RESPIRATION OF SWEET POTATO STORAGE-ROT FUNGI WHEN GROWN ON A NUTRIENT SOLUTION

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INTRODUCTION

Respiration in plants has been a favorite subject for botanical research for many years. Most of the earlier work was done with chlorophyll green plants, or parts of plants, some of the classical studies having been made upon seeds. The results of these researches have given us a better insight into the metalbolism of flowering plants, with some facts regarding the utilization of carbohydrates in respiration. Hasselbring and Hawkins (*9*)[1] in studying the respiration of the roots of sweet potatoes found that the reducing sugars were the immediate source of respiratory material. The cane sugar remained relatively stable when once formed and did not appear to be readily utilized so long as starch and other carbohydrates were present in abundance.

The results of investigations reported by the writers (*23*) show that the fungi responsible for most of the decay of sweet potatoes—namely *Fusarium acuminatum* E. and E. emend. Wollenw., *Sclerotium bataticola* Taub., *Diplodia tubericola* (E. and E.) Taub., *Penicillium* sp., *Mucor racemosus* Fes., *Botrytis cinerea* Pers., *Rhizopus tritici* Saito, and *Sphaeronema fimbriatum* (E. and H.) Sacc.—with the exception of the last-named organism, can utilize glucose as a source of carbon. Not only were these fungi able to use glucose in various amounts, but they also produced certain changes in the hydrogen-ion and osmotic concentrations of the culture medium. Some of the sugar was used in producing mycelium and in supplying energy for carrying on the vital processes of the organisms, although no doubt a part of it was utilized for other purposes.

The investigations reported in this paper were designed to throw further light (1) upon the question of the availability of glucose as a source of carbon for these same fungi, *Sphaeronema fimbriatum* excepted, and (2) upon the amount of the carbohydrate used in respiration as measured by the amount of carbon dioxid (CO_2) given off.

METHODS

In these investigations the fungi studied were grown in Erlenmeyer flasks on a liquid synthetic medium. The apparatus was set up in duplicate,

[1] Reference is made by number (italic) to "Literature cited," p. 225-226.

and parallel experiments were run in most cases. The culture flasks were placed in an incubator held at a constant temperature, and CO_2-free air was pulled through by means of a Richard air pump. The air was passed through three bottles containing pumice stone and concentrated potassium hydroxid (KOH) and one bottle containing concentrated barium hydroxid ($Ba(OH)_2$), the latter being used principally as an indicator to insure that the air was entirely freed of CO_2. The air was finally washed by being pulled through CO_2-free distilled water, from which it was drawn into the culture flasks. The culture flasks and trap bottles for freeing the air of CO_2 and for washing it were placed in an incubator maintained at a temperature of 29° C. From the culture flasks the air was drawn out of the incubator through glass tubing. It was then freed of CO_2 by being drawn through a series of cylinders containing a saturated solution of $Ba(OH)_2$. Fresh air, though not in sufficient amount to interfere with the temperature control, was admitted to the incubator through a small hole about 1 cm. in diameter.

The cultures were grown in 1-liter Jena Erlenmeyer flasks stoppered with 2-holed rubber corks, through each of which were passed two glass tubes which served for the exchange of air. The tubes which admitted the air into the flasks extended to within about 1 inch of the surface of the medium, while the exit tubes barely projected through the corks.

CULTURE MEDIUM

A modification of Czapek's nutrient solution, in which ammonium nitrate (NH_4NO_3) was substituted for sodium nitrate ($NaNO_3$), was used as a substratum. Baker's C. P. dextrose in approximately 10 per cent strength was supplied as a source of carbon. The culture medium was prepared according to a method fully discussed in an article by Weimer and Harter (*23*), to which the reader is referred for complete details.

PREPARATION OF AN EXPERIMENT

Three 1-liter flasks, one to serve as a control, were prepared to contain 300 cc. of the culture medium. After being plugged with cotton they were sterilized by steaming for 20 minutes on three consecutive days. Just before inoculation the cotton plug was replaced by the rubber stopper with glass tubes attached, and the whole was resteamed for 20 minutes. To allow for the expansion on heating and to prevent contamination on cooling a glass tube of 3-mm. bore, about 8 cm. long, with a small bulb about 1 cm. in diameter midway between the two ends, was connected with each of the tubes passing through the cork by means of rubber tubing. The bulb was packed with cotton, which while permitting the passage of air served to filter out all contaminating material. The cotton was left in the bulb throughout the experiment.

