF LOAMY SOIL. (x 160).
PLATE 18.- CONIDIA OF B.BASSIANA GERMINATING IN UNSTERILISED WATER EXTRACT OF LOAMY SOIL AMENDED WITH 1 % DEXTROSE. (x 160).
PLATE 19.- CONIDIA OF B.BASSIANA GERMINATING IN UNSTERILISED WATER EXTRACT OF LOAMY SOIL AMENDED WITH "N" CONCENTRATION OF EXTRACT OF SILK FROM BOMBYX MORI. (x 160).
(GERMINATION AFTER 36 HOURS AT 18°C.)

iii. Test of Fungicidal activity of Inhibitory substances in soil extract.

Results of experiments on pages 181 to 183 have shown that the expression of inhibitory substances might be masked by the presence of stimulatory ones. Such results suggest that conidia are not killed by the inhibitory substances. An investigation was made to determine whether " inhibitors " in the soil extract were fungistatic or fungicidal.

Conidia of B.bassiana and I.farinosa which had lain dormant up to 48 hours under the influence of inhibitory substances of the soil were tested for viability. The humus layers (Zone B) alone were used in this experiment. The experimental procedure was as follows.

Pyridine-washed plain agar was melted, cooled to 40°C., and then used to prepare conidial suspensions. Aliquots of 10 ml. of this conidial suspension were poured into sterile petri dishes and allowed to set. The agar plates were then covered with humus to a depth of 1/4 of an inch. The soils were maintained at 90 % Water Holding Capacity. Controls of seeded agar without humus cover were run. The plates were incubated at room temperature, 21 ± 2°C., for 48 hours. After that period, the soil was carefully removed and the surface of the agar gently washed with distilled water. Disks of agar of uniform sizes (8 mm. in diameter) were

then excised and transferred to petri dishes lined with moist blotting paper. Sample disks were stained with cotton blue and examined to determine percentage germination of the conidia during the period of exposure to the soil (Tables 63 and 65). Germination of the conidia in the presence of the inhibitory substances was very poor, extensive areas in the agar plates being devoid of germinating conidia. Disks of agar, for the subsequent test, were removed from such areas.

0.2 ml. of peptone-dextrose liquid medium (KH_2PO_4, 1 g. ; $MgSO_4 \cdot 7H_2O$, 0.5 g. ; Peptone, 5 g. ; Dextrose, 10 g. ; Distilled water, 1000 ml. ; Rose bengal, 1 : 30,000 ; Aureomycin, 2 µg. / ml.) (Johnson, et. al., 1959) were placed on the agar disks to serve as the nutrient intended to overcome the inhibition of the spores, and incubated at the respective optimum temperatures of the conidia, for 24 hours. After that period, the agar disks were removed and viability of the conidia estimated on basis of percentage germination. Germ tube lengths were also measured. These results are presented in Tables 64 and 66.

Tables 64 and 66 show the great effectiveness of peptone-dextrose medium in annulling fungistasis of conidia of B.bassiana and I.farinosa. The results revealed that inhibited conidia which had lain dormant up to 48 hours under the influence of fungistatic substances of the soil were viable, for while there was germination of only 7.74 and 0.18 per cent in conidia of B.bassiana (Table 63) and I.farinosa (Table 65) respectively, in the agar plates

TABLE 63

Germination of conidia of B.bassiana under the influence of inhibitory substances of soil for a period of 48 hours at 21 ± 2°C.

TREATMENT	No. of conidia examined	% Germination	Mean Germ tube length in μ
Agar covered with humus	2888	7.74	10.7
Control-Agar without humus	2195	68.21	252.8

TABLE 64

Annulment of fungistasis of B.bassiana conidia by added nutrient (Peptone-Dextrose). Percentage germination after 24 hours' incubation at 18°C.

Previous period of contact with soil fungistasis in Hours.	No. of conidia examined	% Germination	Mean Germ tube length in μ
48	2192	100.00	60.3

TABLE 65

Germination of conidia of I.farinosa under the influence of inhibitory substances of soil for a period of 48 hours at 21 ± 2°C.

TREATMENT	No. of conidia examined	% Germination	Mean Germ tube length in μ
Agar covered with humus	2511	0.18	7.3
Control-Agar without humus	3045	14.95	136.8

TABLE 66

Annulment of fungistasis of I.farinosa conidia by added nutrient (Peptone-Dextrose). Percentage germination after 24 hours' incubation at 20°C.

Previous period of contact with soil fungistasis in Hours.	No. of conidia examined	% Germination	Mean Germ tube length in μ
48	2126	97.64	38.7

covered with humus, and the controls with plain agar gave the respective percentage germination of 68.21 and 14.95 per cent, subsequent application of peptone-dextrose liquid medium stimulated 100 and 97.64 per cent germination respectively.

The non-fungicidal nature of inhibitory substances in the soil had also recently been observed by Lingappa and Lockwood (1961). They reported that inhibited conidia of Glomerella cingulata, Penicillium frequentas and Fusarium oxysporum f. lycopersici kept in contact with soil for 5 days were stimulated to germinate with nutrients.

From results obtained, it was evident that the inhibitory substances in humus, were not fungicidal within the period of 48 hours, and that inhibited conidia of B.bassiana and I.farinosa could be stimulated into germination.

It is noteworthy that while the usually poor germination of conidia of I.farinosa in water (10 - 12 %) was previously found to be stimulated to 100 % by yeast-extract and casein hydrolysate, but only to 47 % by dextrose (Tables 39 and 41), the inhibitory effect of soil extract on the fungus's spores could be deviated by a peptone-dextrose medium (Table 66) but not by dextrose (Table 62). It therefore appears that (in both these aspects of spore behaviour) nitrogenous amendments are effective while dextrose is not, or only less so.

Q. CHARACTERISTICS OF THOSE CONIDIA ABLE TO GERMINATE IN WATER.

Although M.anisopliae conidia were capable of attaining a maximum germination of 100 % in water, there was a limit to the proportion of conidia of B.bassiana and I.farinosa which did so. Maximum germination in these species could only be excited with stimulants, such as, yeast-extract and aqueous extract of exoskeleton of Bombyx mori. There must therefore, in a population of conidia, be some physiological differences in certain of them which make germination in water difficult or even impossible, although the spores were still viable and able to germinate under appropriate special conditions.

This partial germination of conidia of B.bassiana and I.farinosa in water indicate a physiologically heterogeneous population. Although conidia of M.anisopliae attain 100 % germination, and apparently suggest a homogeneous population, the usual interval of 24 hours for germination tests, before observations are made, is too long to provide a knowledge of the degree of variation in the latent period of germination of the individual conidia. Generally, two patterns of conidium germination can be hypothesised as criteria for homogeneity or heterogeneity of a conidial population. A large proportion of the conidia in a homogeneous population will germinate within a relatively short time, while those of a heterogeneous population will show a great variation in time for germination. An experiment was therefore

carried out to examine the time needed by individual conidia of B.bassiana, I.farinosa and M.anisopliae to produce germ tubes when incubated in water, over a total period of 32 hours.

The conidia were germinated by the slide method in distilled water at their respective optimum temperatures. The slides were withdrawn at regular hourly intervals and percentage germination of 2,000 to 2,400 observed conidia estimated and the slides returned to the petri dishes. This method provided a way of observing the increase in percentage germination at hourly intervals and the range of latent period of germination of the conidia in the population. The results are presented in Tables 67 to 69 and the accompanying diagrams.

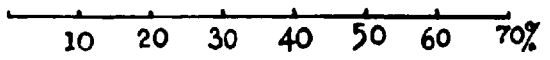
That conidial populations of B.bassiana and I.farinosa are heterogeneous are shown by the data in Tables 67 and 68. The conidia showed great variation in time for germination. Though the earliest germinating conidia of B.bassiana produced germ tubes within 5 hours, some needed as long as 23 hours before germinating, and at no hour between these two extremes did the increase in germination rise substantially. With I.farinosa, the pattern of conidium germination was the same, and there was a gradual increase in germination from the 12th hour, when germination started to the end of the period of experimentation.

Conidia of M.anisopliae, on the other hand, showed a high degree of homogeneity. Within the hours of 8 to 11 of incubation, 82 % of the conidia germinated. (Table 69).

TABLE 67

LATENT PERIOD OF GERMINATION OF INDIVIDUAL CONIDIA IN A POPULATION SAMPLE OF CONIDIA OF BEAUVERIA BASSIANA.

HOURS OF INCUBATION.	PERCENTAGE GERMINATION.	INCREASE % GERMINATION.
1	0.00	0.00
2	0.00	0.00
3	0.00	0.00
4	0.00	0.00
5	1.00	1.00
6	3.26	2.26
7	3.63	1.37
8	4.28	0.65
9	5.30	1.02
10	8.35	3.05
11	10.56	2.21
12	14.89	4.33
13	15.05	0.16
14	17.39	2.34
15	17.75	0.36
16	17.80	0.05
17	23.09	5.29
18	27.18	4.09
19	37.13	9.95
20	41.61	3.48
21	52.96	11.35
22	63.25	10.29
23	68.67	5.42
24	73.17	5.50



PERCENTAGE GERMINATION.

INCREASE PERCENTAGE GERMINATION.

TABLE 68

LATENT PERIOD OF GERMINATION OF INDIVIDUAL CONIDIA IN A POPULATION SAMPLE OF CONIDIA OF ISARIA FARINOSA

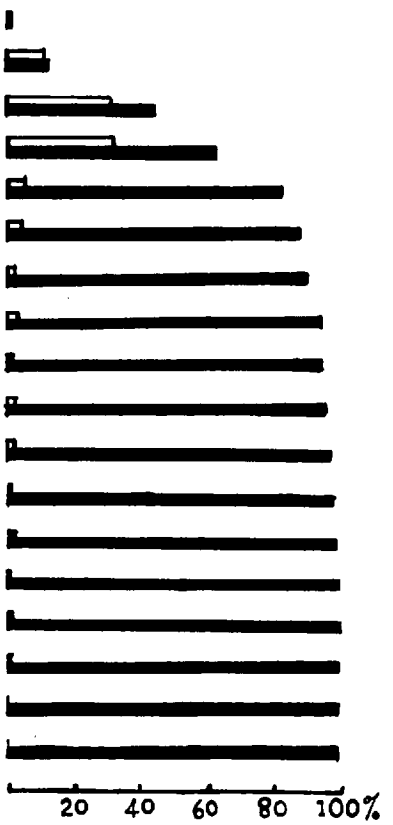
HOURS OF INCUBATION.	PERCENTAGE GERMINATION	INCREASE % GERMINATION
8	0.00	0.00
9	0.00	0.00
10	0.00	0.00
11	0.00	0.00
12	0.00	0.00
13	0.12	0.12
14	0.18	0.06
15	0.34	0.16
16	0.49	0.15
17	1.55	1.06
18	1.86	0.31
19	2.17	0.31
20	2.43	0.26
21	2.81	0.38
22	3.60	0.79
23	6.12	2.52
24	8.49	2.37
25	9.75	1.26
26	10.66	0.91
27	11.15	0.49
28	16.48	5.33
29	16.90	0.42
30	17.17	0.27
31	19.87	2.70
32	20.95	1.08



TABLE 69

LATENT PERIOD OF GERMINATION OF INDIVIDUAL CONIDIA IN A POPULATION SAMPLE OF CONIDIA OF METARRHIZIUM ANISOPLIAE.

HOURS OF INCUBATION.	PERCENTAGE GERMINATION.	INCREASE % GERMINATION.
1	0.00	0.00
2	0.00	0.00
3	0.00	0.00
4	0.00	0.00
5	0.00	0.00
6	0.00	0.00
7	0.67	0.67
8	11.73	11.06
9	44.15	32.77
10	77.11	32.96
11	82.60	5.49
12	87.82	5.22
13	90.50	2.68
14	94.16	3.66
15	94.72	0.56
16	95.89	1.17
17	97.92	2.03
18	98.00	0.08
19	98.38	0.38
20	98.40	0.02
21	98.64	0.24
22	98.65	0.01
23	98.65	0.00
24	98.65	0.00



PERCENTAGE GERMINATION.

INCREASE PERCENTAGE GERMINATION.

These experiments thus show that while conidia of M.anisopliae harvested from 5 days' old cultures are homogeneous, conidia of B.bassiana and I.farinosa from 8 and 21 days' old cultures respectively, are markedly heterogeneous. The experiment described below, was designed to establish which conidia of B.bassiana and I.farinosa were those which, not only germinated in water, but those which germinated first.

After numerous trials, the following method was perfected by which germination capacity of individual conidia of known ages could be studied. Sterile glass cylinders 1 cm. long and 1.5 cm. in diameter were each stood in a separate sterile petri dish and 20 ml. of melted potato dextrose agar poured into the petri dishes, and the glass cylinders adjusted to a central position before the agar solidified. Meanwhile, tiny solidified potato dextrose agar cubes (1 mm. x 1 mm. x 1 mm.) were placed in the centre of sterile glass slides and inoculated with minute fragments of hyphae of the fungus under study. The slides with their loads of inoculated micro-agar cubes were next placed, facing upwards, on the glass cylinders standing in the petri dishes and the dishes closed. The whole set was incubated at 23°C. for 72 hours. The potato dextrose agar in the petri dish maintained a congenial atmospheric humidity for growth of the micro-culture on the slide.

The hyphae of the micro-cultures grew out from the agar and ran in radiating fashion along the surface of the glass slide, sporulating along their lengths. This provided an opportunity of observing the behaviour of individual conidia of known ages.

To study germination of the conidia, the slides were first exposed to air at 23°C. for 2 hours to dry up the mycelium to arrest further growth, and then transferred into humid chambers (plastic boxes, 17.5 x 11.5 x 6.0 cm.). The boxes were incubated at the optimum temperature of the fungus for 18 hours, at the end of which the percentage germination of conidia of identical ages was determined. The results are presented in Tables 70 and 71.

To estimate the ages of the conidia, series of camera lucida drawings were made at regular hourly intervals of hyphae growing under similar experimental conditions as the above, to depict the manner and time of conidial formation (see Figs. 40 - 42).

Phialides of I.farinosa and M.anisopliae produced conidia in chains (Figs. 41 and 42) at the rate of one conidium in approximately 2 and 3 hours respectively. In B.bassiana (Fig. 40) several primordia of phialides appeared along a length of hypha about the same time, and developed at almost the same rate and produced conidia within the same time interval. The age of the group of phialides with their conidia was therefore assessed, instead of the ages of individual conidia. On the average a group of 5 - 6 successive phialides developed and sporulated in 18 hours.

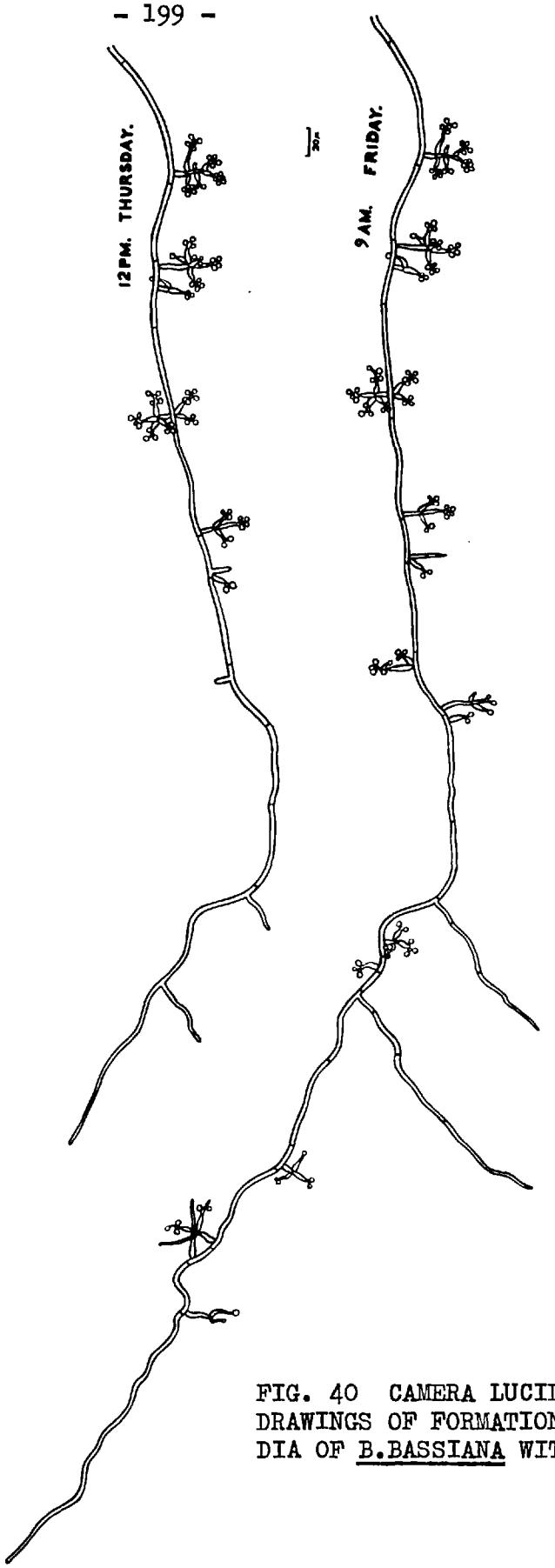


FIG. 40 CAMERA LUCIDA SERIAL DRAWINGS OF FORMATION OF CONIDIA OF B.BASSIANA WITH TIME.

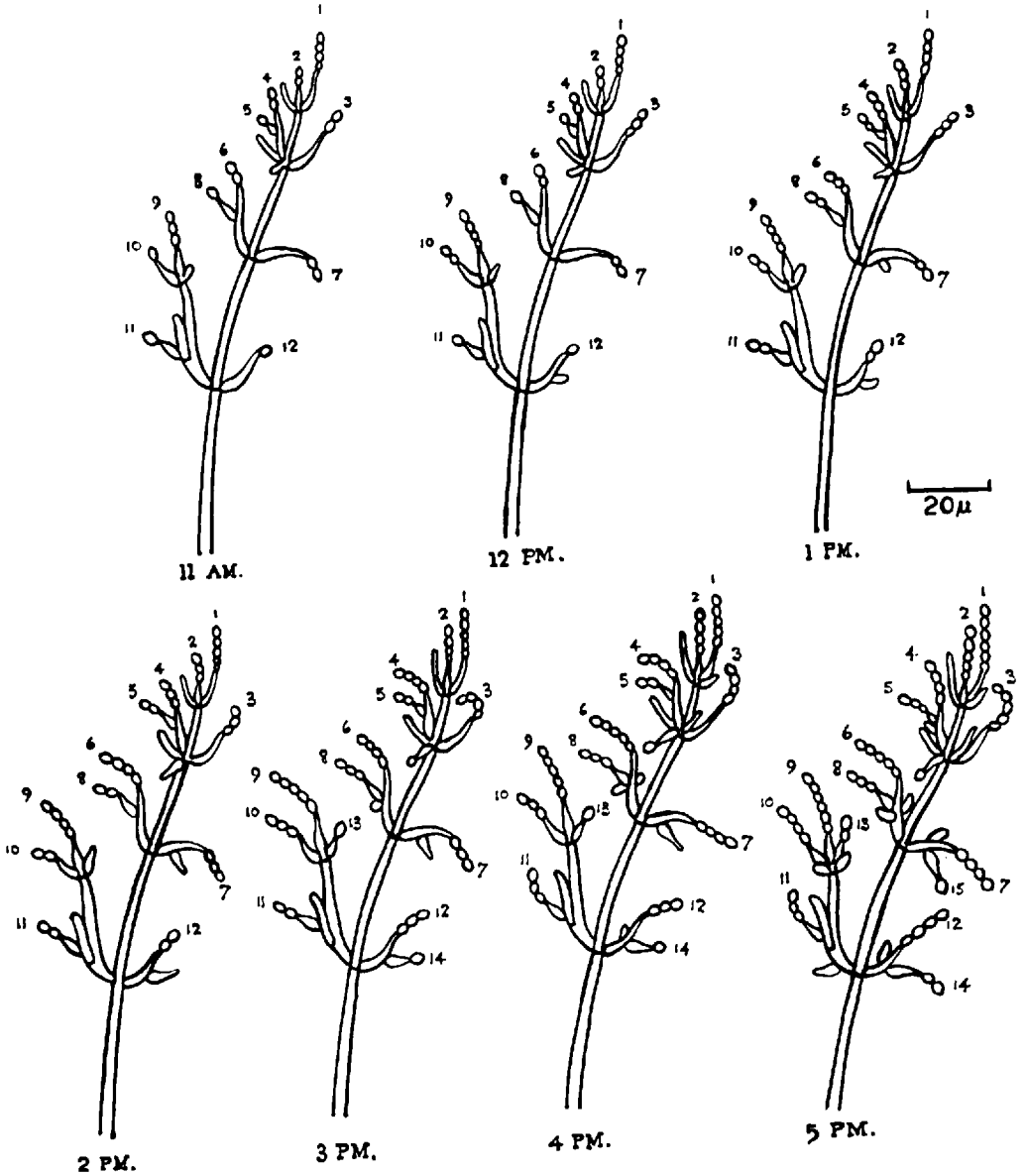


FIG. 41. CAMERA LUCIDA SERIAL DRAWINGS OF FORMATION OF CONIDIA OF I.FARINOSA WITH TIME.

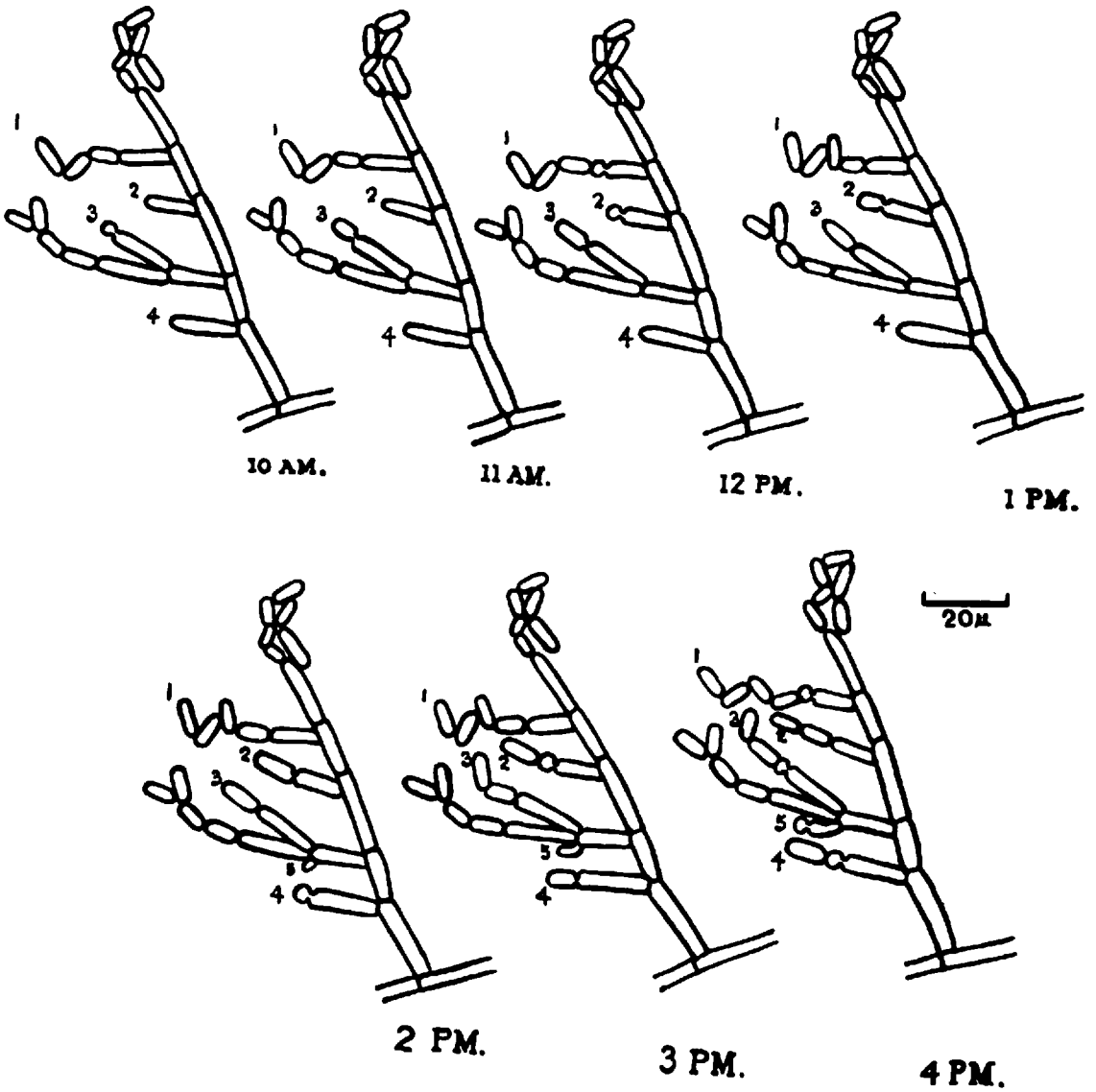


FIG. 42. CAMERA LUCIDA SERIAL DRAWINGS OF FORMATION OF CONIDIA OF M. ANISOPLIAE WITH TIME.

TABLE 70

Germination capacity of conidia of B.bassiana in 18 hour period in relation to age (in hours).

Assessed on observation of 40 identical hyphae

[1]Position of phialide from hyphal apex	[2]Assumed Age of phialides	No. of conidia	% Germination after 18 hours	Mean Germ tube length in μ
1	18 HOURS	611	74.85	49.8
2		706	40.60	28.7
3		692	29.17	22.0
4		668	22.10	21.8
5		601	18.36	19.6
6	36 HOURS	615	10.03	16.8
7		538	10.68	13.7
8		519	7.51	12.4
9		714	4.25	12.3
10		689	8.36	12.5
11	54 HOURS	921	10.43	11.6
12		817	2.60	10.6
13		602	1.54	10.8
14		733	0.64	12.1
15		587	1.76	7.3
16		665	0.00	-

[1] 40 hyphae examined for germination of the spores from the distal 16 phialides of each. Hence data for each phialide position is based on 40 phialides.

[2] "Assumed age" derived from observations on rate of development of representative hyphae (see text).

TABLE 71

Germination capacity of conidia of I.farinosa in 18 hour period in relation to age (in hours).

Results assessed on observation of 50 apparently identical phialides.

Position of conidia from apex of phialide.	Assumed Age of conidia in Hours	% Germination after 18 hours.	Mean Germ tube length in μ
1	2	0.00	-
2	4	0.00	-
3	6	0.00	-
4	8	0.00	-
5	10	3.12	2.4
6	12	5.36	6.7
7	14	13.72	9.5
8	16	18.70	13.0
9	18	31.25	13.9
10	20	37.93	10.2
11	22	55.55	25.8
12	24	70.28	25.0
13	26	36.44	20.6
14	28	17.21	11.8
15	30	10.26	13.7

Results with P.bassiana in Table 70 present observations made on 40 identical hyphae, each bearing a series of 16 phialides, which had developed in about 54 hours. This period was clearly sufficient to lead to the appearance of physiological differences

between the conidia. (Plate 20, Fig. 43). Spores from the youngest group of five phialides possessed the highest ability to germinate in humid air. The youngest cluster of conidia germinated best in the period of test (18 hours), the capacity for germination, in water, waning with "age" (estimated in hours). Very few conidia 36 - 54 hours old germinated. Conidia of B.bassiana were therefore at the best physiological state for rapid germination as soon as actual development of the conidium was completed.

Observations with I.farinosa (Plate 21, Fig. 44) were made on individual conidia. Germination here failed to occur, in the test period (18 hours), during the time just following the attainment of morphological maturity, as assessed by conidial size. There was a period of 8 - 12 hours when hardly any conidia would germinate in water. They germinated best at the age of 18 to 26 hours, after which they very rapidly lost the ability. (Table 71).

Two entirely different physiological states evidently existed in conidia of B.bassiana and I.farinosa. Though the actual age of the individual conidium was not estimated in B.bassiana, it was apparent that they did germinate immediately on reaching morphological maturity, whilst conidia of I.farinosa would not do so, but germinated best at the age of 18 to 26 hours.

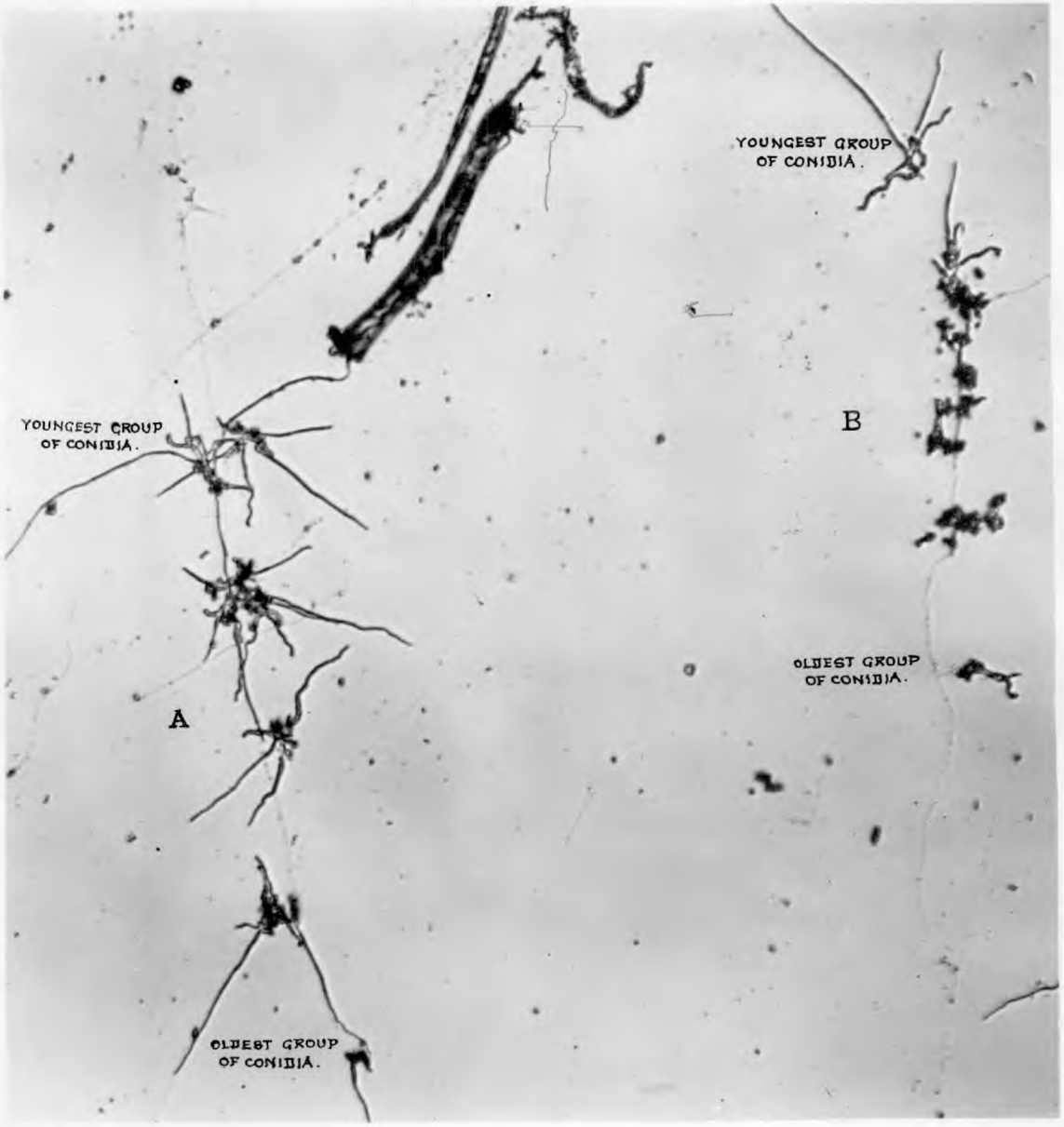


PLATE 20 PHOTOGRAPH SHOWING RADIATING HYPHAE OF MICRO-CULTURE OF B.BASSIANA BEARING GERMINATING CONIDIA. CONVERGING AND DIVERGING ENDS OF RADIATING HYPHAE INDICATE CENTRE AND EDGE OF CULTURE RESPECTIVELY. NOTE GREATER GERMINATION IN YOUNGER GROUP OF SPORES. GERMINATION AFTER 18 HOURS' INCUBATION AT 18°C. AT 100 % RELATIVE HUMIDITY. (x 240).

