PhD 1929

ON THE OCCURRENCE OF "FALSE SECTORS" IN CULTURES OF *FUSARIUM FRUCTIGENUM* FRIES.

by

R. N. Sahai Vasudeva, B.Sc.

On the Occurrence of "False Sectors" in Cultures of *Fusarium fructigenum* Fries.

by
R. N. Sahai Vasudeva, B.Sc.

INTRODUCTORY. In the course of some laboratory experiments in which the growth rates of a strain of *Fusarium fructigenum* were being tested under different conditions of acidity and alkalinity, one of the plates showed the peculiar appearance illustrated in Plate I, Fig. 1. The edge of the colony was very irregular owing to the development of five spreading fan-shaped sectors. When isolations were made from the latter, the five resultant cultures proved to be the same and all were different from the parent strain. The new strain differed from the parent in showing much greater growth of aerial mycelium with corresponding reduction in the amount of sporulation.

The plate concerned contained glucose peptone agar with 0.08% malic acid. Incidentally it should be added that this was the only plate in the series in which the layer of medium was poured shallow. The importance of this point will be emphasized later.

The strain of *Fusarium fructigenum* which gave rise to the above appearance was one (Strain A) which had been studied in detail by Brown (1). This author does not describe or figure sectors of the peculiarly spreading type shown in Plate I, Fig. 1. It was decided, therefore, to investigate the occurrence of such sectors more closely, and in particular to determine whether their appearance could be correlated with the nature and amount of the cultural medium employed.

EXPERIMENTAL. (1) *Fungi and media used.*

Fusarium fructigenum, strain A, was chiefly used in these

experiments. Four other strains were also used in a few tests, with results closely similar to those found to apply for strain A. The purity, i.e. freedom from admixture, of all the stock cultures was guaranteed at the commencement of the work by carrying on each from a single hyphal tip.

The following media were employed:-

(1) Glucose peptone agar:

Glucose	10 grm.
Peptone	2 "
Potassium di-hydrogen phosphate (KH_2PO_4)	1 "
Magnesium sulphate	0.5"
Agar	15 "
Distilled water	1,000 c.c.

(2) Malt agar:

Malt	20 grm.
Agar	15 "
Distilled water	1,000 c.c.

(3) Brown's agar:

Glucose	2 grm.
Asparagin	2 "
Normal potassium phosphate (K_3PO_4)	1.25 "
Magnesium sulphate	0.75 "
Agar	15 "
Distilled water	1,000 c.c.

(2) Depth of Medium as affecting Sector Formation.

The effect of this factor is very clearly marked. It is only in shallow plates that sectors of the markedly diverging type occur. This is in agreement with the results described by Christensen (2) for Helminthosporium sativum.

Plates of varying thickness of medium were poured:- (1) 1 c.m. deep, (2) ½ c.m. deep (3) very shallow. In the case of the last, a few c.c. (about 7) of medium were poured while

still rather warm into the sterilized plate and the latter shaken to spread the medium over the whole surface. The degree of shallowness was somewhat variable, partly on account of the fact that the bottoms of the Petri dishes were not all perfectly flat. In order to eliminate errors due to this variability, a large number of Petri dishes (10-15) were poured in each case.

Table I shows the total number of plates inoculated in a series of experiments and the number showing sectoring.

Table I.

Deep plates		Shallow plates	
No. inoculated	No. with Sectors.	No. inoculated	No. with Sectors
93	0	148	95

Plate I, Fig. 2 illustrates the appearance of Fusarium fructigenum Strain A. on a deep layer of glucose peptone agar. Plate I, Figs. 3 and 4 and Plate II, Fig. 5 show the characteristic lobed or sectored appearance of the fungus when grown on a shallow layer of the same medium. Exactly similar results were obtained with cultures of four other strains (C_{21}, D, D_1 and D_3) of Fusarium fructigenum.

A simple method of demonstrating the effect of shallowness of the medium on the occurrence of sectors is to pour the plate in such a way that the medium grades from very shallow on one side to deep on the other. When such a plate is inoculated at the centre it is found that diverging sectors appear in a high percentage of the plates but only on the side where the medium is shallow. The non-appearance of sectors in some cases is in all probability due to the fungal culture becoming stale and ceasing to grow before its margin has reached a sufficiently shallow region of the plate. The position of the inoculum,

therefore, to some extent determines whether sectors will arise or not. Plate II, Figs. 6, 7 and 8 illustrate the phenomenon just described, the shallow side of the medium being upward in each case.

