Concerning the Characters of Certain Fungi as Exhibited by their Growth in the Presence of other Fungi

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CONCERNING THE CHARACTERS OF CERTAIN FUNGI AS EXHIBITED BY THEIR GROWTH IN THE PRESENCE OF OTHER FUNGI

CHARLES LYMAN PORTER

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INTRODUCTION

In common with all other organisms, fungi are modified by the changes in their environment. The presence of other living forms constitutes a part of the environment, and consequently these affect the morphology and physiology of the fungi; thus, growth is usually checked when two or more fungi are contiguous. Such inhibition may be mutual, or the growth of one individual may be inhibited more than that of the other. The antagonistic action of one fungus toward another may be due to a variety of causes, and results in numerous modifications of the organisms involved. The study of growth changes so induced is the purpose of this paper.

METHODS

In the routine work of determining types of inhibition, the various organisms used were grown on cornmeal agar in petri plates. Effort was made always to have the conditions as nearly uniform as possible. Variations were made from this routine procedure in order to observe the growth phenomena under different conditions.

A. The depth of the medium was varied by permitting the agar to harden in the plates while the plates were tilted. Colonies were then planted in the shallow and deep regions of the agar.

B. The medium was varied as to the amount and kinds of nutrients present, employing washed agar, plain agar, cornmeal agar, dextrose agar, and Brazil-nut agar.

C. The time element was varied by making inoculations at periods ranging from 24 to 129 hours from the time of the initial transfer.

D. The amount of inoculum was varied.

In noting morphological changes, direct observations were made through the microscope. One other method that proved very successful was as follows: Thin layers of agar were carefully poured on cooled sterile cover slips, where the agar hardened immediately. Slips thus prepared were inoculated with two fungi that were to be placed under observation. The inoculations were made so that the developing colonies would not be more than 1 to 2 cm. from each other. As soon as development was well started, the cover slips were mounted on the stage of the microscope and the mi-

croscope was placed in position on the stand of a Bausch and Lomb photo-micro-projection apparatus. In this manner the fungous hyphae were enormously magnified, and even minute changes in appearance and the manner and quality of the change could be quickly noted. Observations were made at short intervals, and the light was not kept on longer than necessary to make observations lest the illumination modify the growth in some manner.

Aseptic seedlings were secured and placed in rag-dolls in the manner suggested by F. L. Stevens (41). The dolls were divided into four lots, the lots being subjected to the following treatments: (1) Several loopfuls of Helminthosporium spores were rubbed on the outside of the cloth surrounding the seedlings, and the whole roll was then dipped into a broth suspension of organism no. 45. (2) Treated as lot one, except that the rolls were not dipped into the broth suspension. (3) Rolls containing the seedlings were dipped into the broth suspension but were not inoculated with the spores of Helminthosporium. (4) Rolls were placed in their tubes without being inoculated with the spores of Helminthosporium or dipped into a broth suspension of organism no. 45. Lots 3 and 4 served as controls.

Pot cultures were secured as follows: Five-inch pots were filled with earth. Cones, one inch in diameter at the top and three and one half inches long, made of thin paper, were filled with earth which had been thoroughly moistened with a broth culture of organism no. 45. These were sunk in the center of the pots. Inside these cones were placed a number of flax seeds that had been so treated as to render them aseptic. The soil outside the cones was inoculated with the spores of *Fusarium lini*. In the control pots the soil in the cones was moistened with sterile broth only.

The staling products of Penicillium growth were secured in the following manner: Sterile orange juice was inoculated with *Penicillium italicum* and kept at room temperature for six weeks. At the end of this time the juice was filtered through a Reichel filter. The filtrate was placed in sterile petri dishes and permitted to stand for several weeks in a sterile desiccator over calcium chlorid. In this time the filtrate was considerably concentrated. The liquid was injected into the oranges with a Leur syringe.

Accession List of Organisms

The following organisms are those made use of in my experiments. Their history so far as it is known may be found in a typewritten thesis filed in the library of the University of Illinois. The organisms are numbered throughout the text to correspond with the numbers of the accession list.

2. *Sclerotium rolfsii*
3. Actinomyces
4. Helminthosporium
5. Pink Fusarium
6. *Rhizopus nigricans*
7. Actinomyces
8. Pink yeast
9. White yeast
10. White yeast
11. *Penicillium glaucum*
12. Bacterium
13. *Penicillium italicum*

14. *Penicillium digitatum*
25. Helminthosporium
26. Helminthosporium
29. Alternaria
30. Mucor
31. Bacteria
32. Bacteria
33. Bacteria
34. Gliocladium
35. *Colletotrichum lindemuthianum*
36. Helminthosporium
37. Helminthosporium
39. Sterigmatocystis
40. Alternaria
42. Penicillium
44. Actinomyces
45. Bacterium[1]
46. Acrothecium
50. Unknown organism
53. Lactobacillus
58. Azotobacter
61. *Acrostalagmus cinnabarinus*
62. *Cytosporium ribis*
68. *Fusarium coeruleum*
70. *Sporotrichium bombycinum*
71. *Sclerotinia libertiana*
72. Botrytis
73. *Fusarium lini*
74. *Bacillus carotovorus*
77. *Fusarium lini*
86. *Bacillus proteus*
87. *Sarcina lutea*
88. *Sarcina aurantiaca*
89. *Pseudomonas violaceus*
90. *Bacillus prodigiosus*
91. *Bacterium alcaligenes*
92. *Bacillus capsulatus*
93. *Actinomyces albus*
94. *Bacillus ramosus*
97. *Bacillus vulgatus*
98. *Bacterium megatherium*
100. *Cladothrix dichotoma*
103. Fusarium
104. *Helminthosporium sativum*
105. *Fusarium lini*
106. Pilobolus-like fungus
108. Alternaria
110. Zygorhynchus
111. Syncephalastrum
112. Cunninghamella
113. *Rhizopus nigricans*, plus race
114. *Rhizopus nigricans*, minus race
115. *Actinomyces tricolor*
116. *Actinomyces albus* var. *ochraceus*
117. *Actinomyces nigrificans*
118. *Phyllosticta solitaria*
120. *Fusarium culmorum*
121. *Gloeosporium piperatum*
122. *Colletotrichum nigrum*
123. *Ustilago violacea*
124. *Ustilago violacea*
125. *Alternaria crassa*
127. *Bacillus mesentericus*
128. Helminthosporium
129. *Fusarium lini*
130. *Fusarium lini*
131. *Fusarium lini*
132. Pythium

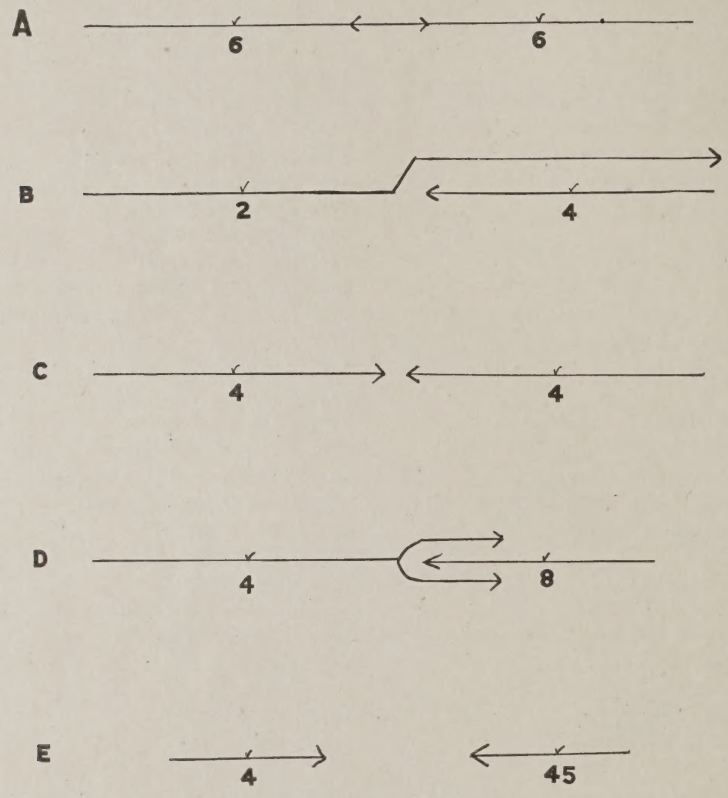
Types of Inhibition

There are many conceivable reactions when the mycelium of one fungus approaches that of another. These theoretical possibilities are nearly all demonstrated by actual examples. Moreover, these examples occur frequently enough and, under properly controlled experiments, regularly enough, to suggest that they may be classified into types which occur whenever the same organisms are made to react with each other. For convenience in discussion, I have described the most prominent and common types of reaction and have designated them by letters.

