On the Germination and Growth of Fungi at various Temperatures and in various Concentrations of Oxygen and of Carbon Dioxide.

BY

WILLIAM BROWN, M.A., D.Sc.

(From the Department of Plant Physiology and Pathology, Imperial College of Science and Technology, London.)

With four Figures in the Text.

INTRODUCTORY.

THE present investigation was carried out under the auspices of the Food Investigation Board of the Scientific and Industrial Research Department, and had for its object the examination of the behaviour of fungi under the conditions prevailing in the practice of fruit storage. There are two methods of storage, known respectively as 'cold storage' and 'gas storage', the former of which is now widely established in practice, while the latter is still in an experimental stage. As the scope of this investigation was to a large extent determined by considerations of a practical nature, a brief account of these two methods may appropriately be given here.

In the ordinary method of cold-storing fruits, where the fruit is subsequently to be used for general household purposes (and not, for example, for wholesale jam manufacture), a temperature of less than 0°C . or thereby is not admissible. In actual practice a temperature of $2-3^{\circ}\text{C}$. is the lowest usually considered safe. No attempt was therefore made to test the behaviour of fungi at temperatures below this limit, and in fact, except for a few experiments, a temperature of 5°C . was the lowest employed.

The gas storage method is based on the retarding action of carbon dioxide on metabolic processes, so that the retarding effect of a lowering of temperature in ordinary cold storage is replaced or augmented by that due to a certain concentration of carbon dioxide in the atmosphere of the store. In practice the carbon dioxide is derived from the respiration of the fruit itself (the concentration being maintained at a steady level by special devices). In view of the fact that the concentration of oxygen in

the atmosphere of the store must not fall so low that anaerobic respiration sets in, it follows that the maximum concentration of carbon dioxide available in practice is something under 20 per cent. In the experiments to be described here the effects of much higher concentrations of carbon dioxide on fungal growth were tested, but special attention was directed to the effects of the lower concentrations admissible in practice.

HISTORICAL.

Numerous statements occur sporadically throughout fungal literature in connexion with the effect of *temperature* on fungal growth. Only the more recent and important papers dealing specially with fungi causing fruit-rot call for mention here.

Schneider-Orelli (1) cites the following fungi as producing fruit-rot: *Penicillium glaucum*, *P. italicum*, *Botrytis cinerea*, *Monilia fructigena*, *Gloeosporium fructigenum*, *G. album*, *Fusarium putrefaciens*, *Cladosporium herbarum*, *Mucor piriformis*, and *Rhizopus nigricans*. He states that with the exception of *P. italicum*, which is the most sensitive of all to low temperature, all the preceding are capable of growth at 4.5° C., and the majority are even capable of slow growth in the neighbourhood of 0° C. The most active parasites are *Botrytis cinerea* and *Monilia fructigena*, and the former does more damage in cold store by reason of its greater capacity for growth at low temperature. *Monilia* tends to disappear from the store as time goes on. The explanation put forward for this is that the low temperature, though not entirely inhibiting the growth of the fungus, is effective in preventing its spore development, in which respect the absence of light is probably an important contributory factor.

Both these fungi, *Botrytis cinerea* and *Monilia fructigena*, are, according to this observer, of special importance in that they are able to attack a great variety of fruits over a wide range in respect of ripeness and over a wide range of temperature. The other fungi listed have more limited parasitic powers. Thus they may only be able to bring about attack when the fruit has reached an advanced stage of ripeness. Of this type is *Penicillium glaucum*, which shows markedly selective action in accordance with the ripeness of the fruit, and which, according to this author, can only attack the late-ripening varieties of home fruits with difficulty, at least for a long time. Its abundant spore-formation and its capacity to attack ripe fruit make it an important parasite during the late period of storage.

Ames (2) determined the cardinal points for temperature (minimum, optimum, maximum) of a number of fungi causing fruit-rot. The minimum temperatures for germination given by this observer are as follows: for *Monilia fructigena* and *Penicillium digitatum*, 1°; for *Rhizopus nigricans*

and *Glomerella rufomaculans*, 3° ; for *Thielaviopsis paradoxa*, $4-5^{\circ}$; and for *Cephalothecium roseum*, $6-9^{\circ}$. The minimum temperature for fructification is in all cases several degrees higher than that for growth.

Edgerton (3) determined the relation of growth to temperature of thirty strains of *Glomerella*. This paper is interesting as illustrating the amount of variation as regards response to different temperatures shown by organisms which are morphologically very similar to each other.

Brooks and Cooley (4) examined in detail the temperature relationships of twelve fungi which produce rot of apple in storage. For tables and graphs of the rate of growth of the various fungi on artificial medium and on apple, the original paper should be consulted. The following results may be quoted here:

1. Spores of *Alternaria* sp., *Botrytis cinerea*, *Penicillium expansum*, and *Sclerotinia cinerea* germinated slowly at 0° on corn-meal agar medium; spores of *Cephalothecium roseum*, *Fusarium radicicola*, *Glomerella cingulata*, and *Pestalozzia funerea* failed to germinate at 0° , but slowly germinated at 5° ; spores of *Aspergillus niger* failed to germinate at 10° .

2. The minimum temperature for the growth of some fungi in the case of inoculations on fruit is higher than that for growth on artificial medium, and in general the inhibitory effect of low temperature on the early phases of growth is more pronounced in the case of inoculations on fruit than for inoculations on artificial medium. Again, in the case of the weaker parasites, the minimum for growth on the fruit is dependent upon the state of maturity of the latter, being lower according as the fruit is in a more advanced stage of ripeness.

3. In the case of *Penicillium expansum* it was noted that the fungus, after starting at ordinary temperatures, was able to continue growth at 0° , whereas it could not initiate attack at the latter temperature. The authors therefore point out the advisability of immediate storage.

In connexion with the last-mentioned point it is interesting to note that Kirchner (5) records a similar phenomenon for the growth of the roots of seedlings.

The effect on growth of *atmospheric composition* has been studied on a large variety of plants and plant parts. The two factors of importance in the present connexion are: (1) the influence of varied concentration of carbon dioxide, (2) the influence of varied concentration of oxygen.

Carbon dioxide. That a concentration of carbon dioxide greater than that normally present in the atmosphere accelerates the growth of higher plants has been claimed by some workers, e. g. by Demoussy (6), Fischer (7), Chapin (8), and Kisselew (9). Brown and Escombe (10), however, failed to observe any appreciable increase of yield brought about by increased concentrations of carbon dioxide, but on the other hand recorded considerable morphological effects produced in this way (see on this point

Farmer and Chandler (11)).¹ In the case of green plants, though Chapin considers carbon dioxide to act here mainly as a stimulant merely, the effect is complicated by its use for photosynthetic purposes, and on that account these results are scarcely relevant to the present investigation.

Fränkel (12) examined the effect of carbon dioxide on the growth of various micro-organisms on liquid or solid culture media. He found that whereas some organisms, e. g. the true beer yeasts, can grow as well in an atmosphere of 100 per cent. CO_2 as in air, the vast majority both of saprophytic and parasitic forms are inhibited by such treatment. He showed that this inhibition was due to the actual presence of carbon dioxide and not to mere absence of oxygen, and that the effect was one of inhibition and not of killing, as the organisms subsequently grew normally when again placed in ordinary air. He also pointed out the marked variations in susceptibility of different individuals in the same culture (quite apart from spore forms), a feature which was also emphasized by Frankland (13).

Lopriore (14), who gives a full account of the earlier literature, showed among other things that the germination of the spores of *Mucor mucedo* was slowed by the action of 10 per cent. CO_2 in the atmosphere, and that undiluted carbon dioxide, though producing total inhibition, did not kill the spores even after an exposure of three months. Sporangium formation was more readily suppressed than spore germination. Lopriore noted a tendency of the mycelium to form swollen cells under the carbon dioxide treatment. He also states that low percentages of CO_2 (1–10 per cent.) accelerate the growth of pollen-tubes.

