The Effect of Air Pressure and Light upon the Germination of the Conidia of *Erysiphe graminis Hordei* Marchal, and the Activity of the Generative Cell in the Production of Conidia.

by

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1. Introduction

In 1940, Brodie and Neufeld (3) published the results of their investigations in connection with Erysiphe Polygoni DC., in which they discussed, among other matters, the failure of the mature conidiam to germinate in situ, i.e., before being dislodged from the parent conidiophore. The authors suggested that the failure of such spores to germinate is based upon two facts, (1) the relatively impervious nature of the conidiophore wall to gases, and (2) the physiological separation of the conidium from the conidiophore by means of a septum. Under these conditions, carbon dioxide produced by the continued respiration of a mature, undislodged conidium would be unable to escape and thus there would result a concentration of carbon dioxide in the spore which would be sufficient to inhibit germination.

In an effort to collect data which might contribute to the support of this theory, the writer undertook an investigation of the relationship between conidial germination and air pressure, using spores of Erysiphe graminis Hordei Marchal instead of spores of Erysiphe Polygoni owing to the ease with which the host plant of the former mildew could be grown.

II. Historical

The demonstration by C.E. Yarwood (18) in 1936, that the conidia of certain species of Powdery Mildew Fungi are able to germinate under conditions of extremely low humidity, opened up an interesting new field of research. Previous investigators had concerned themselves chiefly with taxonomic, cytological and pathological studies of the Erysiphaceae and with the problems of sexuality in this group.

Much of the literature relative to the Physiology of the Powdery Mildews concerns host-parasite relations and will not be included in the present review, the purpose of which is soley to report those investigations bearing directly upon the physiology of the fungi themselves and upon the research reported in this paper. Such literature may conveniently be dealt with in the following order: (1) germination of the conidia and the influence exerted upon it by environmental factors such as light, temperature and relative humidity; (2) the question of the diurnal cycle in the maturation and abstriction of conidia, and (3) the nature of the conidiophore and the relationship between its structure and the process of abstriction of conidia.

The stimulatory effect of light upon the germination of Powdery Mildew conidia has long been recognized. Early references to it are found in the work of Neger (14) and Hammerlund (11), corroborated by the more recent observations of Yarwood (19) Cherewick (4) and the writer himself. Accord-

ing to Neger (14), light stimulates both the germination of conidia and the growth of the germ-tube. Neger stated that germ-tubes always grow in the direction of the light source, i.e., they exhibit positive heliotropism, and that this reaction decreases with the age of the spore. He also differentiated between species of Erysiphe which are sensitive to light and those which are insensitive or neutral. More recently, Yarwood (19) has reported stimulation by light of germination of conidia of Erysiphe Polygoni obtained from red clover, delphinium and cabbage, and Erysiphe graminis from barley. Finally, reference should be made to the work of Cherewick (4) who obtained similar results with conidia of Erysiphe graminis. He pointed out that although light undoubtedly stimulates germination at temperatures at or near the optimum, it may have the reverse effect at temperatures above the optimum.

The results recorded by various workers with respect to the effect of relative humidity upon the germination of Powdery Mildew conidia, are not as clear-cut as those obtained in connection with the effect of light upon germination, since the optimum relative humidity for germination of conidia varies from species to species. Salmon (16) stated that conidia of the Erysiphaceae germinate more readily in a dry atmosphere than in a moist one. This may be true for conidia of some species, but it would be more accurate to state that while conidia of some species are very tolerant of low humidity, better germination is obtained under relatively moist conditions.

With respect to the moisture requirements for conidial

germination, members of the Erysiphaceae fall into two groups: (a) those which are tolerant of low humidity, and (b) those which are intolerant of low humidity. Regarding the former group, Dundas (8) reported that conidia of Erysiphe Polygoni from Phaseolus vulgaris L. germinated at temperatures as high as 28° C and at relative humidities as low as 8%. Yarwood (18) found that conidial germination of this fungus on bean leaves is actually favoured by drying, since he obtained greater germination of conidia at zero humidity (over sulphuric acid) than at 100% relative humidity.

It is interesting to note that many of the fungi which tolerate low humidity germinate well under a wide variety of relative humidity conditions. For example, Brodie and Neufeld (3) reported that Erysiphe Polygoni from Delphinium, Sphaerotheca humuli (DC.) Burr., from rose, Sphaerotheca fuliginea (Schlecht.) Poll. from pansy and Erysiphe graminis Poae Marchal from Poa pratensis germinated equally well at relative humidities ranging from zero to 100% and concluded that, in the matter of germination, mildew conidia are independent of humidity. Again, Yarwood (18) found that, at 22° C, conidia of Erysiphe Polygoni from red clover, Erysiphe graminis Hordei from barley, Erysiphe graminis Tritici Marchal from oats and Erysiphe cichoracearum DC. from sunflower would germinate on dry slides at zero and 100% relative humidity as well as on the surface of water and suspended in water. Similar results were obtained by Dundas (8) with Erysiphe Polygoni on beans.

Of the mildews tested by Yarwood (18) conidia of Erysiphe cichoracearum from sunflower, and Erysiphe graminis from barley proved to be the least able to germinate at zero humidity, giving germination values of 1.5% and 2% respectively. Germination values at zero humidity of the other species tested by Yarwood ranged from 25% to 63%. Yarwood's results are not corroborated by the work of Cherewick (4) who tested conidia of the following varieties of Erysiphe graminis at 18° C and 6° C to determine their ability to germinate under dry conditions: Erysiphe graminis Tritici,- Hordei,- Avenae Marchal and - Agropyri Marchal. His results indicate excellent germination at zero and 100% relative humidity, although germination under saturation conditions was better. This is not in agreement with Clayton's (6) report that conidia of Erysiphe graminis from barley show almost no germination at relative humidities of 88% or below, nor with the observations of Brodie (unpublished data) who has obtained high percentage germination of the conidia of the same mildew under a range of humidity conditions from 30% to 50%.

With respect to the second group of fungi, those which do not tolerate low relative humidities, Hashioka (12), in a paper dealing with Sphaerotheca fuliginea, reported that the optimum relative humidity for germination was 100%, only a trace of germination being obtained at the very low humidities such as those which would obtain in a desicator containing calcium chloride. Similarily, Berwith (1), working with Podosphaera leucotricha (E. and E.) Salm. on apples, found that conidia

germinated only at relative humidities above 90%.

The most accurate investigation of the effect of relative humidity on the germination of mildew conidia was that on Longrée (13) who, by using a special apparatus for the rigid control of temperature, provided very stable conditions of relative humidity. The germination of conidia of Sphaerotheca pannosa (Wallr.) Lev. var. rosae Wor. examined under these conditions was found to be excellent at humidities ranging from 99% to 96.9%, very low at 94.9% and absent below 75%.

Finally, the writer would suggest that since the observations recorded in the literature thus far have, for the most part, been made under conditions that were not always rigidly controlled, a fresh examination of the behaviour of certain species might well be made. At present, one can conclude only that it seems well substantiated that the conidia of certain mildew species do germinate well in very dry air and that a few are apparently quite unable to tolerate low humidity.

Before leaving the question of humidity and conidial germination, reference may be made to the effect of free water upon germination. It is generally agreed that Powdery Mildew conidia do not germinate properly in or upon water. This statement is supported by the reports of a number of workers, among whom may be mentioned Hashioka (12), Berwith (1), Clayton (6), Graf-Marin (10) and Corner (7). Both Graf-Marin and Hashioka have reported that water not only inhibits germination but

affects the structure of the germ-tube itself. To quote Graf-Marin, germ tubes produced are "long and slender .... growing more or less straight and unseptate until the reserve material of the conidium is exhausted." (Ref. page 8). Hashioka made the same observation with reference to conidia of Sphaerotheca fuliginea but failed to find any indication of the septum described by Graf-Marin.

That the degree of tolerance of low humidity of any mildew may vary with the temperature has already been demonstrated by Yarwood (18) for Erysiphe Polygoni. Therefore, the possible effect of temperature must be borne in mind in attempting to evaluate the literature reviewed above.

Careful studies of the effect of temperature upon germination have been made from time to time. Generally, the optimum germination temperature for Powdery Mildew conidia has been found to be low. According to Graf-Marin (10), the optimum temperature for germination of spores of Erysiphe graminis on barley is 12° C, although spores germinate well at temperatures as low as 5° C. The best temperature for the most rapid growth of germ tubes is 21° C. Germination is definitely impaired above 25° C.

Working with freshly collected conidia of Podosphaera leucotricha in moist chambers, Berwith (1) established that the optimum for germination of that species lies between 19° C and 22° C. According to Longree (13), conidia of Sphaerotheca Pannosa possess a minimum germination temperature of 3° C to 5° C, an optimum ranging from 21° C to 25° C, and a maximum of 33° C. In a

subsequent experiment, she found that during the first 13 hours, the optimum temperature for germination was 27.5° C. During the next 98 hours the optimum dropped to 21.5° C. It is obvious, therefore, that not only may the optimum temperature for germination shift from one test to the next but that it tends to decrease with lapse of time.

Cherewick (4) found that conidia of Erysiphe graminis from barley germinated well at 3° to 20° C while the maximum temperature for germination was 35° C. Like Longrée, he found that the optimum temperature was not clearly defined, varying from 15° to 20° C on bright days, to 10° C on dull days when germination was weak.

Periodicity in spore production and liberation induced by the diurnal fluctuations of light intensity from day to night is known for a considerable variety of fungi. In some of the Erysiphaceae, at least, such a diurnal cycle is evident, involving not merely the production of conidia but the ability of these to germinate under standard control conditions.

In 1936, Yarwood (19) demonstrated a diurnal cycle in Erysiphe Polygoni on field-clover plants which was shown in the maturation and dissemination of conidia, in the division of the generative cell, and in the formation of appressoria. According to Yarwood, the generative cell of the conidiophore of Erysiphe Polygoni divides once a day in the late afternoon, the distal daughter cell developing into a single conidium which is abstricted between 8 a.m. and 12 M, and the proximal daugh-

ter cell continuing to function as the generative cell. The dissemination of conidia was found to take place during the latter part of the daylight period while appressoria developed principally during the light period.

In 1939, Childs (5) established diurnal cycles for Erysiphe cichoracearum on sunflower, cucumber, aster and rose; for Sphaerotheca pannosa on rose, Podosphaera leucotricha on apple, Erysiphe Polygoni on bean and Oidium enonymi-japonici on Euonymus Japonica L. In the case of the non chain-forming mildews Erysiphe Polygoni on bean and Oidium enonymi-japonici on Euonymus, the diurnal cycle proved to be similar to that established by Yarwood for Erysiphe Polygoni on clover, one conidium being formed each day, the period of abstriction occurring between 10 a.m. and 2 p.m. Conidia of the mildews which form spores in chains, were formed between 2 to 4 p.m. and 6 to 8 a.m. and were abstricted between 6 to 8 a.m. and 2 to 4 p.m. In connection with the nature of the generative cell of Erysiphe cichoracearum, Childs, on the basis of microscopic examination of stained conidiophores, concluded that both the basal cell and the one above it function generatively.

In 1913, Foex[9] published his researches upon the development of the conidiophore of Sphaerotheca humuli in which he discussed the behaviour of nuclei. According to Foex, a swelling occurs on a hypha just above a nucleus. The nucleus proceeds into the swelling and is cut off from the main

hypha by a transverse septum. The nucleus then divides, and the two daughter nuclei are separated by a septum which divides the elongating hypha into two cells. The upper cell re-divides to form two daughter cells which eventually develop into conidia while the lower cell continues to elongate giving rise, from time to time, to a cell which will later divide to form two elements which, in turn, develop directly into conidia. This description closely parallels that given by the present writer of the behaviour of the generative cell of Erysiphe graminis Hordei in a later section (ref. page37).

An extensive description of nuclear behaviour during conidiophore development in Sphaerotheca mors-uvae and Microsphaera Astragali has been given by Bezssonof (2).

Many of the details of the morphology of the conidiophore and the conidia have been studied by Brodie and Neufeld (3) in Erysiphe Polygoni and by Brodie (3a) in Erysiphe graminis. In the former species, the protoplasm is continuous from the conidiophore to the conidium, passing through a perforated septum which is formed by inward growth of a ring of wall material. When the conidium is mature, the pore in the septum is plugged and the conidium becomes passively dislodged by a mechanism not fully understood.

In Erysiphe graminis, Brodie was able, by using a special staining method, to demonstrate photographically the continuity of protoplasm through transverse septa separating adjoining conidia.

Endeavouring to correlate the facts presented in the present review of the literature and to explain his own results and the results of others, Brodie (3 ,3a) has put forward a theory concerning the unusual behaviour of mildew conidia with respect to humidity relations. This theory will be referred to later in the present paper where it is more properly applicable.

