The Development and Structure of the Conidia of *Erysiphe Polygoni* DC. and their Germination at Low Humidity.

by

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I. Introduction

Although much is known concerning the morphology, taxonomy, cytology and life-history of members of the fungus family Erysiphaceae, there are relatively few references in the literature to the germination of the conidia. None of these suggest that there is anything unusual about the germination process, being concerned chiefly with the manner of development of the germ-tube.

In 1936, C. E. Yarwood (23), in a paper published in Phytopathology, claimed that the conidia of Erysiphe Polygoni and certain other powdery mildews are capable of germinating at low relative humidities, even approaching zero.

It has generally been recognized that fungus spores germinate only under conditions of high humidity and that, in many instances, they must be in actual contact with water. Yarwood's work, therefore, presented a problem of great importance, the solution of which might be expected to throw light upon the epidemiology and control of the mildews and upon the physiology of these parasites.

Yarwood made no attempt to explain the tolerance of the mildew conidia of low humidity and no real confirmation of his observations has been offered up

to the present. In 1937, Dr. H. J. Brodie of the University of Manitoba carried out some preliminary experiments with conidia of Erysiphe Polygoni taken from cabbage. This work was not published, but it showed clearly that Yarwood had not been mistaken: a high percentage of germination of mildew conidia was obtained under conditions of extremely low humidity.

A further investigation of the peculiar behaviour of mildew conidia was undertaken by the writer in an attempt to corroborate Yarwood's work and to find some explanation for the tolerance of the mildew spores of low humidity.

II. Historical

Prior to the publications of Yarwood (22,23), there are but few references in the literature which contain sufficient data relevant to the present problem to warrant a detailed review in this paper.

Early workers concerned themselves chiefly with the taxonomy and morphology of the Erysiphaceae. In Volume I of the Selecta Fungorum Carpologia of the brothers Tulasne (20) there occur descriptions of sixteen species of powdery mildews accompanied by splendid copper-plate illustrations.

As regards the germination of the conidia, the authors merely mention that germinating spores were seen frequently in the material examined.

In 1902, Neger (15) published the results of his study of the germination of mildew spores under different environmental conditions. He showed that certain features are more or less typical of the germ-tubes of each species. These features are: the place of emergence of the germ-tube from the spore, the length of the germ-tube and the way in which it branches. Each of these features was shown to vary over a wide range and to be affected by light and temperature. Similar views were expressed by Hammerlund (12) and Corner (8).

It seems to have been observed generally that the conidia of powdery mildews do not germinate well, or only very scantily, in water. Among those who have reported this fact in a wide range of mildew species are Neger (15), Foëx (10), Sawada (19), Woodward (24), Graf-Marin (11) and Corner (8). That the germ-tubes produced in water may actually be longer than those produced in moist air, has been shown by Graf-Marin (11), Corner (8) and others. Sawada (19) reported that the conidia of some species of Phyllactinia and Uncinulopsis are killed by immersion in water.

It has been pointed out by Beeley (2), Blumer (5) and Hammerlund (12) that dry conditions favor the development of the powdery mildew mycelium and encourage the pro-

duction of conidia. Beyond mentioning that the Erysiphaceae are unusual in this respect, none of these investigators realized the significance of their observations and they did not carry out critical studies of the influence of humidity upon the germination of conidia.

As reported by the workers mentioned above, the optimum temperature for germination of most mildew conidia is about 25°C. Exceptional in this respect are Oidium Tuckeri (24) with 25°C. - 28°C. as its optimum, and Sphaerotheca pannosa with 30°C.

A considerable amount of work has been done on the process of maturation of the conidia. Some reference to this work may now be made, inasmuch as the present writer has studied the formation of conidia in Erysiphe Polygoni.

For Sphaerotheca Humuli on vegetable marrow, Massee (14) stated that the liberation of conidia occurs chiefly at night. Hammerlund (12) reported that in Erysiphe communis (E. Polygoni), from one to six conidia are developed on each conidiophore each day, and that the conidia are forcibly discharged.

In species which produce their conidia in chains, Foëx (9), showed that the basal cell and the one above it divide to produce the conidia (Sphaerotheca Humuli). Blumer (4) claimed that in Erysiphe Cichoraceorum and Sphaerotheca spp. the basal cell is the one from which the conidia are formed.

In 1936, Yarwood (22) showed that in Erysiphe Polygoni on clover there is a diurnal cycle manifest in the maturation of the conidia and also in their germinability. Each conidiophore forms one conidium during a twenty-four hour period and the spore is liberated passively about noon. Yarwood obtained the highest percentage of germination of mildew conidia when these were removed from clover from about mid-day to four o'clock. Germinability decreased with the onset of darkness and reached a minimum in the early morning.

It was also shown that light has a definite stimulatory effect upon germination; conidia collected during the high phase of the germination cycle germinated almost as well in darkness as in light, whereas conidia collected in the low phase of the germination cycle were greatly stimulated by light during germination. These facts were supported by field experiments in which inoculations with clover mildew conidia during the light portion of the day were more successful than inoculations made at night.

More recently, Childs (7) has demonstrated a similar diurnal cycle for a number of mildews which produce their conidia in chains as well as for non-chain-forming mildews.

Working with Erysiphe Polygoni from several different host plants, E. Cichoracearum from sunflower and Sphaerotheca pannosa from rose, Yarwood (23), in 1936, found that at 22°C. germination of mildew conidia was good at relative humidities ranging from 100% to approximately

zero. In many experiments, the percentage germination was as high at zero relative humidity as at 100%.

Although as much as 65% germination was observed at zero relative humidity, the conidia were found to have shrivelled and died at the end of thirty hours, whereas at higher humidity a much smaller proportion of conidia had shrivelled in the same period. In other words, the mildew conidia were tolerant of low humidity to the extent of being able to germinate, but they were eventually injured more quickly by dry air than by moist air. The tolerance of low humidity was also shown to decrease with increase of temperature.

Yarwood claimed that the conidia decrease as much as 24% in volume during germination. In contrast, conidia of Colletotrichum trifolii, Sclerotinia fructicola and Cincinnobolus cesatii did not germinate without being in actual contact with water, and increased greatly in volume during germination.

From field experiments, it was concluded that the best infection of clover, bean, cabbage, barley, cantaloupe, delphinium and mustard was obtained when the inoculation was made at low relative humidity.

Yarwood's general conclusion may be quoted: "Though, under certain conditions, better development of Erysiphe Polygoni has resulted under conditions of low than those of high humidity, the writer believes that high humidity is not, in itself, markedly injurious to the powdery mildews studied..... Rather, the writer believes

that the forms studied can develop luxuriantly over a wide range of relative humidities and that they are especially well adapted, in contrast with most parasitic fungi, to very dry atmospheric conditions."

Not all species examined by Yarwood showed tolerance of low humidity. Erysiphe Polygoni from mustard, E. graminis from barley, E. Cichoracearum from sunflower and Sphaerotheca pannosa from rose were considerably less tolerant of low humidity than other species and strains tested.

Berwith (3) studying Podosphaera leucotricha, the apple powdery mildew, claimed that a high humidity is necessary both for the germination of the conidia and the infection of apple seedlings.

In 1937, Hashioka (13) showed that, in Formosa, the conidia of Sphaerotheca fuliginea on cucurbits are not tolerant of low humidity in the matter of germination. However, this investigator demonstrated that more abundant formation of conidia occurs at low humidity than at high and that the later stages of the infection process are also favored by low humidity.

