THE RELATION BETWEEN VEGETATIVE VIGOR AND REPRODUCTION IN SOME SAPROLEGNIACEAE[1]

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HISTORICAL INTRODUCTION

Advances in science have been made by workers arriving at new points of view, as witness the work of Mendel, Darwin, deVries and others. So also the writings of Klebs during the last twenty-five years have served to open up a field, the cultivation of which is already exercising a profound influence on biological thought. Klebs was not the first to suggest that the forms, to the development of which a plant might at any one time devote its energies, were conditioned by external factors acting upon the plant; but it will be neither necessary not desirable to review the older literature. This has been adequately done by Klebs in his various papers. (See especially '00, '03, '04.)

The idea underlying all of Klebs's work is that every species has a sum total of potentialities constituting the specific nature of that species, but that the chemical and physical conditions prevailing at any one time within the organism determine which of the particular forms that the species is capable of producing shall appear, and that these inner conditions may be controlled, to an extent at least, by regulating the external conditions. In other words the changes in form which we witness in the course of the development of an individual are the results of chemical and physical interactions.

Since 1890, when Klebs ('90) showed that the formation and discharge of zoospores in Hydrodictyon could be encouraged by providing certain conditions, he has established the truth of this principle in the case of other algae ('96), of fungi ('98, '99, '00), and also for flowering plants ('03, '04). Other workers have built upon

[1] Contribution 150 from the Botanical Department of the University of Michigan. This work was begun at Heidelberg, Germany, under the direction of Prof. Dr. Georg Klebs, and was continued at the University of Michigan under the direction of Dr. C. H. Kauffman. To both of these inspiring instructors the writer wishes to return his thanks and to them he desires to express his appreciation of the mental stimulus he owes to their discussions and suggestions.

the foundations laid by Klebs and the present writer believes that the proposition that the succession of forms in the development of any plant is conditioned by environment, needs no further support than that furnished by the work of Klebs, Vöchting ('00), Kauffman ('08), Freund ('08), Obel ('10), and Raciborski ('96).

It has seemed worth while, however, to answer a question raised in the writer's mind by a study of Klebs's paper on *Saprolegnia mixta*. In this paper Klebs describes the production of sporangia and oogonia on a mycelium transferred from one solution to another, and states that such a mycelium, to react properly, must have been "well nourished." He does not define more exactly what he means by this term. Obel ('10) also emphasizes the need of a vigorous mycelium and uses a solution of peptone, sucrose and salts instead of the pea extract favored by Klebs. Obel agrees with Freund ('08), who states that, in algae, the conditions favoring the production of reproductive bodies depend upon the preceding conditions of growth and Obel states that this conclusion is confirmed by an examination of the Saprolegniaceae ('10, p. 427). Freund does not, however, give the results of any experiments to show the importance of the vigor of growth in the algae, and apparently makes the statement as a general one, meaning that the alga must be in good health and not in poor condition.

Neither Obel, Klebs nor Kauffman gives any record of experiments on this point. In his paper on *S. mixta* ('99) Klebs repeatedly refers to the necessity for a well nourished mycelium and states (p. 585) that during vegetative growth a part of the nutritive substance is stored, to be used later when reproduction shall have commenced. Further on the same page the statement is made that the production of oogonia requires a nutritive plasma (Nährplasma) of a somewhat different composition from that needed for the production of sporangia. Klebs fully recognized that the chemical nature of the solution to which a mycelium is transferred, is important. He found that haemoglobin and leucin were the substances most favorable to the production of oogonia and that the addition of certain phosphate salts increased both the number of oogonia and of antheridia. Kauffman ('08), in his work with *S. hypogyna* and with two cultures of *S. mixta*, did not find any marked increase in the number of oogonia due to the addition of salts, and the number of antheridia in the case of *S. mixta* was as marked in the solutions containing magnesium sulphate, as in those containing phosphates (l. c., p. 368). However, neither

studied the effect of various foods consumed by the mycelium before transfer.

The Problem

The question was raised therefore, "What is a 'well nourished' mycelium?" and naturally the question of what constitutes a good nutritive solution followed. This work was commenced at Heidelberg, under the direction of Prof. Dr. Georg Klebs, who agreed with the writer that a culture medium, in which the fungus can attain the greatest dry weight in a given time under favorable conditions, is generally to be considered the best nutritive medium. The question which the writer set himself to answer therefore, is the following: Will a nutritive medium in which a species of water mold produces the greatest amount of dry matter in a given time also give to that mycelium when transferred to a suitable medium, the power to form more reproductive organs, than are produced by a mycelium grown in a poorer medium, measured by dry weight of mycelium produced? Or, in other words, is there a direct and necessary relation between vegetative growth and reproductive capacity?

Generally in cultures of other fungi the reproductive bodies, when formed under these conditions, are produced while the mycelium remains in or on the culture medium in which its vegetative growth has taken place; not so with the Saprolegnias. If satisfactory results in the production of sporangia or of oogonia are to be obtained, these plants must be transferred from the solution in which they have been grown, to another and weaker solution. When the plant is transferred to the second solution, it finds itself in a new environment. This may differ from the old merely in concentration and in the absence of the poisonous products of metabolism, which have accumulated in the old solution, or it may differ from the latter in chemical composition. As Klebs has pointed out ('99), whatever the chemical nature of the second solution may be, it must, if oogonia are to be formed, be of such character and concentration, that a slight growth can take place. If the solution is too poor in food, sporangia will appear and perhaps exhaust the mycelium, while if the food is too good vegetative growth will prevent reproduction.

If the protoplasm produced by the consumption of all kinds of foods is alike we may expect that the nature of the changes observed after transfer will depend wholly on the new environment; but if the food used during vegetative growth can affect the character of the

protoplasm, as well as the weight of the product, such effects should become evident when mycelia, grown in different culture media, are transferred to solutions of identical composition and concentration. The new and uniform environment acting upon the protoplasmic product of varying environments may be expected to bring out differences if any exist. Data will be presented in this paper to show that, in some cases at least, such an effect, not to be measured in terms of dry weight produced, has resulted from the use of special culture media and that there is no direct and necessary relation between weight of mycelium and the production of oogonia.

It was realized at the outset that there were two aspects of the problem—the quantitative and the qualitative. Either a nutritive substance of one kind might affect the organism because of its greater abundance, or substances of various kinds might affect especially the reproductive capacity or the vegetative growth.

Solutions were therefore prepared with these two aspects in view, different concentrations of peptone being used on the one hand and various carbohydrates and salts on the other. The number of oogonia produced on the mycelia after transfer, together with the weight of mycelium in the duplicate lots left in the nutrient solutions, make up the evidence on which the conclusions reached are based; this evidence can best be presented in a series of tables and as tables are rather uninteresting reading, the writer has pointed out briefly after each table the conclusions that seem warranted by the evidence, leaving a general discussion of these conclusions to be given at the end of the paper. The chief contributions which it is believed are made by the present work to our knowledge of the physiology of these forms are the recognition of a minimum concentration of food necessary for a full development of the plant, and the fact that above this minimum an increased concentration during vegetative growth does not necessarily increase the reproductive capacity of the fungus. It developed further, as will be shown in the following pages, that certain carbohydrates are readily used while others can not be utilized, and that of those that are used some are of more value to the plant for the formation of reproductive organs than others.

Material

The material used for this study consisted of four species of Saprolegniaceae, selected from among a larger number isolated and studied.

Two of these belonged to the genus Saprolegnia. *S. ferax* (Gruith) Thuret was collected at Heidelberg in the fall of 1911; *S. monoica* Pringsh. was secured from alga material out of pools near Ann Arbor. *Achlya racemosa* Hildebr. was collected at Heidelberg and was unfortunately lost in a fire which destroyed a large part of the botanical laboratories at Ann Arbor in the spring of 1913, at which time also a large number of notes and other records were lost. The records of this species are therefore incomplete. *Achlya prolifera* De Bary was obtained from alga material out of pools near Ann Arbor. Physiological studies on several other species, together with descriptions of some new species and remarks in regard to certain points of relationships, will be discussed in other papers.

Methods

For isolating the forms Kauffman's ('08) method of single spore cultures was strictly followed. While the writer is not disposed to say that men like Maruzio ('94) and Obel (l. c.) did not work with pure cultures it seems more than likely that the results claimed to have been gotten by Lechmere ('11) may be due, in part at least, to mixed cultures. Moreover, a writer with so little appreciation of the worthlessness of sporangia and gemmae as specific characters,[2] can hardly be taken seriously when he states that the sporangia characteristic of six different genera were found in one supposedly pure culture. While Lechmere says that he used Kauffman's method he has apparently quite overlooked the fact that the essential part of this method is the isolation of one spore; it does not appear that he attempted to do this in his work.

All the cultures of each species made in connection with the present problem were descended from an original single spore and if there was the slightest reason to doubt the purity of a culture, a single spore culture was again made and carried through on a fly, so that the specific characters might be compared with the original. All the species that were not being used for experimental work were kept alive on flies or in pea extract. In either case cultures can be set aside for two or three months without any danger of loss.

[2] See l. c., p. 176, where Lechmere concluded that two species of Saprolegnia were identical because of the similarity of habit, gemmae, and shape and peculiarities of the sporangia, although the one produced oogonia readily and the other did not produce them at all.

For the experiments cultures were made in Erlenmeyer flasks of Jena or of resistance glass except in the few cases that will be noted, the flasks containing 100 or 200 cc. of culture fluid. This medium was inoculated by placing in it small bits of pea agar in which the fungus was actively growing. Pea agar was used instead of the beef gelatin used by Kauffman as it was found more convenient, both to make and to handle. It is easily made by putting about 100 peas in a liter of water together with sufficient agar to make the strength desired. This is heated in an autoclave for an hour at about 15 to 20 pounds pressure and, after filtering, is ready for use. It has been found a good culture medium, and has a further advantage over gelatin in not becoming soft in warm weather and in not being liquefied by the action of the fungus. When a set of cultures was to be inoculated the bits of agar were cut as nearly of the same size as possible, about 1 square millimeter in size. After having grown for the necessary length of time the entire mass of mycelium was placed in a dish of sterile distilled water and allowed to stand for half or three quarters of an hour. It was then cut into pieces of as nearly the same size as possible and usually of about one square centimeter in area. These pieces were transferred to fresh sterile distilled water and were sometimes washed a third time before being placed in the solutions in which sporangia or oogonia were to be made. For the latter solutions glass capsules containing 25 cc. of solution were used. All of these cultures were in duplicate, and sometimes in quadruplicate.

Condition of the mycelium.—For studying the effect of food previously consumed, on a mycelium after transfer to haemoglobin, or to some other suitable medium for the production of oogonia, or to water for the production of sporangia, it is important that the mycelium be actively growing, and that, in all the experiments, it shall be as nearly as possible in the same physiological condition. If a flask containing 100 cc. of pea extract be inoculated, the resulting growth will reach the surface after four or more days, depending upon the species. When the surface of the liquid is reached, a mat is formed, which, after a few days, may become quite thick and firm. At this stage the mycelium is in a very different condition from that in which it is just before reaching the surface. In the latter case the hyphae are all actively growing, the tips being, of course, the most active; when a mat has formed, and especially if the mat has been allowed to become thick, the lower parts of the mycelium are dead or dying while the

parts in the mat have taken on something of a resting condition. If such a mat is cut up and the pieces placed in another solution, new growth must take place in that solution before sporangia or oogonia can be formed, but if the mycelium is used earlier the hyphae not only grow but respond at once to the stimulus of the new environment by the production of appropriate organs. It was found that the most advantageous stage at which to use a mycelium was just as the mat started to form, for at that stage masses of actively growing hyphae, all in uniform condition, can be readily secured.