Inoculations were made with a loop of a heavy spore suspension in distilled water or with a bit of mycelium from a young and vigorous cul-

ture growing on Irish potato cylinders or stems of *Melilotus alba* Desr. Before the culture flasks were inserted into the apparatus, air was pulled through the series of trap bottles and cylinders for about one hour to make sure (1) that all of the CO_2 was being removed from the incoming air and (2) that there was no leak through the connections.

At the termination of each experiment the mycelium was filtered into a tared alundum crucible washed with distilled water and dried to constant weight in a vacuum oven at 60° C. The percentage of glucose present in the control as well as that remaining in the two culture flasks was determined by a Fric saccharimeter, from which the total amount reduced was calculated. The culture and control flasks were weighed at the beginning and at the close of the experiment, and from these data corrections were made for loss of water due to evaporation or other causes.

DETERMINATION OF CO_2

The fungi differed markedly in the rapidity at which they grew in the culture solution. Some of the organisms, as *Rhizopus tritici*, for example, grew rapidly from the outset, and CO_2 was evolved in from two to three days. Some of the other fungi, on the other hand, grew slowly and gave off no CO_2 for a week or more. As soon as a precipitate appeared in the $Ba(OH)_2$ solution the CO_2 was determined for each 24-hour period thereafter to the end of the experiment.

In the determination of the CO_2 evolved the excess $Ba(OH)_2$ was neutralized by the addition of hydrochloric acid (HCl), with the use of thymol blue (thymol sulphonphthalein) as an indicator. The precipitate ($BaCO_3$) was then dissolved by adding an excess of *N*/1 HCl. After the total volume of the solution had been determined, an aliquot portion (usually 25 cc.) was titrated against *N*/10 sodium hydroxid (NaOH) with brom phenol blue (tetra bromo phenol sulphonphthalein) used as the indicator. The number of cubic centimeters of *N*/10 NaOH used to neutralize 25 cc. of the solution was multiplied by the total volume of solution, which gives the equivalent of the excess acid. The excess acid was then converted into its equivalent of *N*/1 HCl, and this amount was deducted from the total number of cubic centimeters of HCl required to dissolve the precipitate. The figure thus obtained was multiplied by the factor 0.022, the equivalent in CO_2 of 1 cc. of *N*/1 HCl.

In the preliminary experiments the two indicators, phenolphthalein and methyl orange, usually used in titrations of this nature were tried, but neither gave a satisfactory end point. Scales (*18*) experienced like difficulties in titrations of a similar kind and found that thymol blue and brom phenol blue both had a very sharp end point. Thymol blue in the presence of $Ba(OH)_2$ and $BaCO_3$ gives a brilliant blue color, which changes to a muddy green at the neutral point, to a lemon-yellow in a slight excess of acid, and to a pink in a strong acid solution. Upon the

addition of brom phenol blue the solution changes to a deep blue color and when slightly acid to a lemon-yellow.

EXPERIMENTAL DATA

RATE OF RESPIRATION

The rate of respiration of the different fungi expressed in CO_2 production is shown by the curves in figure 1, where the abscissae represent days and the ordinates the amount of CO_2 produced daily in grams.