From the evidence presented above, we may conclude that at least certain members of the Erysiphaceae are remarkable in that their conidia possess the ability to germinate under conditions of very low humidity, in this respect being unlike other fungi so far as is known.

No explanation for this peculiar characteristic of the powdery mildews has been offered. The results of the writer's investigations, recorded in the following pages, throw some light upon the problem, although they cannot be said to present its complete solution.

III. Materials and Methods

Erysiphe Polygoni DC., obtained from Polygonum aviculare on the Fort Garry campus of the University of Manitoba during the summers of 1938-39 and 1939-40, was the organism most extensively used in the present writer's studies. Other species were also tested and will be mentioned elsewhere.

The mildewed leaves were gathered about midday when the maximum germinability of the conidia is manifest as was shown by Yarwood (22).

Germination tests were carried out as soon as possible after collection of the material, the conidia being placed in diffuse light in the laboratory at temperatures of from 20°C. - 23°C.

In testing the germination of conidia at different humidities, clean cover-slips were placed at the bottom of a large crock and the mildewed leaves shaken so as to allow the spores to settle on the cover-slips. In this way, a uniform distribution of conidia was obtained.

A series of twelve chambers providing a range of relative humidity from zero to 100% was set up by using large Petri plates sealed with vaseline. Each dish was half filled with a saturated solution of a salt, according to the directions by Spencer (18). The slides or cover-slips bearing spores to be tested were supported above the salt solution on glass rings. Another series was set up using Van Tieghem cells as containers, the spores being suspended from a cover-slip placed over the glass ring of the cell.

In the tables consulted for the various salt concentrations (18) the temperature at which the salts are to be saturated was given as 20°C. All germination tests were carried out at 22°C., and there would therefore be some discrepancy between the relative humidity figure given by the salt at 20°C. and that present in the experimental chambers held at 22°C. This discrepancy would probably not be great and in any case is unimportant since, as will be shown later, no significant variation in percentage germination is brought about by change in humidity.

It was thought that, even in the presence of a humidity-regulating solution, the air in a small chamber might not be uniform as to its moisture content. Yarwood (23) countered this objection by placing the Petri plates on an incline and rotating them so that the air and the solution were agitated. Under these conditions, Yarwood obtained results strictly comparable to those obtained where the Petri plates

were not rotated. In this research, at the lowest humidities used, a further precaution was taken to make certain that the air in which the conidia germinated was actually very dry. In a separate experiment, air was bubbled through two tall wash towers containing concentrated sulphuric acid before being admitted to the germination chamber.

In all experiments concerned with relative humidity, each germination percentage was based on counts of two hundred conidia. In some material, it was noted that a very small proportion of the spores had germinated before the experiment had begun. In no instance, however, was the proportion higher than 0.5 to 1 per cent and it was neglected in making the final count. Some shrivelled (doubtless dead) spores were nearly always present in the freshly gathered material, but never more than 3 - 5% of these were present. The germination percentages were based on counts of all conidia in the fields examined.

Special apparatus was used for testing the germination of the conidia in various gases. Inconsistency of results at one period of the investigation led to the discovery that vulcanized rubber is highly toxic to mildew spores, both ordinary rubber tubing and rubber stoppers being involved. In the apparatus illustrated below, therefore, ground-glass stoppered vessels were employed and connections made only from pure gum-rubber tubing.

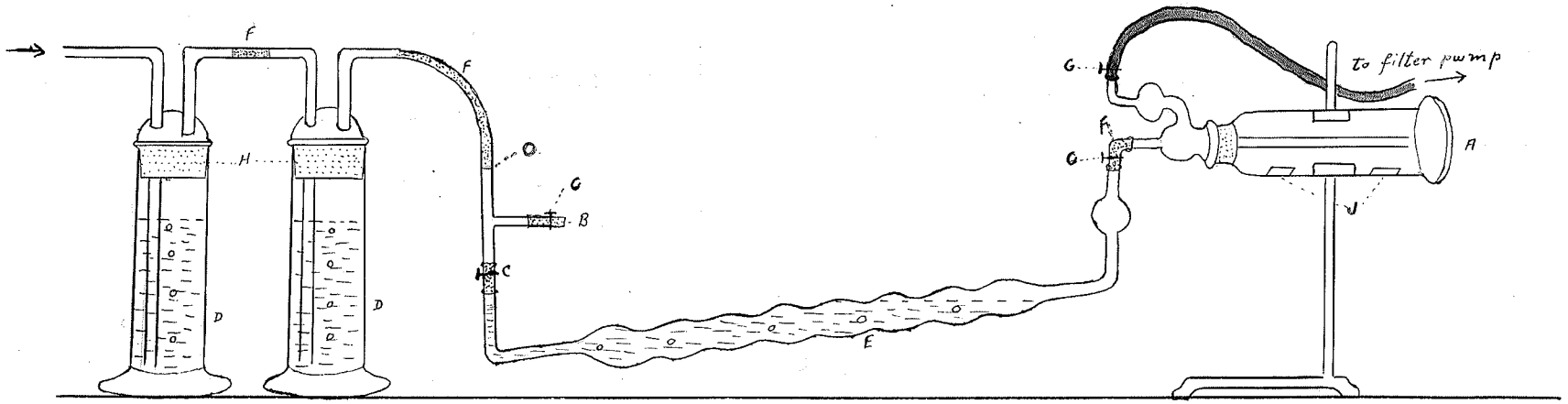

Fig. 1. Apparatus for testing germination of mildew conidia in dried air, carbon dioxide and nitrogen.

A, germination chamber containing spore-laden cover-slips (J); B, Connection for adding carbon dioxide; C, stop-cocks; D, wash bottles for sulphuric acid or pyrogallol; E, wash tube containing boiled distilled water; F, pure gum rubber tubing; H, ground-glass stoppers. O; point at which combustion tube was connected.

In studying the structure of the conidiophore, it was found that internal details were revealed by the use of a very simple staining method. Various stains were used, but such satisfactory results were obtained by the simple immersion of fresh mildew material in a five per cent solution of iodine in potassium iodide that this technique was adopted. Infected leaves were folded so that the conidiophores of the mildew projected out beyond the leaf. The leaf was immersed in iodine solution for five minutes and examined in water on a slide. The protoplast stained a dark brown and did not plasmolyze. Septa were easily made out in material so treated.

IV Investigations

Observations on the Development of the Conidiophore and Conidia.

Because of its possible bearing on the mechanism of germination, the mode of formation of the conidia was studied both from living and stained material of Erysiphe Polygoni on Delphinium. Using the iodine stain described above it was possible to make the following observations.

The young conidiophore is at first terete with a blunt apex (Fig. 2), and it is aseptate. A slight swelling then appears in the apical region and, within the cell wall, a ring of shiny material forms, becoming more

and more clearly defined until it is almost as readily distinguishable as the annular thickenings in angiosperm vessels (Fig. 3). Considering the simple nature of the stain, it was surprising to find that excellent examples of various stages in the development of the septum were to be had in every preparation.

Material is apparently added to this ring toward the inside, until a disc is formed. The centre of the disc remains perforate and provides a pore which, although not distinguishable in the living material, shows up splendidly when the conidiophore is immersed in iodine solution. The cell wall and the septum swell markedly if the preparation is slightly warmed, and the distinctness of the pore leaves nothing to be desired.