Temperature.—Temperature is a factor of the utmost importance. Klebs states that a temperature of about 18 degrees Centigrade is the optimum for *S. mixta*, but this is certainly not true for *S. ferax*. It has not been possible to determine the optimum for each of the various species studied, but enough was learned to warrant the statement that the temperature requirements of the different species differ. In *Achlya racemosa* a high temperature favors the production of oogonia and inhibits the formation of sporangia, while the temperature at which oogonia are produced with certainty by *S. ferax* is in the neighborhood of 15 degrees. *S. monoica* seems to do about as well at 18 degrees as at 15; but at 20 degrees or over there is a distinct decrease in the number of oogonia. *Achlya prolifera* seems to prefer a temperature of 18 to 20 degrees; at lower temperatures as well as at higher, 22 to 24 degrees, the production of oogonia is uncertain. Unfortunately it was not possible to determine the optimum temperatures from lack of apparatus by means of which constant low temperatures could be maintained. For the purposes of the present work, however, it was sufficient to maintain the temperatures near the optimum for each species. It is interesting in this connection to note that Peterson ('10) in his study of the Danish freshwater Phycomycetes states that *A. racemosa* produces oogonia with difficulty, and only at low temperatures. In the writer's experience *A. racemosa* produces oogonia very readily and particularly so at relatively high temperatures.

Method of expressing the number of oogonia.—Klebs, in comparing the value of different substances for the production of oogonia expressed the number of oogonia by the Roman figures I, II, and III, the relative number of oogonia present being in that order; Kauffman and Obel merely stated that few or many oogonia were present, while Horn ('04) followed the precedent set by Klebs. Such a method

does very well when one wants only to say that a food is poor or very good, but obviously if the number of oogonia formed is to be used as a measure of the value of a food, a more exact method of determining that number is necessary. In the present work the method of Klebs was used to some extent, especially in the earlier part of the work, but it soon became evident that this was not sufficiently accurate. When the value of various foods for vegetative growth is to be determined, the mycelium is dried and weighed at the conclusion of the experiment, but it is not practicable to weigh the oogonia produced; these were therefore counted. For this purpose the mycelium was removed from the dish in which it was growing and laid on the inside of a Petri dish cover. Here it was carefully spread out by means of a jet of water from a pipette till it lay as a circular flat mass, the older, and thicker, portion at one end or near the middle and the younger portions forming a fringe around this.

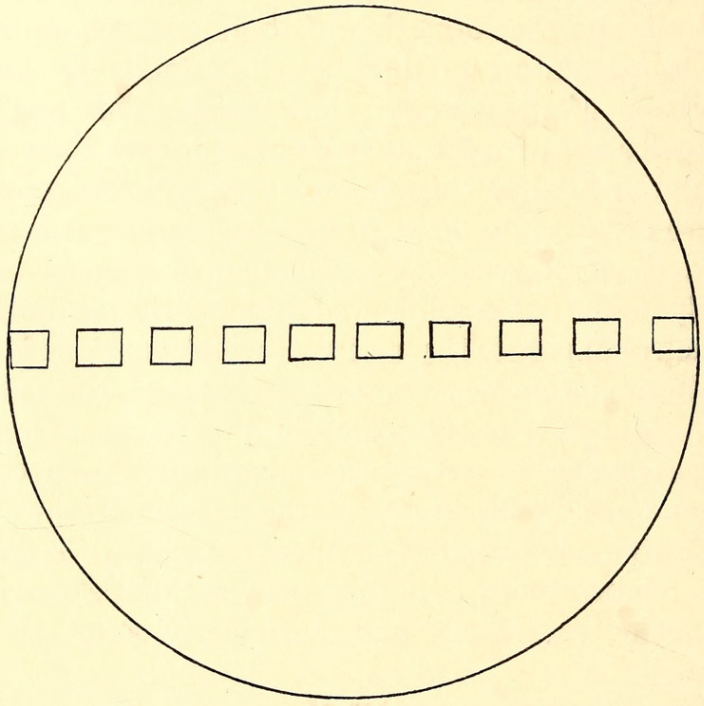

FIG. 1. Diagram showing the arrangement of the spaces in which the oogonia were counted.

The bottom of the Petri dish was then inverted and laid on the mycelium, so that the whole lay flat and firm between the two halves of the Petri dish. A square ruled micrometer had been fitted to the

eyepiece of a Leitz compound erecting body and the number of oogonia in a square, having an area of 25 square millimeters, was determined under a Leitz number two objective. The oogonia are, of course, not uniformly distributed over the mycelium, and in order to find the average number, the oogonia in ten squares were counted, beginning at one edge and following a line across the mass. As a rule the oogonia are more numerous a little back from the edge than in the middle, but this is not always the case.

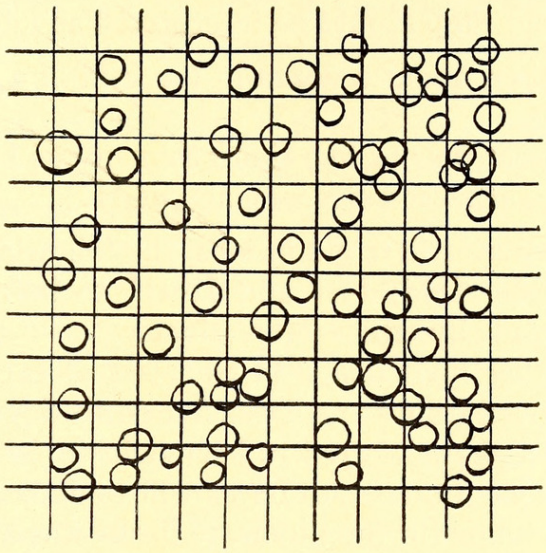

FIG. 2. Diagram showing the arrangement of the oogonia in one of the spaces. Magnified about 100 times.

The diagrams, text figures 1 and 2, show the method of counting and Table I gives some counts showing the distribution of the oogonia on the mycelium. This matter will be referred to again.

TABLE I

Illustrating Method of Counting Oogonia. Each Number Represents the Oogonia in One Square of 25 Square Millimeters. Oogonia out of Haemoglobin 0.075 *Percent or Haemoglobin* 0.05 *Percent*

From Set III

Peptone and levulose	40,	85,	63,	52,	81,	87,	144,	115,	82,	37—	Ave. 78.6
Cross count	14,	41,	26,	145,	92,	76,	76,	86,	66,	49—	" 67.1
Duplicate	44,	80,	88,	72,	79,	85,	70,	76,	76,	85—	" 75.8
From pea	3,	10,	11,	12,	78,	76,	60,	61,	72,	37—	" 42.1
Cross count	4,	8,	13,	15,	22,	79,	122,	97,	37,	27—	" 42.4
Duplicate	6,	65,	89,	80,	57,	75,	66,	23,	13,	19—	" 49.3

From Set VIII

Peptone and levulose.....42, 84, 97, 87, 120, 72, 135, 150, 209, 191—Ave. 118.7

The advantage of this method lies in the fact that the mycelium studied is practically all in one plane of focus, only a slight turn of the focusing screw being required to enable the observer to count every oogonium. That such counting of oogonia was extremely tedious and time consuming is quite obvious; consequently the quicker method of Klebs was also used. In the following tables wherever the Roman numerals are used they should be interpreted as follows:

I. A fair number, about a dozen in the field of a Leitz number two objective.
II. Many, at least four or five times as many as I.
III. Very many, at least twice as many as II.

To express intermediate gradations, the following have been used: 0–I, a few in each field; I–II and II–III to indicate numbers not properly expressible by either figure alone.

Transferring the mycelium.—In each set of experiments the mycelium was removed from some flasks five to six days after inoculation, washed, cut up and transferred to haemoglobin for the production of oogonia, or to some other solution as will be indicated in the proper place. The mycelium in the duplicate flasks was allowed to grow for some time longer and was then washed, dried at 100 degrees and weighed. The length of time the fungus was allowed to grow before being weighed was, of course, precisely the same for every lot of a series; with the exception of series I this time was thirty days. The capsules containing the haemoglobin or other solution were always in duplicate, and sometimes in quadruplicate, and were kept at a temperature as nearly uniform as possible. This varied roughly between 13 and 20 degrees, the extremes being only occasionally reached; usually the temperature was between 15 and 18 degrees. The first experiments were made at Heidelberg and the mycelium was not weighed, nor were the oogonia counted. The later experiments were made at Ann Arbor.

Klebs showed that the most important condition for the production of oogonia by *S. mixta* was a gradual decrease in the food supply and that for the formation of sporangia a sudden, or at least a rather rapid decrease in the concentration of the food, was necessary. To secure oogonia, then, the mycelium must be placed in such an environment

that, while no rapid growth takes place, the formation of sporangia shall be almost or entirely prevented. It is evident that a mycelium, exhausted by the formation of sporangia, cannot produce the number of oogonia that it could have produced under other conditions. Haemoglobin, in concentrations to suit, offers such a medium, but unfortunately the commercial article is not of uniform purity, always leaving more or less of an insoluble precipitate. Klebs ('99, p. 520) states that in 0.05 percent haemoglobin only vegetative growth took place and that even in 0.01 percent sporangia were but sparingly produced. The forms of *S. mixta* collected by the writer formed sporangia readily in 0.05 percent haemoglobin and some even in 0.075 percent, and this was also true of *S. ferax* and of *S. monoica*. While Klebs's form may have developed sporangia less readily than those which the writer used, the difference in the results is doubtless due largely to the relative purity of the haemoglobin. As a consequence concentrations thought to be the same are not always so, and, owing to the delicate balance between growth and sporangia formation existing in a weak solution, a slight difference may result, either in the formation of many sporangia, and in the consequent weakening of the mycelium, or in an undue amount of growth with the attendant formation of the poisonous products of metabolism. In either case the formation of oogonia will be interfered with.

Purified water.—The earliest tests made in Heidelberg brought out the fact that distilled water from the chemical laboratory had a harmful effect on the mycelium. Horn ('04) had already observed this and had attributed it to the presence of copper, or of some other metal in the water. He found that when common distilled water was re-distilled out of hard Jena glass, it lost its poisonous properties. This was not found to be so in the present case. Distilled water was twice re-distilled out of hard Jena glass, but, although the latter was better than the distilled water as it was received from the chemical laboratory, it was by no means as good as freshly collected rain water. The action of the distilled water was not uniform, sometimes wholly preventing the formation of sporangia, while at other times some sporangia were formed but did not discharge normally. This was also found to be true sometimes of the rain water, which, though often satisfactory, was not infrequently quite as toxic as the distilled water. Much of the work first done had to be discarded because of this unreliable action of the water. The use of carbon black as described by

Livingston ('05) was then tried and it was found that this gave uniform and satisfactory results. Out of the many records available on this point only one table will be given to show the effect of the water on the formation of sporangia. Tap water was also found to be quite as unsatisfactory as distilled water.

TABLE II

Achlya racemosa, Grown Three Days in Culture Solution N[3]*; Transferred to Water for the Formation of Sporangia*

1	2	3	4
	Sporangia in 12 Hrs.	In 24 Hrs.	48 Hrs.
Distilled water from chemical laboratory.....	0, Mycelium dying.	Dead.	
Distilled water shaken up with carbon black.....	I + discharged.	III	Exhausted.
Distilled water re-distilled and filtered through carbon black.....	I + discharged.	III	Exhausted.
Double distilled rain water.	Few formed, none discharged.	Few discharged.	Some gemmae.
Double distilled rain water filtered through carbon black.....	II + discharged.	III	Exhausted.

Similar results were secured with other species. All species are not equally sensitive to the water, however, but all gave more uniform results when water purified with carbon black was used. In all the following experiments, therefore, whenever the ability of a mycelium to produce sporangia was to be tested purified water was used.