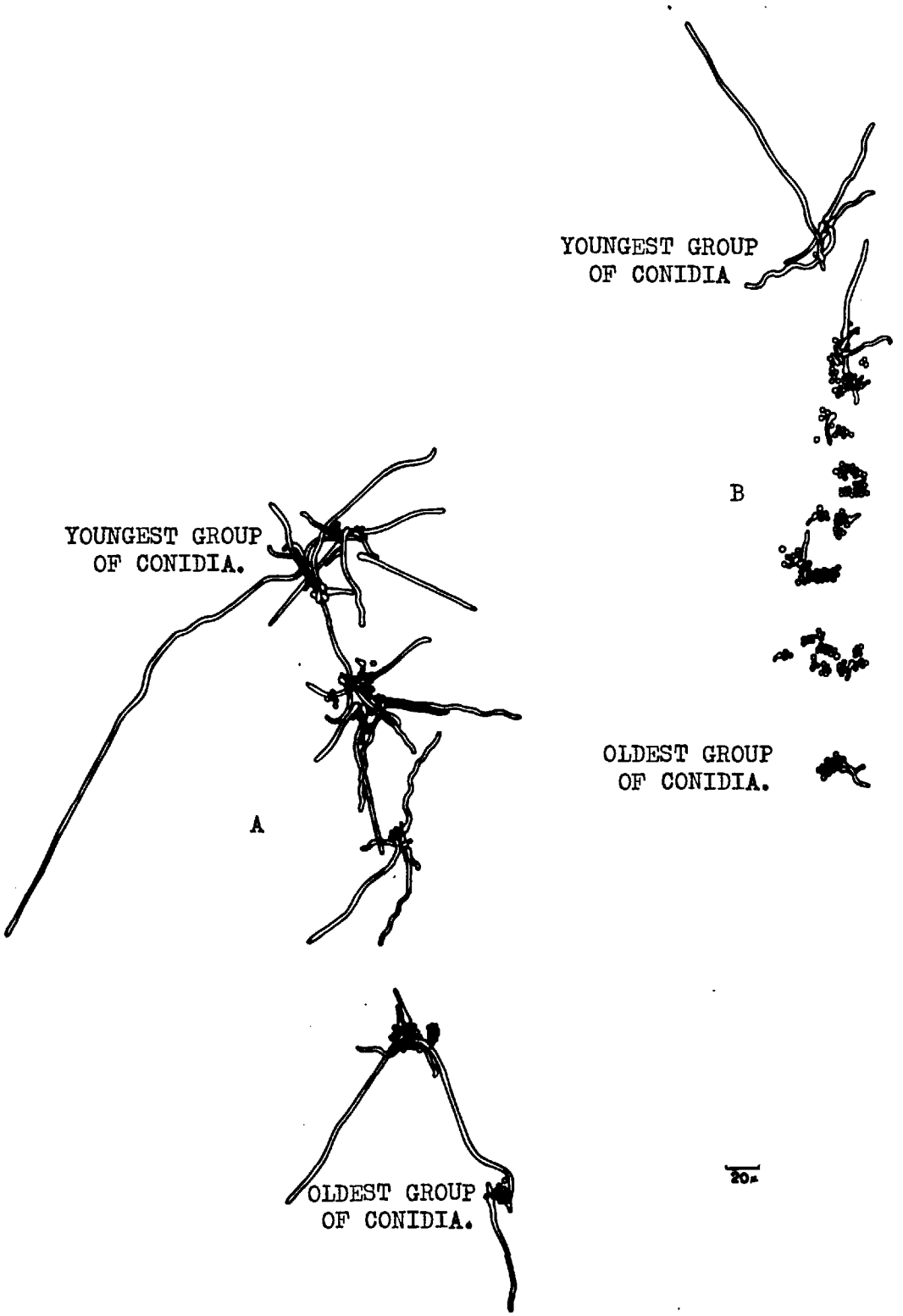


FIG. 43 CAMERA LUCIDA DRAWING OF PLATE 20. (PAGE 205).
CONIDIA OF B.BASSIANA.

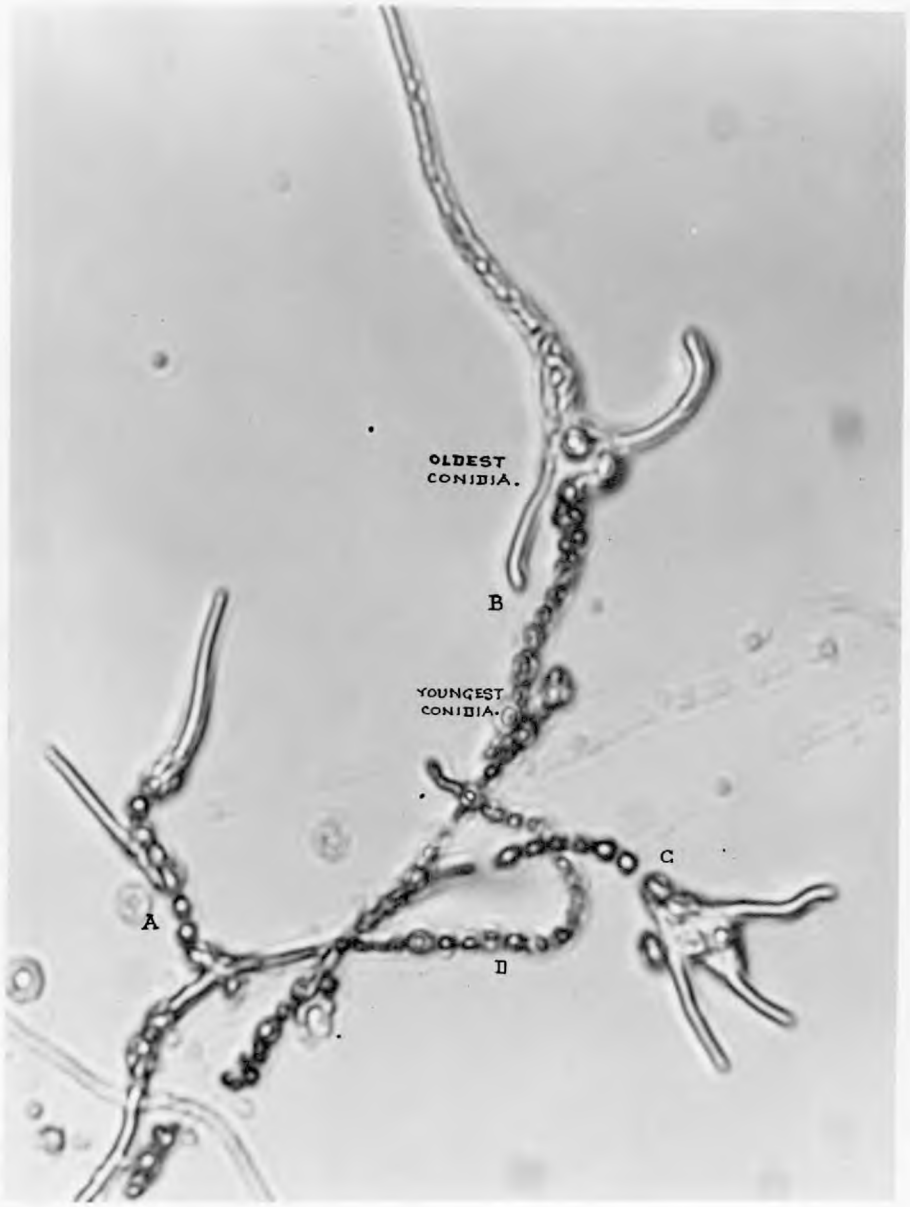


PLATE 21 PHOTOGRAPH OF CHAINS OF CONIDIA OF I.FARINOSA DEPICTING RELATION OF AGE OF SPORE TO CAPACITY TO GERMINATE AT 100 % RELATIVE HUMIDITY AT 20°C. GERMINATION AFTER 18 HOURS' INCUBATION. NOTE GREATER GERMINATION IN OLDER CONIDIA. (x 1000).

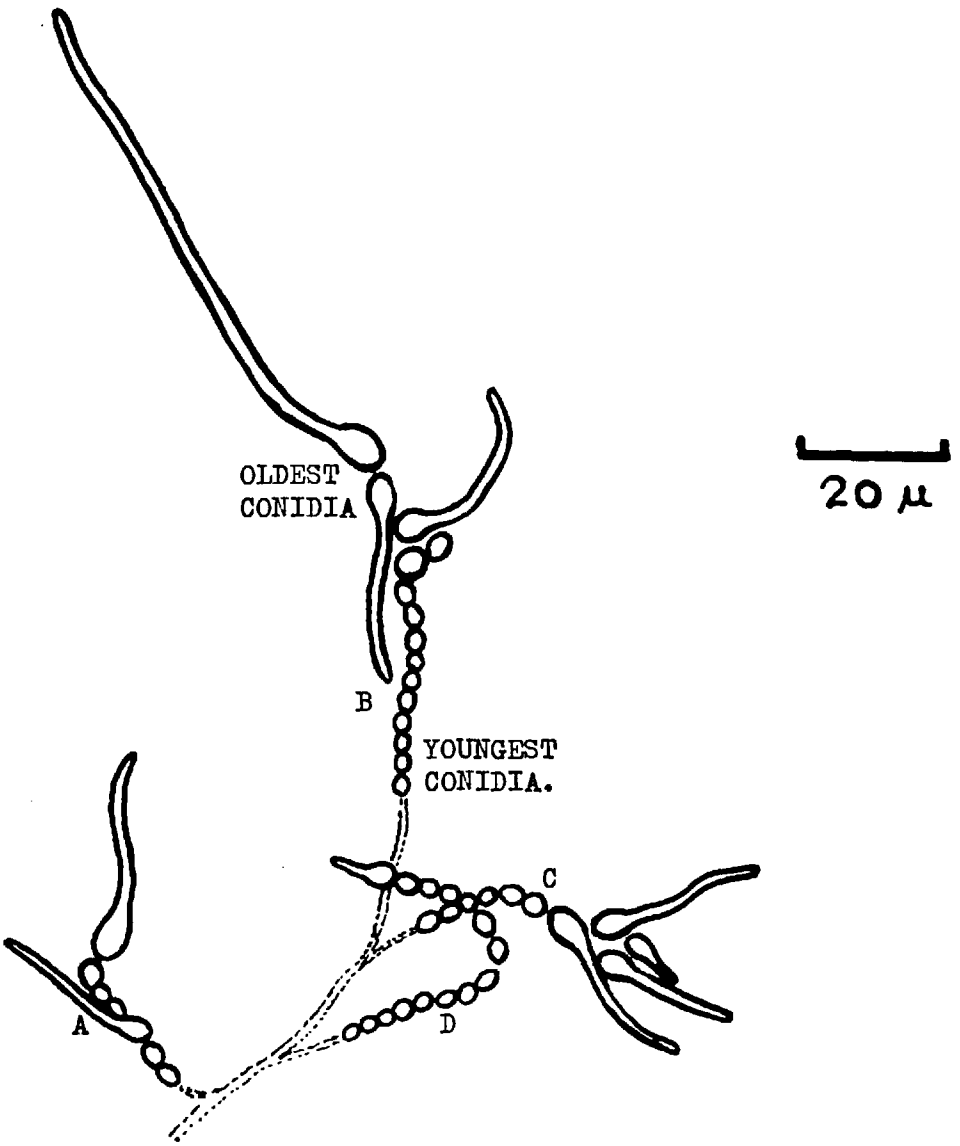


FIG. 44 CAMERA LUCIDA DRAWING OF PLATE 21. (PAGE 207).
CONIDIA OF I.FARINOSA.

These age effects might account for the low percentage germination achieved with B.bassiana and I.farinosa conidia in water. Because M.anisopliae conidia usually showed almost 100 per cent germination in water (usually, about 97 % in 24 hours), and they practically did so together (within 8 - 11 hours), it appeared likely that such aging effect would not exist in the spores of this species.

Germination of M.anisopliae conidia of known ages, up to 144 hours was therefore studied. The results are presented in Table 72 and depict the germination ability of the conidia in relation to age. A conidium of M.anisopliae was produced in approximately 2 - 3 hours (Fig. 42). The results show that conidia of different ages, within the range 1 to 120 hours, were equally capable of germinating in water (see Plate 22 and Fig. 45). The results thus conformed with the above prediction.

TABLE 72

Germination capacity of conidia of M. anisopliae in 18 hour period in relation to age (in hours).

Assessed on observation of 50 apparently identical phialides.

Position of conidial group from phialide apex.	Assumed Age of group of 10 conidia in Hours	% Germination after 18 hours	Mean Germ tube length in μ
1	1 - 20	100.00	26.3
2	21 - 40	99.42	23.2
3	41 - 60	99.00	25.1
4	61 - 80	99.75	25.6
5	81 - 100	100.00	25.8
6	101 - 120	100.00	23.9

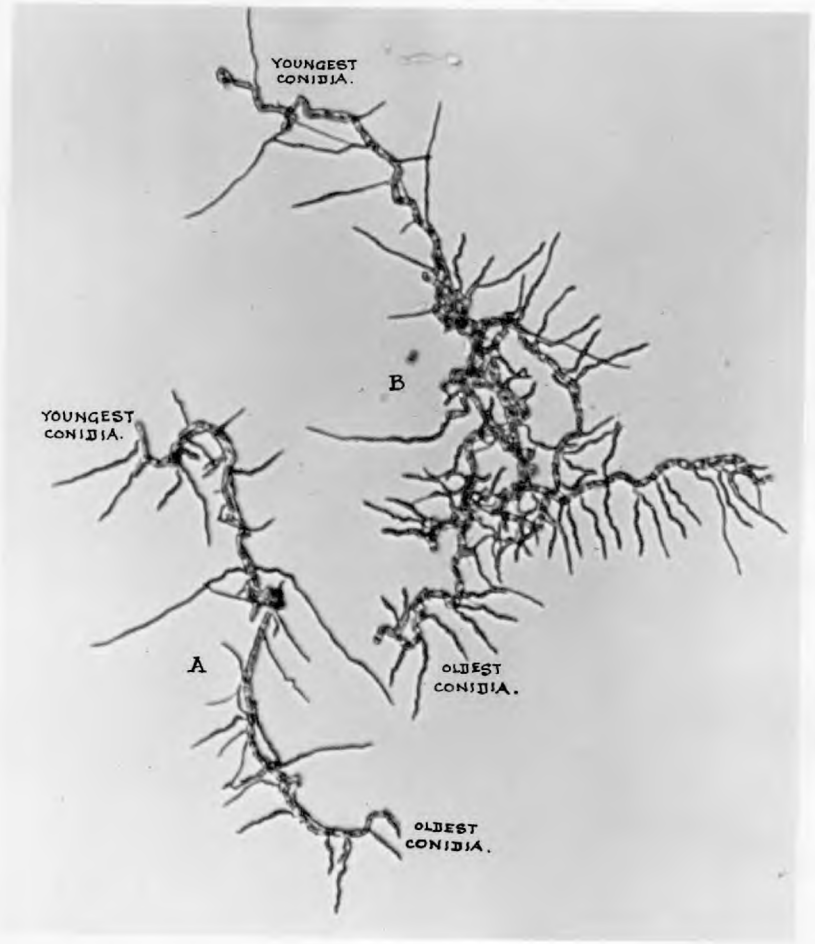


PLATE 22 PHOTOGRAPH OF CHAINS OF CONIDIA OF M.ANISOPLIAE DEPICTING UNIFORM CAPACITY OF THE SPORES (1 - 5 DAYS OLD) FOR GERMINATION AT 100 % RELATIVE HUMIDITY AT 25°C. GERMINATION AFTER 18 HOURS' INCUBATION. (x 320).

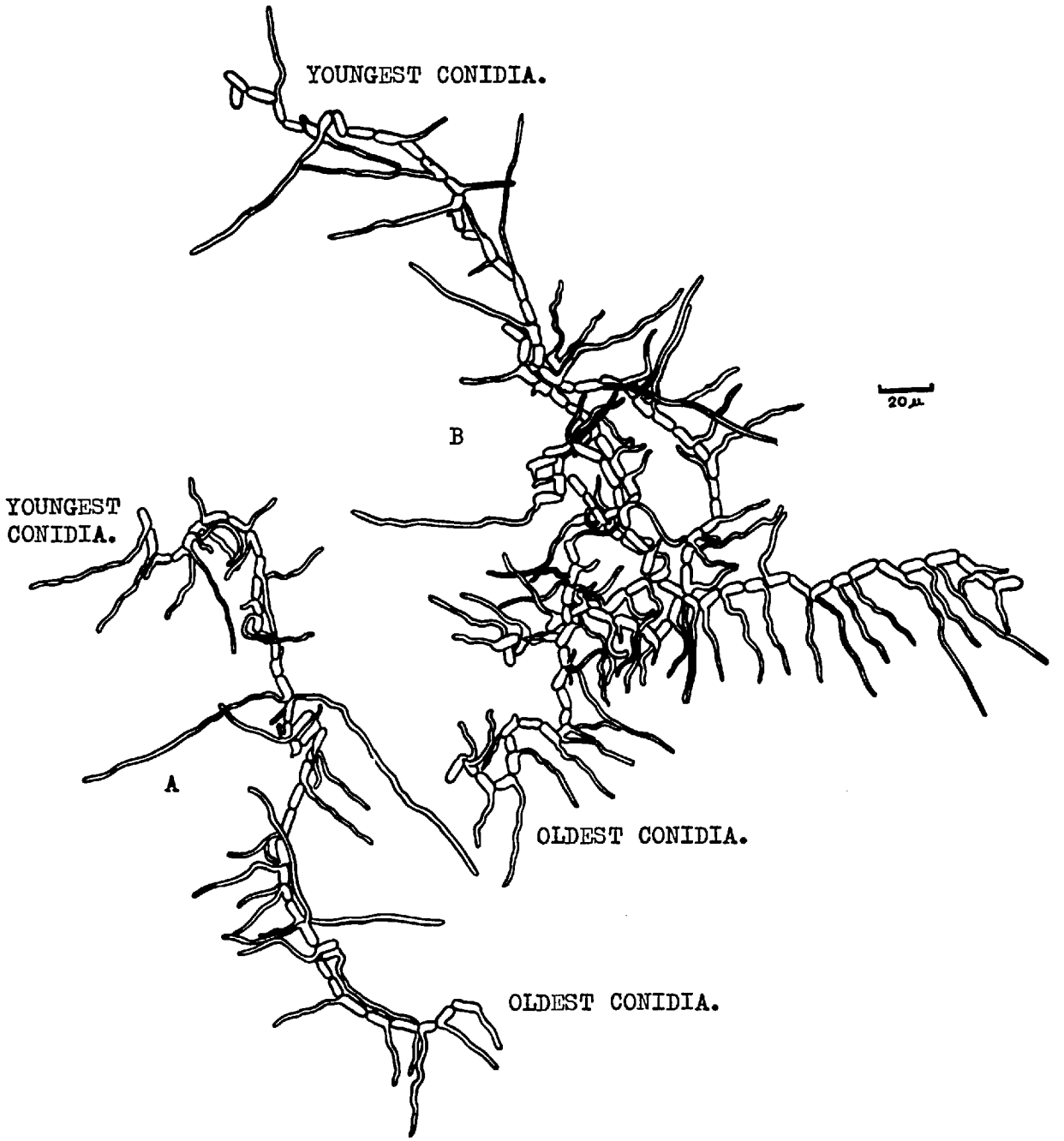


FIG. 45 CAMERA LUCIDA DRAWING OF PLATE 22. (PAGE 211).
CONIDIA OF M.ANISOPLIAE.

R. LONGEVITY OF CONIDIA UNDER DIFFERENT TEMPERATURE AND HUMIDITY CONDITIONS IN LIGHT AND DARK.

The length of life of fungal spores, as measured by their ability to germinate after various periods, is affected in the main, by environmental conditions of humidity, temperature and light. At any given relative humidity, increasing the temperature generally decreased the viability of fungal spores, and lower temperatures, above freezing, favour longevity (Gottlieb, 1950 ; Cochrane, 1958). The relationship between relative humidity and viability does not appear to be as simple, and four main sorts of relationship have been reported, viz. :

(1) 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 fungus spores.

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

(3) 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 Glocosporium musarum survived longest at higher humidities (60 - 80 % R.H.) than at lower humidities (0 - 20 % R.H.)

(4) Teitell (1958) reported that viability of conidia of Aspergillus flavus was preserved longest at 0 and 85 % R.H. and lost quickest at 75 % R.H.

Fergus and Schein (1960) found that light had a deleterious effect on viability of stored ascospores of Urnula craterium, and spores exposed to light died quicker than those kept in darkness.

The longevity of conidia of B.bassiana, I.farinosa and M.anisopliae was studied under different temperature and humidity conditions, in light and dark. Conidia of I.farinosa and M.anisopliae were removed in the dry state into clean watch glasses and spread out to form a very fine layer in the watch glasses. Cultures of B.bassiana were flooded with minute quantities of sterile distilled water and aliquots of the suspension formed were poured into clean watch glasses and left for 1 - 2 hours at 23°C. to dry. The watch glasses with their loads of conidia were placed over saturated aqueous solutions of NaCl and $MgCl_2.6H_2O$, in screw-capped glass jars (5 cm. high and 7 cm. in diameter). These salt solutions provided respective relative humidities of approximately 75 and 33 % (Wexler and Hasegawa, 1954) (see Table 1 for precise R.H. values at different temperatures). Other

jars held dry Calcium chloride to provide a nominal zero humidity. The jars were sealed and stored in incubators with continuous lighting and set at 8, 18 and 25°C. Two sets of experiments were set up in each case. Jars of one set were completely wrapped with black paper to keep the conidia in continuous darkness and the other set left uncovered to expose the conidia to continuous light. The strength of lighting maintained in the 8, 18 and 25°C. - incubators were 16, 78 and 30 foot-candles respectively.

Viability was determined after desired intervals, by germinating samples of conidia transferred from the jars onto Potato dextrose agar at the optimum temperature for the particular fungus for a period of 24 hours, after which, percentage germination was determined and germ tube lengths measured. When a conidium sample registered zero germination, samples from that particular jar were used in the next two consecutive germination tests to confirm the zero results, before tests were discontinued. The experiments were run over a total period of 635 days (from the 15th of May, 1961 to 9th February, 1963). The results for B.bassiana are presented in Tables 73 - 81 and summarised graphically in Figs. 46a, b and c. ; those for I.farinosa are in Tables 82 - 90 and in Figs 47a and b. Those for M.anisopliae are presented in Tables 91 - 99 and in Figs. 48a and b.

i. Survival of Conidia of B.bassiana.

TABLE 73

The longevity of conidia of B.bassiana stored at 25°C. at a constant Relative Humidity of 0 % in LIGHT of 30 ft.-candles and in DARK.

(Percentage Germination after 24 hours on PDA[1] at 18°C.)

STORAGE INTERVAL IN DAYS	LIGHT			DARK		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
0	2618	100.00	158.1	2618	100.00	158.1
14	1619	100.00	155.5	2730	100.00	161.0
28	1516	94.39	140.0	1565	98.21	127.5
56	1549	74.95	89.8	2700	97.19	159.4
84	2329	20.99	11.2	1804	74.72	67.4
112	2254	7.28	6.1	2834	73.08	35.2
140	2127	0.61	5.3	2004	69.81	27.1
168	1719	0.00	-	1654	74.48	34.1
196	2861	0.00	-	1572	62.53	23.9
252	1427	0.00	-	2172	34.71	24.9
308	-	-	-	2286	27.95	15.2
364	-	-	-	2112	28.98	16.8
455	-	-	-	1850	18.97	16.0
545	-	-	-	2721	0.18	7.5
635	-	-	-	2330	0.00	-

[1] Potato Dextrose Agar.

TABLE 74

The longevity of conidia of B.bassiana stored at 25°C. at a constant Relative Humidity of 33.2 % in LIGHT of 30 ft.-candles and in DARK.

(Percentage Germination after 24 hours on PDA at 18°C.)

STORAGE INTERVAL IN DAYS	LIGHT			DARK		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
0	2618	100.00	158.1	2618	100.00	158.1
14	1650	99.21	154.0	1574	99.89	159.2
28	1682	82.45	81.1	1808	81.80	107.3
56	1782	36.92	15.6	2315	60.36	41.7
84	2055	0.00	-	3002	35.04	11.1
112	1918	0.00	-	3103	14.38	9.1
140	1651	0.00	-	2918	2.47	4.6
168	-	-	-	1688	3.70	4.4
196	-	-	-	2680	0.00	-
252	-	-	-	1954	0.00	-
308	-	-	-	2025	0.00	-

TABLE 75

The longevity of conidia of B.bassiana stored at 25°C. at a constant Relative Humidity of 75.8 % in LIGHT of 30 ft.-candles and in DARK.

(Percentage Germination after 24 hours on PDA at 18°C.)

STORAGE INTERVAL IN DAYS	LIGHT			DARK		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
0	2618	100.00	158.1	2618	100.00	158.1
14	1820	80.00	88.7	2689	90.02	159.2
28	2058	0.19	62.0	2213	8.59	47.4
56	2556	0.00	-	2408	0.00	-
84	2425	0.00	-	2583	0.00	-
112	2180	0.00	-	1892	0.00	-

TABLE 76

The longevity of conidia of B.bassiana stored at 18°C. at a constant Relative Humidity of 0 % in LIGHT of 78 ft.-candles and in DARK.

(Percentage Germination after 24 hours on PDA at 18°C.)

STORAGE INTERVAL IN DAYS	LIGHT			DARK		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
0	2618	100.00	158.1	2618	100.00	158.1
14	3016	99.67	168.2	1913	99.80	160.9
28	2746	98.69	101.6	1728	98.96	152.8
56	2962	96.21	82.4	1878	94.03	91.5
84	3106	85.90	89.9	2002	95.55	97.2
112	2642	74.79	61.7	2722	85.74	81.2
140	2228	36.04	19.5	2050	82.60	83.6
168	2974	13.16	10.9	2938	72.36	60.9
196	2597	1.56	4.8	2618	67.22	46.8
252	1854	0.00	-	2342	62.93	47.1
308	1733	0.00	-	2715	52.93	20.9
364	1898	0.00	-	2233	40.82	21.7
455	-	-	-	1800	28.99	20.5
545	-	-	-	2292	23.14	22.1
635	-	-	-	2181	15.00	8.4

TABLE 77

The longevity of conidia of B.bassiana stored at 18°C. at a constant Relative Humidity of 33.7 % in LIGHT of 78 ft.-candles and in DARK.

(Percentage Germination after 24 hours on PDA at 18°C.)

STORAGE INTERVAL IN DAYS	LIGHT			DARK		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
0	2618	100.00	158.1	2618	100.00	158.1
14	3042	99.50	159.0	1874	94.61	156.8
28	2870	97.42	164.0	2598	82.23	144.3
56	1978	44.51	58.5	2485	48.25	57.5
84	2344	37.75	17.5	2359	47.05	19.7
112	1897	0.89	9.9	2668	15.30	12.7
140	1701	0.00	-	2162	1.30	4.5
168	2448	0.00	-	2587	0.00	-
196	2567	0.00	-	1950	0.00	-
252	-	-	-	2086	0.00	-

TABLE 78

The longevity of conidia of B.bassiana stored at 18°C. at a constant Relative Humidity of 75.4 % in LIGHT of 78 ft.-candles and in DARK.

(Percentage Germination after 24 hours on PDA at 18°C.)

STORAGE INTERVAL IN DAYS	LIGHT			DARK		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
0	2816	100.00	158.1	2816	100.00	158.1
14	2720	96.70	82.0	1925	98.82	146.2
28	2682	0.00	-	2300	29.93	19.4
56	2339	0.00	-	2568	0.19	11.8
84	2373	0.00	-	2481	0.00	-
112	-	-	-	2116	0.00	-
140	-	-	-	2061	0.00	-

TABLE 79

The longevity of conidia of B.bassiana stored at 8°C. at a constant Relative Humidity of 0 % in LIGHT of 16 ft.-candles and in DARK.

(Percentage Germination after 24 hours on PDA at 18°C.)

STORAGE INTERVAL IN DAYS	LIGHT			DARK		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
0	2618	100.00	158.1	2618	100.00	158.1
14	1630	99.75	159.0	1893	100.00	157.8
28	2602	99.88	161.7	1882	99.11	151.7
56	1956	98.79	160.0	1722	98.02	163.5
84	1859	98.92	106.6	1994	98.04	126.5
112	2488	95.82	80.5	2312	97.83	91.0
140	2530	92.56	45.4	1923	97.50	82.3
168	2384	98.74	68.2	2636	97.43	85.4
196	2798	90.14	56.8	2050	97.41	65.4
252	1816	94.27	48.7	2068	96.81	86.2
308	2680	91.42	42.9	1899	98.42	80.3
364	2061	94.76	48.1	2220	99.46	88.8
455	2193	89.74	48.0	1932	99.45	69.5
545	2773	79.06	46.6	2140	95.79	73.0
635	2150	52.88	31.0	2622	90.42	66.4

TABLE 80

The longevity of conidia of B.bassiana stored at 8°C. at a constant Relative Humidity of 34.3 % in LIGHT of 16 ft.-candles and in DARK (Percentage Germination after 24 hours on PDA at 18°C.)

STORAGE INTERVAL IN DAYS	LIGHT			DARK		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
0	2618	100.00	158.1	2618	100.00	158.1
14	1684	99.76	159.2	1900	99.81	159.0
28	2031	99.09	158.7	2892	99.52	155.6
56	1953	98.85	155.0	2688	99.35	158.3
84	1899	97.58	93.9	2298	99.56	106.7
112	1934	68.51	49.7	2702	96.71	65.4
140	1785	41.23	32.3	3268	95.38	63.8
168	2422	46.73	34.3	2666	94.88	61.1
196	2608	10.74	13.1	2500	86.40	49.5
252	2152	0.56	7.4	1938	89.99	58.0
308	2255	0.00	-	2090	92.15	64.7
364	1883	0.00	-	2073	97.11	60.0
455	2071	0.00	-	2454	24.55	62.6
545	-	-	-	2851	26.49	33.8
635	-	-	-	2169	8.25	14.3

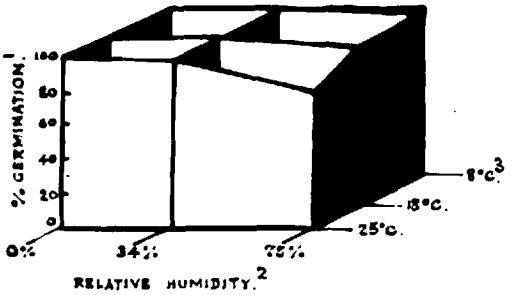
TABLE 81

The longevity of conidia of B.bassiana stored at 8°C. at a constant Relative Humidity of 75.2 % in LIGHT of 16 ft.-candles and in DARK

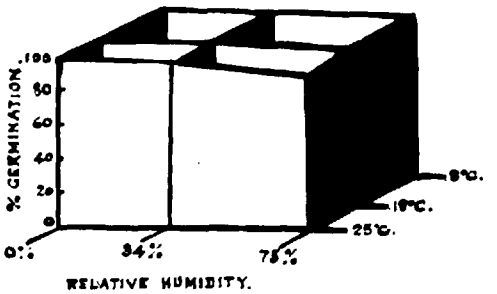
(Percentage Germination after 24 hours on PDA at 18°C.)

STORAGE INTERVAL IN DAYS	LIGHT			DARK		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
0	2618	100.00	158.1	2618	100.00	158.1
14	1789	96.09	126.8	1761	98.07	157.1
28	2492	81.71	70.2	2080	81.85	81.2
56	3070	84.41	79.8	2019	84.60	89.0
84	1715	51.25	59.3	1871	83.57	78.1
112	2626	33.05	27.4	2394	68.83	59.8
140	1959	21.45	18.6	2035	22.46	23.1
168	2516	17.08	26.0	2750	20.33	27.3
196	2074	0.05	23.5	2566	0.51	26.3
252	2592	0.00	-	1854	0.43	22.7
308	1944	0.00	-	2062	0.00	-
364	2008	0.00	-	1987	0.00	-
455	-	-	-	2453	0.00	-

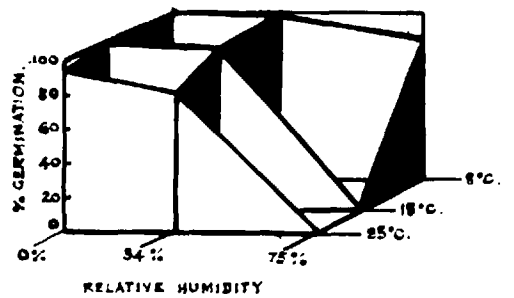
FIG. 46a. EFFECT OF RELATIVE HUMIDITY AND TEMPERATURE ON VIABILITY OF CONIDIA OF B.BASSIANA EXPOSED FOR 14, 28, 56 AND 84 DAYS TO A SERIES OF RELATIVE HUMIDITIES IN LIGHT AND DARK AT 8, 18 AND 25°C.