The nature of the septum in fungi has been discussed fully by Buller (6). The pore in the septum of the conidiophore of Erysiphe Polygoni seems in every way similar to what Buller has described for other fungi. To the writer's knowledge no conclusive demonstration of the septal pore has hitherto been given for a member of the family Erysiphaceae. In a paper concerned with the cytology and sexuality of Erysiphe Polygoni, Allen (1) stated that she believed adjoining mycelial cells to be connected by cytoplasmic strands and she illustrated (loc. cit. Pl. I, H) such a cytoplasmic connection through a septum. She also stated that she believed cytoplasmic

streaming occurs in the mycelium of Erysiphe Polygoni. The writer has been unable to observe the streaming of cytoplasm in living material, although there seems no reason to doubt that such streaming occurs.

Just how the perforate septum (Fig. 3) undergoes modification as the conidium is matured, the writer was unable to determine. However, the end cell of the conidiophore does swell and becomes the conidium which, when mature, remains attached to the conidiophore only by a minute point of contact (Fig. 4).

A detached conidium of Erysiphe Polygoni bears a minute shiny papilla at one end (Fig. 6) and the parent conidiophore is also papillate after the conidium has been dislodged (Fig. 5). Such careful observers as the Tulasnes (20) failed to illustrate the papilla in the conidium of the Erysiphaceae and other workers do not seem to have mentioned it. The writer believes this papilla to have special significance in the process of germination as will be shown later.

The conidium first formed on a young conidiophore bears only one papilla, that developed at its proximal end. Since a papilla is also developed on the conidiophore at the end attached to the conidium, the second spore bears two papillae, one at each end. In a mass of spore material, one can find some conidia with one papilla and some with two.

The papilla appears as a highly refractive structure. The conidium wall is very much thickened in the

papilla region and the writer suggests that the papilla may possibly be formed as a result of the plugging of the perforate septum present in the immature conidium.

As stated above, Hammerlund (12) claimed that the conidia of E. Polygoni are forcibly discharged. Yarwood (22) denied this claim, stating that conidia are passively liberated. Careful observations were made of the conidiophores and conidia on living material of E. Polygoni. Young mildewed leaves of Polygonum aviculare and Delphinium were folded on glass slides with the undisturbed conidiophores on the upper surface of the leaves projecting beyond the edge of the folds. The conidiophores and conidia could be readily observed with both low and high power objectives of the microscope.

The writer has been unable to observe any forcible discharge of the conidia. Continuous watching of mature conidia for periods of twenty-four hours showed that they remain attached to the conidiophores unless disturbed by shock or a breath of air. It would appear that, in nature, they must be dislodged by air currents, rain, or other disturbances on the leaves of the host plant.

Further proof of this point was obtained by suspending mildewed leaves from the top of a covered glass dish and placing a slide underneath the leaves. After two days, during which time the dish was undisturbed, no spores settled on the glass slide.

Structure of the Conidium.

The Protoplast. The conidium of Erysiphe Polygoni is an ovoid colourless spore measuring 30 μ by 15 μ on the average. As stated above, it may have a papilla at one end or at both.

The protoplast is reticulate and contains globules that appear like vacuoles, these being from 1/5 to 1/4 the width of the protoplast. Distributed evenly throughout the reticulum and occurring commonly at the junctures of the granular cytoplasmic strands, are small globules of highly refractive nature. Nuclei were not seen, but no attempt was made to stain them.

When living conidia are immersed in a 0.2% solution of neutral red, certain points may be noted which form a sharp contrast to the appearance of conidia of other fungi. When conidia of Fusarium culmorum, Botrytis cinerea and Macrophoma sp. were immersed in neutral red, the stain entered the spores at once and accumulated rapidly in typical vacuoles of various sizes. In all three species, the total volume occupied by stain-containing vacuoles was large, representing the bulk of the spore contents. In contrast, the mildew conidia behaved very differently in the same stain. Dead, shrivelled conidia absorbed the stain instantly, the entire spore contents becoming red. Turgid conidia at first did not absorb the stain at all. If they were allowed to stand in neutral red for several hours, a

small amount of stain penetrated the spore wall as germination began. By the time (2 - 3 hours) a germ-tube had become visible, considerable stain had entered the spore. The part of the conidium which first showed stain was always the papilla. It became pink and then a faint colouration appeared in that part of the protoplast nearest the papilla.

After several hours, the stain spread through the spore. However, the large globules which appeared like vacuoles did not absorb neutral red. By comparison with the stain-absorbing vacuoles of the other fungi mentioned, it was concluded that the large globules in the protoplast of Erysiphe conidia are not true vacuoles. At least it would appear that they must contain some material which has no affinity for the neutral red. On the other hand, the stain accumulated in considerable concentration in the small refractive globules. These may therefore be true vacuoles of watery nature, or consist of some material having an affinity for neutral red. (Fig. 8).

From these observations, the writer has concluded that the spore wall of the ungerminated mildew conidium is relatively impervious to water. As germination proceeds, the permeability of the papilla probably increases for stain and (?) water appear to enter at the papilla end and to spread slowly from there. The large "vacuole" globules are probably not of watery nature as they do not absorb neutral red. The small

refractive bodies may be of watery nature as it is in them that the stain accumulates. It will be shown later that the protoplast shrinks but slightly during plasmolysis, and it would seem justifiable to conclude that the mature conidium contains very little water prior to germination.

During germination, the neutral red stains the germ-tube, locating there in large typical vacuoles.

The Cell Wall. The wall of the conidium is thin (1 - 1.5 μ thick except at the papilla end), smooth and colourless. Sudan III accumulates slightly on the outer surface of spores immersed in it, but the wall itself is not markedly stained. It is possible that there is a layer of some waxy material on the outside of the spore wall. This suggestion is also borne out by the following evidence. Ungerminated turgid conidia do not, as stated, take up neutral red. If, however, dry spores are immersed in petroleum ether for a moment, allowed to dry thoroughly and then washed with neutral red, the latter stain at once colours the conidia heavily. Possibly the waxy covering is dissolved by the petroleum ether rendering the spore wall permeable to neutral red solution.

No perforation in the wall is discernible at the papilla end when spores are examined with oil immersion lenses and it is not thought that any perforation is present. Rather the papilla, despite the thickness of the wall at that

point, represents a permeable spot in a spore wall which is elsewhere relatively impervious to water.

Osmotic Pressure of the Conidium

It seemed possible that the tolerance of mildew conidia to low humidity during germination might be correlated with a high osmotic pressure of the conidia. Accordingly, an endeavour was made to determine the osmotic pressure of mildew conidia by the plasmolytic method.

As reported by Thatcher (19) in 1939, few data are available for osmotic values of fungus spores and mycelium. In the following table are given the most important of these:

Table I

Osmotic Pressure of Fungi

Organism	Part	O.P. in atmospheres	Investigator	Year
Pythium debaryanum	Mycelium (?)	54	Hawkins & Harvey (19)	1919
Puccinia triticina	Uredinospores	49.5	Ronsdorf (16)	1934
Puccinia simplex	Uredinospores Germ-tubes	30.4	Ronsdorf (16)	1934
Uromyces fabae	Uredinospores Germ-tubes Haustoria	44.25 21.9	Thatcher (19) "	1939 "
Uromyces caryophyllinus	Haustoria	18.6	"	"
Botrytis cinerea	Hyphae	29.8	"	"
Sclerotinia sclerotiorum	Hyphae	23.5	"	"

In determining the osmotic pressure of conidia of Erysiphe Polygoni, an endeavour was first made to obtain plasmolysis in sucrose solutions. To the writer's amazement, the spores were found to remain unplasmolyzed in the strongest concentrations of sugar used. The conidia were able to germinate readily in a 5 M. solution of sucrose.