According to Plummer (15) the ammonifying bacteria are very insensitive to the action of carbon dioxide, 60 per cent. of this gas in the atmosphere having no appreciable effect on the rate of ammonification.

Kidd (16), working with seeds of *Brassica alba*, found that the germination of the latter was inhibited by comparatively low concentrations (10 per cent.) of carbon dioxide. The retarding effect of a given concentration of carbon dioxide was dependent upon the pressure of oxygen present, being greater the lower the oxygen pressure. Kidd also describes acceleration of growth caused by a sufficiently low concentration of carbon dioxide.

Oxygen. Out of the very voluminous literature dealing with the effect of oxygen pressure on plant growth, it is only necessary here to cite the work of Porodko (17), in which maximal and minimal concentrations of oxygen for a number of micro-organisms are given. From this work it appears that aerobic organisms are very insensitive to changes in oxygen pressure. Thus for *Penicillium glaucum* the maximum pressure at which

¹ It is, however, possible that some of the effects recorded may have been due to impurities in the carbon dioxide employed.

the fungus will grow is as high as 3.5 atmospheres, while the minimal pressure is well below 1 per cent. of an atmosphere. In some cases the minimal oxygen pressure is extraordinarily low, as for example 0.00016 per cent. for *Bacillus subtilis*. While obligate anaerobes have a very low oxygen maximum (0.003 per cent. for *Clostridium butyricum*), facultative anaerobes were found by Porodko to be as resistant to increased oxygen pressures as obligate aerobes, and in some cases more so.

The general result of the work just cited, which is in agreement with earlier work on this subject, was to indicate that variations in the oxygen pressure would be found to be of little importance in connexion with the purposes of the present investigation.

Finally, mention may be made of the work of Berghaus (18) and of Chlopin and Tammann (19), dealing with the effect of varying gas-pressure upon the growth of micro-organisms. According to these investigators the effect of mere varying pressure of a neutral gas (hydrogen or nitrogen) upon the growth of micro-organisms is negligible within very wide limits.

EXPERIMENTAL METHOD.

The various temperature conditions were obtained by means of ordinary Hearson incubators in the case of the higher temperatures (15–25°), and for lower temperatures (2–10°) an automatically regulated small 'Isko' refrigerator was used. The temperature of the latter was controlled electrically by means of a toluene regulator and a relay which stopped and started a pump circulating sulphur dioxide. It was constant to within half a degree on either side of the mean value.

As regards the setting up of fungal cultures in the various atmospheres, the technique described by Kidd (16, p. 411) was followed throughout. Large glass containers of $3\frac{1}{2}$ –5 litres capacity (of the type employed as desiccators) were used as receptacles for the fungal cultures. The cultures intended for study, together with a little water to check evaporation, were placed in these, the lids luted down with 'resin cerate', and the required concentration of carbon dioxide in the internal atmosphere made up after the manner described by Kidd—that is, the containers were attached to an air-pump, the enclosed air exhausted to the necessary amount as measured by a manometer (7.5 cm. Hg vacuum for 10 per cent., 15 cm. for 20 per cent., &c.), and the pressure again brought back to normal by the introduction of carbon dioxide gas from a cylinder. The latter gas on analysis gave 98 per cent. purity, the remaining 2 per cent. not being absorbable in either alkali or in alkaline pyrogallol, and therefore being presumably nitrogen.

The composition of the atmosphere in the containers was systematically tested and checked by analysis. For this purpose a large size of Haldane gas apparatus was used. By this means the percentage of carbon dioxide

is readily determined to within 0.1 per cent. With care, much greater accuracy is possible, but this was rarely required. From numerous analyses it was found that with ordinary care the carbon dioxide content of the experimental atmospheres as set up in the manner above described never varied by more than $\frac{1}{2}$ per cent. from the value intended.

Determinations of the oxygen content were only made occasionally, and were carried out by means of the same apparatus.

The following is a list of the fungi used at one time or other in the course of the present investigation, together with a statement of the source from which they were derived:

<i>Botrytis cinera</i>	Originally obtained from Centraalbureau voor Schimmelcultures, Holland.
<i>B. parasitica</i>	Isolated from onion.
<i>Aspergillus repens</i>	All stock laboratory cultures.
<i>Mucor</i> sp.	
<i>Rhizopus nigricans</i>	
<i>Penicillium glaucum</i>	
<i>Monilia cinerea</i>	All isolated from rotted apples.
<i>Fusarium</i> sp.	
<i>Phoma roseola</i>	
<i>Alternaria grossulariae</i> ¹	
<i>Sphaeropsis malorum</i>	

and a *Botrytis cinerea*, also obtained from apple, and which appeared to be identical with the other culture of this organism.

Stock cultures of these fungi were grown at laboratory temperature in diffuse light on sloped tubes of potato agar.

Experiments dealing with the effect on Germination of various concentrations of Carbon Dioxide and Oxygen.

The general nature of the effects produced was worked out in the first instance with spores of *Botrytis cinerea*. A series of experiments was carried out showing the relation between the amount of germination and the following three factors: (1) concentration of carbon dioxide in the atmosphere; (2) density of spore suspension; (3) concentration of nutrient.

The effect on germination (determined quantitatively as percentage of spores germinating or as the average length of the germ-tube produced) of the second and third of the factors mentioned was known from earlier work to be as follows. The amount of germination in a given time increases with concentration of the nutrient up to a certain limit.² The amount of germination in a given nutrient decreases with increasing density of spore

¹ Identified as such by Horne. Ann. Appl. Biol., vii. 190, foot-note 3, 1920.

² Cf. Brown, this volume, p. 108.

suspension; this effect is most strikingly shown in the case of lower concentrations of nutrient.

In the following tables (I-III) the nutrient employed was a turnip extract obtained by extracting the juice of turnips boiled without any addition of water. This full-strength extract is denoted by T.E., and the various dilutions with water as T.E./10, &c.

The different densities of spore suspension are denoted by m, m/10, m/100, where m denotes a suspension containing 0.1 c.c. of wet spores in 10 c.c. liquid. In order to obtain these suspensions, spores were washed from a plate culture of *Botrytis* after the manner described in an earlier paper¹ and freed from mycelium by filtering through muslin. The spore suspension was then centrifugalized about six times in changes of water in order to complete washing, then finally centrifugalized in a graduated tube and the volume of spores determined. This being known, the various dilutions in the various concentrations of nutrient could be made up.

The germination tests were carried out as follows: Drops of the various suspensions were placed on clean glass slides, the latter placed on a rack in a moist container, and the required atmospheric conditions set up as already described.

These tests were carried out at ordinary laboratory temperature (15-18°), the various containers being placed side by side, so that they were under similar temperature conditions throughout. From the method of preparing the spore suspensions complete absence of contamination could not be guaranteed, but as the tests were of short duration, any accidental slight contamination was of no importance.

TABLE I.

Botrytis cinerea.

Percentage of Germinated Spores after 20 Hours' Germination (based on at least 100 counts in each case).

Spore Density.	Nutrient.	Air.	10 % CO ₂ .	20 % CO ₂ .	30 % CO ₂ .
m/100	H ₂ O	37	5	0	0
	T.E./10,000	72	47	0	0
	T.E./1,000	90-100	75	9	0
	T.E./100	90-100	74	25	0
m/10	H ₂ O	0	0	0	0
	T.E./10,000	3	0	0	0
	T.E./1,000	51	25	2	0
	T.E./100	80	63	18	0
m	H ₂ O	0	0	0	0
	T.E./10,000	0	0	0	0
	T.E./1,000	0	0	0	0
	T.E./100	36	23	0	0
	T.E./10	84	64	8	0

Table II gives the average length of germ-tube in the same experiment, this quantity being determined by measuring by means of a micrometer the lengths of all the germ-tubes shown by the counted spores, and dividing

¹ W. Brown: Ann. Bot., xxix. 319, 1915.

the total length by the total number of spores counted. This quantity gives a better representation of the picture presented than does a mere count of the percentage of germinated spores.