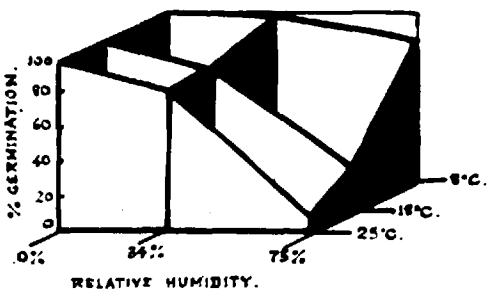
LIGHT 14 DAYS



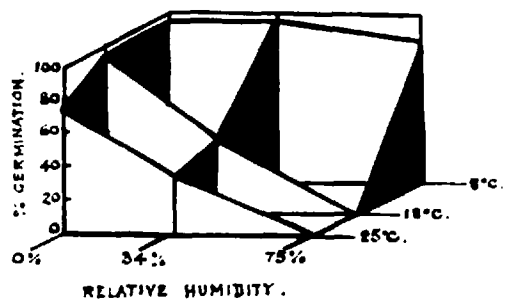
DARK



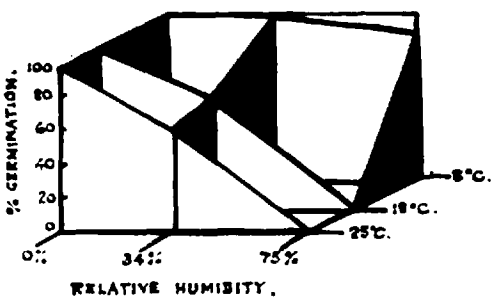
LIGHT 28 DAYS



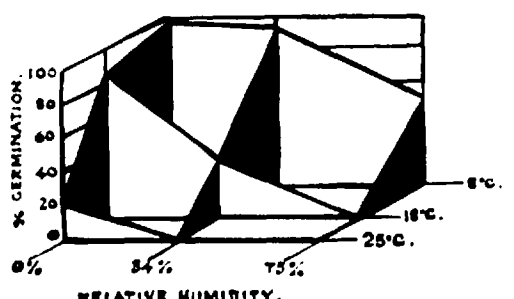
DARK



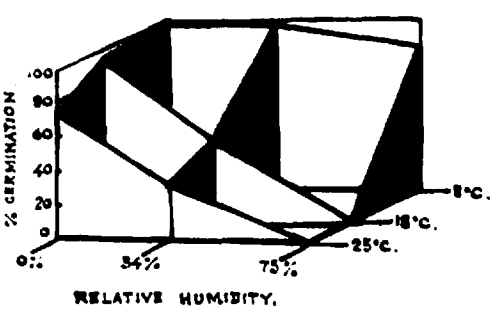
LIGHT 56 DAYS



DARK



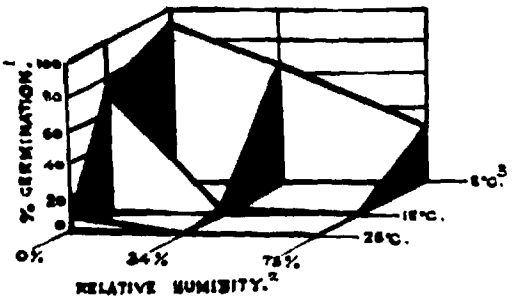
LIGHT 84 DAYS



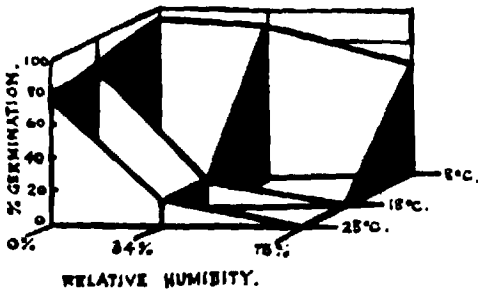
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[1] % VIABILITY : [2,3] HUMIDITY AND TEMPERATURE OF STORAGE.

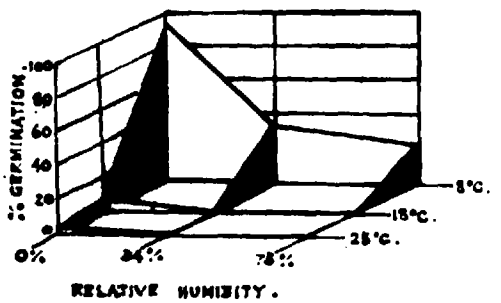
FIG. 46b. EFFECT OF RELATIVE HUMIDITY AND TEMPERATURE ON VIABILITY OF CONIDIA OF B. BASSIANA EXPOSED FOR 112, 140, 168 AND 196 DAYS TO A SERIES OF RELATIVE HUMIDITIES IN LIGHT AND DARK AT 8, 18 AND 25°C.



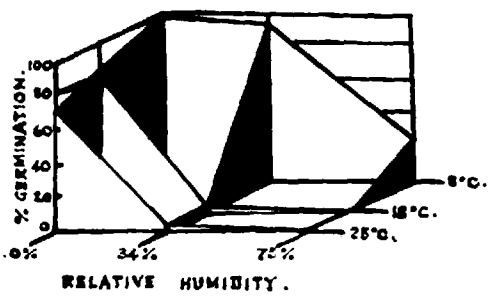
LIGHT 112 DAYS



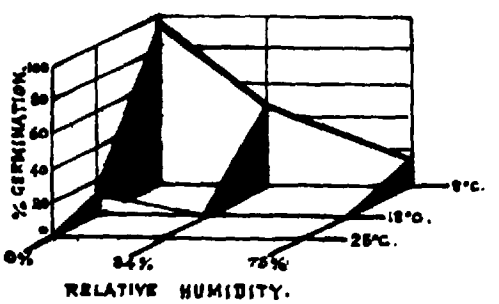
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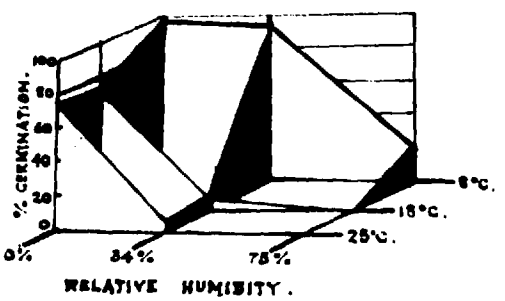
LIGHT 140 DAYS



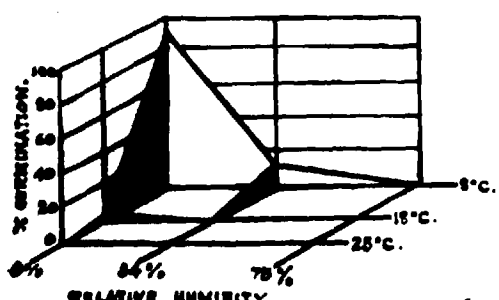
DARK



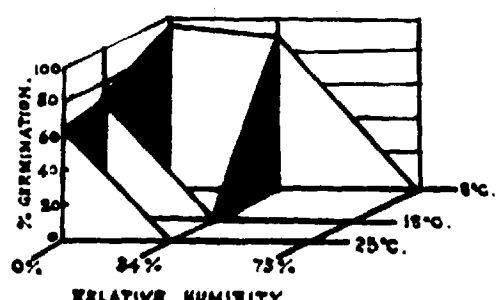
LIGHT 168 DAYS



DARK



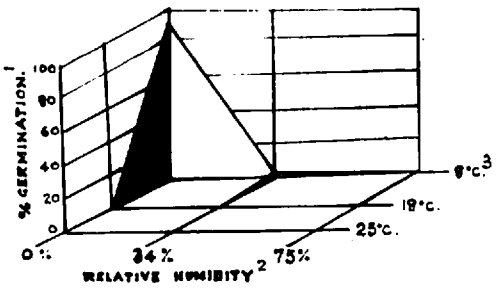
LIGHT 196 DAYS



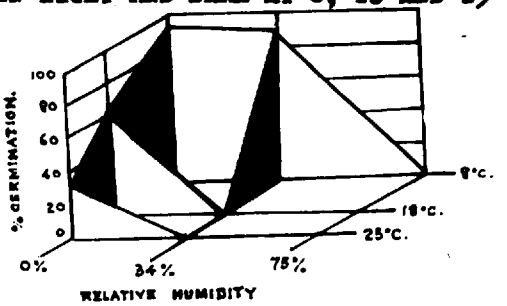
DARK

[1] % VIABILITY : [2,3] HUMIDITY AND TEMPERATURE OF STORAGE.

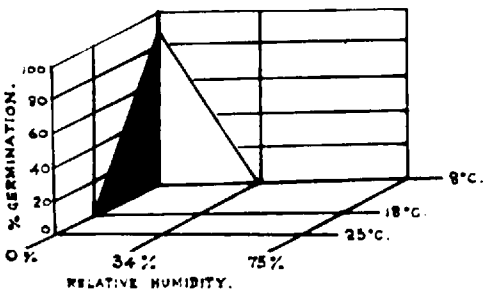
FIG. 46c. EFFECT OF RELATIVE HUMIDITY AND TEMPERATURE ON VIABILITY OF CONIDIA OF B.BASSIANA EXPOSED FOR 252, 308, 364 AND 455 DAYS TO A SERIES OF RELATIVE HUMIDITIES IN LIGHT AND DARK AT 8, 18 AND 25°C.



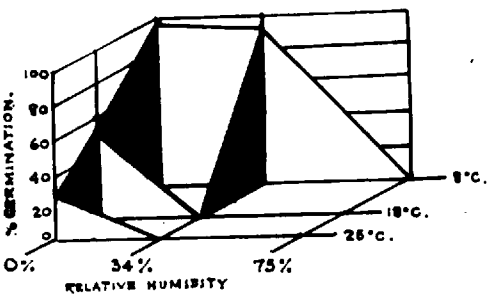
LIGHT 252 DAYS



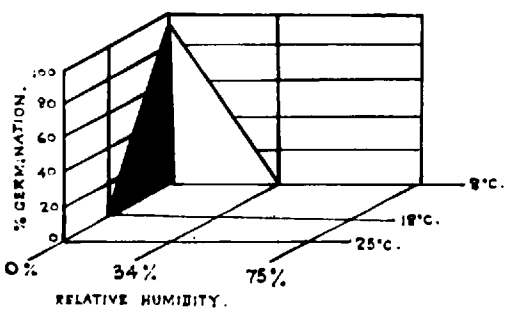
DARK



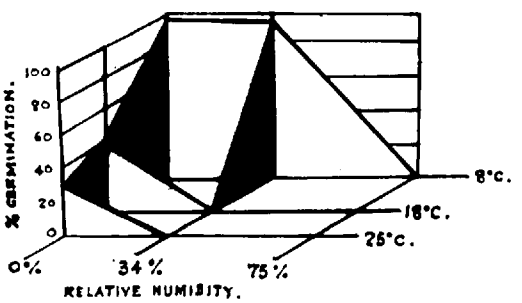
LIGHT 308 DAYS



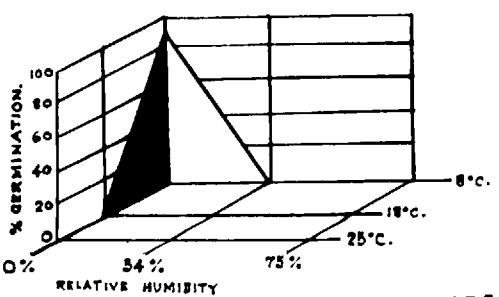
DARK



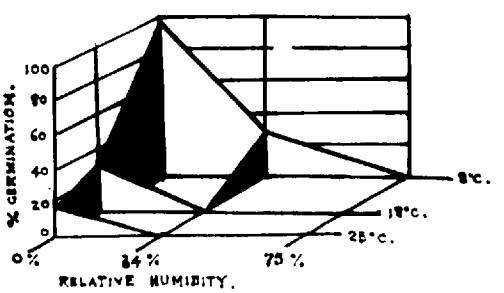
LIGHT 364 DAYS



DARK



LIGHT 455 DAYS



DARK

[1] % VIABILITY : [2,3] HUMIDITY AND TEMPERATURE OF STORAGE.

Response of the stored conidia of B.bassiana to temperature (Tables 73 - 81 and Figs. 46a, b and c - since degree of viability was determined on the basis of percentage germination of conidial samples, percentage viability in all pertinent graphs in this thesis is presented as percentage germination to correspond to data in Tables of results) generally resembled the findings of Steinhaus (1960). The humidity of storage was unspecified in Steinhaus' account, so closer comparison is not possible. The more elaborate experiments here conducted reveal in greater detail the pattern of aging in conidia of B.bassiana. The relative importance of the three environmental variables can be assessed from the data.

At each humidity level in both light and dark, conidia died first at 25°C., followed some time later, by conidia stored at 18°C., whilst at 8°C. the conidia survived many times longer. While at 8°C. 90 per cent survived for 635 days at 0 % R.H. in darkness (Table 79), only 15 per cent survived for the same period at 18°C. (Table 76) and at 25°C. (Table 73), all had died by then.

At each temperature level, the longevity of the conidia directly depends on the humidity of storage. The higher the humidity the sooner the spores lose viability. Taking a typical example, at 25°C. in darkness, all conidia held at 75.8 % R.H. (Table 75) died within 56 days, conidia at 33.2 % R.H. (Table 74) survived for 168 days whilst those at 0 % R.H. (Table 73) lived for as long as 545 days.

Conidia under identical temperature and humidity conditions lived longer in darkness than when exposed to light. This effect was more clearly displayed where the humidity and temperature allowed a fairly long conidial survival. Thus at 25°C. and at 0 % R.H. (Table 73) where the conidia remained viable for a long period, the effect of light was striking. The conidia exposed to light (30 ft.-candles) were all dead within 168 days, whilst it needed 545 to 635 days for those in the dark to die. The cursory observations of Toumanoff (1933) and Gösswald (1939) that viability of B.bassiana conidia was well conserved when protected from the sun, is thus amplified and confirmed.

Finally, the extreme capacity for long survival of the conidia was worthy of note. The last germination test made, after 635 days' storage, showed that 90 and 53 per cent of the conidia held at 8°C. at 0 % R.H., in dark and light respectively, were still viable (Table 79). In some other treatments, also, smaller percentages of the conidia survived (Tables 76 and 80) for periods in excess of 635 days.

The progressive diminution, with passage of time, of the proportion of conidia which remain germinable is accompanied by a reduction in the size of germ tubes produced by the germinating conidia within the test period of 24 hours at 18°C. on Potato Dextrose Agar (The standard germination test procedure adopted in this experiment). In certain treatments (eg. see Table 80, Dark)

a considerable reduction in germ tube length may occur without much change in percentage germinability. It is not, from the data, possible to deduce whether the shorter germ tubes produced by the spores stored for relatively long periods are the result of late emergence of germ tubes from the spore, or slow growth of the germ tubes (they are on a nutrient medium), or a combination of both. Experiments reported below (page 272) investigate these points.

ii. Survival of Conidia of I.farinosa

TABLE 82

The longevity of conidia of I.farinosa stored at 25°C. at a constant Relative Humidity of 0 % in LIGHT of 30 ft.-candles and in DARK.

(Percentage Germination after 24 hours on PDA at 20°C.)

STORAGE INTERVAL IN DAYS	LIGHT			DARK		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
0	1982	99.60	141.4	1982	99.60	141.4
14	2950	47.02	26.5	3155	70.64	49.2
28	2992	0.67	2.4	1729	6.42	18.7
56	2629	0.00	-	2191	4.70	8.5
84	1711	0.00	-	1852	1.29	2.8
112	2077	0.00	-	2653	0.00	-
140	-	-	-	2200	0.00	-
168	-	-	-	1998	0.00	-

TABLE 83

The longevity of conidia of I.farinosa stored at 25°C. at a constant Relative Humidity of 33.4 % in LIGHT of 30 ft.-candles and in DARK.

(Percentage Germination after 24 hours on PDA at 20°C.)

STORAGE INTERVAL IN DAYS	LIGHT			DARK		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
0	1982	99.60	141.4	1982	99.60	141.4
14	2184	0.00	-	2057	0.00	-
28	2675	0.00	-	1966	0.00	-
56	2725	0.00	-	1888	0.00	-

TABLE 84

The longevity of conidia of I.farinosa stored at 25°C. at a constant Relative Humidity of 75.4 % in LIGHT of 30 ft.-candles and in DARK.

(Percentage Germination after 24 hours on PDA at 20°C.)

STORAGE INTERVAL IN DAYS	LIGHT			DARK		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
0	1982	99.60	141.4	1982	99.60	141.4
14	2178	0.00	-	1881	0.00	-
28	1776	0.00	-	1823	0.00	-
56	2689	0.00	-	1926	0.00	-

TABLE 85

The longevity of conidia of I.farinosa stored at 18°C. at a constant Relative Humidity of 0 % in LIGHT of 78 ft.-candles and in DARK.

(Percentage Germination after 24 hours on PDA at 20°C.)

STORAGE INTERVAL IN DAYS	LIGHT			DARK		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
0	1982	99.60	141.4	1982	99.60	141.4
14	2221	69.51	58.6	2590	83.52	74.5
28	2367	40.67	23.7	2185	82.84	61.1
56	4122	3.27	8.7	3044	24.08	31.6
84	2665	0.00	-	2108	1.54	5.1
112	2025	0.00	-	2530	0.00	-
140	1926	0.00	-	1790	0.00	-
168	-	-	-	2114	0.00	-

TABLE 86

The longevity of conidia of I.farinosa stored at 18°C. at a constant Relative Humidity of 33.8 % in LIGHT of 78 ft.-candles and in DARK.

(Percentage Germination after 24 hours on PDA at 20°C.)

STORAGE INTERVAL IN DAYS	LIGHT			DARK		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
0	1982	99.60	141.4	1982	99.60	141.4
14	1810	4.42	26.2	2745	11.51	47.4
28	3431	3.29	17.6	2204	4.58	28.2
56	3364	0.86	10.1	2594	2.27	8.2
84	2020	0.00	-	2624	0.00	-
112	2044	0.00	-	1862	0.00	-
140	1898	0.00	-	2161	0.00	-

TABLE 87

The longevity of conidia of I.farinosa stored at 18°C. at a constant Relative Humidity of 75.4 % in LIGHT of 78 ft.-candles and in DARK.

(Percentage Germination after 24 hours on PDA at 20°C.)

STORAGE INTERVAL IN DAYS	LIGHT			DARK		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
0	1982	99.60	141.4	1982	99.60	141.4
14	1835	0.00	-	2885	0.00	-
28	2344	0.00	-	2534	0.00	-
56	2150	0.00	-	2062	0.00	-

TABLE 88

The longevity of conidia of I.farinosa stored at 8°C. at a constant Relative Humidity of 0 % in LIGHT of 16 ft.-candles and in DARK.

(Percentage Germination after 24 hours on PDA at 20°C.)

STORAGE INTERVAL IN DAYS	LIGHT			DARK		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
0	1982	99.60	141.4	1982	99.60	141.4
14	2638	98.47	95.2	2671	99.28	129.0
28	2751	78.77	82.1	2184	90.38	111.8
56	2339	36.46	44.1	3474	81.81	84.3
84	2222	20.25	29.7	2757	81.58	80.9
112	2601	0.11	8.2	4159	65.52	53.8
140	2172	0.27	6.5	2000	67.00	61.0
168	1880	0.32	5.1	2030	56.74	35.3
196	2920	0.73	9.9	2558	52.93	28.5
252	1803	0.40	4.6	2322	20.56	10.2
308	2200	0.21	4.5	2456	13.50	3.5
364	2172	0.00	-	1828	13.92	3.0
455	2080	0.00	-	2378	12.27	3.0
545	2571	0.00	-	2255	0.44	2.4
635	-	-	-	2198	0.00	-

TABLE 89

The longevity of conidia of I.farinosa stored at 8°C. at a constant Relative Humidity of 34.3 % in LIGHT of 16 ft.-candles and in DARK.

(Percentage Germination after 24 hours on PDA at 20°C.)

STORAGE INTERVAL IN DAYS	LIGHT			DARK		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
0	1982	99.60	141.4	1982	99.60	141.4
14	1916	66.34	46.3	2381	96.83	96.0
28	2286	50.61	27.9	2465	88.41	68.0
56	2114	8.47	11.0	2227	39.99	27.6
84	2299	1.14	1.7	2730	9.58	6.2
112	2626	0.00	-	2580	1.51	2.3
140	1961	0.00	-	1955	0.51	3.0
168	2089	0.00	-	2095	0.05	2.4
196	-	-	-	2110	0.49	4.7
252	-	-	-	2372	0.04	2.5
308	-	-	-	2017	0.00	-
364	-	-	-	2161	0.00	-
455	-	-	-	2375	0.00	-

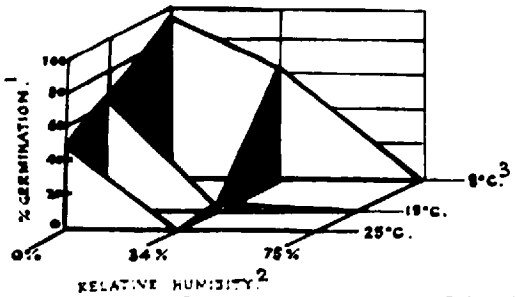
TABLE 90

The longevity of conidia of I.farinosa stored at 8°C. at a constant Relative Humidity of 75.2 % in LIGHT of 16 ft.-candles and in DARK.

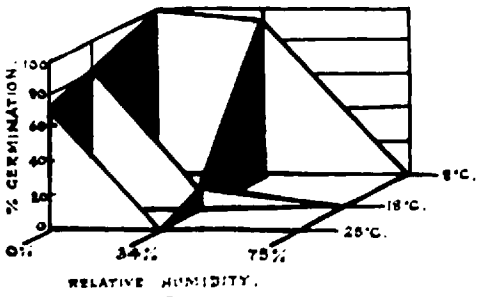
(Percentage Germination after 24 hours on PDA at 20°C.)

STORAGE INTERVAL IN DAYS	LIGHT			DARK		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
0	1982	99.60	141.4	1982	99.60	141.4
14	1822	0.00	-	2767	0.00	-
28	2536	0.00	-	3025	0.00	-
56	2117	0.00	-	2777	0.00	-

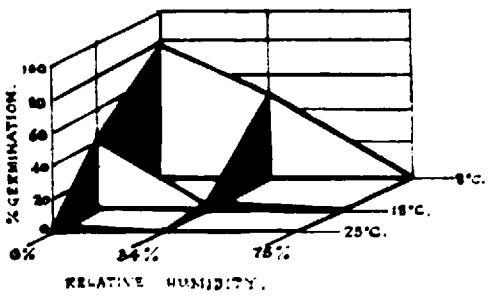
FIG. 47a. EFFECT OF RELATIVE HUMIDITY AND TEMPERATURE ON VIABILITY OF CONIDIA OF I.FARINOSA EXPOSED FOR 14, 28, 56 AND 84 DAYS TO A SERIES OF RELATIVE HUMIDITIES IN LIGHT AND DARK AT 8, 18 AND 25°c.



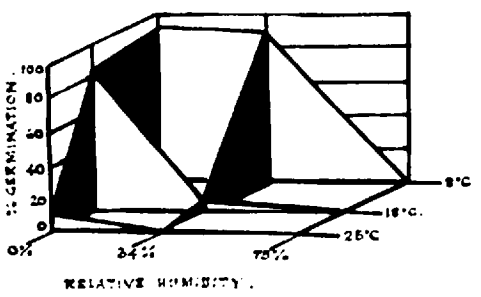
LIGHT 14 DAYS



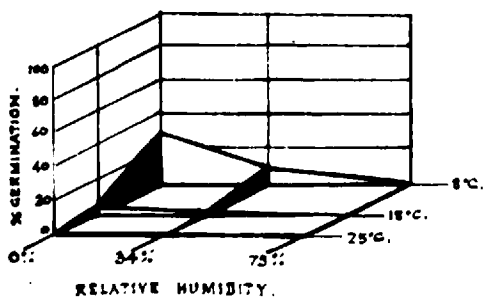
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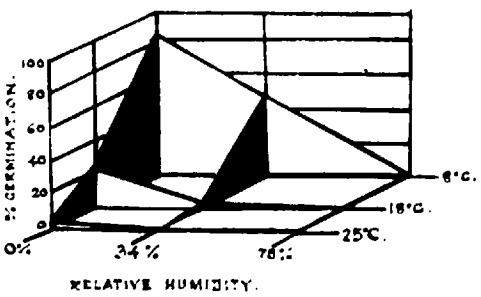
LIGHT 28 DAYS



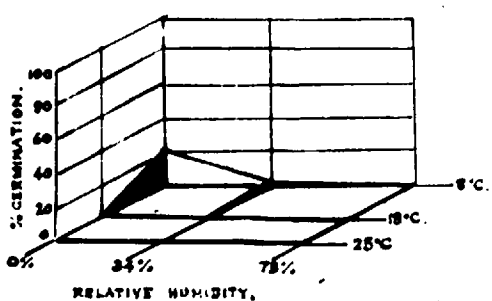
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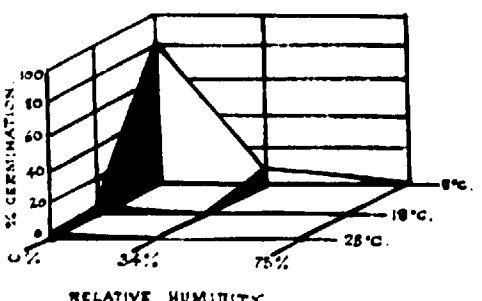
LIGHT 56 DAYS



DARK



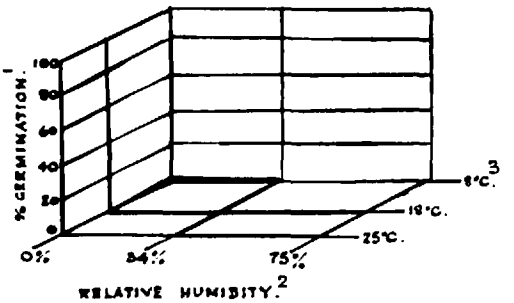
LIGHT 84 DAYS



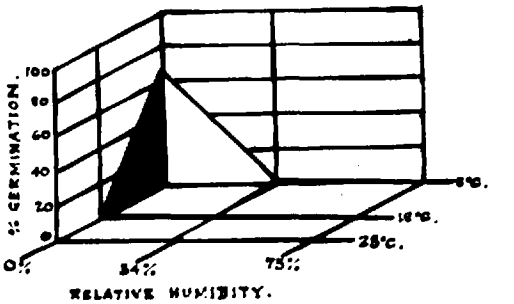
DARK

[1] % VIABILITY : [2,3] HUMIDITY AND TEMPERATURE OF STORAGE.

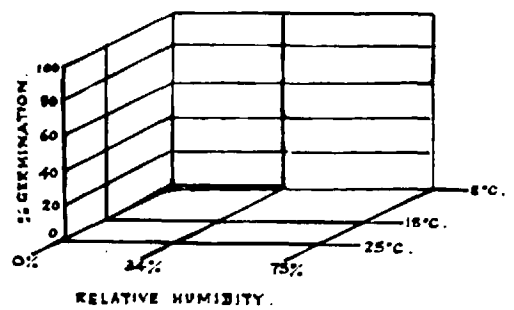
FIG. 47b. EFFECT OF RELATIVE HUMIDITY AND TEMPERATURE ON VIABILITY OF CONIDIA OF I.FARINOSA EXPOSED FOR 112, 140, 168 AND 196 DAYS TO A SERIES OF RELATIVE HUMIDITIES IN LIGHT AND DARK AT 8, 18 AND 25°C.



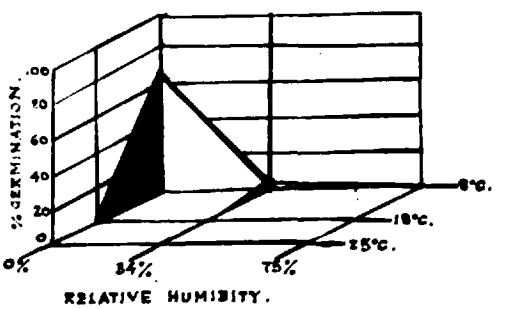
LIGHT 112 DAYS



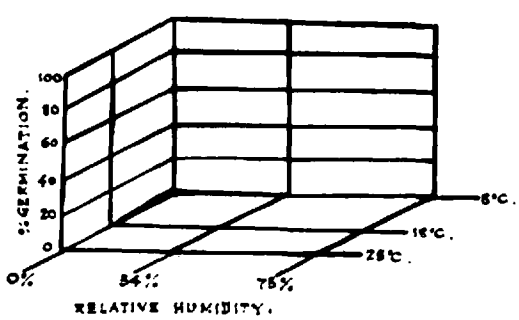
DARK



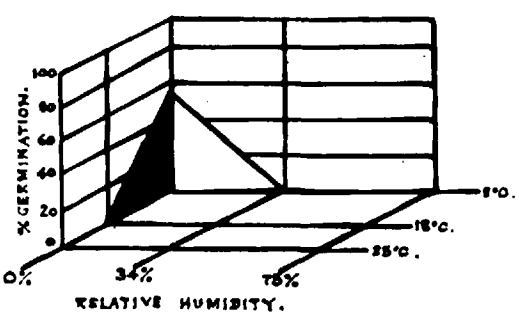
LIGHT 140 DAYS



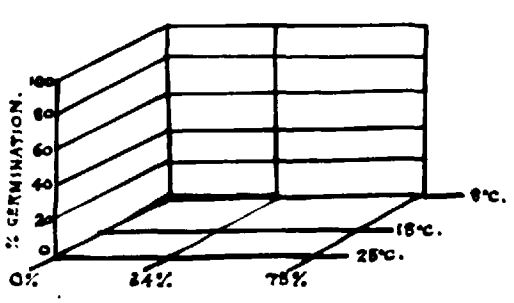
DARK



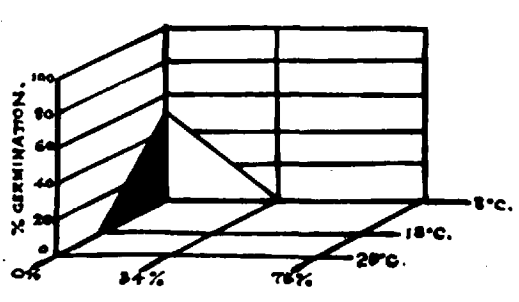
LIGHT 168 DAYS



DARK



LIGHT 196 DAYS



DARK

[1]% VIABILITY ; [2,3]HUMIDITY AND TEMPERATURE OF STORAGE.

The results of viability tests for I.farinosa are presented in Tables 82 to 90 and summarised graphically in Figs. 47a and b. Viability of conidia of I.farinosa shortened its duration with increase in temperature. For example, when conidia were stored in dark at 0 % R.H., none was viable on the 112th day in samples held at 25°C. (Table 82) and at 18°C. (Table 85), whilst 65 per cent was still viable at 8°C. (Table 88).

Humidity was a potent factor. Conidia were preserved best at low humidities and lost viability with increasing rapidity as the humidity rose. The conidia were extremely sensitive to the higher humidities and at all temperatures (8, 18 and 25°C.) conidia held at 75 % R.H. were completely dead within 14 days (Tables 84, 87 and 90).

Conidium vigour was adversely affected by light. The injurious effect of light, as with B.bassiana, was most obvious under humidity and temperature conditions which permitted considerable degree of survival. The effect was very clear in conidia stored at 0 % R.H. Thus conidia held for 196 days at 8°C. gave 0.73 per cent germination if stored in light (16 ft.-candles) and 53 per cent survival in darkness (Table 88).

Though conidia of I.farinosa were fairly long lived at 0 % R.H. at 8°C., where there was 12 per cent survival after 455 days in samples kept in darkness (Table 88), under other humidity and temperature conditions the conidia were rather short lived.

As with B.bassiana, storage led not only to reduced percentage germination, but also to the shortening of the germ tubes, as observed at the end of the germination tests (24 hours). Also, storage sometimes reduced germ tube length before it appreciably depressed percentage germination (see Table 88, light and dark columns). It is reasonable to expect that actual death of the conidia, through storage, would be preceded by a reduction in the vigour of the spores, reflected in germ tube growth. However the exact basis of the observed shortening was not here revealed ; it is studied below (page 272).

iii. Survival of Conidia of M.anisopliae.

TABLE 91

The longevity of conidia of M.anisopliae stored at 25°C. at a constant Relative Humidity of 0 % in LIGHT of 30 ft.-candles and in DARK.

(Percentage Germination after 24 hours on PDA at 25°C.)

STORAGE INTERVAL IN DAYS	LIGHT			DARK		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
0	1624	100.00	416.0	1624	100.00	416.0
14	2572	99.43	392.4	2548	99.87	408.7
28	2267	91.00	293.4	2060	99.85	423.1
56	1678	18.53	52.6	1955	26.91	110.4
84	2580	0.00	-	2392	0.00	-
112	2027	0.00	-	2788	0.00	-
140	1968	0.00	-	2993	0.00	-

TABLE 92

The longevity of conidia of M.anisopliae stored at 25°C. at a constant Relative Humidity of 33.2 % in LIGHT of 30 ft.-candles and in DARK.

(Percentage Germination after 24 hours on PDA at 25°C.)

STORAGE INTERVAL IN DAYS	LIGHT			DARK		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
0	1624	100.00	416.0	1624	100.00	416.0
14	2485	91.64	376.6	1909	99.11	371.1
28	1850	33.19	14.5	2962	74.47	43.7
56	2403	0.00	-	1744	0.00	-
84	2533	0.00	-	2290	0.00	-
112	2172	0.00	-	2380	0.00	-

TABLE 93

The longevity of conidia of M.anisopliae stored at 25°C. at a constant Relative Humidity of 75.8 % in LIGHT of 30 ft.-candles and in DARK.

(Percentage Germination after 24 hours on PDA at 25°C.)