The reason for the non-plasmolysis in sucrose solution is not clear. However, it was noticed that the conidia shrink appreciably in strong sucrose solutions and it may be that the shrinkage of the spore as a whole masked the shrinkage of the protoplast.

Solutions of potassium nitrate were then used, and plasmolysis was observed at high concentrations. Solutions were made up differing by .05 M. and varying from 1 M. to 3.56 M. (saturation) in concentration.

Conidia on slides were irrigated with plasmolyte until a point was found at which plasmolysis was incipient in some of the spores. Not all the conidia in any sample were plasmolyzed at this point, and it was always necessary to use stronger solutions in order to obtain the maximum proportion of plasmolyzed spores. The concentration half way between that required to produce incipient plasmolysis and that required to produce the maximum proportion of plasmolyzed spores was taken as the average isotonic point for the sample. One hundred conidia were counted in obtaining each percentage.

The highest proportion of plasmolyzed conidia observed was 39%. Since in the same sample, the germination was 60%, about 20% of the viable conidia remained unplasmolyzed. Whether or not plasmolysis of the remainder could have been obtained by using higher concentrations of plasmolyte is not known. The saturation point for potassium nitrate at the temperature of the experiment was about 3.56 M., and salts capable of giving a higher concentration were not used.

It should be noted, however, that above 2.92 M. the rise in percentage of plasmolyzed conidia was slow.

Table II

Plasmolysis of Conidia of Erysiphe Polygoni by Potassium nitrate.

Incipient Plasmolysis	Maximum Plasmolysis	Average Isotonic Point
1.32 M.	3.56 M.	2.44 M.
1.67 M.	3.52 M.	2.56 M.
1.92 M.	2.92 M.	2.41 M.
1.67 M.	3.52 M.	2.56 M.
Average 1.65 M.	3.38 M.	2.49 M.

The average isotonic point for the four determinations is 2.49 M. This figure will be used below in calculating the osmotic pressure.

It is interesting to note that the plasmolysis observed was normal in that the protoplast always returned to its original volume when water was substituted for the

plasmolyte. That the potassium nitrate is not toxic to the conidia was shown by their ability to germinate in concentrations of that salt up to the strength inducing plasmolysis. The shrinkage of the protoplast was small in all instances and was slow, presumably owing to the relative impervious nature of the conidium wall and the fact that liquids must pass into the conidium via the papilla. Even at high concentrations of plasmolyte, the protoplast did not assume a spherical shape nor even show any marked reduction in volume (Fig. 9). This is interpreted as additional evidence that the protoplast contains very little water which could be withdrawn during plasmolysis.

Calculation of Osmotic Pressure

A molar solution of a non-electrolyte at standard temperature and pressure is capable of exerting an osmotic pressure of 22.4 atmospheres. Applying a correction for the temperature obtaining during the determinations given above, we have:

$$\text{Osmotic Pressure} = \frac{22.4 \times 300}{273} \text{ for 1 M. non-electrolyte.}$$

A 2.49 molar solution (See Table II) would therefore have an osmotic pressure of: $\frac{22.4 \times 300 \times 2.49}{273}$ atmospheres

But we are dealing with an electrolyte and a correction must be made for the degree of dissociation of this electrolyte. This correction is represented by $(1 + \alpha)$ where "α" is the dissociation constant.

At 22°C. the value of "α" for potassium nitrate at a concentration of 2.49 M. is .52

The final value for the average osmotic pressure would then be:-

$$\frac{22.4 \times 300 \times 2.49 \times (1 + .52)}{273}$$

which equals 93.16 atmospheres.

Calculating the other cardinal points in the same way, we have then, for Erysiphe Polygoni the following data:-

Minimum osmotic pressure . . . 64.17 atmospheres

Average osmotic pressure . . . 93.16 atmospheres

Maximum osmotic pressure . . .121.47 atmospheres

The maximum osmotic pressure as determined by the writer using the plasmolytic method is seen to be higher than any yet recorded for fungus cells.

Whether or not this high osmotic pressure is an aid to mildew conidia in taking up water during germination is difficult to say. Since it will be shown later in this paper that the conidia of Erysiphe Polygoni are capable of germinating as well at zero relative humidity as at 100%, it would appear that they are independent, in the matter of germination, of their ability to take up water. Further, the question of the taking up of water during germination may be one of imbibition rather than osmotic pressure.

Osmotic Pressure During Germination

Tests were made to determine whether or not the osmotic pressure of the conidia falls during germination. Fresh conidia were dusted on to dry slides and allowed to germinate on the laboratory table. Four times, at intervals of fifteen minutes, they were tested for plasmolysis. They were tested a fifth time one hour and forty-five minutes from the beginning of the experiment. At the time of the last test, 69% had germinated.

Table III

Average Isotonic Point During Germination

Time on dry slide	Average Isotonic Point
15 minutes	1.89 M.
30 "	1.99 M.
45 "	1.94 M.
60 "	1.99 M.
105# "	1.99 M.

Conidia had germinated 69%.

It will be seen that the conidia were plasmolyzed with about the same concentration of plasmolyte throughout the period of germination..

However, germ-tubes which were produced on dry slides in four hours(germ-tubes were about 1/3 length of spore) could be plasmolyzed by a solution of 1.67 M. concentration. Although there does not seem to be any appreciable fall in osmotic pressure up to the time of appearance of the germ-tube, the value in the older germ-tube is slightly lower than in the ungerminated conidium.

Germination of the Conidia

Under the various conditions described below, the conidia of Erysiphe Polygoni were found to germinate on the average in one hour and forty-five minutes. The minimum time recorded was one hour and fifteen minutes. This time is extremely short compared with that required by the spores of most fungi for germination, which is usually between twelve and twenty-four hours.

The germ-tube emerges slightly to one side of the middle of one end of the conidium and is generally straight and terete. As has already been noted by other workers (Neger and Hashioka), the germ-tube tends to be long and straight when growing in moist air whereas in dry air it is short and often convoluted at the apex (Fig.22,23).

The writer has heard the criticism made of Yarwood's investigations that the germ-tubes observed by that worker developed under conditions of low humidity might be abnormal swellings on the spores. This idea is entirely incorrect, for the writer has observed the development of thousands of normal germ-tubes on spores held in dry air.

Conidia of Erysiphe Polygoni from Delphinium and Polygonum aviculare, Sphaerotheca Humuli from rose, S. Humuli v. fuliginea from pansy and Erysiphe graminis from Poa pratensis were all subjected to germination tests under conditions of humidity ranging from zero(approximately) to 100%, according to the method described above. The

temperature during the experiment varied but slightly from 22°C. although no attempt was made to control it. In estimating the percentage of germination, two hundred conidia were counted in each test, the counts being made at the end of three hours. The results of these tests are given in Table IV.

The results fully uphold Yarwood's contention, for it will be seen from the Table that (except for the two varieties of Sphaerotheca Humuli) the mildews examined germinate as well at low humidity as at high. In no case was there a clear-cut optimum humidity and the writer agrees with Yarwood in concluding that mildew conidia are tolerant of a wide range of humidity as regards germination or, to put it in another way, are independent of humidity.