TABLE II.

*Botrytis cinerea.**Average Length of Germ-tube after 20 Hours' Germination.*

Spore. Density.	Nutrient.	Air.	10 % CO ₂ .	20 % CO ₂ .	30 % CO ₂ .
m/100	H ₂ O	0.6	0.04	0	0
	T.E./10,000	1.45	1.0	0	0
	T.E./1,000	4.0	2.5	0.1	0
	T.E./100	6.8	3.3	0.25	0
m/10	H ₂ O	0	0	0	0
	T.E./10,000	0.05	0	0	0
	T.E./1,000	1.16	0.45	0.01	0
	T.E./100	5.0	2.6	0.25	0
m	H ₂ O	0	0	0	0
	T.E./10,000	0	0	0	0
	T.E./1,000	0	0	0	0
	T.E./100	0.52	0.5	0	0
	T.E./10	3.35	2.50	0.15	0

We see from the above tables that, within the limits of nutrient, density of spore suspension, and concentration of carbon dioxide employed, the following factors act in retardation of germination: (1) dilution of nutrient, (2) increase in density of spore suspension, (3) increased concentration of carbon dioxide. The effect of these various factors is more strongly shown in Table II than in Table I. Thus, to take a particular instance:

With m/100 spore suspension and in T.E./100 nutrient, the effect of 20 per cent. CO₂ as compared with air is to reduce the percentage of germination from about 100 to 25, i.e. to one-fourth; whereas the corresponding effect on the average germ-tube length is from 6.8 to 0.25, i.e. to one-twentyseventh. This is, of course, due to the fact that the smaller percentage of spores which have germinated in 20 per cent. CO₂ have on the average much shorter germ-tubes than have the corresponding ones in air.

On comparing the suspensions of spores in water with those in the various turnip extracts, it is seen that the relative retarding effect of a given concentration of carbon dioxide is greater in the former case. The magnitude of this differential effect depends on the time at which the measurements are made and tends to become less as time goes on. Nevertheless, that the effect persists even after long time will be seen later, when it is shown that inhibition of germination of *Botrytis* spores is produced by 30 per cent. CO₂ when the spores are sown in water, whereas a concentration of somewhat over 50 per cent. is required to stop germination of the spores when they are sown in nutrient.

The retarding effect of density of spore suspension on germination is theoretically interesting, though in regard to infection by the spores it is

probably of no practical importance, seeing that such a density of suspension as that represented by m is very much greater than any that would ever be met with in practice. For densities such as $m/100$ and less, this mass effect arising from the spores becomes less pronounced, and for the dilute suspensions which are typically used for germination and infection studies its effect is more or less negligible.

In the foregoing experiments the concentration of *oxygen* was not kept absolutely constant in the different cases. Taking ordinary air as containing 20 per cent. oxygen, the concentration of the latter in the container set up with 10 per cent. CO_2 would be approximately 18 per cent. ($20 \text{ per cent.} \times 90 \div 100$), and the corresponding oxygen concentrations for the experiments with 20 per cent. and 30 per cent. CO_2 would be 16 per cent. and 14 per cent. respectively. The following tables show that such variations of oxygen concentration are of negligible importance, and, more generally, that the germination of *Botrytis* spores is independent of oxygen concentration over a wide range.

The various oxygen concentrations were made up by suitable admixture of atmospheric air and of oxygen or nitrogen from cylinders. The method of procedure was that employed for the carbon dioxide atmospheres. The symbols used in the following table are the same as in the preceding tables.

TABLE III.

*Effect of Oxygen on Germination of Botrytis Spores.*A. *Effect of Increased Oxygen.*

% Oxygen.	% Germination of spores in water.		
	$m/100.$	$m/10.$	$m.$
21	75	59	30
30	65	47	30
50	45	23	0
80	45	25	0

B. *Effect of Decreased Oxygen.*

% Oxygen.	Spores in water.			Spores in T.E.* / 10.		
	$m/100.$	$m/10.$	$m.$	$m/100.$	$m/10.$	$m.$
21	15	3	0	83	79	20
5	20	4	0	84	70	20
1	0	0	0	20	—	5

It will be observed that the amount of germination in a given time diminishes with increasing concentration of oxygen, but only very slowly, so that even in water quite a good germination results in 80 per cent. oxygen. Again, as the oxygen pressure is diminished, no appreciable effect is shown until very low concentrations of oxygen (*c.* 1 per cent.) are reached. These results were in accordance with anticipation based on the statements of previous workers already cited, and they showed that, as far as the present investigation was concerned, the effects of changes of oxygen pressure on spore germination could be ignored. In subsequent experiments dealing with the rate of growth of fungal colonies this point was

again examined, as it was thought conceivable that a diminution of oxygen pressure which would not affect the germination of spores might affect the rate of growth of a large fungal colony. Experiments were carried out to compare the growth of *Sphaeropsis* colonies in containers in air at atmospheric pressure with that of similar colonies maintained in air at 40 per cent. of the normal pressure. No definite difference could be established between the former growing at 20 per cent. oxygen pressure and the latter at 8 per cent. It was therefore concluded that, for all the purposes of the present investigation, oxygen pressure was a factor of no importance within the limits laid down by the practical aspects of the problem. As regards the effect of the composition of the atmosphere on the germination and growth of fungi, attention was therefore directed solely to the carbon dioxide constituent.

The general nature of the retarding action of carbon dioxide on germination having been studied as above described, experiments were now set up to determine what concentrations of carbon dioxide were necessary to inhibit the germination of fungal spores under various conditions of nutrition. Parallel series of germination tests were set up, in the one case in water and in the other in a good nutrient. For the latter a turnip extract of one-fifth full strength was used. These experiments were carried out at laboratory temperature.

The general type of result will be seen from the following table. The various preparations were set up in the usual way in moist containers. The table describes the progress which has been made in germination after seven days from the start of the experiment.

TABLE IV.

Spores sown in T.E./5.

<i>Fungus.</i>	20 % CO ₂	30 %.	40 %.	50 %.	60 %.
<i>Botrytis parasitica</i>	weft	weft	strong germination	c. 50 % germination	Very occasional papilla
<i>B. cinerea</i> . . .	"	"	good germination	none	none
<i>Monilia cinerea</i> .	"	"	"	rare germination	none
<i>Mucor</i> sp. . . .	long germ- tubes	short germ- tubes	none	none	none
<i>Rhizopus nigricans</i>	weft	weft	weft	good germination	rare germination

Penicillium glaucum and *Fusarium* sp. were the only two fungi which were found to germinate strongly in 60 per cent. CO₂.

It must be observed that the time of seven days allowed for germination is purely arbitrary, and that though in some cases germination had not taken place in this time, it does not follow that some germination would not eventually have occurred.

In all cases it was observed that the carbon dioxide treatment did not appear to harm the fungi in any way, for they germinated normally and

with more or less their usual vigour when again brought into normal atmospheric conditions. Thus the question whether a given concentration of carbon dioxide produced absolute inhibition could only be determined by experiments lasting a very long time. The incubation time for germination in a good nutrient in the neighbourhood of 18° varies for the different fungi, being as low as 4–6 hours for normal *Botrytis* spores and as much as about one day for spores of *Aspergillus repens*. For most of the fungi used the time required for germination in air at ordinary laboratory temperature is about eight hours, and thus the period of seven days chosen in the above experiment is about 20 times the normal incubation period for germination. A fungus, therefore, which fails to germinate under the conditions of the experiment in a period 20 times its normal germination period may be looked upon as completely inhibited to all intents and purposes.