STORAGE INTERVAL IN DAYS	LIGHT			DARK		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
0	1624	100.00	416.0	1624	100.00	416.0
14	1885	99.25	425.0	2579	99.62	413.0
28	1878	94.61	218.3	1761	98.07	350.0
56	2832	15.74	27.7	2276	19.33	33.1
84	2240	0.00	-	2455	0.00	-
112	2782	0.00	-	1977	0.00	-
140	2648	0.00	-	2080	0.00	-

TABLE 94

The longevity of conidia of M. anisopliae stored at 18°C. at a constant Relative Humidity of 0 % in LIGHT of 78 ft.-candles and in DARK.

(Percentage Germination after 24 hours on PDA at 25°C.)

STORAGE INTERVAL IN DAYS	LIGHT			DARK		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
0	1624	100.00	416.0	1624	100.00	416.0
14	1685	99.64	424.7	1884	99.74	431.5
28	1788	96.09	225.2	2694	98.16	323.2
56	2716	45.34	124.6	2760	64.16	111.8
84	2592	0.58	10.1	3272	6.27	28.3
112	2003	0.00	-	2878	0.00	-
140	2860	0.00	-	2780	0.00	-
168	2735	0.00	-	2041	0.00	-

TABLE 95

The longevity of conidia of M.anisopliae stored at 18°C. at a constant Relative Humidity of 33.8 % in LIGHT of 78 ft.-candles and in DARK.

(Percentage Germination after 24 hours on PDA at 25°C.)

STORAGE INTERVAL IN DAYS	LIGHT			DARK		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
0	1624	100.00	416.0	1624	100.00	416.0
14	2082	99.43	421.5	1984	99.74	420.1
28	2165	71.28	153.3	2821	79.84	165.7
56	1867	0.00	-	2706	0.00	-
84	2227	0.00	-	2256	0.00	-
112	1983	0.00	-	2580	0.00	-

TABLE 96

The longevity of conidia of M.anisopliae stored at 18°C. at a constant Relative Humidity of 75.4 % in LIGHT of 78 ft.-candles and in DARK.

(Percentage Germination after 24 hours on PDA at 25°C.)

STORAGE INTERVAL IN DAYS	LIGHT			DARK		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
0	1624	100.00	416.0	1624	100.00	416.0
14	3040	99.54	426.7	3016	99.67	419.2
28	2764	99.26	435.8	2726	98.84	314.2
56	2722	94.28	214.4	2690	91.22	220.4
84	1908	69.55	107.9	2786	71.16	102.8
112	2820	2.48	4.4	2898	22.15	25.1
140	2059	0.00	-	2376	0.00	-
168	1882	0.00	-	2019	0.00	-
196	2519	0.00	-	2550	0.00	-

TABLE 97

The longevity of conidia of M. anisopliae stored at 8°C. at a constant Relative Humidity of 0 % in LIGHT of 16 ft.-candles and in DARK.

(Percentage Germination after 24 hours on PDA at 25°C.)

STORAGE INTERVAL IN DAYS	LIGHT			DARK		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
0	1624	100.00	416.0	1624	100.00	416.0
14	2574	99.81	416.9	2631	99.75	424.5
28	2675	99.76	425.8	2550	99.27	418.2
56	2532	98.96	324.6	2678	98.69	316.4
84	2134	85.98	257.6	2105	98.38	241.8
112	2344	77.98	140.4	2186	96.16	228.0
140	2164	43.16	106.8	2956	85.79	113.5
168	2788	26.69	107.3	2343	81.46	118.9
196	2182	3.89	67.5	2860	54.19	107.4
252	2038	0.69	29.4	2838	6.13	82.1
308	1970	0.00	-	2233	0.55	31.5
364	2100	0.00	-	1984	0.31	10.2
455	2272	0.00	-	3209	0.00	-
545	-	-	-	2317	0.00	-

TABLE 98

The longevity of conidia of M. anisopliae stored at 8°C. at a constant Relative Humidity of 34.3 % in LIGHT of 16 ft.-candles and in DARK.

(Percentage Germination after 24 hours on PDA at 25°C.)

STORAGE INTERVAL IN DAYS	LIGHT			DARK		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
0	1624	100.00	416.0	1624	100.00	416.0
14	2750	99.49	414.7	2330	99.65	385.8
28	1879	88.07	126.2	2665	94.83	223.4
56	2015	70.03	107.6	2767	80.53	123.0
84	2607	41.81	67.2	1996	52.05	82.3
112	2182	18.79	62.0	2824	34.56	77.6
140	2446	5.21	15.2	1893	9.91	16.6
168	3108	0.26	15.1	2902	1.83	16.5
196	2871	0.00	-	2489	0.00	-
252	1907	0.00	-	2733	0.00	-
308	2279	0.00	-	2865	0.00	-

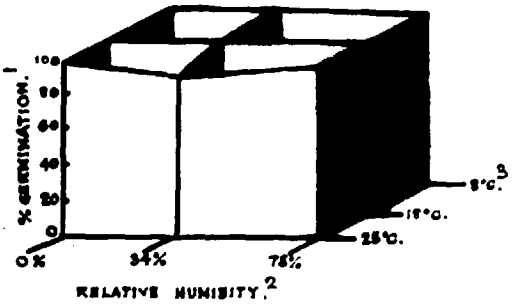
TABLE 99

The longevity of conidia of M.anisopliae stored at 8°C. at a constant Relative Humidity of 75.2 % in LIGHT of 16 ft.-candles and in DARK.

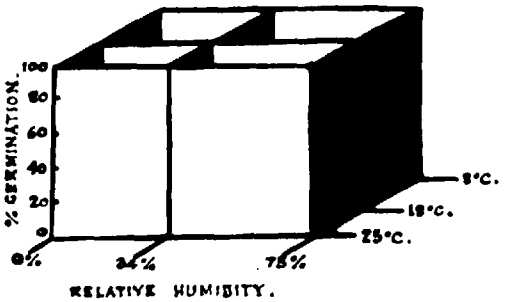
(Percentage Germination after 24 hours on PDA at 25°C.)

STORAGE INTERVAL IN DAYS	LIGHT			DARK		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
0	1624	100.00	416.0	1624	100.00	416.0
14	2371	99.70	420.9	2684	99.76	427.0
28	2702	99.41	319.6	2610	99.57	420.9
56	2745	98.62	246.3	2500	99.38	414.7
84	2875	98.45	128.0	2103	99.14	416.2
112	2522	92.31	104.0	2708	94.60	322.0
140	2161	91.93	143.2	2643	89.45	214.5
168	2870	96.73	103.9	3022	95.37	164.7
196	2270	90.68	97.7	2980	95.90	105.6
252	2034	88.00	96.1	2030	91.52	98.0
308	2133	84.53	93.9	2205	86.80	99.1
364	2772	84.76	87.1	2930	85.00	92.8
455	2946	57.66	61.5	2092	75.16	70.5
545	2070	0.00	-	2625	0.00	-
635	2240	0.00	-	2639	0.00	-

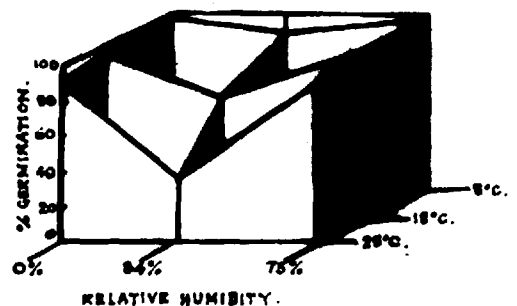
FIG. 48a. EFFECT OF RELATIVE HUMIDITY AND TEMPERATURE ON VIABILITY OF CONIDIA OF M.ANISOPLIAE EXPOSED FOR 14, 28, 56 AND 84 DAYS TO A SERIES OF RELATIVE HUMIDITIES IN LIGHT AND DARK AT 8, 18 AND 25°C.



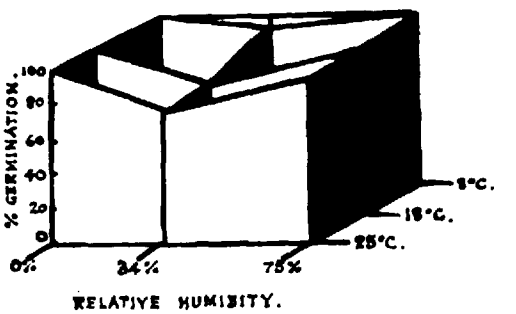
LIGHT. 14 DAYS.



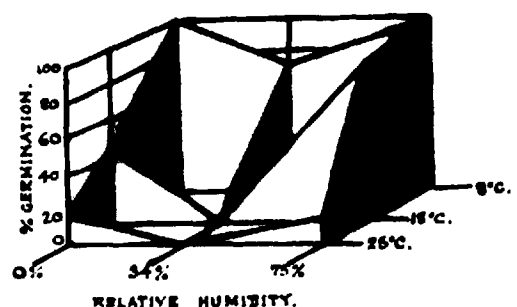
DARK.



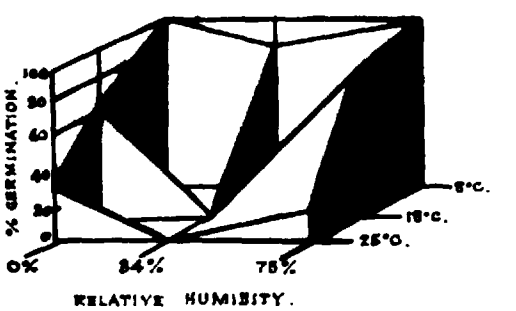
LIGHT 28 DAYS.



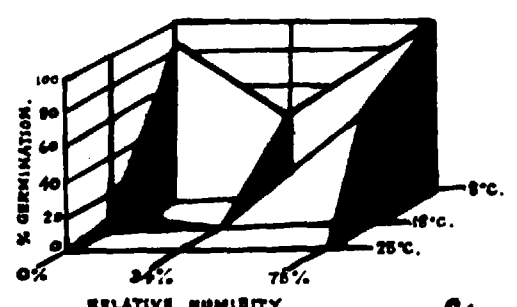
DARK.



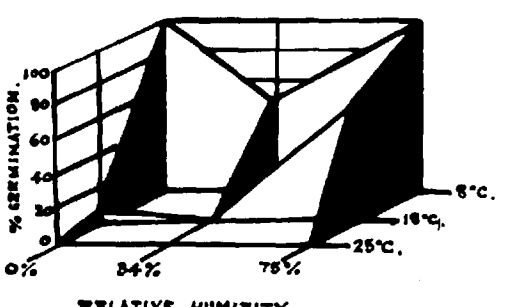
LIGHT. 56 DAYS.



DARK.



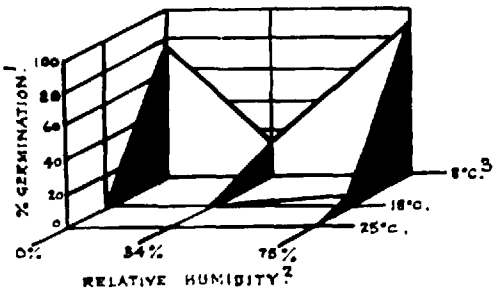
LIGHT 84 DAYS.



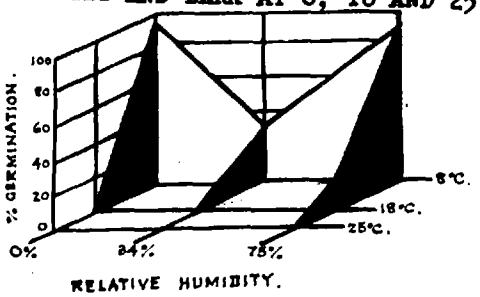
DARK.

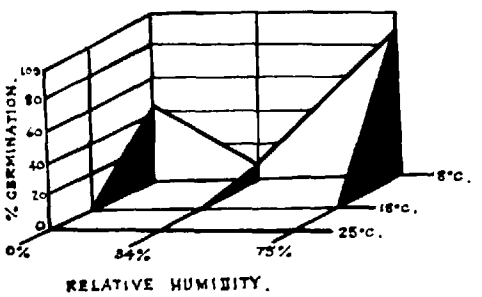
[1] % VIABILITY : [2,3] HUMIDITY AND TEMPERATURE OF STORAGE.

FIG. 48b. EFFECT OF RELATIVE HUMIDITY AND TEMPERATURE ON VIABILITY OF CONIDIA OF M.ANISOPLIAE EXPOSED FOR 112, 140, 168 AND 196 DAYS TO A SERIES OF RELATIVE HUMIDITIES IN LIGHT AND DARK AT 8, 18 AND 25°C.

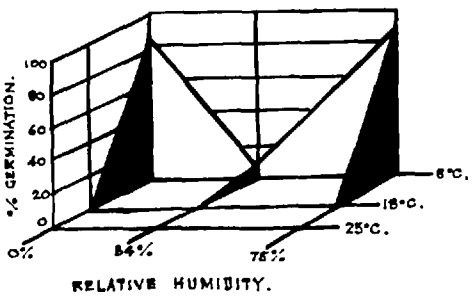


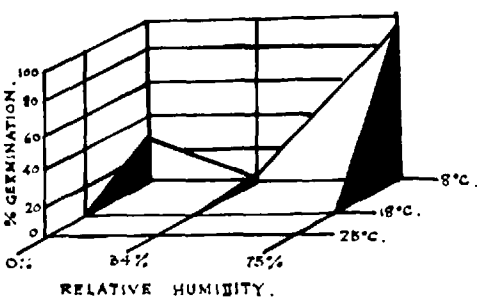
LIGHT 112 DAYS DARK



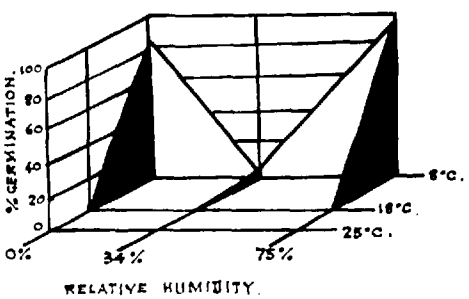


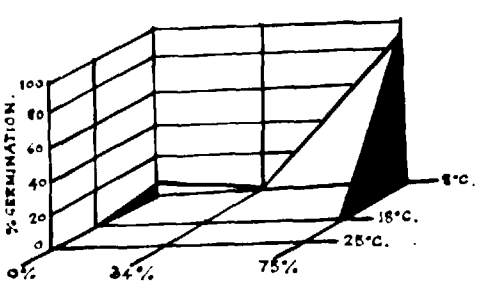
LIGHT 140 DAYS DARK



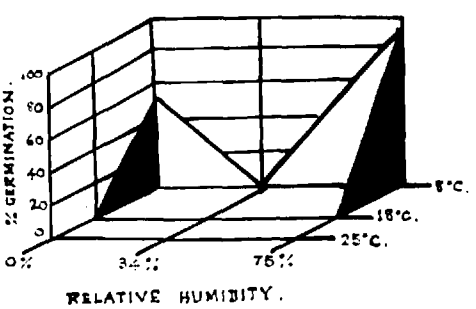


LIGHT 168 DAYS DARK





LIGHT 196 DAYS DARK



[1]% VIABILITY ; [2,3]HUMIDITY AND TEMPERATURE OF STORAGE.

The results of viability tests with M.anisopliae are presented in Tables 91 to 99 and summarised graphically in Figs. 48a and b. The response to temperature of conidia of M.anisopliae at a particular humidity level, was the same as in conidia of B.bassiana and I.farinosa. Higher temperatures hastened loss of viability whilst lower temperatures conserved conidium vigour.

The pattern of response of conidia of M.anisopliae to humidity was quite different from the pattern observed in experiments with conidia of B.bassiana and I.farinosa. It can be seen from the results (Tables 91 - 99, Figs. 48a & b) that by the 28th day of exposure, the viability of the conidia at approximately 33 % R.H. at all three temperatures was markedly reduced, whereas relative humidities of zero and 75 % had only slight effects or none at all, depending on the temperature of incubation. Within 56 days, all the conidia at 25°C. and 33 % R.H. (Table 92) were dead, whereas conidia exposed to zero (Table 91) and 75 % R.H. (Table 93) both at 25°C., retained approximately 20 per cent viability. At 18°C., again, all the conidia at 33 % R.H. (Table 95) were non-viable, whilst conidia at zero % R.H. (Table 94) showed on the average 55 per cent survival, and those at 75 % R.H., 92 per cent survival (Table 96). At 8°C., 34.3 % R.H. reduced viability to approximately 75 per cent in 56 days (Table 98) whilst relative humidities of zero (Table 97) and 75 % (Table 99)

had negligible effect. There was approximately 99 per cent survival at these two humidities. It appeared that atmospheric humidity at or close to 33 % R.H. had an unusual effect on the conidia of M.anisopliae, leading to relatively rapid loss of viability at all temperatures tested. Unlike the previous two species spores of M.anisopliae survived well at high humidities as well as low.

At the two humidities, zero and 75 % R.H., where conidia survived longer that at 33 % R.H., viability was apparently lost at the same rate at 25 and 18°C. (Tables 91, 93, 94 and 96) ; but at 8°C. where the viability of the conidia was well preserved, differences now became apparent. 75 % R.H. seemed to favour longer conidium preservation than 0 % R.H. (Tables 97 and 99).

Light served slightly to accelerate death of the conidia, though this effect was much less evident at high humidities (75 %) than low (zero). (see Table 97, 140 - 196 days' storage in Light and Dark).

The longest survival of conidia of M.anisopliae (Table 99) was for a period between 455 and 545 days. Since the conditions of storage were not stated in the report of Glaser (1926), no direct comparison could be made with his results, in which the conidia were stated to have survived in a dried state for 3 years. Masera (1957) asserted that at 25 - 29°C. the conidia lived for approximately 4 months. Viability of the conidia stored at 25°C. agreed with the report of Masera.

As with B.bassiana and I.farinosa, storage led not only to reduced percentage germination, but also to the shortening of the germ tubes, as observed at the end of the germination tests (24 hours). The exact basis of the observed shortening of the germ tubes in stored spores is studied below (page 272).

The points along the humidity range used were too scanty to allow the building of a complete picture of the pattern of response of the conidia of M.anisopliae. A subsequent experiment was therefore designed, after the original experiment had run for some time, to verify and extend the results of the original experiment.

The experiment was partially repeated, setting up one series of humidity chambers, in darkness only, at 8, 18 and 25°C., adding two more humidities of approximately 12 and 54 % R.H. to the original three. The relative humidities of 12 and 54 % were provided by saturated aqueous solutions of $LiCl.H_2O$ and $Na_2Cr_2O_7.2H_2O$ respectively (Wexler and Hasegawa, 1954). This experiment was run over 120 days, and viability was estimated as in above experiment, at regular 30 day - intervals. The results are presented in Tables 100 - 102 and in Fig. 49.

The previously observed response of the conidia to humidity was confirmed in this second experiment (Tables 100 - 102, Fig. 49). 54 % R.H. appeared to accelerate death even more than 34 % R.H. Conidia at 54 % R.H. died first (all within 30 days),

TABLE 100

The longevity of conidia of M.anisopliae stored at 25°C. at constant Relative Humidities of 0, 12.0, 33.2, 53.8 and 75.8 % in DARK.

(Percentage Germination after 24 hours on PDA at 25°C.)

Storage interval in Days.	% Relative Humidity	No. of conidia examined	% Germination	Mean Germ tube length in μ
30	0.0	2794	98.67	411.7
	12.0	2490	98.15	422.5
	33.2	2900	50.26	78.2
	53.8	2526	0.00	-
	75.8	2510	98.00	556.9
60	0.0	2108	64.80	75.5
	12.0	2312	51.47	66.3
	33.2	2004	0.00	-
	53.8	2201	0.00	-
	75.8	3128	26.93	44.4
90	0.0	2848	0.00	-
	12.0	2798	0.00	-
	33.2	2881	0.00	-
	53.8	2895	0.00	-
	75.8	2450	0.00	-
120	0.0	2220	0.00	-
	12.0	2851	0.00	-
	33.2	2992	0.00	-
	53.8	2785	0.00	-
	75.8	2015	0.00	-

TABLE 101

The longevity of conidia of M.anisopliae stored at 18°C. at constant Relative Humidities of 0, 12.5, 33.7, 57.0 and 75.4 % in DARK.

(Percentage Germination after 24 hours on PDA at 25°C.)

Storage interval in Days.	% Relative Humidity	No. of conidia examined	% Germination	Mean Germ tube length in µ
30	0.0	2624	98.32	559.2
	12.5	2442	98.69	573.5
	33.7	2026	63.38	39.8
	57.0	3122	0.19	7.1
	75.4	2402	99.91	671.7
60	0.0	2964	66.69	87.8
	12.5	2246	52.70	70.3
	33.7	2300	0.00	-
	57.0	2119	0.00	-
	75.4	2555	68.87	117.8
90	0.0	2674	10.91	32.7
	12.5	2312	0.00	-
	33.7	2171	0.00	-
	57.0	2002	0.00	-
	75.4	2567	18.00	44.2
120	0.0	2413	0.00	-
	12.5	2177	0.00	-
	33.7	2984	0.00	-
	57.0	2108	0.00	-
	75.4	2975	0.00	-

TABLE 102

The longevity of conidia of M.anisopliae stored at 8°C. at constant Relative Humidities of 0, 13.6, 34.3, 58.4 and 75.2 % in DARK.

(Percentage Germination after 24 hours on PDA at 25°C.)

Storage interval in Days	% Relative Humidity	No. of conidia examined	% Germination	Mean Germ tube length in μ
30	0.0	2612	99.85	642.4
	13.6	2170	99.90	650.4
	34.3	2318	99.56	630.2
	58.4	2258	95.48	629.8
	75.2	2164	100.00	739.1
60	0.0	2304	98.03	427.8
	13.6	2330	97.85	408.1
	34.3	2240	96.43	246.9
	58.4	2477	41.31	53.8
	75.2	2590	100.00	443.7
90	0.0	3112	73.52	284.0
	13.6	2386	49.81	226.9
	34.3	2410	39.09	146.1
	58.4	2076	13.87	36.5
	75.2	2196	99.91	378.7
120	0.0	2178	37.00	129.1
	13.6	2860	6.77	98.3
	34.3	3140	5.60	18.2
	58.4	2512	3.26	16.6
	75.2	3226	99.78	359.9

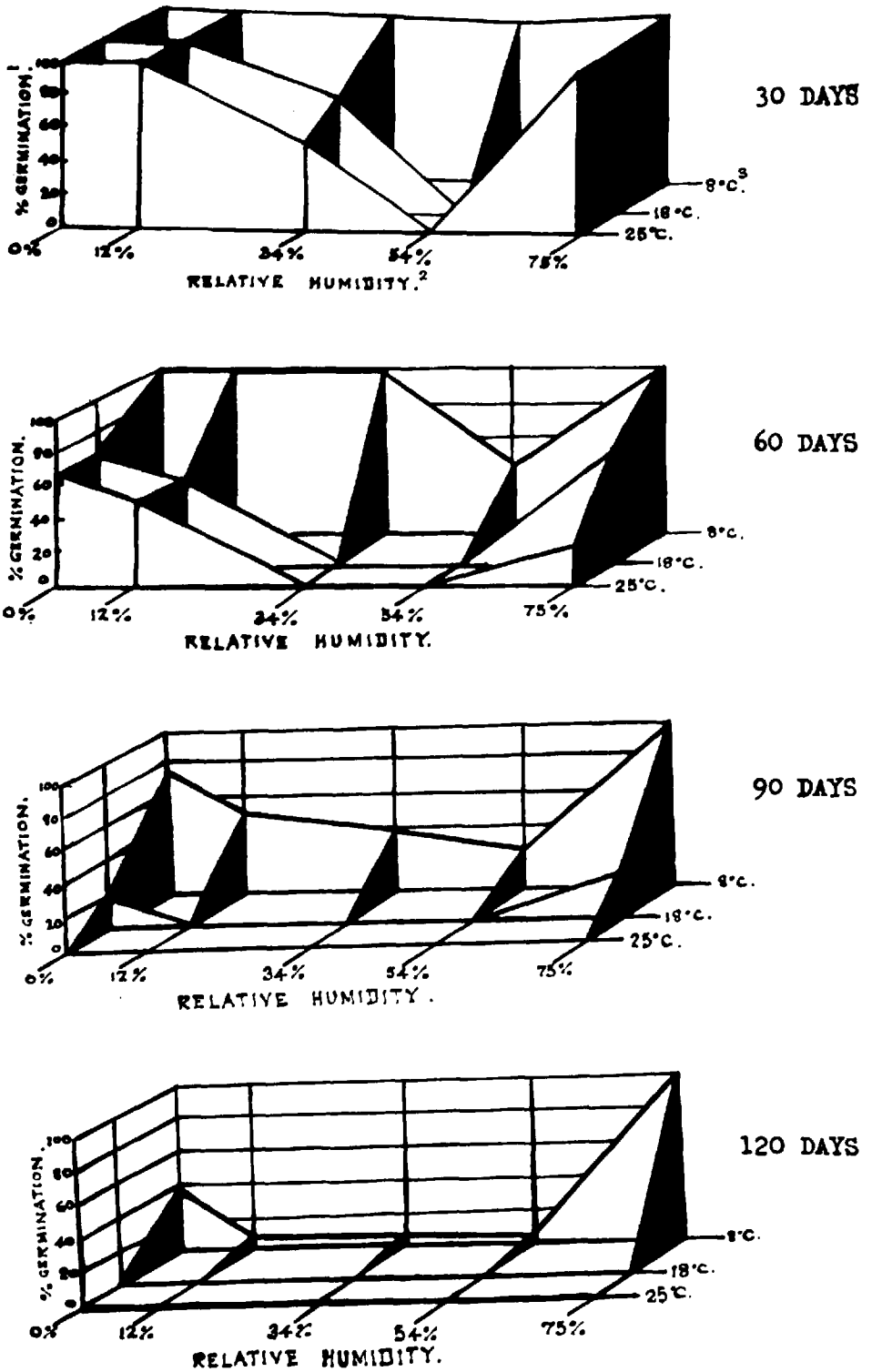


FIG. 49 EFFECT OF RELATIVE HUMIDITY AND TEMPERATURE ON VIABILITY OF CONIDIA OF M.ANISOPLIAE EXPOSED FOR 30, 60, 90 AND 120 DAYS TO A SERIES OF RELATIVE HUMIDITIES IN DARK AT 8, 18 AND 25°C.

[1] % VIABILITY : [2,3] HUMIDITY AND TEMPERATURE OF STORAGE.

followed within the next 30 days by conidia at 34 % R.H. Conidia were preserved for considerably longer periods at 0, 12 and 75 % R.H. The minimum in the viability - R.H. curve was first located at 54 % R.H., then broadened with increased exposure time towards particularly the lower humidities (Fig. 49).

The highest relative humidity of 75 % used in these experiments was considerably below the least relative humidity of 96 % capable of supporting conidium germination in M.anisopliae. (see Table 25). It was considered desirable to conduct a further experiment to provide information on the fate of conidia stored at humidities between 75 and 96 % R.H. An experiment was set up for this purpose. Relative humidities employed and salts used to produce them, were : zero % (Dry calcium chloride), 12.0 % ($LiCl.H_2O$), 33.2 % ($MgCl_2.6H_2O$), 53.8 % ($Na_2Cr_2O_7.2H_2O$), 75.8 % (NaCl), 80.3 % ($(NH_4)_2.SO_4$), 92.0 % (KNO_3) and 96.9 % (K_2SO_4). (Wexler and Hasegawa, 1954). Conidia exposed to these humidities were incubated in the dark at only 25°C. for 84 days, and viability estimated as usual at selected intervals. The results are presented in Table 103 and in Fig. 50.

The data in Table 103 show very clearly that conidia of M.anisopliae were preserved very well at humidities above 75 % R.H. At 96.9 % R.H. the conidia started to germinate; consequently no data on duration of viability could be gathered. Conidia at 75.8,

TABLE 103

The longevity of conidia of M.anisopliae stored at 25°C. at constant Relative Humidities of 0, 12.0, 33.2, 53.8, 75.8, 80.3, 92.0 and 96.9 % in DARK.

(Percentage Germination after 24 hours on PDA at 25°C.)

Storage interval in Days	% Relative Humidity	No. of conidia examined	% Germination	Mean Germ tube length in μ
7	0.0	2486	100.00	452.7
	12.0	2555	99.94	434.3
	33.2	2602	82.36	149.5
	53.8	3080	32.47	76.3
	75.8	3128	99.87	299.3
	80.3	3076	99.93	319.0
	92.0	3320	99.88	400.0
14	0.0	2308	99.65	417.4
	12.0	2111	99.90	403.0
	33.2	2020	68.47	43.8
	53.8	3202	0.40	17.4
	75.8	2150	99.82	299.8
	80.3	2174	99.82	320.1
	92.0	2346	99.91	333.1
28	0.0	2500	99.00	342.4
	12.0	2412	99.08	305.2
	33.2	2193	0.00	-
	53.8	2002	0.00	-
	75.8	3120	97.82	318.5
	80.3	2740	97.93	334.7
	92.0	1936	98.02	349.3

TABLE 103 (cont'd)

The longevity of conidia of M.anisopliae stored at 25°C. at constant Relative Humidities of 0, 12.0, 33.2, 53.8, 75.8, 80.3, 92.0 and 96.9 % in DARK.

(Percentage Germination after 24 hours on PDA at 25°C.)

Storage interval in Days	% Relative Humidity	No. of conidia examined	% Germination	Mean Germ tube length in μ
56	0.0	2088	59.77	112.7
	12.0	2175	51.28	69.1
	33.2	2632	0.00	-
	53.8	2359	0.00	-
	75.8	1912	2.71	18.5
	80.3	2900	2.94	34.1
	92.0	2740	56.97	126.9
84	0.0	2332	0.00	-
	12.0	2175	0.00	-
	33.2	2233	0.00	-
	53.8	2870	0.00	-
	75.8	2352	0.00	-
	80.3	2433	0.00	-
	92.0	2289	0.00	-

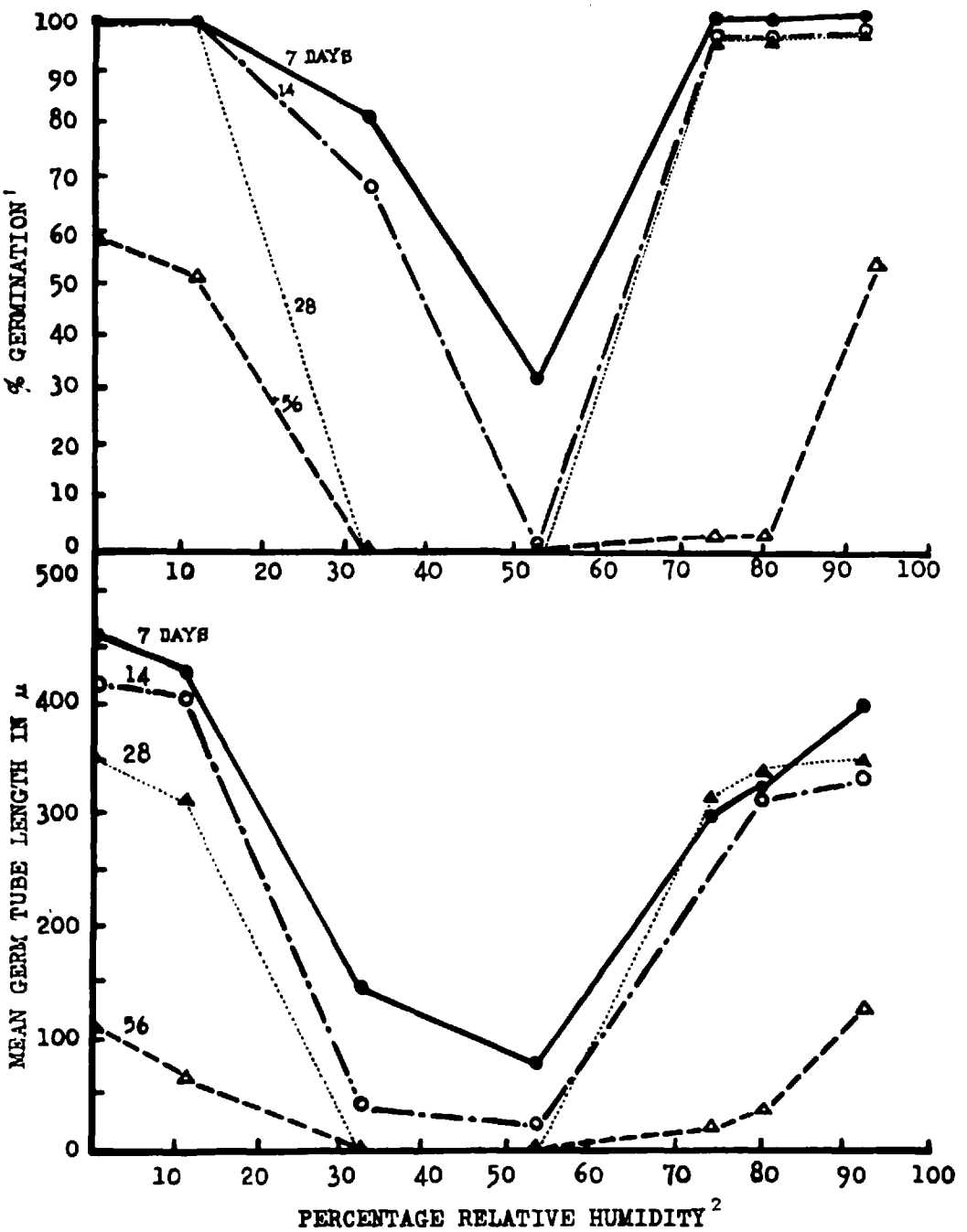


FIG. 50. EFFECT OF RELATIVE HUMIDITY ON VIABILITY OF CONIDIA OF M. ANISOPLIAE DURING STORAGE AT 25°C. FOR 7, 14, 28, 56 AND 84 DAYS . CONSTANT RELATIVE HUMIDITIES WERE PROVIDED BY SATURATED AQUEOUS SALT SOLUTIONS.

