STUDIES IN THE GENUS *COLLETOTRICHUM*

II. Physiological Studies on *Colletotrichum falcatum* Went.

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Colletotrichum falcatum which causes the red-rot of sugarcane has a world-wide distribution being present in all countries where this crop is cultivated. In this paper are recorded the observations made on some aspects of the behaviour of the fungus in culture.

Materials and Methods

The fungus was isolated from a thick cane variety called *Poovan* which is usually subjected to heavy infestations of 'red-rot' in Madras. The fungus was grown on solid and liquid media. Liquid media were synthetic and were employed to determine the dry weight of the fungus growth. 50 c.c. of the medium were placed in 100 c.c. Erlenmeyer flasks. After a definite period the growths were removed from the flasks, filtered through gooch crucibles and washed in several changes of distilled water. The crucibles were dried to constant weight by keeping in steam ovens. They were then ignited for 2-3 hours and the weights determined after ignition. The difference between the two weighings gave the dry weight of the fungus. The weight of the fungus ash being negligible was not taken into consideration. The cultures were run in triplicates and the average measurements taken. The inoculum consisted of equal quantities of spore suspensions or similar pieces of culture.

The Richards' solutions used in these experiments did not contain ferric chloride. The media were sterilised at 10 lbs. pressure for half an hour. pH determinations were made by the colorimetric method. The cultures were incubated at 26° or 29° C. Except where otherwise stated 150-200 spores were measured to arrive at average dimensions.

Sugarcane setts were inoculated in the following manner. The setts had three nodes. The ends were dipped in molten paraffin as soon as they were cut. Just above the lowest node a bore was made to 2/3 the thickness of the sett with a sterilised cork-borer. The inoculum was introduced into the hole and the plug replaced in position after cutting off a portion to make room for the inoculum. Melted paraffin was smeared over the plug. The

setts were then placed in washed moist sand and kept at laboratory temperature for a definite period. Afterwards they were taken out, split into two and the length and breadth of the reddened area measured. The virulence index was calculated by multiplying the ratio of lesion width to sett diameter by the ratio of lesion length to length of internode (Abbott, 1938).

The enzymes produced by the fungus were determined in different ways. The capacity of the fungus to grow on certain media was taken as an index of the formation of particular enzymes in the mycelium of the fungus (Uppal and Kulkarni, 1937). A stock medium containing magnesium sulphate ·5 gm., K_2HPO_4 1 gm., KCl 1·5 gm., ferrous sulphate ·01 gm., agar 20 gm. and distilled water 1000 c.c. was prepared. To this were added different compounds for testing particular enzymes. Another method followed was to grow the fungus in Richards' solution for two weeks and remove the fungus mats. These mats were then washed several times in sterile water, dried between filter-papers and then kept inside a desiccator for 10 days. The dried mat was then mixed with a small quantity of sterile water and silver sand and ground well in an agate mortar. The ground material was placed in 20 times the quantity of sterile distilled water, shaken well and allowed to stand for 24 hours at 15° C. The extract was filtered and some drops of toluene added to the filtrate (Venkatarayan, 1936). This extract was used for testing enzyme reactions and is referred to as 'crude enzyme extract'.

Growth on Media

The fungus grows well on a number of media and the nature of the growth on some of them is given below:

TABLE I

Growth of C. falcatum *on Different Media*

Medium	Initial pH	Diameter on 6th day in cm.	Nature of growth
French bean agar ..	4·7	6·75	Good, grey mealy aerial growth, zonation visible, numerous pink powdery masses of spores, setæ rare.
Quaker oats agar ..	4·5	6·52	Good, grey fluffy aerial growth with plenty of pink spore masses; black stromatoid bodies with setæ formed.
Richards' agar	4·5	4·4	Grey aerial growth less than above, numerous pink spore masses, black stromatoid bodies formed, setæ rare or absent.
Brown's agar	5·5	5·0	Thin growth, greyish white, aerial, pink spore masses less than in the above media, no setæ.
Soil-extract agar	6·25	3·72	Poor sparse growth, no spore masses or setæ.
Leaf-mould-extract agar ..	6·25	3·9	Do.

Quaker oats and french bean agars form the best media for the cultivation of the fungus; where a transparent synthetic medium is necessary Richards' agar may be used with advantage. The mycelium is made up of hyaline and brown hyphæ with numerous chlamydospores on the sides of the growths; but in Brown's, soil-extract and leaf-mould-extract agars the hyphæ are mostly hyaline with few chlamydospores. Setæ are rarely formed.

H-ion concentration of medium and growth.—The effect of different H-ion concentrations on the growth of the fungus was determined by growing the fungus in Richards' solution of different pH values at laboratory temperatures. The final pH of the media and the dry weight of the growth were determined.