(3) Composition of Medium as affecting Sector Formation.

The experiments in this connection were carried out with the glucose peptone medium. Various modifications of this medium were prepared and used for shallow plate cultures.

On the complete medium as given on page 2, sectors were freely formed. When the magnesium sulphate constituent was omitted, there was no obvious effect on the amount of growth or the frequency of sector formation. When the sugar was omitted, the vigour of growth was reduced, but sector formation occurred though not so frequently as on the complete medium. When the medium was deprived of peptone, growth was reduced to a thin scanty film, with no formation of sectors.

The most striking results were obtained in the experiments in which the phosphate constituent was modified. In the complete medium, acid potassium phosphate is present. When this is omitted, the general amount of growth is not markedly reduced (probably on account of traces of the essential elements present in the agar), but sector formation is almost completely suppressed. Out of 147 cultures examined, sectors appeared in only two cases. A similar result is obtained when the acid phosphate is replaced by the acid sulphate.

When potassium phosphate (KH_2PO_4) is replaced by sodium phosphate (NaH_2PO_4), sectors occur as freely as on the complete medium. Thus the important constituent is the phosphate radicle. When the di-acid phosphate (KH_2PO_4) is replaced by either of the less acid salts (K_2HPO_4 or K_3PO_4) growth comparable to that on the standard medium is obtained, but no diverging sectors occur. When either of the latter phosphates is used together with a suitable concentration of malic acid, sectors arise as ordinarily. Control plates in which the phosphate is omitted

and to which malic acid is added show no sectors. It is thus clear that the factors which determine the appearance of sectors are a sufficiency of phosphate and a certain range of acidity.

Table II gives a summary of the results obtained in connection with the replacement of the di-acid phosphate constituent of the glucose peptone medium.

Table II.

KH_2PO_4 replaced by:-	No. of Plates inoculated.	No. of Plates showing sectoring.
1. K_2HPO_4	51	0
2. K_2HPO_4 + malic acid (0.1 - 0.16%)	73	43
3. K_3PO_4	50	0
4. K_3PO_4 + malic acid (0.16%)	20	10
5. Malic acid (0.08 - 0.16%)	28	0
6. NaH_2PO_4	24	9
7. $KHSO_4$	20	0

The effect of a certain degree of acidity on sector formation was well shown in some experiments in which a glucose peptone medium was made up to different degrees of acidity or alkalinity by addition of malic acid or sodium bicarbonate. The medium as given on page 2 is acid, 100 c.cs, requiring approximately 0.067 g. of sodium bicarbonate for neutralisation. Nine modifications of the medium were made up, one of these being definitely alkaline, one neutral and the remainder showing a range of acidities.

Table III gives the number of plates showing sectoring in two such experiments. In the first experiment, four plates, in the second, ten plates of each medium were used. All the plates were poured with shallow layers of media.

Table III.

100 c.c. Basal medium (Glucose Peptone) to which is added:-

	0.16 g. $NaHCO_3$ (alk.)	0.067 g. $NaHCO_3$ (neut.)	Malic Acid					
			0	0.005	0.01	0.02	0.04	0.08	0.16
Exp. I	0	0	3	4	4	3	2	0	0
Exp. II	0	0	4	5	5	3	3	2	0

(4) Nature of the sectors produced.

In the case illustrated in Plate I, Fig. 1, all the sectors proved on isolation to belong to a new strain clearly different from the parent. A considerable percentage of such sectors, however, have proved on examination merely to yield the parent form. The following data will illustrate this point.

Twenty two cultures showing sectors on glucose peptone agar (some of the plates showing more than one) were examined by transferring portions of the growing edges of the sectored areas to fresh plates of Brown's synthetic agar and comparing with cultures of the parent strain. Altogether 33 transfers from the growing edges of sectors were made. Of these, 13 gave the parent form, the remaining 20 being saltants.

Sixteen of the saltant colonies were again transferred by taking a mass inoculum from the growing edge. The second series of cultures gave the following results:-

Saltant remained saltant in 10 cases.

Saltant reverted to parent in 4 cases.

Saltant gave rise to mixed parent and saltant cultures in 2 cases

Of seven similar transfers of the parent form six remained true to type, and one showed admixture with a saltant form.

In another experiment in which sectors arising on Malt agar were tested, nineteen transfers from the margins of sectors were made. Thirteen of these gave saltant strains, while the remaining six gave the parent form. Of the thirteen saltants

six remained, as far as could be seen, pure on a second transfer, four gave mixed colonies, and three gave the parent form only.