¹% VIABILITY ; ²HUMIDITY OF STORAGE.

80.3 and 92.0 % R.H. showed mutually similar degrees of viability in the first 28 days of storage. 39 per cent of the conidia at 92.0 % R.H. were still viable after 56 days, whereas conidia at 75.8 and 80.3 % R.H. showed respective viability of 2.71 and 2.94 per cent.

Pattern of loss of viability of conidia at zero, 12.0, 33.2 and 53.8 % R.H. followed closely results of the previous experiments. The depression in the survival curve (Fig. 50) appeared first at 53.8 % R.H. and broadened in both directions, though at first chiefly towards the lower humidity end of the scale.

Longevity of conidia of M.anisopliae stored in atmospheres of different relative humidities maintained by Glycerol and Sulphuric acid solutions.

Although it seemed unlikely that there would be any effect from the saturated salt solutions used in the above experiments, other than through the relative humidity of the atmosphere above the solutions, the experiment was partially repeated using, aqueous glycerol (Johnson, 1940) (see Table 2) and sulphuric acid (Solomon, 1952) (see Table 3) solutions to provide desired relative humidities. The conidia were incubated in the dark at 25°C. for 7 days, after which viability of the conidia was determined as above. The results are presented in Tables 104 and 105 and in Fig. 51.

After 7 days, the viability of conidia at 44.3 % R.H. in atmospheres controlled with aqueous glycerol solutions (Table 104) was markedly reduced, whilst viability was practically unimpaired by exposure within the same period to relative humidities of 0.0, 16.9, 73.4, 85.2 and 92.8 %. Conidia of M.anisopliae in atmospheres with humidities maintained by glycerol solutions thus showed a similar pattern of survival to those in the earlier experiments, viz., conidia died quicker at mid-humidities, and were preserved for comparatively longer periods at higher and lower humidities.

TABLE 104

Longevity of conidia of M.anisopliae stored in atmospheres of different relative humidities maintained by GLYCEROL SOLUTIONS for 7 Days at 25°C.

(Percentage Germination after 24 hours on PDA at 25°C.)

% Relative Humidity	No. of conidia examined	% Germination	Mean Germ tube length in μ
0.0	2324	100.00	498.6
16.9	2250	100.00	363.3
33.8	2354	97.20	178.1
44.3	2194	61.08	95.8
62.4	2612	94.02	307.9
73.4	2359	99.72	362.6
85.2	2477	99.75	373.1
92.8	2957	100.00	500.2

TABLE 105

Longevity of conidia of M.anisopliae stored in atmospheres of different relative humidities maintained by SULPHURIC ACID SOLUTIONS for 7 Days at 25°C.

(Percentage Germination after 24 hours on PDA at 25°C.)

% Relative Humidity	No. of conidia examined	% Germination	Mean Germ tube length in μ
5.0	2710	99.89	440.8
10.0	3060	99.74	460.0
15.0	3252	99.87	434.6
20.0	2848	98.31	316.9
25.0	2776	96.54	259.9
30.0	2068	58.08	56.0
35.0	2620	50.13	40.5
40.0	2936	0.00	-
45.0	2702	0.00	-
50.0	2814	0.06	1.4
55.0	2282	47.94	136.2
60.0	2994	50.38	135.7
65.0	2868	90.29	361.2
70.0	3020	95.49	360.7
75.0	3016	98.80	448.6
80.0	2985	99.95	452.6
85.0	2221	99.81	447.4
90.0	2190	99.96	496.7

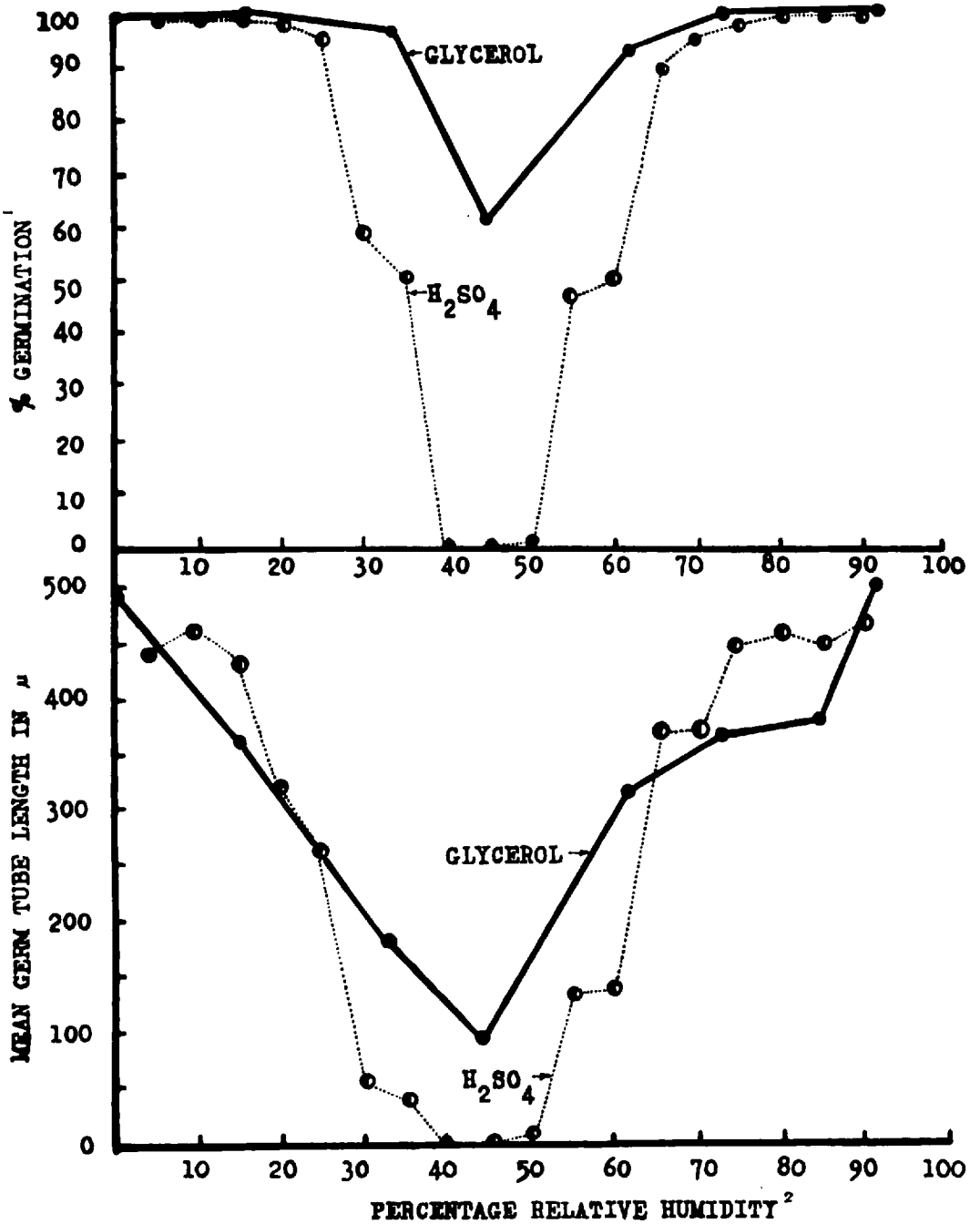


FIG. 51. VIABILITY OF CONIDIA OF M.ANISOPLIAE STORED IN ATMOSPHERES OF DIFFERENT RELATIVE HUMIDITIES CONTROLLED WITH AQUEOUS GLYCEROL AND SULPHURIC ACID SOLUTIONS AT 25°C. FOR 7 DAYS .

[1] % VIABILITY : [2] HUMIDITY OF STORAGE.

Similar pattern of survival was presented by conidia stored in atmospheres whose humidities were controlled with H_2SO_4 solutions. All the conidia were dead at the end of the incubation period of 77 days, in samples exposed to 40 and 45 % R.H., whilst conidia stored at 5, 10, 15, 20, 75, 80, 85 and 90 % R.H. showed viability over 98 per cent in each case (Table 105).

It was observed that conidia in atmospheres whose humidities were maintained by H_2SO_4 solutions lost viability quicker, at least over the unfavourable range (40 - 50 % R.H.), than did conidia in atmospheres with humidities controlled by either saturated salt solutions or aqueous glycerol solutions, within the same period. Whether fumes from the H_2SO_4 had an unfavourable effect on the conidia, and so augmented the humidity effect, or whether the H_2SO_4 absorbed some critical volatile metabolite, with the same effect, cannot at present be decided.

Table 105 and Fig. 51 show that the adverse effects on percentage germination of storage at certain humidities are accompanied by adverse effects on subsequent germ tube growth. The mean germ tube length appears to be a growth measurement more sensitive to storage condition than percentage survival.

iv. Investigation of the effect of Aging on Germination.

(a) Delay in germination.

Conidia which had been stored for long periods, in the longevity tests, often showed, when germinating on potato dextrose agar, shorter germ tubes, at the end of the germination test (24 hours) than those produced by younger conidia under the same conditions. Since the conidia were germinated on a nutrient medium, depletion of endogenous nutrients within the old conidia on long storage seemed unlikely to be the cause of the difference in germ tube lengths. The more probable explanations were that there was either delayed germ tube production, or impaired functioning of the conidium and it's emergent germ tube with age.

An experiment was therefore set up to examine the first supposition. The latent period of germination of approximately one year old conidia was compared with that of young conidia on potato dextrose agar. 364 day old conidia of B.bassiana, which had previously been stored at 25°C. in the dark at zero % R.H., and I.farinosa and M.anisopliae conidia, which had been kept for the same length of time at 8°C. and at zero % R.H., were used. Conidia taken from fresh cultures represented the young conidia. The young and old conidia were germinated at the optimum temperatures appropriate for the species and examined at regular hourly intervals for the first signs of

germination. The results are presented in Table 106.

TABLE 106

Latent Period of Germination of Young and Old Conidia of B.bassiana, I.farinosa and M.anisopliae sown on Potato Dextrose Agar.

FUNGUS	Temperature of Incubation.	Age of conidia in Days	Latent period of germination in Hours
B.BASSIANA	18°C.	Young - 8	6
		Old - 364	17
I.FARINOSA	20°C.	Young - 21	11
		Old - 364	23
M.ANISOPLIAE	25°C.	Young - 5	5
		Old - 364	19

The latent periods of germination of old and young conidia showed very significant differences (Table 106). Age led to a marked delay in germ tube production. Old conidia needed at least 11 hours more than the young conidia to produce germ tubes. It is coincidental that the old conidia used had previously been stored at zero % R.H., where it might be argued that the dehydrated conidia needed a longer time to absorb sufficient water to initiate germination metabolic processes. Delayed germination was however not a result of water relations of the conidium, for,

M.anisopliae conidia which were preserved for long periods (over 400 days) at 75.2 % R.H. at 8°C. (Table 99) suggested by their shorter germ tubes that conidia kept at high humidities still show this habit of delayed germination. Delayed germination in old conidia might have been imposed by interaction of several factors, for example, slowed rate of activation or production of metabolic enzymes, etc.

b. Comparative growth and sporulation of monospore cultures of Young and Old Conidia.

Results from the previous experiment showed that old conidia were slow to produce germ tubes. The second possibility that metabolic functioning was impaired by aging and so contributed to short germ tube growth was examined by comparing the rate of growth and degree of sporulation of monospore cultures from Young and Old conidia (ages as stated in Tables 107 to 109).

The conidia in very low concentration (about 60 spores per ml. of water) were first sown on pyridine-washed plain agar, and single germinating conidia (with about 10 μ long germ tubes) were transferred to synthetic medium agar plates. This germination prior to the experiment served to eliminate differences caused by young and old spores taking different lengths of time to produce germ tubes. The medium was made up as follows : Glucose, 10g. ; NH_4-tartrate, 2g. ; $MgSO_4 \cdot 7H_2O$, 1g. ; KH_2PO_4, 1g. ; $FeSO_4 \cdot 7H_2O$, 0.01g. ; Agar, 20g. ; water, 1000ml. The inoculated plates were incubated at the respective optimum temperatures of the fungi, and diameters of the cultures measured at regular 5 day - intervals. Degree of sporulation of cultures of B.bassiana and M.anisopliae was determined on the 25th day of incubation, following the procedure outlined on page 29. Coremia produced by cultures of I.farinosa were excised and weighed on the 25th day. The results were analysed statistically. The results are presented in Tables 107 - 109.

TABLE 107

Comparative growth and sporulation of monospore cultures from Young and Old conidia of B.bassiana on Synthetic nutrient medium at 18°C.

Source of Monospore Culture	MEAN DIAMETER OF 5 CULTURE PLATES IN MM. Incubation period in Days					Mean Degree of Sporulation (conidia per ml. of water)
	5	10	15	20	25	
Young conidium 8 Days old	15.8	28.0	40.7	55.1	63.2	15.18×10^6
Old conidium 364 Days old	16.1	29.1	41.0	54.4	62.1	14.79×10^6

TABLE 108

Comparative growth and sporulation of monospore cultures from Young and Old conidia of I.farinosa on Synthetic nutrient medium at 20°C.

Source of Monospore Culture	MEAN DIAMETER OF 5 CULTURE PLATES IN MM. Incubation period in Days					Mean Degree of Sporulation (Wt. in GM. of coremia/plate)
	5	10	15	20	25	
Young conidium 21 Days old	14.6	26.8	38.1	50.6	59.7	2.00
Old conidium 364 Days old	14.2	24.6	36.6	51.4	60.5	1.97

TABLE 109

Comparative growth and sporulation of monospore cultures from Young and Old conidia of M.anisopliae on Synthetic nutrient medium at 25°C.

Source of Monospore Culture	MEAN DIAMETER OF 5 CULTURE PLATES IN MM. Incubation period in Days					Mean Degree of Sporulation (conidia per ml. of water)
	5	10	15	20	25	
Young conidium 5 Days old	10.1	22.8	27.9	35.3	42.7	52.68×10^6
Old conidium 364 Days old	11.7	22.0	28.0	35.9	41.7	51.02×10^6

Results of the experiments (Tables 107 - 109) showed that aging apparently had no effect on the metabolic activities of the conidia. The cultures from old and young conidia grew at equal rates, and the degrees of sporulation were closely similar in each pair. There was no statistically significant difference between the growth rates (at $P = 0.05$ and $P = 0.01$) of the cultures at any stage of growth and degree of sporulation (at $P = 0.05$ and $P = 0.01$) between cultures of old and young conidia.

S. STUDIES ON THE ALTERATIONS INDUCED IN CONIDIA OF M.ANISOPLIAE STORED AT CRITICAL MID-HUMIDITIES

It has been suggested (Cochrane, 1958) that in the type of survival - pattern exemplified by conidia of B.bassiana and I.farinosa, the metabolism of the conidia is accelerated at high humidities with a consequent depletion of some essential reserve materials, thus leading to early death. On the other hand, desiccation of the conidia is thought to slow down metabolic activities, and so prolong the life of the conidia.

The response of M.anisopliae conidia to humidity suggests that a special sort of mechanism underlies the effect, different from that in B.bassiana and I.farinosa. A series of experiments was therefore conducted in which attempts were made to gain some insight into the way middling relative humidities shortened the life of conidia.

i. (a) Effect of washing with wetting-agents on response of the conidia to humidity.

Because conidia of M.anisopliae have been observed, throughout this work to be very difficult to wet, it was suspected that they possessed a waxy coat. The degree to which this waxy covering might have impeded water loss or gain by the conidia and subsequently affected the water relations in the conidium , was not known. An experiment was therefore designed to find how a change

in the wettability of the conidial surface might affect response of the conidia to humidity. Conidia of M.anisopliae were washed with wetting-agents at 200 ppm. : Gemex Z11, Nonidet P42 and Manoxol OT (see foot-note), and later dried without rinsing and used in longevity tests. Washing of the conidia lasted exactly 5 minutes. Washed conidia were air-dried at 23°C. for 1 - 2 hours. The conidia were placed in humidity chambers, where relative humidities were maintained with H_2SO_4 (Solomon, 1952) and stored at 25°C. in the dark for 4 days. (This short period of storage over H_2SO_4 was adequate to show the effects of relative humidity on storage). Conidia in control experiments were stored without previous washing. Viability was determined in the usual way, by estimating percentage germination of conidia grown on potato dextrose agar at 25°C. for 24 hours, at the end of the storage period. The results are presented in Tables 110a and b and in Fig. 52.

GEMEX Z-11 - Cationic.

MANOXOL OT - Tolerant to metallic ions.

NONIDET P42 - Anionic.

TABLE 110a

Longevity of conidia of M. anisopliae pre-washed with wetting agent and stored at different relative humidities for 4 days at 25°C.

(Percentage Germination on PDA after 24 hours at 25°C.)

% RELATIVE HUMIDITY	CONTROL UNWASHED CONIDIA			CONIDIA WASHED WITH 200 ppm. MANOXOL OT		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
5	1998	100.00	476.9	2600	99.88	469.6
15	2211	100.00	476.6	2636	99.87	487.1
25	2815	100.00	474.2	2716	99.77	459.7
35	2896	99.68	363.4	2576	99.61	348.4
45	2108	49.90	140.5	2556	91.38	229.9
55	2324	94.32	201.1	2098	25.50	88.9
65	2360	98.31	410.0	2156	33.74	117.1
75	2416	100.00	430.4	2580	52.97	183.6
85	2165	100.00	495.4	2356	98.44	371.9
95	2199	100.00	564.1	2922	99.86	479.8

TABLE 110b

Longevity of conidia of M.anisopliae pre-washed with wetting agent and stored at different relative humidities for 4 days at 25°C.

(Percentage Germination on PDA after 24 hours at 25°C.)

% RELATIVE HUMIDITY	CONIDIA WASHED WITH 200 ppm. GEMEX Z-11			CONIDIA WASHED WITH 200 ppm. NONIDET P 42		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
5	2608	99.63	529.5	2568	99.88	527.8
15	2611	99.62	555.3	2866	99.79	498.1
25	2752	99.77	479.6	2667	99.76	484.8
35	3188	99.62	230.6	2987	99.78	491.6
45	2908	1.52	38.1	2654	56.15	134.7
55	3128	1.53	49.6	2042	0.00	-
65	2072	4.69	44.3	2356	0.00	-
75	2860	5.62	48.0	2870	4.99	97.6
85	3032	97.89	233.8	2376	98.05	383.8
95	2464	99.63	522.1	2522	99.62	500.8

The results presented in Tables 110a and b reveal that the unfavourable relative humidity for unwashed conidia was 45 %, as in the previous experiments, but that the conidia of M.anisopliae washed with the wetting agents showed a very significant shift and broadening of the unfavourable R.H. from 45 % to 55 - 75 % (Fig. 52).

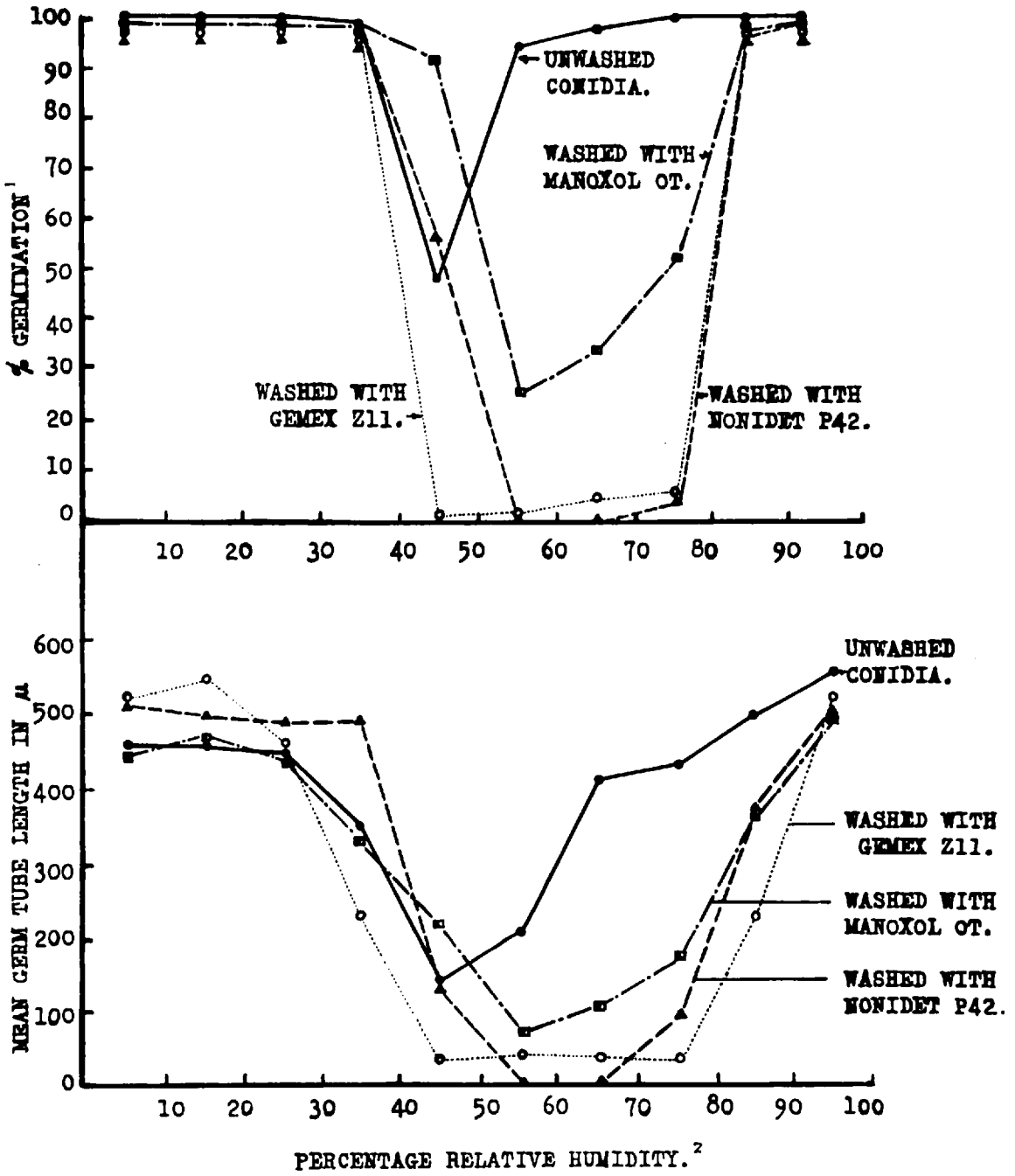


FIG. 52. VIABILITY OF CONIDIA OF M.ANISOPLIAE PRE-WASHED WITH SURFACTANTS AND STORED AT DIFFERENT RELATIVE HUMIDITIES FOR 4 DAYS AT 25°C.

[1] % VIABILITY : [2] HUMIDITY OF STORAGE.

(b) Effect of different concentrations of Gemex Z11.

Although the distinctive pattern of survival of conidia of M.anisopliae persisted on pre-washing with wetting agents, the critical humidity shifted towards the higher humidities. An experiment was set up to investigate the influence of the concentration of the wetting agent. The above experiment was repeated, using Gemex Z11, selected arbitrarily from the three washing agents, at concentrations of 1, 10, 100, 1000 and 10000 ppm. Controls with unwashed and water-washed conidia were run. Conidia stored for 4 days at different relative humidities, controlled by H_2SO_4 solutions (Solomon, 1952) in darkness at 25°C., were then tested for viability as above. The results are presented in Tables 111a, b, c and d.

The results of the experiments (Tables 111a, b, c and d) were in complete agreement with the results of the previous ones (Tables 110a and b). Conidia in all treatments showed high survival at the more extreme humidities and lost viability most rapidly at the mid-humidities. Water and the different concentrations of Gemex Z11 effected identical shifts of the unfavourable humidity towards the high humidities. Generally, the unfavourable humidity was around 65 - 75 % R.H., in the washed conidia, in contrast to 45 % R.H. in unwashed conidia.

It was obvious that washing with water alone was enough to effect the shift in the unfavourable humidity range.

TABLE 111a

Longevity of conidia of M.anisopliae pre-washed with Gemex Z11 and stored at different relative humidities for 4 days at 25°C.

(Percentage Germination on PDA after 24 hours at 25°C.)

% RELATIVE HUMIDITY	CONIDIA WASHED WITH WATER			CONIDIA WASHED WITH 1ppm. GEMEX Z11		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
5	2500	99.84	380.9	2816	98.86	387.4
15	2086	99.75	336.3	1892	99.79	348.7
25	2553	92.29	332.6	2980	91.82	216.9
35	2146	89.59	117.9	1726	82.16	140.7
45	2477	33.02	74.7	1938	60.68	135.7
55	2269	23.43	42.0	2055	30.41	65.9
65	2595	0.00	-	2156	0.00	-
75	1957	0.00	-	2268	0.00	-
85	2968	31.71	286.4	2144	52.12	217.6
95	2288	97.45	399.2	2406	99.75	442.1

TABLE 111b

Longevity of conidia of M.anisopliae pre-washed with Gemex Z11 and stored at different relative humidities for 4 days at 25°C.

(Percentage Germination on PDA after 24 hours at 25°C.)

% RELATIVE HUMIDITY	CONIDIA WASHED WITH 10 ppm. GEMEX Z11			CONIDIA WASHED WITH 100ppm. GEMEX Z11		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
5	3168	99.74	399.2	1714	99.30	478.0
15	2804	99.72	317.8	1878	99.77	325.2
25	2736	90.79	288.0	2964	88.39	284.5
35	2555	80.36	152.0	2159	86.90	130.0
45	2628	21.51	31.[illegible]	1882	37.31	55.4
55	2171	19.21	30.[illegible]	1836	38.97	36.5
65	2157	0.00	-	2058	0.00	-
75	1973	0.00	-	1864	0.00	-
85	2071	28.50	286.2	2200	41.12	290.2
95	2674	97.37	435.1	2114	98.01	417.8

TABLE 111c

Longevity of conidia of M.anisopliae pre-washed with Gemex Z11 and stored at different relative humidities for 4 days at 25°C.

(Percentage Germination on PDA after 24 hours at 25°C.)

% RELATIVE HUMIDITY	CONIDIA WASHED WITH 1000 ppm. GEMEX Z11			CONIDIA WASHED WITH 10000ppm. GEMEX Z11		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
5	2690	99.53	392.1	1812	99.89	407.6
15	2270	99.29	378.4	1950	99.70	425.9
25	2588	88.54	392.8	2068	98.63	440.7
35	2462	76.95	243.7	1962	90.31	232.4
45	2005	61.29	96.5	2175	82.47	145.7
55	2762	53.77	58.4	2538	82.97	75.8
65	1872	0.00	-	2830	0.28	23.5
75	1994	0.00	-	2371	0.35	18.9
85	2486	37.21	172.6	1918	71.33	286.4
95	2696	100.00	383.3	2049	99.92	453.8

TABLE 111d

CONTROL

Longevity of unwashed conidia of M. anisopliae stored at different relative humidities for 4 days at 25°C.

(Percentage Germination on PDA after 24 hours at 25°C.)

% RELATIVE HUMIDITY	No. of conidia examined	% Germination	Mean Germ tube length in μ
5	2210	100.00	500.5
15	2425	99.64	488.6
25	2005	99.85	492.7
35	1972	99.02	354.0
45	1903	2.68	43.4
55	2170	35.32	102.4
65	2509	95.28	400.3
75	2121	99.49	446.3
85	2634	99.88	475.7
95	2100	99.92	512.5

c. Conidia on the threshold of germination.

The above experiments showed that washing with water induced some changes in the conidium, which effected a shift of the unfavourable humidity range from 40 - 45 % to 55 - 75 % R.H.

Washed conidia took 1 to 2 hours at 23°C. to dry. One possible effect of washing during the period when the conidia were in the process of drying but still mostly wet, was the possible initiation of germination activities. Gottlieb and Caltrider (1963) reported that enzyme synthesis in germinating conidia of Ustilago zeae was initiated several hours before germ tubes appeared. Conidia of M.anisopliae possess a latent period of germination of 5 hours, and a period of 1 to 2 hours would probably be enough to evoke initial germination activities. The longevity at different humidities of conidia which had previously been exposed to conditions conducive to germination was therefore examined, to test whether initiation of germination activities in the conidia was responsible for the observed shift in the unfavourable humidity.

Conidia of M.anisopliae were shaken in water on shaking machine in a constant temperature room of 25°C. for 2 and 4 hours respectively and then quickly dried under a stream of cool dry air. The apparatus used for drying the conidia is shown below in Fig. 53. Air was drawn through a series of dry calcium chloride tubes (X_1 - X_5) and conducted into a spore chamber. The spore

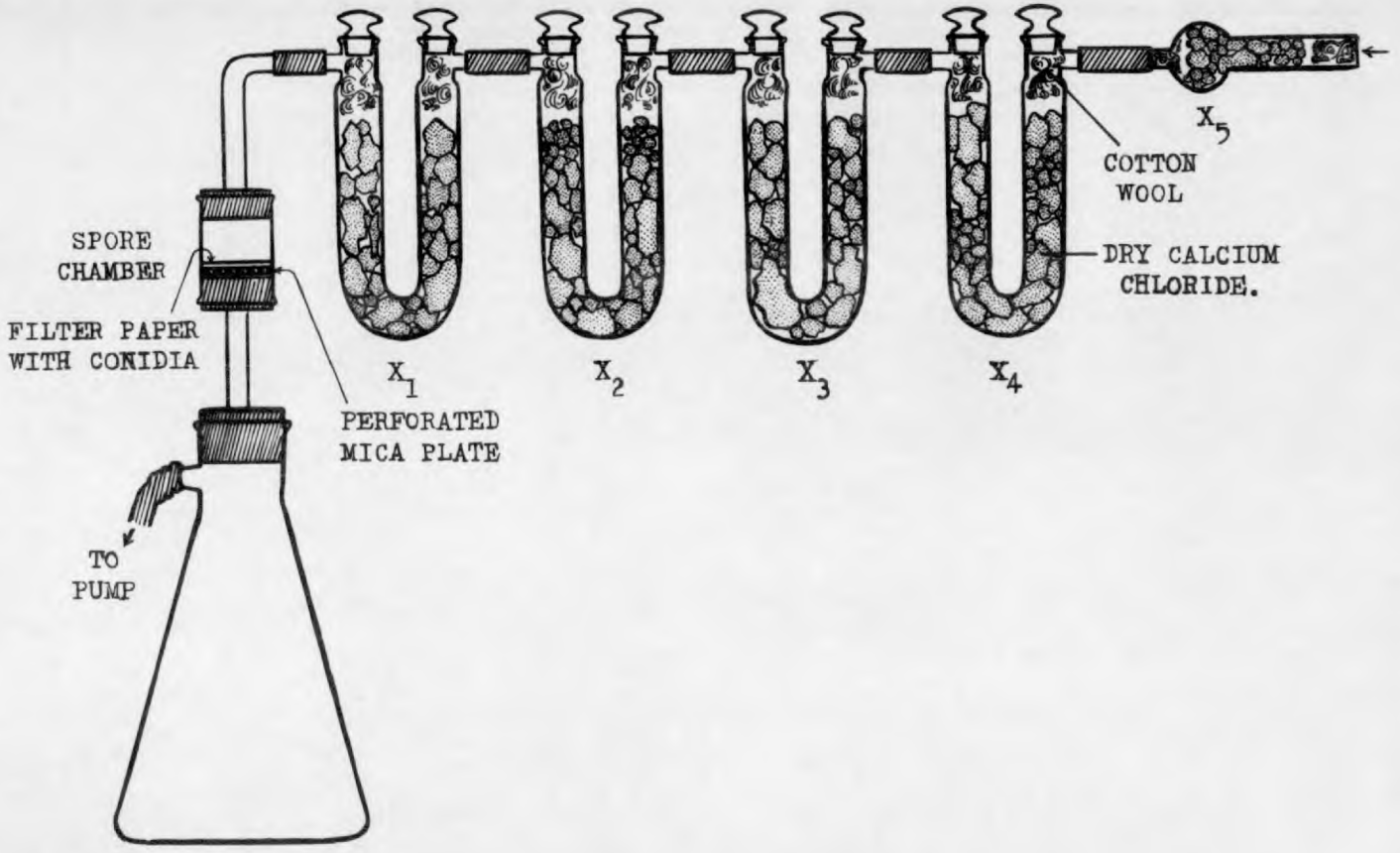


FIG. 53. SPORE DRYING APPARATUS.

chamber held a disc of filter paper with a deposit of wet conidia. The conidia were always completely dried within 2 minutes.