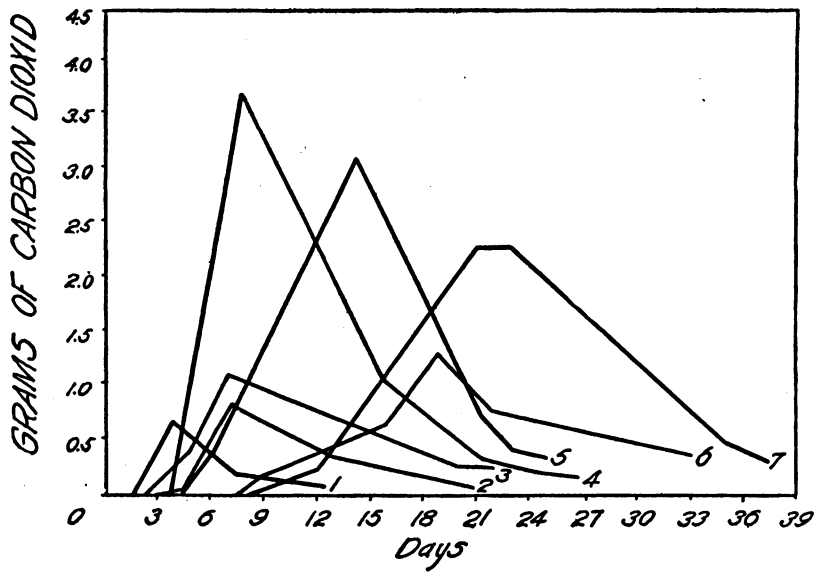

FIG. 1.—Rate of respiration of various fungi: 1, *Rhizopus tritici;* 2, *Diplodia tubericola;* 3, *Mucor racemosus;* 4, *Penicillium* sp.; 5, *Sclerotium bataticola;* 6, *Fusarium acuminatum;* 7, *Botrytis cinerea.*

The figures used in plotting these curves are in most cases the averages of two parallel tests. One familiar with the physiology and growth of fungi is aware that the same nutrient medium, the same temperature, and other environmental factors do not meet the requirements of all fungi equally well. In order to make the results comparable, a uniform standard of conditions for all the organisms was established as nearly as possible. They were all grown in an incubator maintained at a constant temperature of 29° C. in the dark.

An inspection of the curves shows that three organisms—namely *Penicillium* sp., *Botrytis cinerea,* and *Sclerotium bataticola* produced a quantity of CO_2 in excess of 2 gm. in a single day, while the four remaining organisms produce a relatively small amount. The data show also that those organisms which grew rapidly produced a comparatively small amount of CO_2, reaching their maximum in a short time after the culture flasks were inoculated and declining steadily thereafter. *Rhizopus*

tritici illustrates this point. On the second day it produced a measurable amount of CO_2, the quantity steadily increasing up to the fourth day. Thereafter it gradually decreased and practically ceased entirely by the twelfth day. *Penicellium* sp., *B. cinerea*, and *S. bataticola*, on the other hand, grew slowly and did not give off a measurable quantity of CO_2 for several days following inoculation. The evolution of CO_2 rapidly increased thereafter and continued a considerable number of days before it ceased entirely.

Except with *Botrytis cinerea*, the day of maximum production of CO_2 was followed by a sharp decline, which continued to the close of the experiment. *B. cinerea* differed from all the other organisms in that there was a period of two or three days when the maximum CO_2 production remained about constant. However, it is probable that if determinations had been made in less than 24-hour periods an apex in the curve would have been shown for this fungus.

DRY WEIGHT OF MYCELIUM

A direct comparison of the dry weight of the different organisms is not possible for reasons that can be well understood. As previously pointed out, the fungi were allowed to grow as long as a measurable quantity of CO_2 was given off. This varied from 11 to 30 days, so that some fungi had a much longer time to form mycelium. Furthermore, some fungi will produce a much larger quantity of dried material than others under identical conditions; in fact, it was shown by Kunstmann (*14*) that in duplicate cultures of the same fungus two and one-half times as much dried material might be produced in one flask as in another.

An examination of Table I and the curves in figure 1 will show that although the experiments with *Botrytis cinerea* ran for 38 days, *Penicillium* sp. produced about 11 per cent more dry weight in 10 days less time and that *Sclerotium bataticola* produced a greater dry weight in 25 days than *B. cinerea*, which grew 12 days longer. *Mucor racemosus* and *Rhizopus tritici* were grown 23 and 12 days and produced 1.13 and 0.94 gm. of dry weight, respectively. A comparison of *M. racemosus* with *S. bataticola* shows an even more striking difference, the ratio of dry weight being approximately 1 to 5.5.

TABLE I.—*Glucose reduced and dry weight and CO_2 formed by certain sweet potato storage-rot fungi, together with the numerical expression of the interrelationship existing between the glucose changed and the resulting products as well as between the products themselves*

Organism.	Solution.	Dry weight of mycelium produced (in grams).	Glucose remaining at the end of the experiment (in percentage by weight).	Amount of glucose reduced (in grams).	Total amount of CO_2 (in grams).
Fusarium acuminatum	Control		9.2		
	Inoculated	1.607	.55	27.510	15.524
Sclerotium bataticola	Control		9.18		
	Inoculated	6.068	.09	27.771	29.327
Diplodia tubericola	Control		9.1		
	Inoculated	1.972	6.35	7.628	6.066
Penicillium sp	Control		9.2		
	Inoculated	6.251	0	30.791	32.523
Mucor racemosus	Control		9.1		
	Inoculated	1.127	1.5	25.762	12.837
Botrytis cinerea	Control		9.6		
	Inoculated	5.377	.05	27.271	32.714
Rhizopus tritici	Control		9.2		
	Inoculated	.938	7.93	4.345	2.718

