COMPETITION IN FUNGI

I. A Study of the Growth Reactions of Non-Parasitic Fungi in Associated Culture

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Introduction

The earlier works on mixed culture are mostly studies on growth reactions of fully developed mycelia in a given medium. The present work was undertaken to study the influence of one fungus upon another in paired and associated cultures in media variously modified, with a view to gaining an insight into the nature of competition in fungi.

In nature, competition occurs whenever more than one fungi associate together, and the success or failure of a fungus depends upon the chemical and physical nature of the habitat and upon the environment. It must also depend, to some extent, upon the effect the fungi exert upon one another. A study of the latter aspect is the main objective of this paper.

Growth reactions in artificial culture are not necessarily true indications of what may occur on host in nature (Machacek, 1928), but nevertheless, it was thought that studies in simpler media are likely to throw more light on general problem of competition, particularly with regard to saprophytes, than studies using complex host tissues.

This paper which is the first of a series deals with the growth reactions primarily of two non-parasitic fungi, *Fusarium* and *Dendryphiella,* in associated cultures. The detailed investigation was restricted to these two fungi only since it was found that a larger number of combinations would make the work unwieldy. Similar experiments with parasitic organisms will follow in subsequent papers of the series.

Material and Method

Six fungi were utilized for preliminary observation. The fungi differed both in the microscopic and macroscopic characters, and these afforded an

easy means of distinguishing the competing strains in mixed growths. Out of these six only two fungi were chosen for a detailed investigation. The names of the fungi together with a very short description of their macroscopic characters are given below.

1. *Fusarium* sp. .. Mycelium white, moderately fluffy, non-zoning, submerged mycelium very little.
2. *Dendryphiella* sp. .. Mycelium dark, fluffy, non-zoning, submerged mycelium abundant.
3. *Phoma* sp. .. Mycelium white, fluffy, zoning with distinct dark-red rings. Visible as light band at the top very clearly in the substratum.
4. *Helminthosporium* sp... Mycelium greyish-white, slightly fluffy, zoning, submerged mycelium very little.
5. *Monilia* sp. .. Mycelium dark-grey, moderately fluffy, zoning with rings of darker grey colour. Submerged mycelium very little.
6. *Gibberella* sp. .. Mycelium whitish-grey, mostly superficial, thin growth, non-zoning.

The actual combinations in which the fungi were paired are given along with the experimental details.

The method of investigation consisted in inoculating a given plate of nutritive medium with two fungi (members of a pair) and comparing the growth rates and the relative area occupied by the competing fungi with those of the controls.

The strains were inoculated at different degrees of proximity, *viz.*, 2 cm. apart, adjacent (two inocula touching each other) and mixed, with individual controls.

For purposes of getting the average rates of growth and also for verification of results replicates of three petri-dishes were used in each kind of inoculation with two controls for each of the strains.

The growth rates of the competing fungi and of the controls were noted every twenty-four hours together with other features of interest. The growths arising from mixed inocula were examined under binocular microscope whenever required.

The standard synthetic medium used throughout the experiment as basal medium, except where otherwise stated, had the composition : glucose

2·0 gm., potassium nitrate 2·0 gm., magnesium sulphate 0·75 gm., potassium phosphate 1–25 gm., potato starch 10·0 gm., shredded agar 15·0 gm. and distilled water 1 litre.

The detailed work on *Fusarium* and *Dendryphiella* was carried out in the standard medium variously modified by the addition of N/10 hydrochloric acid and malic acid in acid series, sodium carbonate and sodium hydroxide in alkali series, in different percentages; the details of which are given in appropriate places.

Petri-dishes 2 cm. deep and 11 cm. in diameter were used in all cases. The different media were poured 1·5 cm. deep to enable to make necessary observations in the substratum as well. The usual methods of sterilizing petri-dishes and media were employed.

At the commencement of the work the purity of the various fungi was assured by taking monohyphal cultures and maintaining stock cultures in tubes of standard synthetic medium.

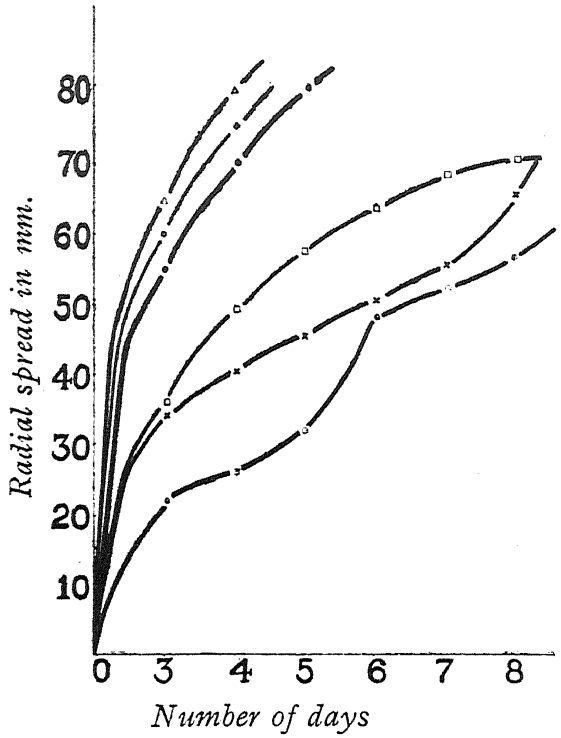
For purposes of inoculations equal amount of inocula were used from the peripheral regions of the cultures of four or five days growth in standard media and for mixed inocula cultures these were thoroughly mixed by means of a scalpel and then inoculated.

All operations were carried out in aseptic condition.

The work was done at temperatures ranging between 18–20° C.

Preliminary Observations

As has been already stated six strains were utilized for the experiment. The monohyphal cultures of all these six strains were grown in standard synthetic medium in order to compare their growth rates and their morphological characters. A short account of the macroscopical characters of each of the strains has already been given; the rate of growth for different strains is given graphically in Text-Fig. 1.



▲ *Monilia* sp.
▫ *Fusarium* sp.
× *Dendryphiella* sp.
◦ *Phoma* sp.
○ *Gibberella* sp.
• *Helminthosporium* sp.

TEXT-FIG. 1.—Graphs showing the radial spread in mm. of the fungi *Monilia* sp., *Gibberella* sp., *Dendryphiella* sp., *Helminthosporium* sp., *Phoma* sp. and *Fusarium* sp. in standard synthetic medium.

It will be seen from Text-Fig. 1 that *Monilia* has the fastest growth rate followed by *Gibberella, Helminthosporium, Fusarium, Dendryphiella* and *Phoma* in descending order.

For experiment on competition the strains were paired in such a way that each member of a pair had mycelium of different colour, to enable easy detection of hyphæ in mixed growth. The combinations employed were as follows :—

1.	*Fusarium*	..	white, non-zoning.
	and		
	Dendryphiella		dark, non-zoning.
2.	*Fusarium*	..	white, non-zoning.
	and		
	Gibberella	..	greyish-white, non-zoning.

3.	*Phoma*	..	white, zoning.
	and		
	Helminthosporium	..	greyish-white, zoning.
4.	*Phoma*	..	white, zoning.
	and		
	Monilia	..	dark-grey, zoning.
5.	*Phoma*	..	white, zoning.
	and		
	Dendryphiella	..	dark, non-zoning.

These six strains in the above five combinations were inoculated 2 cm. apart, adjacent and mixed, in standard synthetic medium plates. Controls were kept.

The results show that in the combinations employed the growth rates of the associated cultures were not much different from those of the control strains.

In adjacent and mixed cultures the faster strain usually enveloped the slow growing strain and the later appeared as sectors. For example in adjacent and mixed cultures of *Dendryphiella* and *Phoma*, *Dendryphiella* which is slightly fast growing occupied the major portion of the growth.

Mixed and adjacent cultures of *Dendryphiella* and *Phoma* were, however, exceptions. Although the former was decidedly fast-growing, it was the slow growing *Phoma* that dominated, and *Helminthosporium* appeared as small sectors in a major growth of *Phoma*.

The appearance of sectors as found in the above mixed cultures in isolated areas is interesting. Obviously these had their origin in the original inoculum, but their continuity was difficult to establish. Among the other features of interest were the behaviour in regard to the formation of colour and zonation in the mixed growths.

Colour.—In the region where the young hyphæ of *Monilia* and *Phoma* arising from inocula planted 2 cm. apart came in contact with each other, there a few millimetres inside the growing edge of *Phoma* culture, was formed a single dark-red band very clearly visible in the substratum. As the faster *Monilia* colony started encircling the *Phoma*, the dark-red band appeared at the region of fresh hyphal contact, while the colour became fainter at the first place of appearance. The red band gradually moved further up where the younger hyphæ of *Monilia* had come in contact with younger hyphæ of *Phoma*.

When the entire *Phoma* colony was enveloped by *Monilia* and the younger hyphæ of the latter occupied a position directly opposite to the first place of contact with *Phoma* the band moved to the new place of contact, the colour at the original place having disappeared.

The formation of colour at the line of contact of two colonies is well known but the subsequent disappearance as noted here is peculiar.

The fresh, younger hyphæ of *Monilia* induces the formation of the dark-red band in *Phoma* colony. This must be due to the interaction of the staling products of the two colonies. The subsequent disappearance of the colour band may be attributed to the excess of a chemical substance due to its accumulation or to the formation of a new chemical by the older mycelium.