The dried conidia were then stored in humidity chambers of different relative humidities, controlled with H_2SO_4 solutions (Solomon, 1952) and incubated at 25°C. in the dark for 4 days, after which viability was determined in the usual way.

Two control experiments were run, one with unwashed conidia, the second with conidia washed in such a way as to eliminate the likelihood of a biochemical change incident to germination. The conidia were washed with water at 0°C. for exactly 1 minute and then quickly dried with the spore drying apparatus (Fig. 53). The entire process of washing and drying took exactly 4 minutes. The results are presented in Tables 112a and b.

Conidia of M. anisopliae kept in water for 4 hours at 25°C., where conditions were maintained long enough to induce germination activities, showed the same pattern of survival, with a shift of the unfavourable humidity to 55 - 75 % R.H., as conidia washed at 0°C. for a minute (Tables 112a and b), whereas the unfavourable humidity occurred at 45 % R.H. in experiments with unwashed conidia.

Possible initiation of germination activities on wetting was evidently not responsible for the shift in the unfavourable humidity range. The effect was clearly caused by the act of washing in water.

TABLE 112a

Longevity of conidia of M.anisopliae brought to the threshold of germination and stored at different relative humidities for 4 days at 25°C.

(Percentage Germination on PDA after 24 hours at 25°C.)

% RELATIVE HUMIDITY	CONTROL UNWASHED CONIDIA			CONTROL CONIDIA WASHED AT 0°C. FOR 1 MINUTE		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
5	2991	100.00	476.9	2172	96.86	471.4
15	3212	100.00	476.7	1920	91.25	415.4
25	2055	99.75	474.2	2148	91.25	355.9
35	2698	99.50	360.4	1892	91.06	227.3
45	2008	33.00	140.5	1968	77.48	158.7
55	2432	65.32	201.1	2573	58.32	90.2
65	2063	99.00	317.5	1959	49.41	97.1
75	1964	100.00	410.0	2068	53.55	182.8
85	2561	100.00	484.4	2044	94.36	321.5
95	1991	100.00	524.1	2241	99.82	491.4

TABLE 112b

Longevity of conidia of M.anisopliae brought to the threshold of germination and stored at different relative humidities for 4 days at 25°C.

(Percentage Germination on PDA after 24 hours at 25°C.)

% RELATIVE HUMIDITY	CONIDIA WASHED FOR 2 HOURS.			CONIDIA WASHED FOR 4 HOURS.		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
5	2103	99.71	422.0	2198	99.64	500.1
15	2245	99.28	401.4	2308	99.48	405.7
25	2334	98.78	362.1	1821	99.34	337.6
35	2794	98.66	218.6	2484	99.37	299.8
45	2364	58.66	129.7	2845	58.00	187.9
55	2020	36.24	60.3	2249	37.01	85.8
65	2185	40.33	58.9	3300	33.45	77.8
75	2048	56.79	150.3	2046	48.23	136.4
85	2892	87.11	318.4	2888	75.63	225.7
95	2733	100.00	509.5	2592	99.69	487.4

d. Hysteresis effect.

In the previous experiments where conidia were washed, the conidia were subsequently dried to a level below the water content of fresh unwashed conidia. Experiments with fresh unwashed conidia and washed ones thus involved the use of conidium samples of two different initial levels of moisture. The possibility existed that conidia stored at mid-range humidities equilibrated with those humidities in the one instance by losing water, in the other by absorbing it. Perhaps the difference between the unfavourable relative humidities for washed and unwashed conidia reflected a hysteresis effect in some substance or structure critically involved in spore survival, whose characteristics depended on whether it reached a certain state of hydration from a moister or drier initial condition.

Conidia were washed with water for a minute and then dried under a stream of cool dry air. A second conidium sample was also washed with water and centrifuged at 6,000 revolutions per minute for 5 minutes. The conidial sediment was then transferred onto a disc of filter paper to absorb the excess moisture. These two conidial samples with obviously two originally different moisture levels were incubated at different relative humidities, maintained with H_2SO_4 solutions (Solomon, 1952) in the dark at 25°C. for 4 days. The viability of the conidia was determined after that period in the usual way, by evaluating percentage germination of

conidia sown on potato dextrose agar for 24 hours at 25°C. The results are presented in Tables 113a and b.

It was evident from the results in Tables 113a and b, that the shift in the unfavourable humidity range, to 55 - 75 % R.H. was exhibited by conidia of either treatment, whereas unwashed conidia showed a critical humidity of 45 - 55 % R.H. It seemed therefore that hysteresis effect was possibly not responsible for the shift of the critical (unfavourable) humidity range.

TABLE 113a

CONTROL

Longevity of unwashed conidia of M.anisopliae stored at different relative humidities for 4 days at 25°C.

(Percentage Germination on PDA after 24 hours at 25°C.)

% RELATIVE HUMIDITY	No. of conidia examined	% Germination	Mean Germ tube length in μ
5	2033	99.98	392.4
15	2172	99.53	376.0
25	3125	99.60	338.9
35	2444	97.13	250.0
45	3001	41.00	94.9
55	2232	49.75	148.6
65	2188	93.83	212.4
75	1972	99.95	402.3
85	2361	100.00	439.5
95	2752	100.00	533.0

TABLE 113b

Longevity of conidia of M.anisopliae of originally different water content stored at different relative humidities for 4 days at 25°C.

(Percentage Germination on PDA after 24 hours at 25°C.)

% RELATIVE HUMIDITY	CONIDIA WASHED AND DRIED AT 0.00 % R.H.			CONIDIA WASHED AND WATER DRAINED ON FILTER PAPER.		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
5	2168	98.89	455.9	2198	98.45	474.1
15	1755	98.20	401.5	2180	98.17	433.6
25	1788	98.24	367.5	2060	95.34	374.7
35	1974	98.16	293.6	1921	93.56	322.1
45	2276	78.21	201.0	1913	63.81	212.1
55	2013	42.33	161.8	1856	41.81	90.6
65	2169	35.13	85.3	2028	52.07	104.2
75	1848	55.32	101.5	2140	68.04	180.1
85	2780	84.72	353.5	2252	88.39	398.1
95	2292	99.82	497.0	2621	99.58	498.1

ii. Working Hypothesis.

The above experiments showed that washing the conidia of M.anisopliae with water modified the response of the stored conidia to humidity. As a working hypothesis, it was presumed that there was a humidity-sensitive membrane on or in the wall or around the conidium's cytoplasm, which contained hygroscopic colloidal materials, and the property of which was modified by washing. This membrane was supposed to critically maintain the vitality of the conidium by controlling the entrance or escape of gases which regulate the metabolic activity of the conidium and hence the duration of it's viability. This critical membrane was presumed to be more permeable at the mid-humidities and there was free diffusion of gases with incidence of high metabolic activities, which led to more rapid loss of essential reserve materials and loss of viability. At the higher and lower humidities, the membrane was less permeable, and either oxygen was excluded from the conidium or carbon dioxide or other volatile metabolites retained within the conidium. In either alternative, metabolic activities will be considerably lowered with possible conservation of spore vigour. Experiments were therefore carried out to find how closely these suppositions approximate to real occurrences in the conidia.

(a) Washing conidia of M.anisopliae with salts of the Hoffmeister ion (Lyotropic) series.

On the supposition that the humidity-sensitive membrane

contained hygroscopic colloidal materials, and that washing produced a shift in the unfavourable humidity range by modifying their physical properties, experiments were made in an attempt to alter the degree of swelling or contraction of the hypotheticated components of the humidity-sensitive membrane so as to alter it's permeability.

The degree of swelling of colloids is known to be influenced by salts according to their position in the Hoffmeister ion (Lyotropic) series[1]. (Meyer and Anderson, 1939). An attempt was therefore made to alter the presumed swelling of the critical humidity-sensitive membrane by washing the conidia with salts, at concentrations of 0.1, 0.01 and 0.001 Molal, selected for their position in the Hoffmeister ion (Lyotropic) series (see foot-note).

Conidia of M.anisopliae were in the first instance washed with salts, with univalent cations : Ammonium nitrate, ammonium sulphate,

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[1]Two lyotropic series of cations, and one of anions, are generally recognised, as follows :

Univalent cations : Li - Na - NH_4 - K - Rb - Cs.

Bivalent cations : Mg - Ca - Sr - Ba.

Anions : CNS - I - NO_3 - Br - Cl - SO_4.

As arranged above the series represent (reading from left to right) the order of their increasing effectiveness in checking the imbibition / of water by kelp stipe.

caesium iodide, caesium sulphate, lithium iodide, lithium sulphate, potassium nitrate, potassium sulphate, sodium nitrate and sodium sulphate. Washed conidia were centrifuged at 6,000 revolutions per minute for 5 minutes and the conidial deposits placed on filter paper to absorb the excess moisture and incubated at different relative humidities (controlled with H_2SO_4 solutions, Solomon, 1952) at 25°C. for 7 days. Control experiments were run with unwashed conidia , and conidia washed with plain water. Viability of the conidia was determined after that period, by germinating the conidia on potato dextrose agar at 25°C. for 24 hours. The results are presented in graph form in Figs. 54a, b and c., and in Table 114 (abstract of data on viability of conidia stored at 75 % R.H.).

The results (Figs. 54a, b and c.) show that washing the conidia with water and different concentrations of salts, with the exception of sodium nitrate and sodium sulphate, not only failed to alter the unique survival pattern of conidia of M.anisopliae, but also, did not rectify the shift of the unfavourable humidity range, from 45 - 55 % R.H. to 55 - 75 % R.H., characteristic of washed conidia. Though sodium nitrate and sodium sulphate also failed to alter the basic survival response of stored M.anisopliae conidia to humidity, conidia washed with these salts showed more or less similar response to that of unwashed conidia (Fig.54c,) Table 114), and conidia washed with these salts and stored at

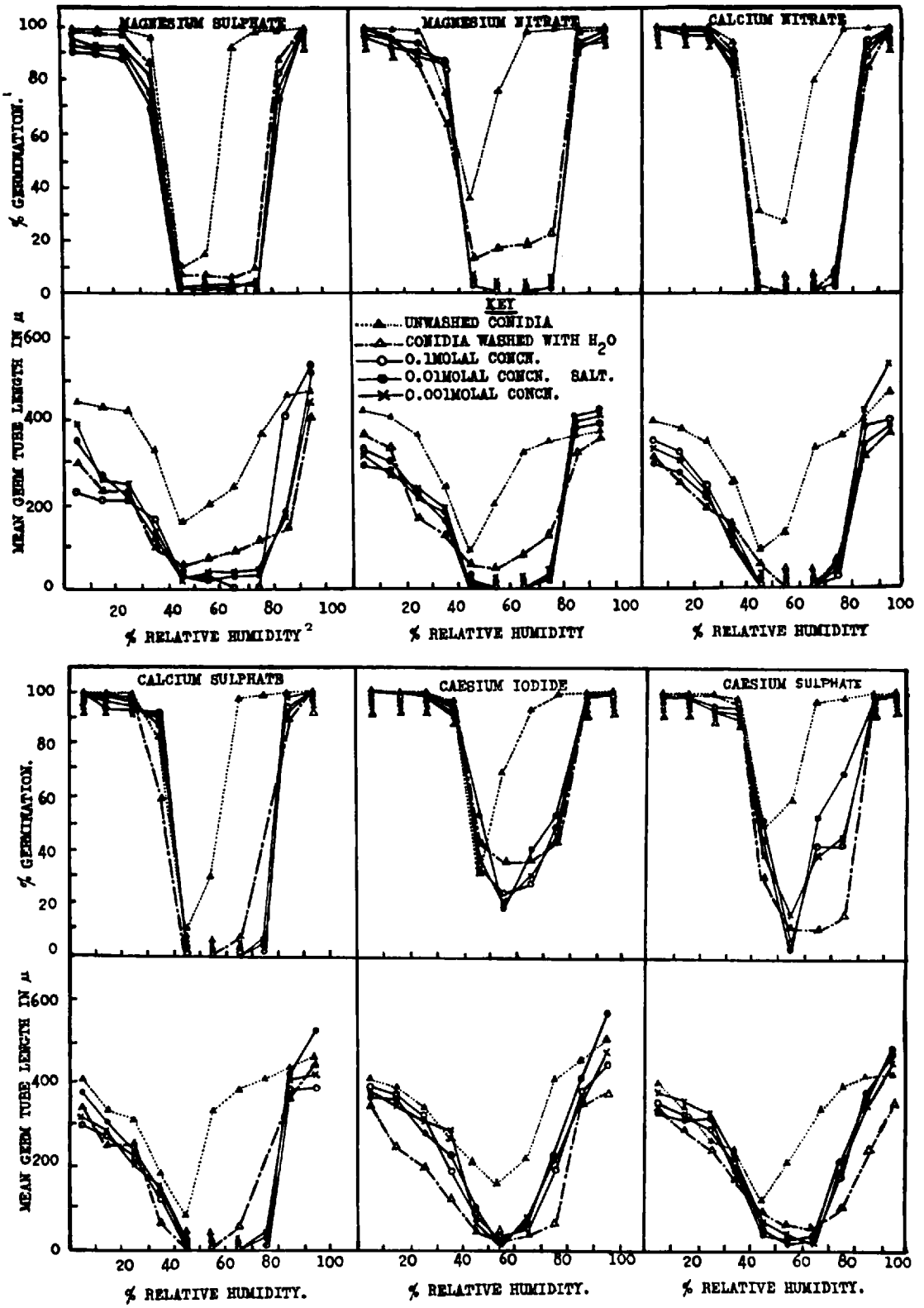


FIG 54a. VIABILITY OF CONIDIA OF M.ANISOPLIAE PRE-WASHED WITH SALTS (WITH IONS OF THE "LYOTROPIC" SERIES) AND STORED AT DIFFERENT RELATIVE HUMIDITIES FOR 7 DAYS AT 25°C.

[1] % VIABILITY : [2] HUMIDITY OF STORAGE.

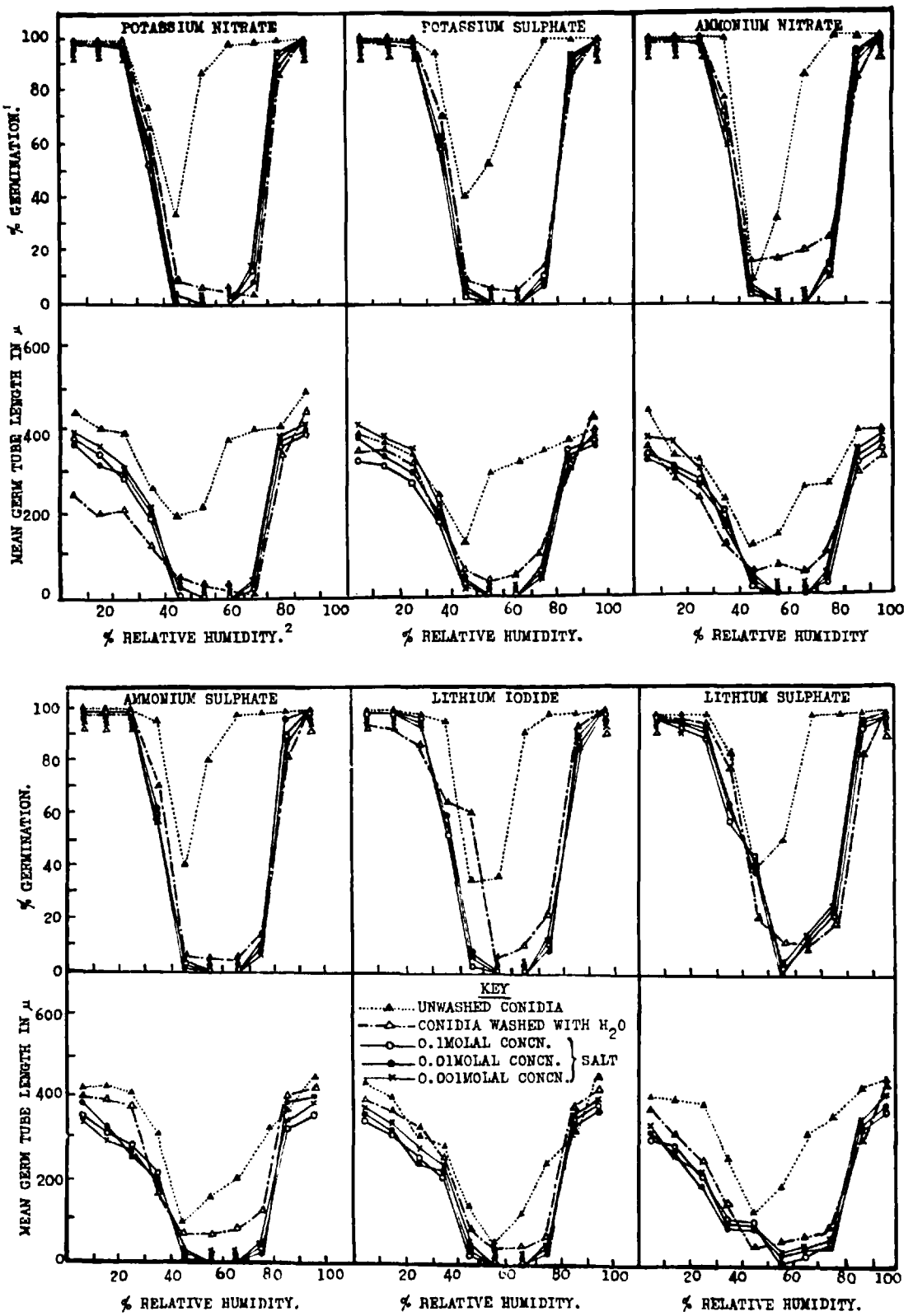


FIG. 54b. VIABILITY OF CONIDIA OF M.ANISOPLIAE PRE-WASHED WITH SALTS (WITH IONS OF THE "LYOTROPIC" SERIES) AND STORED AT DIFFERENT RELATIVE HUMIDITIES FOR 7 DAYS AT 25°C.

[1] % VIABILITY : [2] HUMIDITY OF STORAGE.

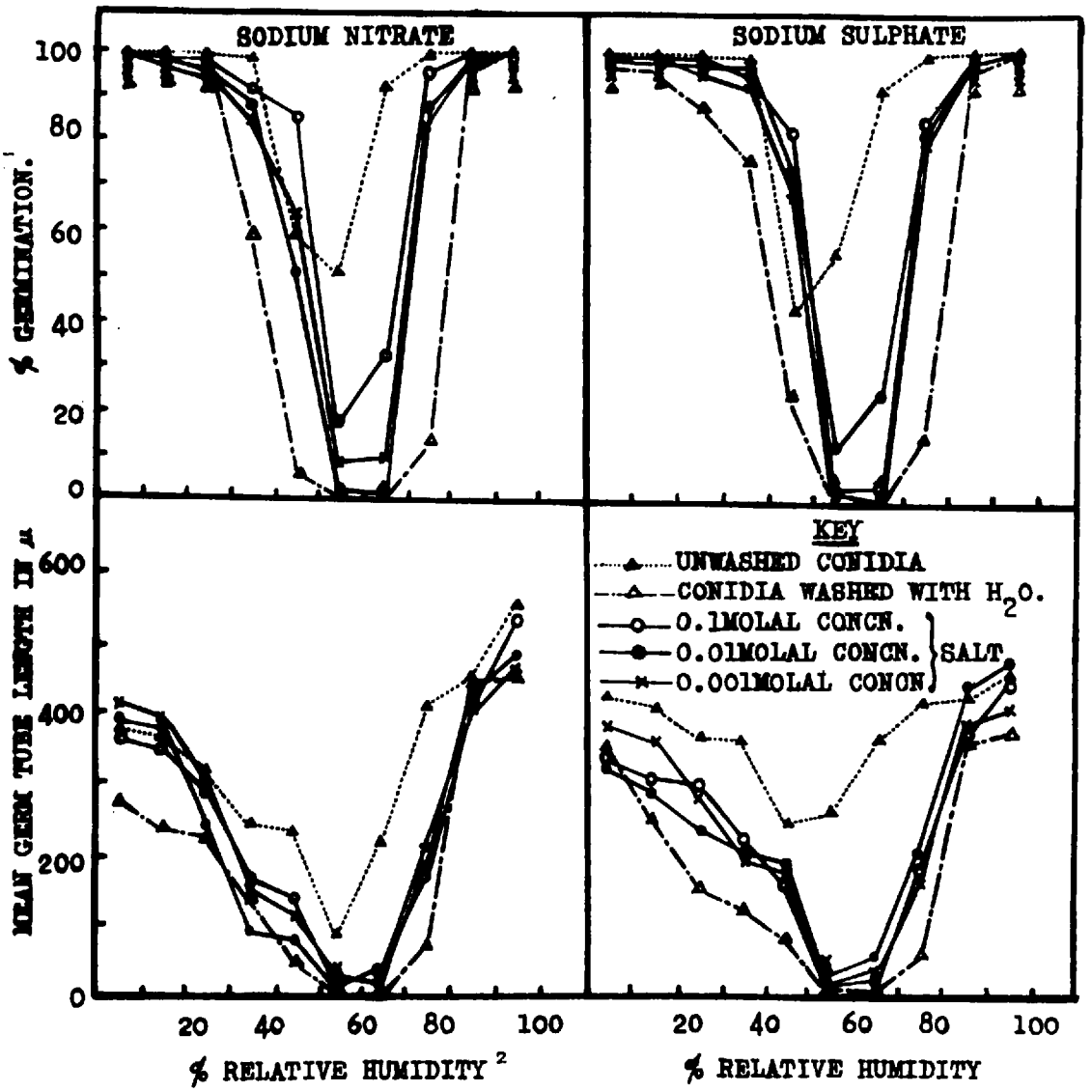


FIG. 54c. VIABILITY OF CONIDIA OF M.ANISOPLIAE PRE-WASHED WITH SALTS (WITH IONS OF THE "LYOTROPIC" SERIES) AND STORED AT DIFFERENT RELATIVE HUMIDITIES FOR 7 DAYS AT 25° C.

[1] % VIABILITY : [2] HUMIDITY OF STORAGE.

75 % R.H. retained a high degree of viability, closely similar to that of unwashed conidia. Apparently, addition of sodium to the spores partially nullified the effect of washing. Probably sodium replaced a factor removed from the conidia by washing.

Because of this effect of sodium, salts (with bivalent cations) : calcium nitrate, calcium sulphate, magnesium nitrate and magnesium sulphate were tried in a subsequent experiment. These were chosen for their positions in the bivalent cation series, which are parallel to the position of sodium in the univalent cation series. These salts however did not have any effect on the conidia as portrayed by spores washed with sodium nitrate and sodium sulphate. (Fig. 54a and b.). Conidia washed with these salts showed a shift of the unfavourable humidity (55 - 75 % R.H.) charactoristic of washed conidia.

TABLE 114

Abstract of data of Fig. 54 : Viability of conidia of M.anisopliae pre-washed with salts (of Lyotropic series) and stored at 75 % Relative Humidity for 7 days at 25°C.

(Percentage germination on PDA after 24 hours at 25°C.)

SALT	CONTROL		CONCENTRATION OF SALT		
	Unwashed conidia.	Conidia washed with plain water	0.1 Molal	0.01 Molal	0.001 Molal
	% Germination	% Germination	% Germination	% Germination	% Germination
Ammonium nitrate	99.52	15.62	11.72	12.32	9.72
Ammonium sulphate	99.46	7.89	8.72	8.33	9.00
Caesium iodide	99.72	55.24	49.65	53.47	48.32
Caesium sulphate	99.90	51.58	43.08	53.84	44.65
Calcium nitrate	99.00	0.18	0.00	0.00	0.00
Calcium sulphate	99.02	0.00	0.00	3.42	0.00
Lithium iodide	99.62	8.25	10.12	8.98	9.25
Lithium sulphate	99.34	1.59	22.76	25.42	24.89
Magnesium nitrate	99.64	10.92	0.50	2.60	1.85
Magnesium sulphate	99.10	8.33	0.00	2.69	4.22
Potassium nitrate	99.33	11.12	12.72	11.51	8.88
Potassium sulphate	99.05	10.62	10.00	7.29	9.51
Sodium nitrate	99.50	12.16	96.04	86.14	82.73
Sodium sulphate	99.74	16.29	84.62	83.42	79.85

(b) Effect of High level of Carbon Dioxide.

It was suggested in the working hypothesis that, at the higher and lower humidities, there is the exclusion or retention of gaseous or volatile regulators of respiratory activity. Clearly the possibility to be investigated first was that gaseous respiratory participants, viz., carbon dioxide and/or oxygen, were involved.

The possibility that retention of carbon dioxide by a humidity-sensitive membrane slowed down respiratory activities in conidia stored at higher and lower humidities, and so prolonged their viability, was investigated in the following experiment. If such a system operated in the conidia, the usual survival response should not be demonstrable in spores stored in a high carbon dioxide atmosphere.

Humidity chambers (glass jars, 5 cm. high and 7 cm. in diameter) were prepared as usual, holding H_2SO_4 solutions to provide the relative humidities (Solomon, 1952). Tiny specimen tubes, 15 mm. high and 8 mm. in diameter, holding 1 ml. sodium bicarbonate solution, 1 Molal concentration, were stood in the humidity chambers before they were sealed. Each humidity jar held 2 tubes of $NaHCO_3$ solution and sample of conidia of M.anisopliae. The stored conidia were incubated at 25°C. in the dark for 4 days, after which viability was determined in the usual way, by germinating the conidia on potato dextrose agar at 25°C. for 24 hours. Control humidity jars held no $NaHCO_3$ solutions. On evaporation, the $NaHCO_3$ solution

released carbon dioxide into the ambient atmosphere to raise the level above normal atmospheric concentration. (The $NaHCO_3$ solutions in the humidity jars were found to have completely evaporated at all humidities used at the end of the incubation period.). The results are presented in Table 115.

TABLE 115

Longevity of conidia of M.anisopliae stored in High CO_2 Atmospheres of different relative humidities for 4 days at 25°C.

(Percentage Germination on PDA after 24 hours at 25°C.)

% RELATIVE HUMIDITY	CONIDIA IN HIGH CO_2 ATMOSPHERE			CONTROL CONIDIA IN NORMAL AIR		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
5	2033	99.82	466.2	2105	99.95	392.9
15	2174	99.84	466.1	2516	98.93	370.7
25	2392	99.45	469.7	2233	98.03	323.4
35	2180	99.68	458.4	2364	91.87	261.3
45	2195	99.77	471.0	2377	24.29	60.6
55	2072	99.62	461.3	2075	42.85	62.9
65	2004	99.83	482.1	2265	89.62	241.3
75	2034	99.96	496.6	2280	99.02	331.0
85	2246	99.98	490.2	2336	100.00	480.8
95	2433	99.94	498.3	2314	100.00	518.5

The results of the experiments (Table 115) were clear. Conidia of M.anisopliae stored in atmospheres of high CO_2 at the different relative humidities retained viability to the same degree. Survival was beyond 99 per cent at all humidities, whereas conidia in normal air showed the usual rapid loss of viability at 45 to 55 % R.H. The presence of an elevated concentration of CO_2 (exact concentration not known in this experiment) had a significant influence on survival of M.anisopliae conidia.

In order to confirm this result, similar experiments to the above were performed, the conidia being stored in high CO_2-atmospheres for 7 and 14 days respectively. The results are presented in Tables 116 and 117.

The results fully confirmed those of the previous experiment in Table 115.

TABLE 116

Longevity of conidia of M.anisopliae stored in High CO_2 Atmospheres of different relative humidities for 7 days at 25°C.

(Percentage Germination on PDA after 24 hours at 25°C.)

% RELATIVE HUMIDITY	CONIDIA IN HIGH CO_2 ATMOSPHERE			CONTROL CONIDIA IN NORMAL AIR		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
5	2216	99.64	473.9	2100	99.00	347.2
15	2080	99.42	416.7	2108	99.02	305.8
25	2159	99.63	425.9	2114	98.37	266.4
35	2308	99.48	418.9	2133	78.65	125.0
45	2136	99.62	434.2	2235	0.00	-
55	2161	99.81	427.2	2417	8.00	40.6
65	2300	99.83	433.4	2302	91.62	175.8
75	2111	99.81	461.3	2332	98.95	288.7
85	1943	99.79	490.6	2236	99.35	326.3
95	2456	99.84	551.6	2085	99.29	389.7

TABLE 117

Longevity of conidia of M.anisopliae stored in High CO_2 Atmospheres of different relative humidities for 14 days at 25°C.

(Percentage Germination on PDA after 24 hours at 25°C.)

% RELATIVE HUMIDITY	CONIDIA IN HIGH CO_2 ATMOSPHERE			CONTROL CONIDIA IN NORMAL AIR		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
5	2621	99.28	380.6	2724	99.56	389.9
15	1875	99.20	387.9	2860	98.74	386.6
25	2388	96.08	252.9	2544	98.89	288.9
35	1890	95.24	239.1	2980	63.09	127.5
45	1992	95.03	264.6	2470	0.00	-
55	1977	95.05	237.9	2032	0.00	-
65	2146	97.50	357.6	2344	18.25	34.0
75	2568	99.37	396.3	2772	76.77	180.9
85	2188	99.26	383.5	2197	93.49	347.8
95	2236	99.46	403.8	1969	99.59	392.5

The effect of high CO_2 level on duration of viability was consistent with the view that, with increased CO_2 concentration in the ambient atmosphere, the respiration rate decreased in the conidia, with resultant conservation of essential reserve materials

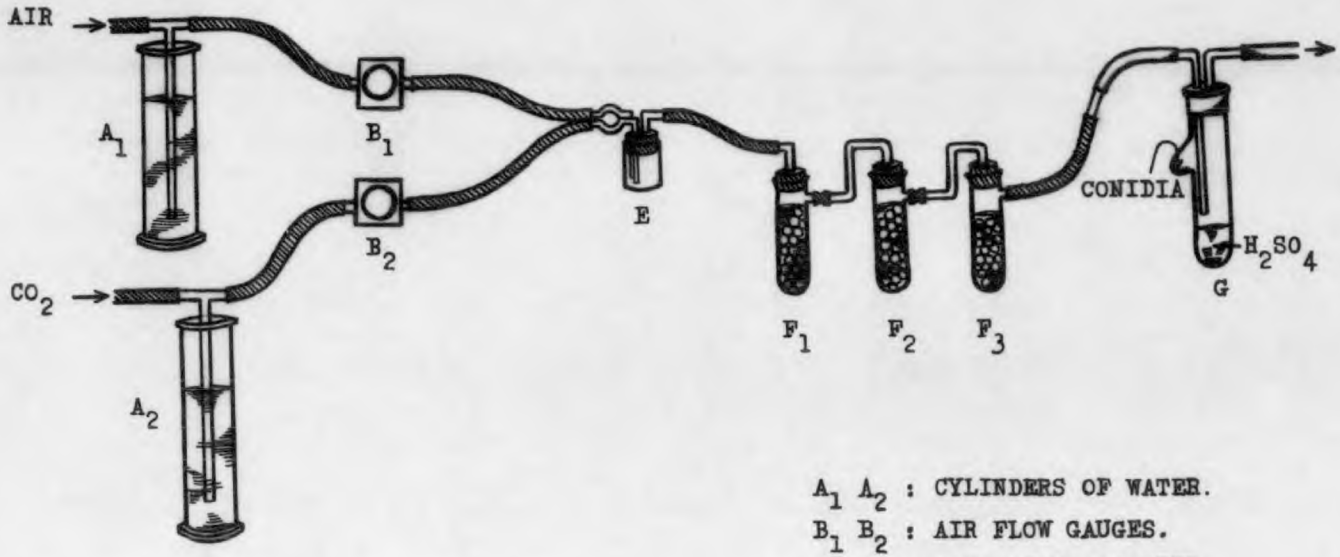
in the conidia and conserved viability of the conidia. The retarding effect of CO_2 on respiration has indeed been recognised for a long time (Kidd, 1915). On the other hand, reduced respiration rate imposed by the high CO_2 level, might have acted by lowering the rate of production of deleterious metabolites in the conidia.

(c) Effect of known levels of High CO_2 concentration.

The rapid loss of viability of M.anisopliae conidia at mid-humidities could be prevented by unknown high atmospheric CO_2 concentrations. In order to determine the level of CO_2 capable of exercising this effect, experiments with known levels of CO_2 were conducted, using, industrially prepared gaseous carbon dioxide, obtained from the British Oxygen Company.

Using the apparatus shown in Fig. 55 below, humidity chambers holding conidia of M.anisopliae were filled with gas mixture of known CO_2 concentration and stored at 23°C. for 7 days, after which viability of the conidia was determined as in the above experiments, by germinating them on potato dextrose agar for 24 hours at 25°C.

The desired CO_2 concentration was obtained by mixing pure CO_2 gas and normal air (CO_2 content 0.04 %) flowing at different known rates in a mixing chamber (E) (10 cm. high and 2.5 cm.



A_1 A_2 : CYLINDERS OF WATER.
B_1 B_2 : AIR FLOW GAUGES.
E : GAS MIXING CHAMBER.
F_1 F_2 F_3 : BUBBLING TUBES HOLDING H_2SO_4 SOLUTION TO EQUILIBRATE MIXED AIR TO DESIRED % R. H.
G : INCUBATION CHAMBER.