TABLE II

Growth at Different H-ion Concentrations

Initial pH	pH after 23 days	Weight of fungus in gm.	Nature of growth
3·6	5·3	·1551	Grey, floating growth.
4·6	6·4	·3531	Thick grey growth, floating and submerged.
5·0	6·4	·2993	Do.
5·9	6·1	·1512	Less than the above.
6·6	6·6	·0827	Do.
7·4	6·8	·0292	Thin growth, light grey floating and submerged.
7·9	7·3	·0257	Little growth, mostly submerged.

The best growth occurs between pH 4·5 and 5·0, but the fungus is capable of growing over a wider range. Abbott (1938) noticed that the optimum was at 5·5 but he came to this conclusion from measurements of the diameters of growths on solid media. The pH is shifted differently according to the initial value but in most cases the final reading comes to be between pH 6 and 7.

The progressive shift in the pH value of the medium as a result of the fungus growth was determined at intervals by growing the fungus in Richards' solution of normal strength. Alongside with this, the effect of half strength of the medium on the growth and shift of pH was also noted.

In the first week the growth in the two strengths appear to be equal but from the second week onwards the growth in the normal solution is much more, almost double that in the half strength medium. But the shift in the pH is almost similar without relation to the strength of the solution or the amount of growth. After the second week the shift in the pH is not marked. After the third week there is a slight decrease in the weight of the mycelial growth in both the series.

TABLE III

Progressive Shift of pH Value of the Medium

No. of days' growth	NORMAL STRENGTH			HALF STRENGTH		
	Initial pH	Final pH	Weight of fungus in gm.	Initial pH	Final pH	Weight of fungus in gm.
7	4·3	5·5	·0338	4·7	5·7	·0370
14	4·3	6·4	·2611	4·7	6·3	·1713
22	4·3	6·4	·5388	4·7	6·8	·2624
30	4·3	6·7	·5141	4·7	7·0	·2151

The filtrates were tested with Nessler's reagent when a brown precipitate was formed indicating the presence of ammonia in the solution. Boyle (1924) has stated that NH_3 is produced by some fungi during growth. The controls did not show any precipitate.

Temperature and Growth.—The fungus was grown in peptone agar in Petri dishes kept at different temperatures. The diameters of the growths were measured and the results are given below:

TABLE IV

Growth Characters of C. falcatum *at Different Temperatures*

Temperature in °C.	Diameter on 7th day in cm.	Average spore length in μ	Range in μ	Nature of growth
10	No growth	..	..	..
15	2·1	19·9	12–32	Thin white powdery growth, spores abnormal.
20	5·2	23·5	12–40	Thin whitish aerial and greyish olive submerged growth.
23	7·2	24·1	12–44	Pearl grey aerial growth; vinaceous fawn spore masses.
26	7·3	..	..	Pearl grey aerial browth with plenty of pale vinaceous fawn spore masses over most of the surface.
30	7·6	27·6	16–44	Pearl grey aerial growth with numerous shell pink spore masses over the whole surface.

From the above table it is found that 30° C. is the optimum among the temperatures tried. At 10° C. there was no growth but when the plates were removed and placed in the laboratory (about 28° C.) growth commenced and spread over the plate. The size of the spore is affected by temperature. The spore length increases from 15° C. onwards and at about 30° C. the normal size is attained. The shape of the spore produced at 15° C. is abnormally

curved. The intensity of sporulation increases with the temperature in the range studied.

Spores were kept in hanging drops at different temperatures for 24 hours and the correlation between temperature and percentage of germination and germ tube length was observed. 50 Measurements of germ-tube length were made.

TABLE V

Temperature and Germination of Spores

Temperature in °C.	Percentage of germination	Average germ-tube length in μ	Range in μ
10	..	..	..
15	5	76·4	40–108
20	50	138·4	52–216
26	82	163·5	120–280
32	100	191·5	160–338
37	84	71·8	24–128

Among the several temperatures tried, the percentage of germination and the growth of the germ tube in 24 hours are best at 32° C. Abbott (1938) has recorded 30–32·5° C. as the optimum for *C. falcatum*. Though germination at 37° C. is as good as at 26° C. the growth of the germ tube at the former temperature is much less. There is progressive reduction in germination and germ-tube length as the temperature falls below 32° C.

Humidity and spore size.—Infected sugarcane setts were kept exposed in the laboratory (about 60%) and inside moist chambers (100% humidity) for a week. Spores from the two series were measured to note the influence of humidity on spore length. The average spore size from the two series is the same ; those from setts exposed to laboratory air being 28·3 × 4·9 μ and those from setts inside the moist chamber 28·4 × 5·0 μ.

Slides containing air-dried spores were placed inside desiccators containing saturated solutions of $NaHSO_4$, $NaNO_2$, $NaClO_3$, KBr and Na_2SO_4, $7H_2O$ and distilled water in another desiccator capable of maintaining 52%, 66%, 75%, 84%, and 95% and 100% relative humidity respectively (Riker and Riker, 1936). When examined after 24 hours, germination was visible only in spores kept at 100% relative humidity and not in others.