To determine whether the use of this carbon black would introduce an unknown food element into the water some carbon black was carefully washed by shaking it up with a small quantity of water, which was then filtered off. Mycelium was transferred to this water but in no case did any growth result, though sporangia were at once formed showing that the water contained no food.

Culture media.—With the exception of pea extract, only synthetic media were used. Many of the various natural media, such as ants' eggs, larvae, and so forth, might have been used and might have given interesting results. It was desired, however, in the first place to use, so far as possible, only media of known composition; and in

[3] This solution contained 0.1 percent peptone, 1 percent sucrose and 0.1 percent salts.

the second place to weigh the mycelia. Any insoluble substances would introduce an element of error by adhering to the fungus hyphae. When pea extract was used this was prepared by sterilizing about six peas with a hundred cubic centimeters of distilled water. If carefully sterilized, the peas did not break up. Any flasks in which the peas had broken up were discarded for these experiments, as the loose starch grains would surely adhere to the fungus and increase the weight. Peptone was used as a source of both nitrogen and carbon and in a few cases haemoglobin was used. Asparagin and leucin were tried but the growth was so poor that these substances were not considered satisfactory; Klebs ('99, p. 520–523) also found that growth took place in lower concentrations of peptone than of leucin. The latter is very expensive to use for so large a number of experiments as had been planned, and is moreover of a rather uncertain quality. In the course of the work there was occasion to try the leucin from three prominent German makers and no two were found to give like results. Synthetic leucin was once used, but proved to be so toxic that little growth took place in it. Carbohydrates were added as an additional source of carbon. At first sucrose alone was used, but later maltose, levulose and dextrose were added. For salts, potassium nitrate, magnesium sulphate, and potassium phosphate, either mono- or di-basic were added. Wherever in the tables the term "salts" is used, the above salts, in concentration to total 1/100 or 1/200 molecular, are meant. In the earliest experiments these salts were used in a strength of 0.1 percent or 0.05 percent but later molecular weights only were used. In some cases each salt was used separately.

Experimental

Experiments with Achlya racemosa

This species offers advantageous material because it will produce in water oogonia with oospores and antheridia. It is not necessary to transfer to another food solution; it is only necessary to prevent the too rapid formation of sporangia. This can be done by placing the mycelium in a temperature of 24 degrees, at which temperature few sporangia are formed, while the formation of oogonia is not interfered with. The mycelia for the following experiments were grown for seven days in 50 cc. of the following solutions.

(1) Peptone 0.1 percent, sucrose 1 percent, salts 0.1 percent; (2) peptone 2 percent; (3) peptone 1 percent; (4) peptone 0.5 percent;

(5) peptone 0.1 percent; (6) peptone 0.05 percent; (7) peptone 0.01 percent; (8) peptone 0.1 percent, salts 0.1 percent; (9) peptone 0.1 percent, salts 0.05 percent; (10) peptone 0.1 percent, sucrose 1 percent; (11) peptone 0.1 percent, sucrose 0.5 percent.

At the end of seven days a mat had been formed on the surface of the solutions, numbered 1, 8 and 9 while the quantity of mycelium was in the following order, numbers 1, 8, 9, 2, 3, 4, 10, 11, 5, 6, and 7.

As before stated, no weighings were made for this species, but careful notes were taken of the apparent mass and condition. From these it appears that the complete solution, such as was suggested by Obel, solution number 1, produced the best growth; that a mat was formed only in those solutions containing salts and that those solutions were much better for growth than those containing the same amount of peptone and sugar, but no salts. There was very little difference between the mass of mycelium produced by solutions of 0.5 percent or 1 percent peptone or by peptone and sucrose, but the mass of mycelium out of 2 percent peptone was many times greater

TABLE III

Achlya racemosa. Mycelia Grown Seven Days in Media Given in Column 2. Transferred to Water at 18°

1	2	3 Sporangia, 1 Day.	4 Sporangia, 2 Days.	5 Oogonia, 1 Day.	6 Oogonia, 2 Days.	7 Oogonia, 5 Days.
1	Peptone 0.1%, sucrose 1%, salts 0.1%	II–III	II[4]	0–I	I	I[5]
2	Peptone 2%	II	II	0–I	0–I	0–I
3	Peptone 1%	II–III	I	0–I	I	II
4	Peptone 0.5%	II	III	I	I	II
5	Peptone 0.1%	0–I	0	I–II	II	III
6	Peptone 0.05%	0	0	II	II–III	III
7	Peptone 0.01%	Very few	0	II	III	III oogonia mostly empty
8	Peptone 0.1% + salts 0.1%	II–III	0–I	I	III	III
9	Peptone 0.1% + salts 0.05%	II+	I	0	I	I
10	Peptone 0.1% + sucrose 1%	0	0	I	II	III
11	Peptone 0.1% + sucrose 0.5%	0	0	II	II	III oogonia mostly empty

[4] The number of sporangia given for the second day is in addition to those first recorded.

[5] The number of oogonia recorded for each day represents the number then present and not a number in addition to those previously recorded.

than that out of 0.01 percent peptone, as was to be expected. After being washed the mycelia from these solutions were transferred to water at the laboratory temperature, about 18° C. Sporangia were readily produced and Table III shows the relative number of sporangia and oogonia from mycelia grown in various culture media.

With the exception of the mycelium out of 2 percent peptone, there is roughly an agreement between the mass of mycelium and the number of sporangia formed, and it is clear that a poorly nourished mycelium will not make many sporangia. The addition of salts to the peptone not only increased the vegetative growth but also the number of sporangia, a result not secured with sugar. So far as could be observed the mycelium from the 0.5 percent peptone developed as many sporangia as did that from 1 percent or from 2 percent, while that from 0.1 percent showed a falling off. This would seem therefore to be the critical concentration, below which a still more decided falling off in the number of sporangia is to be noted. The same mycelium developed both sporangia and oogonia in water, and the

TABLE IV

Achlya racemosa. Mycelia Grown Seven Days in Media Under Column 2. Transferred to Water at 24°

1	2	3	4	5	6	7	8
	Media.	Sporangia, 1 Day.	Sporangia, 2 Days.	Oogonia, 1 Day.	Oogonia, 2 Days.	Oogonia, 5 Days.	Remarks.
1	Peptone 0.1%, sucrose 1%, salts 0.1%.....	Few	0	II	III	III	Many without oospores.
2	Peptone 2%.....	I	I	I	I	II	Oogonia larger than in No. 1 and well filled.
3	Peptone 1%.....	0–I	0–I	II	II	III	Oogonia large, mostly with one large oospore.
4	Peptone 0.5%....	I–II	0	II–III	III	III	
5	Peptone 0.1%....	Very few	0	II–III	III	III	As in number 3.
8	Peptone 0.1%, salts 0.1%.....	0	Rare	II–III	III	III	Some empty oogonia.
9	Peptone 0.1%, salts 0.05%....	0	0	II–III	III	III	About 20% empty.
10	Peptone 0.1%, sucrose 1%....	0–I	0	III	III	III	About 30% empty.
11	Peptone 0.1%, sucrose 0.05%..	0	0	III	III	III	About 50% empty.

table shows that there was no agreement between mass of mycelium and number of oogonia, which fact may, however, have been due to the exhaustion of the mycelium by reason of the sporangia produced.

By keeping the mycelia after transfer to water at a temperature of 24°–25° C. the production of sporangia could be almost wholly prevented. This was done and the result is given in Table IV. The cultures used in this case were duplicates of those used for the previous experiment.

It will be noted that at this temperature the production of sporangia was almost inhibited. The number of oogonia present at the end of five days was in every case great; but it was also noted that the oogonia on mycelium from 1 percent and from 0.1 percent peptone were large, though containing but one oospore, while in all other cases, except the mycelium from 2 percent peptone there were many empty oogonia, sometimes as many as 50 percent. In this series the largest number of oogonia, containing oospores, was produced by the mycelium having the smallest mass.

From the same culture series another set, now seventeen days old, was taken and placed in water. After five days the number of oogonia found was as shown in Table V.

TABLE V

Achlya racemosa. Mycelia Grown Seventeen Days in Media Under Column 2. Transferred to Water at 18°

1	2 Media	3 Oogonia in 5 Days
3	Peptone 1%	III, oospores 2–3 in an oogonium.
4	Peptone 0.5%	III, large, 2–4 oospores.
5	Peptone 0.1%	II–III large oospores.
6	Peptone 0.05%	II, smaller than in 0.1 and few oospores.
7	Peptone 0.01%	I–II, few with oospores.
8	Peptone 0.1%, salts 0.1%	II–III, large and good. There have also been many sporangia.
9	Peptone 0.1%, salts 0.05%	0–I, there have been many sporangia.
10	Peptone 0.1%, sucrose 1%	II, small, but with oospores.
11	Peptone 0.1%, sucrose 0.5%	II, mostly with oospores.

The table shows that, while above a certain concentration there is no relation between mass of mycelium and the number of oogonia produced, there is a concentration below which the lack of food is manifested in the smaller number of oogonia formed. In the case of

Achlya racemosa, this limit lies between 0.1 percent and 0.05 percent peptone. Above this limit there was an increase in the growth, whether the concentration of the peptone only was increased, or whether salts or sugar were added, but there was no corresponding increase in the number of oogonia produced. Results similar to those given in the above tables were secured from other tests and we may conclude that for *Achlya racemosa* there is a concentration of food which, while it will enable the fungus to make some growth, will so reduce its vigor as to interfere markedly with the production of sporangia and of oogonia. Above this critical concentration the effect of the food will be marked by an increase in weight without a corresponding increase in the number of sporangia or of oogonia formed when the mycelium is transferred to water. For the production of the maximum number of sporangia this concentration lies above 0.1 percent peptone while for the production of oogonia it lies between this and 0.05 percent peptone.

Experiments with Saprolegnia ferax (Gruith) Thuret

This species was collected at Heidelberg in the fall of 1911. One spore was isolated, and all the cultures made during the past three years have been with material derived from this original culture. This species was first clearly separated from other members of the "ferax group" as *S. Thureti* by DeBary in 1881 ('81) when he called attention to the fact that antheridia were scarcely ever found. Later ('88) he distinguished *S. mixta* as having antheridia on about one half of the oogonia and as having a more slender mycelium than that of *S. ferax*. A careful study of the form collected as number 25 failed to disclose antheridia and a comparison of this form with another collected as number 17 led to the conclusion that number 25 should be identified as *S. ferax* while number 17 had the characters of *S. mixta*. Humphrey ('92) has discussed the nomenclature of these species and since his point seems well taken the name *S. ferax* (Gruith) Thuret is used here instead of *S. Thureti* as this species was called by DeBary. The fact that antheridia were found later has been recorded in a paper read before the Michigan Academy of Science at the meeting of April 1915, together with a discussion of the "ferax group" of the genus Saprolegnia (see Pieters, '15).

S. ferax furnishes favorable material, since the oogonia are quite large and are readily produced. Klebs had already shown that

S. mixta produced oogonia readily in 0.05 percent haemoglobin and Kauffman that *S. hypogyna* produced oogonia readily under similar conditions. Preliminary tests showed that *S. ferax* behaves like the above species and in all subsequent work haemoglobin, either alone or with salts and sugars, was used as the transfer solution when it was desired to test for oogonia. Unfortunately commercial haemoglobin is not of uniform purity and it sometimes happened that a solution made up to 0.05 percent was stronger or weaker than expected and thus either encouraged unusual growth or the formation of sporangia. Either event worked disadvantageously for the production of oogonia.