Type A (text fig. 1, *A*). Mutually intermingling. It might be assumed that this would be a common type, but few really good examples are found. Perhaps the best is afforded by the intermingling hyphae of two colonies of Rhizopus. Organism no. 5 grown against no. 5 also presents a fairly good example of this type. It seems, however, but rarely possible for two organisms to occupy equally well the same area at the same time.

[1] The index number of this organism was determined and reads 5131–52120–1333.

Type B (text fig. 1, *B*). Growth superficial over the contending organism. The underlying organism is always greatly inhibited. An example is supplied by organism no. 2 grown with no. 4. No. 4 is the inhibited organism.



TEXT FIG. 1. Types of inhibition. *A*, mutually intermingling. *B*, overgrowing. *C*, slight inhibition. *D*, growth around. *E*, inhibition at a distance.

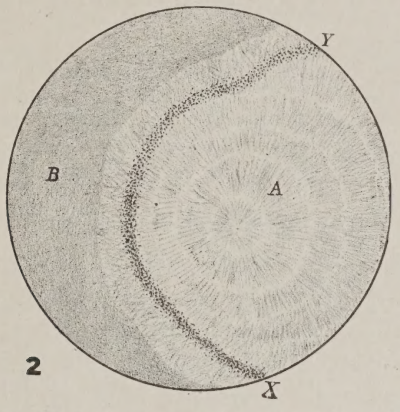
Type C (text fig. 1, *C*). Slight inhibition. Both organisms are inhibited but approach each other until almost in contact, when growth ceases. In such cases the space between the two colonies, while very narrow, is clearly marked. This is the prevailing type when any organism is grown with another individual of the same species.

Type D (text fig. 1, *D*). Growth around the contending organism. The Helminthosporiums react in this manner when grown with organisms numbers 8, 9, or 10.

Type E (text fig. 1, *E*). Mutual inhibition at considerable distance. Organisms nos. 31, 33, and 34 so inhibit any of the Helminthosporiums. These three organisms when grown with organisms nos. 5, 30, 32, and 35 produce this type of inhibition. The power to inhibit strongly which is

shown by nos. 31, 33, 34, and 45 will be a fruitful field of study for future investigations. They can inhibit growth in many fungi at a distance of 100 mm., and perhaps at greater distances.

Very often a fungus may show for a time complete checking of growth when in the presence of another fungus. Later, growth will appear to be resumed. This growth on closer examination will be seen to be in the deeper part of the medium, which is apparently less affected by the inhibiting organism. The surface growth when once checked usually does not again resume activity. A case of this sort is illustrated by text figure 2.



TEXT FIG. 2. *A*, colony of Alternaria. *B*, bacterial growth. *XY* represents the line upon which the Alternaria colony was temporarily inhibited.

COLONY CHANGES

Inhibition modifies a fungus in many ways evident to the eye. Physiological changes also probably occur, but these will not be discussed at this time. Changes that occur vary directly with the inhibition. All organisms that do not overspread the surface of the contesting fungus are restricted in growth. Helminthosporium when alone in a plate would normally continue to grow until the surface of the medium is occupied. If any other organism is placed in the plate with the Helminthosporium, the growth of the latter is greatly restricted. Two spores of Helminthosporium may germinate in the same plate and the resulting colonies may each cover approximately half the area, with a narrow but definite streak of unoccupied agar between them. If organism no. 31, no. 33, or no. 34 is planted in a petri plate with Helminthosporium, the latter forms a colony with a curved margin, the concave side toward the antagonistic organism.

Some fungi are almost entirely inhibited in growth by the presence of other organisms in the same petri plate. This reaction is illustrated by organisms nos. 3, 7, 8, 9, or 10 in the presence of Helminthosporium, or of any other fungus that develops mycelium rapidly. Thus we see that antagonism greatly modifies the shape and size of the colony.

Changes in color are quite often associated with other inhibitional changes. Color may be limited to a very narrow band or may pervade the entire mycelium.

Organism no. 12, a bacterial form, causes a narrow (1-mm.) line along the proximal border of an Helminthosporium colony with which it is grown. Organism no. 5 causes a similar red line to appear in a colony of organism no. 4 when these two are grown together in a petri plate. Organism no. 5, when grown by itself, has a light pinkish tinge. The depth of this color is modified by the organism grown with it; it may fade out entirely or become bright red, with all gradations between according to the species of the antagonistic organism.

Antagonism stimulates spore-production. Along the line of impending contact between colonies spores are more numerous than elsewhere. No case was observed in which spore-production was decreased, although such spore-production is more apparent with some combinations than with others.

Morphological Changes

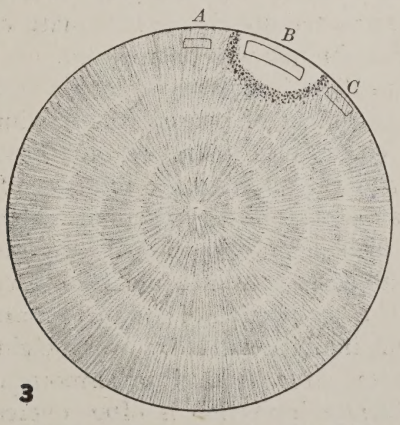
When Helminthosporium is growing in pure culture on cornmeal agar under normal conditions of moisture and temperature, the aërial mycelium at the edge of the colony is of uniform diameter, infrequently branched, and straight. If a culture of organism no. 45 is introduced several centimeters away from the periphery of the colony, the following morphological changes occur: when the hyphae of Helminthosporium are within 2 centimeters of the bacterial colony, growth slackens and eventually ceases. Branches are given off from the hyphae in every direction and these become gnarled and twisted, and bubble-like enlargements of varying size appear in this portion of the mycelium with almost instantaneous suddenness. The swollen segments may be regarded in some cases as extremely dwarfed branches, but often they appear as enlargements in the existing hyphae. So great is the distortion thus produced that often the species is not recognizable. The multiplication of branches piles up the hyphae along the line of impending contact, which appears to the eye as a black streak. The effect is often heightened by excessive spore-formation along this line. In addition to these distortions, the hyphae have a tendency in their general direction of growth to avoid the zone of influence controlled by organism no. 45 and to grow around it. Elliot (12), working with Alternaria and a bacterial form which he designated as "X," found distortions practically identical with those which I have described for Helminthosporium, and I have obtained similar results with Alternaria when inhibited by my organism no. 45. No. 45 produces strong inhibitory effects under the conditions described in all filamentous fungi experimented with except the Phycomycetes, which are little affected.