Organism.	Solution.	Grams of CO_2 given off per gram of glucose reduced.	Dry weight of mycelium (in grams produced by 1 gm. of glucose).	Coefficient of respiration: sugar (in grams reduced per gram CO_2 formed).	Economical coefficient: sugar used (in grams to produce 1 gm. dry weight).	Respiratory quotient: dry weight (in grams formed per gram CO_2 given off).
Fusarium acuminatum	Control					
	Inoculated	0.567	0.058	1.77	17.11	0.103
Sclerotium bataticola	Control					
	Inoculated	1.056	.218	.95	4.58	.207
Diplodia tubericola	Control					
	Inoculated	.795	.258	1.26	3.86	.325
Penicillium sp	Control					
	Inoculated	1.056	.203	.95	4.93	.192
Mucor racemosus	Control					
	Inoculated	.498	.044	2.01	22.86	.088
Botrytis cinerea	Control					
	Inoculated	1.200	.197	.83	5.07	.164
Rhizopus tritici	Control					
	Inoculated	.625	.216	1.59	4.63	.345

UNCHANGED GLUCOSE REMAINING IN THE SOLUTION

With the exception of *Diplodia tubericola* and *Rhizopus tritici* the fungi either utilized directly practically all the glucose originally present in the solution or converted it into other substances (Table I, columns 4 and 5), the former utilizing about 2.8 and the latter 1.3 per cent. The percentage of glucose consumed should be carefully compared with the dry weight of mycelium shown in column 3. *Fusarium acuminatum* and *Sclerotium bataticola* utilized all but 0.55 and 0.09 per cent, respectively, of the glucose and produced 1.61 and 6.1 gm. of mycelium. In

other words, *S. bataticola* made nearly four times as much dry weight in nine days less time by the utilization of practically the same amount of sugar as *F. acuminatum*. *Penicillium* sp., on the other hand, utilized all the glucose and formed 6.25 gm. of mycelium, while *Mucor racemosus* consumed 25.8 gm. of sugar in making 1.13 gm. of dry weight. A study of the figures shows that there is no uniformity among the fungi as regards the glucose utilized and dry weight produced, even if an account is taken of the length of time they were grown.

CO_2 PRODUCTION

In column 6 is shown the total amount of CO_2 produced by each fungus during the time of the experiment. A comparison of the total CO_2 given off with the dry weight produced and sugar utilized will not be necessary in all cases, but attention will be called to a few outstanding examples. *Sclerotium bataticola* gave off nearly twice as much CO_2 as *Fusarium acuminatum* and yielded nearly four times as much dry weight, while *Botrytis cinerea* and *Rhizopus tritici* produced 32.71 and 2.72 gm. of CO_2, respectively, and formed 5.38 and 0.94 gm. of dry weight. Here also a résumé of the figures shows that there is little or no uniformity in the relation of CO_2 evolved to the glucose consumed and dry weight produced.

RELATION OF CO_2 GIVEN OFF TO GLUCOSE REDUCED

The amount of CO_2 given off in grams for every gram of sugar reduced is shown in column 7. It will be seen that for *Sclerotium bataticola*, *Penicillium* sp., and *Botrytis cinerea* more than 1 gm. of CO_2 is produced for each gram of sugar consumed. In all the other cases it is less than unity. It should be noted that these are the three fungi which produced the largest amount of dry weight and are among those which consumed the most glucose. If extremes are contrasted it will be seen that *B. cinerea* and *Mucor racemosus* produce approximately 1.2 and 0.5 gm. of CO_2, respectively, for each gram of sugar utilized. While some reasons for these differences will be suggested later, attention should be called to the fact that *B. cinerea* continued to give off an appreciable amount of CO_2 some days longer than *M. racemosus*. Botrytis, Penicillium, and Sclerotium consumed all or practically all the sugar, so that it is not unlikely that other compounds were formed from the sugar which may have been utilized as a source of carbon.