The different tests could not of course be carried on at the same time and hence were subject to variations in temperature. Klebs showed that a temperature of 18° C. was favorable for the production of oogonia in *S. mixta* but under the conditions prevailing in the laboratories of the University of Michigan such temperatures are almost impossible to maintain. The room temperature is generally 20° C. and above, temperatures at which oogonia are not readily produced. Some tests were therefore carried on in the basement of a private house while others were conducted in a small closet built in from a window in the botanical laboratory and opening to the outside by a swinging window. By doing the work in the winter and by using an electrically heated incubator, fairly satisfactory temperatures could be maintained but not exact enough to reach any conclusions as to the optimum temperatures for reproduction. The following record will, however, show the difference between the number of oogonia produced at lower and at higher temperatures. Mycelia

TABLE VI

Saprolegnia ferax. Mycelium Out of Media Under Column 1. *Transferred to Haemoglobin and to Haemoglobin and Salts at Different Temperatures. Average Number of Oogonia in* 25 *Square Millimeters*

1	2	3	4	5
Medium	20°–23° C. Haemoglobin 0.05%	14°–18° C. Haemoglobin 0.05%	20°–23° C. Haemoglobin 0.05%, Salts 0.1%	14°–18° C. Haemoglobin 0.05%, Salts 0.1%
Peptone 2%	13	70	—	198
Peptone 1%	19	67	15	142
Peptone 0.1% + Knop's Sol. 0.05%	15	176	5	157
Peptone 0.2% + sucrose 1%	5	51	4	163
Peptone 0.1% + sucrose 0.1%	21	59	15	155

from various solutions were transferred February 14, 1913 to 0.05 percent haemoglobin and to haemoglobin and salts, one set being kept in the laboratory where the temperature ranged from 20° to 23° C. and often higher while another set was kept in the basement of the writer's home at a temperature varying between 14° and 18° C.

Production of sporangia.—The optimum temperature conditions for the production of sporangia are quite different, a high temperature causing the sporangia to develop more rapidly than a low one. Klebs found this to be the case in *S. mixta* also. At a temperature of 28° C. *S. ferax* formed young sporangia in five and one-half hours and these were discharged in seven hours from the time the mycelium was put into water. A duplicate lot from the same culture, kept at laboratory temperature (about 20° C. at that time), formed young sporangia in seven hours and these were discharged in nine hours. The maximum temperature at which sporangia were formed in this species was 30°+ C. at which some swarming took place in six and three quarter hours but the spores left the sporangium slowly and were evidently feeble. In another test duplicate lots of mycelium were placed in water at 28° C., at laboratory temperature 18°–20° C., and in an ice box where the temperature was between 6° and 8° C. Table VII shows at the same time the effect of the higher temperature on the production of sporangia and also the relative number of sporangia formed in mycelia out of different foods.

TABLE VII

S. ferax. Mycelia Out of Media Under Column 1. *Transferred to Water at Different Temperatures for the Production of Sporangia*

1	2	3	4	5
	At 28°. 16 Hrs.	18°–20°. 16 Hrs.	6°–8°. 16 Hrs.	6°–8°. 22 Hrs.
Peptone 0.1%, sucrose 1%, salts 0.1%	III	III	II, none discharged	III, just discharging.
Peptone 2%	III	III	I, none discharged	I, none discharged.
Peptone 0.5%	II+	II+	I, none discharged	I, a few discharged.
Peptone 0.1%	II+	II+	I, none discharged	I–II, a few discharged.
Peptone 0.05%	I	I	0	0–I, a few discharged.
Peptone 0.01%	0–I	0	0	0, a few discharged.
Peptone 0.1% + sucrose 1%	III	II	0–I, none discharged	I–II, discharging.
Peptone 0.1% + salts 0.1%	III	III	0–I, none discharged	I, discharging.

The fact that no examination was made before sixteen hours explains why there is no apparent difference between the number of sporangia produced at 20° and at 28° C. It may also be noted how the higher temperature helps to conceal the difference in strength between the well- and the poorly-nourished mycelia. When many sporangia are formed at high temperatures it is really impossible for the observer to make a close comparison between them. At the low temperature, however, the difference in vigor is more clearly brought out. Here again, as noted before for *Achlya racemosa*, the difference between mycelia grown in 2 percent, 0.5 percent, and 0.1 percent peptone is not very marked though the first had food at a concentration twenty times greater than the last. But as soon as the concentration of the food falls below 0.1 percent the effect is quite marked; the critical concentration lies between 0.1 and 0.05 percent.

Production of oogonia.—It will be necessary before presenting the results of the experiments in which oogonia were counted to discuss more fully than was done earlier the reliability of the method of counting the oogonia. When a mycelium is spread out between two glass plates the oogonia are sometimes scattered quite uniformly over the surface of the plate, but more often the largest number of oogonia lie near but not just at the margin and again near the thickest part of the mass, which is the original piece put into the dish of haemoglobin. When the oogonia are uniformly scattered the count can be made along any line, but sometimes the observer must select a line that will lie across an average field or must count along a second line at right angles to the first. It may be asked whether such a count will accurately represent the number of oogonia on a given mycelium. The answer to this is that with few exceptions two counts of oogonia on lines at right angles to each other agree closely. It may also be asked whether such a count means anything, whether it is not rather wholly fortuitous and whether another lot under the same conditions might not show a very different number. It must be admitted that there is an element of uncertainty here which the writer would have been glad to eliminate. Living forms do not always behave with apparent uniformity; cases occurred in which the mycelium in one dish of a pair showed many oogonia while on the duplicate not one was to be found. Such instances however were exceptional. As a rule the number of oogonia was nearly the same in each dish of a pair as will be seen from the tables and it is believed that

where duplicate pieces of mycelium placed under identical conditions give similar results, such results may be assumed to correctly represent the response of the plant to these conditions. In Tables VIII and IX a number of figures are given to show that counts at right angles to each other agree substantially and that the counts from duplicate dishes also agree pretty closely. While the duplicate dishes were not always counted, because of the great amount of labor involved in this work, they were always examined and when the number of oogonia was strikingly different from that in the dish counted this fact was noted. In all other cases the oogonia were either counted or the fact noted that the two dishes were in substantial agreement. No oogonia were counted that did not contain oospores. Table VIII has been compiled from the record of Series III transferred November 19, 1914. In no case is there a great difference between the counts at right angles to each other, and only in numbers 6, 7 and 12 is there any considerable difference between the number of oogonia in the two dishes of a set. Such a difference was often due to contamination of one dish by bacteria, but whatever the cause, whenever there was as great a variation as is shown by numbers 6, 7 and 12, the highest average was used in the final tables.

TABLE VIII

S. ferax. Mycelia Out of Media Given Under Column 2. *Transferred to* 0.075 *Per cent Haemoglobin. All Sugars in the Culture Media* 1/50 *Molecular. Temperature* 16°–18° *C. All Figures Given to the Nearest Whole Number*

1	2	First Dish			Second Dish			9
		3	4	5	6	7	8	
	From Medium	First Count	Cross Count	Average	First Count	Cross Count	Average	Average of All
1	Pea	42	42	42	49	—	49	46
2	Peptone 0.1%	19	—	19	23	—	23	21
3	Peptone 0.2%	49	44	47	45	—	45	46
4	Peptone 0.5%	54	41	47	27	31	29	38
5	Peptone 1%	41	39	40	43	—	43	41
6	Peptone 0.1% + sucrose	25	34	29	11	15	13	21
7	Peptone 0.1% + maltose	65	65	65	25	28	27	46
8	Peptone 0.1% + dextrose	29	28	29	28	—	28	28
9	Peptone 0.1% + levulose	79	67	73	76	—	76	74
10	Peptone 0.2% + sucrose	19	26	22	36	32	34	28
11	Peptone 0.2% + maltose	46	46	46	40	—	40	43
12	Peptone 0.2% + dextrose	23	25	24	8	13	11	17
13	Peptone 0.2% + levulose	33	29	31	55	—	55	43
20	Haemoglobin 0.1%	23	22	23	21	—	21	22

In Table IX another record is given to show that there is substantial agreement between the number of oogonia found on mycelia from like origin and that mycelia grown in different media may vary in their capacity to produce oogonia. It is not urged that the figures obtained have the value of those commonly secured in a seed test, for example, for there are too many unknown and uncontrollable factors; but they are offered as a more exact statement of the relative number of oogonia produced on various mycelia of a given series than could be made in any other way.

TABLE IX

S. ferax. Part of Record from Set I Showing Agreement Between Count and Cross Count. Mycelia in 0.05 *Percent Haemoglobin. Temperature* 16°–18° *C. Figures to Nearest Whole Number*

1	2	3	4	5
		1st Count	Cross Count	Average
1	Pea	40	44	42
2	Peptone 1%	37	31	34
	" 1% duplicate	43	45	44
3	Peptone 0.5%	36	32	34
4	Peptone 0.2%	27	34	31
	" 0.2% duplicate	26	23	25
5	Peptone 0.1%	16	16	16
6	" + sucrose	19	17	18
7	" + maltose	43	38	41
8	" + sucrose + salts	27	28	28
9	Peptone + maltose + salts	44	40	42
10	Peptone 0.2% + sucrose	55	52	54
11	" 0.2% + maltose	45	50	48
12	" 0.2% + " + salts	64	47	56
13	" 0.1% + salts	32	33	33
14	" 0.2% + "	32	38	36

Record of Series I, October, 1914.—For this experiment the food solutions were contained in tubes each holding 50 cc. of liquid. These were inoculated and allowed to grow for seven days and transferred to 0.05 percent haemoglobin October 1, 1914. At the time the mycelium from one tube was transferred to haemoglobin, that from a duplicate was washed, dried and weighed, and a third tube was left for forty-eight days and the contents then washed, dried and weighed.

Table X gives the complete record of this test. Unfortunately a rather large number of sporangia were produced in the haemoglobin, especially by the mycelium from pea and from peptone 1 percent and this interferred materially with the production of oogonia. In the

following discussion the record of these two will be disregarded as being probably too greatly affected by the production of sporangia.

TABLE X

Series I. Saprolegnia ferax. Mycelia Out of Media Under Column 2, Transferred to 0.05 *Percent Haemoglobin; Sugars and Salts* 1/100 *Molecular Except as Noted. Weight in Milligrams of Mycelia Out of* 50 *cc. Liquid. Record of Sporangia and Oogonia Produced. Figures for Number of Oogonia to Nearest Whole Number*

1	2	3 Weight, 7 Days.	4 Weight, 48 Days.	5 Sporangia in Water, 7 Hrs.	6 Sporangia in Water, 24 Hrs.	7 Sporangia, Haemoglobin, 3 Days.	8 Oogonia, 10 Days.
1	Pea	0.018	0.056	III	III	III, oogonia started	42
2	Peptone 0.1%	0.002	0.005	II	II–III	I+	16
3	" 0.2%	0.003	0.011	I–II	II	I+	30
4	" 0.5%	0.004	.....	II+	II–III	I–II	34
5	" 1%	0.0036	0.037	I–II	II–III	II–III	36
6	" 0.1%, sucrose	0.0035	0.007	0–I	I+	0, oogonia started	18
6*m*	" 0.1%, maltose	0.0044	0.033	II	II–III	I	41
7	" 0.1%, sucrose and salts	0.0054	0.010	0–I	I+	I–II	28
7*m*	" 0.1%, maltose and salts	0.004	0.033	I	II–III	0–I	42
8	" 0.2%, sucrose	0.0054	0.013	0–I	II	0–I	53
8*m*	" 0.2%, maltose	0.005	0.036	0–I	II	II	43
9	" 0.2%, sucrose and salts	0.006	0.020	Few	I–II	II	51
9*m*	" 0.2%, maltose and salts	0.005	0.044	Few	II–III	I–II	50
10	" 0.1%, salts	0.002	0.0066	0	I–II	I	36
11	" 0.2%, "	0.0023	0.007	Few	I–II	I	28
12	" 0.2%, " 1/333 molecular	0.003	.....	0	I–II	0	43

If these records are arranged in two groups, the first containing those in which 16–36 oogonia were found in 25 square millimeters, and the other those with 41–53 oogonia per square it will be noted that the first group contains those culture media without sugar, except for numbers 6 and 7, in which sugar was used. The second group includes all those with sugar except 6 and 7 and also one (12) to which no sugar was added. See Table XI.