In carrying out these observations, an interesting behaviour was noticed in the case of germ-tubes of *Rhizopus nigricans*. In 50 per cent. CO_2 spores of this fungus showed universal germination, but the germ-tubes had a peculiar appearance. Instead of the normal long straight cylindrical hyphae of uniform diameter the germ-tubes here appeared as short stunted structures of very irregular shape. In optical section they presented a very ragged outline, and their diameter, though varying widely in different parts, was much in excess of the normal. The same appearance was observed in the germ-tubes in 60 per cent. CO_2 , but not in those in 40 per cent. These germ-tubes were strongly reminiscent of the cell swellings in species of Mucorineae described by various workers (Ritter, Wehmer)¹ as 'giant cells' (*Riesenzellen*). The latter have been shown to be due to the accumulation of excess of acid in the culture solution, and it appears probable that the formative factor in the present case is acidity due to the high pressure of carbon dioxide. On removal of these distorted germ-tubes to ordinary air, they at once continued their growth in the normal fashion.

The following table gives the concentrations of carbon dioxide which were found to stop germination at $15\text{--}18^{\circ}$ over a period of seven days:

TABLE V.

Fungus.	Spores sown in T.E./5.	Spores sown in water.
<i>Aspergillus repens</i>	40	—
<i>Mucor</i> sp.	40	—
<i>Botrytis cinerea</i> (both strains)	50 +	20–30
<i>Monilia cinerea</i>	50 +	20
<i>Phoma roseola</i>	50 +	20
<i>Alternaria grossulariae</i> . . .	50–60	>30
<i>Rhizopus nigricans</i>	60	20
<i>Botrytis parasitica</i>	60 +	20
<i>Sphaeropsis malorum</i>	60–70	30
<i>Fusarium</i> sp.	80 +	30
<i>Penicillium glaucum</i>	80–95	>30

¹ See on this point Ritter: Jahrb. f. wiss. Bot., lii. 351–403, 1913. Wehmer: Ber. deut. bot. Gesell., xxxi. 257–68, 1913. Lopriore: l. c.

Of all the fungi tested, *Penicillium glaucum* is the most insensitive to carbon dioxide. When we remember that, in the case of an atmosphere which gave 95 per cent. CO_2 on analysis, the bulk of the remaining 5 per cent. is nitrogen, it follows that limited supply of oxygen was now probably entering in as a factor. It is fairly doubtful, therefore, if *Penicillium glaucum* can be inhibited entirely by carbon dioxide alone—that is, within the limits of one atmosphere pressure. This again illustrates the very marked resistance of this organism to external conditions.

The effect of nutrient in relation to the inhibition of germination by carbon dioxide is again shown in the above table.

Speaking generally, we may say that the concentration of carbon dioxide which inhibits germination of fungal spores at ordinary temperatures lies in the neighbourhood of 20–30 per cent. when the spores are sown in water, and in the neighbourhood of 50–60 per cent. when the spores are sown in a good nutrient. These concentrations are not admissible in the practice of the gas storage method, and therefore inhibition of germination of the fungal spores cannot be realized under practical conditions. It remains to examine the degree of retardation which can be effected by concentrations which do not exceed 20 per cent., and especially to determine what effect as regards retardation can be brought about by the combined action of moderate concentrations of carbon dioxide and of low temperature.

The remaining part of the experimental work may be divided into three sections, dealing with the combined action of carbon dioxide and low temperature upon (a) germination of fungal spores, (b) rate of growth of fungal colonies on artificial media, (c) rate of growth of specific fungi on certain fruits.

A. Rate of Germination of Fungal Spores at different Temperatures and in different Concentrations of Carbon Dioxide.

Experiments were carried out with spores of *Botrytis cinerea*, *B. parasitica*, *Penicillium glaucum*, and *Fusarium* sp. Sowings of spores were made on the following media:¹ potato agar, apple gelatine, prune gelatine, and Richard's solution with agar. These media were liquefied by heat and just before solidification were mixed with a few drops of a dense spore suspension, and then drops of the medium spread out on glass slides. The latter were then set up in moist containers and germination carried out in air and in air containing 10 per cent. and 20 per cent. CO_2 . One series was run at 15° and another at 5°. At intervals the average length of the germ-tubes was determined by micrometer counts. The carbon dioxide atmospheres

¹ The nutrient media were prepared in the usual way. Agar media had 1.5 % agar; gelatine media had 15 % gelatine. In the case of Richard's solution with agar, the nutrient and the agar require to be heated separately and mixed when nearly cool. The various plant decoctions were prepared according to the conventional standards suggested by Duggar (Fungous Diseases of Plants, p. 24, 1909).

were analysed at each measurement, and in no case was there found a deviation of more than 0.5 per cent. from the concentration required.

The incubation period and the rate of germination were found to vary to some extent with the different media employed, but the results obtained were of the same type for each medium and for each fungus. The following table gives the figures obtained for *Penicillium* on the two media, potato agar and apple gelatine:

TABLE VI.

Average Length of Germ-tube (in micrometer divisions).

	At 15°.	12 hrs.	16 hrs.	20 hrs.	23 hrs.	36 hrs.
Potato Agar.	{ Air	0.0	0.36	2.40	4.72	weft
	{ 10 % CO ₂	0.0	0.0	0.32	1.12	12.2
	{ 20 % CO ₂	0.0	0.0	0.0	+	4.84
Apple Gelatine	{ Air	0.0	0.70	2.44	5.12	weft
	{ 10 % CO ₂	0.0	+	0.44	1.10	12.0
	{ 20 % CO ₂	0.0	0.0	+	0.26	5.68

	At 5°.	1 day.	2 days.	3 days.	4 days.	5 days.	6 days.	7 days.	8 days.	9 days.
Potato Agar	{ Air	0.0	0.0	0.62	2.96	7.44	—	—	—	—
	{ 10 % CO ₂	0.0	0.0	0.0	0.0	0.82	2.96	8.84	—	—
	{ 20 % CO ₂	0.0	0.0	0.0	0.0	0.0	0.0	0.22	1.46	3.84
Apple Gelatine	{ Air	0.0	0.0	0.72	2.52	7.52	—	—	—	—
	{ 10 % CO ₂	0.0	0.0	0.0	0.0	0.12	—	—	—	—
	{ 20 % CO ₂	0.0	0.0	0.0	0.0	0.0	0.0	0.58	2.14	4.68

The above figures illustrate clearly a feature of the germination of fungal spores—that from the moment germination has begun, growth proceeds at a constantly increasing rate (at any rate for some time). Thus if we plot the average germ-tube length as ordinate against the time as abscissa, the curve of growth is convex to the x-axis.

The general quantitative effect of the two factors, low temperature and concentration of carbon dioxide, separately and combined, is perhaps better seen by comparing the time necessary for the average germ-tube to reach a given length in the different cases. Choosing a length = 5, and dealing with the germination tests on potato agar in the preceding table, we arrive at the following results:

Average germ-tube = 5 is reached in:

At 15° in Air	c. 24 hrs.
„ 10 % CO ₂	24–36 hrs.
„ 20 % CO ₂	36 hrs.
At 5° in Air	c. 4½ days
„ 10 % CO ₂	c. 6½ days
„ 20 % CO ₂	9 (+) days

These figures illustrate the magnitude of the effect produced. Even in the case of this very resistant fungus and in abundant nutrient, the effect of a 10° drop from ordinary temperature, combined with 10–20 per cent. CO₂, slows down the rate of growth in the early phases 6–9 times.

This method of measurement was, however, somewhat laborious and ill suited to the needs of the problem. Apart from the practical difficulty of carrying out the measurements sufficiently rapidly so as not to interfere too seriously with the various conditions of the experiment, there is the fundamental objection that the method of counting is severely restricted to the early phases of germination. When the average germ-tube reached a length of about ten divisions on the scale used, the method was no longer available except at the expense of excessive labour. It was therefore discarded in favour of the following one, which was much simpler to carry out and which at the same time gave results of much wider applicability and probably of more interest from the practical point of view.