111. Materials and Methods.

Erysiphe graminis Hordei: Marchal, Race 8 (4), was used in all investigations, the mildew being maintained upon pots of barley (variety OAC-21) stored in a Wardian case in the laboratory. The host plants were grown in an environment of continuous light, in order to prevent the physiological resistance to the mildew which the plants develop when grown in the diffuse light of the laboratory (15, 17). A 600-Watt electric light bulb, set in a white reflector and suspended at a distance of one foot above the center of the Wardian case, provided satisfactory illumination.

Collection of Spore Samples: Six glass microscope slides were placed at the bottom of a tall bell jar, and heavily infected leaves were shaken so as to allow the dislodged conidia to settle upon the slides; by this method, fairly uniform spore samples were obtained. Three of these samples were subjected to the conditions of the experiment, the other three being kept as a control.

Method of Counting Spore Samples: The number of conidia and the number of germ tubes in each field was determined, a total of three hundred conidia being counted on each slide. Dense fields were avoided wherever possible because of the difficulty of counting accurately. Since three slides were used in the experiment and control respectively, approximately nine hundred conidia were counted for each series. From the total number of conidia and the total number of germ-tubes in each series, the percentage

of conidia that germinated under control and experimental conditions could be calculated. In order to avoid inconsistencies caused by variations from day to day in the uniformity of the spore samples, all germination values will be expressed as percent of control.

Fixing of Spore Samples: In order to exclude a possible error due to additional germination during the counting period, spore samples, after being allowed to germinate under experimental and control conditions, were fixed by exposing them for one minute to the fumes of glacial acetic acid in a desiccator. This prevented further germination but did not cause either the spores or the germ tubes to shrivel. Since each counting period lasted for at least three-quarters of an hour, spore samples were stored in a petri-plate moist-chamber in order to prevent their desiccation.

IV Investigations

The Effect of Variations in Pressure upon Germination of Conidia of Erysiphe graminis Hordei

According to the theory put forward by Brodie and Neufeld (3) to account for the non-germination of powdery mildew spores *in situ* and the ease with which they germinate after being abstricted from the parent conidiophore, the passage of gases through the papilla is of the utmost importance in germination. Their account of the theory may be quoted in part as follows:

"The septum which abstricts the terminal conidum ..... becomes entirely closed and the conidium may be supposed to be separated physiologically from the conidiophore. After separation, the continued respiration within the conidium might do two things: (1) increase the internal concentration of carbon dioxide and, at the same time, (2) decrease the internal concentration of oxygen. It has already been shown in this paper that the conidium wall is relatively impervious to water except at the papillate end. If it be assumed that it is also relatively impervious to gases, then, as long as the conidium remains attached to the conidiophore, either the accumulation of carbon dioxide within the mature conidium or the low concentration of oxygen might prevent its germination." (ref. page 55).

It follows that any condition which would facilitate the escape of carbon dioxide from the protoplast *via* the papilla

might be expected to enhance germination and, conversely, any condition which would render the escape of carbon dioxide more difficult might be expected to depress the germination activity of the conidia.

With this in mind, the effect of variations in air pressure upon the germination of mildew conidia was studied and will now be dealt with.

(1) Effect of Reduced Pressure

Materials and Methods: The apparatus devised for the germination of conidia at various degrees of reduced pressure is illustrated in Plate I. Fig. 1. The two germination chambers, D and E, were immersed in a constant temperature bath, B supplied with cold running water from an inlet tube at the bottom and maintained as closely as possible to 20°C.[1] The experimental chamber, D, was connected on the one hand to a mercury manometer, C, and on the other, through the trap, A, to

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1 It has been shown by Cherewick and Gräf-Marin (4, 10) that the optimum temperature for germination of conidia of Ersiphe graminis lies between 10° - 20°C. Since the writer's experiments were performed during the heat of the summer when the temperature of the laboratory ranged anywhere from 23° - 29°C (75° - 85°F), it was necessary, in the absence of apparatus for accurate maintenance of low constant temperatures, to devise some means of keeping the germinating conidia within the optimum temperature range. This was done by adjusting the temperature of the water bath to 18°C by means of ice and cold running water from the inlet tube of the bath, and by constant attention, keeping it as near to this temperature as possible. Although slight variations in temperature from 18° - 22°C could not be avoided, the effect of such a drift upon the accuracy of the results obtained would be obviated by the fact that the germination of any sample was always recorded as percent of control at precisely the same temperature.

Plate I

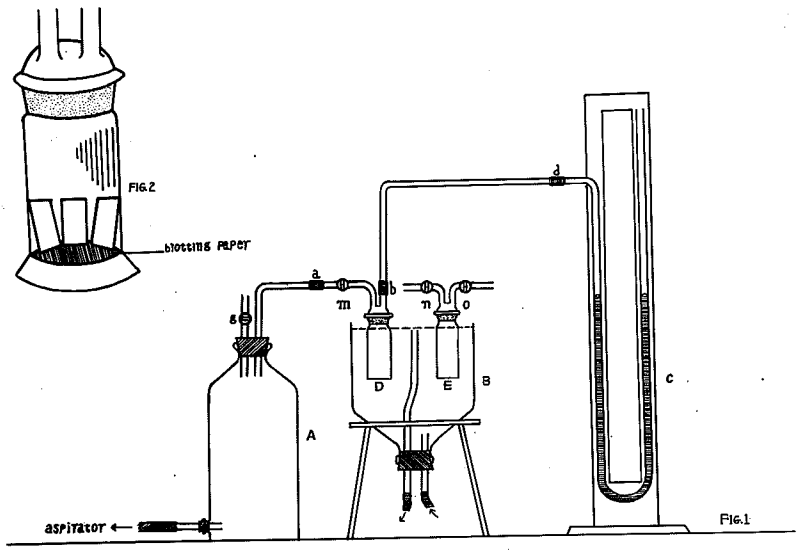

FIG.1

a powerful water aspirator. Fig. 2 shows a germination chamber in detail and illustrates the manner in which spore-laden slides were arranged for an experimental run. A disc of moist blotting-paper, placed at the bottom of each chamber, ensured a saturated atmosphere which would be comparable in all experiments.[1]

Due to the toxic effect of vulcanized rubber upon mildew conidia (3), ground-glass stoppered vessels and gum-rubber connections[2] only were employed. The bung of the trap and the pressure-tube leading to the aspirator were of vulcanized rubber since, under the conditions of the experiment, no vitiated air could pass from the trap into the experimental chamber.

The standard procedure in making an experimental run was as follows: Slides, dusted with conidia in the manner previously described, were placed in their respective chambers as shown in Plate I, Fig. 2. It has been observed by Brodie (unpublished data) that even a slight increase in pressure, such as that produced in a bottle by the forcing home of the stopper, may be sufficient to depress the germinability of conidia; therefore, when adjusting the ground-glass stoppers of the two chambers, the writer was careful to leave the experimental and control systems in free communication with the outer air by removing the bung and

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[1] It may be assumed that this method of obtaining comparable humidity conditions is used in all subsequent experiments in the thesis whenever germination chambers are employed.

[2] Gum rubber tubing was employed in all experiments where rubber connections were required. If, in any instance, vulcanized rubber was used, it will be specifically stated to that effect.

opening taps m, n and o. When the glass stoppers of the germination chambers had been fitted into place, the taps of the control chamber were closed and the rubber bung forced firmly into the neck of the trap. As soon as the manometer indicated that the aspirator had produced the desired reduction in pressure, the tap, m, was closed, thus cutting off the germination chamber and the manometer from the rest of the system.

Since it had been noticed that germination of conidia appeared to be greatest during the hours immediately preceding and succeeding noon,[1] conidia were allowed to germinate under the above conditions from 11 A.M. until 1 p.m.

Results:

Table I

Effect of Reduced Pressure upon Germination of Mildew Conidia at approximately 18° C.

Pressure (mm.hg)	Germination (percent Control)
704	96
696	102
647	103
645	100
635	106
608	126
571	111
570	119
567	117
540	111
500	98
494	93
348	92
344	85
340	89
196	81
186	77
94	43
60	5
50	0

[1] Footnote on P.18

Table II

Effect of Reduced Pressure upon Germination of Mildew Conidia at approximately 18° C.

Pressure (mm. Hg)	Germination (Percent Control)
724	98
716	104
690	109
683	113
660	117
660	105
616	108
596	97
503	89
501	93
416	92
346	95
249	91
190	75
142	62
82	53
34	6

Discussion: The tables given above indicate that the germination of conidia is enhanced over a narrow range extending, in Table I, from 696-540 mm; and from 716 - 616 mm. in Table II. Below these limits, germination falls, at first gradually and then more rapidly to zero. It is interesting to note that when these values are expressed graphically, (Plate II) they show a definite, linear relationship.

Each graph may be divided arbitrarily into three regions: The first, the region of enhanced germination, is that in which the curve rises to a maximum and then declines.

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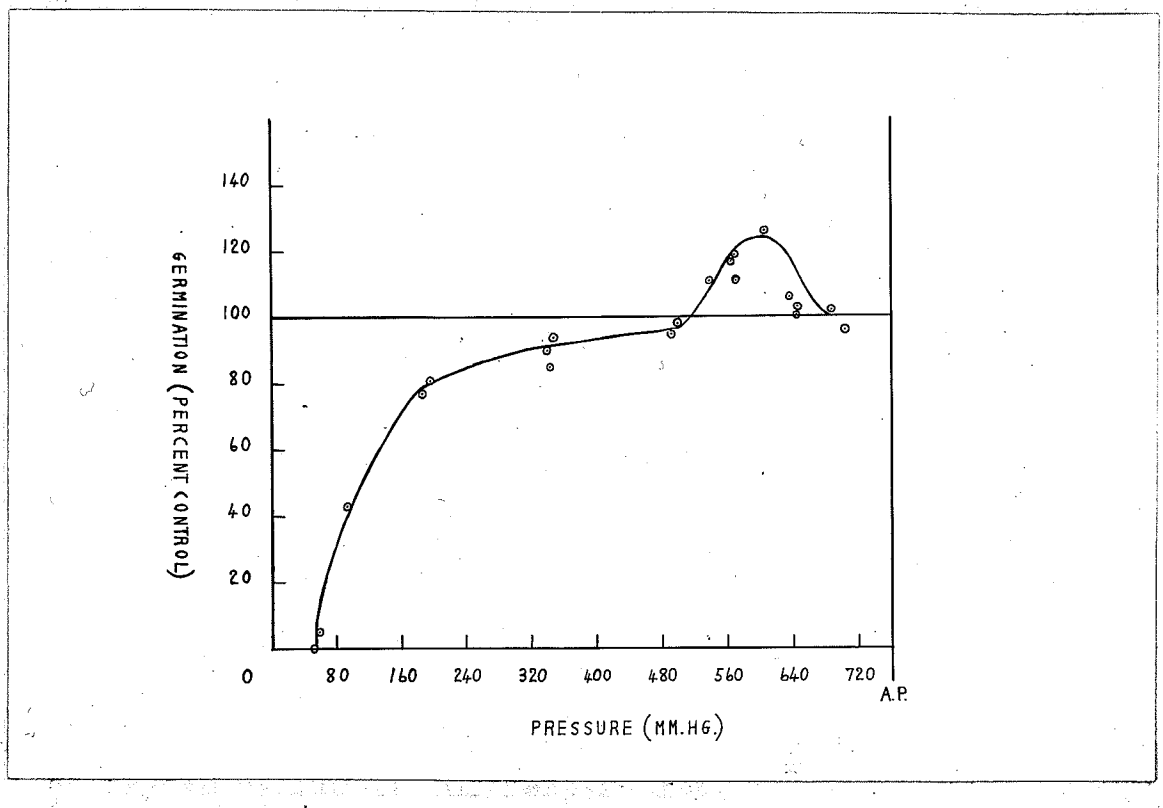
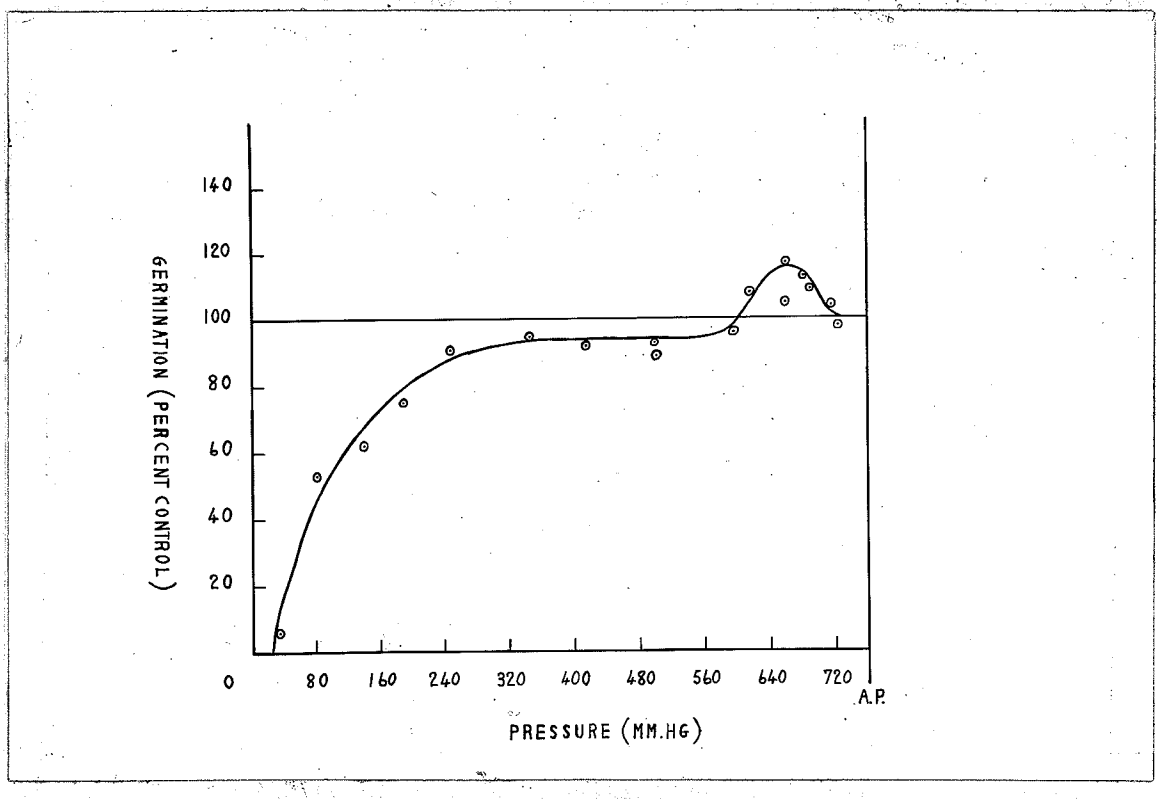
[1] Neger (14), Hammerlund (11) and Cherewick (4), as well as the writer have shown that light stimulates germination. It is not surprising, therefore, that germination should be at its maximum during the hours of maximum light intensity.