The weights for the seven-day-old mycelia are, as a rule, higher in the second than in the first group and this is even more striking

TABLE XI

Series I. Saprolegnia ferax. Record of Table X Arranged in Two Groups According to the Number of Oogonia Per Square of 25 Square Millimeters. Weights in Milligrams

1	2	3	4	5
No.	Number of Oogonia	Weight in 7 Days	Weight in 48 Days	Increase in Weight
Group I:				
2.....	16	.002	.005	×2.5
6.....	18	.0035	.007	×2
7.....	28	.0054	.010	×1.85
11.....	28	.0023	.007	×3
3.....	30	.003	.011	×3.3
4.....	34	.004	——	—
10.....	36	.002	.0066	×3.3
Group II:				
6*m*.....	41	.0044	.033	×7.5
7*m*.....	42	.004	.033	×8
8*m*.....	43	.005	.036	×7
12.....	43	.003	——	—
9*m*.....	50	.005	.044	×9
9.....	51	.006	.020	×3.3
8.....	53	.0054	.013	×2.5

when the weights of the mycelia forty-eight days old are compared. Number 12 has the one disturbing record in this series. With a small weight, it combines a rather large number of oogonia. From the weight of mycelium as well as from the composition of the food, it was to be expected that this lot would appear in a group with numbers 2, 3, 10, and 11. The explanation of the peculiar behavior of this lot doubtless lies in the fact that no sporangia were produced in haemoglobin. As has already been shown, the suppression of sporangia, in a solution of this sort, reacts very favorably on the formation of oogonia, but why no sporangia were formed in this case it is impossible to say. A study of the record in Table XI would seem to show an agreement between weight of mycelium and the number of oogonia produced. But this is true only as showing a trend toward a minimum food requirement. In group I, for example, 0.1 percent peptone is clearly too poor a food to produce vigorous mycelium and the addition of sucrose alone does not add to the vigor of growth. When, however, salts are added, or higher concentrations of peptone used, the number of oogonia at once approaches that of the lower numbers in the second group. The separation of the groups along the line between 36 and 41 oogonia is, of course, arbitrary and only

for the purpose of illustration. Differences as great as between 28 and 41 might well be found between two counts of the same dish, but no two duplicate dishes would, without contamination or other accident, show as great a difference as that between 28 and 53.

While the upward trend in the number of oogonia, with the increase in the food furnished, is evident, there is no corresponding increase in the weight of mycelium. It will be noted also that the amount of increase in the weight of mycelium between seven and forty-eight days is by no means uniform and a study of this feature brings out a rather interesting point. In Table XII these data are arranged in the order of the increase in weight of each mycelium due to the growth from the seventh to the forty-eighth day.

TABLE XII

Series I. S. ferax. Record of Table X Arranged in the Order of the Increase in Weight, Due to Growth Between Seven and Forty-eight Days

1	2	3	4	5	
Record Number	Amount Increase	Weight 7 Days	Weight 48 Days	Number of Oogonia	
7.....	×1.85	.0054	.010	28	Group I. Average number of oogonia 29. Average excluding No. 8—21.
6.....	×2	.0035	.007	18	
2.....	×2.5	.002	.005	16	
8.....	×2.5	.0054	.013	53	
11.....	×3	.0023	.007	28	Group II. Average number of oogonia 36.
10.....	×3.3	.002	.0066	36	
3.....	×3.3	.003	.011	30	
9.....	×3.3	.006	.020	51	
8*m*.....	×7	.005	.036	43	Group III. Average number of oogonia 47.
6*m*.....	×7.5	.0044	.033	41	
7*m*.....	×8	.004	.033	42	
9*m*.....	×9	.005	.044	50	

Of the first group, those showing an increase in weight of not more than 2.5 times, number 8 is the only one credited with a large number of oogonia; of the second group number 9 is credited with a much larger number of oogonia than the others. The third group differs strikingly in the much greater increase in weight shown by all the mycelia. Reference to Table X will show that the culture medium for all those in group III contained maltose, and that this group includes only those in which maltose was added to the culture medium.

The record of numbers 2 and 6 may be compared since in both cases 0.1 percent peptone was available, but sucrose had been added

to solution 6. It is apparent that the fungus was not able to utilize sucrose in the presence of this strength of peptone while it made free use of maltose as shown by the record of number 6*m* which also contained 0.1 percent peptone but to which maltose had been added instead of sucrose. Numbers 8 and 9 contained the same amount of sucrose as 6 had and in addition 0.2 percent peptone, and 0.2 percent peptone and salts respectively, and on these mycelia were developed the largest number of oogonia of any lot in the series. This large number of oogonia cannot be accounted for by the higher percentage of peptone alone nor by the presence of salts alone, as then numbers 3 and 11 should have given comparable results. It is evident that in numbers 8 and 9 the sucrose was used but the weight record indicates the use of only a small part of it. During the first seven days' growth the fungus was very well nourished as shown by the fact that the weights at the end of that time are among the highest. This rate of growth, however, was not maintained as is shown by the small increase in weight. The explanation is doubtless that in numbers 8 and 9 part of the sucrose was changed to invert sugar owing either to too prolonged heating at high pressure while sterilizing, or to the presence of salts in the solution together with a high temperature. If this is true the fungus would find invert sugar present as soon as new growth began; development would then be rapid in the next few days or as long as the invert sugar lasted. Upon the exhaustion of the invert sugar growth would slow down and the final weight would be less than that from solutions containing more available carbohydrates than sucrose. Further, if the fungus were removed before the invert sugar present was consumed it would be in the same physiological condition as it would have been if all the carbohydrate consisted of invert sugar.

These theoretical considerations are supported by the facts of the experiment. After seven days numbers 8 and 9 had made as large a growth as any lot, but at the end of forty-eight days the total increase was 2.5 times the weight at the end of seven days, as compared with 7–9 times the weight in the case of 8*m* and 9*m*. Growth was at first rapid, then slowed down; the invert sugar was used up. That the mycelium at the end of seven days was in good condition is shown by the large number of oogonia produced.

At the end of seven days and while the fungus in solutions 8 and 9 was in the full enjoyment of an abundant supply of invert sugar the

mycelium was transferred to haemoglobin; that this mycelium was then in good condition is shown by the large number of oogonia produced. We may then conclude that invert sugar is an especially valuable food for the subsequent production of oogonia; that sucrose cannot be utilized by this fungus, but that maltose is used and is a good food both for vegetative growth and for the production of oogonia.

(In series II the oogonia rarely developed oospores. This was due probably to contaminated haemoglobin. The entire set was discarded.)

Series III.—This series was prepared to repeat the work in series I and also to determine if possible which of the two sugars present in sucrose, dextrose or levulose, was most used by the fungus. Solutions containing salts as well as sugars were also prepared and cultures grown in them, but since the large number of cultures thus set up would make it impracticable to handle all at one time those with salts were transferred in a separate lot. For some reason not determined, all of the mycelia from the solutions containing salts failed to make oogonia or at most produced but a few oogonia each. Since this result was obviously due to some abnormal condition the entire lot was discarded and the results with the sugars alone are presented.

Table XIII gives all the data for this series.

TABLE XIII

Series III. Saprolegnia ferax. Mycelium Out of Culture Media in Column Two. Grown Seven Days Before Transfer to 0.075 *Percent Haemoglobin and Thirty Days in* 200 *cc. Liquid Before Weighing; Weights in Milligrams. Oogonia Count Recorded to Nearest Whole Number. Temperature* 15°–18° C. *Sugars All* 1/50 *Molecular*

No.	Food	Weight	Record After 2 Days		Oogonia After	
			Sporangia	Oogonia	3 Days	17 Days
1	Pea	0.041	I+	II	III	46
2	Peptone 0.1%	0.0172	0–I	0	Few	21
3	" 0.2%	0.0376	I	0–I	I	46
4	" 0.5%	0.079	II	0	0–I	38
5	" 1%	0.124	II	0	I	41
6	" 0.1% + sucrose	0.024	I–II	0	0–I	29
7	" 0.1% + maltose	0.068	II	0	I	65
8	" 0.1% + dextrose	0.023	II	0	0–I	28
9	" 0.1% + levulose	0.061	I	I	II–III	73
10	" 0.2% + sucrose	0.0404	II	0	I	28
11	" 0.2% + maltose	0.099	II	I	II	43
12	" 0.2% + dextrose	0.0474	II	0	Few	24
13	" 0.2% + levulose	0.065	II	Starting	0–I	55
20	Haemoglobin 0.1%	0.0199	I–II	0	Few	22

It will be noted that not so many sporangia were formed in the 0.075 percent haemoglobin as in the 0.05 percent used in series I, and that the numbers of sporangia were nearly the same in all the dishes, so that any influence on the production of oogonia can be disregarded. This table shows, as did Table X, that there is no constant relation between the amount of dry weight a culture medium will produce and the number of oogonia formed by the mycelium after transfer. In only one case do these figures agree; the mycelium out of 0.1 percent peptone is at the bottom of the list in both instances. The greatest weight was made by mycelium out of 1 percent peptone which was seventh in the production of oogonia. Mycelium out of solution number 9 had the largest number of oogonia and was sixth in weight. The second heaviest mass of mycelium was out of solution number 11 and this was sixth in the production of oogonia. The results secured in the first experiment are therefore confirmed so far as concerns this point. The value of maltose is also confirmed, and the great difference between the value of dextrose and levulose in the conditions of this experiment is brought out. Dextrose added to peptone increased the weight of mycelium slightly, but the number of oogonia not at all, while the addition of levulose produced a mycelium with a tendency toward the production of oogonia quite out of proportion to its value for vegetative growth.

In the course of this work many tests were made to determine the effect on the production of oogonia when salts or sugars were added to the haemoglobin. If it is true that levulose is an especially favorable form of carbohydrate for the production of oogonia, this fact should also be brought out if levulose is added to haemoglobin. This was done in a number of cases and the results confirm the conclusion that for *S. ferax* levulose has an especial value for the production of oogonia.

In Table XIV the results of one test are brought together.

TABLE XIV

Saprolegnia ferax. Mycelium Out of Pea Extract in Haemoglobin and in 0.025 *Percent Haemoglobin and sugars. In Every Case Concentration of Sugar Equals* 1/200 *Molecular. Figures Represent the Number of Oogonia Formed in* 25 *Sq. Mm. of Mycelium After Nine Days*

Haemoglobin 0.025%	Haemoglobin 0.05%	Haemoglobin + Dextrose	Haemoglobin + Levulose	Haemoglobin + Maltose	Haemoglobin + Sucrose
21	34	49	129	14	92

It will be noted that here sucrose has also had a marked effect, due doubtless, as before suggested, to the inversion of a part of the sucrose. In dextrose the growth was short and dense but did not fill the dish, while in maltose the growth was very dense and rapidly filled the dish with a mass of delicate hyphae. Possibly a lower concentration of maltose might have given better results. The growth in the haemoglobin-levulose solution was open but vigorous; oogonia appeared earlier than in the other dishes and the number formed was so much greater than the number produced by mycelium of the same origin in haemoglobin alone or in haemoglobin and dextrose that there can be no mistaking the conclusions that are indicated. The fact that these results confirm those secured when levulose was offered as a food during vegetative growth only makes them especially significant.