In a further series of tests, cultures were grown on glucose peptone agar and transfers made from the growing edge of sectors, from the base of sectors, from the edge and from the centre of the parent colony and the resultant cultures compared. A typical set of results is shown in Table IV.

Table IV.

Origin of Inoculum	No. of Inocula taken.	Saltant	Parent
Edge of sectors	15	9	6
Centre of parent colony	9	2	7
Edge of sectors	8	8	0
Base of sectors	8	4	4
Edge of sectors	30	6	24
Edge of parent colony	30	10	20

Transfers from the edge of the sectored regions give a higher percentage of saltant strains than transfers from within the body of the culture, but it is remarkable that sectors can occur, transfers from which do not give a higher percentage of saltants than are obtainable by culturing from the edge of the typical part of the culture.

It is clear from the foregoing that there is a great deal of admixture in these sectored cultures and that there are sectors which may be described as "false" inasmuch as they do not appear to be essentially different from the normal part of the culture, though differing very considerably in appearance. In other cases sectors which are indistinguishable in appearance from the preceding, regularly give rise to new strains when examined in the same way.

The parent culture is obviously tending to throw off saltants

repeatedly and these may be obtained by reculture from any part of the parent. Though the striking divergent sectors may appear on the shallow side, there is clear evidence that saltant areas are present on the deep side, though they may not give rise in the parent culture to clearly marked sectors. The device of growing the cultures on a shallow medium appears therefore to serve the purpose of magnifying the difference between saltant and parent. It cannot be claimed that the shallow medium is the cause of saltation as such.

The appearance of highly exaggerated sectors, as illustrated in this paper, is probably capable of explanation in terms of staling phenomena. Under the conditions of culture on shallow plates and on certain slightly acid media, any portion of the growing margin which slightly outstrips the neighbouring parts gets a certain advantage and this advantage becomes cumulatively greater as time goes on. It advances farther and farther ahead, and by the fact of so doing tends still more to repress the growth of the neighbouring parts of the margin. On a deep medium the same appearance cannot arise as there is there a greater development of fungal mycelium with correspondingly great liberation of staling products. On this account the whole plate is rendered unsuitable for further growth, and thus the formation of strongly out-growing sectors is prevented. On a very shallow medium there is a minimal liberation of staling products so that conditions are favourable for the continued growth of any part of the margin which is slightly in advance of the remainder..

From these considerations it is clear that in those cases where the sector proves to be a saltant strain, there may be very little difference in growth rate between parent and saltant. In fact a number of tests have failed to establish any definite difference in growth rate between the parent strain and some saltants derived from it. This feature is more pronounced still where the sector proves on isolation to be merely the parent,

(e.g. in Pate II, Figs. 7 and 8). In spite of the very pronounced sectored appearance shown in these figures, which would seem to indicate the presence of a rapidly growing saltant strain, no difference whatever was observable between the normal and sectored portions when these were subsequently compared.

It might be suggested that there is a difference in growth rate between the sectored and normal portions, but that it is so small as to be masked by the experimental errors of determination. The question, therefore, arises whether this small difference can be increased by selective culture through a number of generations. For this purpose the following experiment was carried out.

From such a plate as is illustrated in Plate II, Fig. 7 a transfer was made from the edge of the sector to a shallow plate of the glucose peptone medium. The resultant colony again produced a similar diverging sector. The process was repeated a third time, and again a similar sector arose. The latter was then carefully compared with the stock parent culture and no difference appeared. There is thus no evidence of intensification of small differences after three generations. This type of experiment has been repeated a number of times, with the same result in each case. Provisionally, therefore, one must consider that such sectors are false in that there are no grounds for believing that they represent saltant strains.

SUMMARY.

1. Under suitable conditions, viz. on shallow plates of medium containing an acid phosphate, certain strains of Fusarium fructigenum regularly give rise to strongly diverging sectors.

2. Many of these sectors do not represent saltant strains.

On isolation and reculture they prove to be identical with the parent.

REFERENCES.

1. Brown W.: Studies in the Genus Fusarium. IV. On the Occurrence of Saltations. Ann. Bot., 1926, XL, pp. 223-43.

2. Christensen, J.J.: Physiologic Specialization and Parasitism of Helminthsporium sativum. Univ. of Minn. Agr. Exp. Sta., 1926, Tech. Bull. 37, pp. 1-101.

EXPLANATION OF PLATES.

In all cases the fungus is strain A of Fusarium fructigenum, Fries. and the medium is glucose peptone agar.