Neither of the two varieties of Sphaerotheca Humuli are tolerant of extremely low humidity, no germination being observed below 32.5% and but little below 90%. However, as compared with the requirements of most fungi, the figure is remarkably low. Neither the rose nor the pansy mildew are usually found in hot dry locations and it is not surprising that they should be less tolerant of low humidity than the other kinds tested, all of which may be found in open, dry situations.

Table IV---Percentage germination of mildew conidia under different conditions of humidity at 22°C.

Organism	Relative humidity in germination chamber - per cent											
	0	2.5	4.5	7.5	10.5	20	32.5	54.7	79.5	90	95	100
Erysiphe Polygoni from Delphinium	36 21 23	46 16 23	20	24 18 21	27 28 39	21 18 39	31 37 38	24 34 31	41 26 32	43 29 36	52 27 29	12 31 29
Erysiphe Polygoni from Polygonum	33	6	1	4	28	16	2		2	4	0	7
Sphaerotheca Humuli from Rose	0	0	0	0	0	0	4		0?	1	6	14
Sphaerotheca Humuli v. fuliginea from Pansy	0	0	0	0	0	0	6		0?	22	14	24
Erysiphe graminis	22	16	14	11	15	31	11		20	13	12	10

Germination of Conidia in a Current of Dried Air

When, in previous experiments, conidia were germinated in closed vessels over concentrated sulphuric acid, it was always possible that some time might elapse before conditions of low humidity were really established in the germination chamber. Thus, if the laboratory air had a relative humidity of 50% at the time of the test, the conidia would begin their germination at that humidity and would be subjected to low humidity only after the acid had withdrawn moisture from the air of the germination chamber. Since the conidia germinate in a very short time, this lag in the establishment of low humidity might lead to serious error.

In order to leave no possibility of doubt regarding the ability of the conidia of Erysiphe Polygoni to germinate in very dry air, the following experiment was performed.

Freshly gathered conidia on cover-slips were placed in the chamber "A" of the apparatus shown in Fig. 1. The tall wash bottles were connected to the apparatus and these were filled with concentrated sulphuric acid. Air was drawn through the whole apparatus immediately after the spores were placed in the germination chamber. The air, bubbling very slowly through the two sulphuric acid wash towers must have been very desiccated when it reached the conidia. The air originally present in the germination chamber which might have contained considerable moisture

was removed at once in this experiment and one could be certain that the conidia were subjected to dry air from the beginning of the test.

Under these conditions, the following results were obtained for germination of the conidia in a current of dried air.

Table V

Germination of Mildew Conidia in a Current of Dried Air

Test No.	Time	Percentage of Conidia Germinating
1.	3 hours	43
2.	3 hours	31
3.	4 hours	41
4.	3 hours	27

This experiment proves beyond doubt that the conidia of Erysiphe Polygoni are able to germinate in extremely dry air.

Constancy of Volume of Conidia during Germination

Yarwood (23) stated that he had observed a shrinkage of as much as 24% in the volume of turgid conidia when these were germinating in an atmosphere of 80% relative humidity. The writer made a special effort to obtain confirmation of this finding, with negative results.

It had been found that excellent germination may be obtained when the conidia are merely caused to fall on to dry slides and allowed to germinate exposed to the air of the laboratory. Conidia of Erysiphe Polygoni from delphinium and from Polygonum aviculare were dusted on dry slides and then placed on the stage of the microscope. A suitable field, showing several healthy (turgid) conidia was chosen and careful camera-lucida drawings were made of these under the high power at intervals of about twenty minutes. In another series of observations, measurements were taken with an ocular micrometer at intervals.

During the time the conidia were germinating (up to three hours), the writer failed to observe any change in volume of the dozens of conidia examined (Fig. 13 - 19). It was suggested to the writer that depression of the conidia along the short axis might take place without resulting in change in outline seen from above. However, spores were found which had landed on one end and these were watched carefully. Here too, no change in outline could be observed (Fig. 10 - 12)

Tests were also made at zero and 75% relative humidity with the same results.

The writer is forced to conclude that, in his material, there was no change in the volume of the spore as indicated by its outline seen under the microscope.

Yarwood's measurements, from which he deduced that shrinkage takes place, were made after 5, 10 and 24 hours. The writer has rarely found germinated conidia of this fungus to remain alive for more than four to five hours. After about four hours, growth ceases (Fig. 19 and 20) and one observes a marked change in the appearance of the protoplast: it becomes denser and darker. At about this time, a marked wrinkling of the spore wall is evident and the whole conidium shrinks (Fig. 20 and 21). It seems certain that the conidium is dead, however, before this shrinkage occurs.

Shrivelling of Conidia at Various Humidities

Yarwood (23) showed that, at low humidity, the proportion of conidia that were shrivelled (and doubtless) dead) at the end of thirty-hour germination tests was progressively greater as the temperature was increased. In other words, although the mildew are tolerant of low humidity to the extent of being able to germinate, they are eventually injured by dry air more quickly than by moist air at higher temperatures (up to 22°C.).

The writer's experience confirms Yarwood's work on this point. In Table VI below are shown the percentages of shrivelled conidia found at the end of germination tests at 22°C.

Table VI

Percentage of Shrivelled Conidia of Erysiphe Polygoni at Various Humidities at 22°C.

Humidity	Percentage of Conidia Shrivelled at End of Three Hours
0	77
2.5	77
7.5	76
10.5	54
20.0	47
32.3	26
54.7	32
79.5	25
90.7	24
95.0	25
100.0	15

Non-Germination of Mildew Spores In Situ

Having established the fact that the spores of some powdery mildews are independent of moisture for germination, the question naturally arises: are the conidia capable of germinating in situ? In nature, mildew conidia produced on conidiophores above the surface of the leaf might be expected to germinate as soon as they are produced.

Examination of the illustrations of such careful observers as the brothers Tulasne (20) fails to reveal any spores germinating while still attached.

Several times the writer kept material of Erysiphe Polygoni under constant observation for a period of twenty-four hours. The mildewed leaves were folded so that the conidiophores projected into the air

and could be readily examined under the low power of the microscope.

As a result of these observations, it can be stated definitely that the conidia do not germinate as long as they remain attached to the conidiophores.

One has only to shake the conidia on to a dry slide to cause them to germinate, and it is necessary to offer some explanation as to why they remain ungerminated while attached to the conidiophores.

At first it was believed that, as long as the conidia remain attached, some chemical substance from the conidiophore might inhibit germination. To test this hypothesis, a large quantity of mildew mycelium was carefully scraped from infected leaves and added to a small quantity of distilled sterile water. The mycelium was thoroughly macerated in the water. Fresh conidia were then suspended in the water and macerated mycelium. As control experiments, conidia were suspended in sterile distilled water and in water containing macerated unifected host tissue. The conidia germinated to the extent of 45% in all experiments, showing that there is no chemical substance present in the mycelium to inhibit the germination of conidia.

Next, the matter of maturity was considered. It has been shown that the conidia become disjoined from the conidiophores by shock or wind and it was thought that possibly they may be immature until just before they are

able to be dislodged. If this were so, conidia artificially detached prior to maturity could not germinate when detached.

A mildewed leaf was held firmly with forceps and then shaken violently in order to dislodge all conidia possible. The leaf was then examined under the low power of the microscope and several conidiophores bearing firmly attached conidia were found. By means of careful micromanipulation, these immature conidia were removed. They were dislodged only after considerable probing with the needles of the micromanipulator. Despite the fact that none of the ten conidia so obtained was mature enough to drop from its conidiophore, nine of the ten germinated after being detached.