The results with *S. ferax* support the general conclusions arrived at in the experiments on *A. racemosa*. The weight of mycelium secured from any culture is no measure of the number of oogonia which that mycelium may be expected to produce when transferred to a suitable environment. There is however a minimum concentration of food at and below which both the weight of the dry matter and the number of oogonia decrease. This minimum is about at 0.1 percent peptone for the production of both sporangia and oogonia.

Levulose is especially valuable as a food for the formation of oogonia; dextrose is not used as freely and sucrose not at all unless previously inverted by other agencies, while maltose is readily used.

Experiments with S. monoica

S. monoica was used because it normally has an antheridium on each oogonium and oogonia are freely produced, even under somewhat adverse conditions. This species is closely related to *S. ferax*, and experiments with it might be expected to confirm the conclusions drawn from the results of previous experiments. It was also thought that some light might be thrown upon the kind of food that is best for the production of antheridia. Cultures were made in flasks containing 200 cc. of liquid in the same manner as for *S. ferax* series III; after growing five days these cultures were transferred to 0.05 percent haemoglobin.

Table XV gives the complete record for this set.

TABLE XV

Series VIII. S. monoica. Mycelium From Media Under Column 2, Transferred to 0.05 Percent Haemoglobin. Weight of Mycelium in Milligrams after Thirty Days' Growth in 200 cc. Liquid. In Numbers 6–17 the Amount of Peptone is Always 0.1 Percent, of Sugar 1/50 Molecular, and of Salts 1/200 Molecular

No.	Food	Weight, Milligram	Sporangia 2 Days	Oogonia 10 Days	Antheridia, Percent	Weight of Mycelium Out of Haemoglobin, Milligram
1	Pea	.0398	II	80	21	.0157
2	Peptone 0.1%	.0146	I	170	6	Lost
3	" 0.2%	.0256	I	146	9	.0109
4	" 0.5%	.0561	I–II	79	9.5	.0132
5	" 1%	.0863	II	108	7	.0138
6	" + sucrose	.015	II	62	6	.0120
7	" + maltose	.0598	II	58	12	.0144
8	" + dextrose	.0281	II	96	10	.0128
9	" + levulose	.0581	II	119	20	.0152
14	" + sucrose + salts	.3645	II	79	6	.0191
15	" + maltose + salts	.2151	0–I	52	15	.0219
16	" + dextrose + salts	.1810	II	105	13	.0188
17	" + levulose + salts	.2100	II	103	64	.0186

A striking feature of this record is the very large number of oogonia found on the mycelium from 0.1 percent and from 0.2 percent peptone. The preliminary tests showed that in this species mycelium from 0.1 percent peptone produced as many oogonia as that from 1 percent but such a large number as recorded in Table XV was quite unexpected. In this series the tests in haemoglobin were conducted in quadruplicate. Before making the final records all dishes were carefully examined and the number of oogonia in each was found to be practically the same. Probably the large number of oogonia is connected with the smaller number of sporangia in these lots, for, as has been said, a mycelium producing few sporangia is in better condition to produce oogonia than one that has produced many sporangia. This fact introduces an element of uncertainty into this work that is always present and must be taken into consideration in interpreting the results. Leaving the record of numbers 2 and 3 out of consideration it is apparent that levulose is a food favorable for the formation of oogonia out of proportion to its value for vegetative growth, and that sucrose with 0.1 percent peptone alone is probably not available to the plant.

The weight of mycelium out of solution number 6 is practically the same as that out of number 2. When salts are added to peptone and sucrose the number of oogonia produced is materially increased and the weight is much greater than from solutions without salts. As before suggested, this is probably due to the formation of invert sugar from the sucrose by the action of high temperature and salts.

There is evidently no relation between weight of mycelium and number of oogonia even when the record of numbers 2 and 3 is discarded. The weight of mycelium was about equal out of solutions 4, 7 and 9 but the number of oogonia varied from 58 on mycelium out of solution 7 to 119 out of solution 9. Again, mycelia out of solutions 15 and 17 show practically the same weight but vary in the number of oogonia recorded from 52 to 103. For vegetative growth *S. monoica* does not use dextrose as freely as it does maltose and levulose, agreeing in this with *S. ferax*. The difference in weight is striking and appears consistently in all the records, but it is doubtful whether it is safe to draw more than the most general conclusions from the record of the number of oogonia, since this species produces oogonia more readily and abundantly than any other species the writer has isolated. In general, however, the conclusions previously drawn seem warranted by the results of this experiment also. The minimum food required for an abundant supply of oogonia is less than that demanded by *S. ferax*, the critical concentration evidently being below 0.1 percent peptone, but there is no uniform relation between weight of mycelium and number of oogonia. The value of levulose is also confirmed.

Klebs found that when *S. mixta* was placed in 0.05 percent haemoglobin oogonia were readily formed but they were not accompanied by antheridia. With the same treatment *S. monoica* gave similar results although some oogonia with antheridia were always present. The number of oogonia accompanied by antheridia was determined by counting 100 or 200 or sometimes a larger number of oogonia under a 16 millimeter objective and recording the number to which antheridia were attached. While the figures cannot be considered final there is a consistent trend toward an increase in the number of antheridia when the culture medium has contained a form of invert sugar, especially levulose. Number 14 is the only exception to this. In this solution much of the sucrose was probably converted into invert sugar and if so the number of antheridia should approach that in the case of the solutions with invert sugar. The larger number of antheridia present

on mycelium grown in the presence of levulose is especially interesting, since when this sugar is added to haemoglobin the resulting growth produces oogonia nearly all of which are accompanied by antheridia, while when dextrose is substituted for levulose the oogonia are larger but only a few of them have antheridia.

In this series mycelium from each culture solution was transferred to four dishes of haemoglobin and especial care was taken to have these transferred masses of as nearly the same size as possible, as it was intended to weigh the mycelium from the haemoglobin after the final oogonium record. This was done to see whether there was any relation between the number of oogonia and mass of mycelium produced in the haemoglobin. The column at the right in Table XV shows the total weight of the mycelium in the four dishes. It is true that slight differences in the masses originally placed in the haemoglobin probably did exist but when it is remembered that the figures represent the total weight from four dishes after several days growth, it will be seen that the small piece of mycelium originally put into the haemoglobin could in no case have exceeded one milligram in weight. The difference in weight between any two such masses therefore would be represented at most by one unit in the fourth decimal place and could have no influence on the general result. Here again the evidence confirms the conclusion previously reached that there is no relation between the weight of mycelium and the number of oogonia produced by it. On March first another series was set up with *S. monoica* to confirm the results obtained by series VIII. Peptone and sugars only were used and the resulting data are presented in Table XVI. The cultures in this series were made in Joseph Kavalier Bohemian glass flasks.

TABLE XVI

Series IX. S. monoica. Mycelia Out of Solutions Under Column 2. Transferred to 0.05 *Percent Haemoglobin After Five Days. Weighed After Thirty Days in* 200 *cc. Solution. Weight in Milligrams. Number of Oogonia in* 25 *Square Millimeters. Sugars All* 1/50 *Molecular*

1	2	3	4	5
	Media	Weight in Milligrams	Sporangia 3 Days	Oogonia 8 Days
1	Peptone 1%	.0980	0	89
2	" 0.1%	.0244	A few	48
3	" 0.1% + dextrose	.0270	A few	52
4	" 0.1% + levulose	.0196	A few	85
5	" 0.1% + maltose	.0773	A few	57
6	" 0.1% + sucrose	.02675	A few	45

In this series the small weight of the mycelium out of peptone-levulose is difficult to account for. Unfortunately the duplicate flask failed of inoculation, and the weight represents but one culture instead of an average of two as in the other cases. The number of oogonia on the mycelium out of levulose-peptone was however again greater than that on mycelia out of other solutions containing sugars and was practically the same as that out of 1 percent peptone. Maltose was freely used for vegetative increase but the mycelium out of maltose-peptone did not develop a large number of oogonia.

At the same time that the cultures recorded in Table XVI were set up, another series was planned to determine what effect salts added to the peptone alone, or to peptone and sugars, would have on the vegetative growth or on the number of oogonia. The time available was insufficient for repeating this experiment and the results are offered as suggestive rather than as proof of the conclusions that seem to be warranted.

Table XVII gives the results of this experiment. The mycelia out of the solutions marked with an asterisk were overheated during the experiment, and the number of oogonia formed cannot be regarded as fairly representing the capacity of each mycelium to produce oogonia. The figures have, therefore, been omitted.

It would appear from the record that the addition of phosphate salts, and, to a much less degree, potassium nitrate, stimulates growth while calcium nitrate, potassium sulphate, and magnesium sulphate have no marked effect. The only conclusion that seems fully justified by the somewhat uncertain results of one trial, however, is that phosphates exert a marked effect both on the amount of dry matter and on the number of oogonia produced. Comparing numbers 5 to 10 with 16 and 17 it will be seen that while in the latter the use of phosphates with peptone alone has increased the weight materially over that when peptone only was used (21), it still falls short of the weight of the mycelia out of the solutions containing sugar, peptone and phosphates. The number of oogonia produced on the various mycelia is, however, of the same order of magnitude in all cases, in which a phosphate was used, and this number is much larger than that obtained when other salts were used (compare 19 and 20).

The conclusions seem warranted, therefore, that in the case of *S. monoica* also there is no necessary relation between weight of mycelium and the number of oogonia that may be produced; that

levulose is especially adapted to the production of oogonia and that phosphate salts influence the number of oogonia produced to a greater degree than they do the increase in weight of mycelium.

TABLE XVII

Series IX. S. monoica. Mycelia Out of Culture Media in Column Two, Transferred to 0.05 *Percent Haemoglobin After Seven Days' Growth, and Weighed After Growing Thirty Days in* 200 *cc. Solution. Weights in Milligrams. Sugars All* 1/50 *and Salts All* 1/200 *Molecular; Peptone* 0.1 *Percent*

1	2	3	4	5
No.	Media	Weight	Sporangia	Oogonia
1	Peptone + dextrose + KNO_3.....	.0423	0	41, oogonia small
2	" + dextrose + $CaNO_3$....	.0281	0	80
3	" + levulose + KNO_3.....	.03675	0–I	93
4	" + levulose + $CaNO_3$....	.02095	0–I	40
5	" + dextrose + KH_2PO_4..	.2011	0–I	94
6	" + levulose + KH_2PO_4...	.2084	0	79, large and well filled
7	" + dextrose + NaH_2PO_4 .	.13835	0	90, many small ones
8	" + levulose + NaH_2PO_4..	.17145	0–I	89, mostly small ones
9	" + dextrose + $Ca(H_2PO_4)_2$	.15954	0–I	110
10	" + levulose + $Ca(H_2PO_4)_2$	.1093	I	93, large well filled
11	" + dextrose + KSO_4.....	.0291	0–I	*
12	" + levulose + KSO_4.....	.01855	Few	*
13	" + dextrose + $MgSO_4$....	.0404	"	*
14	" + levulose + $MgSO_4$....	.02225	0–I	54
15	" + KNO_3.....	.0183	Few	*
16	" + KH_2PO_4.....	.0426		111
17	" + NaH_2PO_4.....	.0401		101, small
18	" + $Ca(H_2PO_4)_2$.....	.0416		*
19	" + KSO_4.....	.0213		68
20	" + $MgSO_4$.....	.0276		57, small
21	" 0.1%.....	.0244		48

Experiments with Achlya prolifera

Several sets of experiments were carried on with this species along the same lines as those before described. The results however were not satisfactory but are presented because so far as they may be accepted at all they seem to confirm most of the conclusions arrived at by a study of the previously considered species. The oogonia could not be counted as the mycelium was so thickly matted that many oogonia could be but dimly seen and the distribution of the

oogonia was often so irregular that it was not possible to secure a fair average. The number of the oogonia was therefore estimated. The most important cause of the lack of good results with this species is that no entirely satisfactory solution was found to which the mycelium could be transferred for the formation of oogonia. Early experiments showed that haemoglobin was worthless, oogonia were formed but no oospores were ever developed. Many experiments were then made with peptone and sugars as suggested by Obel ('10) and also by placing masses of mycelium into pure water or water to which salts had been added.