Plate I.

Figs. 1, 3 and 4 - showing highly lobed, sectored appearance on shallow plates.

Fig. 2 - showing appearance on deep plate.

Plate II.

Fig. 5. - similar to Figs. 1, 3 and 4 above.

Figs. 6-8 - showing sector-like outgrowths on the shallow sides of obliquely poured plates.

PLATE I.

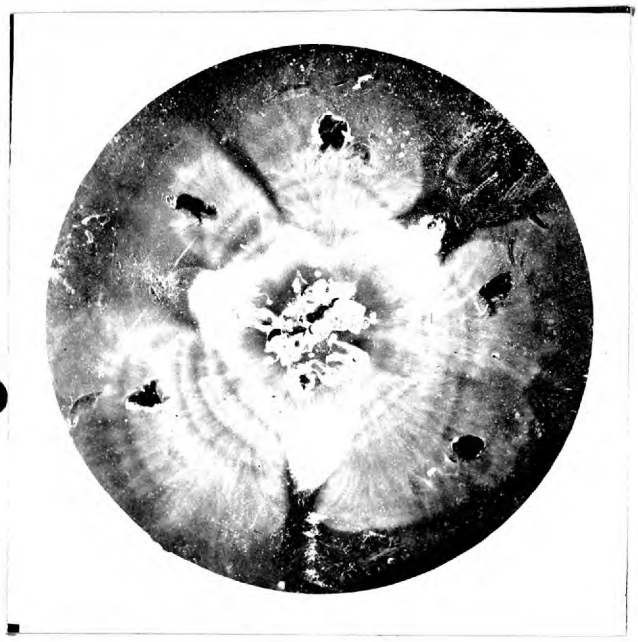

Fig.1.

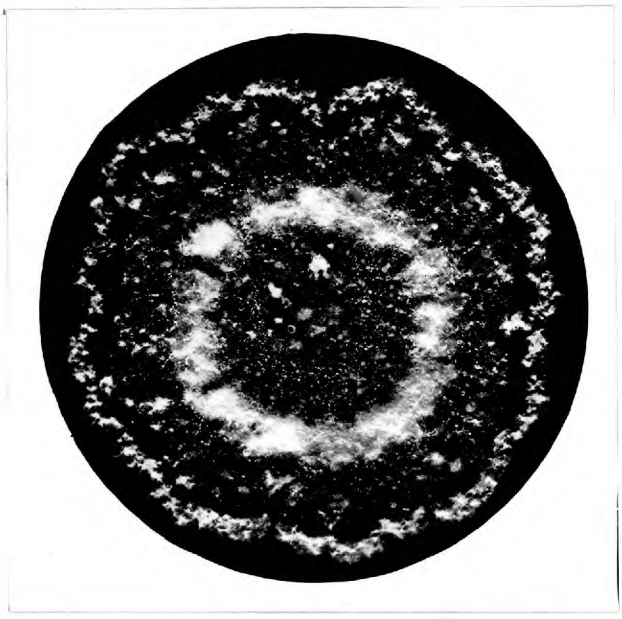

Fig. 2.

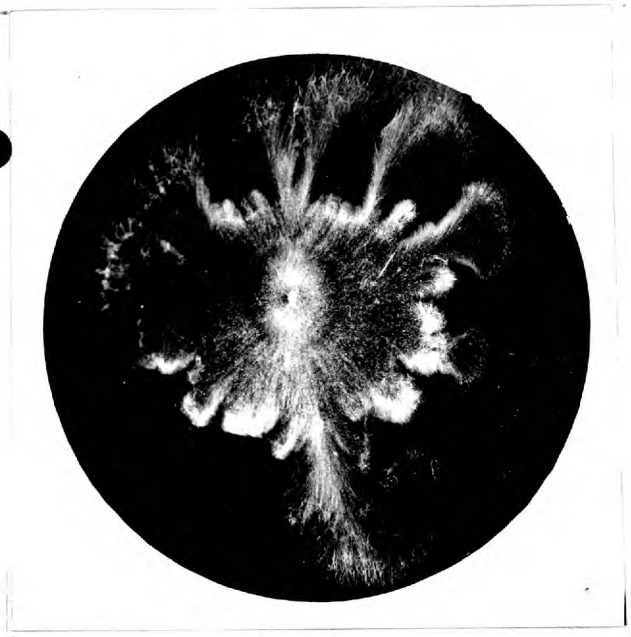

Fig.3.