Zoning.—In normal cultures *Phoma* and *Helminthosporium* produce zonation although *Dendryphiella* does not. When *Phoma* and *Helminthosporium* were inoculated adjacently to form a mixed growth *Helminthosporium* appeared as sectors. In the mixed growth the zonation of both *Phoma* and *Helminthosporium* persisted ; the red concentric rings of the former forming almost an unbroken ring with dark bands of the latter. When the growth arose from the mixture of inocula, the important point observed was the complete absence of zonation in *Phoma* and the persistence of the same in *Helminthosporium.* The identical results were obtained with *Phoma* combined with *Dendryphiella.* In adjacent cultures *Phoma* zoned very well as in controls but in mixture culture zonations in *Phoma* were singularly absent.

Detailed Observations

With a view to making a detailed investigation it was decided to concentrate on two strains, that is one pair of strains only, instead of the five pairs used for preliminary observation. In order to find out the best reacting strains all the six strains were inoculated individually in 0·1, 0·25, 0·5, 1·0 and 2·0 per cent. malic acid in standard synthetic medium. *Dendryphiella* and *Fusarium* having proved more sensitive to the acids, were chosen for the investigation.

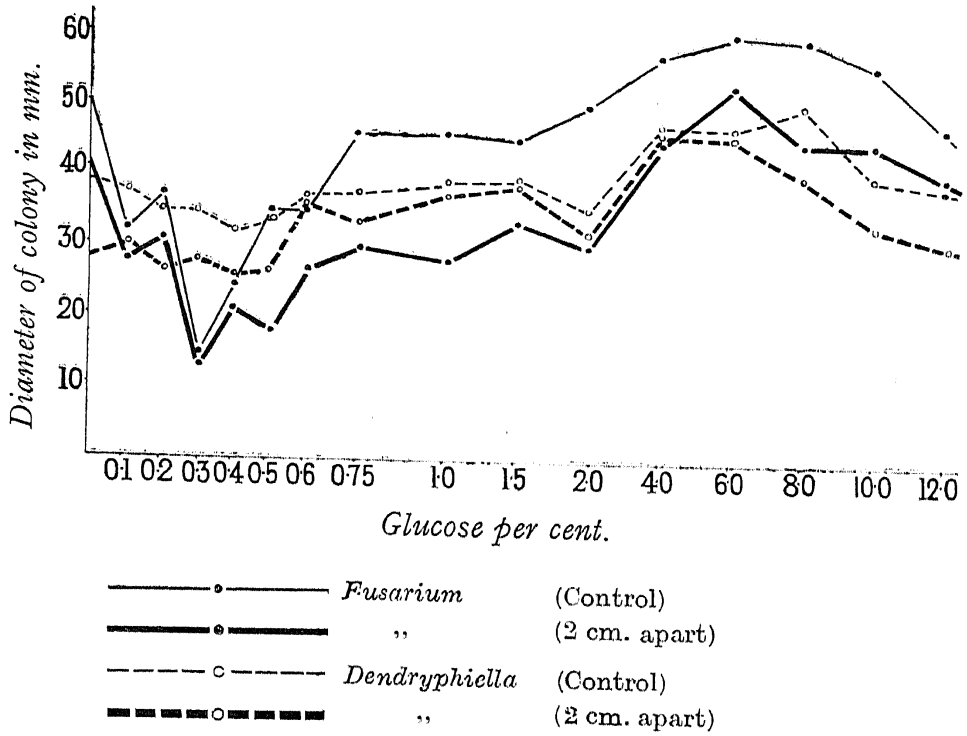
As has already been seen *Fusarium* and *Dendryphiella* are also easily distinguishable macroscopically by the striking difference in the mycelial colour—*Fusarium* being white and *Dendryphiella* dark-grey. Microscopically the difference lay in the hyphal characters and branching. Further profuse sporulation occurred in *Dendryphiella* where its growth met that of *Fusarium.* This last character particularly facilitated the detection of the boundary of

the *Dendryphiella* growth, especially in the case of partial and complete suppression. For these experiments the fungi were inoculated 2 cm. apart and adjacently.

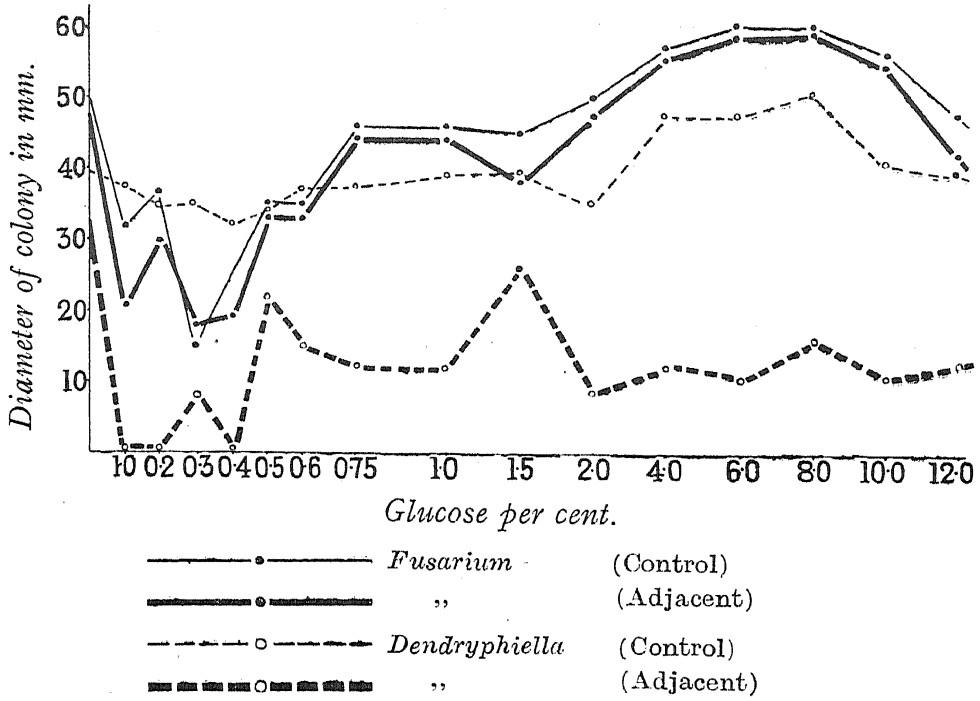
Glucose series.—The combined effect of various salts in the standard medium having failed to yield much result, individual salts like glucose and potassium nitrate were tried in different concentrations using agar as the basal medium.

Glucose was added to 1·5 per cent. agar in the strengths 0·1, 0·2, 0·4, 0·5, 0·6, 0·75, 1·0, 1·5, 2·0, 4·0, 6·0, 8·0, 10·0 and 12·0 per cent. steamed instead of autoclaved for sterilization and plated. *Fusarium* and *Dendryphiella* were inoculated 2 cm. apart and adjacent controls were kept.

The results are given in Text-Figs. 2 and 3 where the diameters of six days growths are plotted against the glucose concentration for 2 cm. apart and adjacent cultures respectively.



TEXT-FIG. 2.—Graphs showing the behaviour of *Fusarium* sp. and *Dendryphiella* sp. in associated culture and in control in glucose series. Inocula placed 2 cm. apart.



TEXT-FIG. 3.—Graphs showing the behaviour of the two fungi *Fusarium* sp. and *Dendryphiella* sp. in associated culture and in control in glucose series. Inocula placed adjacently.

It will be seen from Text-Fig. 2 that in the cultures where the fungi had been inoculated 2 cm. apart :

1. *Fusarium* and *Dendryphiella* are inhibited in the presence of each other as compared to the controls. Of these, however, *Fusarium* shows a greater inhibition than *Dendryphiella.*

2. At lower concentrations of glucose 0·1–0·6 per cent. *Fusarium* has a lower growth rate than *Dendryphiella* both in control and in paired cultures. At 0·75 per cent. *Fusarium* accelerates and throughout the higher concentrations employed, the one in control remains by far the fast growing.

3. In completing cultures at concentrations 0·3 to 4·0 per cent. *Dendryphiella* has a faster growth rate than *Fusarium,* but beyond 4·0 it is the other way round.

4. At concentrations 0·1 to 0·6 per cent. *Fusarium* and *Dendryphiella* colonies do not meet when inoculated 2 cm. apart and the mycelia of the two growths thin out as they approach each other.

It will be seen from Text-Fig. 3 that in adjacent culture :

1. Throughout the series *Fusarium* and *Dendryphiella* are inhibited in the presence of each other as compared with the controls except in the case

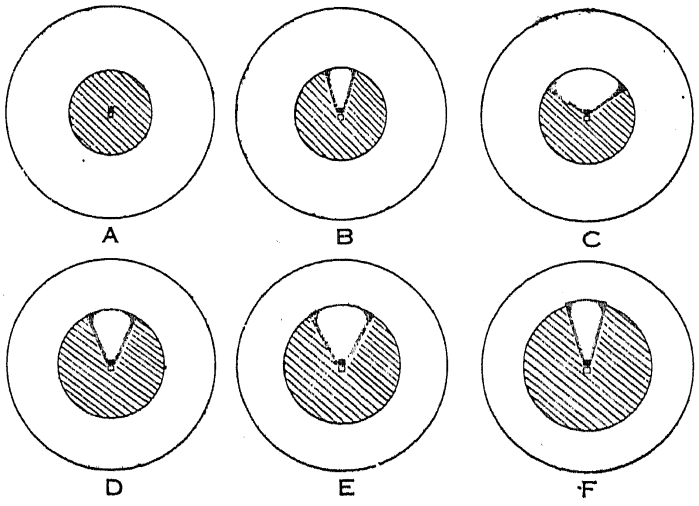
of *Fusarium* at 0·3 per cent. Of these however, *Dendryphiella* is very markedly inhibited by *Fusarium*.