FIG. 55. DIAGRAM OF APPARATUS FOR FLUSHING INCUBATION CHAMBER WITH MIXED AIR OF KNOWN CARBON DIOXIDE CONCENTRATION.

in diameter). Rates of flow of the gases were regulated by flow gauges (B_1 : B_2) which had been previously calibrated with a flow-meter. The gas mixture in the mixing chamber was then conducted into vessels arranged in series (F_1 : F_2 : F_3) holding H_2SO_4 solutions of known densities. The mixed air was bubbled through to adjust the humidity to the desired level, before entering the humidity chamber (G). The humidity chamber contained the conidia in a side arm and H_2SO_4 solution in the main chamber, identical in density to that in the bubbling tubes, to maintain the desired constant relative humidity. The gas mixture was passed through the chamber for 5 minutes, and the outlet and inlet of the chamber then simultaneously sealed with metal clips. The humidity chamber was then detached and the end of the tubes also sealed with glass plugs, before storage. Polythene tubings were used to avoid CO_2 leakage from the humidity chamber.

The carbon dioxide concentrations of gas mixtures were determined by absorbing the CO_2 in Baryta solution in Pettenkofer tubes and titrating against standard HCl, following the procedure outlined by Loomis and Shull (1937).

The results are presented in Tables 118 to 120 and in Fig. 56.

The results in Tables 118 to 120 show that in all three series of controls, the conidia at the mid-humidities died with rapidity; in all three sets, at 45 % R.H. viability was below 50 per cent (Fig. 56). In atmospheres with 18.20 and 7.28 per cent CO_2 ,

TABLE 118

Longevity of conidia of M.anisopliae stored in atmospheres of 18.20 % CO_2 at different relative humidities for 7 days at 23°C.

(Percentage Germination on PDA after 24 hours at 25°C.)

% RELATIVE HUMIDITY	CONIDIA IN ATMOSPHERE OF 18.20 % CO_2			CONTROL CONIDIA IN NORMAL AIR		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
5	3080	99.64	488.5	2672	99.92	402.1
15	2988	99.39	442.0	2909	95.00	343.6
25	2570	99.22	430.5	3010	94.35	313.7
35	2511	96.05	376.7	3411	35.06	128.9
45	2568	98.44	432.4	3459	32.39	77.6
55	2506	97.20	408.0	2715	86.00	377.9
65	2862	99.37	440.2	3032	99.00	398.8
75	2730	100.00	469.1	2791	99.31	406.6
85	2491	100.00	500.3	2560	100.00	418.6
95	2665	100.00	503.9	2888	100.00	501.6

TABLE 119

Longevity of conidia of M.anisopliae stored in atmospheres of 7.28 % CO_2 at different relative humidities for 7 days at 23°C.

(Percentage Germination on PDA after 24 hours at 25°C.)

% RELATIVE HUMIDITY	CONIDIA IN ATMOSPHERE OF 7.28 % CO_2			CONTROL CONIDIA IN NORMAL AIR		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
5	1858	100.00	435.7	1994	99.36	400.0
15	1947	99.43	414.1	2035	98.91	371.3
25	2148	99.44	381.1	1920	98.75	369.9
35	2310	97.55	388.2	1871	95.72	247.2
45	1890	97.94	273.1	1940	32.63	91.4
55	2160	98.61	372.8	2255	50.47	126.7
65	2352	99.89	373.1	2088	94.95	323.4
75	2819	99.29	419.4	2100	99.29	369.9
85	2466	100.00	432.6	2040	100.00	389.2
95	2595	100.00	446.9	2123	100.00	429.2

TABLE 120

Longevity of conidia of M.anisopliae stored in atmospheres of 0.52 % CO_2 at different relative humidities for 7 days at 23°C.

(Percentage Germination on PDA after 24 hours at 25°C.)

% RELATIVE HUMIDITY	CONIDIA IN ATMOSPHERE OF 0.52 % CO_2			CONTROL CONIDIA IN NORMAL AIR		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
5	2226	100.00	592.0	2018	100.00	456.3
15	2170	99.35	579.9	2197	99.30	449.4
25	2069	99.46	531.2	2224	96.40	436.2
35	2175	97.93	380.2	2390	94.05	340.7
45	2304	73.96	262.7	2601	25.82	92.5
55	2140	80.00	390.3	2256	39.89	156.2
65	2205	92.00	406.8	2174	67.94	180.7
75	2346	100.00	569.4	2240	98.75	378.5
85	1969	100.00	565.9	2392	100.00	473.9
95	2275	100.00	582.6	2147	100.00	480.3

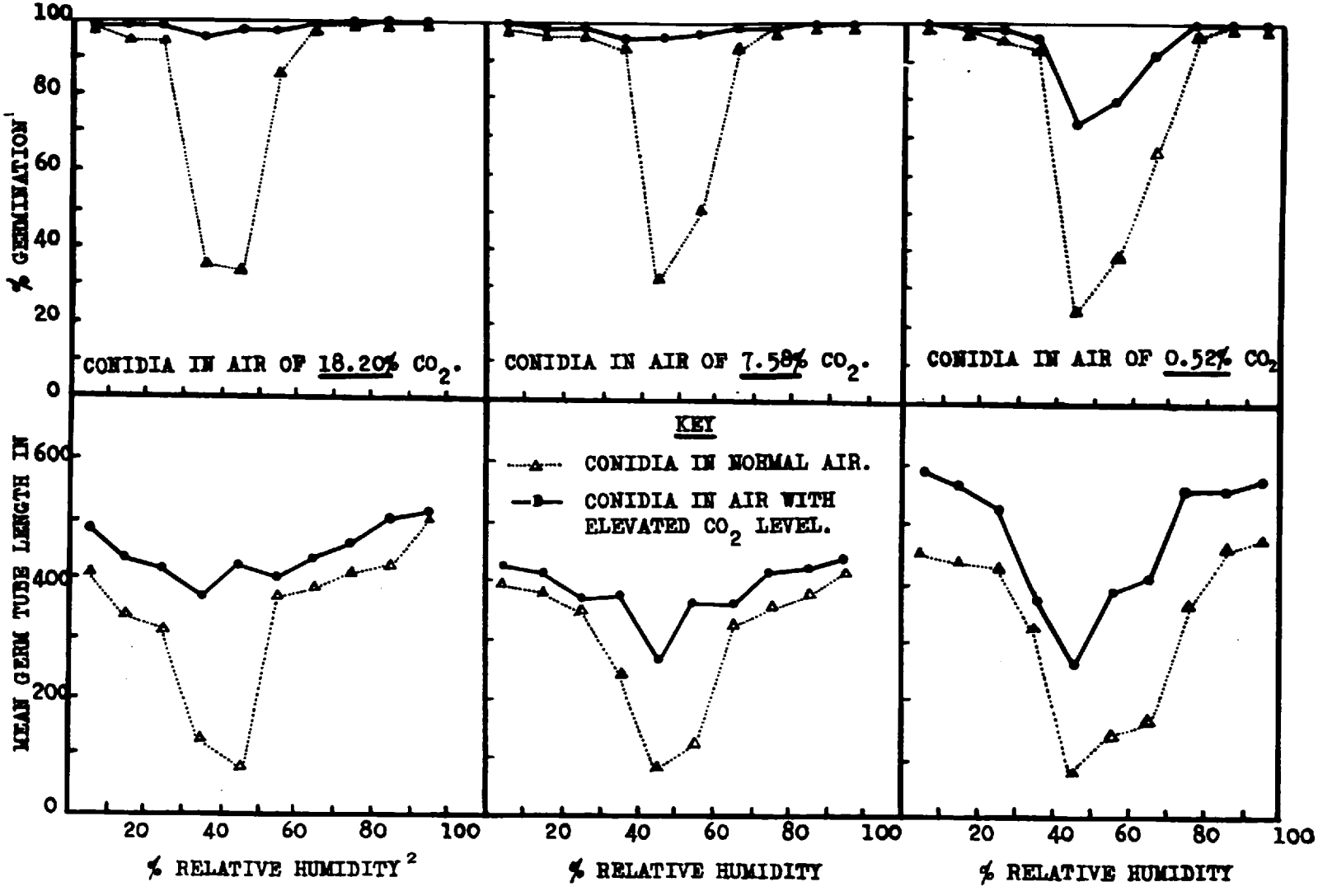


FIG. 56. EFFECT OF ELEVATED CO_2 CONCENTRATION ON SURVIVAL OF CONIDIA OF M.ANISOPLIAE STORED AT DIFFERENT RELATIVE HUMIDITIES AT 23°C. FOR 7 DAYS.

[1] % VIABILITY : [2] HUMIDITY OF STORAGE.

viability of the conidia, after 7 days' storage, was above 96 per cent at all humidities. As the concentration of CO_2 decreased the protection afforded against loss of viability also diminished, and, in an atmosphere of 0.52 per cent CO_2, viability of the conidia at 45 and 55 % R.H. fell to 73 and 80 per cent respectively. Both of these figures, however, were considerably higher than those of 25.82 and 39.89 per cent respectively in the control with normal air.

The minimum CO_2 concentration below which, the protection against rapid loss of viability at the mid-humidities disappears, was not determined in this investigation. Indeed, the protection afforded by CO_2 appears to diminish gradually with CO_2 concentration rather than cease suddenly at a certain level.

In the previous experiments it had been noted that germ tube length was a more sensitive index of changed vigour of conidia than percentage germination. The results in Table 118 show that though the unfavourable effect of median humidities on germinability is almost, but not quite, suppressed, there is still an adverse effect on germ tube length (presumably a manifestation of an effect on the latent period of germination).This is more marked in Table 119 (7.28 % CO_2) (Fig. 56).

Thus while CO_2 greatly retards the manifestation of the adverse effect of humidities near 45 % R.H., it does not altogether prevent this effect. The data all indicate that humidities close to 45 % R.H. are least favourable for survival, even though the mortality of conidia, in the course of 1 or 2 weeks' storage, at these humidities

amounts only to 3 or 4 per cent.

(d) Effect of Exclusion of Oxygen.

Air with elevated CO_2 concentration of 0.52 % and above prevented rapid loss of viability in conidia of M.anisopliae at the unfavourable mid-humidities. The effect of humidity on survival of the conidia in an oxygen-free atmosphere was next examined.

The experiment was set up in ways similar to those outlined above. Humidity chambers (see Fig. 55) with conidia of M.anisopliae in the side arm and H_2SO_4 solutions (to maintain desired relative humidities, Solomon, 1952) in the main chamber, were each flushed with pure Nitrogen gas, obtained from the British Oxygen Company, for 5 minutes and sealed. The nitrogen gas was first passed through alkaline pyrogallol solution (a mixture of equal volumes of 10 % Na_2CO_3 and 10 % pyrogallol solutions) to absorb contaminant oxygen, before being conducted into the humidity chamber. Control experiments were run with conidia in normal air (about 21 % Oxygen). The humidity chambers were sealed and stored at 25°C. for 7 days, after which viability of the conidia was determined by germinating them on potato dextrose agar at 25°C. for 24 hours. The results are presented in Table 121.

While conidia incubated in normal air (about 21 per cent O_2) showed the usual rapid loss of viability at 45 % R.H., where only

TABLE 121

Longevity of conidia of M.anisopliae stored in O_2- free atmospheres of different relative humidities for 7 days at 25°C.

(Percentage Germination on PDA after 24 hours at 25°C.)

% RELATIVE HUMIDITY	CONIDIA IN OXYGEN-FREE ATMOSPHERE			CONTROL CONIDIA IN NORMAL AIR		
	No. of conidia examined	% Germination	Mean Germ tube length in μ	No. of conidia examined	% Germination	Mean Germ tube length in μ
25	3310	100.00	553.7	3052	99.02	466.0
35	2023	100.00	549.2	2691	99.25	444.2
45	2644	100.00	558.0	3821	48.16	54.8
55	2982	100.00	550.7	2539	91.28	294.5
65	2557	100.00	551.0	2338	99.00	339.9
75	2121	100.00	600.9	3676	99.43	502.9

48 per cent of the conidia were viable at the end of the period, there was complete survival of conidia in the oxygen-free atmospheres (100 % Nitrogen) at all humidities used in the experiment.

The prevention of early death of the conidia at unfavourable mid-humidities appeared more complete in oxygen-free atmospheres than with high concentrations of CO_2 (18.20 % CO_2), for the data in Table 121 show that germ tube length - normally a more sensitive indicator of deterioration of vigour of spores than is percentage germination - was essentially uniform in all treatments.

iii Respiration of conidia of M.anisopliae at different relative humidities.

Evidence had been gathered from the numerous previous experiments reported above, to support the view that quicker loss of viability of M.anisopliae conidia occurred at mid-humidities because of a more rapid rate of respiratory activity. The prolongation of conidium life by high concentrations of CO_2 or exclusion of O_2 could be interpreted as a result of a slowing down of respiration. Direct measurements of respiration of M.anisopliae conidia at different relative humidities were therefore considered desirable. Respiration was studied by the Warburg manometric method (see pages 23 and 24).

Since KOH solutions (see Table 3) were to be used to maintain the relative humidities in the Warburg flasks, as well as to absorb CO_2, a preliminary experiment was carried out to examine survival of the conidia at different relative humidities controlled by KOH solutions (Solomon, 1952). Conidia of M.anisopliae were therefore stored in humidity chambers holding KOH solutions which provided desired humidities (Solomon, 1952). The conidia were incubated at 25°C. in darkness for 5 days, after which viability of the conidia was determined in the usual way, by germinating the conidia on potato dextrose agar at 25°C. for 24 hours. The results are presented in Table 122 which shows the usual pattern of survival of M.anisopliae conidia in storage at different relative humidities.

TABLE 122

Longevity of conidia of M.anisopliae stored at different relative humidities maintained with KOH SOLUTIONS, for 5 days at 25°C.

(Percentage Germination on PDA after 24 hours at 25°C.)

% RELATIVE HUMIDITY	No. of conidia examined	% Germination	Mean Germ tube length in μ
15	2062	99.73	430.1
25	2318	99.13	341.5
35	2216	99.41	306.0
45	2177	52.74	161.2
55	2270	57.45	169.7
65	2228	94.37	324.5
75	2965	99.75	460.36
85	2159	99.73	461.1
95	2599	99.76	500.1

Having established that the expected pattern of survival was shown by conidia at different relative humidities controlled by KOH solutions, the respiration studies were proceeded with. The methods used are described above (pages 23 and 24). The results are presented in Table 123. Results of experiments with conidia in aqueous suspension are the averages of 6 trials, and those for conidia in a dry state at the different relative humidities are averages of 3 trials.

Data in Table 123 show that conidia of M.anisopliae stored at relative humidities of 25 to 75 % consumed measurable quantities of oxygen, the volume of oxygen taken up increasing with increase in

TABLE 123

Oxygen uptake by conidia of M.anisopliae in aqueous suspension and in dry state at different relative humidities at 25°C.

RESPIRATORY MEDIUM.	OXYGEN UPTAKE µl. per mgm. dry wt. conidium[1]					
	TIME IN HOURS					
	1	2	3	4	5	6
Water	3.56	6.87	9.96	12.75	15.56	-
75 % R.H.	0.39	0.96	1.38	1.77	2.22	2.48
65 % R.H.	0.16	0.27	0.38	0.46	0.53	0.57
55 % R.H.	0.08	0.14	0.21	0.27	0.32	0.34
45 % R.H.	0.00	0.02	0.04	0.061	0.069	0.071
35 % R.H.	0.01	0.04	0.06	0.085	0.085	0.087
25 % R.H.	0.00	0.00	0.00	0.017	0.017	0.018

[1] Conidia subsequently oven-dried at 60°C. for 24 hours.

humidity, with the greatest respiration in liquid water in which germ tubes were ultimately formed.

Several erratic results were shown in preliminary tests by conidia at 45 and 55 % R.H., no doubt some of the conidia had become moribund within the period of experimentation, and readings of oxygen consumption by conidia at these humidities in Table 123 might most likely be the respiratory index of fewer live conidia than assumed.

Result of this experiment evidently does not support the supposition that higher respiration rates occur in the conidia stored

at the mid-humidities as suggested by experiments with high CO_2 concentrations and O_2-free air. These results are fully discussed below.

V. GENERAL DISCUSSION.

The economic importance of entomogenous fungi lies in their ability to destroy noxious insect pests. Though the idea of using these fungi to produce epidemics among insect pests has been accepted for many years, and numerous trials of biological control have indeed been made, there still lingers a distinct reluctance to and an antipathy for the idea of using micro-organisms to control insect pests from some quarters. Scepticism is, in part, understandable when it is remembered that many of the highly advertised attempts of the past resulted in discouraging failure. The disappointment in a few hastily tried attempts to control insects with certain micro-organisms has blinded many to the potentialities that this method has, when based on a firm scientific foundation and a thorough knowledge of the numerous factors involved. Unquestionably, the greatest stumbling block to the widespread use of fungi to combat insect pests is the lack of knowledge of the circumstances which influence the establishment of parasitic relations between the fungi and their insect hosts.

The object of the present study was to determine more precisely the factors which affect survival and germination of the conidia of three polyphagous entomogenous fungi - Beauveria bassiana, Isaria farinosa and Metarrhizium anisopliae as a contribution, chiefly to the study of the problem of using disease-producing organisms in the biological control of insect pests, but also to that of the

behaviour of conidia in general.

The results of the present work showed that different proportions of the conidia of the three fungi were capable of germinating in free water. M.anisopliae conidia, at the optimum temperature (25°C.) germinated very well in water, attaining a consistent average germination of 96 per cent in 24 hours. Conidia of B.bassiana and I.farinosa within the same period, and at their respective temperatures, attained an average germination of 60 and 10 - 12 per cent respectively. Conidia of B.bassiana germinated very well at low temperatures, and altogether germinated over the temperature range of 4 to 25°C., with an optimum at 18°C. (see Table 7). The optimum temperature obtained was slightly lower than those reported by other workers. Arnaud (1927) found an optimum temperature of 22°C., and Schaerffenberg (1957) and Hart and MacLeod (1955) claimed still higher optima of between 20 and 30°C., and 28°C. respectively. I.farinosa conidia differed in the range of temperature over which they germinated according to their origin. Although conidia of I.farinosa from the coremia and of the Paecilomyces-state both germinated best at 20°C., conidia from the coremia germinated over the range of 10 to 25°C. (see Table 8), whilst those of the Paecilomyces-state germinated over the narrower range of 15 to 25°C. (see Table 9). Conidia of I.farinosa of the Paecilomyces-state further showed in general, a smaller percentage germination. Later studies on viability (see

pages 231 - 243) revealed that conidia of I.farinosa are extremely short lived at the higher humidities likely to prevail in culture vessels. Since the coremia normally appear in the cultures at least 10 to 12 days after the production of the conidia of the Paecilomyces-state, conidia of the Paecilomyces-state are therefore on average approximately 14 to 20 days older than the youngest conidia on the coremia. This difference in age might well have led to the observed differences in percentage germination. It is however possible that fundamental physiological differences may exist between the two sorts of conidia.

Conidia of M.anisopliae germinated over the temperature range of 15 to 35°C. with an optimum at 25°C. (see Table 10). Vouk and Klas (1932) found the optimum temperature for conidium germination to be 24 - 26°C. for conidia of M.anisopliae, a result which agreed with the optimum temperature observed in this work. Conidia of M.anisopliae germinated over a higher range of temperature than those of B.bassiana and I.farinosa, which might possibly be a reflection of the origins of the isolates. The isolate of M.anisopliae used was of tropical origin (from Trinidad), whilst the other two were isolated in England.

Continued studies in relation to temperature showed that the incomplete germination of B.bassiana and I.farinosa conidia in water was not rectified by prolonging the incubation period to 144 hours, when percentage germination failed to rise above

73 per cent and 30 per cent respectively (see Tables 11 and 12) at the optimum temperatures, unlike conidia of M.anisopliae where at the optimum temperature a maximum germination of 100 per cent occurred within 48 to 72 hours, and at the end of the period of experimentation of 144 hours a wide temperature range of 15 to 25°C. had led to 100 per cent germination (see Table 13). In all three fungi, the percentage germination-time curves at temperatures far from the optimum temperatures were distinctly sigmoid. Because of the relatively large intervals (24 hours) between observations, the curves nearer the optimal temperatures were not evidently sigmoid but have nevertheless been drawn as such (see Figs. 5 - 7).

Temperatures around 35°C. affected the morphology of the germ tubes of M.anisopliae. The proximal portions became swollen (see Text-Fig. 4, Fig. 19). The basis of this morphogenetic effect was not established. Similar phenomena have been observed by other workers in other fungal spores germinating under extreme environmental conditions. For example, Bonner (1948) found that germinating conidia of Aspergillus niger at low relative humidities of 70 - 78 %, produced extraordinarily enlarged germ tubes which occasionally tended to be spherical.

Results of experiments in this investigation are in contrast to those reported by Gabriel (1959) that conidia of M.anisopliae were incapable of germinating in pure water, and that conidia of B.bassiana barely did so. Gabriel stated that he obtained good

germination in both with dilute NaCl solution. It has not been possible to discover why he failed to obtain germination in water. A tentative explanation is that different strains of M.anisopliae and B.bassiana had been used. The existence of different physiological activities among strains of same species of fungus is not unknown (Wolf and Wolf, 1947). NaCl had contrasting effects on conidia of B.bassiana and M.anisopliae in this work. A concentration of 1×10^{-3} Molal of NaCl enhanced germination of conidia of B.bassiana to about 90 per cent (see Table 37), whereas concentrations from 1×10^{-7} to 0.5 Molal inhibited germination in conidia of M.anisopliae (see Table 43).

Conidia of B.bassiana studied in this work consisted of 68 per cent globose and 32 per cent oval types. MacLeod (1954), in a taxonomical study, recognised only two Beauveria species - B.bassiana and B.tenella - on the basis of conidial size and shape, and he concluded that the other 12 so-called Beauveria species were simply strains of these two species. However all cultures of both were found to be heterogeneous in spore shape - the very criterion that justified their taxonomic separation. 57 per cent of the spores of B.bassiana were globose, while among those of B.tenella, only 2 per cent were. The remaining proportions in each case, were oval in shape. The isolate studied here was typical B.bassiana. Conidia of I.farinosa and M.anisopliae were oval and cylindrical in shape respectively, and the observed

dimensions were similar to most of the reported measurements (Petch, 1924 ; 1926 ; Smith and Brown, 1957 etc.).

Apart from the study of early stages of germination of conidia of B.bassiana by MacLeod (1954) and a brief observation by Brown and Smith (1957) on germinating conidia of I.farinosa, our knowledge of germination of the conidia of these three species is scant. Swelling of the conidia was a prominent feature of all the conidia about to germinate. The fastest germinating conidia of B.bassiana produced initials of germ tubes in 5 hours at 18°C., when grown in water. MacLeod (1954) had stated that conidia of B.bassiana showed a latent period of germination of 8 hours when grown on Sabouraud-maltose agar. The latent period of germination of M.anisopliae conidia was approximately similar to that of B.bassiana conidia. Germinating conidia were observed first after 5 - 6 hours. Conidia of I.farinosa, on the other hand, took 12 hours before producing germ tubes. This agreed with the report of Brown and Smith (1957). Germ tubes of all three species were initially non-branching, hyaline and continuous. They emerged from specific sites (ends of the conidia) in conidia of I.farinosa and M.anisopliae (see Text-Fig. 3 and 4), but those of B.bassiana emerged apparently randomly from the surface of the conidium (see Text-Figs. 1 and 2). These germ tubes when sustained entirely by the endogenous food reserves of the conidia attained strikingly similar maxima. Lengths of 1300, 1121 and 1326 μ were attained by

conidia of B.bassiana, I.farinosa and M.anisopliae respectively (see Fig. 20) in approximately 120 hours. This behaviour of the conidia might be of some significance in nature. The conidia in the presence of sufficient moisture would not only successfully germinate in a non-nutritive environment, but would also be sustained by endogenous substrates for long periods (between 4 to 5 days).

It has been recognised since at least 1860 (Hoffman, 1860) that strong light inhibits germination of some fungal spores. A number of reports - too many to ignore - suggest that spore germination in some species is increased by light or even requires light (Cochrane, 1958). Marsh, Taylor and Bassler (1959) on the other hand, summarised numerous reports that light had no influence on germination of fungal spores. There was no indication from the present investigation that light had any effect, favourable or otherwise, on germination of conidia of B.bassiana, I.farinosa and M.anisopliae.

The lowest relative humidity permitting conidia of B.bassiana to germinate at the optimum temperature (18°C.) lay below 93.8 % R.H., but was above 80.6 % R.H. (see Table 23). At their respective optimum temperatures, conidia of I.farinosa germinated at 97.2 % R.H., but not at 93.2 % R.H. (see Table 24) and those of M.anisopliae germinated at 96.9 % R.H. but not at 92.0 % R.H. (see Table 25). Though more conidia of M.anisopliae germinated

in free water than in saturated air, with I.farinosa and B.bassiana, slightly more germinated in air at 100 % R.H. than in free water. The ability of the conidia of the three fungi to germinate in atmospheres of high humidity, particularly between 90 and 100 % R.H., had been reported earlier by other workers (Arnaud, 1927 ; Averna-Sacca, 1936 ; Hart and MacLeod, 1955 ; Kornbluth, 1961 ; Masera, 1957 ; Müller-Kögler, 1942, 1948 ; Steyaert, 1927). It is obvious from the narrow range of humidity capable of supporting germination that the humidity of the atmosphere in the field is going to be a factor limiting the activity of the fungi, and successful establishment among an insect population will take place only under sufficiently protracted periods of high humidities.

With regards to growth rates of the germ tubes, the latter were found always to grow fastest in free water, and next fastest in atmospheres of 100 % R.H. The rates of growth fell with decreased relative humidity (see Tables 26 - 28). It therefore appears likely that in nature, high relative humidity not only leads to the mobilisation of a larger number of infective units in an insect's environment, it also accelerates their growth rate, and presumably, therefore their likelihood of infecting in a given period of time. This humidity relationship will thus be of importance in nature in connection with the establishment of an epizootic of a mycosis in an insect population.

There is a variability among fungi in their response to the

interaction of temperature and humidity, which indicates that no simple temperature-humidity relationship exits for fungi. For example, Bonner (1948) found that the optimum temperatures for spore germination of Aspergillus niger were near to 40°C. at 93 % R.H. and near 30°C. at 100 % R.H. For this species the optimum temperature for germination changed with different humidities. Tomkins (1927) on the other hand, found that in Alternaria citri and Trichoderma lignorum, the optimum temperature for spore germination was the same for all relative humidities. Similar characteristics to those observed by Tomkins (1927) were shown by the conidia studied in this work. The percentage of conidia germinating at each relative humidity was always highest at the same temperature, and the range of humidity which allowed germination was greatest at this optimum temperature (see Tables 29 - 31). At lower temperatures, higher humidities are required for spore germination than at more nearly optimal temperatures.

Sown on buffered pyridine-washed agar, at their respective optimum temperatures, conidia of B.bassiana and M.anisopliae germinated over a very wide pH range, and best at pH 5.0 and pH 5.0 to 7.6 respectively (see Tables 32, 34 and 35). The acid limit for germination of conidia of B.bassiana was not established, for the conidia germinated at the lowest pH tested (pH 2.2), but the alkaline limit was found to be between pH 11.7 and 12.0. M.anisopliae conidia germinated over the range of pH 2.6 to 12.0.

Similar wide ranges of pH tolerance have also been observed in other fungi, for example in germinating spores of Gloeosporium musarum (Goos and Tschirsch, 1962). By contrast, I.farinosa conidia germinated over the rather narrow range of pH 2.6 to 7.0, with an optimum at pH 4.0 (see Table 33).

It is most unlikely that pH is a limiting factor in germination of conidia of B.bassiana and M.anisopliae under natural environmental conditions, for example, as a factor of ecological significance in soil infection. On the other hand, environmental pH may prove to be a factor limiting the activity and distribution of I.farinosa. There appear to be insufficient data available to construct a clear picture of the natural distribution and abundance of these three species of parasitic fungi . However the host records in Charles' (1940) list of North American entomogenous fungi show B.bassiana (sensu MacLeod, 1954) on 124 species of insect hosts, M.anisopliae on 63 and I.farinosa on 8. There is thus this suggestion of more restricted occurrence of I.farinosa, for which pH tolerance could be in part responsible.

An interesting feature was the double maximum showed by conidia of I.farinosa germinating at different pHs on washed agar buffered with McIlvaine's buffer solutions (a mixture of Na_2HPO_4 and citric acid). There was a major peak at pH 4.0 and a minor one at pH 6.0, with a minimum at pH 5.0 in between. This sort of phenomenon has been observed in various physiological functions of plants in

general in relation to pH. Webb (1919) was apparently the first to call attention to this phenomenon. He found that, when spores of Penicillium cyclopium were germinated in buffer mixtures of H_3PO_4 and NaOH containing mannite, two maxima appeared, with a minimum between, when percentage of spore germination was plotted against the pH of the solutions. He found similar curves for Fusarium sp. and, under certain temperature conditions, for Aspergillus niger. Salter and McIlvaine (1920) found a double-maximum curve for the growth of wheat seedlings in solution cultures, the reaction of which was adjusted with citric acid and NaOH. Hixon (1920) found a double-maximum curve for the germination and seedling growth (in length) of corn, wheat and oats with the minimum at pH 6.0 between the two maxima. Hixon quotes a suggestion by E. J. Cohn that the minimum point is the isoelectric point of a protein of the cell membrane of the seed. The cause of this double maximum is suggested by Arrhenius (1922) as being due to changed intensity of permeability for the different nutrients, or to the fact that the solubility of salt differs at different hydrogen-ion concentrations. Robbins (1923) from results of experiments on the absorption of water by potato-tuber tissue in solutions of different hydrogen-ion concentrations and experiments on the absorption of dyes by plant tissue which had been in contact with solutions of different hydrogen-ion concentration concluded that the cytoplasm acts, in water absorption and

absorption of solutes, like an ampholyte with an iso-electric point. He obtained a double-maximum curve with a minimum between for the absorption of water by plant tissues in solutions of different hydrogen-ion concentration. This offered a satisfactory explanation for the double-maximum curves found in the germination of fungal spores, seed germination and growth of seedlings - physiological mechanisms closely associated with water absorption. Possibly, a similar phenomenon was operating in conidia of I.farinosa ; pH 5.0 being the iso-electric point in water absorption in the conidia.

The double-maximum was not evident in the pH - germination curves of B.bassiana and M.anisopliae. Although there is an increasing body of evidence to indicate that the occurrence of a double-maximum curve is to be expected when the influence of hydrogen-ion concentrations upon various physiological functions or processes of plants is investigated, a considerable amount of work has been reported which shows no such phenomenon. Robbins (1923) suggested that, this should not be considered as necessarily indicating the non-existence of a double-maximum curve. Many conditions may conceivably conceal or obliterate the minimum located between the two maxima. For example, the pH values of the solutions may be separated so far that the minimum is not found or growth conditions, such as temperature, nutrient and salt content of the medium etc., may conceal it.

That water is insufficient for the germination of many fungal spores was recognised very early in the development of mycology, since improved germination could usually be obtained by addition of nutrients to the medium. The pertinent literature has been reviewed by Gottlieb (1950). The response of spores of different fungi is very varied. The requirements for germination are sometimes very specific as in Penicillium digitatum (Gottlieb, 1950), for which peptone and ammonium, but not nitrate, ions are effective. Very few studies however on salt requirements for spore germination have been reported. Throughout this investigation, wholly viable populations of conidia of B.bassiana and I.farinosa, at their optimum temperatures, attained approximately 60 and 12 per cent germination respectively in pure water. 100 per cent germinated only under the influence of exogenous nutrients. On the other hand, M.anisopliae conidia germinated very well in water, achieving on the average 97 per cent germination. Experiments carried out to examine the germination of the conidia in the presence of organic nutrients and mineral salts showed that, response of the conidia of the three species here studied was quite varied. An abstract of the stimulated germination observed is presented here in Table 124.

The rather low germination (60 %) of B.bassiana conidia in water was elevated to (nearly) 100 per cent by a diverse range of materials (see Table 124). This unspecific stimulation is

TABLE 124

Abstract of Data on Stimulated Germination (Tables 36 - 44) in conidia of B.bassiana, I.farinosa and M.anisopliae.