It would appear from this observation that, provided the formation of the conidium is complete, it can germinate whether ready to drop off of its own accord or whether it be artificially dislodged. The idea that the conidia do not germinate *in situ* because they are undergoing a process of maturation is not borne out by this experiment.

Relation of Carbon Dioxide to Germination

In considering the conditions within the mature conidium which might be responsible for its failure to germinate as soon as it is matured, it will be necessary here to re-state certain facts regarding its structure.

The septum which abstricts the terminal conidium is at first perforate, allowing for cytoplasmic connection with the conidiophore below (Fig. 3). Later the septum becomes entirely closed and the conidium is separated physiologically from the conidiophore.

After separation, the continued respiration within the conidium would 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 been shown in this paper that the conidium wall is relatively impervious to water except at the papilla 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.

To test this hypothesis, mildew conidia were first subjected to an atmosphere of air containing an excess of carbon dioxide.

The apparatus used is illustrated in Fig. 1. The wash bottles D, D, were not connected to the apparatus.

Freshly dislodged conidia were placed in the germination chamber "A". Air was then drawn through the chamber and a small proportion of carbon dioxide, approximately one part in ten, passed through water was allowed to mix with the air at point "B" in Fig. 1, passing into and through the germination chamber. The gas mixture

which contained more carbon dioxide than is normally present in the atmosphere was passed slowly through the germination chamber for thirty minutes. Connections immediately on either side of the germination chamber were then closed.

A control experiment was set up using a duplicate apparatus lacking the arrangement for adding carbon dioxide.

After two hours, excellent germination had taken place in the control apparatus while in the presence of added carbon dioxide no spores germinated.

The ungerminated spores were then removed from the germination chamber in which there was added carbon dioxide and they were allowed to stand exposed on the laboratory table. Two hours later, these spores were found to have germinated.

The results of this experiment are presented in the following table:

Table VII

Effect of Carbon Dioxide on Germination of Mildew Conidia

	Percentage Germination		
Trial No.	In Chamber with added CO_2	In Chamber with air	Same Conidia shown in Column 2, two hours after removal from CO_2 Chamber
1.	0	45	43
2.	0	50	55
3.	0	50	44

From these results we may conclude (1) that carbon dioxide in a concentration of approximately 10% prevents mildew conidia from germinating during the two hours required for germination of conidia exposed to atmospheric air; and (2) that conidia which have been held in carbon dioxide for two hours germinate as well after they have been removed from the carbon dioxide as though they had not been subjected to experiment.

The writer hoped to be able to experiment with mixtures of gases to find the critical concentration of carbon dioxide above which no germination could occur. However, much difficulty was experienced at first in obtaining consistent results in the experiment described above. This proved to be due to the extreme toxicity to the mildew spores of the vulcanized rubber used in the form of rubber tubing and stoppers in the apparatus. The source of trouble was not located until late in the work and it was not possible to put the experiment on a quantitative basis. However, the results are so clear-cut as to seem convincing even in their unrefined form.

Relation of Oxygen to Germination

Experiments were carried out to determine whether or not the conidia of Erysiphe Polygoni are capable of germinating in an atmosphere in which the proportion of oxygen is less than that present in ordinary air.

At first an attempt was made to remove oxygen from atmospheric air by passing the latter slowly through three wash bottles containing strong alkaline pyrogallol. The conidia were placed in the germinating

chamber as in the previous experiments and a control apparatus was set up in which washed air passed directly over the conidia without first passing through pyrogallol.

The results of several tests were entirely negative. About 65% germination was obtained in the air which had passed through pyrogallol as well as in the control apparatus. It was thought that the pyrogallol solution might in a short time have lost its ability to absorb oxygen from the air stream, and fresh tests were made in which the air was bubbled through pyrogallol for ten minutes only, which time was considered sufficient for the original air of the germination chamber to have been replaced. The pinch-cocks on either side of the germination chambers were then closed, both on the chamber containing pyrogallol-washed air and on the control chamber.

Again the same result was obtained: as good germination (60%) resulted in two hours in the pyrogallol-washed air as in ordinary air.

The conclusion to be drawn is either that the conidia are capable of germinating in oxygen-free air or that the pyrogallol did not remove sufficient oxygen to cause any checking of germination. The latter seemed the more likely explanation.

A further experiment was conducted in which a heated combustion tube was connected between the pyrogallol wash-bottles and the germination chamber (at "O" in Fig. 1). Into the combustion tube was inserted a roll of freshly reduced copper gauze which was heated as the air from the intake was drawn slowly over it. In the control

apparatus, a combustion tube was heated but no copper gauze was present.

Again negative results were obtained, when the air was passed through the apparatus for ten minutes and twenty minutes in separate experiments.

The conclusion was drawn that sufficient oxygen had still not been removed from the air. Accordingly, conidia were then placed in an atmosphere of "tank" nitrogen.

The gas was allowed to pass through the apparatus for twenty minutes only and the pinch-cocks were then closed on either side of the germination chambers. In the control experiment, the conidia were subjected to ordinary air. This time, conclusive positive results were obtained as shown in Table VIII.

Table VIII

Effect of Nitrogen on the Germination of Mildew Conidia

Trial No.	Percentage Germination		
	In Chamber with N_2	In Control Chamber	Same Conidia shown in Column 2, two hrs. after removal from N_2 Chamber
1	0	12	10
2	2	80	80
3	2	75	75
4	0	27	18
5	6	80	80
6	0	27	30
7	13	80	80

It will be seen that in all trials, germination was prevented or markedly checked. In trials Nos. 1, 4 and 6, absolutely no germination had occurred in two hours in nitrogen, whereas, innthe controls, 12%, 27% and 27% germination respectively was observed.

In all trials, the germination of conidia held for two hours in nitrogen was approximately the same after the conidia had been removed from the presence of nitrogen and allowed to germinate on the laboratory table as though these conidia had not been subjected to experiment.

The small percentages of germination in nitrogen observed in trials Nos. 2, 3, 5 and 7, may be explained by the fact that "tank" nitrogen, i.e. the residue from liquid air, contains as high as 4% oxygen and this, presumably, is sufficient to allow a small proportion of spores to germinate.

From these experiments, it may be deduced (1) that lack of oxygen or a very low concentration of oxygen prevents germination of the mildew conidia; and (2) that, at least for a period of two hours, conidia are not permanently injured by lack of oxygen as shown by the fact that they germinate well when oxygen is provided.

Again, time has not permitted a demonstration of the critical concentration of oxygen below which no germination can occur. This would have to be done using chemically pure nitrogen to which could be added as much oxygen as desired.

The Toxicity of Vulcanized Rubber

During the course of the above experiments, considerable difficulty was experienced at first in obtaining any germination within the apparatus described. Finally it was found that both ordinary rubber tubing and rubber stoppers are extremely toxic to mildew conidia.

The problem was solved by the use of ground-glass connections in the apparatus and pure gum-rubber tubing, when excellent germination within the apparatus was obtained.

The high degree of toxicity of vulcanized rubber observed seemed to warrant some actual tests, and the following experiments were therefore performed.

Spores were suspended on dry cover-slips over Van Tieghem cells. In each cell was placed a small (about 3 mm. in diameter) fragment of rubber stopper. Twenty-four such cells were arranged along with a series of the same number of controls not containing rubber fragments and a series of cells containing fragments of pure gum rubber. The results of this experiment are given below, the figures being the average of the twenty-four tests.