2. *Fusarium* dominated throughout. *Dendryphiella* in addition to being retarded has three points of complete inhibition at 0·1, 0·2 and 0·4 per cent. There is a slight growth at 0·3 per cent. which probably is due to the associated *Fusarium* being slightly less active at that concentration. The almost complete inhibition at concentrations 0·1–0·4 is especially interesting since about that region *Dendryphiella* in the control has a faster growth than *Fusarium*.

3. As in the case of 2 cm.–apart–culture from 0·75 per cent. and beyond *Fusarium* outgrows *Dendryphiella*.

4. In competing cultures *Fusarium* always has a decidedly faster growth rate.

In Text-Fig. 4 is illustrated diagrammatically the dominance of *Fusarium* over *Dendryphiella* in adjacent cultures where Fig. 4 (A) indicates the complete



TEXT-FIG. 4 *A–F*.—Diagrammatic representation of the relative area occupied by *Fusarium* sp. and *Dendryphiella* sp. in various concentrations of glucose. Inocula adjacent.

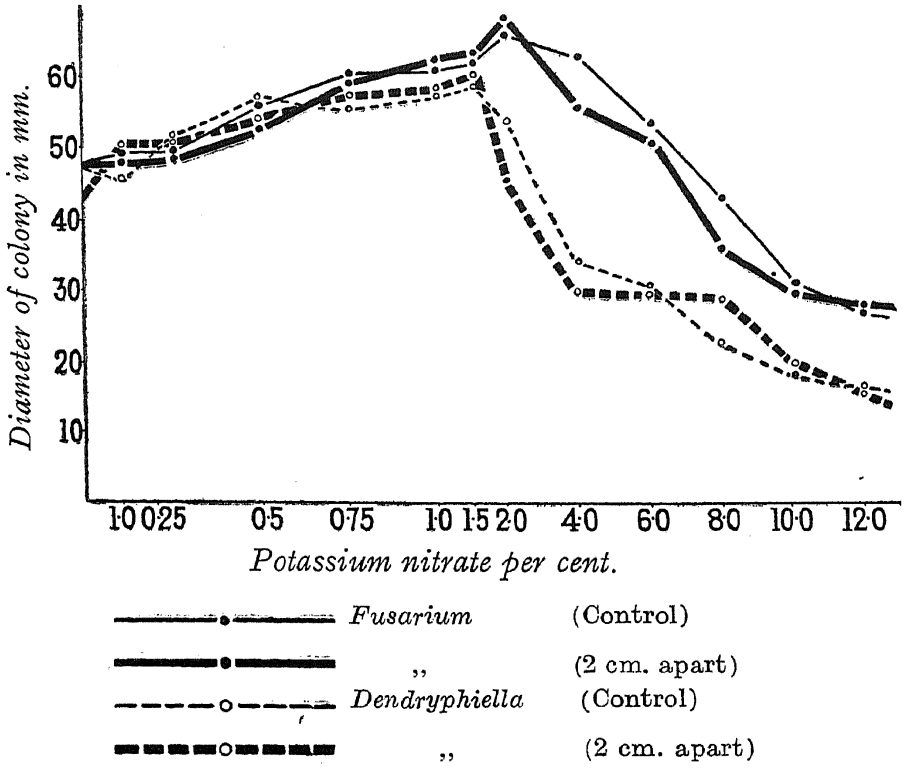
Shaded area represents *Fusarium*.
Unshaded area represents *Dendryphiella*.
Dots indicate sporulation of *Dendryphiella*.

A. *Fusarium* and *Dendryphiella* inoculated adjacent in 0·1, 0·2 and 0·4% glucose showing complete suppression of *Dendryphiella*.
B. Same strains in 0·5% glucose showing the appearance of *Dendryphiella*.
C. Same strains in 1·5% glucose showing an acceleration of *Dendryphiella* growth over that in 0·5%.
D. Same strains in 2·0% glucose showing an acceleration.
E. Same strains in 4·0% glucose showing a retardation of *Dendryphiella*.
F. *Dendryphiella* showing an acceleration over 4·0% with the addition of 8·0%.

inhibition of *Dendryphiella* as seen in 0·1, 0·2, 0·4% glucose; the fluctuations in the relative area occupied by the two strains in other concentrations are shown in Fig. 4 (A–F).

Potassium nitrate series.—Potassium nitrate was next tried with 1·5 per cent. agar in strengths 0·1, 0·25, 0·5, 0·75, 1·0, 1·5, 2·0, 4·0, 6·0, 8·0, 10·0 and 12·0 per cent. *Fusarium* and *Dendryphiella* were inoculated 2 cm. apart and adjacently. Controls were kept.

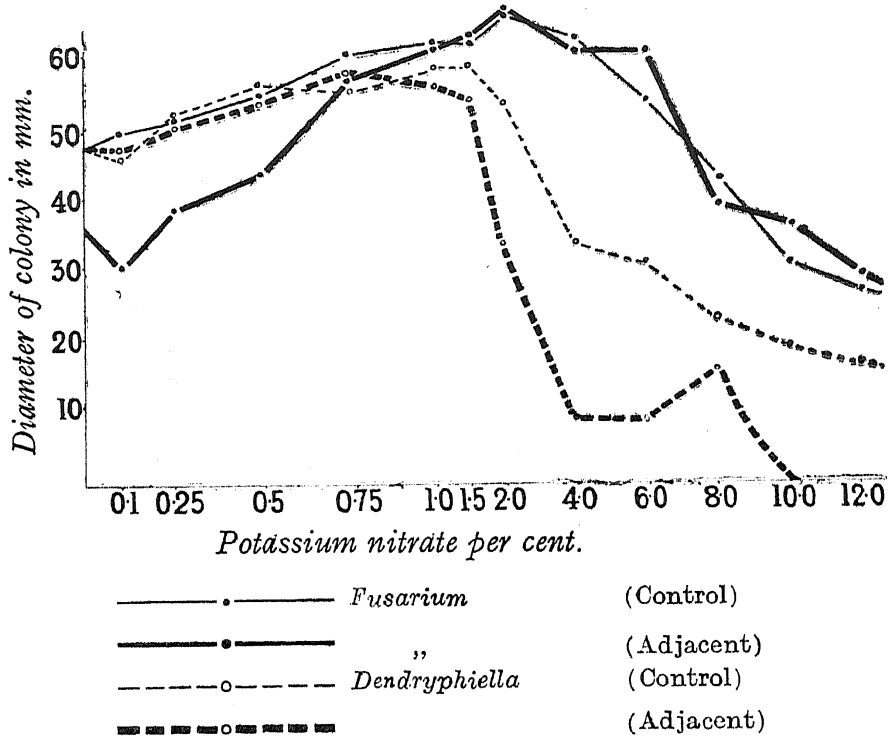
The results are given graphically in Text-Figs. 5 and 6 where the diameters of six days growths are plotted against the potassium nitrate concentrations for 2 cm. apart and adjacent cultures respectively.



TEXT-FIG. 5.—Graphs showing the behaviour of *Fusarium* sp. and *Dendryphiella* sp. in associated culture and in control in KNO_3 series. Incoula placed 2 cm. apart.

It will be seen from Text-Fig. 5 that in cultures 2 cm. apart, there was virtually no difference in the growth rates shown by *Dendryphiella*, *Fusarium* and their controls, up to the concentrations of potassium nitrate 0·1–1·5 per cent. Beyond this concentration *Fusarium* definitely takes upper hand, but nevertheless the fungi in associated cultures have almost similar growth rates to those of the controls, showing that the interaction of the two fungi has resulted into inhibition of growth rates only to an insignificant extent.

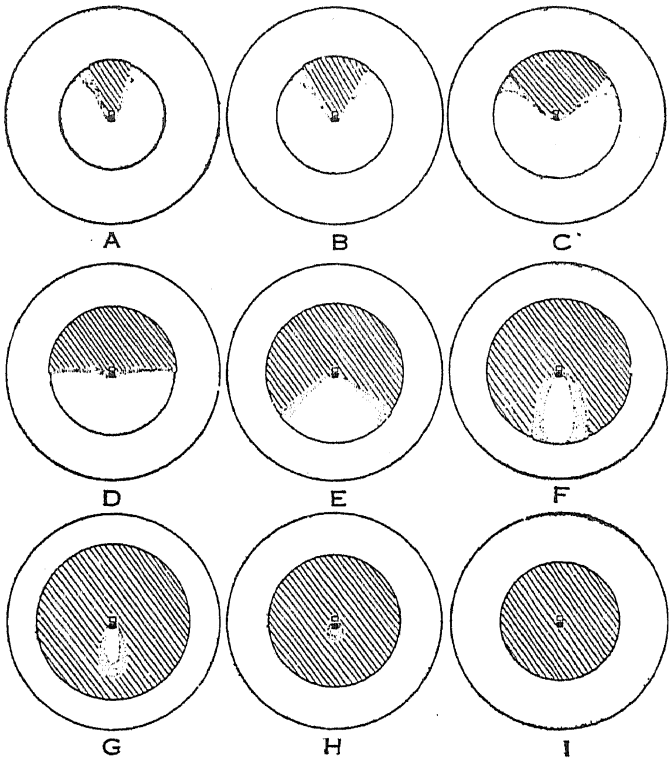
In adjacent culture (Text-Fig. 6) in media up to the concentration of 0·75 per cent. the growth rates of associated cultures and the controls are



TEXT-FIG. 6.—Graphs showing the behaviour of *Fusarium* sp. and *Dendryphiella* sp. in associated culture and in control in KNO_3 series. Inocula placed adjacently.

almost the same except for that of *Fusarium* which shows a definite retardation. Beyond 0·75 per cent. *Fusarium*, both in the control and in associated culture, is much faster than *Dendryphiella*. In concentrations 0·1 to 0·5 per cent. *Dendryphiella* dominates *Fusarium*, they are almost equal at 0·75 per cent. but beyond that concentration *Fusarium* completely dominates *Dendryphiella* as is shown by the difference in the growth rate between the two. At 4 per cent. *Dendryphiella* shows very slight growth which is completely inhibited at 10 per cent. although the control maintains a steady rate of growth. *Dendryphiella* seems to exert no influence up on *Fusarium* at relatively higher concentrations as judged from its rate of growth.