B. *Growth of Fungal Colonies at different Temperatures and in different Concentrations of Carbon Dioxide.*

In these experiments plates of sterile medium, solidified with agar or gelatine, were 'poured' and inoculated at the centre with the fungus to be examined. In the case of *Sphaeropsis malorum*, the inocula consisted of mycelium only, as this fungus was found to sporulate very sparingly. For all the other fungi spore inoculations were in general used. It may be remarked here that, apart from a certain small difference in the amount of growth during the early stages, spore inocula and mycelial inocula gave rise to colonies of very similar appearance and rate of growth. The diameter of the colonies was measured directly by means of a small metric scale, and each measurement was made to the nearest half-millimetre.

As this method is extensively used in a paper dealing with the growth of fungal colonies under various conditions which will shortly appear, a detailed criticism of it will not be attempted here, but will be reserved till that occasion. The procedure is as follows: The plates, which contain a somewhat deep layer of medium (on the average, 1 cm.), are inoculated at the centre, care being taken to make the original inoculations as local as possible. They are then placed, face downwards and with lids off, in moist containers, four being placed in each of the latter. The Petri dishes are piled in column and separated from each other by means of wooden rods in such a way as to allow free access of the air of the container to the surface of each. For each temperature examined a comparison was made of the rate of growth in air, in air containing 10 per cent. CO₂, and in air containing 20 per cent. CO₂. The contents of each container were analysed for its carbon dioxide content at the time of measurement, this being especially necessary in the present series of experiments as the CO₂ content of each vessel tended to increase on account of the respiration of the fungal colonies within. This effect was negligible in the initial stages of each experiment, but became more pronounced as time went on, especially at the higher temperatures. It was desirable not to make the measurements

of growth too frequently, as each measurement involved a certain amount of disturbance of the experimental conditions intended. On the other hand, if the interval between successive measurements was too long, the accumulation of carbon dioxide in the containers tended to upset the experimental conditions aimed at. A compromise had therefore to be effected. From numerous analyses a fairly good idea of the rate at which carbon dioxide was formed in the containers was obtained, and from that it was possible to arrange to make each set of measurements at a time when the CO_2 content of each vessel did not exceed that originally intended by more than a certain percentage; in practice a limit of 2 per cent. was imposed. In the early stages of each experiment the deviation from the theoretical value at each time of measurement was much less than this limit, but towards the end of the experiment this limiting deviation was frequently reached. Thus in referring to growth in air, in 10 per cent. and in 20 per cent. CO_2 , what is really meant is growth in atmospheres containing 0-2 per cent., 10-12 per cent., 20-22 per cent. CO_2 respectively. The effect on growth produced by 2 per cent. CO_2 is small, and in any case what effect there is would only tend to make the results a little less pronounced than otherwise, seeing that the increase in CO_2 content due to respiration was always somewhat more rapid the lower the original CO_2 concentration in the container.

All the fungi listed earlier in the paper were examined and the usual variety of media—potato agar, potato-, apple-, plum-, prune-gelatine, &c.—were employed. A series of experiments was run comparing the rates of growth in air, 10 per cent. and 20 per cent. CO_2 at 15° , 20° , and 25° ; a further series at 10° , 15° , and 20° ; and finally a more thorough comparison made of the rates of growth in the various atmospheres at 5° and 15° . A comparison was also made of the rate of growth of *Botrytis cinerea*, *Monilia cinerea*, *Alternaria grossulariae*, and *Fusarium* sp. in the various atmospheres at ordinary laboratory temperature (15 – 18°) with that at the ordinary cold storage temperature of 3° .

The general nature of the result will be seen by a study of Figs. 1 and 2, which represent the growth in diameter of colonies of *Botrytis*

FIG. 1. Growth of *Botrytis cinerea* on apple gelatine at 15° .

cinerea at 15° and 5° on apple gelatine in air, 10 per cent. and 20 per cent. CO_2 . All the cultures compared were started on the same day on the same medium, as was the invariable practice throughout this work. As the curves of growth at the two temperatures are drawn to different scale, the relative amount of retardation produced by the CO_2 treatment is not obvious at a glance, but an idea of its magnitude may be obtained by comparing the amounts of growth in the CO_2 cultures at times when the air cultures in the two cases have reached the same size. Thus if we read

FIG. 2. Growth of *Botrytis cinerea* on apple gelatine at 5° .

off from the graphs the amount of growth in 10 per cent. and 20 per cent. CO_2 corresponding to a diameter of 8 cm. in the air cultures we get the following figures:

	Air.	10% CO_2 .	20% CO_2 .
At 15°	8.0 (100)	5.5 (69)	3.0 (37.5)
At 5°	8.0 (100)	4.3 (54)	1.1 (14)

The figures in brackets represent the relative amount of growth, that in air being taken as 100 in each case.

It will be observed that at the times when the air cultures at the two temperatures have reached the same diameter the corresponding amount of growth in the CO_2 atmospheres is considerably less at 5° than at 15° . This result may be expressed generally in the following terms: *The relative retarding effect of a given concentration of carbon dioxide is greater at a low than at a higher temperature.*

Strictly speaking, a correction should be made in the above curves for the diameter of the original inoculum, which in the case of *Botrytis* was

about 0.2 cm. The effect of making this small correction is to increase the relative retarding effect (as measured by dividing the growth in CO_2 by that in air), and more particularly is this the case for growth at the lower temperature.

FIG. 3. Growth of *Botrytis cinerea* on potato agar at laboratory temperature (L) and in refrigerator (R).

FIG. 4. Growth of *Fusarium* sp. on potato agar at laboratory temperature (L) and in refrigerator (R).

The effect under consideration becomes still more pronounced on comparing growth at 15° with that at temperatures lower than 5° , e.g. at 3° as in Figs. 3 and 4, which refer to experiments with *Botrytis cinerea* and *Fusarium* sp. on potato agar at laboratory temperature (15 – 18°)

and in a refrigerator maintained at 3° . On examining these curves along the lines indicated above we have the following comparison:

For *Botrytis*:

	Air.	10% CO ₂ .	20% CO ₂ .
At 15°	3.4 (100)	2.0 (59)	1.0 (30)
At 3°	3.4 (100)	0.7 (21)	0.0 (0)

For *Fusarium*:

	Air.	10% CO ₂ .	20% CO ₂ .
At 15°	2.0 (100)	1.8 (90)	1.15 (57.5)
At 3°	2.0 (100)	0.82 (41)	0.36 (18)

Here in fact it is seen that in the case of *Botrytis* the low temperature combined with the depressing effect of 20 per cent. CO₂ is effective in almost stopping growth over a period of 56 days.

Similar curves were obtained for the growth of *Alternaria* and *Monilia* under the same set of conditions.

Considerable attention has been paid throughout this work to the study of the ratio

(growth in a given percentage of CO₂) : (growth in air).

The results arrived at may be stated as follows:

I. In the case of any particular fungus and for a given temperature this ratio tends to increase as time goes on; that is, the amount of retardation is greatest in the earliest phases of growth. For some fungi the ratio tends to reach a steady value, as is shown in the following table, which deals with the growth of *Alternaria grossulariae*.

TABLE VII.

Alternaria grossulariae. Growth on Potato Agar.