RELATION OF DRY WEIGHT TO GLUCOSE CONSUMED

An examination of column 8 shows that the fungi differed greatly in the dry weight formed from 1 gm. of sugar consumed. *Fusarium acuminatum* and *Mucor racemosus* stand out as conspicuous examples of fungi which produce a small amount of mycelium per gram of sugar used, this

being only 0.058 and 0.044 gm., respectively. Contrasted to these, *Diplodia tubericola* formed the most, namely, 0.258 gm., while *Sclerotium bataticola* and *Rhizopus tritici* formed 0.218 and 0.216 gm., respectively.

COEFFICIENT OF RESPIRATION

The number of grams of sugar reduced per gram of CO_2 evolved is shown in column 9. The differences in the case of several of the fungi are very large. *Mucor racemosus* is the most extravagant and *Botrytis cinerea* the most economical user of sugar when the comparison is made on the basis of CO_2 produced, but it may be otherwise when the dry weight of fungus substance alone is considered. Four of the fungi studied require more and three less than 1 gm. of sugar to make 1 gm. of CO_2. Kunstmann (*14*) in working with *Aspergillus niger* v. Tieg. in a 5 per cent solution of cane sugar found a considerable variation in the results obtained from different experiments. In all cases, however, more than 1 gm. of sugar was required to form 1 gm. of CO_2, the variation ranging from 1.05 to 1.98. He further showed that a greater growth of the fungus was accompanied with an increase in respiration, as would be expected, and that the respiration became more rapid as the temperature rose. He showed, furthermore, that the concentration of the cane sugar in the nutrient solution influenced the rate of respiration, it being about 1.5 times as rapid in a 30 as in a 5 per cent solution. In a solution made slightly acid with phosphoric acid (P_2O_5) the quantity of CO_2 evolved by a unit weight of growth was considerably less than in those which remained alkaline.

It is evident that there are a number of factors which might have some influence on the evolution of CO_2 in experiments with a number of fungi. It is not possible to find an environment which would be the optimum for all of them. If one considers the concentration of the solution alone with respect to the source of carbon, which Kunstmann showed to influence respiration, it is clear that a concentration which might be considered optimum for one organism might not necessarily be so for another. In spite of the great ability of fungi to adapt themselves to solutions of high osmotic concentration, it is a well-known fact that some organisms can tolerate a more concentrated solution than others. The Penicillia, for example, grow in a sugar solution of high concentration. The writers found that *Botrytis cinerea* and *Penicillium* sp. grew equally well in a concentration of 38 per cent and 58 per cent, respectively. Bearing in mind the influence the concentration has on respiration one may readily conceive how the quantity of sugar present, which in these experiments was alike for all the organisms, might work to the advantage or disadvantage of the different fungi, at least so far as the amount of sugar required to produce 1 gm. of CO_2 is concerned.

Other factors which conceivably might influence the results are (1) acidity of the solution, (2) spore sowing, (3) light, (4) temperature. As

regards the first of these, it is a well-recognized fact that most fungi prefer a slightly acid medium, and in some cases if the solution is not acid they make it so. Since this subject is to be discussed more in detail in another connection, suffice it to say here that, as with the concentration, it is probable that the degree of acidity of the original solution was not optimum for all the organisms. Kunstmann considered the question of mass inoculation of the culture by spores and found that it exercised very little influence on the rate of respiration. There is at present some difference of opinion as to the influence of light on the respiration of fungi. Kreusler (*13*) and Wehmer (*22*) deny the influence of light on growth and respiration, while Bonnier and Mangin (*1*) and Elfving (*6*) ascribe a retarding effect and Ziegenbein (*24*) a favorable influence on some flowering plants. Probably all fungi will grow in the dark. Janssens and Mertens (*10*) found that the red Torula is influenced by light and behaves like green plants, respiration being apparently more pronounced in the light than in the dark. The writers have also found that certain organisms fruit abundantly when exposed to moderately strong light but remain sterile if grown in the dark. In the writers' present experiments the fungi were exposed to light for only a short period of time each day, when the incubator was opened to adjust the apparatus or to examine the cultures.