Plate II


GERMINATION (PERCENT CONTROL)
140
120
100
80
60
40
20
0
80
160
240
320
400
480
560
640
720
A.P.
PRESSURE (MM.HG.)


GERMINATION (PERCENT CONTROL)
140
120
100
80
60
40
20
0
80
160
240
320
400
480
560
640
720
A.P.
PRESSURE (MM.HG)

The second commences at the point where the curve intersects with the 100 percent line and forms a more or less horizontal plateau. At the pressures obtaining in this region, sufficient oxygen has been removed from the germination chamber to cause a slight depression in germination. In the third region, where low pressures obtain, so little oxygen is present to support respiration that germination falls rapidly to zero.

Summarising, we may say that two facts are indicated by the graphs: (1) that slight reductions in pressure enhance germination and (2) that reduction in pressure beyond a certain critical point brings about a slow and then a more rapid decline in germinative activity due to a steady decrease in the amount of oxygen available for respiration.

In referring to the theory of Brodie and Neufeld (3), viz., that the passage of gases through the papilla is of the utmost importance in germination, the writer went on to suggest that any condition which would facilitate the escape of carbon dioxide through the papilla might be expected to enhance germination. The question that arises, therefore, is this: How does reduction in pressure facilitate the escape of carbon dioxide from the spore?

Let us consider a single spore, germinating in an enclosed space. As soon as carbon dioxide has escaped from the spore, it will begin to diffuse away into the surrounding air and more carbon dioxide will diffuse out through the

papilla to take its place. In this way, there is set up a diffusion gradient from the protoplast into the air of the enclosing chamber. Now, it may be assumed that, at atmospheric pressure, the air molecules are arranged in a certain density. The rate at which carbon dioxide molecules could escape from the immediate vicinity of the papilla would depend upon this density since their progress would be impeded by repeated collisions with the molecules of air. However, in reducing the pressure in a closed system, such as the one employed in the investigation under discussion, a certain quantity of air is removed from the germination chamber and the air remaining behind must therefore expand. As a result of this expansion, the distance between the individual molecules would be greater and the incidence of collisions between the two types of molecules proportionally less. The carbon dioxide molecules would therefore be able to diffuse away more rapidly from the papilla, the diffusion gradient from protoplast to air would be steepened and germinability correspondingly enhanced.

If this theory represents a picture of what actually takes place, then the converse should also be true, that an increase in pressure would depress germination by compressing the air molecules surrounding the spore and thereby slowing down the escape of carbon dioxide molecules from the papilla. The following investigation, therefore, will deal with the germination of spores under conditions of increased pressure.

(2) Effect of Increased Pressure

Materials and Methods: The apparatus used for germinating conidia under conditions of increased pressure was the same as that employed in the previous experiment (Plate I, Fig.1) except for the fact that the experimental chamber was connected to an improvised water pump instead of to an aspirator. This pump (Plate III, Fig.1) consisted of a round, pyrex flask, C, provided with a cork stopper, g, through which passed a tightly-fitting glass tube, the free end of which was connected to the water faucet by means of a short length of rubber tubing, n. Because of the relatively high pressures produced in the system, the stopper, g, and the rubber connections were securely wired whilst the ground glass stopper of the experimental chamber was sealed in position for the duration of the experiment by means of a special device (Plate III, Fig. 2). This consisted of a hinged collar, i, which fitted snugly around the neck of the chamber, just below the projecting lip. A metal clamp, m, fitting over the stopper, was attached to the collar at each end by means of a bolt and winged nut. By tightening the nuts, the clamp could be pressed firmly against the stopper, thus preventing pressure inside the chamber from forcing the stopper out. The collar and clamp were adjusted after sporeladen slides had been arranged in the germination chamber as shown in Plate I, Fig. 2. In order to increase the pressure in the system, water was

Plate III

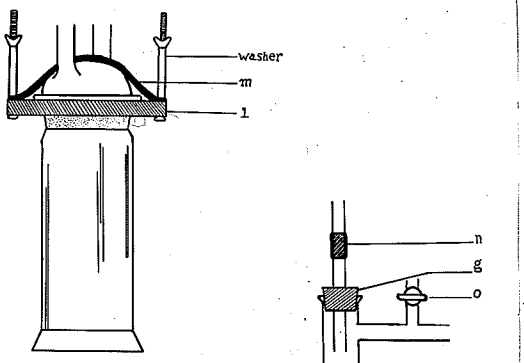


FIG. 2

FIG. 1

forced into C (Fig. 1, Plate III) and when the desired increment had been obtained, the tap m, (Fig.1, Plate I) was closed. As in the previous experiment, germination was allowed to proceed from 11 A.M. to 1 P.M., after which the spores were fixed and counted.

Results:

Table III

Effect of Increased Pressure upon Germination of Mildew Conidia at 18° C.[1]

Pressure (mm.Hg)	Percentage Germination At Increased P.	At Atmospheric P.	Percent Control
780	60	68	88
824	57	63	90
841	42	43	97
940	37	37	100
1038	43	55	78
1114	32	50	64
1117	26	37	70
1155	23	40	57
1233	30	40	75
1256	37	40	92
1267	36	48	70
1316	28	28	100
1364	28	31	90
1378	26	23	113
1385	38	33	118
1405	33	45	73

The major portion of the data presented in Table III indicates that the germination percentages obtained under conditions of increased pressure are lower than those obtained at atmospheric pressure. Only in two cases, viz. at pressures of 1378 and 1385 mm. are the experimental values higher than those of the control.

Discussion: Although there appears to be no doubt

[1] Approximately 18°C.

that germinability is inhibited by an increase in pressure, a comparison of the first and last columns of Table III indicate that germination values, expressed as percent of control, do not decrease with corresponding increases in pressure. Pending further investigation, only a general statement can be made; that over a range of pressures extending from 780 - 1405 mm., an inhibition in germinability is brought about which may be expressed by a series of values ranging from 57 - 100 percent of the control. It is the writer's belief that a series of pressures beyond the scope of the present apparatus will have to be employed before any further decrease in the germinability of conidia is obtained.

A Comparison of the Germination Times of Spores of Erysiphe graminis Hordei under Conditions of Reduced Pressure and Atmospheric Pressure respectively.

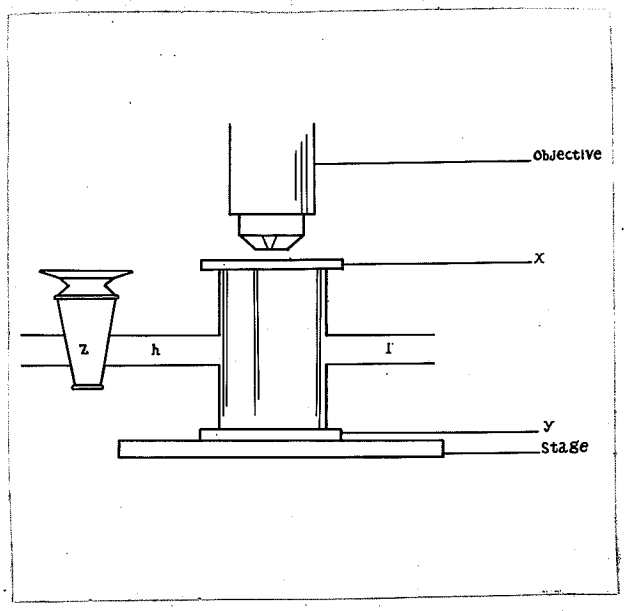
The writer has shown that reduction in pressure, within a certain limited range, definitely enhances the germinability of spores. The question now arises: does such a reduction actually increase the percentage of spores which will germinate upon a given slide, i.e., does it induce spores to germinate which might otherwise have remained dormant? Or is the effect of pressure reduction merely one which allows more spores to germinate within the time limit of the experiment? It occurred to the writer that some light might be thrown upon the problem if a comparison were made of the germination times of

spores under conditions of reduced and of atmospheric pressure respectively. It is to an account of this work that the present section is devoted.

Materials and Methods: By means of the apparatus shown in Plate IV, constant microscopic observation of germinating spores, under conditions of reduced and of atmospheric pressure respectively, was made possible. Two microscopes were arranged side by side and their fields illuminated by a common light source. Upon the stage of each was placed a germination chamber, as shown in the figure. Each chamber consisted of a length of glass tubing, four centimeters long and two-and-a-half centimeters in diameter, the ends of which had been ground level. The tubes, h and i, either connected the experimental chamber to an aspirator and manometer respectively or, in the case of the control chamber, terminated in taps which were closed during an experimental run. The arm of the experimental chamber leading to the aspirator was also provided with a tap, z, by means of which the chamber and the manometer were sealed off from the rest of the system after the desired reduction in pressure had been obtained.

A microscope slide, y, was hermetically sealed to the lower end of the tube or chamber by means of paraffin wax. When an experiment was to be made, a similar slide, x, bearing spores upon its upper surface, was inverted and placed over the upper end as shown in the figure. By coating the ground glass surface with a thin layer of vaseline, a temporary air-tight

Plate IV



seal was made between chamber and slide. Owing to the impossibility of immersing the chambers in a constant temperature bath, all experiments were conducted at room temperature (about 20° C).

RESULTS:

Table IV

Effect of Reduced Pressure upon Germination Time of Mildew Conidia.