The domination of *Fusarium* by *Dendryphiella* in lower concentrations of potassium nitrate (0·1–0·5) and the domination of *Dendryphiella* by *Fusarium* in higher concentrations (1·0–10·0) and complete inhibition of the former by *Fusarium* in still higher concentrations are illustrated diagrammatically in Text-Fig. 7 (A–I).



TEXT-FIG. 7 *A–I.*—Diagrammatic representation of relative area occupied by *Fusarium* and *Dendryphiella* in various concentrations of KNO_3. Inocula adjacent.

Shaded area represents *Fusaruim.*
Unshaded area represents *Dendryphiella.*
Dots indicate sporulation of *Dendryphiella.*

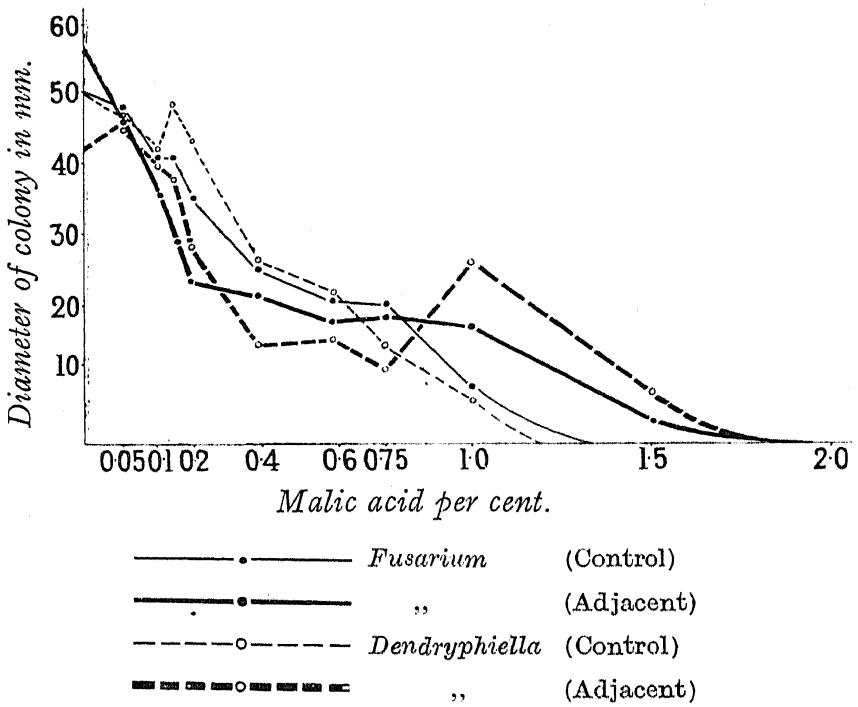
A. *Fusarium* recessive with a sectorial growth in 0·1 % ;
B. *Fusarium* accelerates with addition of 0 .25 % ;
C. *Fusarium* further accelerates with addition of 0·5 % ;
D. *Fusarium* growth is equal to that of *Dendryphiella* growth in 0 ·75 % ;
E. *Dendryphiella* retards and occupies a sector in *Fusarium* growth in 1·5 % ;
F. *Dendryphiella* further retards with addition of 2·0 % ;
G. *Dendryphiella* occupies a still smaller part in 4·0 % ;
H. *Dendryphiella* occupies a very small area in 6 .0 % and 8·0 % ;
I. Entire growth is that of *Fusarium* and there is no growth of *Dendryphiella* with addition of 10·0 and 12·0 % KNO_3 (Diagrammatic).

It is seen from Fig. 7 that *Fusarium* starts as a small sector in 1 per cent potassium nitrate (7A), increases in size with the increase in concentration (7B–7C), attaining the same size as *Dendryphiella* becomes reduced to a very small sector in 4 per cent. (7G), still smaller in 6 per cent. and 8 per cent. (7H) till there is no growth of *Dendryphiella* at 10 per cent. and 12 per cent. (7I, and Plate I, Figs. 16–20).

Acid series.—Malic acid was added to standard synthetic medium in strengths 0·025, 0·05, 0·1, 0·2, 0·3, 0·4, 0·6, 0·75, 1·0, 1·5, and 2·0 per cent. and plated. *Fusarium* and *Dendryphiella* were inoculated adjacent and 2 cm. apart. Controls were kept.

The results are shown graphically in Text-Figs. 8 and 9 where the diameters of six days growths are plotted against acid concentrations for adjacent and 2 cm.–apart-cultures respectively.

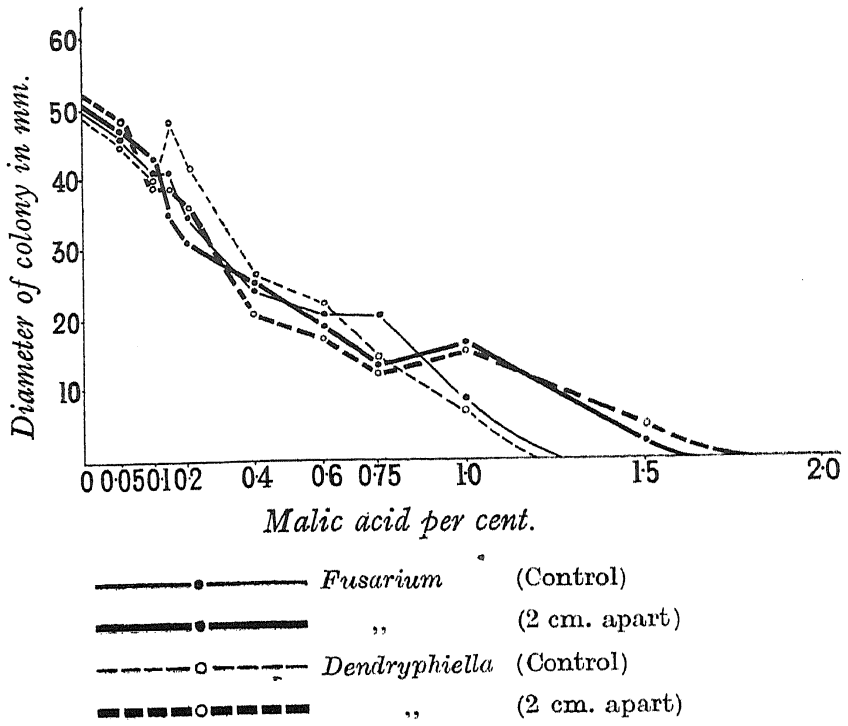
It will be seen from Text-Fig. 8 that in adjacent culture *Dendryphiella* shows a marked increase in growth in 1 per cent. to 1·75 per cent. over *Fusarium* although in lower concentrations, it is either being dominated by



TEXT-FIG. 8.—Graphs showing the two fungi *Fusarium* sp. and *Dendryphiella* sp. in associated culture and in control in Malic acid series. Inocula placed adjacently.

Fusarium or has a similar growth rate. In 2 cm.–apart–culture (Text Fig. 9), throughout the series the two fungi run closely parallel to each other and to the controls.

Far interesting is the fact that the controls of both the strains, *Dendryphiella* and *Fusarium*, stop growth at about 1·5 per cent. acid. But when they are inoculated 2 cm. apart and adjacent, the point of total inhibition is raised from 1·25 to 1·75 per cent. The result demonstrates a marked increase in the tolerance of acid due to the association of the strains.



TEXT-FIG. 9.—Graphs showing the behaviour of *Fusarium* sp. and *Dendryphiella* sp. in associated culture and in control in Malic acid series. Inocula placed 2 cm. apart.