A. prolifera will form oogonia in water as is the case with *A. racemosa*, but unfortunately the formation of oogonia is prevented by a temperature lower than that at which sporangia continue to be freely formed. If however a mass of mycelium is left in the water in which it was placed on being taken from the culture flask, it may find sufficient food present to prevent any free formation of sporangia and thus permit the production of oogonia. This method was therefore used with the others, but the results have not been uniform. Many solutions containing peptone and sugars were tried and at temperatures from 10° to 24° C. A few of the results of these tests are given in Table XVIII; it is thought unnecessary to extend the record as in most of the other cases only vegetative growth took place with an occasional oogonium, sometimes with and sometimes without oospores.

Oogonia were formed under most of the conditions presented in the table but they were seldom well filled, often only a small number of the oogonia containing oospores. The failure to produce oospores appeared generally to be connected with excessive vegetative growth although the specific value of the carbohydrate is clearly seen when the record of numbers 3, 4, 6 and 7, each of which contained 0.01 percent peptone, are compared, or when the record of number 8 is compared with that of number 11. Both of these solutions contained 0.02 percent peptone but when this only was present the fungus made a dense growth which rapidly filled the dish and no oogonia were produced. The addition of sucrose restricted the growth and caused the development of many well filled oogonia In nearly all peptone-sucrose solutions oogonia were freely produced; maltose and dextrose were also generally satisfactory; levulose always encouraged a great growth of delicate hyphae while few oogonia were formed and those were usually empty.

TABLE XVIII

Achlya prolifera. Mycelia Out of Pea and Peptone-Sucrose-Salts Solution into Solutions Under Column 2. Estimated Number of Oogonia and Oospores. The Figures for Oospores at Temperatures Given are Estimated Percentages

1	2	Peptone-Sucrose-Salts, 20°–22° C.		Peptone-Sucrose-Salts, 10°–15° C.		Pea Extract, 20°–22° C.		Pea Extract, 16°–18° C.	
		3	4	5	6	7	8	9	10
No	Media.	Oog.	Oosp.	Oog.	Oosp.	Oog.	Oosp.	Oog	Oosp.
1	Haemoglobin 0.1%.	Few	0	0–I	0				
2	" 0.05%	I–II	0	II	0				
3	Peptone 0.01%....					0–I	Few	I	Few
4	Peptone 0.01% + dextrose M/500					I–II	"	II–III	50
5	Peptone 0.01% + levulose M/500					0–I	"	I–II	Few
6	Peptone 0.01% + maltose M/500					II–III	50	III	50
7	Peptone 0.01% + sucrose M/200					II–III	50	III	90
8	Peptone 0.02%....					Growth only		Great growth only	
9	Peptone 0.02% + dextrose M/1000					I–II	25	I	0
10	Peptone 0.02% + levulose M/250					I	Few		
11	Peptone 0.02% + sucrose M/300					III	95	II	25
12	Peptone 0.02% + sucrose M/500					Great growth only		Great growth only	
13	Peptone 0.03% + dextrose M/300	I	50	I–II	Few				
14	Peptone 0.03% + maltose M/300	II–III	60	0–I	0				
15	Peptone 0.03% + sucrose M/300	III	95	II	50				

The experiment with 0.02 percent peptone and sucrose, number 11, was among the earlier ones and it seemed reasonable to conclude that this was a favorable medium for the production of oogonia and that experiments at room temperature would give reliable results. Series V was therefore planned and carried through, the mycelia being grown in the culture media for seven days and then transferred, after being twice washed, to a solution containing 0.02 percent peptone and 1/300 molecular sucrose. The entire experiment was carried through at room temperature. The results are presented in Table XIX. In all cases more or less growth took place in the peptone-

sucrose solution and in some the formation of oogonia and oospores was all that could be desired. The results, as a whole, however, are not regular enough to warrant the statement that the failure of some mycelia to produce oogonia was due to the preceding conditions of growth. This however does not affect the general value of the results as pointing to the conclusions previously reached. It is true that the mycelia with the largest number of oogonia and oospores are also among those having the greatest weight, but not all of the latter class have developed large numbers of oogonia (see numbers 12, 15, 17), while the records of several numbers show a good development of oogonia together with weights one half to one third as great as those from other lots. When the solutions containing sucrose are not overheated there is clearly little invert sugar; the fungus makes practically no more growth in such solutions than in peptone alone and the number of oogonia is about the same on mycelia from all these solutions; compare the record of numbers 2 and 6, 3 and 10. The weight of mycelium out of levulose-peptone solution was nearly the same as that out of peptone alone and the mycelium out of the former solution responded to the peptone-sucrose environment by an unusually vigorous growth of delicate hyphae which soon filled the dish. This phenomenon was observed in two out of three series of experiments while in a third oogonia were formed on mycelium out of levulose-peptone but these were not nearly so numerous as on other mycelia. The influence of levulose when used as a culture medium for *Achlya prolifera* was certainly against the formation of oogonia. The addition of salts produced a marked increase in growth with all the sugars, but most with the sucrose, doubtless because of the extensive inversion of the sucrose under the action of the high temperature and salts.

Two other series of experiments were made with *A. prolifera*, and in both the results were similar to those recorded for series V. In one series a transfer solution weaker in peptone (0.01 percent) and stronger in sucrose (1/200 molecular) was used in the hope that this would lead to a better development of the oospores. The results were, however, no more uniform than before as only a small proportion of oogonia in any lot contained oospores. In another series various salts were added to the peptone-sugar solutions and to the peptone alone. The salts used were various phosphates, nitrates and sulphates, the same as were used in the experiments with *S. monoica*,

TABLE XIX

Series V. Achlya prolifera. Mycelia Grown Seven Days in Media Shown in Column 2, Transferred to Peptone 0.02 *Percent + Sucrose M/*300. *Weight of Mycelia Grown Fifteen Days in* 200 *cc. of Media Shown in Column 3. Numbers of Oogonia and of Oogonia with Oospores Estimated, the Latter in Percentages*

1	2	3 Weight in Milligrams	4 Oogonia	5 Oogonia with Oospores
1	Pea	.091	II	25%
2	Peptone 0.1%	.025	II	10 "
3	" 0.2%	.0332	II	10 "
4	" 0.5%	.039	II–III	20 "
5	" 1.0%	.0405	II–III	75 "
6	" 0.1% + sucrose	.0270	II	10 "
7	" 0.1% + maltose	.0476	II	5 "
8	" 0.1% + dextrose	.036	0–I	Few
9	" 0.1% + levulose	.0286	0	
10	" 0.2% + sucrose	.0324	I	Few
11	" 0.2% + maltose	.058	II	10%
12	" 0.2% + dextrose	.0412	I	Few
13	" 0.2% + levulose	.0346	0–I	"
14	" 0.1% + sucrose + salts	.1140	II–III	75%
15	" 0.1% + maltose + salts	.0950	I	Few
16	" 0.1% + dextrose + salts	.1234	II–III	90%
17	" 0.1% + levulose + salts	.085	I	40 "
18	" 0.1% + salts	.0466	II	Few
20	Haemoglobin 0.1	.0242	I	10%

see Table XVII. In every case the mycelia grown in solutions containing potassium, sodium or calcium phosphate, either with or without sugar, when transferred to peptone-sucrose solutions grew rapidly and filled the dish with a mass of fine hyphae on which few or no oogonia were formed. Mycelia out of solutions containing nitrates or sulphates, on the other hand, made little growth when transferred to the peptone-sucrose solution and the number of oogonia was recorded as I, II, or III on various mycelia. As in the case of *S. monoica*, the addition of phosphates to the solution increased the vegetative growth markedly, and the great growth without oogonia may be because the peptone-sucrose solution used was not the best for the production of oogonia by this kind of mycelium.

DISCUSSION

In the preceding pages attention has been called to such conclusions as seemed warranted by the record of each set of experiments.

These conclusions will be briefly discussed under the following heads.

1. The relation between the weight of mycelium and the number of oogonia produced.
2. The expression in one environment of tendencies acquired in a former environment.
3. The value of levulose.
4. The value of sucrose.
5. The value of phosphates.

The Relation between Weight of Mycelium and the Number of Oogonia Produced

The question which was stated at the beginning of the paper, whether any constant and necessary relation exists between the vegetative growth and the reproductive activity in several species of the Saprolegniaceae must be answered in the negative. The record of experiments with *Achlya racemosa*, *A. prolifera*, *Saprolegnia ferax* and *S. monoica* all agree in showing that a mycelium out of a relatively poor solution, measured by vegetative growth, may produce more or larger oogonia than are produced on a mycelium from a solution that is much better for vegetative growth. This has, of course, nothing to do with the well-known fact that after a mycelium has been transferred to a new solution, vegetative growth may prevent the formation of oogonia. When a mycelium of *S. ferax* is taken from pea extract and placed in a haemoglobin solution that is stronger than 0.1 percent, few if any oogonia are produced; vegetative growth is excessive and prevents reproduction. Strong vegetative growth and reproduction cannot go on at the same time in the same solution but the problem under discussion is the relation between vegetative growth in one solution and reproduction by such a mycelium when transferred to another solution. It might be expected, a priori, that if a vigorous mycelium will produce a given number of oogonia when transferred to a suitable medium a more vigorous one, that is, one that had made a greater weight of dry matter in a given time in a fixed quantity of solution, would produce a greater number of oogonia. To produce oogonia after being transferred the fungus must draw upon its reserve materials and it might be thought that the dry weight of the mycelium would serve as a measure of the reserve materials available for the production of oogonia; but this was found not to be the case.

When only one food, peptone, was offered in varying concentrations there was a steady increase in the dry weight with the increase in the concentration of peptone but the mycelium out of 0.2 percent, 0.5 percent and 1 percent produced practically the same number of oogonia. See Series II, Table XIII and Series VIII, Table XV. When sugars or sugars and salts were added to the solution the absence of any fixed relation between weight of mycelium and the number of oogonia was even more marked. This does not mean, however, that a mycelium need not be vigorous in the sense in which this term seems to have been used by Klebs, Kauffman and Obel. They evidently meant that a mycelium must have been well enough nourished, and that a poorly nourished mycelium was to be guarded against in experimental work. It is very clear that a fungus can grow in a solution that will nourish too little to enable it to reproduce well; such a solution is to be considered as being below the minimum concentration necessary for satisfactory growth. For *S. ferax* a peptone solution containing 0.1 percent peptone, with or without sucrose provided none of the sucrose has been inverted, represents such a sub-minimum concentration. The minimum concentration for the production of a "well-nourished" mycelium may vary with the species. For *S. monoica* 0.1 percent peptone is enough to produce a well nourished mycelium if the number of oogonia produced be accepted as determining whether or not a mycelium has been well nourished, while *S. ferax* needs a stronger solution. Solutions containing nutrient substances at higher concentrations than the minimum will enable the fungus to produce an increased yield of mycelium but not a proportionate increase in the number of oogonia. Above the minimum concentration an increase in the number of oogonia will only be secured by changing the quality of the nutrient solution.

The Effect of a Given Environment May Not Become Evident Until the Plant Has Been Transferred to Another Environment

Nutritive substances do not, however, all have the same importance for the development of the different parts of the plant. Pfeffer ('97) states this in general terms when he says (Oxford Ed., 1900, p. 387) "the importance of a substance to a plant is not to be measured solely by the amount of growth which it induces."