Expt. No.	Graph No.	Temp. (°C)	Time (minutes)	Number of Germ Tubes at Reduced Pressure	Number of Germ Tubes at Atmospheric Pressure
1	1	20	0	0	0
			15	0	0
			30	1	2
			45	3	3
			60	6	6
			75	7	7
			90	7	8
			105	11	9
			180	15	9
			195	16	11
			210	16	11
2	2	20	0	0	0
			15	0	0
			30	2	0
			45	4	2
			60	6	2
			70	13	3
			90	18	3
			120	22	4
3	3	20	0	0	0
			15	0	0
			30	3	4
			45	6	6
			60	11	6
			75	14	6
			90	25	11
			105	25	12
			115	30	13
			130	33	13

Table IV (Continued)

Expt. No.	Graph No.	Temp. (°C)	Time (Minutes)	Number of Germ Tubes at Reduced Pressure	Number of Germ Tubes at Atmospheric Pressure.
4	4	20	0	0	0
			15	0	0
			30	1	1
			45	7	6
			60	8	6
			75	9	6
			90	14	8
			105	14	8
			120	17	10
			135	18	11
			150	19	13
			165	19	14
			210	20	17
			225	20	19
5	5	20	0	0	0
			15	0	0
			30	3	5
			45	4	5
			60	6	6
			75	12	6
			90	14	6
			105	14	11
			120	18	15
			135	18	15
6	6	20	0	0	0
			15	1	1
			30	2	2
			60	6	4
			75	12	6
			90	12	9
			105	15	9
7	7	20	0	0	0
			75	12	5
			90	18	10
			105	22	10
			120	25	12
			135	27	15
			150	29	15

Table IV (Continued)

Expt. No.	Graph No.	Temp! (°C)	Time (Minutes)	Number of Germ Tubes at Reduced pressures	Number of Germ Tubes at Atmospheric Pressure.
8	8	20	0	0	0
			15	0	0
			30	2	2
			60	7	5
			90	12	10
			120	18	10
			180	23	13
			210	26	16
			285	26	16
9	9	20	0	0	0
			15	3	0
			30	6	1
			45	8	1
			60	10	2
			120	14	7
10	10	20	0	0	0
			15	0	0
			30	1	1
			60	7	6
			90	9	7
			115	11	12
			250	12	12
11	11	20	0	0	0
			15	0	0
			30	0	1
			45	3	1
			75	10	6
			105	17	9
			135	21	12
			195	22	13
			220	24	13
			340	24	15

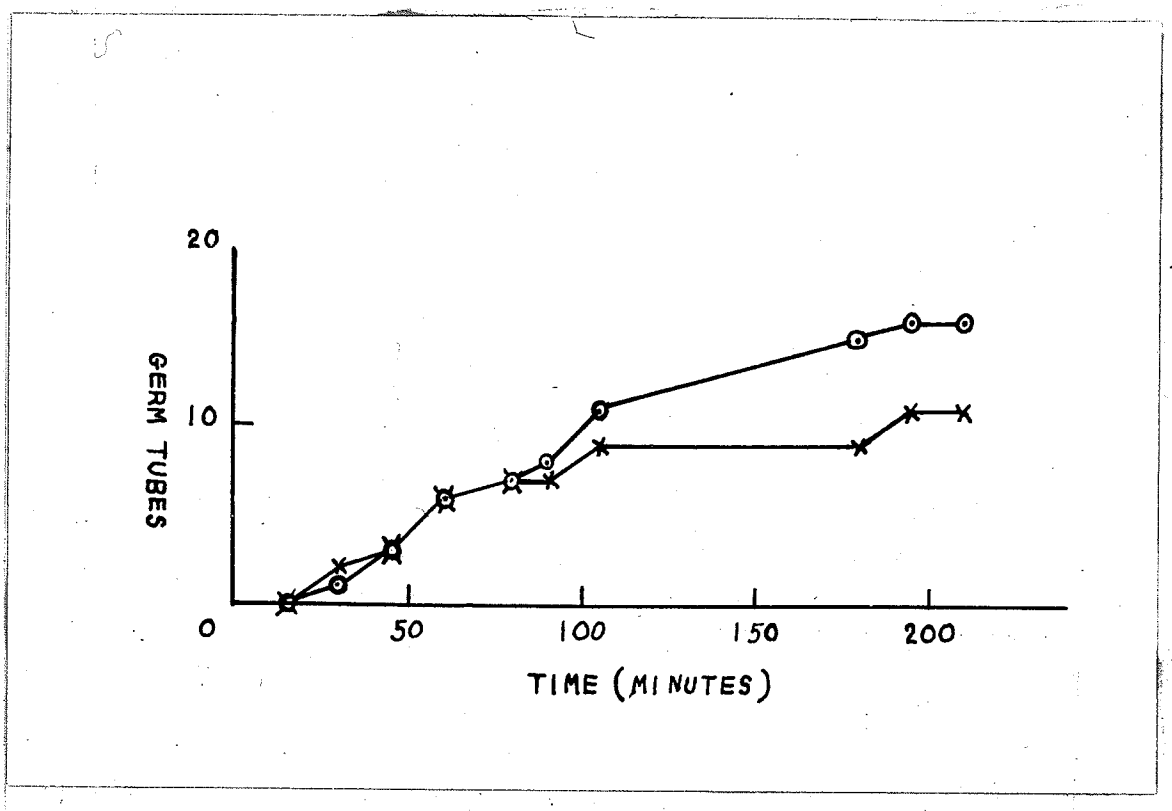
Table IV (Continued)

Expt. No.	Graph No.	Temp (°C)	Time (Minutes)	Number of Germ Tubes at Reduced Pressure	Number of Germ Tubes at Atmospheric Pressure
12	12	20	0	0	0
			15	0	0
			30	3	4
			45	5	5
			90	8	13
			180	23	16
			225	23	17
			300	27	21
			315	27	26
13	13	10.5	0	0	0
			15	0	0
			30	0	0
			45	0	0
			60	0	0
			135	5	4
			165	8	5
			195	16	7
			225	19	9
			255	20	9
			285	21	11
			315	23	12
14	14	9	0	0	0
			30	0	0
			45	0	1
			70	8	6
			105	11	6
			120	12	6
			150	13	6
15	15	9	0	0	0
			15	0	0
			30	0	0
			45	2	0
			60	3	1
			75	4	2
			90	5	2
			105	6	2
			180	6	4
			310	9	9

Table IV (Continued)

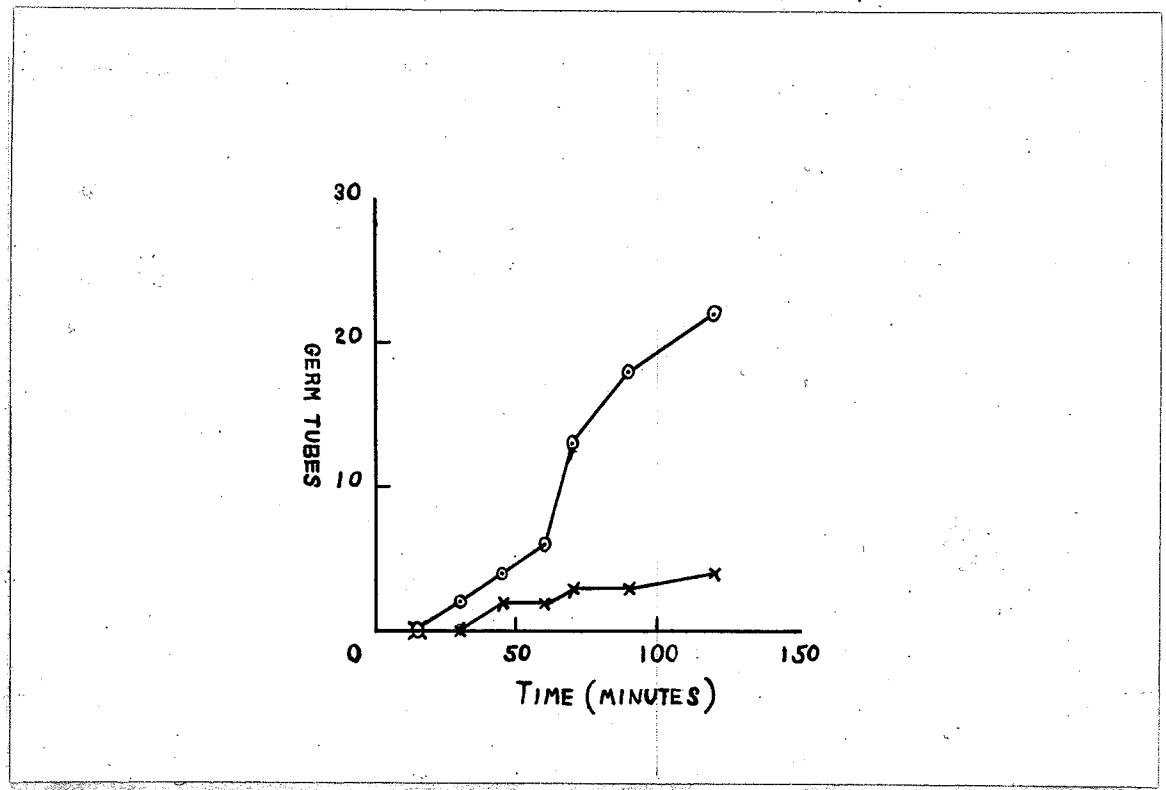
Expt. No.	Graph No.	Temp. (°C)	Time (Minutes)	Number of Germ Tubes at Reduced Pressure	Number of Germ Tubes at Atmospheric Pressure
16	16	8	0	0	0
			15	0	0
			90	6	8
			105	6	8
			135	7	10
			195	10	19
			225	12	20
17	17	8	0	0	0
			15	0	0
			30	0	0
			45	0	0
			60	2	2
			75	4	3
			105	7	9
			135	8	12
18	18	6.5	0	0	0
			15	0	0
			30	0	0
			45	0	1
			60	1	1
			75	1	1
			100	1	1

Plate V



1

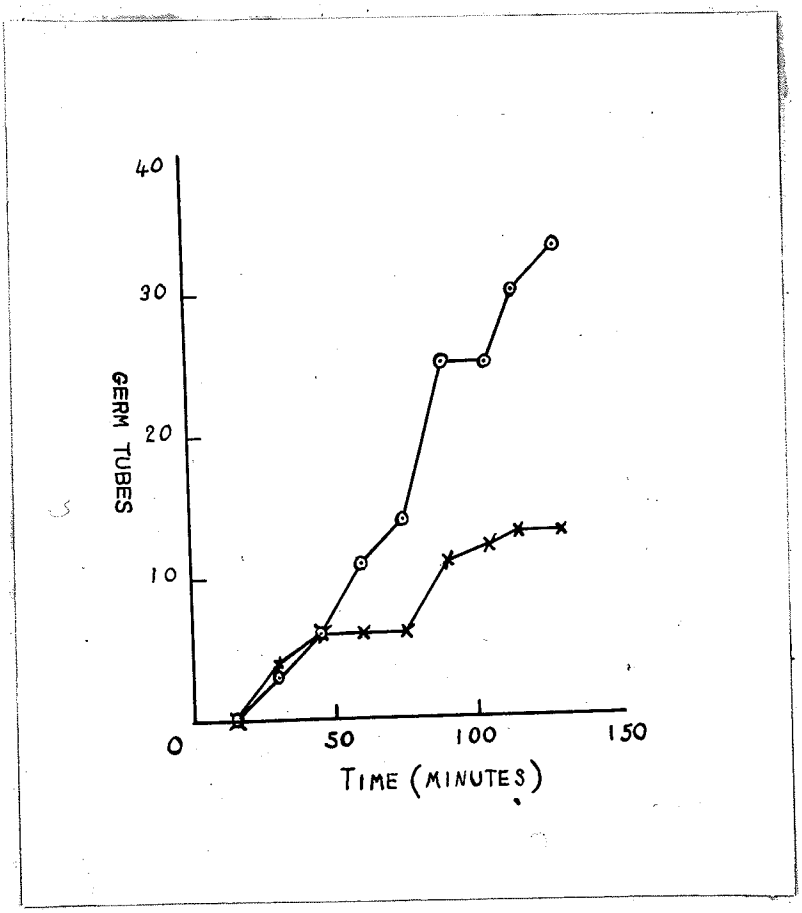
20°C.



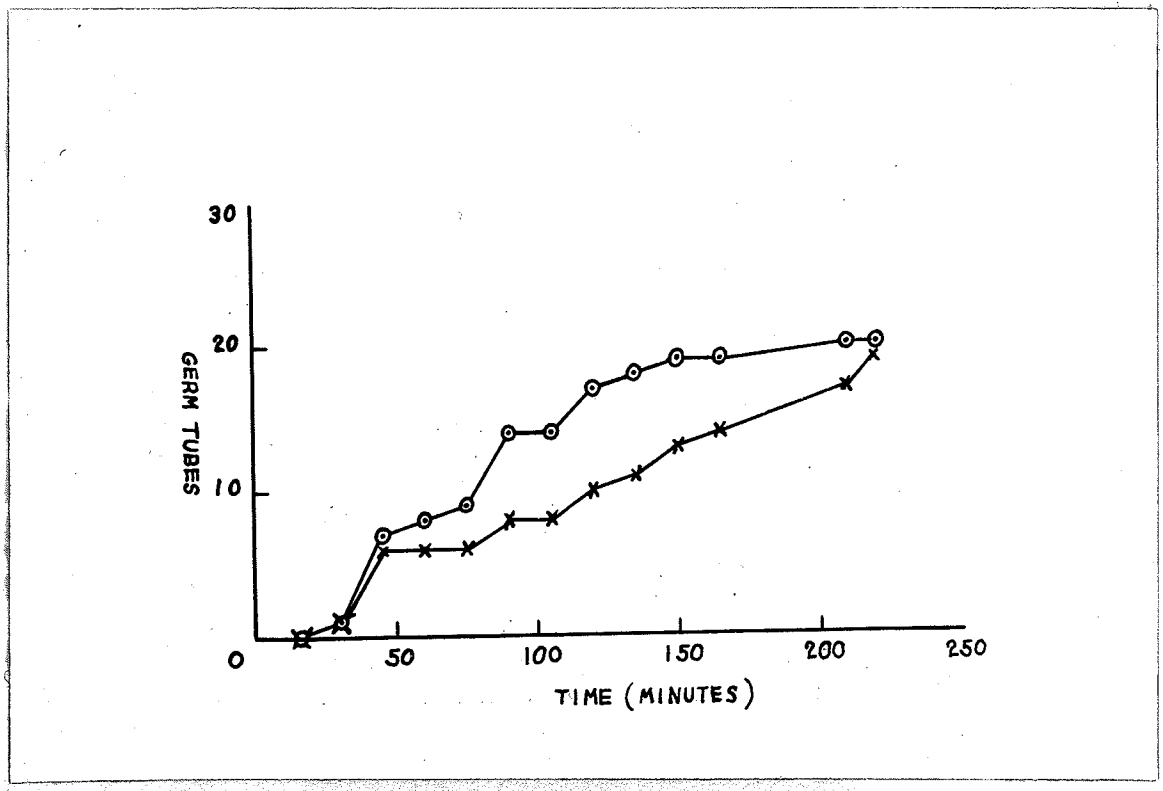
2

20°C.