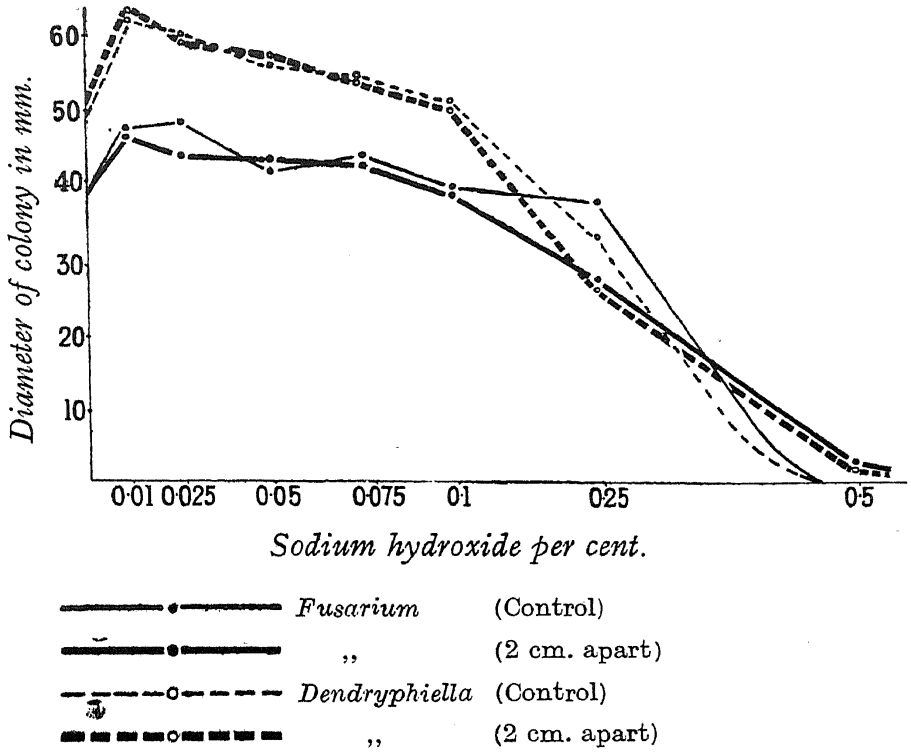
Alkali series.—The effect of alkali on the competing strains was next tried by using sodium hydroxide and sodium carbonate.

Sodium hydroxide.—This was added to standard synthetic medium in the following strengths and plated 0·01, 0·025, 0·05, 0·075, 0·1, 0·25 and 0·5 per cent. Inoculations were made 2 cm. apart and adjacently. Controls were kept.

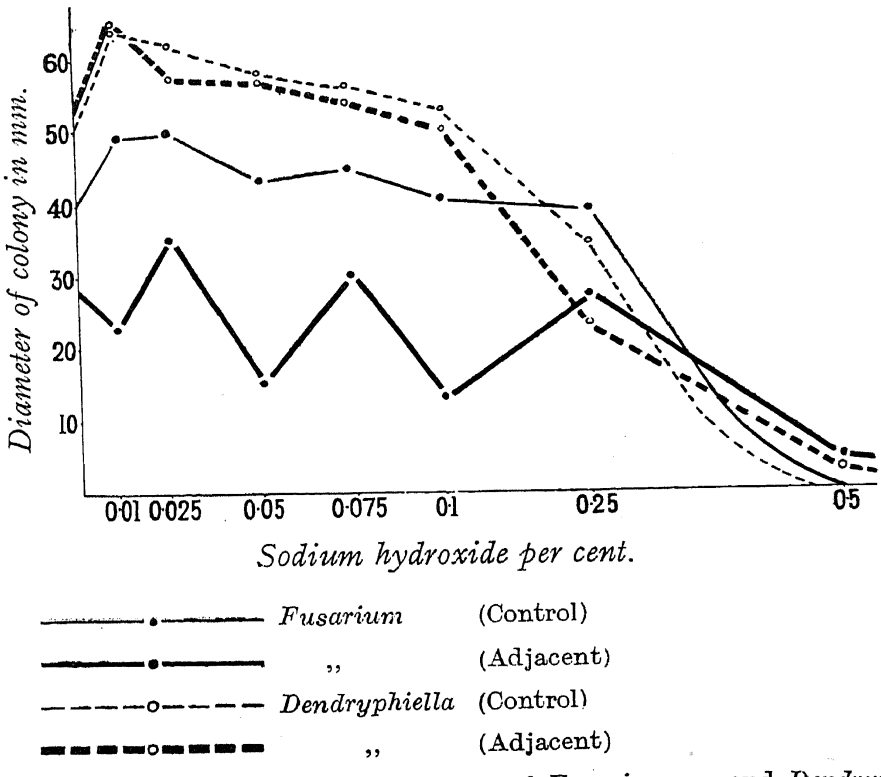
The results are shown graphically in Text-Figs. 10 and 11 where diameters of six days growth are plotted against concentrations of sodium hydroxide for 2 cm. apart and adjacent cultures.

The behaviour of the strains in the series was very interesting. It will be seen from Text-Fig. 10 that in cultures 2 cm. apart *Dendryphiella* dominated in percentages 0·01, 0·025, 0·075 and 0·1, but all the time both growing slower than in controls. At 0·25 per cent. the *Fusarium* so far slower than *Dendryphiella* becomes faster both in 2 cm.-apart-culture and in the control, due to the sudden drop in the growth rate of the latter. At 0·5 per cent. when the controls have ceased to grow both the strains still continue with *Fusarium* somewhat dominating.

In adjacent culture the reactions of the fungi are very similar to that for cultures 2 cm. apart as already explained. In the latter case, however,



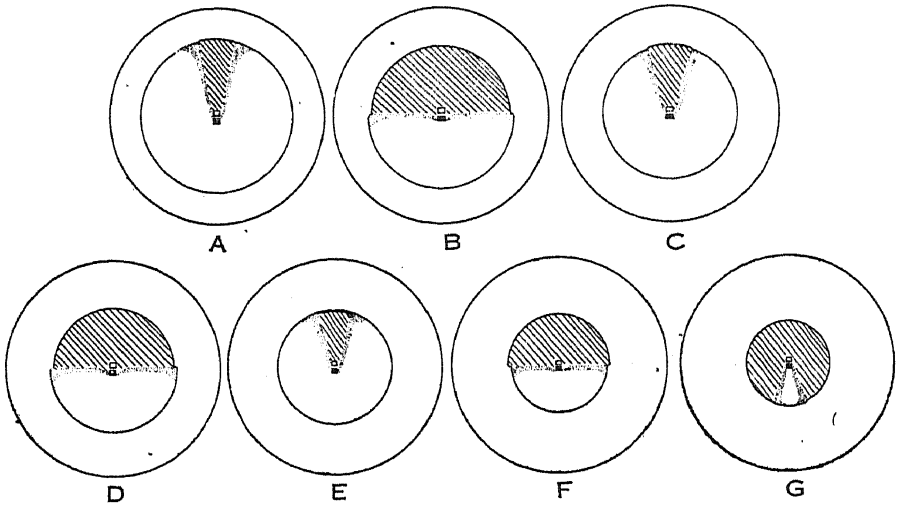
TEXT-FIG. 10.—Graphs showing the behaviour of *Fusarium* sp. and *Dendryphiella* sp. in associated cultures and in control in NaOH series. Inocula placed 2 cm. apart.



TEXT-FIG. 11.—Graphs showing the behaviour of *Fusarium* sp. and *Dendryphiella* sp. in associated culture and in control in NaOH series. Inocula placed adjacently.

Fusarium shows peculiar fluctuations, *viz.*, acceleration at one strength and retardation at the next.

It will be seen from Text-Fig. 12 (A–G) that in a medium containing 0·01 per cent. sodium hydroxide, *Fusarium* is confined to a small sector-like growth in a major growth of *Dendryphiella* [Text-Fig. 12 (A)]. In 0·25 per



TEXT-FIG. 12 *A–G.*—Diagrammatic representation of the relative area occupied by *Fusarium* sp. and *Dendryphiella* sp. in various concentrations of NaOH. Inocula adjacent.

Shaded area represents *Fusarium.*
Unshaded area represents *Dendryphiella.*
Dots indicate sporulation of *Dendryphiella.*

A. *Fusarium* occupies a small sector in a major growth of *Dendryphiella* with the addition 0·01% NaOH to the standard medium;

B. *Fusarium* accelerates with the addition of 0·025%;

C. *Fusarium* retards again with the addition of 0·05%;

D. *Fusarium* accelerates with 0·075%;

E. *Fusarium* again retards with addition of 0·1%;

F. *Fusarium* growth is more than that of *Dendryphiella* with 0·25%;

G. *Fusarium* dominates the growth and *Dendryphiella* occupies a sector at 0·5% NaOH (Diagrammatic).

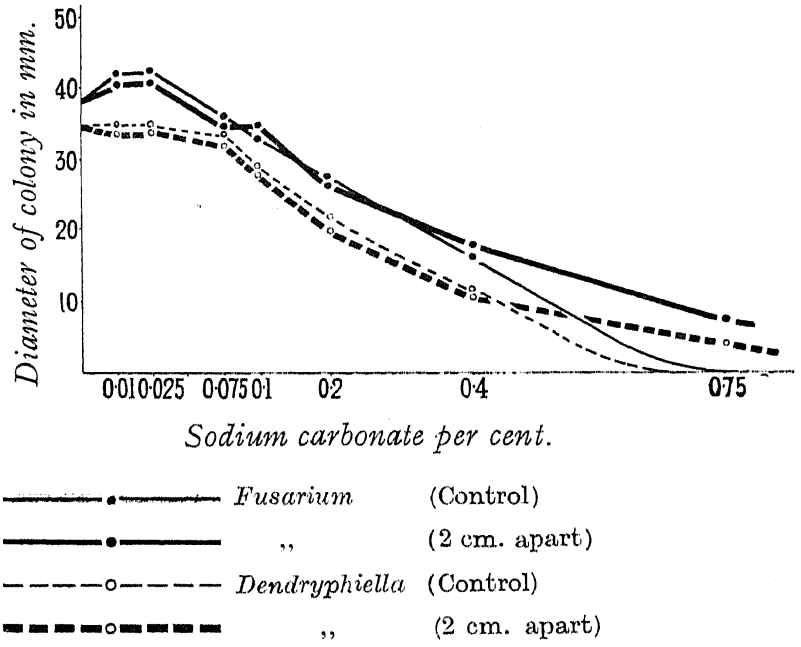
cent. sodium hydroxide the two growths occupy equal area. In 0·05 and 1·0 per cent. *Fusarium* is restricted to a small sector, while in concentration 0·075 per cent. *Fusarium* outgrows *Dendryphiella* and occupies half the area of the entire growth and at 0·5 per cent. dominates *Dendryphiella* completely restricting it to a very small sector.