The greater the inhibition, the greater the distortion. Therefore, the greater the distance through which the reaction takes place, the more marked

are the results. The morphological changes grade from changes in form, size, and structure of the hyphae, and change in direction of growth, to mere cessation of growth with hyphal segments becoming progressively shorter.

The morphological changes described above indicate that there are forces operating capable of producing profound effects on the morphology of the organism involved. In every case in which malformations occur, no morphological differences of diagnostic value could be determined. Three hypotheses suggest themselves as to the causes of these variations: (*a*) The nutrients may be exhausted. (*b*) The distortions may be due to change in the osmotic equilibrium of the medium induced by the metabolic activities of the growth process. (*c*) Certain poisonous products may be created by fungous growth capable of producing malformations and creating a zone through which fungous filaments can not pass. Much work would have to be done in order to determine which of these hypotheses best explains the facts. I am not prepared to give a definite answer to the question, but my experiments have thrown some light that may be of aid in the solution.

It has been explained that organism no. 45 inhibits all Helminthosporiums at a distance of 2 centimeters or more, so that the growth of the latter is checked entirely. If a block of the medium which occupies the space between the two organisms is removed, aseptic precautions being exercised, and placed in another dish occupied solely by an Helminthosporium colony, the filaments of the Helminthosporium will not pass over the block but will be checked sharply in front of it, with characteristic distortions. That the effect was not due to mechanical blockade was proven when sterile blocks of normal agar were placed in proximity to Helminthosporium colonies and were soon overgrown by the advancing hyphae. Furthermore, blocks of agar taken from the vicinity of organism no. 45 caused inhibition with effects as marked as before when the blocks were inlaid rather than placed upon the surface (text fig. 3).



TEXT FIG. 3. Colony of Helminthosporium sharply inhibited in front of *B*, a block of sterile agar taken from the vicinity of organism no. 45. Blocks *A* and *C* are from sterile agar plates and cause no inhibition.

To note the effects of certain chemicals on the growth of Helminthosporium filaments, the following substances were used:

1. Copper sulphate crystals,
2. Phenol crystals,
3. Phenol (melted) full strength,
4. Phenol (melted) one-half strength,
5. Chloramine T crystals,
6. Sodium nitrate,
7. Mercuric chlorid,
8. Glucose,
9. 95 percent alcohol (one drop),
10, 11, 12, 13, 14. Aqueous solutions of copper sulphate of the following strengths: saturated, 75 percent, 50 percent, 25 percent, and 1 percent.

These materials were placed within 2 centimeters of vigorously growing colonies of Helminthosporium no. 20. The chemicals were permitted to act separately in separate plates. Total inhibition was obtained by the use of substances 1, 2, 3, 4, 5, 7, 10, 11, 12, and 13. With substances 6 and 14, slight inhibition was obtained. Substances 8 and 9 caused no inhibition. Where inhibition occurred, except with 6 and 14, distortion of the mycelium was apparent. The distortion was in direct ratio to the strength of the substances used. The distortions were very similar to those previously described when organism no. 45 was used as the inhibiting agent. Those occurring with the use of chemicals very often showed hyphae peculiarly twisted into tight loops. It will be noted that in this experiment those chemicals which are known as powerful germicides and fungicides produced the more marked effects, even though considerably diluted.

Effect of Modifications upon Inhibition

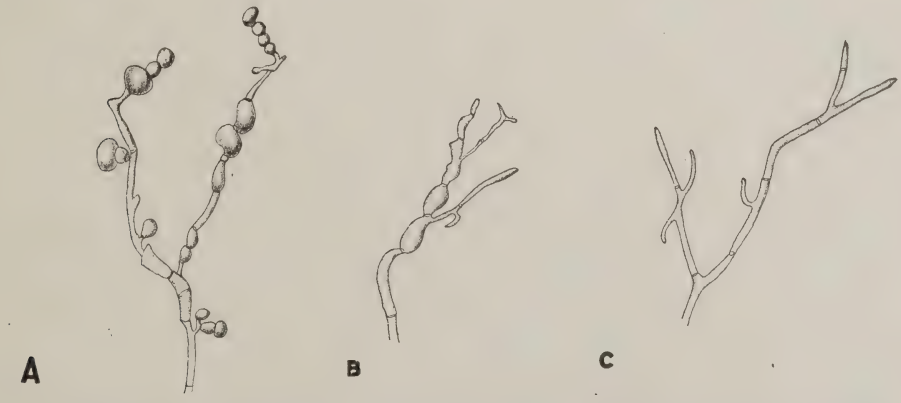
To this point, the phenomena peculiar to antagonism have been considered as occurring under uniform conditions. In order to discover whether variation of conditions materially changes the nature and degree of inhibition, modifications in technique were introduced.

The depth of the medium in a plate was easily varied through all gradations by the simple expedient of pouring the medium in the plates while they were tilted. A colony of a fungus planted in such a plate tended to occupy all sections, and the effect of the presence of other fungi could be tested in both the shallow and the deep portions. Media including cornmeal agar, dextrose agar, plain agar, and washed agar were experimented with in this manner. In all cases in which inhibition occurred normally it was more marked in the shallow portion of the plate than in the deep portions. Evidently the normal inhibitional effect was heightened by the lack of nutrients in the shallow situations. Furthermore, in a normally poured plate the effect of surface inhibition is often overcome by the inhibited fungus sending forward hyphae in the deeper portions of the medium where the inhibitory products make their presence felt to a less degree. In a

shallow medium, however, the inhibitory products penetrate throughout the layer of the medium and the fungus is checked entirely. While the effects are more marked in the shallow portions, they are always the same in character and the inhibitional type is not changed. Spore-formation was more abundant in the shallow portions, and especially in the direction of approaching contact, than was normally the case.

Two types of media were used: one rich in nutrients, the other poor in nutrients. Cornmeal, dextrose, and brazil-nut agars represent the former type, while plain and washed agars are included in the latter class. Many organisms were grown on these media in a manner to test their inhibitional characteristics. The organisms included Helminthosporium, bacteria, Mucors, Alternarias, and yeasts. It was found that, when a fungus was sharply inhibited on normal cornmeal agar, it was better able to contend with its antagonistic neighbor on a medium rich in dextrose. On the other hand, in washed agar the inhibition became even more marked. On media poor in nutrients, the line of demarcation as between two closely related Helminthosporiums became much more distinct and had the same characteristics that it would have on cornmeal agar between two widely divergent forms.

The longer a colony of any organism grows by itself on the surface of a nutrient medium, the larger and more vigorous it becomes. Such a large colony is just as effectually checked by the proximity of another organism as if the two contending forms were of equal age and vigor. Helminthosporium with a 120-hour start is inhibited by organisms nos. 31, 33, 34, and 45, and the inhibition is of the same type as if they had been planted simultaneously.



TEXT FIG. 4. *A*, distortion of an Helminthosporium filament caused by the presence of organism no. 45. *B*, distortion of an Helminthosporium filament caused by *Bacillus ramosus*. *C*, a normal filament of Helminthosporium.

Change in the mass of inoculum has much the same effect as difference in the time of inoculation. If the mass of inoculum is small, the inhibition

may not be as great and the reaction may be slower. There are exceptions to this statement. The smallest amount of inoculum possible, using organisms nos. 31, 33, 34, and 45, produces the same effect as when a mass many times larger is used.

Inhibition Characteristic of Various Groups

The great groups of fungi were studied critically to ascertain whether any of the types of inhibition previously described were peculiar to them.

Thirty-five different species representative of the Schizomycetes were studied. The Schizomycetes are quite variable regarding the nature of their inhibitions. Some of the most powerful inhibitors belong to this class. These cause marked morphological disturbances and may do so even over considerable distances. On the other hand, most of the bacteria studied were quite inert, causing no inhibition, and being covered eventually by the hyphae of even slow-growing filamentous fungi. It appears from my experiments that the spore-formers as a rule are strong inhibitors. Actinomyces likewise exhibited strong inhibitory action against most filamentous fungi.