Plate VI

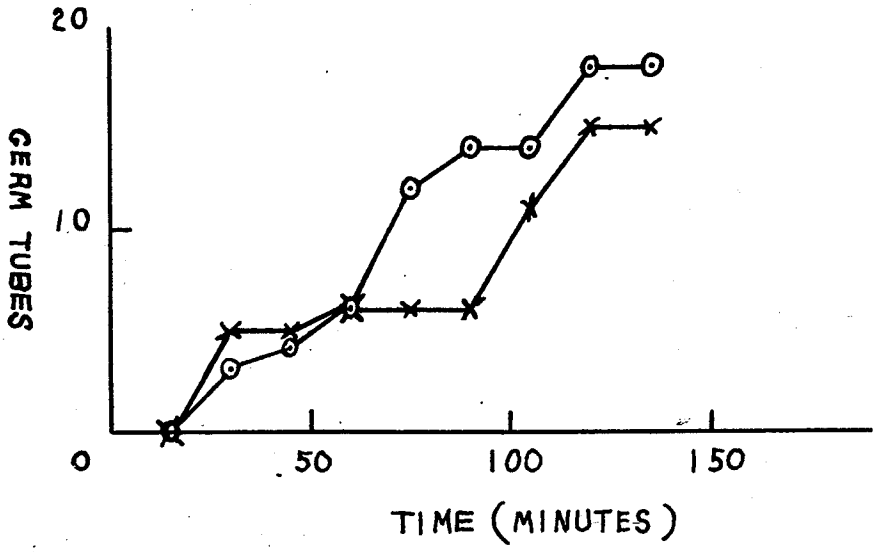


3
20°C.



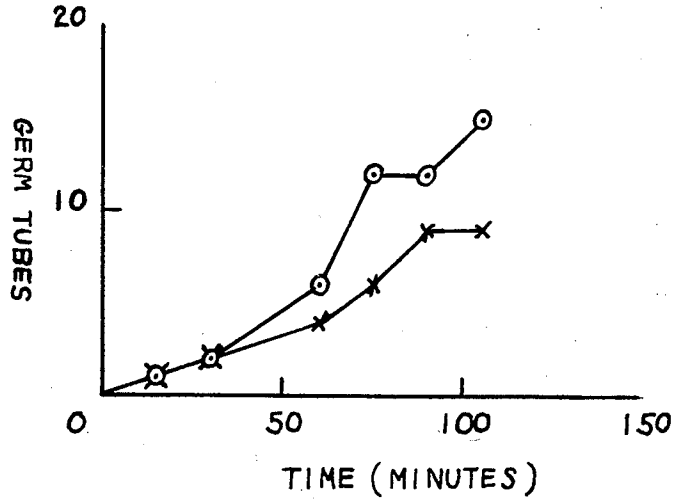
4
20°C.

Plate VII



5

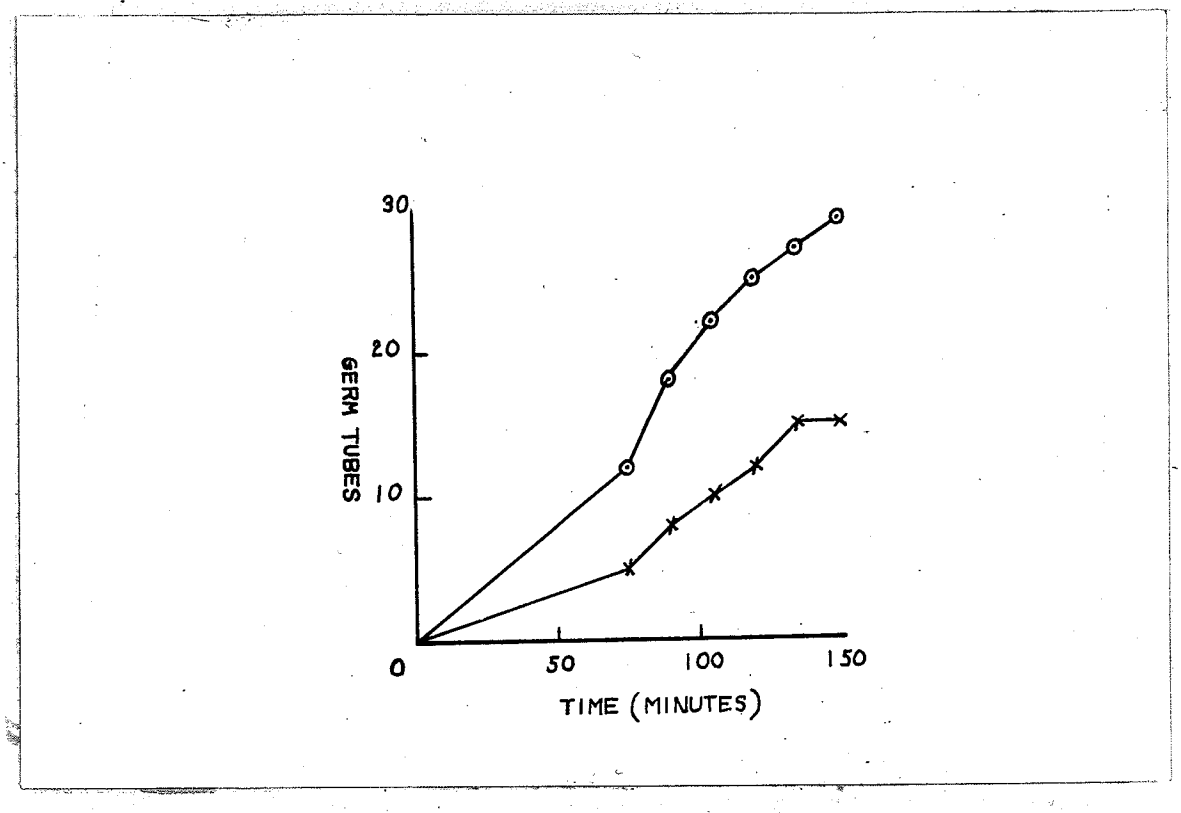
20°c.



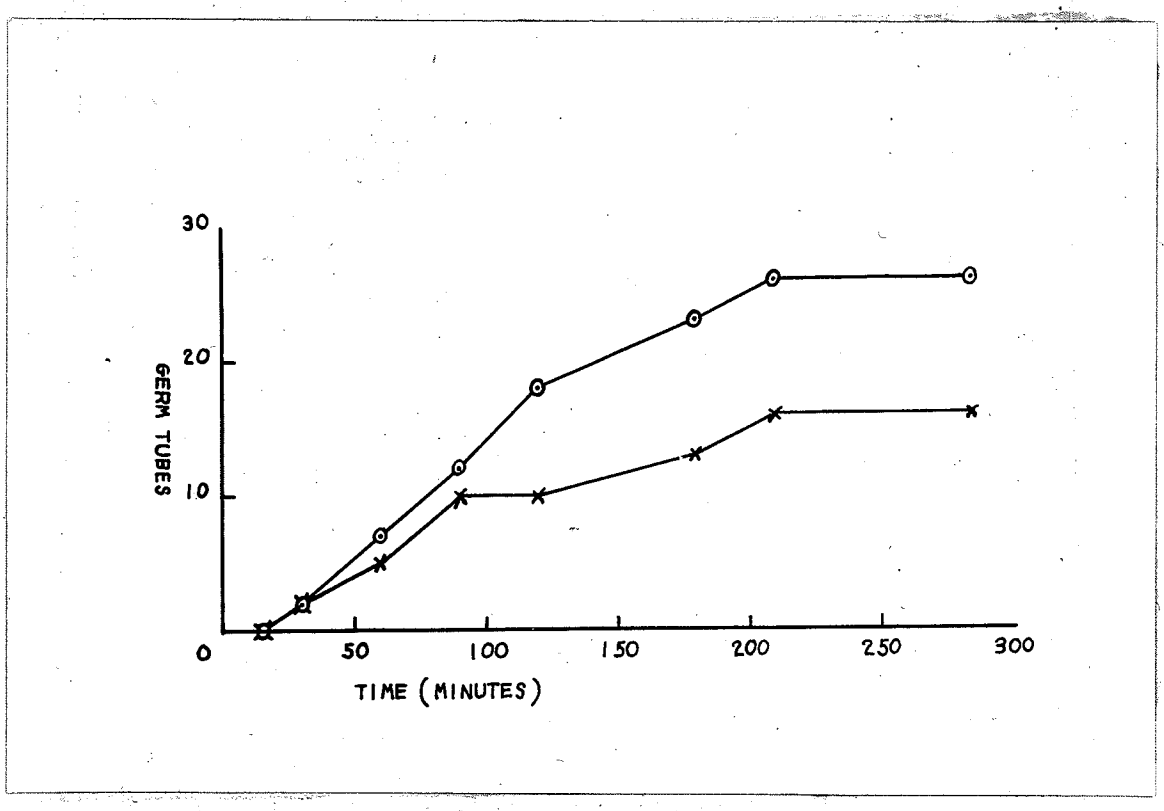
6

20°c.