A comparison of Text-Figs. 11 and 12 will show that in this case there is no correspondence between the relative growth of the two fungi and the

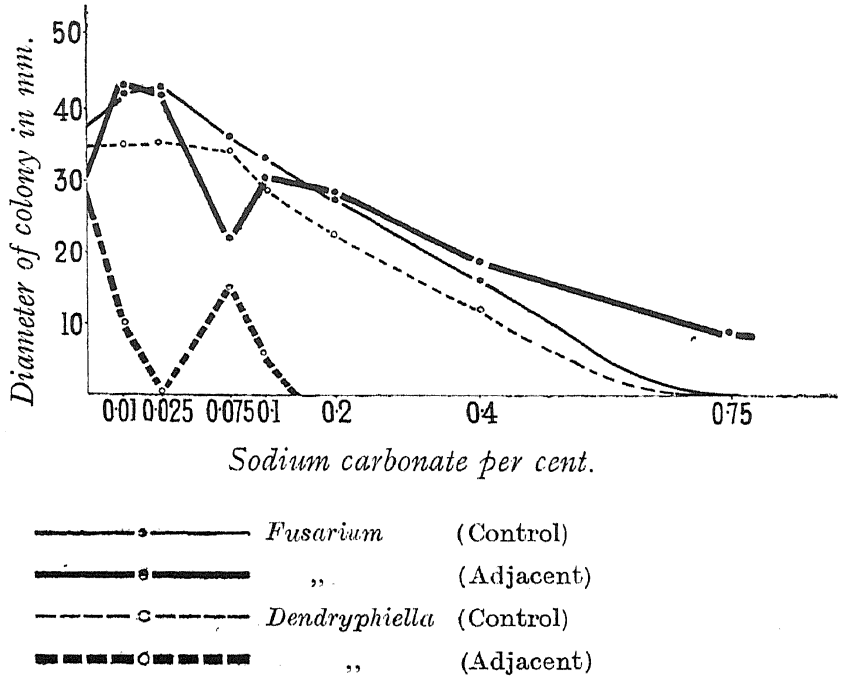
area occupied by them in adjacent cultures. It is seen from Text-Fig. 11 that in all concentrations up to 0·1 per cent. *Dendryphiella* greatly dominates the *Fusarium*, having almost twice the growth rate, in spite of that *Fusarium* at 0·025 and 0·075 per cent. of NaOH occupies area equal to that occupied by *Dendryphiella*; these are the concentrations when *Fusarium* shows accelerated growth.

It is interesting to note that in these series as in the others already mentioned, the associated fungi are capable of growth in higher alkali in adjacent culture and in culture 2 cm. apart while they have ceased to grow in the control plates.

Sodium carbonate.—This was tried in the following percentages with standard synthetic medium 0·01, 0·025, 0·075, 0·1, 0·2, 0·4 and 0·75. The two strains *Fusarium* and *Dendryphiella* were inoculated 2 cm. apart and adjacent. The results are shown graphically in Text-Figs. 13 and 14 where diameters for six days growth of the competing strains and of the controls are plotted against various concentrations of sodium carbonate for 2 cm. apart and adjacent cultures respectively.



TEXT-FIG. 13.—Graphs showing the behaviour of *Fusarium* sp. and *Dendryphiella* sp. in associated culture and in control in Na_2CO_3 series with inocula placed 2 cm. apart.



TEXT-FIG. 14.—Graphs showing the behaviour of *Fusarium* sp. and *Dendryphiella* sp. in associated culture and in control in Na_2CO_3 series. Inocula placed adjacently.

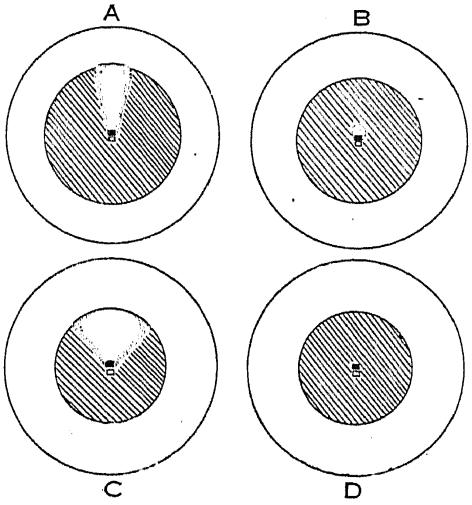
It will be seen from Text-Fig. 13 that in the concentrations of sodium carbonate employed *Fusarium* and *Dendryphiella* growing 2 cm. apart do not show significant difference from the controls upto 0·4 per cent. At 0·75 per cent. however, both the fungi continue to grow while there are no growths in the controls.

Particularly interesting results are seen in adjacent culture (Text-Fig. 14). In media containing 0·01 per cent. and 0·025 per cent. sodium carbonate *Fusarium* has the same growth rate as in the control; at 0·075 per cent. there is a sudden drop. At 0·1 per cent. of sodium carbonate the growth rate shows a marked increase and at 0·2 per cent. it is again virtually the same as that of the control. At 0·4 per cent. the competing *Fusarium* has a slightly higher growth rate and beyond that at 0·75 per cent. where the growth of the controls has completely ceased, it continues to grow.

A comparison of *Dendryphiella* in control and in adjacent culture with *Fusarium* is still more interesting. In adjacent culture *Dendryphiella* has a much slower growth rate. A slight addition of sodium carbonate 0·01 per cent. brings down the rate considerably and at 0·025 the growth of the fungus is inhibited. At 0·075 per cent. *Dendryphiella* shows definite acceleration. *Dendryphiella* is finally inhibited at a concentration beyond 0·1

per cent. sodium carbonate although growth in the control continues till 0·75 per cent.

The results are of great importance. They show a definite inhibitory influence exerted by *Fusarium* on *Dendryphiella*. In sodium carbonate concentration where *Fusarium* is active, *Dendryphiella* is considerably inhibited and with the decrease in the activity of *Fusarium*, *Dendryphiella* shows a corresponding increase. Beyond 0·1 per cent. however, *Fusarium* completely checks the growth of the fungus.



TEXT-FIG. 15 *A-D.*— Diagrammatic representation of the relative area occupied by *Fusarium* and *Dendryphiella* in various concentrations of Na_2CO_3. Inocula adjacent.

Shaded area represents *Fusarium*.
Unshaded area represents *Dendryphiella*.
Dots indicate sporulation of *Dendryphiella*.

A. *Dendryphiella* forms a sector in an entire growth of *Fusarium* at 0·01 % ;
B. Very little growth of *Dendryphiella* seen with the addition of 0·025 % ;
C. *Dendryphiella* accelerates with the addition of 0·075 % ;
D. There is no growth of *Dendryphiella*, the dominant strain being *Fusarium* in percentages 0·1 to 0·75 of Na_2CO_3.

Text-Fig. 15 (A–D) will show diagrammatically the inhibitory influence exerted by *Fusarium* on *Dendryphiella* in sodium carbonate series. At 0·01 per cent. and 0·075 per cent. *Dendryphiella* forms a small sector, at 0·025 per cent. it is restricted to a very small area near the centre, at 0·075 per cent. it occupies a much bigger area and is completely inhibited at 0·1 per cent.

Discussion

A certain amount of work has been done on the growth of fungi in mixed culture. Harder (1911), Cook (1924), Porter (1924), Machacek (1928),

Vanin and Vladimirsky (1932), Endo Sigeru (1932, 1933) and others have worked on different aspects of the problem.

Harder has given a detailed account of changes in the rate of growth, in hyphal structures, of the formation of colouring matter, phenomenon of aversion, etc., in mixed cultures of a number of strains. He has also demonstrated that one mycelium does not kill another. Vanin and Vladimirsky (1932) have shown that in the mixed cultures of *Merulius lacrymans* and *Coniophora cerebella*, at an early stage, the mycelium of each grows normally without interfering with each other. Later the mycelium of *Merulius lacrymans* outgrows that of *Coniophora cerebella* causing a considerable retardation in the growth of the latter. Endo Sigeru (1932) has shown that the presence of micro-organisms is one of the factors controlling the sclerotial formation in *Sclerotium oryzæ-sativæ*. The inhibition and retarding action of one fungus on another in mixed culture have been worked out in detail by Porter (1924). Cook (1924) has made an interesting study on the succession of fungi in artificial culture and Machacek (1928) on the association of phytopathogens.

On the applied side of the problem Endo Sigeru (1933) has found that the antagonistic action of several organisms prevented appearance of a disease due to *Hypochnus Sasakii Shirai*. Vasudeva (1930) has demonstrated that the parasitic activity of a fungus is greatly retarded by the presence of a non-parasitic fungus and Asthana (1936) has found some interesting results on the effect of various fungi on the parasitic vigour and other characteristics of *Botrytis cinerea*.

The problem is approached from altogether a different view-point in this investigation. An attempt has been made here to find out how two fungi will behave in different growth conditions in associated cultures when they are grown contiguously or a little distance apart and how will they influence each other's growth.

It is evident from the various experimental data given that fungi are influenced not only by the change in the composition of the medium but also by the association of one strain with the other. A profound alteration in growth reactions occur when these are grown adjacently and 2 cm. apart in altered condition of the medium.