Table IX

Effect of Vulcanized Rubber on Germination of Mildew Conidia

Percentage Germination after two hours[1]		
In presence of Vulcanized Rubber	In presence of Non-Vulcanized Rubber	In Control
3.5		41
	37	57

[1] Average of twenty-four counts of one hundred conidia each.

This experiment shows that some substance present in vulcanized rubber is highly toxic to the spores of Erysiphe Polygoni. Since non-vulcanized rubber is not toxic and vulcanized rubber contains sulphur, and also since it is a matter of common knowledge that sulphur is a good fungicide for the control of diseases caused by powdery mildews, it would appear probable that the substance present in rubber toxic to the mildew spores is a compound of sulphur.

In the above experiment, that the vulcanized rubber injured the conidia permanently was shown by their failure to germinate after the rubber had been removed from the germination chambers. A search for the toxic principle of vulcanized rubber might lead to the discovery of an excellent means of control of powdery mildews. Although ordinary powdered sulphur is used for this purpose, it must be used in considerable quantities and its toxicity does not seem to be at all of the order shown by vulcanized rubber.

V. Discussion

Unlike the spores of all other fungi, so far as is known, the conidia of certain powdery mildews are capable of germination under conditions of extremely low humidity. Yarwood showed that two strains of Erysiphe Polygoni have a high degree of tolerance of low humidity, and the writer has added three more strains and one other species to the list which now includes:

Erysiphe	*Polygoni*	from	Red Clover	(Yarwood)
"	"	"	Bean	(Yarwood)
"	"	"	*Polygonum*	(Neufeld)
"	"	"	*Delphinium*	(Neufeld)
"	"	"	*Aster sp.*	(Neufeld)
"	"	"	*Poa pratensis*	(Neufeld)

Certain mildews, examined by Yarwood and by the writer have shown much less tolerance of low humidity. These are:

Erysiphe	*Polygoni*	from Mustard	(Yarwood)
"	*graminis*	from Barley	(Yarwood)
"	*cichoracearum*	from Sunflower	(Yarwood)
Sphaerotheca	*pannosa*	from Rose	(Yarwood)
"	*Humuli*	from Rose	(Neufeld)
"	" v. *fuliginea*	from Pansy	(Neufeld)

It is to be expected that certain strains and species of mildew would not be as tolerant of low humidity as others. The rose and pansy mildews are usually found in damp, shaded situations and are physiologically quite unlike *Erysiphe Polygoni* which develops luxuriantly in dry exposed places. The host *Polygonum aviculare* (Knotweed) seems to develop best in well-drained, dry, sunny places and even when most exposed may be heavily infected with mildew.

The writer, however, agrees with Yarwood in concluding not that low humidity is essential to the luxuriant development of mildew; but rather that, unlike other fungi, the powdery mildews are capable of developing

over a wide range of relative humidity and that they are especially well adapted to very dry atmospheric conditions.

From the research recorded in the preceeding pages, the writer concludes that this adaptation is related to the physiological and morphological peculiarities of the mildew conidia. It has been shown that, as it approaches maturity, the conidium becomes cut off from the conidiophore by a septum. The wall of the conidium appears to be relatively impervious to water and probably to gases because of the presence of a thin outer coating of some waxy substance. At the proximal end of the conidium is a papilla which has been shown to provide a permeable spot in the conidium wall.

After maturation, the conidium remains on the conidiophore with its papilla firmly in contact with the conidiophore. The conidium is then sealed, if not hermetically, at least sufficiently to prevent the ingress of oxygen from the outside or the release of carbon dioxide from the inside. Thus it fails to germinate <u>in situ</u> even although, as has been demonstrated in this paper, the relative humidity of the air surrounding the spore may be suitable for germination.

Upon being dislodged from the conidiophore, the seal formed by the firm contact of the papilla with the conidiophore is broken. The end of the papilla is now exposed to the surrounding air and, being more permeable than the rest of the conidium wall, allows carbon dioxide to pass out from the protoplast and oxygen

to pass in.

From the writer's experiments, it is not possible to conclude definitely whether low oxygen concentration or high carbon dioxide concentration is responsible for checking germination *in situ*. However, since it has been shown that some germination can occur in nitrogen containing only 4% or less oxygen, it seems more probable that it is the accumulation of carbon dioxide within the protoplast that checks germination *in situ*.

The fact that conidia of *Erysiphe Polygoni* germinate in high percentage within an hour and a half after being shaken on to a dry slide would seem to be due to the release of carbon dioxide from the protoplast or the entrance of oxygen into it rather than to water uptake. Indeed, the writer is inclined to the belief that water plays no direct part in the commencement of germination in these spores. It cannot, if, as has been shown above, the spores germinate well in a current of air which has been thoroughly dried with sulphuric acid.

When Yarwood's work was first published, the criticism was raised (but apparently not published) that the increase in volume resulting from the spore pushing out a germ-tube must come from the uptake of water during germination, and the problem arose as to how the spore could possibly withdraw water from dry air. It has been shown above that the conidium of *Erysiphe Polygoni* has a very high osmotic pressure. This might possibly account for the ability of the spore to withdraw water from moderately dry air but it does not account for its

germination in a current of extremely dry air.

The protoplast seems unlike that of the spores of Fusarium culmorum, Botrytis cinerea and Macrophoma sp. with which it has been compared. In the latter fungi there are large watery vacuoles which absorb neutral red, while no such vacuoles are present in the ungerminated Erysiphe conidium. The writer believes that very little water is present in the mildew conidium, the protoplast consisting of cytoplasm which is gel-like in consistency.

Upon the release of carbon dioxide from the conidium, respiration would begin if sufficient oxygen were present. Conceivably, as a result, the viscid protoplast would be converted into materials more labile and voluminous and the increase in volume necessary for the production of a germ-tube would come from this source alone.

It has been shown that neutral red begins to enter living conidia when the germ-tube is about one-third the length of the conidium. Possibly, at this time, the sporeling does require water and, under natural conditions, absorbs it. At low humidity on dry slides, the germ-tubes seldom become as long as the conidium and they soon die. This may be due to the lack of water necessary for further development.

Quite regularly, many conidia shrivel early in germination tests at low humidity. One may ask: why, under dry conditions do some conidia germinate while others shrivel and die? One explanation may be that, for some reason, in the conidia that shrivel, the papilla does not

function properly. It may become too permeable and allow the contents of the spore to suffer through drying before normal germination can begin. The fact that neutral red enters the spore more rapidly as germination proceeds would seem to prove that the permeability of the papilla does increase. Dead conidia stain immediately upon immersion in neutral red, which would indicate that the papilla end of the spore becomes entirely permeable when death of the spore sets in.

VI. Summary

1. The conidium of Erysiphe Polygoni is formed by the swelling of the apex of the conidiophore which is, at first, aseptate and later develops a perforate septum.

2. The septum in the conidiophore is formed by a ring of material which is added to inwardly until a disc is formed having a small hole in the centre.

3. A mature conidium bears a minute papilla at one end or it may bear a papilla at each end. When the conidium is abstricted from the conidiophore, the latter also bears a papilla.

4. The conidia are passively discharged, there being no special disjunctor mechanism.

5. The conidial protoplast appears to contain very little water as compared with the protoplast of other fungi examined.

6. Living, turgid conidia take up neutral red extremely slowly, the stain eventually entering at the papilla. The wall of the conidium appears relatively impervious to water.