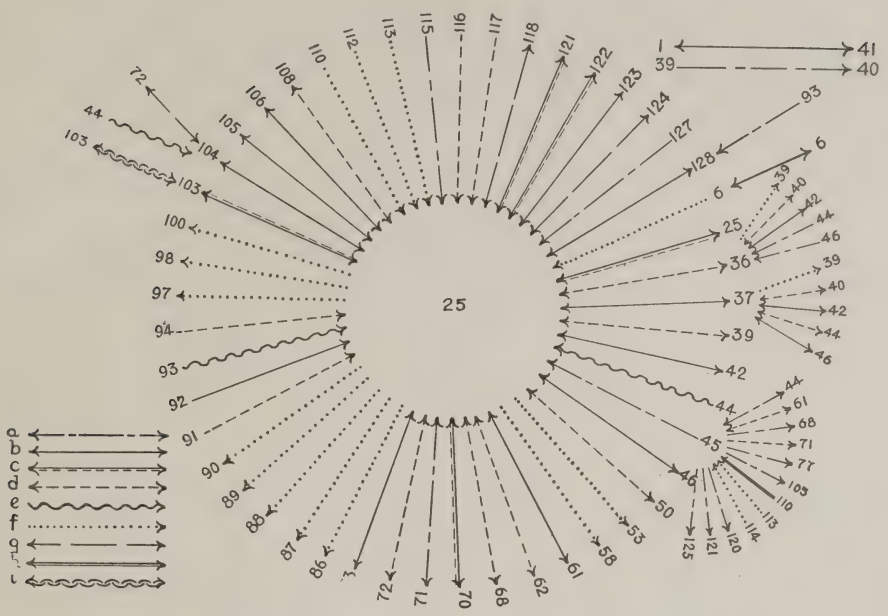
The Phycomycetes have a tendency to grow superficially and to spread rapidly over the surface, burying all other fungous colonies beneath their advancing mycelium. As a rule, they neither cause inhibition, nor are they inhibited.

The Ascomycetes vary somewhat in the nature of their behavior toward each other and in the presence of other organisms. As a rule their inhibitory powers are not great, though some of the yeasts rank high as inhibitors in this class. With respect to these powers no distinction could be made between the conidial and ascigerous forms of the same species.

Few cultures of Basidiomycetes were used because of the difficulty in getting them to grow well in artificial media. Sporidial cultures of two varieties of *Ustilago violacea* were found to be absolutely inert with respect to their inhibitory powers. Moreover, they were but little affected in the presence of other fungous forms, but were invariably overgrown by them.

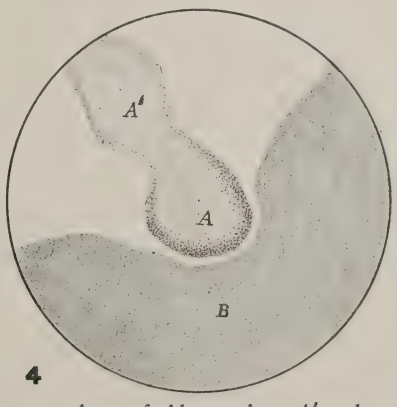
The Fungi Imperfecti, like the Ascomycetes, are quite variable respecting their inhibitory powers. While these powers never appear to be great, yet they are possessed by nearly all of the group to some degree.

Text figure 5 illustrates the nature of the contacts when the various fungi were grown with each other. With but few exceptions, the closer the degree of relationship the less marked is the inhibition between the colonies. If two colonies of Helminthosporium grown from spores obtained from a single pure culture are permitted to grow in the same petri dish, they will eventually grow together with their fibers intermingling, giving only slight evidence of separate contact borders. Two colonies of the same species of Mucor, if they are of the same race, react similarly, as do also two colonies of Alternaria, of Acrostalagmus, of Botrytis, or of Fusarium. If the or-



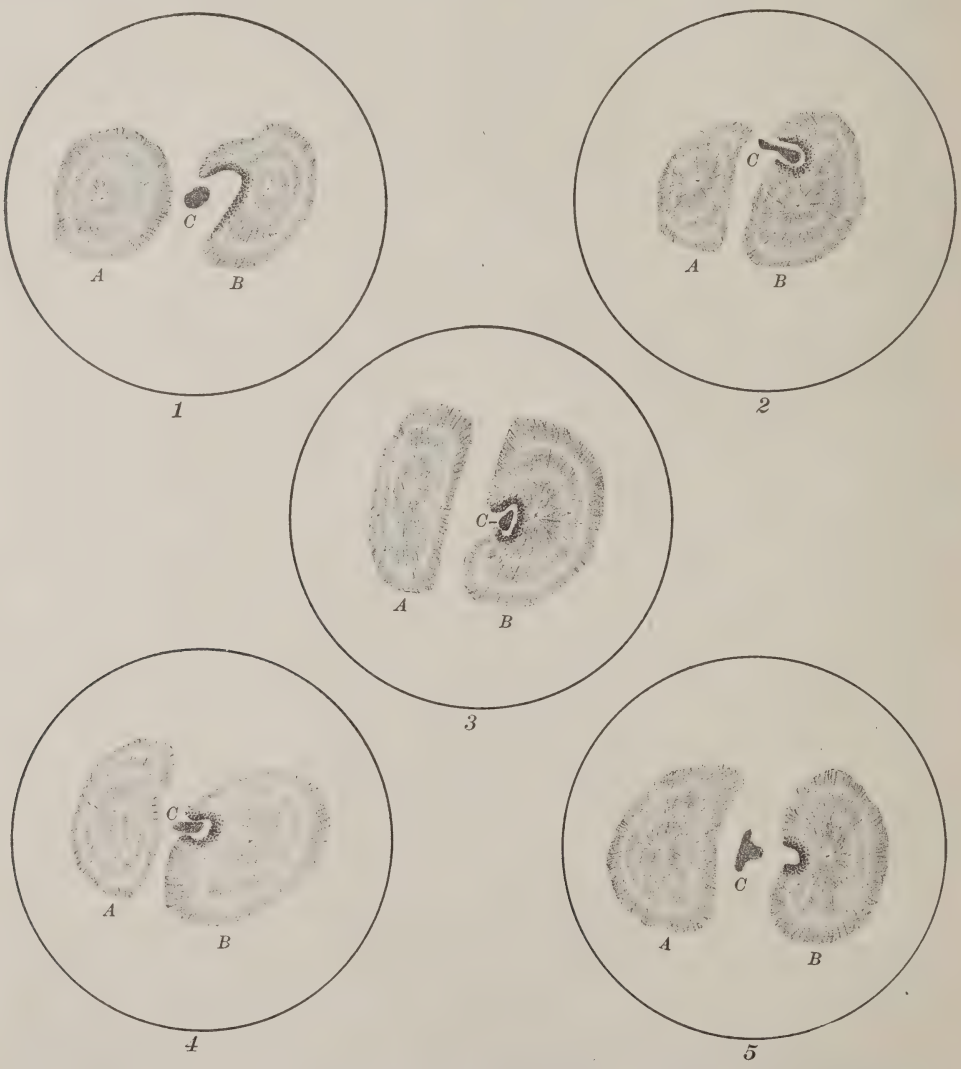
TEXT FIG. 5. Reactions of fungi growing together on cornmeal agar. The figures refer to numbers in the accession list. The arrows point to the direction of the reaction. The symbols are explained as follows: *a*, Antagonistic and producing distortion. *b*, Slightly antagonistic. *c*, Antagonistic at first only. *d*, Complete inhibition without distortion. *e*, Spore-formation increased. Growth checked. No distortion. *f*, Overruns. Arrow points to the colony overrun. *g*, No inhibition. *h*, Grows around. Arrow points to colony grown around. *i*, Mycelium more abundant and raised at contact.

ganisms are of distinct species, they usually betray that fact by some more or less distinct hiatus at contact such as distorted hyphae, color lines, more abundant sporulation, or a wider neutral zone through which the hyphae of the two colonies are unable to pass.