The temperature of 29° C. at which the organisms were grown probably does not represent the optimum for many, if it does for any of these fungi. The results of many investigators have shown that there is a wide range of optimum temperatures between different organisms, some thriving as well at 37° as others at 25°. In fact, there is considerable variation between species of the same genus. Brooks and Cooley (*3*) showed that the apple-rot fungi vary in their temperature requirements, and Edson and Shapovalov (*5*) showed that certain potato-rot and wilt-producing organisms of the potato had different optimum and maximum temperatures for growth. The writers found, for example, that *Rhizopus tritici* grows better at 35° than at 29° and will make a good growth at 40° or above. Some intermediate temperature, therefore, had to be employed which would permit all the organisms to make a good growth. Furthermore, the fact must not be overlooked that the rate of CO_2 production is more or less correlated with the temperature at which the fungus is grown. Ziegenbein (*24*) found that the optimum temperature for respiration of different flowering plants varied from 35° to 45°, and Kunstmann (*14*) and Stoklasa (*19*) report a similar variation for fungi and beetroot, respectively. According to these investigators the maximum rate of respiration correlates closely with the maximum temperature for growth. When the maximum temperature for growth is reached, the rate of respiration declines rapidly.

ECONOMIC COEFFICIENT

Pfeffer (*17*) and Kunstmann (*14*) have termed the numerical relation between the sugar consumed and the dry weight of the substance formed the "economic coefficient." According to Jost (*11*) the theoretical minimum value of the coefficient is about ½, but in reality it has been found to be higher than unity. Kunstmann found in working principally with *Aspergillus niger* that it varied from 1.13 to 3.88 in parallel experiments in a 5 per cent solution of cane sugar. Ono (*16*), on the other hand, obtained values as high as 6.1.

The results obtained by the writers are shown in column 10 of Table I. It will be seen that the economic values for *Fusarium acuminatum* and *Mucor racemosus* are very much higher than any of those given by Kunstmann and Ono, being 17.11 and 22.86, respectively. The five other organisms are fairly consistent in the amount of sugar required to produce 1 gm. of dry substance, all of which, however, are equal to or higher than the maximum given by Kunstmann. In view of these facts, it is quite evident that fungi in general can in no sense be regarded as economic users of sugar. In none of the writers' experiments or in those of Kunstmann and Ono has the minimum value of the economic coefficient fallen below unity.

From the data at hand it is evident that no sweeping generalizations can be made for all fungi. Ono showed that the addition of a small amount of zinc sulphate reduced the "economic coefficient," and Jost points out that the coefficient increases with the progressive development of the fungus and with an elevation of the temperature. Since the progressive development of the fungus influences the coefficient, the element of time would have an important bearing on the results. Although all of these experiments were carried out at the same temperature (29°C.), no doubt the fungi studied did not respond to heat in a similar manner.

RESPIRATORY QUOTIENT

The dry weight of fungus material produced for each gram of CO_2 given off is in all cases considerably less than unity (Table I, column 11). To contrast the extremes, *Rhizopus tritici* formed about four times as much dry material as *Mucor racemosus*. Kunstmann in all his experiments obtained a much higher numerical value of the respiratory quotient with *Aspergillus niger* in a 5 per cent cane sugar solution. In a few cases considerably more than 1 gm. of dry weight was produced for each gram of CO_2 given off. Kunstmann's results are not in every respect comparable, since he used different temperatures in different experiments. The higher temperatures for the most part appeared to lower the value of the "respiratory quotient." However, it may be concluded that in general under experimental conditions considerably more than 1 gm. of CO_2 is given off for each gram of fungus material formed.

These variations are more or less dependent upon the temperature at which they are carried out and the length of time the experiment has run.

DISCUSSION OF RESULTS

PRODUCTS OF FERMENTATION

ALCOHOL.—It is well known that fungi often produce alcohol and various organic acids as fermentation products during respiration. If the oxidation of the sugar was complete, CO_2 and water only would be produced, but results obtained by various workers have shown that other substances are often formed. An extensive literature is extant on the production of alcohol by different fungi in culture, but no attempt will be made to review all or any considerable part of it. Suffice it to say that the results of Brefeld (*2*), Fitz (*7*), and Hansen (*8*) with different species of Mucors, Aspergillus, Rhizopus, and Penicillium, and more recenty Kostytschew (*12*) with *Aspergillus niger* show that alcohol production by fungi is not uncommon. The amount of alcohol produced by the different organisms, according to the authors cited, differs with the medium used, the temperature, and the length of time the organism was grown. *Mucor racemosus* was found by Hansen to produce as much as 7 per cent by volume in 12 months at room temperature, and Fitz showed that *M. mucedo* Bref. would form 0.8 per cent alcohol by weight in 7 weeks at a temperature of 30°C.