FUNGUS	% Germination in water.	Best Stimulated %Germination	Stimulation of Germination by :	
B.BASSIANA	60.58	100.00	Dextrose	(0.25-0.5% conc.)
		98.93	Yeast-extract	(0.2 % conc.)
		97.69	$MgSO_4 \cdot 7H_2O$	(1×10^{-3} Molal)
		96.92	K_2HPO_4	(1×10^{-3} Molal)
		96.58	$(NH_4)_2 \cdot HPO_4$	(1×10^{-5} Molal)
		92.05	Casein hydrolysate	(2.0 % conc.)
		91.46	$CuSO_4 \cdot 5H_2O$	(1×10^{-7} Molal)
		89.55	NaCl	(1×10^{-3} Molal)
		82.00	$Na_2MoO_4 \cdot 2H_2O$	(1×10^{-5} Molal)
		81.64	CaCl	(1×10^{-7} Molal)
		75.19	$MnSO_4 \cdot 4H_2O$	(1×10^{-3} Molal)
I.FARINOSA	11.08	100.00	Casein hydrolysate	(2.0-4.0 % conc.)
		100.00	Yeast-extract	(0.2-4.0 % conc.)
		47.29	Dextrose	(2.0 % conc.)
		37.20	$Na_2MoO_4 \cdot 2H_2O$	(1×10^{-7} Molal)
		28.00	K_2HPO_4	(1×10^{-5} Molal)
		23.93	$MnSO_4 \cdot 4H_2O$	(1×10^{-3} Molal)
		23.84	$(NH_4)_2HPO_4$	(1×10^{-3} Molal)
		21.35	CaCl	(1×10^{-3} Molal)
		17.04	$FeCl_3 \cdot 6H_2O$	(1×10^{-3} Molal)
		16.12	$MgSO_4 \cdot 7H_2O$	(0.5 Molal)
M.ANISOPLIAE	97.12	100.00	Casein hydrolysate	(0.2-4.0 % conc.)
		100.00	Dextrose	(0.125-8.0% conc.)
		100.00	Yeast-extract	(0.2-4.0 % conc.)

not easily understood, for it implies that the dormant metabolism of the non-germinated spore can be reactivated by "pressure" at widely separated points in the cell's metabolic processes. For example, dextrose presumably acts through respiratory path-ways ; casein hydrolysate through N-metabolism (possibly linked with respiratory path-way, i.e., the casein hydrolysate may supply materials which can be respired) ; yeast-extract either acts like casein hydrolysate, or as a source of vitamins, perhaps involved in enzyme synthesis or activity ; and some of the salts presumably as co-factors in some enzyme systems. These possibilities have however not been investigated further.

The situation in I.farinosa is also complex. The data (Table 124) show that various salts enable many spores to germinate which otherwise would not in plain water. However, dextrose and especially casein hydrolysate and yeast-extract, improve germination further. Possibly some of these salts are present in the organic stimulants as trace contaminants, but clearly other components of these materials exert a beneficial effect. Because vitamin-free casein hydrolysate is as effective as yeast-extract, and both much more so than dextrose, it appears probable that amino acids are involved in this effect. Again further investigation is required to elucidate the mechanism of this stimulation.

Only a very small proportion of a population of M.anisopliae conidia fails to germinate in water (about 2 - 3 %). These few

can be led to germinate by organic supplements. Because dextrose is as effective as yeast-extract and vitamin-free casein hydrolysate, the effect is probably due to respirable materials present.

The sensivity of conidia of M.anisopliae to salts in the environmental solution, which inhibit germination, is remarkable. For example, a concentration of 1×10^{-5} Molal of almost all salts tested inhibits germination of M.anisopliae conidia, while having no effect at 1×10^{-7} Molal.

Koidsumi (1957) showed the existence of an antifungal action in extractable lipids from the epicuticle of silkworms, an effect he attributed chiefly to free medium-chain saturated fatty acids (presumably caprylic or capric) which occur in the cuticle. It was here shown (see Tables 45 - 47) that conidia of B.bassiana, I.farinosa and M.anisopliae were stimulated into total germination (100 %) by aqueous extracts of egg shell, cuticle of pupa, silk of pupal cocoon and cuticle of imago of Bombyx mori (silkworm). The effect was two-fold : the elevation of percentage germination to 100 per cent by these extracts ; and the production of substantially longer germ tubes. Experiments carried out with aqueous extract of silk from the cocoon indicated that the stimulatory substances in these extracts were spontaneously released into the aqueous medium, i.e., were not produced as a result of fungal action on the cocoon. Although these extracts enhanced germination

and germ tube growth, they appeared to exercise no consistent chemotropic stimulus. A remarkable feature of the data in Tables 48 to 50, on studies of chemotropic stimulus of the extracts, was the regular occurrence of greater numbers of germ tubes in classes A and C (growing towards and away from the source of stimulant, respectively) than in B and D. Taking as an example the data on M.anisopliae germ tubes, which are critically identical to the others, the sum of germ tubes in classes A and C for the entire experiment were 2052 and 2036 respectively, whilst those of classes B and D totalled 1392 and 1399 respectively (see Table 50). One may tentatively speculate that such a result might reflect the existence of tropic responses, in which the sign of the response critically depends on either the concentration of the excitant, or the characteristic of the individual responding spore, or on both. Unfortunately, the available data do not permit this possibility to be examined.

The aqueous extract of silk from Bombyx mori was found to be able to withstand much dilution without loss of stimulatory power. A dilution of 1/64th of the standard extract still increased germination of I.farinosa conidia from 10 to 60 per cent. The stimulant was , further, found to be thermostable ; autoclaved extract (at 15 lb. per sq. in. steam pressure for 20 minutes) was as potent as non-autoclaved extract. When adjusted to different pH levels with McIlvaine's buffer solutions (a mixture of Na_2HPO_4

and citric acid), it was found to widen the optimun range of pH for conidium germination in I.farinosa, so that 100 per cent germinated from pH 3.0 to 7.0. Further at pH 8.0, where conidia in the unamended buffer solution failed to germinate, 98 per cent germinated in the buffered extract (see Table 54). Neither the acid nor the alkaline limits for conidium germination in the buffered extract was determined in this work.

This result serves to emphasize that the range of pH tolerated by the germination process is critically dependent on other factors in the environment, and that the ranges described above (pages 103 - 112) are known to be valid only for the conditions under which they were established. It therefore would not be wise to uncritically translate the results of these experiments into conditions in the field.

Various amino acids have been identified by several workers in aqueous extracts of exoskeleton of insects (Richards, 1950). The stimulatory property of the extracts was therefore suspected as being due to constituent amino acids. That this might be so was supported by the unspecific nature of the stimulant, for it enhanced the germination of spores and promoted greater germ tube growth of non-entomogenous fungi - Aspergillus niger, Botrytis allii, Cladosporium herbarum, Fusarium culmorum, Penicillium chrysogenum, Penicillium digitatum and Rhizopus stolonifera. (see Table 57). Association of the stimulatory property of the extracts with the

constituent amino acids was demonstrated, when the amino acids in aqueous extract of silk from Bombyx mori (Glycine, leucine, serine, threonine and tyrosine) were separated by paper chromatography (Plate 8), and used in germination tests. These tests showed that the degree of stimulation of germination in conidia of I.farinosa by the separated amino acids was more or less proportional to the concentration of these acids, and not due to any particular ones. The effect appears therefore to be an unspecific response to the provision of unspecific nutrients. Results of numerous chromatograms testing for the presence of carbohydrates in the aqueous extract of silk from Bombyx mori were negative. It can be inferred from these findings that amino acids form very good nutrients for germinating conidia of B.bassiana, I.farinosa and M.anisopliae, supporting the results obtained in germination tests with casein hydrolysate and yeast-extract. Stimulation by these two substrates may be attributed to the general occurrence of amino acids in them, rather than to specific ones. In common with other instances of biological stimulation (Brown, 1946), the stimulant in the extract of silk from Bombyx mori was not specific to entomogenous fungi.

How important this phenomenon is to entomogenous fungi in the field is dependent among other things on how widespread is the existence of water-soluble stimulants in the outer layers of insects in general.

Substances in water extracts of soils in which B.bassiana and I.farinosa had been found depressed germination of the conidia of these fungi (see Table 58). However the soil extracts became stimulatory when filtered or heat sterilised, without adding nutrients (see Tables 59 and 60). This phenomenon has also been found in other investigations on soil extracts (Dobbs and Hinson, 1953 ; James, 1958 ; Lingappa and Lockwood, 1961). All evidence shows that stimulatory and inhibitory substances exist at the same time in soil, with the latter more often predominating. The effects of the inhibitory substances in the soil extracts were annulled by the addition of dextrose and extract of silk from Bombyx mori.

Although antibiotic-producing micro-organisms are known to be normal soil inhabitants (Leithy and Monib, 1959 ; Abd-el-Malek, Monib and Zayeb, 1961) and great quantities of antibiotics can be extracted from their cultures on laboratory media, and, further, the production of antibiotic substances in soil can be stimulated in various ways, the depressant effect of soil extract on conidium germination has been attributed to the existence of a general fungitoxicity in soil and soil solutions in this investigation, as distinct from the effects of specific antibiotics. This fungitoxicity has been investigated in detail (Dobbs and Hinson, 1953 ; Jefferys and Hemming, 1953 ; Stover, 1958 etc.). The above interpretation was based on the uninhibited germination which

occurred after the addition of amendments, and also on the effects of either filtering or heat-sterilising the extracts : both of which normally nullified the general fungitoxicity of soil. No attempt was made in this work to find out the particular micro-organisms which were responsible for the production of the inhibitory conditions in the soil.

These inhibitory substances were fungistatic but not fungicidal. Conidia of B.bassiana and I.farinosa which had remained dormant for 48 hours under the influence of the inhibitory substances of the soil were later stimulated into germination with nutrient medium. Similar observations have been made by Lingappa and Lockwood (1961). They stimulated spores of Glomerella cingulata, Penicillium frequentans and Fusarium oxysporum f. lycopersici, which had lain dormant under the influence of the inhibitory substances for 5 days, into germination with nutrients.

Because the conidia of these insect-parasitising species are affected by the fungistatic properties of the soil, and further, because this fungistasis is capable of modification or elimination by other factors in the environment (eg. nutrients), it is not possible to predict the behaviour of such conidia if added to the soil in attempts at biological control.

It was possible, as described above, to increase the percentage germination of conidia by suitable chemical stimulation. However the reason why only a certain proportion of the population should

be able to germinate in water alone was not known. Evidently the conidial population was heterogeneous. It was found that the age of the conidia was closely associated with their capacity to germinate, in water. Practically 100 per cent germination was attained in water by conidia of M.anisopliae from 5 day-old cultures, suggesting that conidia of varied ages of 1 to 4 days old, germinate equally well in water - depicting a homogeneous population. This supposition was substantiated by experiments (see Plate 22). The conidia of M.anisopliae were germinable immediately on attaining mature size, and fully retained this ability over a period of, at least, 120 hours (maximum period of experimentation).

The data in Table 70 show that 75 per cent of B.bassiana conidia would also germinate in water immediately on formation, but this proportion rapidly diminished with age (in hours) and beyond the age of 40 hours, the conidia germinate very tardily in water (Plate 20). In any conidial sample, it appears that the conidia which germinated in water were the younger ones, with the youngest in this group possessing the greatest ability to germinate. From the data in Table 70, one would expect a mixed population of spores of different ages to average only about 10 to 15 per cent germination, yet in practice, spores harvested from plate cultures averaged about 60 per cent germination. This difference suggests that the aging effect manifested in Table 70 is more exaggerated than that which occurs in an undisturbed petri dish. Possibly the culture

technique employed (viz., mycelium from a piece of nutrient agar spreading over, and sporulating on a barren glass surface) was responsible, or perhaps the 2 hours desiccation employed to kill the vegetative hyphae before testing the germinability of the spores accounted for the effect.

Conidia of I.farinosa on the other hand, would germinate best in water 18 to 26 hours after attaining mature size, and few or none germinated in water before or after this period (Plate 21). Data in Table 71 suggest that a mixed population of spores would average around 15 per cent germination. This approximates to the 10 to 12 per cent observed in mixed population of spores from coremia produced in cultures. Possibly spore production on a coremium rising from the substrate is closely paralleled by the slide culture technique used to obtain the data in Table 71.

Changes in germinability of spores during the first hours of their existence may reflect permeability changes in the spore walls and membranes, as well as changes in enzyme system. Such a disappearance of particular enzyme activities would be comparable with the reversed procedure of reappearance of enzyme activities during the first few hours of germination (see Gottlieb and Caltrider, 1963). However no experiments were made to investigate the bases of the germinability changes.

These differences in behaviour of the three sorts of conidia in respect of age emphasize how different can be the behaviour of

different species, and how necessary it is to examine each individually. It is not possible to generalise.

Very great differences have been found in the survival potential of different kinds of fungal spores. The viability of all spores decreases with time, and rate of loss of vigour is dependent on the inherent characteristics of the spore and upon environmental conditions, especially, temperature, humidity and light (Gottlieb, 1950 ; Cochrane, 1958). References from the literature had already been mentioned in the pertinent section in this thesis (pages 213 - 214).

Temperature greatly influences the longevity of spores, and long survival is correlated with low temperature, while high temperatures shorten this period. The effect of particularly high temperature on viability is probably due to the gradual denaturing of the proteins of the cell protoplasm. It is likely that moderately high temperatures increase the respiration rate of the quiescent spore, with consequential early depletion of essential reserve materials resulting in it's death.

Humidity, too, influences the length of time during which spores retain their vitality. Unlike the uniform temperature-viability relationship, fungal spores portray at least four types of response to the humidity of storage (see pages 213 and 214). The underlying effect of humidity on the survival of the conidia differ in each survival pattern.

Radiation shortens the period of viability of some spores, (Dillon-Weston and Halnan, 1931 ; Wolf, 1934) and blue light appears to be the most active portion of the visible spectrum (Dillon-Weston and Halnan, 1932). One protective mechanism against injurious rays is offered by the pigments of some spores, and many correlations between colour of spores and resistance to radiation have been made (Duggar, 1936). Dillon-Weston and Halnan (1931, 1932) showed that white and orange spores of Puccinia gramini tritici are more easily killed by ultraviolet light than are grey and red spores.

Generally, conidia of B.bassiana, I.farinosa and M.anisopliae lost viability more rapidly when exposed to light (with highest intensity of 78 foot-candles) than when kept in darkness. The deleterious effect of light was however more accentuated in conidia of B.bassiana and I.farinosa than in those of M.anisopliae (see Tables 73 - 99). It would be interesting to find out the wave-lengths most injurious to the conidia. The extent to which the green walls of M.anisopliae conidia shielded the protoplasm from the damaging effect of light, and were responsible for the lesser sensitivity to light, is not known.

In common with all observed fungal spores in storage, conidia of all three species survived longest at the lowest temperature (8°C.) of experimentation and lost viability most rapidly at the highest temperature used (25°C.). It has often been suggested

that the early death of spores of fungi at higher temperatures is caused by accelerated respiratory rate with attendant exhaustion of vital reserves and/or denaturation of proteins of the conidia, but there appears to be little direct experimental evidence for this. Although respiration will be increased by higher temperatures, the associated decrease in longevity might not be entirely due to the greater loss of essential reserves, but also, may be associated with the accumulation of certain more or less toxic products of respiration. Analogy is here being drawn to conditions observed in stored seeds. Curtis and Clark (1950) reported instances of marked increase in acidity in seeds, kept at higher temperatures, resulting from increased respiration. Increase in acidity was said to be caused by accumulation of toxic metabolites, which may hasten cell death, perhaps by hastened protein coagulation. Curtis and Clark (1950) further suggested that other products of respiration may be toxic for other reasons. The highest temperature employed here (25°C.) was not high enough to cause denaturation of the proteins of the protoplasm within the period in which viability was lost in some of the conidia. For example, conidia of I.farinosa lost viability within 14 days at 25°C., at 75 % R.H.

Humidity was found to be a factor powerfully influencing survival. Two types of response to humidity during storage, was found among the conidia of the three fungi studied. Conidia of B.bassiana and I.farinosa survived longest at zero % R.H. and

lost viability more rapidly with rise in humidity. Conidia of B.bassiana were evidently more long-lived than conidia of I.farinosa. After 635 days' storage, conidia of B.bassiana stored at 8°C. and at zero % R.H. showed 52.88 and 90.42 per cent viability according to whether in light or darkness respectively. No conidia of I.farinosa stored under the same conditions lived for more than 308 days in the light and 545 days in darkness. These conditions were the most congenial conditions for survival. Desiccation of the conidia of B.bassiana and I.farinosa under these conditions might have slowed metabolic activities considerably and consequently prolonged the life of the conidia. High moisture content of the spore, such as must exist at higher humidities, probably influences the rate at which viability is lost in a manner similar to that of higher temperatures. There is presumably an increase in respiration with an exhaustion of reserve materials or a resultant accumulation of certain toxic metabolites.

In view of the results obtained here on the longevity of conidia of B.bassiana and I.farinosa, it seems improbable that the conidia of these fungi introduced naturally or artificially into the soil, with fairly high water content, would remain viable for long under soil moisture conditions. Time of application of the conidia, in attempted biological control, presumably would have to coincide with the susceptible phase of the insect they are intended to infect. However it is possible that the very

fungistasis which apparently prevents germination in the soil might itself prolong the survival of the conidia. Survival under fungistasis has not been studied. It remains to be established whether these fungi are capable of a saprophytic phase in the soil, whereby they could bridge the interval between entering the habitat, and finding a suitable host, by means of a vegetative phase, instead of persistence as ungerminated spores.

M.anisopliae conidia showed a pattern of response to humidity which was quite different. The only other fungal spore known to show this response is that of Aspergillus flavus (Teitell, 1958). Mid-humidities were found to be less favourable for survival of M.anisopliae conidia than lower and higher relative humidities. Thus the conidia always lost viability first at about 40 to 55 % R.H., whereas the viability of the conidia was well conserved at more extreme humidities, eg., 0.0 to 25, and 75 to 95 % R.H. Of these two extreme humidity ranges, the higher humidities (75 - 95 % R.H.) appeared to conserve conidium vigour better than the lower (0.0 - 25 % R.H.). Under the best humidity and temperature conditions (8°C. and 75 % R.H.- see Table 99) the conidia lived for over 545 days.

Because of the ability of the conidia of M.anisopliae to endure long periods of high humidities, they stand a good chance of remaining viable for considerable periods on introduction into the soil.

Though year-old conidia of B.bassiana, I.farinosa and M.anisopliae were slow to germinate (see Table 106), metabolic activity of cultures raised from these conidia was unimpaired. For they grew at similar rates, and sporulated to the same degree, as cultures from comparatively young conidia (less than 24 days old). Naqvi and Good (1957) found similar delayed germination in old conidia of Monilinia fructicola. They failed to restore its rate with several nutrients and vitamins. Delayed germination could be the result of several factors. Among these are rehydration of dried conidia, and more important, the synthesis of essential enzymes incident to germination, which might perhaps take a longer time in older conidia.

Attempts were made to obtain a knowledge of the effect of relative humidity on stored conidia of M.anisopliae which leads to their more rapid death at mid-humidities. Because the conidia of M.anisopliae were wetted only with difficulty, it was suspected that there were waxy materials on the spore coat, and that these might have been involved in the curious humidity response of the spores. However, washing the spores with wetting agents failed to alter the humidity response, except for a non-specific shift in the position of the unfavourable humidity range, a shift which could be produced equally well by washing with water alone. Thus, washing shifted the unfavourable mid-humidity range from 45 - 55 % relative humidity to 55 - 75 % relative humidity. It has not been

possible to find reasons for this shift. It was established that it was not caused by :

(a) either the type or the concentration of wetting agent used, as pure water had the same effect as different concentrations of the wetting agent (Gemex Z-11).

(b) a hysteresis effect, due to the conidia approaching the storage relative humidity from a different direction (wetness) after washing.

(c) Starting germination processes during washing and then arresting them before storage, for conidia on the threshold of germination (4 hours' immersion in water at 25°C.) showed exactly the same survival/R.H. response as conidia washed at 0°C. for 1 minute.

This was followed by a series of studies based on the hypothesis outlined on page 296, that humidity causes permeability changes in a humidity-sensitive membrane in the conidium, lowering gas permeability of this membrane at lower and higher humidities and increasing it, and thereby increasing the metabolic rate, at the median unfavourable humidities. On the supposition that the membrane contained hygroscopic colloidal materials, and that washing produced a shift in the humidity/viability response by modifying their physical properties, experiments were made in an attempt to alter the degree of swelling or contraction of the hypothetical components of the humidity-sensitive membrane so as to alter its permeability. This

was done by washing the conidia with different salts selected for their position in the Hoffmeister ion (Lyotropic) series.

The results (Fig. 54 and data abstracted from this, and presented in Table 114) showed that of 14 salts of univalent and divalent cations and anions tested, $NaNO_3$ and Na_2SO_4 alone showed some restitution to washed spores of the humidity-viability characteristics of unwashed spores. Even so, this restitution was incomplete. The data may be interpreted as showing that, washing removes ions from a relevant system in or on the spore which influence the humidity-viability characteristics, and that these ions can be replaced by sodium. They might indeed be sodium ions themselves.

Results of experiments carried out after this on the storage of conidia of M.anisopliae in Oxygen-free atmospheres (100 % Nitrogen), and in atmospheres with elevated Carbon dioxide concentration (18.20 % CO_2), showed that these prevented the rapid loss of viability of the conidia at mid-humidities. Conidia under these conditions at relative humidities ranging from 5 % R.H. to 95 % R.H. showed virtually uniform survival, after 7 days' storage, of over 99 per cent. At lower CO_2 levels (7.58 % and, more particularly, 0.52 %) the familiar humidity-viability characteristic again began to appear, but were much reduced in magnitude in comparison with the control treatments (see Fig. 56). The influence of O_2 and CO_2 on respiration is well known . Respiration

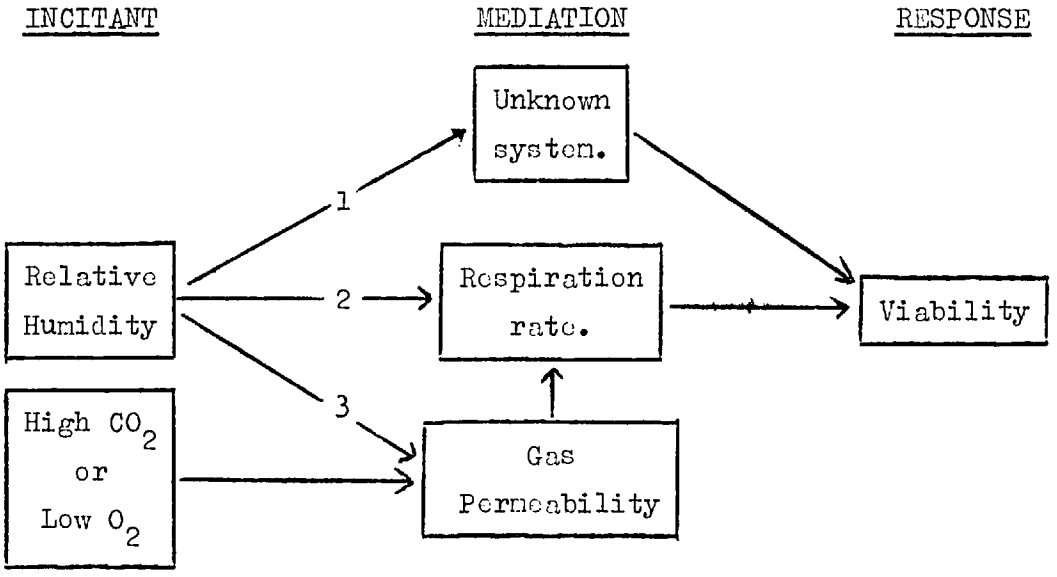
rate in general drops with either reduced O_2 concentration or increased CO_2 level (Curtis and Clark, 1950). Probably such relationship occurred in these experiments.

Although the high CO_2 concentration and absence of O_2 prevented the rapid death of the conidia at the median humidities, it cannot be claimed with certainty that retention of CO_2 in the conidia or exclusion of O_2 or both were the causes of the long survival at the higher and lower humidities. The presence of such a membrane as hypothecated has yet to be demonstrated. Further, the CO_2 and O_2 effects do not prove that the response of the spores to storage humidity is a direct result of different rates of respiration, i.e., more rapid at the mid-humidities in conidia with the supposedly more permeable membrane, and slower in conidia at lower and higher humidities with less permeable membranes. Indeed, the results of studies of respiratory activities of conidia at different relative humidities do not support the supposition that the conidia respired most vigorously at the unfavourable median humidities. The data show a gradual rise in respiratory rate (O_2 consumed) with increase in humidity throughout the range tested, viz., 25 to 75 % R.H. (see Table 123).

However, it is not absolutely certain that the method used was sufficiently sensitive, or precise in it's environmental control to detect differences in respiratory rate of the order expected. Warburg respirometry presents difficulties in ensuring

that the atmospheric humidity presented to each spore in the system is the same, and is at the desired % R.H. In the absence of proof that the spores actually employed in the experiment showed the germination characteristics one associates with storage at a range of different relative humidities, a doubt will remain about the respiratory rates observed for the spores stored under the required humidity conditions. It is probable that a more refined method could be developed using a quantitative radioactive tracer technique.

The data on the humidity-viability characteristics of conidia of M.anisopliae permit the following alternative hypotheses to account for the relationship, and which could be the basis of future experimentation. : -



Concerning the three alternatives, the evidence at hand is largely compatible with the belief that permeability to gases is the mediatory system by which relative humidity affects viability, but this assumes that a membrane of the postulated permeability characteristics can exist, and that respiration rate is critical in affecting viability. Neither assumptions have been proven, though both seem reasonable. Direct testing of this hypothesis is thus called for. If the above finding (Table 123) that respiration rate does not conform to the assumption is a true one, then one is forced to the belief that an unknown system mediates the humidity-viability effect.

VI SUMMARY

1. Cultures of B.bassiana, I.farinosa and M.anisopliae sporulated best on V-8 agar, boiled rice and yeast-extract agar respectively.

2. The optimum temperatures for germination of B.bassiana, I.farinosa and M.anisopliae conidia were 18, 20 and 25°C. respectively. Conidia of B.bassiana germinated in water from 4 to 25°C., those of I.farinosa from 10 to 25°C., and M.anisopliae from 15 to 35°C., in 48 hours. These limits were not exceeded when the incubation period was extended to 144 hours.

3. In confirmation that B.bassiana and M.anisopliae conidia could germinate in water, they were successfully germinated in rain water, metal- and glass-distilled water ; when collected by either flooding the parent cultures or in the dry state ; and when sown at different conidial densities.

4. Shape and size of the conidia have been examined :

   (a) On average, 68 % of the conidia of B.bassiana were globular, and the rest oval. Globular conidia were 2.1 - 2.8 μ in diameter, and oval conidia measured 2.1 - 2.4 x 2.8 - 4.7 μ. Oval secondary conidia were sometimes formed on germ tubes of primary conidia germinating in water or humid air on glass slides. These secondary conidia measured 1.5 - 2.5 x 3.5 - 4.6 μ.

(b) Conidia of I.farinosa were ellipsoidal and measured 1 - 2 x 2 - 3 μ.

(c) Conidia of M.anisopliae were uniformly cylindrical and rounded at the ends ; 2.2 - 2.6 μ wide and 4.7 - 9.4 μ long.

5. Germ tubes were initially simple, hyaline and continuous. They emerge randomly on the conidium in B.bassiana, but appear at specific positions in conidia of I.farinosa and M.anisopliae.

6. Respective latent periods of germination of conidia of B.bassiana, I.farinosa and M.anisopliae were, under specified conditions, 5, 12 and 6 - 7 hours.

7. Abnormal morphological development of germ tubes of M.anisopliae at 35°C. in water are described.

8. Germ tubes of B.bassiana occasionally produced oval secondary conidia in distilled water and in humid air (96 - 100 % R.H.)

9. Nutrients within the conidia allowed maximum germ tube length of 1300, 1121 and 1326 u in conidia of B.bassiana, I.farinosa and M.anisopliae respectively.

10. Light had no effect on germination of conidia of all three fungi.

11. Conidia of B.bassiana germinated between 93.8 and 100 % R.H. at

the optimum temperature (18°C.).

12. Conidia of I.farinosa failed to germinate below 97 % R.H. at the optimum temperature (20°C.).

13. M.anisopliae conidia germinated at and above 96.9 % R.H. at its optimum temperature (25°C.).

14. In all three fungi germ tubes in free water grew faster than in humid air. Rate of growth generally fell with decrease in atmospheric humidity.

15. As atmospheric relative humidity decreased the range of temperature over which germination was possible narrowed.

16. Conidia of B.bassiana, I.farinosa and M.anisopliae germinated best at pH 5.0, 4.0 and 5.0 - 7.6 respectively. The acid limit for B.bassiana conidia lay beyond pH 2.2 ; the alkaline limit was between pH 11.7 and 12.0. Conidia of I.farinosa germinated from pH 2.6 to 7.0. Conidia of M.anisopliae germinated at the most alkaline reaction of pH 12.0 used. The acid limit was between pH 2.2 and 2.6.

17. Of 10 mineral salts tried, all except $MgSO_4.7H_2O$ depressed germination of conidia of M.anisopliae to varying degrees. All on the other hand, stimulated conidium germination in B.bassiana and I.farinosa, with the exception of $FeCl_3.6H_2O$ and ZnCl which

inhibited germination in conidia of B.bassiana, and $CuSO_4 \cdot 5H_2O$ and NaCl which suppressed germination of conidia of I.farinosa.

18. Casein hydrolysate, yeast-extract and dextrose enhanced conidium germination in all three fungi.

19. Aqueous extracts of egg shell, silk of pupal cocoon, cuticle of pupa and adult of Bombyx mori (silkworm) stimulated germination and germ tube growth of all three fungi.

20. The extract of silk from Bombyx mori was inherently stimulatory ; no action of the fungi was necessary.

21. The extract of silk from Bombyx mori was highly effective on dilution, even to 1/64th of the original strength.

22. The stimulatory substances of the extracts of the exoskeleton of Bombyx mori had no general chemotropic effect on the hyphae. An inconsistent effect on germ tube growth was however indicated.

23. The stimulant in extract of silk from Bombyx mori was thermostable and effective over a wide range of pH.

24. Analysis of the aqueous extract of silk from Bombyx mori by paper chromatography revealed the presence of 5 amino acids : glycine, leucine, serine, threonine and tyrosine.

25. The individual consituent amino acids of aqueous extract of silk

highly stimulated germination of conidia of I.farinosa.

26. The response to aqueous extract of silk was not specific to entomogenous fungi alone. The extract stimulated spores of Aspergillus niger, Botrytis allii, Cladosporium herbarum, Fusarium culmorum, Penicillium chrysogenum, Penicillium digitatum and Rhizopus stolonifera.

27. Non-sterile water extract of their native soils, depressed germination of conidia of B.bassiana and I.farinosa.

28. The inhibitory properties of the soil extracts were annulled by : filteration, heat-sterilisation, the addition of dextrose or aqueous extract of silk from Bombyx mori.

29. The inhibitory substances of the soil extract were not fungicidal ; inhibited conidia of B.bassiana and I.farinosa were subsequently stimulated to germinate with peptone-dextrose medium.

30. Conidial samples of B.bassiana and I.farinosa from respectively, 8 and 21 day old cultures are heterogeneous, the individual conidia requiring different periods to produce germ tubes, whilst M.anisopliae conidia from 5 day old cultures are homogeneous and possess similar latent periods of germination.

31. Conidia of B.bassiana germinated best in water immediately on

attaining mature size, and thereafter gradually decreased with age. Few conidia would germinate without stimulation at the age of 40 hours.

32. I.farinosa conidia within the first 8 - 10 hours after attaining mature size would not germinate in water. They germinated best in water when 18 - 26 hours old, and quickly lost the ability thereafter.

33. Conidia of M.anisopliae germinated in water immediately on attaining mature size, but unlike B.bassiana conidia, the ability to germinate in water was fully retained over at least 120 hours.

34. The length of life of the conidia was affected by environmental conditions.

(a) Light had a deleterious effect.

(b) At any given relative humidity, increasing the temperature generally decreased viability of the conidia, and lower temperatures favoured long survival.

(c) B.bassiana and I.farinosa conidia lost viability most rapidly at high humidities and were preserved longest at the lower humidities.

(d) Conidia of M.anisopliae exhibited a singular response to relative humidity during storage. Viability of the conidia was lost most rapidly at intermediate relative humidities

(40 - 50 % R.H.) and most slowly at the extreme relative humidities (0 - 25 and 75 - 95 % R.H.).

35. The time for conidia to form germ tubes lengthened with age.

36. Monospore cultures of old and young conidia grew at similar rates and sporulated to the same degree.

37. Conidia of M.anisopliae washed with wetting agents, to render them more wettable, still showed the unique pattern of survival when stored at different relative humidities, but the critical unfavourable humidity however shifted from 45 - 55 % R.H. to 55 - 75 % R.H.

38. Conidia of M.anisopliae washed with salts of the Hoffmeister ion series also showed the unique pattern of survival, with the conidia dying more rapidly at median humidities and surviving longer at higher and lower humidities. Conidia washed with almost all these salts still showed the shift in the unfavourable mid-humidity range from 45 - 55 % R.H. to 55 - 75 % R.H. Of 14 salts tested $NaNO_3$ and Na_2SO_4 alone showed some restitution to washed spores of the humidity-viability characteristics of unwashed spores.

39. High carbon dioxide concentration almost suppressed the early death of the conidia of M.anisopliae at the critical mid-humidities.

40. Exclusion of oxygen from the humidity chambers by using 100 % Nitrogen also suppressed the early death of conidia of M.anisopliae at the critical median humidities.

41. Respiration rates (oxygen consumed) of M.anisopliae conidia held at 25 to 75 % R.H. were measured with a Warburg Apparatus. Generally, volume of oxygen consumed increased with rise in relative humidity.

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