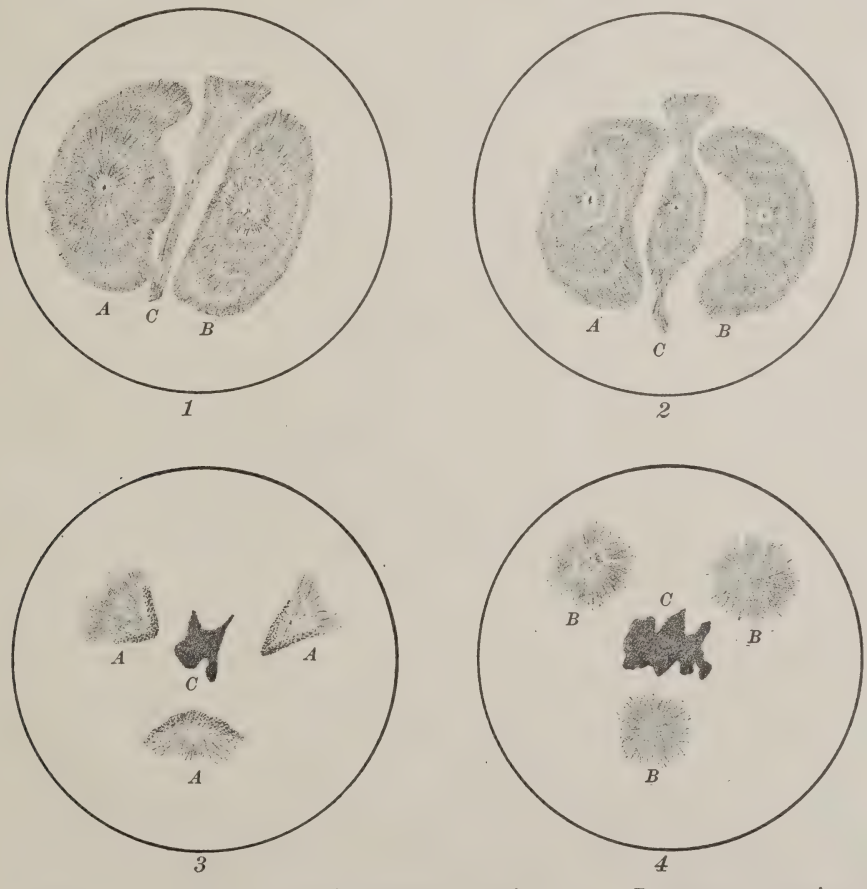
TEXT FIG. 6. *A*, parent colony of Alternaria. *A'*, sub-colony of *A*. *B*, bacterial form inhibiting *A*. No inhibition between *A* and *A'*.

When the relationship is more distinct than that of species, the line of demarcation is usually still more marked. Text figure 6 illustrates this point. In this figure, *A* represents a parent colony of Alternaria. *A'* is a sub-colony of *A*, and they grow together, merging so perfectly that it is impossible to tell where one colony leaves off and the other begins. On the other hand, the line between *A* and *B*, a bacterial form, is made clearly evident by the increased sporulation at contact. The rule cannot be applied further than this, since one can not say that members of two orders are more antagonistic than are the members of two families. After reaching certain limits, degrees of antagonism are not distinct.



TEXT FIG. 7. In every case *A* represents organism no. 25; *B* represents organism no. 26. In *1*, *C* = organism 31; in *2*, *C* = 8; in *3*, *C* = 32; in *4*, *C* = 12; and in *5*, *C* = 10.

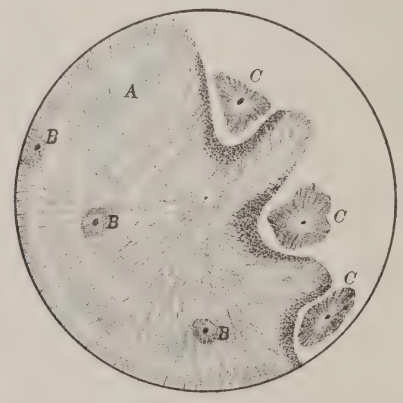
Two different fungi may not respond alike in the presence of other forms. This may be of diagnostic aid even when the fungi concerned are as closely related as varieties of the same species. Two varieties of *Helminthosporium teres*, nos. 25 and 26 of the accession list, illustrate this possibility. The colonies of these Helminthosporiums differ somewhat in appearance, zonation being more marked in the latter than in the former. The organisms were both inhibited by a number of organisms with which they were grown, but reacted differently with most of them. The difference was so great that one had no difficulty in distinguishing between the two varieties, judging solely from the reactions due to antagonism. Text figures 7 and 8 illustrate the differences described. When organisms nos. 25 and 26 were grown in the presence of organism no. 31, a bacterial colony, no. 25 is not inhibited while inhibition in no. 26 is characterized by cessation of growth and greater spore-formation. In the presence of organism no. 8, a yeast, no. 25 is inhibited somewhat, as may be seen by the fact that toward the yeast colony



TEXT FIG. 8. In every case *A* represents organism no. 25; *B* represents organism no. 26. In *1*, *C* = 5; in *2*, *C* = 29; in *3* and *4*, *C* = 33.

no. 25 presents a straight rather than a rounded outline. No. 26 is inhibited more sharply and with spore-formation increased. In the presence of organism no. 32, a bacterial form, much the same relations exist as with the case of the yeast just described. These relations are also much the same when the two Helminthosporiums are grown in the presence of organism no. 12, another bacterial form, or of organism no. 10, a yeast. Both organisms nos. 25 and 26 are inhibited by organism no. 5, a Fusarium, although in this case the growth of no. 25 is more sharply checked than is the growth of no. 26. Little difference in inhibition is to be noted when the two are grown together in the presence of organism no. 29, an Alternaria. In the presence of organism no. 33, an arborescent-growing bacterium, no. 25 is sharply inhibited at a considerable distance with cessation of growth, distortion of hyphae, and increased spore-formation toward the sides presented to the bacterial colony. On the other hand, no. 26 showed no evidence of inhibition in the presence of organism no. 33. Summarizing, no. 25 is inhibited markedly only by organisms nos. 5, 29, and 33. No. 26 is markedly inhibited by organisms nos. 31, 8, 32, 12, 10, 5, and 29. This experiment was repeated many times, invariably with the same results as described above and as shown by text figures 7 and 8.

Reference to the diagram (text fig. 5) reveals illustrations of the same sort. In this diagram attention is called particularly to reactions exhibited



TEXT FIG. 9. Pestalozzia inhibited by *C*, Penicillium colonies, but not by *B*, Penicillium colonies of a species different from *C*.

by organisms nos. 36 and 37. In this instance Acrothecium caused distortion, the threads being twisted into knots when this fungus was in contact with organism no. 36, an Helminthosporium; no. 37, another Helminthosporium, was slightly inhibited by the Acrothecium but without visible distortion. In this case no. 37 caused more abundant spore-formation in the Acrothecium colony. Actinomyces caused both Helminthosporiums to be inhibited, but distortion of filaments was produced only in no. 36. With

organism no. 25, another Helminthosporium, growth was completely stopped in no. 36 with practically no intermingling of hyphae. Growth of no. 37 was slowed somewhat in the presence of organism no. 25, but the hyphae mingled to a considerable degree.