Obviously it is not possible to determine with any degree of accuracy by present chemical methods just what a fungus does in a solution as complex as Czapek's nutrient medium. The writers' experiments showed that alcohol was produced to a limited extent by four of the fungi studied—namely, *Fusarium acuminatum*, *Rhizopus tritici*, *Diplodia tubericola*, and *Mucor racemosus*. *R. nigricans* and *M. racemosus* were shown to be alcohol producers by other investigators, the former to a very limited extent, and the latter in considerable quantity. So far as the writers are aware, no one has reported the production of alcohol by *F. acuminatum*, *R. tritici*, or *D. tubericola*. If alcohol was produced by the other fungi it was either utilized by the fungus or formed in such a limited amount that it could not be detected by the method employed. The results from which the conclusions were drawn, although not unqualified proof, were determined by the following method: One hundred cubic centimeters of the solution were neutralized with magnesium carbonate ($MgCO_3$). Fifty cubic centimeters were then distilled off, and from this a 25-cc. fraction was taken. The iodoform test was applied to the last distillate. A positive test was obtained in most cases only upon warming. The second distillate in all cases, when the iodoform test indicated the presence of alcohol, had a lower specific gravity than water. The same tests were carried out with the control solutions with negative results.

In no case was enough CO_2 produced to account for all the glucose used up, so that the question of what became of the remainder of the glucose naturally suggested itself. Attention already has been called to the fact that many fungi produce alcohol in nutrient solutions. According to Jost (*11*) a 10 per cent solution of alcohol is usually injurious to fungi, while a 2 to 4 per cent is usually nutritive. *Sclerotium bataticola*, *Penicillium* sp., and *Botrytis cinerea* did not produce alcohol according to the iodoform test, and yet they used up all or nearly all the glucose. While the writers have no proof to offer, it is possible that alcohol was formed by these organisms which was utilized by the fungi as a source of carbon.

Positive iodoform tests were obtained for *Rhizopus tritici*, *Diplodia tubericola*, *Fusarium acuminatum*, and *Mucor racemosus*, while *Botrytis cinerea*, *Sclerotium bataticola*, and *Penicillium* sp. gave negative results. It will be seen that the last three organisms produced more than 1 gm. of CO_2 for each gram of glucose used, while the first group gave off considerably less. The distillate from the solution on which *M. racemosus* grew gave an especially heavy precipitate of iodoform crystals.

Under the conditions of the experiment *Rhizopus tritici* and *Diplodia tubericola* produced only a trace of alcohol. A separate experiment was conducted to test further the ability of the former organism to form alcohol. Flasks were prepared in triplicate, with the same medium as in the previous experiments and 10 per cent glucose as the source of carbon. All three of the flasks were inoculated, two being stoppered with rubber corks and one with a cotton plug. A normal growth of mycelium took place in the flask stoppered with cotton, but in the other two flasks the mycelium was abnormal in appearance, less luxuriant, and mostly submerged. Distillations were made from all the solutions, and as indicated by the iodoform test an abundance of alcohol was formed in the flasks stoppered with corks, while only a mere trace could be detected in the flask plugged with cotton. It would seem then that *R. tritici* will produce alcohol much more readily when growing on the medium used in the experiments recorded above when a reduced supply of oxygen is available and intermolecular respiration is thereby induced. The concentration of the CO_2 would be increased also under these conditions. In the respiration experiment, as indicated by the amount of CO_2 produced per gram of sugar used, it is possible that considerable alcohol was formed and possibly a part of it was carried off by the air current continually passing through the flask. However, some of the sugar may have gone to form other organic substances.