7. As determined by the plasmolytic method, the conidia of Erysiphe Polygoni have an osmotic pressure of 93.16 atmospheres. This value remains constant during the early stages of germination but falls slightly as the germ-tube approaches one-third the length of the spore.

8. Conidia of Erysiphe Polygoni from Delphinium and Polygonum aviculare, Sphaerotheca Humuli from rose, S. Humuli v. fuliginea from pansy and Erysiphe graminis from Poa pratensis were tested for tolerance of low humidity. Good germination at low humidity was obtained for all except the Sphaerotheca species.

9. The conidia of Erysiphe Polygoni germinated well (about 35%) in a current of air dried by passage through sulphuric acid.

10. No shrinkage during germination was observed. Shrivelling and collapse of the conidia occur after death.

11. A higher percentage of conidia shrivel at low humidity than at high, temperature being constant.

12. The conidia do not germinate *in situ*. Mere detachment of the conidia is sufficient to cause germination in ordinary air.

13. In air containing approximately 10% carbon dioxide, the conidia failed to germinate. After removal from the gas they germinated perfectly in ordinary air.

14. In nitrogen (not absolutely pure) no germination occurred. The conidia germinated well in ordinary air after the experiment.

15. Vulcanized rubber is highly toxic to the conidia of *Erysiphe Polygoni*. A small piece in a large closed dish with dry spores was sufficient to inhibit germination and injure the spores permanently.

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Explanation of Plate I

All figures are those of *Erysiphe Polygoni*

Fig. 2. Young aseptate conidiophore. x 800.

Fig. 3. Conidiophore showing perforate septum and cytoplasmic connection. x 800.

Fig. 4. Mature conidium attached to conidiophore. x 800.

Fig. 5. Conidiophore showing papilla after conidium has become dislodged. x 800.

Fig. 6. Detached conidium showing papilla. x 800.

Fig. 7. Unstained conidium. x 1200.

Fig. 8. Conidium stained with neutral red showing the stain accumulated in the small refractive globules. x 1200.

Fig. 9. Plasmolyzed conidium. x 800.

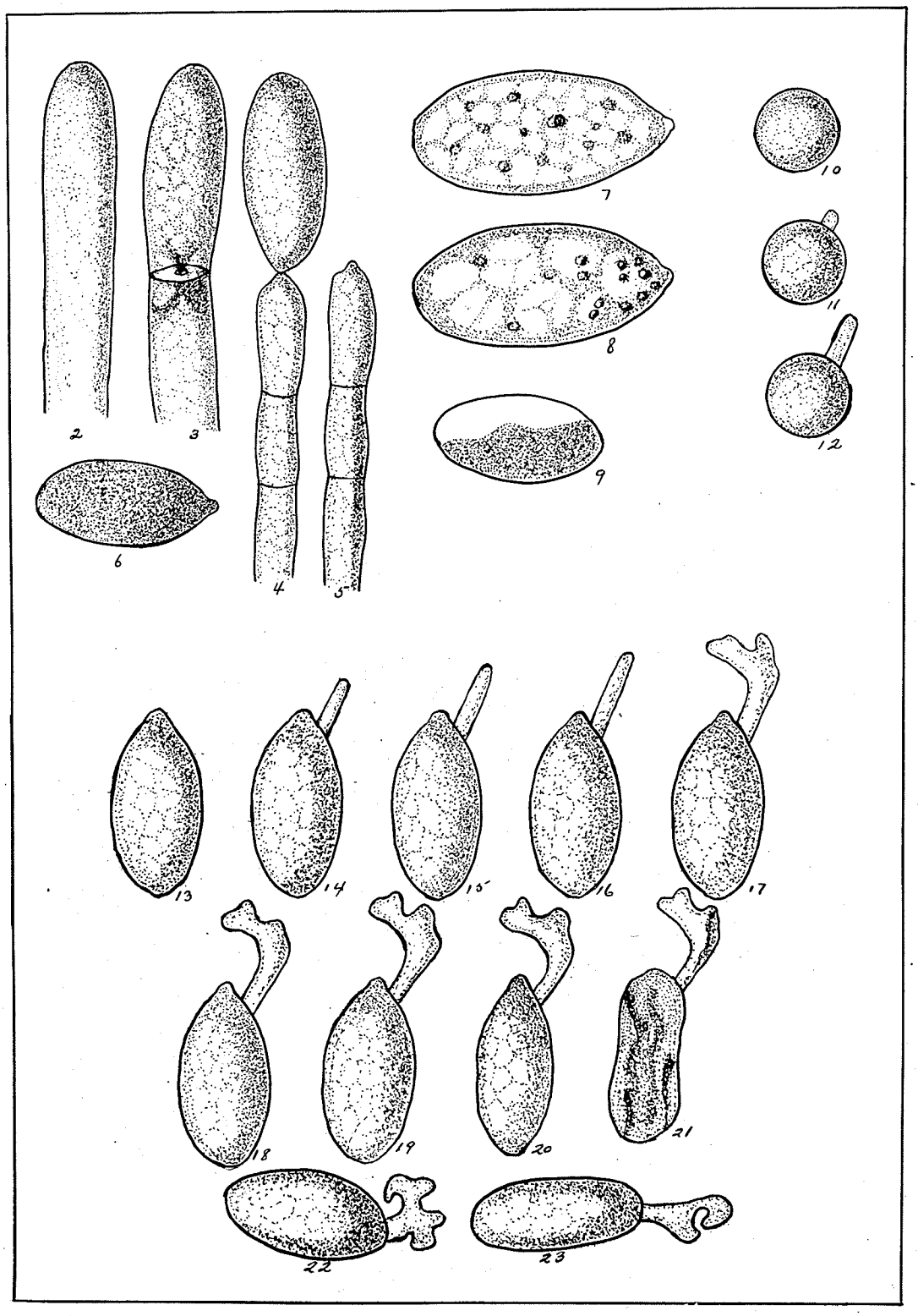
Fig. 10. End-view of conidium. x 800.

Fig. 11 and 12. End-views of germinating conidium. x 800

Fig. 13 - 21. Series of camera-lucida drawings showing various stages of development of a germinating conidium. x 800.

Fig. 22 and 23. Conidia germinating in dry air showing convolutions at the apex. x 800.

Plate I



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THE UNIVERSITY OF MANITOBA

Winnipeg, Canada,
October 5th, 1940.

Dr. Margaret Newton,
Dominion Rust Laboratory.

Dear Sir:

The Graduate Studies Committee has appointed you a member of the Committee of Examiners to report upon the Master's thesis submitted by

C. C. Neufeld

The regulations state that a total of 600 marks shall be assigned to the major subject, including the thesis. The general practise is to give 400 out of the 600 to the thesis, though this proportion is subject to change at the will of the department.

Copies of the thesis are submitted in an unbound condition in order to permit alterations and corrections desired by the Committee of Examiners before they are finally bound.

You are requested to return your copy direct to Mr. W. B. H. Teakles, Assistant Registrar, and the Chairman will submit the Committee's report on the thesis to the undersigned at an early date. Members of the Committee of Examiners are listed below, the first named acting as chairman.

Yours truly,

G. M. Brownell,
Secretary, Graduate Studies Committee.

Committee:

Dr. H. J. Brodie
Professor William Leach
Dr. Margaret Newton