At 20° .	2 days.	3 days.	5 days.	7 days.	8 days.
Air	1.21 (100)	2.10 (100)	3.93 (100)	6.05 (100)	6.85 (100)
10% CO ₂	1.00 (83)	1.83 (88)	3.65 (93)	5.18 (86)	6.15 (90)
20% CO ₂	0.70 (58)	1.38 (66)	2.58 (66)	3.78 (62)	4.42 (64)
At 5° .	3 days.	5 days.	7 days.	10 days.	16 days.
Air	0.31 (100)	1.10 (100)	1.87 (100)	3.14 (100)	5.20 (100)
10% CO ₂	0.09 (29)	0.48 (44)	0.96 (51)	1.63 (52)	3.10 (58)
20% CO ₂	0.0 (0)	0.10 (9)	0.34 (18)	0.73 (23)	1.45 (27)

At both temperatures there is a tendency for the ratio of growth in CO₂ to that in air to increase. That the ratio is smallest at the beginning is well shown in the experiment at 5° , and is obviously related to the fact that in the early stages there is a period during which the air cultures show fairly considerable growth while the CO₂ ones have not as yet germinated. In such a case the ratio of growth in CO₂ to that in air is zero, but it begins to increase the moment the CO₂ cultures spread beyond the limits of the original inoculum. The same effect takes place at the higher temperature, but in the present experiment the first measurement was made too late to show the rapid rise of the ratio in the early stages. At both temperatures the ratio tends to reach a steady value. This has been

reached at the higher temperature by about the fifth day; at the lower temperature it has not been quite reached by the sixteenth day, though inspection of the table shows that the daily increase in the ratio is getting less and less. At the higher temperature the ratios of growth in 20 per cent. CO₂: growth in 10 per cent. CO₂: growth in air, tend to reach the limiting values 65: 85-90: 100, whereas the corresponding ratios for growth at 5° are in the neighbourhood of 30: 60: 100.

These tables again illustrate the effect of a lowering of temperature on the retardation of growth produced by a given concentration of carbon dioxide.

In the case of some fungi, and especially when they are sown on certain media, the ratio of growth in CO₂ to that in air goes on increasing with time without showing any tendency to reach a limiting value, at least within the limits of size to which the colonies have been studied. In such cases as these the growth of colonies in the CO₂ concentrations finally exceeds that in ordinary air, especially at higher temperatures. The following table dealing with the growth of *Fusarium* will illustrate this point:

TABLE VIII.

Fusarium. Growth on Potato Agar.

At 15°.	3 days.	5 days.	7 days.	8 days.
Air	1.23 (100)	2.23 (100)	2.98 (100)	3.3 (100)
10% CO ₂	1.1 (85)	2.4 (108)	3.5 (118)	4.04 (121)
20% CO ₂	0.95 (73)	2.1 (94)	3.3 (111)	3.8 (115)
At 5°.	7 days.	14 days.	21 days.	35 days.
Air	0.4 (100)	1.6 (100)	2.2 (100)	3.85 (100)
10% CO ₂	0.1 (25)	0.8 (50)	1.3 (59)	2.5 (65)
20% CO ₂	0.0 (0)	0.05 (3)	0.18 (8)	0.7 (18)

The present case brings out best of all the effect of temperature on the retarding action of carbon dioxide. Corresponding to a colony diameter of 3.85 at the lower temperature, there is very considerable retardation in the CO₂ atmospheres, whereas at the higher temperature the colonies in 10 per cent. and 20 per cent. CO₂ are distinctly ahead of the colonies in air for some time before the latter reach that value. It is not proposed to discuss this effect here, as it will be dealt with more fully in a later paper, but it may be stated that it is related to the marked 'staling' which takes place in the air cultures. The main feature of 'staling' in the present case is the development of alkalinity by the fungus, and this is partly counteracted by the action of carbon dioxide. Hence while the air cultures at 15° become progressively slower in growth, the CO₂ cultures keep up a steady rate of growth for a longer time, with the result that the initial retardation produced by carbon dioxide is sooner or later converted into an acceleration.

As will be shown more fully in a subsequent paper, the particular

effect just described is more markedly shown by cultures on potato agar, though it is also seen on other media. Also it is met with in the case of other fungi which show marked staling on potato agar. Such fungi are, in addition to *Fusarium*, *Sphaeropsis malorum* and more markedly *Colletotrichum Lindemuthianum*. The effect is not seen with fungi such as *Alternaria grossulariae*, *Botrytis cinerea*, &c., which show no such marked development of alkaline reaction on potato medium.

2. On comparing different fungi with each other in respect of the amount of retardation produced by a given concentration of carbon dioxide, it was seen that some are more susceptible than others. One could thus arrange the various fungi in a series of diminishing susceptibility, and this series was roughly the same as that given on p. 283, which is based on the concentration of carbon dioxide necessary to inhibit spore germination. In constructing such a table on this principle, one is limited to a consideration of the early phases of growth, as the staling effect by and by comes in as an important disturbing factor. However, it is quite possible to show that at ordinary temperatures a given concentration of carbon dioxide exerts a greater retarding effect on *Botrytis cinerea* than on *Sphaeropsis malorum*, at all stages of growth on all the media tested. When, on the other hand, the experiment is repeated at 5° the reverse is found to be the case. Whereas *Botrytis* colonies grow at 5° in 20 per cent. CO₂, *Sphaeropsis* colonies under these conditions are practically inhibited. The explanation of this is probably to be found in the different temperature minima of the two fungi. The minimum for *Botrytis* is very near 0°, and that for *Sphaeropsis* was shown to lie between 3° and 5°. The greater effectiveness of 20 per cent. CO₂ in the case of the latter fungus is probably correlated with the fact that the experimental temperature is approaching very close to the minimum for the fungus. It is reasonable to suppose that within a few degrees of the minimum temperature a moderate concentration of carbon dioxide is effective in producing not merely retardation but total inhibition of growth. Thus the region of total inhibition by 20 per cent. CO₂ is already reached at 5° in the case of *Sphaeropsis*, whereas a lower temperature has to be sought in the case of *Botrytis*.

3. The amount of retardation of growth at a low temperature produced by a given concentration of carbon dioxide is different according as the colonies have been grown throughout at the low temperature or were started at a higher temperature. The quantitative effect produced here appears to depend considerably on the particular medium used. The following table refers to *Botrytis cinerea* inoculations on apple gelatine :

(a) *B. cinerea* grown throughout at 5°.

Diameter of growth after 23 days :

Air.	10% CO ₂ .	20% CO ₂ .
7.6 (100)	3.35 (44)	0.85 (11)

(b) Cultures grown for two days in air at 20°, then transferred to 5°, some in air, some in 10 per cent. and 20 per cent. CO₂.

Diameter of growth in 14 days after transference:

Air.	10 % CO ₂ .	20 % CO ₂ .
6.75 (100)	5.2 (77)	2.65 (39)

In the latter case the retarding action of carbon dioxide is less than in the former, and this is obviously due to the fact that in the latter case, by germinating the spores in air at a high temperature, they were taken past the stage at which the retarding effect of carbon dioxide on them is greatest.

A similar behaviour to that of *Botrytis* was shown by *Monilia* and *Fusarium* on apple gelatine.

With potato agar, very diverse results were obtained with different fungi. *Botrytis cinerea* behaved in the same way on this medium as on apple gelatine. On the other hand, *Penicillium* and *Sphaeropsis*, after being grown for two days at 20° and then transferred to 5°, had their growth in air stopped entirely for a long time at the latter temperature, whereas they continued to grow slowly in 10 per cent. CO₂. This complicated behaviour is again without doubt associated with the fact that potato agar is a medium which lends itself with particular readiness to staling by development of alkalinity.

C. Rate of Growth of certain Fungi on Fruit at different Temperatures and in different Concentrations of Carbon Dioxide.

Experiments were carried out on the rate of attack of apple by *Botrytis cinerea* and *Monilia cinerea*, and of orange by *Penicillium glaucum*. The fungi were introduced through artificial punctures in the skin of the fruit. Parallel series were run at 15° and 5°. Throughout the experiment it was necessary to open the containers every second day at the higher temperature and every four days at the lower temperature, and the atmospheric composition was made up afresh, this by reason of the respiration of the fruit. At the end of the experiment the amount of rotting in the case of apple was determined by weighing before and after removal of the rotted portion, and in the case of orange by counting the percentage of loculi affected by the fungus. The latter method did not appear to be very satisfactory. The following table gives the percentage of rotted tissue:

TABLE IX.