Plate VIII

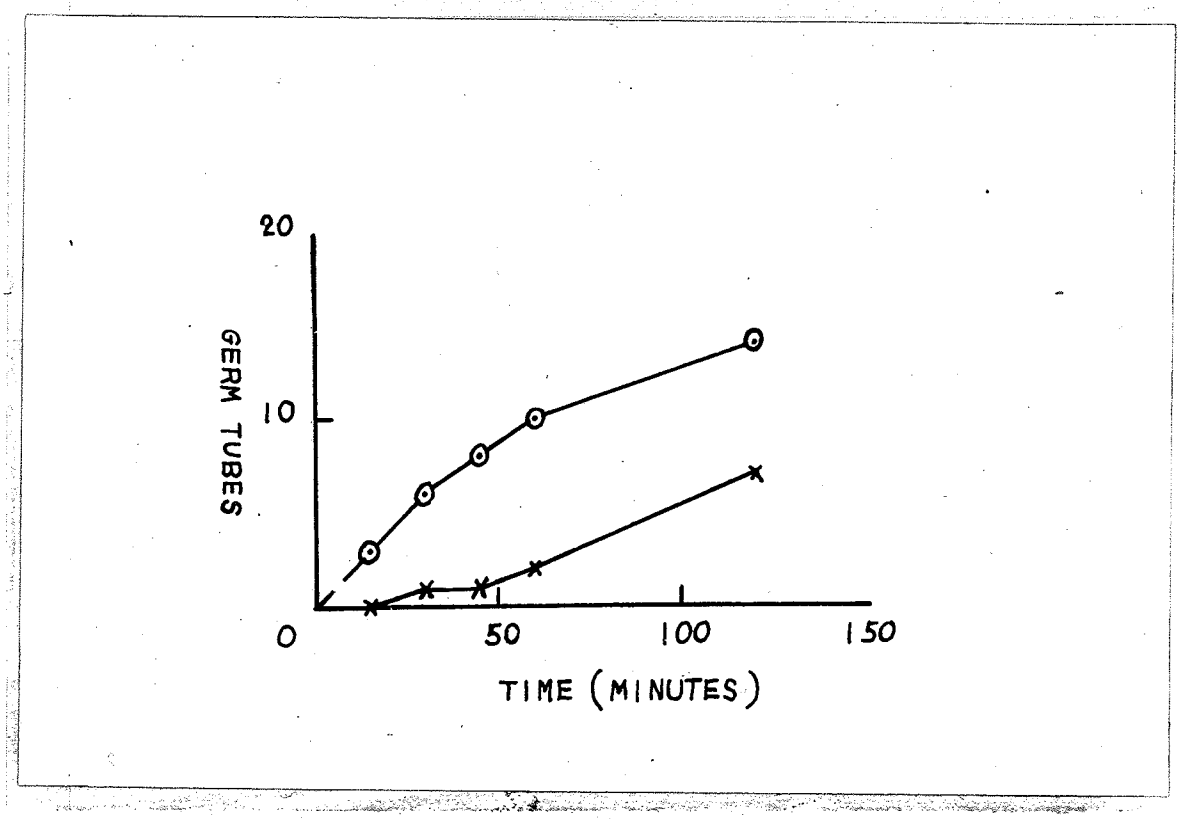


7
20°C



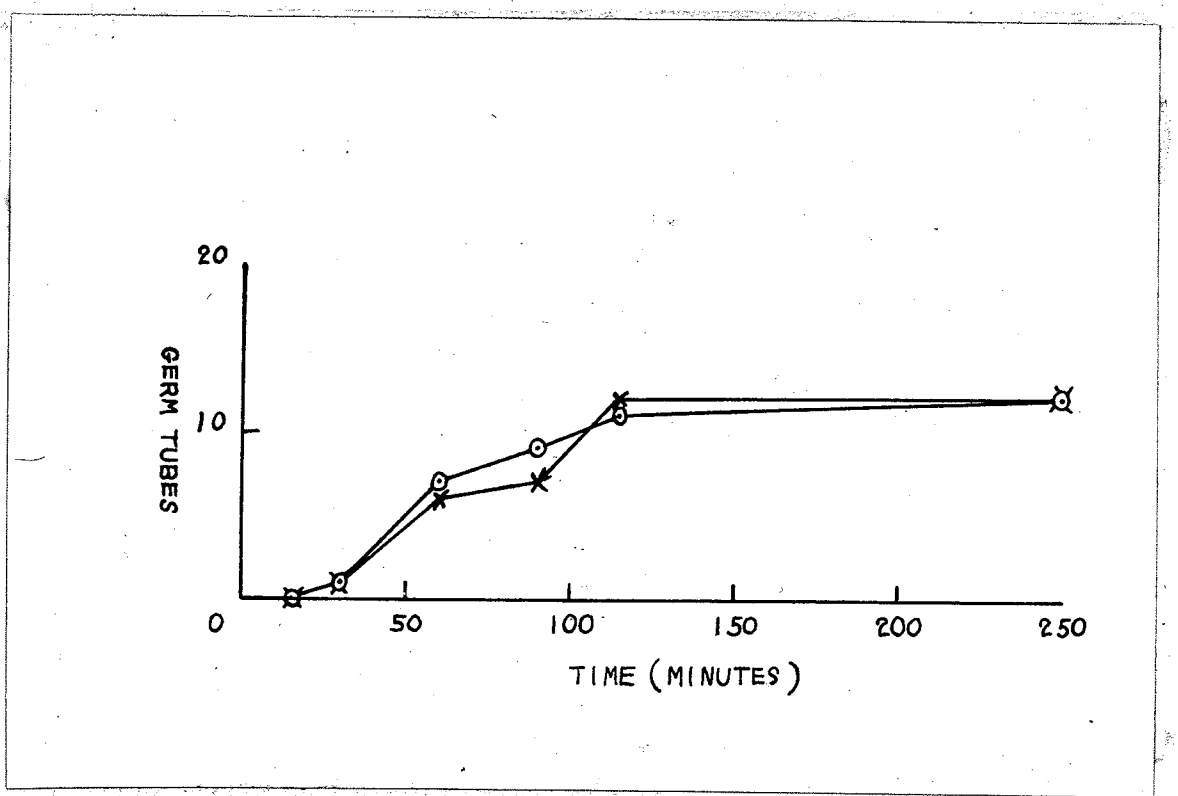
8
20°C.

Plate IX



9

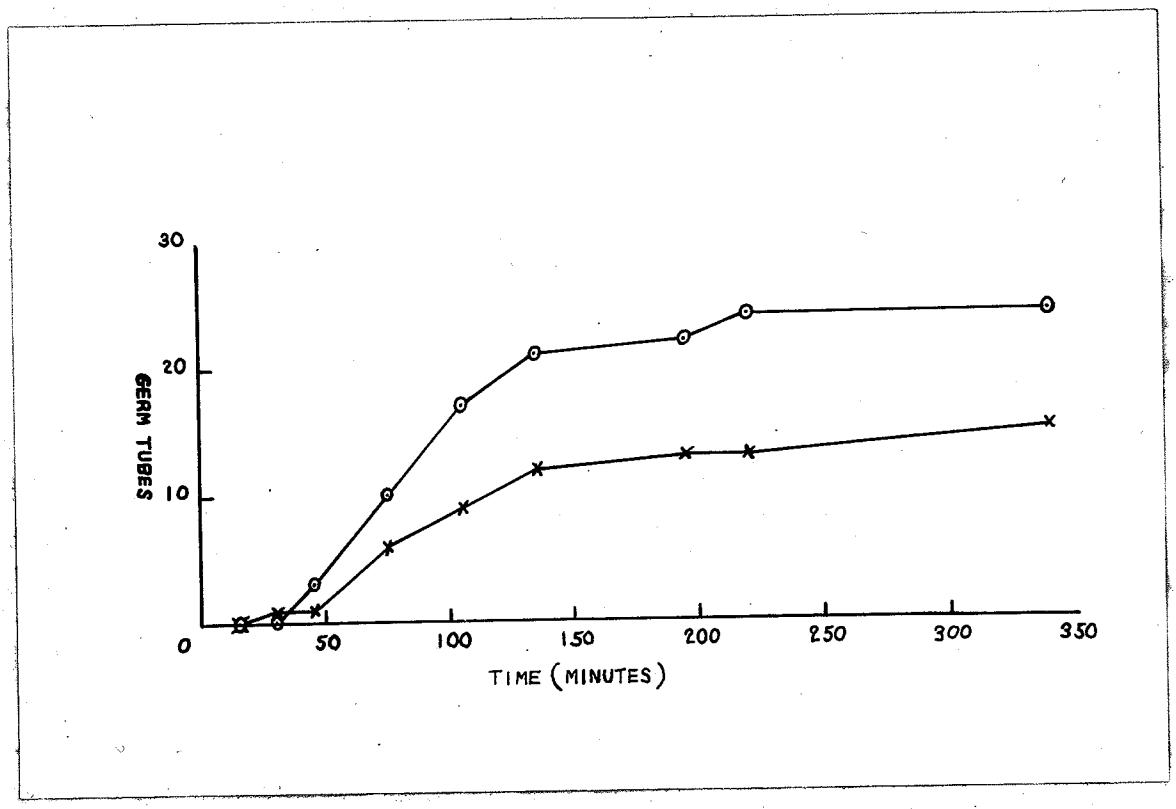
20°C.



10

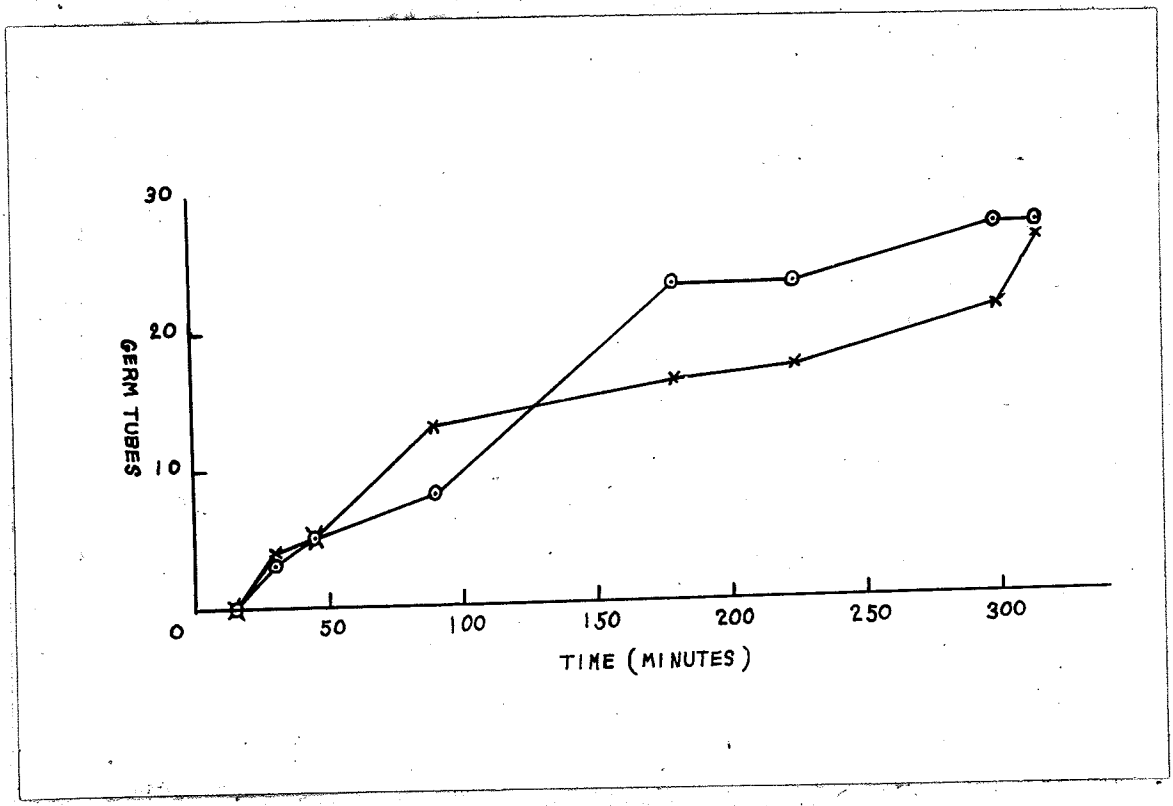
20°C.

Plate X



11

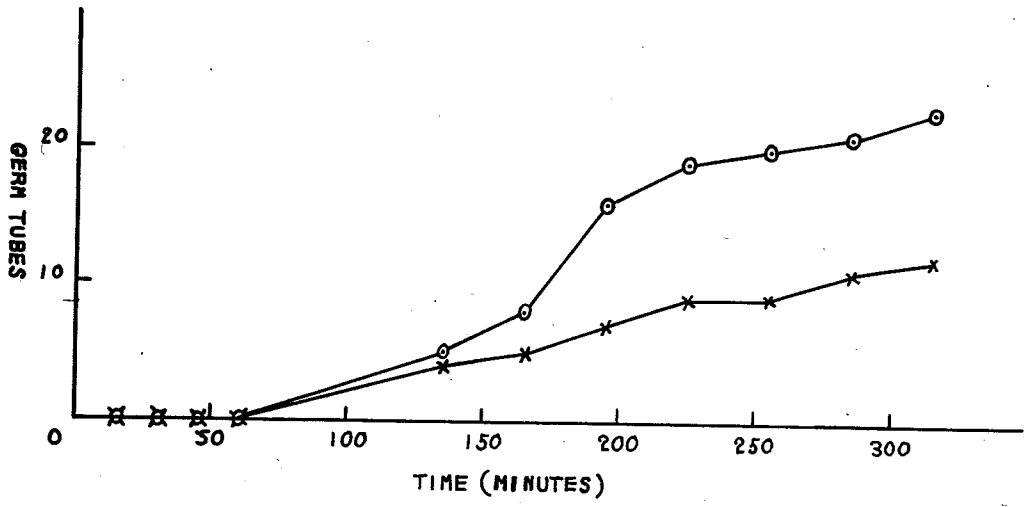
20°C.



12

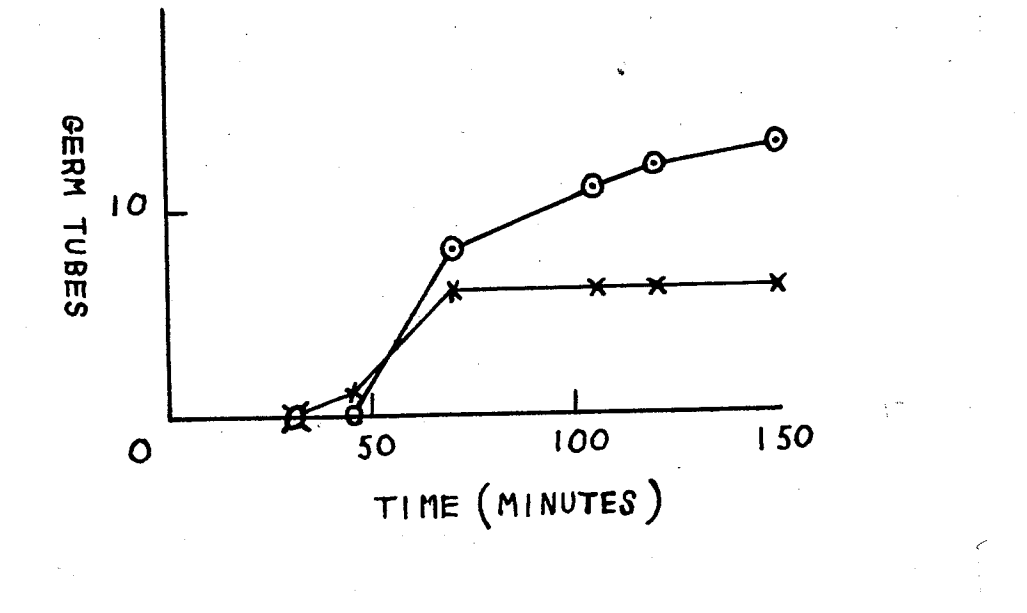
20°C.