Text figure 9 shows a culture of Pestalozzia. This became contaminated with two races or species of Penicillium. Three colonies of one kind of Penicillium caused no inhibition of the Pestalozzia. The other Penicillium, of which there were also three colonies, completely inhibited the Pestalozzia with increased sporulation and a piling up of the hyphae. These two kinds of Penicillium are clearly differentiated by the nature of their reaction with the Pestalozzia.

Biological Equilibrium

Inasmuch as organism no. 45 was found to possess such extreme inhibitory powers, an effort was made to discover whether this organism might be of some practical importance in checking the growth of other fungi upon plants parasitized by them.

In the first of this series of experiments, sterile wheat seedlings were grown in rag-dolls and the cloth around the seedlings was heavily inoculated with Helminthosporium spores. One half of the rag-dolls prepared in this manner had previously been immersed in a broth culture of organism no. 45. Table 1 gives the results of this experiment.

Table 1. *Protection afforded wheat seedlings from attacks by Helminthosporium, using organism no. 45 as the protecting agent*

	October 25			November 3			December 2			December 18		
	Infected	Uninfected	Percent Infected	Infected	Uninfected	Percent Infected	Infected	Uninfected	Percent Infected	Infected	Uninfected	Percent Infected
Protected by no. 45....	1	9	10	1	9	10	14	28	33.3	3	20	13.04
Unprotected.....	4	6	40	5	50	28	15	65.1	20	9	9	68.9
Without either Helminthosporium or organism no. 45.....	0	10	40	0	10	0	0	35	0.0	0	25	0.00
Without Helminthosporium, with organism no. 45.....	0	10	0	0	10	0	0	36	0.0	0	30	0.00

The percentages given in this table would indicate that a certain amount of protection was given to the wheat seedlings by the presence of organism no. 45.

The next experiment was made to determine whether organism no. 45 would protect plants under more natural conditions than existed in the foregoing experiment. It was decided to attempt the protection of flax seedlings grown in pots from attacks of *Fusarium lini*. *Fusarium lini* grows

well in the soil, quickly infects and kills the plants, and in plate cultures was inhibited by organism no. 45, although not so strongly as are the Helminthosporiums. In this experiment the protected seedlings had their bases surrounded by cores of earth heavily impregnated with a broth culture of organism no. 45 and separated from the surrounding earth containing *Fusarium lini* by paper cones. The controls were surrounded by cores of sterile earth, also in paper cones. The earth in these cones was saturated with a sterile broth solution in order to have the physical conditions the same. All the plants in this experiment succumbed to Fusarium wilt. The controls, however, in every case showed indications of the wilt before the protected plants did. An examination of the protected plants indicated that the roots had penetrated the paper cones and were growing into the unprotected regions containing Fusarium. This fact probably accounts for the absence of protection exhibited by the inhibiting organism in this case. Further experimentation on this phase has been inconclusive because of difficulty in securing virulent strains of *Fusarium lini.*

Experiments were made to determine whether organism no. 45 was as strongly inhibitory to *Fusarium lini* in soil as on culture plates. Soil thoroughly moistened with beef bouillon was packed into test tubes. The lower third of the tube was inoculated with *Fusarium lini*, the middle third was moistened with a broth culture of organism no. 45, and the top third was sterile earth. In the controls, the middle third of the tube was filled with sterile earth. Samples were removed from time to time from the top third of each tube and plated. After a month, *Fusarium lini* had not penetrated to the top third of earth in any tube where the intervening third was impregnated with a suspension of organism no. 45. In most of the controls, after an interval of two to three weeks *Fusarium lini* could be detected by cultural methods in the upper third of earth.

Plate cultures of earth were made, the earth being moistened with beef bouillon and then packed while damp into plates to the depth of a half centimeter or more. Transfers were made from pure cultures of fungi to the surface of the earth on these plates in the same manner as one would inoculate plates of agar. Most fungi grew nearly as well, although perhaps more scantily, on such plates as on plates containing cornmeal agar. *Fusarium lini* was as sharply inhibited by organism no. 45 on such a plate as on ordinary media. In fact, all fungous combinations tried reacted in much the same way as illustrated in text figure 5. On plain earth moistened with sterile water, most fungi grew so scantily that their inhibitions could only with difficulty be determined. On non-enriched earth, organism no. 45 grows so slowly that it is doubtful whether it would be very useful in checking the normal soil flora.

Potter's (29) suggestion that plants may be protected from fungous attacks by injections of the metabolic by-products of the fungus concerned was thought worthy of testing at this time because it supports the idea that

inhibition is due to products secreted or excreted by the fungus rather than to the exhaustion of nutrients. Oranges were injected with the by-products of the growth of Penicillium and were then inoculated with Penicillium. Growth of the Penicillium was sharply inhibited at the line of injection and but slowly and imperfectly worked its way over the line. Oranges that had not been injected with the staling solution were quickly and entirely covered with the Penicillium.

Since there exists within the soil such a very close association between rootlets and fungi, the possibility exists that one may be very much affected or even considerably modified by the other. In order to determine how far this might be true, aseptic seedlings of barley, oats, wheat, and rye were placed in close proximity to colonies of Helminthosporium and of bacteria growing in petri dishes upon cornmeal agar. The seedlings were placed in such a manner that the tips of the rootlets of one and the sides of the rootlets of the other were presented to the fungous colony. The following possibilities were kept in mind: (1) Inhibition or stimulation of the fungus; (2) Inhibition or stimulation of root-hair production; (3) change of direction of growth of the rootlet tip. After exposure for 48 hours there were no noticeable effects either upon the fungus or upon the rootlets (Plate VI, fig. 2).

Discussion

Smith (40) summarizes the possibilities existent when two or more organisms are grown in close proximity as antagonistic, indifferent, or favorable. Zeller and Schmitz (48) enumerate the possibilities as stimulating, inhibiting, overgrowing, and non-influencing.

Garré (15), de Freudenreich (10), Laws and Andrews (21), Remy (37), Horrocks (19), and Frost (13), all working with *Bacillus typhosus*, have demonstrated that it may be inhibited in the presence of several other bacterial organisms.

The antagonism of protozoa toward bacterial forms has been put to practical use in the purification of water and sewage. In this instance, however, the inhibitory action may be due to the actual ingestion of the bacteria by the other organisms involved. Purdy and Butterfield (32), Razetto (35), Huntemuller (20), Stokvis (45, 46), and Olitsky (27) have cited such examples.

It has frequently been demonstrated during my experiments that some of the most profound inhibitory effects are exhibited by bacteria toward fungi. This fact has been noted by Ravn (33, 34), Elliot (12), and Reinhardt (36).

The antagonism which certain fungi exhibit against other fungi of the same or of different species has been noted in the literature by Blakeslee (3), Edgerton (11), Stevens and Hall (42, 43), Crabill (8), Reinhardt (36), Fulton (14), Zeller and Schmitz (48), F. L. Stevens (41), N. E. Stevens (44),

and among the higher fungi the same phenomenon is noted by Shantz and Piemeisel (38).

The references to well authenticated instances in which fungi have stimulated growth of some sort or have been otherwise beneficial to each other are not so numerous. Nevertheless, such references are made by F. L. Stevens (41), Stevens and Hall (43), Shear (39), Zeller and Schmitz (48), Reinhardt (36), Ward (47), de Bary and Woronin (9), Manns (24), Pringsheim (31), and Nikitinsky (25).