	Air.	10 % CO ₂ .	20 % CO ₂ .
At 15°. After 10 days.			
<i>Botrytis cinerea</i> . .	14.6	1.8	0.4
<i>Monilia cinerea</i> . .	63.5	23.3	13.7
<i>Penicillium glaucum</i>	53	52	45
At 5°. After 28 days.			
<i>B. cinerea</i>	13.3	1.0	0.05
<i>M. cinerea</i>	45.9	11.4	1.0
<i>P. glaucum</i> (35 days)	50	—	26

A comparison of the figures obtained at the two temperatures of the experiment again shows that the relative retarding effect of a given concentration of carbon dioxide is accentuated by lowering the temperature. The comparatively high degree of retardation produced by carbon dioxide on the rate of *Botrytis* attack as compared with that of *Monilia* would seem to be correlated with the less vigorous parasitic action of the former on apple.

DISCUSSION.

As the main part of the foregoing experimental work deals with the rate of increase in *diameter* of fungal colonies, a brief discussion of the method of measurement adopted is necessary here, though a fuller criticism is reserved for a future occasion.

For the present investigation (as for many others of a physiological nature) the really important quantity to be determined in connexion with fungal growth is the amount of work done, i.e. the amount of chemical transformation carried out. This quantity, which would be very difficult to determine directly, is in all probability closely related to the amount of growth made by the fungus. There is thus a rough proportionality between the amount of chemical transformation effected by the fungus and the amount of fungal mycelium. The question now arises as to the relation between the amount of mycelium in a fungal colony and the diameter of the latter. As between different media, or even different concentrations of the same medium, there is no relation between the amount of mycelial growth and the diameter. On some media a given fungus may produce a dense web with slowly advancing margin, and on others a thin colony with rapidly increasing diameter. In such a case measurement of the diameter is of no value in determining the amount of growth made by the fungus in the different cases.

When, however, comparisons are made between growths under different conditions on the same medium, definite conclusions as to the amount of mycelium can be drawn from a measurement of the diameter of the colony. With any particular medium, the type of growth produced by the same fungus is very similar under the different conditions of experiment, whether in air or in concentrations of carbon dioxide, or whether at laboratory temperature or in cold store. With fungal colonies growing on the same medium, the diameter of the colony is a measure of the amount of growth in the sense that a greater diameter represents a greater amount of mycelium than does a smaller diameter, and conversely.

The exact relation between diameter of colony and amount of mycelium is not certain, and almost certainly varies with the age of the colony. If the colony grew spherically on the medium (or on the fruit) the amount of mycelium would be more or less proportional to the cube of the diameter, but, as is noted by Brooks and Cooley (4), the colony always spreads more

rapidly tangentially than in a direction normal to the surface, and therefore the amount of mycelium will be proportional to the diameter raised to a power intermediate between two and three. Thus it will be seen that if the foregoing results are expressed in terms of 'amount of mycelium in the colony', the effects become more marked in each case, even if we only assume that the amount of mycelium is proportional to the square of the diameter.

The main conclusion arising from the experimental work detailed in this paper is that the retarding effect of a given concentration of carbon dioxide on germination and growth is greater at a low than at a high temperature. The question arises as to what causes this differential effect can be assigned. A similar effect was obtained by Kidd for the action of carbon dioxide on the germination of seeds, and the nature of the action briefly discussed. As is pointed out by Kidd, the important factor is probably the tension of carbon dioxide within the living protoplasm, and this is controlled to a large extent by the pressure of carbon dioxide in the surrounding atmosphere. For the case of a fungus growing within the tissue of a fruit such as an apple, the factors which influence the carbon dioxide content of the fungal hyphae (respiration of the fruit, respiration of the fungus, rate of gaseous diffusion, &c.) are so various and complicated that any discussion of them would be useless. However, the differential retarding effect under consideration was shown not only for the rate of attack of fruit, but also for the rate of growth of fungus colonies on artificial media, in which case the phenomenon is obviously simpler, and to which case the discussion may be confined.

The concentration of carbon dioxide within a fungal hypha growing in a given atmosphere of carbon dioxide depends upon:

1. The solubility of carbon dioxide in water.
2. The rate of respiration within the hypha and the rate of gaseous diffusion across the hyphal membrane.

The solubility of carbon dioxide in water at 5° is to that at 15° in the ratio 1.4 : 1 (according to Landolt-Börnstein). If the concentrations of carbon dioxide in the growing hyphae at the two temperatures stand to each other in this ratio, then it is difficult to see that the retarding effects obtained can be completely explained on this ground. On reference to the tables given in the present paper it will be seen that the retarding effect of a given concentration of carbon dioxide at 5° is in many cases very much greater than that shown at 15° . Furthermore, the relative retardation shown varies from fungus to fungus on the same medium, and is strongly influenced by the amount of nutrient present and by the proximity of the temperature under consideration to the minimum temperature for the particular fungus. It is difficult, therefore, to see how these various effects can all be explained on the grounds of this one factor.

The effect of respiration would be to make the concentration of carbon dioxide within the hypha somewhat greater than that in the nutrient medium immediately surrounding the hypha. The actual difference in concentration would depend on the rate of production of carbon dioxide within the hypha and its rate of diffusion through the hyphal membrane, both of which are accelerated by increasing temperature. According to Kidd, one is probably not far wrong in assuming that the variations of the two factors with temperature neutralize each other, so that the carbon dioxide content of the growing hypha would be approximately the same at different temperatures. It does not appear, therefore, that the differential retarding effects described in this paper can be explained on the basis merely of differential carbon dioxide content of the hyphae under the different experimental conditions.

Summarizing the above argument, we may say that the greater retarding effect of a given concentration of carbon dioxide at a low than at a high temperature can be partly explained on the basis of the greater solubility of carbon dioxide in water at the low temperature, but over and above that other physiological factors come into play, the precise nature of which is at present unknown.

Whatever be the physiological explanation of the various results obtained, it is possible to put forward an hypothesis that brings all the experimental results into a coherent form. The more important factors influencing growth are as follows:

1. *Nutrient.* The rate of growth increases with the concentration of the nutrient up to certain limits.
2. *Temperature.* The rate of growth increases with rise of temperature up to a certain limit, and as growth is nil at a minimum temperature, the rate of growth is obviously some direct function of $(t-t_0)$, where t is the particular temperature and t_0 is the minimum temperature.
3. *The amount of growth already made.* The relation of growth to this factor is complex, but up to a certain point it is such that the rate of growth increases with the amount of growth already made.
4. *Density of spore suspension of the fungus.* This factor becomes prominent in the case of spore sowings in weak nutrient and acts in such a way that growth diminishes with increasing density of spore suspension.
5. *The percentage of carbon dioxide in the atmosphere.* As far as can be seen this is always a retarding factor in the case of fungal growth, except in cases where alkaline 'staling' takes place and accelerated growth is the result.

Considering the first four factors which constitute the environmental conditions of the fungus from the point of view of the carbon dioxide treatment, we may say that the energy of growth is proportional (in some ratio) to the concentration of nutrient, to the temperature, to the amount

of growth already made, and to the diluteness of spore suspension (all within limits). All the experimental results obtained will then fall under the following general rule, viz. *that the carbon dioxide retarding factor has greatest effect when the energy of growth is small*. This general statement will cover all the experimental results obtained for the retarding effect of carbon dioxide on growth at different temperatures, in different concentrations of nutrient, and at different stages of growth. It will also include certain results obtained by comparing the retarding effect of carbon dioxide on the growth of some fungi on living fruit with that on artificial medium. On referring to Table IX, it will be seen that at 15° a given concentration of carbon dioxide more effectively retards the parasitic attack of *Botrytis cinerea* on apple than is the case with attack by *Monilia cinerea*. On artificial media both fungi were seen to be about equally sensitive to the action of the fungus. Now *Botrytis* is a weaker parasite on apple than is *Monilia*, and though a large number of factors may play a part in producing this result, one can state generally that *living* apple tissue is a poorer nutrient for *Botrytis* than for *Monilia*. Thus the figures shown in Table IX can be brought into line with the general rule that the retarding effect of carbon dioxide is greater the poorer the nutritive value of the substrate for the particular fungus. Some of the results of Brooks and Cooley already cited will also be seen to be interpretable along the lines of the general rule here put forward.