Where a fast-growing strain was paired with a relatively slow-growing one, it was usually the faster strain that outgrew and enveloped the slower one, but reverse condition was also found where the slow-growing *Phoma* strain in mixed and adjacent cultures had completely dominated the faster *Helminthosporium* restricting it to small sectors.

It is in the detailed investigation of the behaviour of *Fusarium* and *Dendryphiella*, however, that more interesting results were obtained.

The domination of one strain over another as seen from the extent of area occupied by the competing strains of *Fusarium* and *Dendryphiella* in a mixed growth arising from inoculation either put adjacently or 2 cm. apart, in various concentrations of the chemicals employed is very instructive.

Fusarium is only slightly faster than *Dendryphiella* in standard medium. Nevertheless in almost all the series so far employed, glucose, potassium nitrate, sodium carbonate and sodium hydroxide as the concentration was increased *Fusarium* gradually dominated *Dendryphiella* in adjacent culture. In the highest concentration of potassium nitrate and sodium carbonate employed the growth was all *Fusarium* to the total inhibition of *Dendryphiella*.

The details of reaction in different series, however, differed to a large extent. In glucose, for example, there were three inhibition points at relatively low concentrations of 0·1, 0·2 and 0·4 per cent. where *Dendryphiella* was totally suppressed. But at 0·2 per cent., however, there was a certain amount of growth of *Dendryphiella*, which probably is due to the associated *Fusarium* being slightly less active at that concentration.

In subsequent concentrations, *Dendryphiella* occupied a relatively small sectorial area throughout and was never again completely suppressed even in the highest concentration employed. In sodium carbonate series too the major growth of *Fusarium* contained a sector of *Dendriphiella* whose area fluctuated in higher concentrations. *Dendryphiella* was completely inhibited in concentrations 0·15–0·75 per cent.

In potassium nitrate series it is the *Fusarium* that appeared as a small sector, which gradually became larger as the concentration of potassium nitrate increased finally suppressing the entire growth of *Dendryphiella*.

The result in sodium hydroxide series, however, was more complicated. *Fusarium* appeared as a small sector at the lowest concentration. But instead of a steady increase in the area with the increase in the concentration of sodium hydroxide there was alternate rise and fall. A semi-circular area in one concentration was followed by a small sector in the next till at 0·5 per cent. *Fusarium* dominated the growth and restricted *Dendryphiella* to only a small sector.

A comparison of the results indicates that on the whole, the reaction of one strain against another is more pronounced in adjacent than in 2 cm.–apart–culture.

The influence of Fusarium and Dendryphiella upon each other is well manifested in the difference between the growth rates of the strains in associated culture on one hand and their controls on the other. The growth rate of a fungus depends upon the chemicals constituting the media and their concentrations, but the reaction of the competing strains to the media is also profoundly modified by the association of one fungus with the other. The modification in growth rate results in either retardation or acceleration.

The acceleration of growth rates of the competing fungi in associated cultures was primarily observed in higher concentration of chemicals. For example, both *Fusarium* and *Dendryphiella* showed acceleration over their controls beyond 0·4 per cent. sodium hydroxide in adjacent culture, and in the same concentration of sodium carbonate in cultures 2 cm. apart. In malic acid series it was found only in concentration as high as 0·75 per cent. and over. Comparatively lower concentration may also bring out the same result. Thus *Fusarium* showed faster growth rate in adjacent culture beyond 0·1 per cent. sodium carbonate.

The acceleration of growth *raises the final inhibition point of the strains.* The strains in these cases maintained their growths in concentrations where the controls had ceased to grow. It was found in all the series except that of glucose. For example, in malic acid series while the controls failed to grow at about 1·25 per cent. both *Fusarium* and *Dendryphiella* continued to grow at 1·5 per cent. and had respectively diameters of 3·0 mm. and 7·0 mm. in adjacent culture and 2·5 mm. and 5·0 mm. in 2 cm.–apart–culture. Similarly in sodium hydroxide series at 0·5 per cent. while control had failed to grow altogether, *Fusarium* and *Dendryphiella* had diameters 3·0 mm. and 2·0 mm. respectively in cultures 2 cm. apart, and 5·0 mm. and 3·0 mm. respectively in adjacent culture. In sodium carbonate again at 0·75 per cent. in 2 cm.–apart–culture *Fusarium* and *Dendryphiella* had diameters of 7·0 mm. and 4·0 mm. respectively while controls showed no growth. At the same concentration, in adjacent culture diameter of *Fusarium* proved to be 9·0 mm. against no growth of control, *Dendryphiella* had stopped growth at 0·15 per cent. sodium carbonate.

The retardation of growth rates of the competing fungi was usually observed. in the lower concentrations and sometimes throughout the series.

In malic acid there was retardation for both the strains in concentrations between 0·01–0·75 per cent. In sodium hydroxide, the strains growing 2 cm. apart had a slightly slower growth rate in lower concentrations; in adjacent culture only *Fusarium* was strongly retarded below 0·4 per cent. In sodium carbonate in 2 cm.–apart–culture, *Fusarium* and *Dendryphiella*

were both retarded below 0·4 per cent. while the limit for the same in adjacent culture was 0·1 per cent. The degree of retardation, however, varied. This was evident in all concentrations of potassium nitrate. In glucose series too, in adjacent culture, there was a slight retardation throughout. *Dendryphiella* on the other hand, showed slight fall in growth rate in 2 cm.–apart–culture which became very marked in adjacent culture with inhibition points at 0·1, 0·2 and 0·4 per cent. concentrations.

The retardation culminating in the complete suppression of *Dendryphiella* by *Fusarium* occur at 0·15 per cent. sodium carbonate while the diameter of the control is 25·0 mm. There is a similar and equally striking inhibition of *Dendryphiella* at 10 per cent. potassium nitrate when the control fungus shows a diameter of 20·0 mm.

It is possible that the media in the neighbourhood of the growths become modified, either to the advantage or to the disadvantage of the strains by the staling substances produced by them and the observed differences in the growth rates are due to the differential reaction of the strains to these modified media. Or as it has been suggested by Machacek (1928) the differences may be due to unequal assimilation of available food from the media causing the starvation of one organism.

That these factors play an important role in the growth reactions of fungi is well known, but it is not improbable that actual contact (Das Gupta, 1934) or the mere presence of hyphæ may, to a certain extent, be responsible for some of the observed results.

At particular strengths of sodium carbonate (0·2, 0·4 and 0·75 per cent.) in adjacent cultures the retarding influence of *Fusarium* was so great that *Dendryphiella*, in spite of having a favourable growth rate in the controls, is totally suppressed by *Fusarium*. In such a condition where there was no growth of *Dendryphiella* the accelerated growth of *Fusarium* over its control could not possibly be due to diffusion of staling products from *Dendryphiella*. It may be concluded therefore that at least in certain media even the mere presence of an inoculum of *Dendryphiella* placed adjacently to that of *Fusarium* has a striking effect on the acceleration of growth of the latter.

It is apparent from the study of the results obtained from different series of experiments that growth reaction of associated non-parasitic fungi as represented by two strains under consideration, are extremely variable. They show various degrees of tolerance and inhibition. Machacek (1928) distinguished several types of association on the basis of mutual reaction, *viz.*, (1) one organism causes complete inhibition of its associates; (2) associated organisms are mutually tolerant on each other and so on. Results here

show, however, that no hard and fast rule can be laid down ; in fact, inhibition, tolerance, etc., as shown by the fungi are but functions of the chemical composition of the growth medium which are profoundly modified by the interaction of the organisms upon each other.

Summary

In order to ascertain the nature of growth reactions in associated cultures of non-parasitic fungi six strains were used in five different combinations.

Fusarium sp. and *Dendryphiella* sp. ; *Fusarium* sp. and *Gibberella* sp. ; *Phoma* sp. and *Helminthosporium* sp. ; *Phoma* sp. and *Monilia* sp. ; *Phoma* sp. and *Dendryphiella* sp.

The paired strains were inoculated mixed, adjacent and 2 cm. apart in media differing in composition of acid, alakli and nutritive chemicals and their reactions were studied. Detailed work was done only with one pair *Fusarium* sp. and *Dendryphiella* sp. Some of the more interesting results are given below :

In the majority of cases usually the faster strain of a pair enveloped the slower one and the latter appeared as sectors. But *Phoma*, a slow growing strain, was found to dominate the faster strain of *Helminthosporium*.

Dendryphiella and *Fusarium* showed differential reaction to the various media employed. There was also a pronounced influence of each fungus on the other as judged from the relative area occupied by these in mixed growth as well as from their growth rate.

The domination of one strain over another as indicated by the extent of area occupied by the competing strains of *Dendryphiella* and *Fusarium* in adjacent and 2 cm.-apart-culture varied with the composition of media employed. Generally in lower concentrations *Fusarium* appeared as major growth with *Dendryphiella* as sector. In some cases *Fusarium* appeared as a small sector in the major growth of *Dendryphiella*. Ultimately, however, in all the series in relatively higher concentrations, it was always the *Fusarium* that dominated the growth ; sometime to the complete inhibition of *Dendryphiella*.

The accelerating influence of *Fusarium* and *Dendryphiella* upon each other was manifest at comparatively higher concentrations and rarely at lower, where *Fusarium* and *Dendryphiella* both accelerated over their controls both in adjacent and 2 cm.-apart-culture. The immediate, important result of the acceleration was the *raising of the final inhibition point of the strains* in almost all the series. Here the individual strains in associated

cultures continued to grow in media where controls had ceased to grow altogether.