Plate XI



13

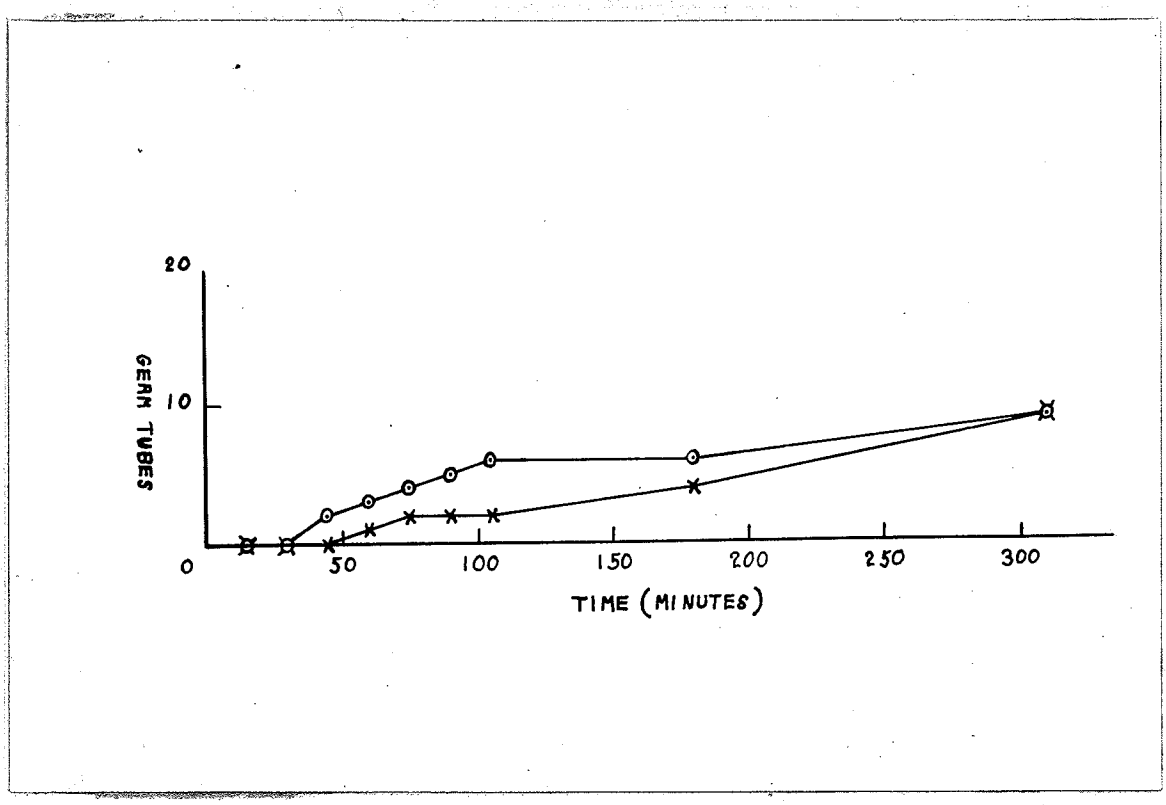
10.5°C.



14

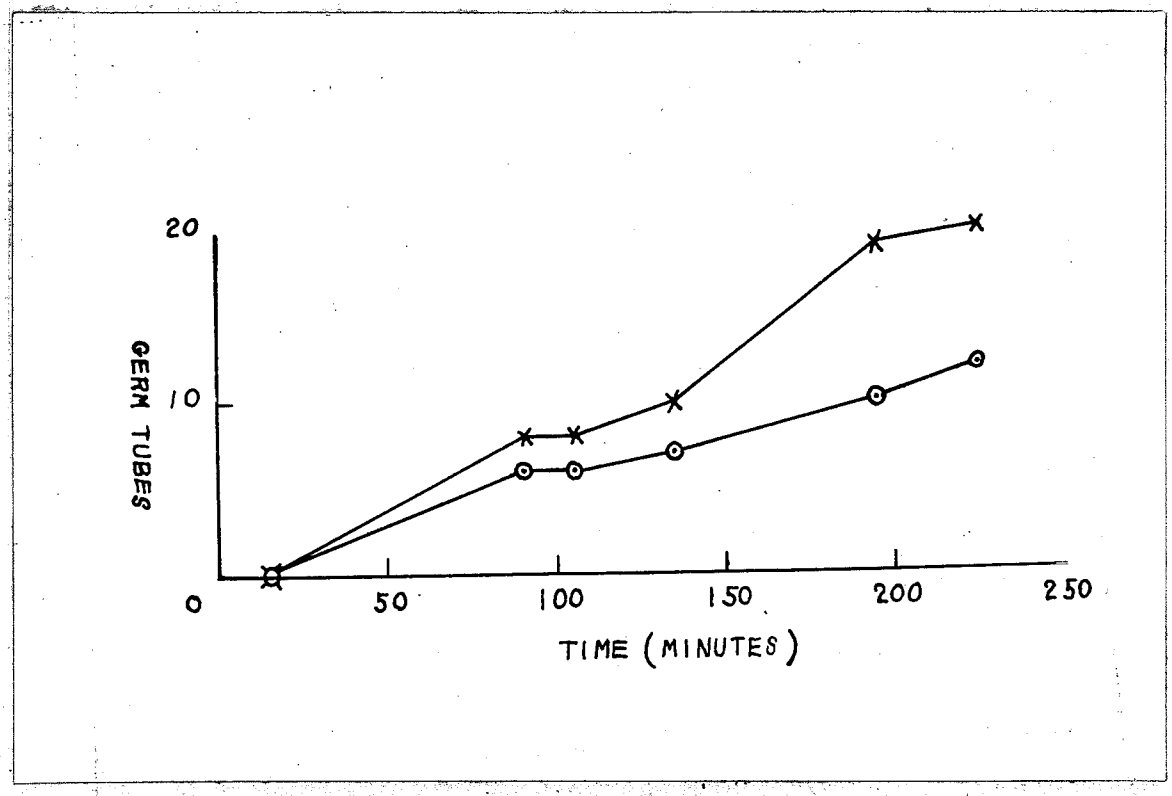
9°C

Plate XII



15

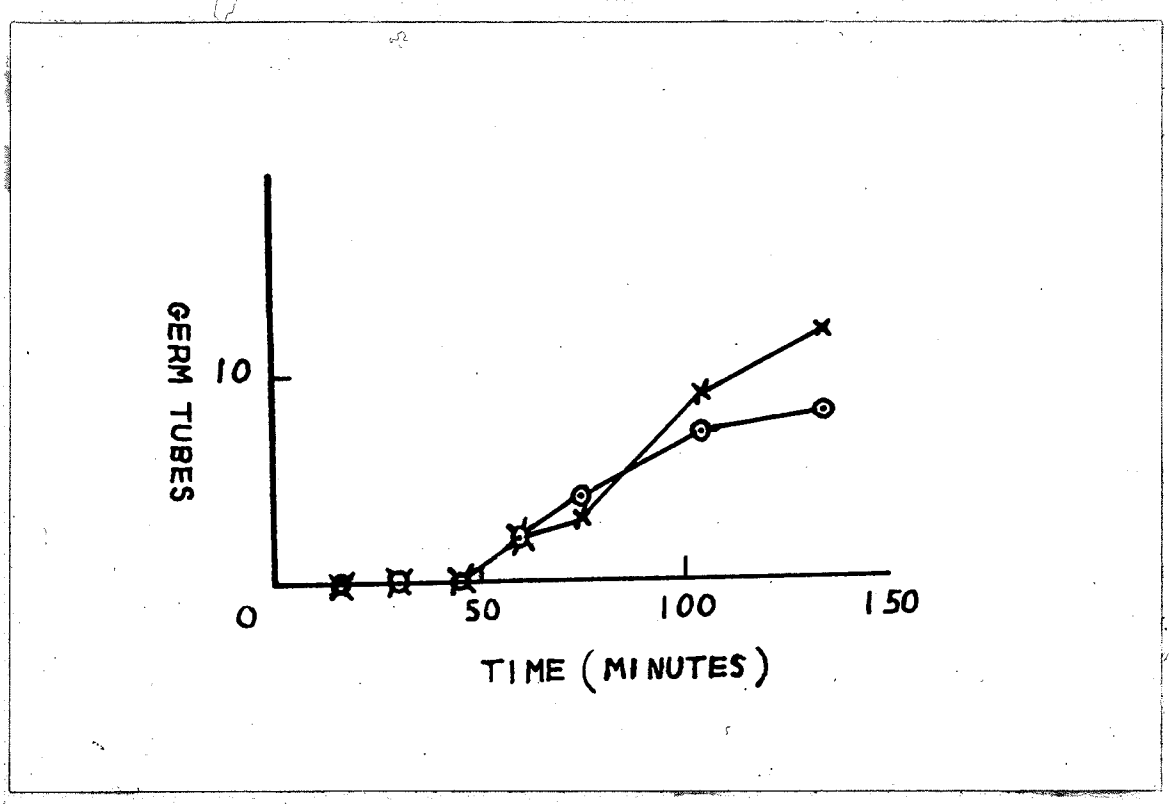
9°c.



16

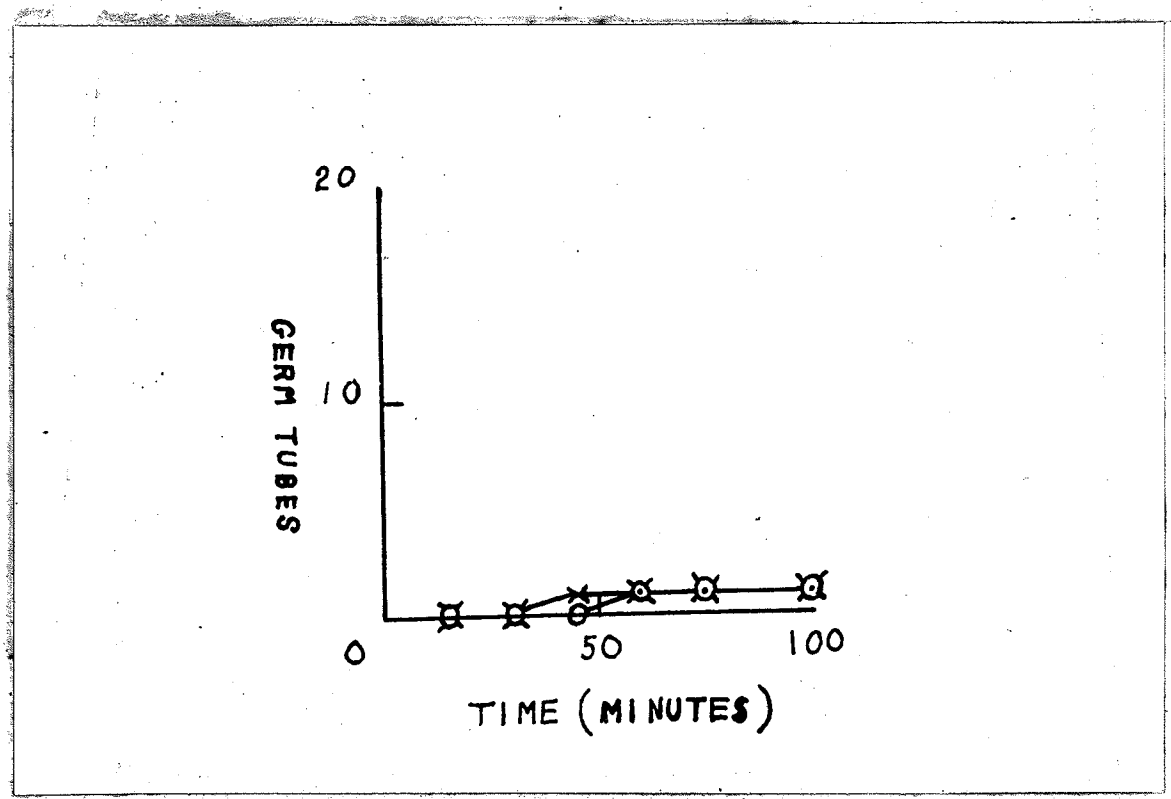
8°c.

Plate XIII



17

8°C.



18

6.5°C.

From an examination of the data set forth in Table IV, it is evident that at 20° C, conidia germinate more rapidly under conditions of reduced pressure than at atmospheric pressure. The same condition obtains at 10.5° C and 9° C. At 8° C, the situation is reversed and the control germinates more rapidly, while at 6.5° C, germination has dropped so low that there does not appear to be any appreciable difference between the experiment and the control.

Discussion: An examination of the graphs (Plates V - XIII) will illustrate what has been stated above. The curve representing conidial germination at 20° C under conditions of reduced pressure rises rapidly during the first two or three hours of germination while a more gradual rise is shown by the curve for the control during the same period. As a result, the percentage germination in the control, at the end of three hours, is less than that of the experiment. This accounts for the results obtained in the previous experiment in which the effect of reduced pressure upon germination was investigated. Now let us consider what happens when germination is allowed to proceed indefinitely. The experimental curve begins to flatten out and finally attains a maximum value, at which point no further germination can take place in the experimental chamber. The control curve continues its gradual rise and eventually reaches its maximum at which point the two curves tend to meet. This tendency of the two curves to come together is illustrated in graphs 4, 5, 10, 12 and 15.