The following practical considerations which arise from the foregoing work may now be briefly described:

1. Within the limits of carbon dioxide concentration admissible in practice, carbon dioxide is not so important a factor in reducing the amount of fungal growth as is temperature. At ordinary temperatures a concentration of carbon dioxide as great as 20 per cent. is not so effective in controlling fungal growth as is a drop of 10° without carbon dioxide.

2. The efficiency of the carbon dioxide treatment depends on the amount of nutrient available for the fungus. A concentration of carbon dioxide of 10–20 per cent. would give a good effect on spores devoid of nutrient and a much less effect on spores which had free access to nutrient, and less still when attack had already started. The nutritive conditions obtaining on the surface of stored fruit will obviously depend on a number of factors—the particular kind of fruit, the soundness of the fruit, &c. Where the fruit is injured in any way, nutrient will always be available to any fungal spores which happen to be present, but even under the best conditions of storage a certain amount of nutrient will always be available to fungal spores (at least in the case of apple), as it has been shown in unpublished work of the writer that certain volatile products of the apple have a very distinct accelerating effect on spore germination. The food available to the fungus is therefore never negligible. As regards the effect

of the carbon dioxide treatment on the germination of fungal spores on fruit in store, one would expect a result intermediate between that obtained for germination in water and germination in a good nutrient.

3. The efficiency of the carbon dioxide treatment is enhanced by lowering the temperature. Certain cases have been alluded to where a moderate concentration (10–20 per cent.) of carbon dioxide at ordinary temperature accelerates fungal growth, and though this result was only obtained for growth on certain artificial media, it is not at all certain that a similar effect might not arise if the storage of certain fruits or vegetables were attempted at ordinary temperatures in carbon dioxide. The present experiments thus indicate that, as far as concerns fungal attack in the store, the gas storage method is to be considered as an aid to the ordinary method of cold storage, and as in no way replacing the latter.

SUMMARY.

1. Within very wide limits, variation of oxygen pressure has little effect on the germination and growth of the ordinary fruit-rot organisms such as *Botrytis*, *Fusarium*, and *Alternaria*.

2. The germination and growth of these organisms is retarded by carbon dioxide. The retarding action of carbon dioxide on germination and growth is more marked the lower the temperature, the weaker the nutrient in which the fungal spores are sown, and, to a less degree, the greater the density at which the spores are sown.

3. The concentration of carbon dioxide which inhibits germination at ordinary temperature was determined for the commoner fruit-rot organisms both for germination in water and in nutrient.

4. Graphs and tables are given of the rates of growth of a number of fungi at various temperatures and in various concentrations of carbon dioxide.

5. The experimental results indicate that the gas storage method is most effectively used in combination with the ordinary cold storage method, and that it will give the best results when no attack of the fruit has begun previous to storage, and when conditions are such that a minimum of nutrient is available to fungal spores on the surface of the fruit.

LITERATURE CITED.

1. SCHNEIDER-ORELLI, O.: Versuche über die Wachstumsbedingungen und Verbreitung der Fäulnispilze des Lagerobstes. Centr. f. Bakt., II xxxii. 161–9, 1912.
2. AMES, A.: The Temperature Relations of some Fungi causing Storage Rots. Phytopath., v. 11–19, 1915.
3. EDGERTON, C. W.: Effect of Temperature on *Glomerella*. Phytopath., v. 247–59, 1915.
4. BROOKS, C., and COOLEY, J. S.: Temperature Relations of Apple-rot Fungi. J. Agr. Res., viii. 139–64, 1917.

5. KIRCHNER, O.: Über das Längenwachstum von Pflanzenorganen bei niederen Temperaturen. Beitr. z. Biol. d. Pflanzen, iii. 335-64, 1883.
6. DEMOUSSY, E.: Végétation dans les atmosphères riches en acide carbonique. Comptes Rendus, cxxxix. 883-5, 1904.
7. FISCHER, H.: Pflanzenernährung mittels Kohlensäure. Gartenflora, lxi. 298-307, 1912.
8. CHAPIN, P.: Einfluss der Kohlensäure auf das Wachstum. Flora, xci. 348-79, 1902.
9. KISSELEW, N.: Über den Einfluss des gegen die Norm erhöhten Kohlensäuregehalts auf die Entwicklung und Transpiration der Pflanzen. Bot. Centr. Beih., I. xxxii. 86-96, 1914.
10. BROWN, H. T., and ESCOMBE, F.: The Influence of varying Amounts of Carbon Dioxide in the Air on the Photosynthetic Process of Leaves and on the Mode of Growth of Plants. Proc. Roy. Soc., B. lxx. 397-413, 1902.
11. FARMER, J. B., and CHANDLER, S. E.: On the Influence of an Excess of Carbon Dioxide in the Air on the Form and Internal Structure of Plants. Proc. Roy. Soc., B. lxx. 413-23, 1902.
12. FRÄNKEL, C.: Die Einwirkung der Kohlensäure auf die Lebenstätigkeit der Mikroorganismen. Zeitschr. f. Hyg., v. 332-62, 1889.
13. FRANKLAND, P. F.: Über den Einfluss der Kohlensäure und anderer Gase auf die Entwicklungsfähigkeit der Mikroorganismen. Zeitschr. f. Hyg., vi. 13-22, 1889.
14. LOPRIORE, G.: Über die Einwirkung der Kohlensäure auf das Protoplasma der lebenden Pflanzenzelle. Jahrb. f. wiss. Bot., xxviii. 531-626, 1895.
15. PLUMMER, J. K.: Some Effects of Oxygen and Carbon Dioxide on Nitrification and Ammonification in Soils. Bull. Cornell Agr. Exp. Sta., cclxxxiv. 305-30, 1916.
16. KIDD, F.: The Controlling Influence of Carbon Dioxide in the Maturation, Dormancy, and Germination of Seeds. Parts I and II. Proc. Roy. Soc., B. lxxxvii. 408-21, 609-25, 1914. Part III, *ibid.*, B. lxxxix. 136-56, 1915.
17. PORODKO, T.: Einfluss der Sauerstoffspannung auf pflanzliche Mikroorganismen. Jahrb. f. wiss. Bot., xli. 1-64, 1904.
18. BERGHAUS, W. H.: Über die Wirkung der Kohlensäure, des Sauerstoffs und des Wasserstoffs auf Bakterien bei verschiedenen Druckhöhen. Arch. f. Hyg., lxii. 172-200, 1907.
19. CHLOPIN, G. W., and TAMMANN, G.: Einfluss hoher Drucke auf Mikroorganismen. Zeitschr. f. Hyg., xlv. 171-204, 1903.

Brown, William. 1922. "On the germination and growth of fungi at various temperatures and in various concentrations of oxygen and of carbon dioxide." *Annals of botany* 36, 257–283.

<https://doi.org/10.1093/oxfordjournals.aob.a089805>.

View This Item Online: <https://www.biodiversitylibrary.org/item/234428>

DOI: <https://doi.org/10.1093/oxfordjournals.aob.a089805>

Permalink: <https://www.biodiversitylibrary.org/partpdf/319041>

Holding Institution

Smithsonian Libraries

Sponsored by

Biodiversity Heritage Library

Copyright & Reuse

Copyright Status: Not in copyright. The BHL knows of no copyright restrictions on this item.

This document was created from content at the **Biodiversity Heritage Library**, the world's largest open access digital library for biodiversity literature and archives. Visit BHL at <https://www.biodiversitylibrary.org>.