The retarding influence of *Fusarium* and *Dendryphiella* upon each other was evident in the lower concentrations. It was more pronounced in adjacent culture. *Dendryphiella* was nore influenced by *Fusarium* than *Fusarium* by the former. In glucose, in adjacent culture, *Fusarium* totally inhibited the growth of *Dendryphiella* at three low concentrations (0·1, 0·2 and 0·4 per cent.). The most interesting was the complete inhibition of *Dendryphiella* by *Fusarium* at 0·15 per cent. sodium carbonate and 10·0 per cent. potassium nitrate in adjacent cultures although in control *Dendryphiella* showed diameter of 25·0 mm. and 20·0 mm. respectively.

There is a suggestion that the presence of a mere inoculum of one fungus may also, in certain cases, modify the growth reaction of another.

I wish to express my grateful thanks to Dr. S. N. Das Gupta for suggesting the problem and offering me ready guidance and criticism throughout.

LITERATURE CITED

Asthana, R. P. .. " Antagonism in fungi as a measure of control in ' Red-leg ' disease of Lettuce," *Ann. Bot.*, 1936, **4**, 201–07.

Cook, M. T. .. " Succession of fungi on culture media," *Amer. Journ. of Botany*, 1924, **11**, 94–99.

Das Gupta, S. N. .. " Studies in the genera *Cytosporina*, *Phomopsis* and *Diaporthe*. *VI*. On the conversion of one strain of *Diaporthe perniciosa* into another," *Phil. Trans. Roy. Soc. Lond.*, 1934, Ser. B, **223**, No. 497, 121–61.

Endo Sigeru .. " Studies on the antagonism of micro-organisms. II. Growth of *Hypochnus Sasakii Shirai*, as influenced by the antagonistic action of other organisms," *Bull. Miyasaki College of Agri. and Forestry*, 1932, **4**, 133–85.

———— .. " Studies on the antagonism of micro-organisms. IV. Growth and pathogenicity of *Sclerotium oryzæ sativæ* Sawada in the presence of other organisms," *ibid.*, 1933, **5**, 51–75.

Harder, R. .. " Ueber der Verhalten von Basidiomyceten und Ascomyceten in Mischkulturen," *Naturw. Zeitschr. f. Forestu. Landw.*, 1911, Nos. 3–4.

Machacek, J. E. .. " Studies on the association of certain phytopathogens," *MacDonald College, McGill Univ. Tech. Bull.*, 1928, No. 7.

Porter, C. L. .. " Concerning the characters of certain fungi as exhibited by their growth in the presence of other fungi," *Amer. Journ. of Bot.*, 1924, **11**, 168–88.

Vanin and Vladimirsky .. "On the biology of the fungus *Merulius lacrymans* and *Coniophora cerebella*," *Bulletin of the Leningrad Institute for Controlling Farm and Forest Pests*, 1932, No. 3, 57–74.

Vasuveda R. Sahai .. "Studies in the physiology of parasitism. XII. On the effect of one organism in reducing the parasitic activity of another," *Ann. Bot.*, 1930, 44, 557-64.

DESCRIPTION OF PLATE FIGURES

FIGS. 16-20.— Photographs showing the behaviour of *Fusarium* sp. and *Dendryphiella* sp. when grown adjacently in various concentrations of KNO_3. The darker colony represents *Dendryphiella*. × $\frac{7}{9}$.

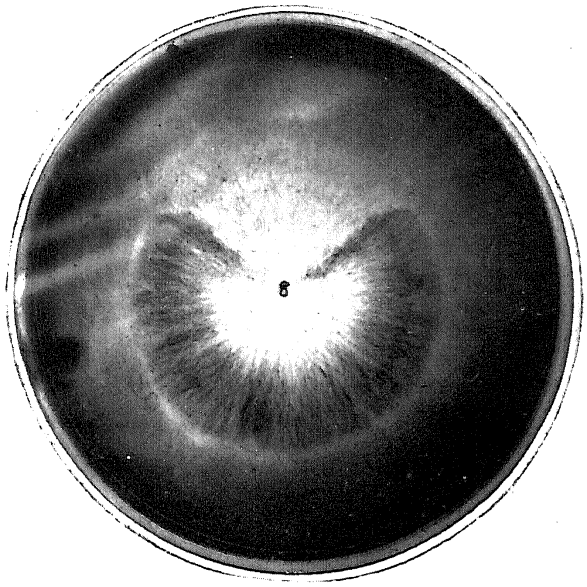
FIG. 16. 0.25% KNO_3. *Dendryphiella* occupies major area and *Fusarium* a small sector.

FIG. 17. 0.75% KNO_3. *Dendryphiella* and *Fusarium* occupying equal area.

FIG. 18. 1% KNO_3. *Fusarium* occupying major area.

FIG. 19. 2% KNO_3. *Fusarium* occupying still greater area and *Dendryphiella* forming a sector.

FIG. 20. 6% KNO_3. *Fusarium* completely dominating. *Dendryphiella* restricted to a small growth near the inoculum (dark).



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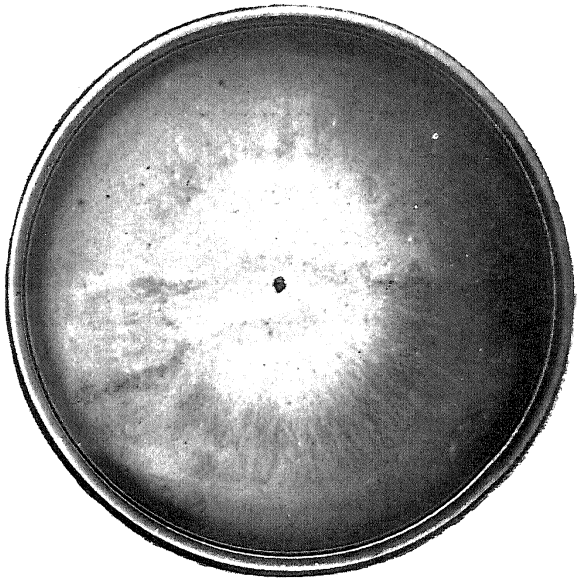

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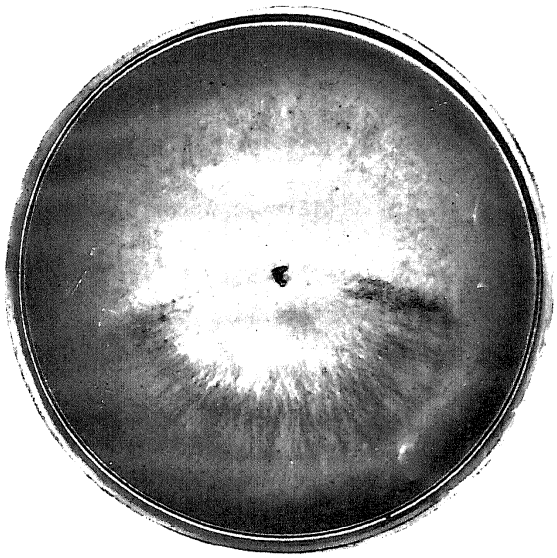

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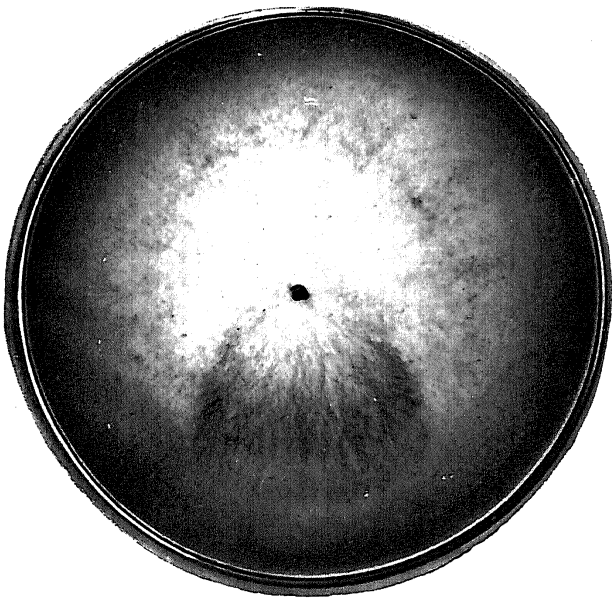

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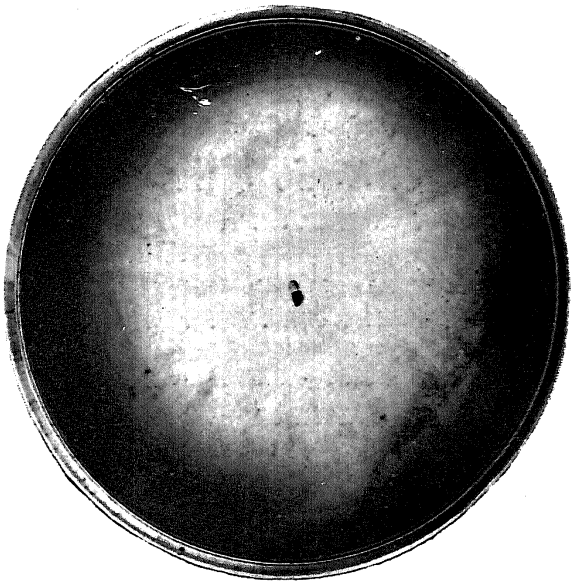

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