These effects, both antagonistic and stimulative, have a very direct bearing upon the members of a mixed culture and the nature of succession in such a culture. Pringsheim and Nikitinsky have expressed such views in articles already cited (31, 25). This idea is also upheld by Gwynne-Vaughan (16), Hesler (18), Smith (40), and Heinemann (17).

The explanation of effects produced upon fungi in mixed cultures may be divided into two classes: (1) The nutrients of the medium may have become exhausted; (2) Products are formed which are detrimental or beneficial to further growth. Obviously when the nutrients are exhausted growth will be checked, but Leisegang (22) is one of the few who would explain the entire inhibitional phenomenon in this manner. On the other hand, many advocate the theory that during growth organisms give off materials that may be inhibitory or stimulatory to themselves or to other members of the flora. Gwynne-Vaughan (16), Clark (7), Brown (4), Balls (1), Fulton (14), Lutz (23), and Chambers (6) are advocates of the latter theory.

By taking advantage of the products produced as mentioned in the preceding paragraph, fungous growth may be inhibited or stimulated to the advantage of man. Those who have done this experimentaily are Potter (29, 30), Picardo (28), and Beauverie (2). Norton (26) believes that such experiments have no practical value.

Judging from my own experiments and from the literature on this subject, it would seem possible that under certain circumstances the knowledge of the relationships of fungi could be used in controlling the growth of these organisms to our advantage.

Summary

1. The inhibitions exhibited by fungi may be grouped into five classes.

2. Helminthosporium was inhibited by various chemicals in a manner similar to that caused by other fungi.

3. The inhibiting qualities of a fungus may be of aid in identification of species.

4. The richer the medium in nutrients the less marked were the inhibitions.

5. The inhibitions varied but slightly with changes in the amount of inoculum, in time of inoculation, or in depth of medium.

6. A common cause of the inhibitory action in the cases studied was determined to be the presence of some product formed during growth.

7. Seedlings were protected measurably from infection by Helminthosporium, using organism no. 45.

8. Flax seedlings were measurably protected from Fusarium, which could only with difficulty pass a layer of earth heavily infected with the inhibitor.

9. Roots of seedlings and root hairs gave no tropic response in the presence of fungi.

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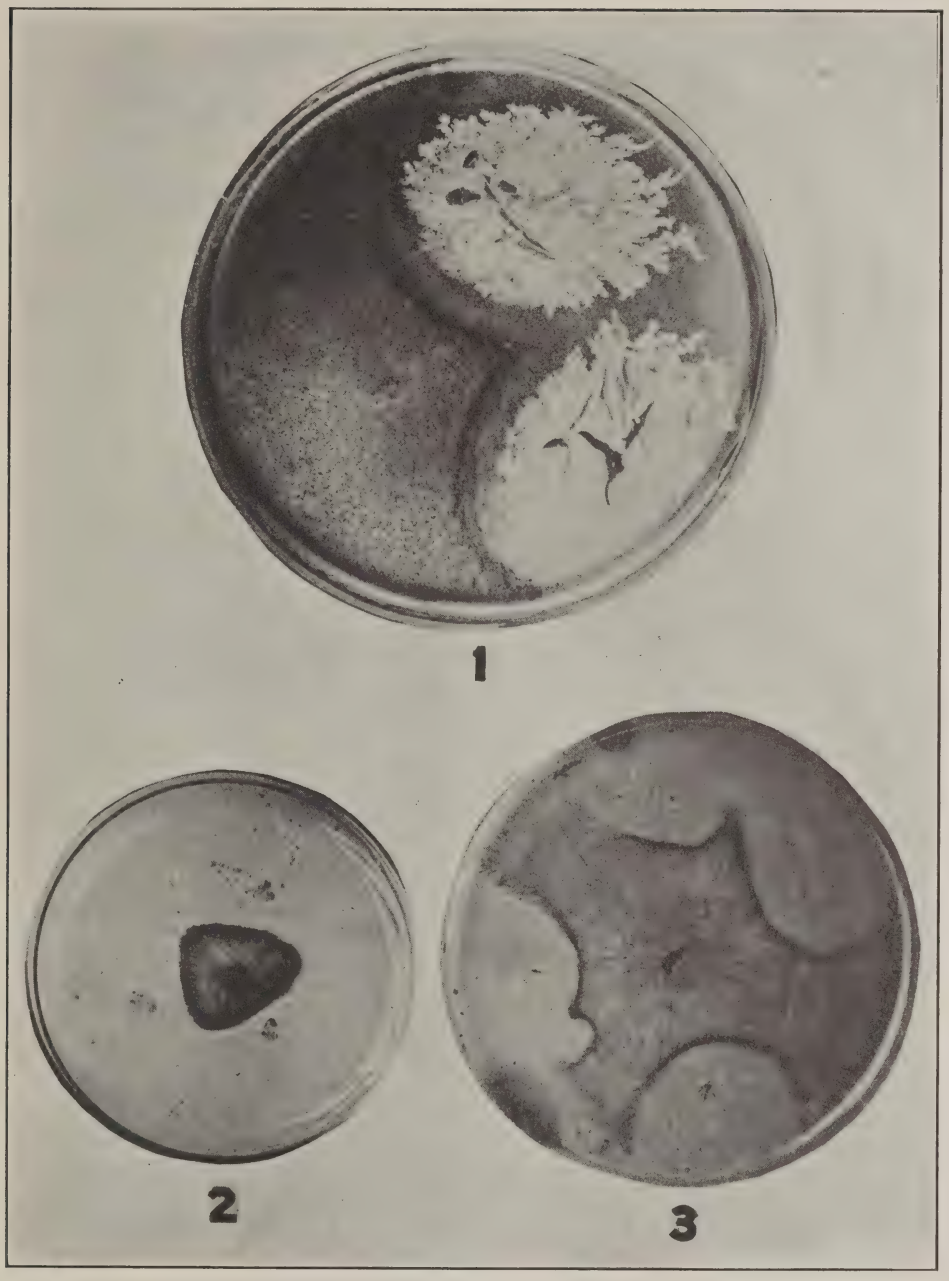