The writer realizes that the tendency of the experimental curve to rise more rapidly than that of the control has not been always equally displayed and that, in several graphs, the ascendency of the experimental curve over the control curve is only slight. This irregularity may be accounted for by the fact that observations of germinating conidia were necessarily confined to a single field. Now, in examining slides of germinating conidia, it has been observed that there are present on every slide a few fields in which the percentage germination of conidia is low. It is obvious, therefore, that the presence of such a field would, in this instance, not only give a false picture of germination but introduce a serious error into the results, i.e., the limitation value of the control in this experiment is that it, too, covers only a very limited field.

The Effect of Light upon Germination of Conidia of Erysiphe graminis Hordei at 19°, 25°, and 30° C.

The effect of light upon the germination of conidia of members of the Erysiphaceae has been investigated by Neger (14) Hammerlund (11), Yarwood (19), and more recently by Cherewick (4). In each case, light was found to exercise a stimulative effect upon germination, a fact which is supported by the present experiment undertaken by the writer, an account of which is given below.

Materials and Methods: A large bell jar served as

a constant temperature bath in which the germination chambers were immersed at an equal distance from the light source which consisted of a 60-watt frosted light bulb and silvered reflector, set at a distance of two feet from the side of the bath. One of the chambers was made lightproof by covering its entire surface with several layers of black adhesive tape rendered water-proof by means of a thin layer of wax. All germination tests were performed during the winter in a darkroom where the temperature was never above 18° C, therefore permitting accurate temperature control of the bath by means of a thermostatic heater. The latter provided a temperature which was constant to within one-tenth of one degree centigrade while a mechanical stirrer kept the water in constant circulation, thereby maintaining an evenly distributed temperature. Slides bearing conidia were arranged at the bottom of each chamber upon discs of moist blotting paper. Germination was allowed to proceed from 11 a.m. to 1 p.m. after which the spores were fixed and counted.

Results:

Table V.

Effect of Light upon Germination of Mildew Conidia at 19°, and 25° and 30° C respectively.

Temperature (°C)	Percentage Germination of Spores in Total Darkness	in Light.
19	19	38
	21	35
	24	29
	18	32
25	38	46
	10	17
30	2	3
	3	2
	4	4

From the data presented in Table V. the following statements regarding the effect of light and temperature upon germination of conidia may be made: At 19° and 25° C conidial germination was definitely stimulated by light while at 30° C light had no effect.[1] This is probably due to the fact that 30° C is so far above the optimum temperature for germination as reported by Graf-Marin and Cherewick (10, 5) that any stimulative effect of light is screened out by the inhibitive effect of temperature.

---

[1] Cherewick (4) reported that light exercised a depressing effect upon germination at this temperature.

A Study of the Formation and Abstriction of Conidia of Erysiphe graminis Hordei over a Twenty-Four Hour Period, with a view to establishing the Possibility of a Diurnal Cycle

In certain powdery mildews, there is a periodicity in the formation and abstriction of conidia. For example, it has been shown by Childs (5) that in the chain-forming mildew, Erysiphe cichoracearum, formation of conidial chains commences at approximately 4 p.m. and continues throughout the night. A period of rapid abstriction follows, commencing at 8 a.m. and continuing until 4 p.m., after which the process of maturation re-commences.

An examination of the literature reveals that very little information is available concerning the possibility of the existance of a diurnal cycle in Erysiphe graminis Hordei. Both Yarwood (19) and Cherewick (4) give brief references to the effect that they were unable to discover any such cycle but make no mention as to what methods were employed during the course of their investigations. It was considered advisable, therefore, to re-investigate the entire matter and it is to an account of such an investigation that the following section will be devoted.

Materials and Methods: Observations of developing conidiophores were made under conditions which approximated natural conditions as closely as possible. A leaf, still attached to the plant which was itself growing in earth, was laid upon a glass slide upon the stage of the microscope and

then folded in such a way as to cause a cluster of conidiophores to project beyond the folded edge, in which position they could easily be observed by the high-power objective. In this way, it was possible to focus upon a single conidiophore and to observe, in detail, its growth during the twenty-four hour period. The principal value of the method outlined above lies in the fact that it was possible to make accurate observations of the living fungus as it developed upon an actively-growing leaf. No attempt was made to control environmental conditions. The microscope was set up close to a window where it received a maximum amount of light in the daytime. No artificial light was permitted in the laboratory during the night with the exception of a small microscope lamp which was turned on during the few seconds necessary to make an observation. Due to the withering effect of heat upon the conidiophores, all observations were made at moderate temperatures, i.e., 68° - 70° F.

Results:

Table VI

Activity of the Generative Cell

First Trial.

8.15 p.m. - 12.15 a.m.:	1 cell produced in 4 hours.)		
12.15 a.m. - 3.30 a.m.:	1 " " " 3½ ")		
3.30 a.m. - 10.15 a.m.:	1 " " " 5¾ ")		
10.15 a.m. - 1.00 p.m.:	1 " " " 2¾ ")	Total: 5 cells in 24 hours.	
1.00 p.m. - 6.45 p.m.:	1 " " " 6¾ ")		
6.45 p.m. - 8.15 p.m.:	0 " " " 1½ ")		

Second Trial

7.30 p.m. - 9.30 p.m.:	1 cell produced in 2 hours	)
9.30 p.m. - 5.30 a.m.:	1 " " " 8 "	)
5.30 a.m. - 9.30 a.m.:	1 " " " 4 "	)Total: 4 cells
9.30 a.m. - 3.30 p.m.:	1 " " " 6 "	) in 24 hours.
3.30 p.m. - 7.30 p.m.:	0 " " " 4 "	)

Third Trial.

5 p.m. - 7 p.m.:	1 cell produced in 2 hours.)	
7 p.m. - 9 p.m.:	1 " " " 2 hours.)	Total: 5 cells
9 p.m. - 5 a.m.:	1 " " " 8 " .)	in 26 hours.
5 a.m. - 1 p.m.:	1 " " " 8 " .)	
1 p.m. - 7 p.m.:	1 " " " 6 " .)	

FOURTH Trial

5 p.m. - 11 p.m.:	1 cell produced in 6 hours.)	
11 p.m. - 3 a.m.:	1 " " " 4 ")	Total: 5 cells
3 a.m. - 11 a.m.:	1 " " " 8 ")	in 26 hours.
11 a.m. - 1 p.m.:	1 " " " 2 ")	
1 p.m. - 7 p.m.:	1 " " " 6 ")	

Discussion:

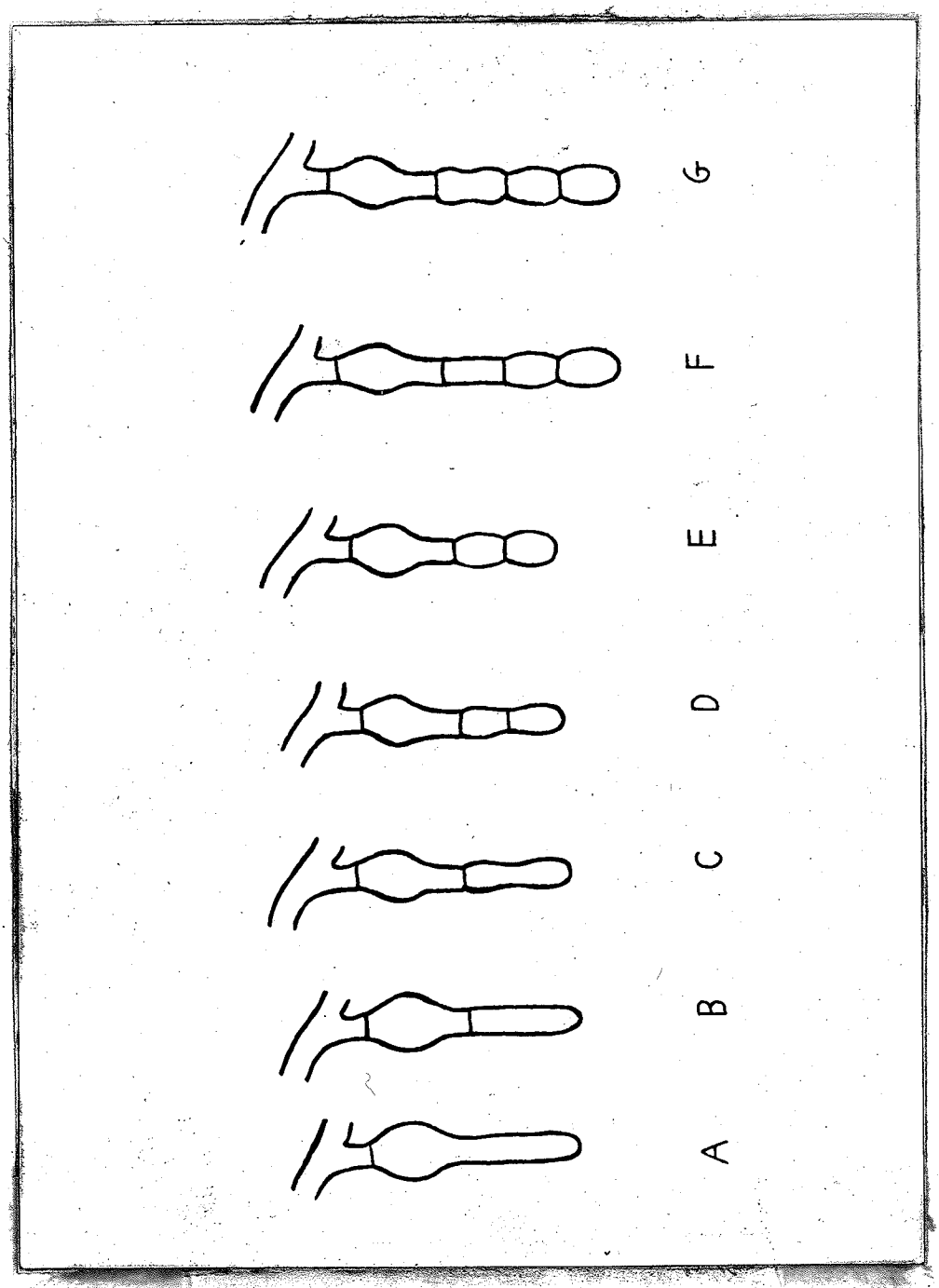
(1) Abstriction: There appears to be no diurnal fluctuation in the process of abstriction. Continuous observation over the twenty-four hour period suggested that abstriction of mature conidia may take place at any time during that period and that abstriction is passive, conidia being out off either in chains or singly.

(2) Nature of the Generative Cell and Formation of

Conidia: The basal, bulbous portion of the conidiophore functions as the generative cell. It grows forward to produce a cylindrical hypha which is cut off from the swollen portion by the formation of a transverse septum (Plate XIV, A - B). The cylindrical cell thus produced by the division of the generative cell, soon becomes slightly swollen at each end and undergoes transverse division into two daughter cells of equal size. Each of these becomes swollen and develops into a conidium (Plate XIV, C - G). The generative cell then elongates and divides and the process is repeated.

(3) Activity of the Generative Cell: On the basis of the data presented in Table VI, it may be concluded that the generative cell elongates and divides approximately once every four to five hours. However, the writer was unable to find any evidence of a diurnal fluctuation in such division. The results of this experiment, therefore, are in entire agreement with those of Yarwood (19) and Cherewick (4), neither of whom were able to find any evidence of a diurnal cycle in connection with the abstriction of conidia.

Plate XIV



V. Summary.

1. At pressures below atmospheric pressure, and not lower than 618 mm., conidia of Erysiphe graminis Hordei germinate in higher percentage than under the prevailing atmospheric pressure. Below this value, at moderately low pressures, germination is depressed slightly and falls off rapidly at low pressures.
2. Increase in pressure depresses the germinability of conidia, but germination values do not decrease with corresponding increments in pressure.
3. Slight reduction in pressure shortens the time required for maximum germination to be reached upon a given slide.
4. Light stimulates germination of conidia at temperatures at or near the optimum. At temperatures sufficiently above the optimum, the stimulatory effect of light upon germination is screened out by the inhibitory effect of temperature.
5. No diurnal cycle is manifest in Erysiphe graminis Hordei, continuous observation of growing conidiophores over the twenty - four hour period indicating complete absence of periodic fluctuation both in the abstriction of conidia and in the activity of the generative cell. The generative cell is the basal, bulbous portion of the conidiophore.
6. Abstriction of conidia is passive, conidia being abstricted either singly or in chains.

VI. Acknowledgement.

The writer wishes to express his indebtedness to Dr. H. J. Brodie, assistant professor of Botany of the University of Manitoba, who suggested the problem and who was a constant source of help and inspiration during the course of the investigation.

VII. Literature Cited.

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