ORGANIC ACIDS.—The nutrient solution with which these experiments were conducted was slightly acid at the beginning. In a paper by Weimer and Harter (*23*) it was shown that all of these same organisms when growing in a 10 per cent glucose solution increase the P_H value of the solution and some of them to a considerable extent. It has been hither-

to shown by many investigators that fungi frequently increase the acidity of the substratum. Wehmer's (*20*, *21*) work in this direction is significant. He found that *Aspergillus niger* rendered the solution at first acid. The acidity (oxalic acid) gradually increased to a maximum and declined once more during the next few weeks to zero, the solution finally becoming alkaline. The fungus was found to decompose free oxalic acid at the higher temperatures. He likewise showed that the amount of acid formed was not necessarily associated with the quantity of fungous growth produced. The acid is found only when the substratum gives no acid reaction and when the organism is cultivated in sugar, proteid, glycerin, oil, and salts of organic acids. Wehmer also found that *Citromyces glaber* Wehm. can utilize the citric acid which it has produced. Furthermore, although this organism will tolerate a concentration of 20 per cent citric acid, it only produces enough to render the substratum 4 per cent acid. In a discussion of his results he calls attention to the fact that the only acids formed by Aspergillus or Penicillium in notable quantities are oxalic and citric. *Botrytis cinerea* and *Rhizopus nigricans* and some other fungi produce oxalic acid only in traces and only in a nearly neutral medium. An abundant carbohydrate supply and calcium salts, such as calcium phosphate or carbonate, favor its production. Kunstmann (*14*) found that oxalic acid was produced in all the media used by him but that at the end of the experiment it never exceeded 0.05 gm. in 100 cc. of solution. Molliard (*15*), however, found that *Sterigmatocystis nigra* v. Tieg. produced both citric and oxalic acid, together or alone in the medium in which saccharose was used as the source of carbon. Citric acid was more abundantly produced than oxalic, but both increased gradually up to the end of the experiment. The acid production of a large number of species of Penicillium was studied by Currie and Thom (*4*), who found that it was formed in varying amounts by all of them and in a large quantity by one species in particular, *Penicillium oxalicum* Currie and Thom. According to these investigators the oxalic acid produced is not an end product. It reaches its maximum in 8 to 12 days and then declines. The results of the writers' experiments show that in no case was enough CO_2 produced to account for all the sugar consumed. Undoubtedly some of the sugar was converted into other compounds, and in this connection alcohol, aldehydes, and organic acids suggest themselves as the most likely. The presence of alcohol was demonstrated in the culture solution in which the fungus grew in the case of four organisms and not in the controls. In another paper it was shown that these same organisms growing in the solution used in these experiments increased the hydrogen-ion concentration. It seems, therefore, that alcohol or acids or both may have been produced in the solutions.

SUMMARY

(1) The following fungi can utilize glucose as a source of carbon: *Fusarium acuminatum, Sclerotium bataticola, Diplodia tubericola, Penicillium* sp., *Mucor racemosus, Botrytis cinerea,* and *Rhizopus tritici.*

(2) *Penicillium* sp., *Botrytis cinerea,* and *Sclerotium bataticola* produced a maximum of a little more than 2 gm. of CO_2 in a single day. The other fungi formed a relatively small amount. The organisms which grew rapidly produced a comparatively small amount of CO_2 and reached their maximum in a short time after the culture flasks were inoculated. In all cases the respiration was measured as long as CO_2 was given off in any measureable quantity.

(3) The three fungi, *Penicillium* sp., *Botrytis cinerea,* and *Sclerotium bataticola,* which grew slowly, produced a relatively large amount of dry material and consumed all or nearly all of the glucose. The reverse is true of the other organisms.

(4) The quantity of CO_2 evolved does not necessarily correlate with the amount of dry material formed or with the amount of glucose reduced. Some organisms (*Mucor racemosus* and *Fusarium acuminatum*) which produced a comparatively small quantity of dry material reduced a large amount of sugar.

(5) Three organisms evolved more than 1 gm. of CO_2, the others considerably less, for each gram of glucose reduced.

(6) The dry weight of material per gram of glucose consumed is in all cases considerably less than unity.

(7) The "coefficient of respiration" varies from 0.83 to 2.01, the "economic coefficient" from 3.86 to 22.86. The "economic coefficients" of *Fusarium acuminatum* and *Mucor racemosus* (17.11 and 22,86, respectively) are several times higher than that of any of the other fungi studied. They are also higher than the values given by other investigators.

(8) The quantity of CO_2 evolved was not equivalent to the theoretical amount that might have been produced from the sugar consumed. Other investigators have shown that alcohol is formed by *Mucor racemosus,* but the writers have demonstrated for the first time that it is produced by *Fusarium acuminatum, Rhizopus tritici,* and *Diplodia tubericola.* It was previously shown by the writers that these same organisms when growing in a 10 per cent solution of glucose increase the acidity of the solution. It is therefore probable that some of the glucose was utilized in the production of alcohol and acids.

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