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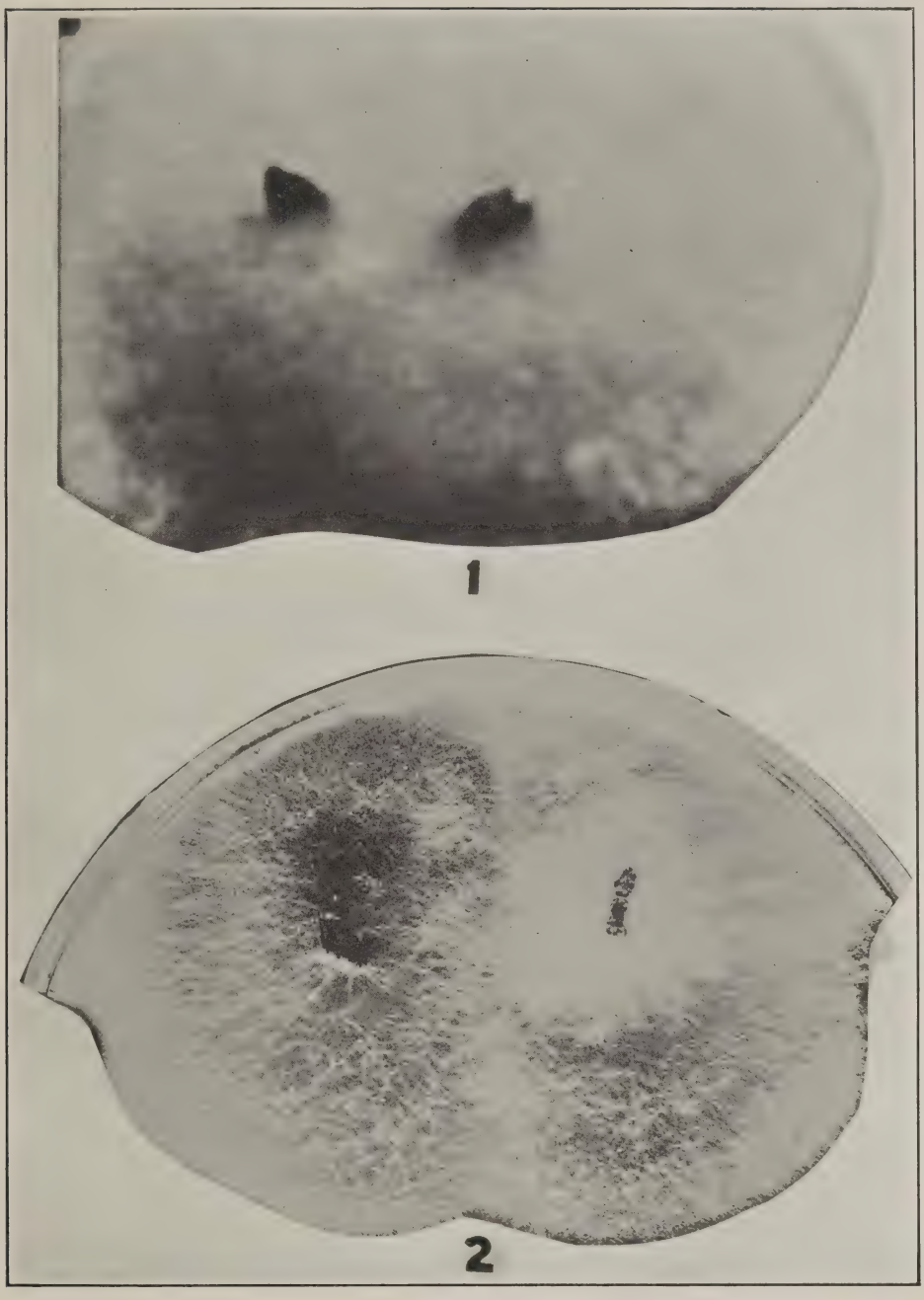

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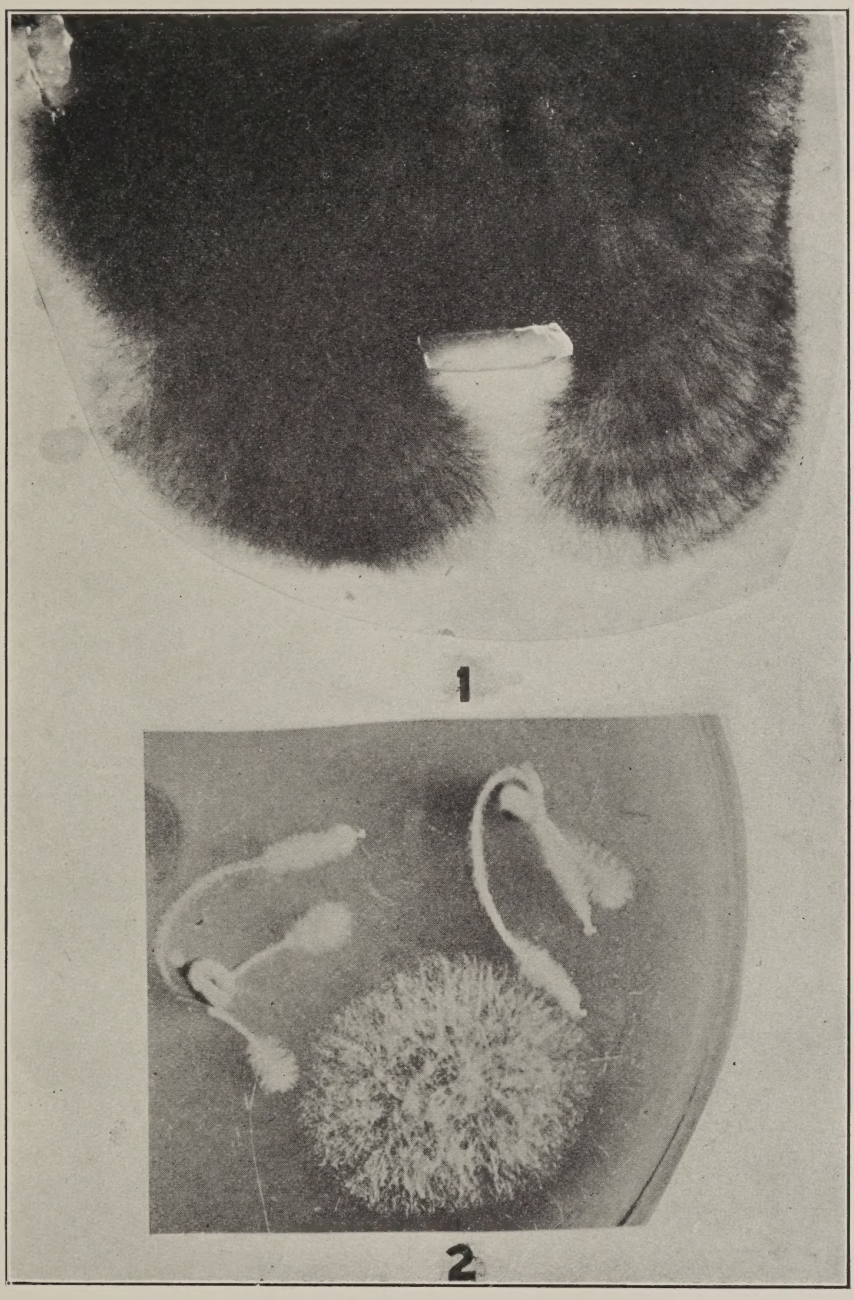

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EXPLANATION OF PLATES

PLATE IV

FIG. 1. Alternaria inhibited by two colonies of organism no. 45.

FIG. 2. Helminthosporium in the center inhibited on three sides by Actinomyces.

FIG. 3. Helminthosporium in center inhibited by four small colonies of organism no. 45.

PLATE V

FIG. 1. Nature of reaction when two colonies of the same species grow together. Organism no. 25 of the accession list.

FIG. 2. Nature of the reaction when two species of Helminthosporium grow together. Organisms nos. 25 and 37 of the accession list.

PLATE VI

FIG. 1. Helminthosporium inhibited by a block of sterile agar taken from the vicinity of organism no. 45.

FIG. 2. Indifference of wheat rootlets to a colony of Helminthosporium.


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VITA

The writer of this thesis was born at Mackinaw, Illinois, February 22, 1889. His early education, including his high school training, was received in the schools of that community. In 1907 he entered the Illinois Wesleyan University at Bloomington, receiving the degree of B.S. from that institution in 1911. In the spring of 1912 he entered the University of Illinois, graduating in 1913 with the degree of A.B., doing most of his work in the College of Agriculture. From 1914 to 1916 he was instructor in biology at Parsons College, Fairfield, Iowa. From 1916 to 1919 he served as head of the Department of Biology at Fairmount College, Wichita, Kansas. He served this college as registrar in 1918. The summer and latter part of 1918 was spent in military service. In 1919 he again entered the University of Illinois to do graduate work in the Department of Botany. He was assistant in botany at the University of Illinois 1919–1921, including the summer terms of 1920 and 1921. He received the degree of Master of Arts in 1921. From September, 1921 to September, 1922 he was engaged part time as botanist in the Natural History Survey and part time in graduate work. The summer of 1922 was spent in the field in the interests of the Natural History Plant Disease Survey. The university year of 1922–1923 was devoted to full-time graduate work.