While there is as yet no evidence that new species can be produced by changing the conditions, we can safely say that a plant is the

creature of its environment in the sense that the potentialities of the species may be called forth in greater or less degree, or the expression of them may be retarded by the conditions under which it grows. The effect produced may be due to the environment as a whole or to certain factors. Klebs and others have already proved that conditions determine what phase of the plant's growth shall appear and it has been shown in the preceding pages that these phases may be influenced by the conditions prevailing during vegetative growth. The plant therefore not only responds to the immediate environment but is molded by it in ways that can find expression only after that environment has been changed for another.

While growing vegetatively a mycelium may acquire a tendency toward the development of sporangia and oogonia and such a tendency may be carried over to the new environment into which the mycelium is transferred. This fact was recognized by Klebs who said ('99, p. 585) that in all investigations on the production of sporangia the nutrition conditions of the mycelium have a specific importance as upon them depends the ability of the mycelium to respond to the change in nutrition by the formation of reproductive organs. Klebs further states that these two forms of reproduction make different demands on the protoplasm (Nährplasma), both quantitative and qualitative. So far as the present studies show, the minimum concentration of peptone needed to grow a mycelium that will develop sporangia or oogonia is about the same, but the addition of sugars favored the production of oogonia and that of salts the production of sporangia. In this connection reference may be made to some observations not given in the data above. Mycelium of *Achlya prolifera* grown in solutions of peptone and potassium nitrate, when transferred to water developed sporangia more freely than that from any other solution and the sporangia were strikingly large, with dark walls. A similar observation was made on *Dictyuchus monosporus*, mycelium of which from solutions with potassium nitrate developed large dark sporangia, while that out of solutions with mono-potassium phosphate formed small light colored sporangia. This phenomenon was observed a number of times and proved quite constant and characteristic.

The view that the fungus may, during vegetative growth, acquire a tendency toward the development of oogonia irrespective of the vigor of growth is strongly supported by the results obtained with

levulose as a food as well as by those with phosphates. The fact that the effect of both levulose and phosphates was different on *Achlya prolifera*, from what it was on *Saprolegnia* serves to support this view and to show that the specific characters of different plants will react differently to similar environmental conditions. The close relationship existing between *S. ferax* and *S. monoica* made it reasonable to expect that similar responses would be made by these species to like conditions. But *Achlya* is, in habit and in choice of habitat, very different. As Petersen ('10) has pointed out, the Achlyas, though growing on dead animal matter, are frequently found on decaying plant parts, habitats not affected by the Saprolegnias.

It seems probable that the specific response of the plants grown in levulose and in phosphates may be due to a storage of reserve materials which are drawn upon as soon as the fungus finds itself in a poorer nutrient medium. Klebs suggests this but seems also to hint at a more intimate change in the character of the plasma. It is sufficient for the present purpose to point out the fact that the specific effect of one solution may be to awaken an appropriate reaction when the mycelium is transferred to another solution.

The Value of Levulose

The results of the experiments on both *Saprolegnia ferax* and *S. monoica* support the statement that levulose is used more readily by these species than other sugars except maltose and that it has a much greater effect in developing a tendency toward the production of oogonia than maltose has. This effect on the vegetating mycelium is confirmed in the case of both maltose and levulose by the effect of dilute solutions into which mycelia are transferred for the production of oogonia. In this case also maltose promotes vegetative growth to the almost entire exclusion of oogonia while levulose encourages the production of both oogonia and antheridia. The remarkable effect of levulose on some species was shown in the case of a still undescribed form which was collected as number 74. The details of the experiments with this form will be given in another paper but here it may be said that while, during almost two years of cultivation on natural and synthetic media, no oogonia were produced, these appeared when a mixture of leucin and levulose was used. The result was undoubtedly due to the levulose, as leucin alone or with any other carbohydrate or with salts failed to produce a like effect.

An apparent exception to the statement that levulose is as good a food for vegetative growth as maltose is found in the results shown in Table XVI. At the completion of this series (IX) it seemed difficult to account for the small amount of dry weight out of the peptone-levulose solution compared with that out of peptone-dextrose and peptone-maltose. On comparison of the conditions under which the various series of experiments had been made, it developed that the levulose used in the earlier experiments had been from a certain lot from Kahlbaum. The supply being exhausted and no more from the same source being available, the solutions for series IX were made up with levulose from Schuckardt. It seemed probable therefore that the smaller weight could be accounted for by the difference in the quality of the levulose. To test this the small amount remaining in the original Kahlbaum bottle was carefully collected and two lots of two times 100 cc. each of a solution containing 0.1 percent peptone and 1/50 molecular levulose were made up, one set with the Kahlbaum and one with Schuckardt levulose. After being inoculated with *S. monoica* the solutions were allowed to stand 20 days and the mycelia were then dried and weighed. The average weight of one lot out of the Kahlbaum levulose was .0212 milligrams, and out of the Schuckardt lot .0082 milligrams; practically the same degree of difference as that existing between the weights of mycelia out of peptone-levulose solutions recorded in Tables XV and XVI. It was not possible to undertake an analysis of the sugars from these two sources but the organism clearly recognized a difference between them. This experience perhaps suggests one of the reasons for the sometimes contradictory results secured by different workers. The reactions of living things to their surroundings are extremely delicate. Not only must the inherent characters of different species be considered, but in each species there are most probably a number of strains each of which makes its own peculiar response to a given set of conditions. Add to this the effects produced on a certain organism by supposedly chemically pure substances from different manufacturers and the conditions are present for varying results from which conclusions may be drawn leading to endless controversy. Possibly the result which Klebs reported, that *S. mixta* made a better growth in sucrose, dextrose and maltose than in levulose may have been due to the use of a poorer levulose than that which the writer used in the earlier series.

The Value of Sucrose

The records of the tests with solutions containing sucrose have been difficult of interpretation throughout the work. Under the discussion of Table X it was pointed out that the close agreement in the weight between the mycelium out of 0.1 percent peptone and that out of peptone and sucrose, as compared with the results when maltose was used, showed that little if any sucrose was utilized by the plant. The mycelium out of solution 8, however, showed not only a considerable increase in weight, but also in the number of oogonia formed over that out of No. 11, the difference between the two solutions being the addition of sucrose in number 8.

The results recorded in Table XIII are similar, the fungus growing in a solution containing sucrose having made little more weight than that grown in peptone alone, and the number of oogonia formed on the mycelium out of sucrose and peptone is nearly the same as that from the mycelium out of peptone alone. At the time the experiments were carried on the writer was not aware of the work of Noel Deerr ('10) on the effect of high temperatures on the inversion of sucrose. Deerr found that when sugar solutions in pure water were heated in an autoclave to a temperature of 110° no invert sugar was formed but that above that temperature a rapid increase in inversion with equal increments of temperature took place. Salts, as the nitrates of potassium, sodium and others, and the sulphates, as that of magnesium, increased the amount of inversion. The above facts offer an explanation of the results obtained with sucrose. The solutions used were always sterilized in an autoclave at a temperature of about 112°–120° C., but occasionally the temperature rose to 125° C. According to Deerr's results a small amount of invert sugar should have been made in practically every solution in which sucrose was used and it will be noted that the weights secured from mycelia out of peptone and sucrose are consistently a trifle greater than those from mycelia out of peptone alone. When salts were added, and occasionally when peptone and sucrose alone were present, a more marked increase in weight resulted.

In order to determine whether invert sugar was formed under the conditions prevailing in the above described experiments a series of solutions was made up containing in each case 0.1 percent peptone and 1/50 molecular sucrose. One set was sterilized at 110° C., one at 115° C. and the other at 125° C. Another series was also prepared

in which the following salts were added to the peptone and sugar, magnesium sulphate, potassium nitrate, and di-hydrogen potassium phosphate, each salt being present in 1/600 molecular. A third series was like the second except that mono-hydrogen potassium phosphate was substituted for the di-hydrogen. The two series containing salts were sterilized at 110°, 115°, and 125° C. These solutions and temperatures were selected because they represent the conditions of the foregoing experiments and not for the purpose of making a detailed study of the reactions involved; quantitative determinations of the amount of invert sugar present in each lot after being sterilized for 30 minutes were made by Mr. R. B. Harvey, PhC. Of the solutions sterilized at 110° C. those with peptone and sugar alone and with the mono-hydrogen phosphate showed no inversion, while in that with the di-hydrogen phosphate 1.2 percent of the sucrose had been inverted. Sterilized at 115° C. the peptone-sugar solution and the solution containing mono-hydrogen potassium phosphate showed a trace of inversion, not enough to express in percentage, while in that containing the di-hydrogen 1.9 percent of the sucrose had been inverted. When the temperature at which the solution was sterilized went up to 125° C. 1.2 percent of the sucrose had been inverted in the solution containing peptone and sucrose only, 0.6 percent in that containing mono-hydrogen potassium phosphate, and 4.7 percent in that with di-hydrogen potassium phosphate. The fact that more inversion took place when the di-hydrogen potassium phosphate was used is interesting because this was used in practically all of the solutions made up for the experiments with Saprolegnias. On the whole the results correspond to those obtained by Deerr: at 110° C. there was no inversion, while at the higher temperatures an increasing amount of sucrose was inverted and when salts, including the di-hydrogen potassium phosphate were used a considerable amount of invert sugar was present before the solution was inoculated. Invert sugar was therefore available but varied in amount in different solutions and under different conditions.

We may conclude that *S. ferax* does not contain invertase and cannot make use of sucrose. In sterilized culture media, however, there is sure to be more or less invert sugar present owing to the inversion caused by heating and by the presence of salts. What has been shown to be true of *S. ferax* is equally true of the other Saprolegniaceae so far as they have been studied by the writer.

The Value of Phosphates

No attempt was made to determine the need of the fungus for inorganic nutriment. This would have been difficult, if not impossible, with peptone as the source of nitrogen, as the peptone itself contains a large percentage of total salts. An analysis of the Witte peptone used showed 2.78 percent total salts and a qualitative test revealed the presence of phosphates, sulphates, chlorides, and of potassium, sodium, magnesium, calcium, aluminum, and of ferric iron ($K_3(Fe\text{-}Cu)_6$), so that it was manifestly impossible to exclude the presence of all of these salts except the one wanted for a particular experiment. However, the tests in which certain salts were added to the culture medium showed that with an abundant supply of phosphates *Saprolegnia* developed a greater capacity for the production of oogonia than when other salts were added, and this irrespective of whether potassium, sodium, or calcium phosphate were used. This result is in general agreement with the conclusions of Klebs, that the phosphates favored the production of oogonia, though Kauffman secured as good results from some of the other salts as from the phosphates.

In *Achlya prolifera* the increase in growth due to the phosphates was marked, but in this case the mycelium may have carried over sufficient reserve material to produce a vigorous growth in the peptone-sucrose solution, and thus prevented the formation of oogonia.

Summary

1. There is no constant and necessary relation between vegetative growth and sexual reproduction when the food offered exceeds the minimum concentration necessary for the given species.

2. The minimum concentration of food necessary varies with the species but lies, in general, in the neighborhood of 0.1 percent peptone for the production of both sporangia and oogonia.

3. While growing vegetatively a mycelium may develop tendencies that may affect the number and character of the reproductive organs produced under subsequent and different conditions.

4. Of the carbohydrates used maltose and levulose are especially useful for vegetative growth and the latter has an especial value for the production of oogonia.

5. Sucrose is probably not used by species of Saprolegnia or of Achlya unless it is first inverted by some other agency.

6. Phosphates in the culture solution tend to increase the reproductive capacity of the fungus.

7. In preparing a culture solution for the Saprolegniaceae due regard must be paid to the fact that the conditions present during vegetative growth may affect the mycelium qualitatively as well as quantitatively. Not only is a vigorous mycelium needed but the composition of the medium must be accurately given for each experiment if workers are to arrive at comparable results.

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