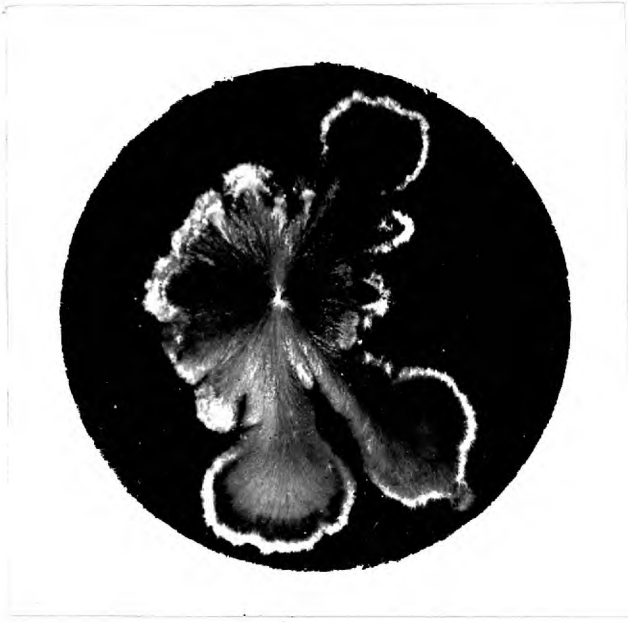

Fig.4.

PLATE II.

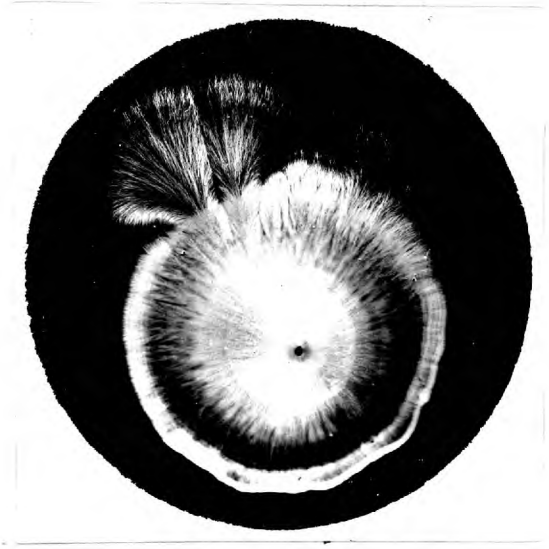

Fig. 5.

Fig. 6.

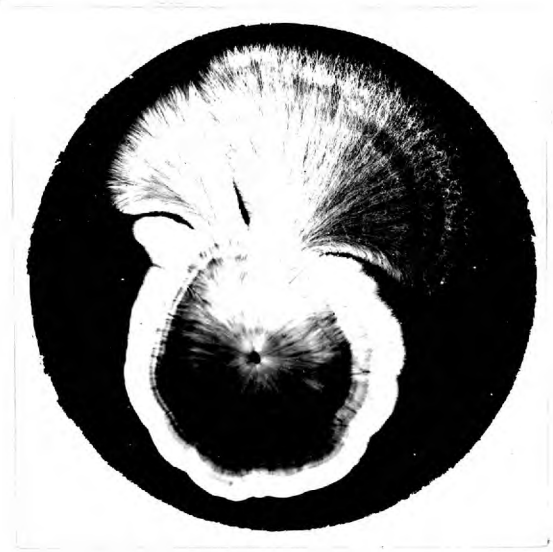

Fig. 7.

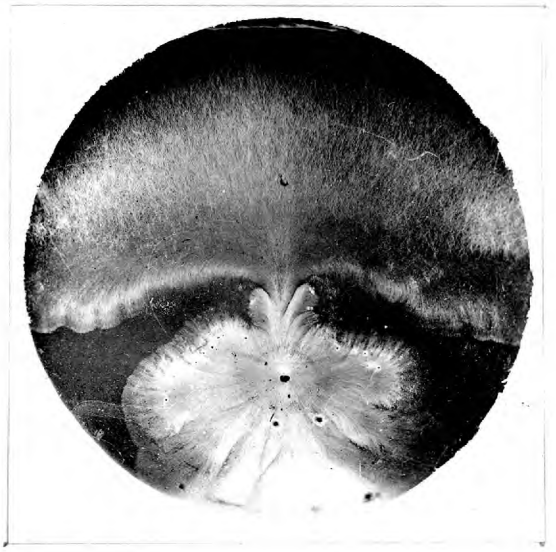

Fig. 8.