Utilization of carbohydrates.—A stock medium containing KNO_3 10 gm., KH_2PO_4 5 gm., $MgSO_4$ $7H_2O$ 2·5 gm., and water 1000 c.c. was prepared. Two per cent. by weight of different carbohydrates were added to this solution in order to test the capacity of the fungus to utilise different sources of carbon. For the preparation of solid media 2% agar was added.

TABLE VI

Carbohydrates in Relation to Linear Growth of Fungus Colony on Solid Media

Source of carbon	Diameter in 12 days in cm.	Spore length		Remarks
		Range in μ	Average in μ	
Sucrose	8·0	16–40	28·12	Thick growth, soft woolly, pale olive grey, central portion raised, numerous pink spore masses.
Glucose	5·6	16–40	28·2	Pearl grey aerial growth not so thick as above, pink spore masses in the centre.
Starch (soluble) ..	7·2	16–40	29·96	Pale gull grey, soft fluffy, centre raised, not so thick as in glucose, pink spore masses in the middle.
Lactose	8·4	16–40	27·8	Less than in starch, a mixture of white, grey and chamois, soft plushy, spore production medium without prominent spore masses.
Maltose	4·2	..	..	Thin aerial, woolly chamois growth, spores scanty.
Cellulose	Not measureable	..	..	Very thin growth with few spores.
Arabinose	2·5	..	..	Very thin growth, light olive grey no spore masses.
No carbohydrate ..	Not measureable	..	..	Few radiating hyphæ.

TABLE VII

Carbohydrates in Relation to Growth in Liquid Media

Source of carbon	pH of control	pH after 18 days' growth	Dry weight of growth in gm.
Sucrose	4·3	6·8	·3234
Glucose	4·2	6·1	·1435
Starch	4·6	5·0	·0174
Maltose	4·4	5·3	·0115
Lactose	4·4	4·8	·0088
Cellulose	4·4	4·4	..
No carbohydrate ..	4·6	4·6	..

In both the series sucrose gives the best growth. Incidentally it is also brought out that linear measurements do not always give a correct picture of the amount of growth in media differing in composition, since it is not possible to differentiate between densities of growth. Lactose gives the largest diameter but the mat being very thin the quantity of growth is very poor. Being a parasite on sugarcane, it is natural that the best source of

carbon for this fungus is sucrose. Spore measurements show that there is no significant difference in the length of spores produced by injesting carbon from different sources. But there is marked difference in the intensity of sporulation sucrose producing profuse sporulation.

To the stock medium 2½%, 5%, 10% and 15% glucose and 2% agar were added to find out the effect of different concentrations of carbohydrate on the growth of the fungus.

TABLE VIII

Concentration of Glucose and Growth

Concentration %	Diameter in 8 days in cm.	Intensity of sporulation	Nature of growth
2½	8·8	+++	Good aerial greyish white growth; numerous pinkish spore masses.
5	9·0	+++++	Best growth; more spore masses.
10	4·8	++	Less growth and less of spore masses.
15	4·6	++	Less growth and less of spore masses.

Sporulation is best at 5% and diminishes as concentration increases. The amount of growth also decreases with increase in concentration above 5%.

Utilisation of nitrogen sources.—The influence of varying amounts of nitrate in the medium, on the growth of the fungus, was investigated. Richards' solutions with 0, 5, 10, 15 and 20 gm. of potassium nitrate per 1000 c.c. were prepared. To these 2% agar was added.

TABLE IX

Different Amounts of Nitrate and Growth

Amount of nitrogen	Diameter in 9 days in cm.	REMARKS
No nitrogen ..	10·0	Very thin light grey, with minute pink spore masses near margin; least sporulation.
5 gm. KNO_3 ..	10·2	Thick growth, aerial, mealy, olive grey; spore masses in patches of pink; stromatoid bodies many.
10 gm. ,, ..	9·1	Thick, mealy, grey and white, spore masses in plenty in the centre; stromatoid bodies many.
15 gm. ,, ..	8·6	Thick, mealy, light grey with numerous pink spore masses; profuse sporulation.
20 gm. ,, ..	8·3	Not so thick as above; whitish with islands of grey growth; spores less profuse than in 10 gm.

In the medium with no nitrogen, the growth is very thin and the diameter is no measure of the amount of growth. The highest amounts of growth and sporulation were in dishes containing media with 10 and 15 gm. nitrate. The spore masses formed large pink patches over the growth.

The capacity of the fungus to utilise nitrogen from different sources was studied. A basic medium containing glucose 10 gm., KH_2PO_4 1·75 gm., $MgSO_4$. H_2O 0·75 gm., agar 15 gm., and water 1000 c.c. was prepared to which were added KNO_3, KNO_2, $(NH_4)_2SO_4$, urea, asparagin and peptone respectively as the different nitrogen sources. 5 gm. of KNO_3 were added to 1000 c.c. of medium. The other substances were used in quantities calculated to contain an equivalent amount of nitrogen.

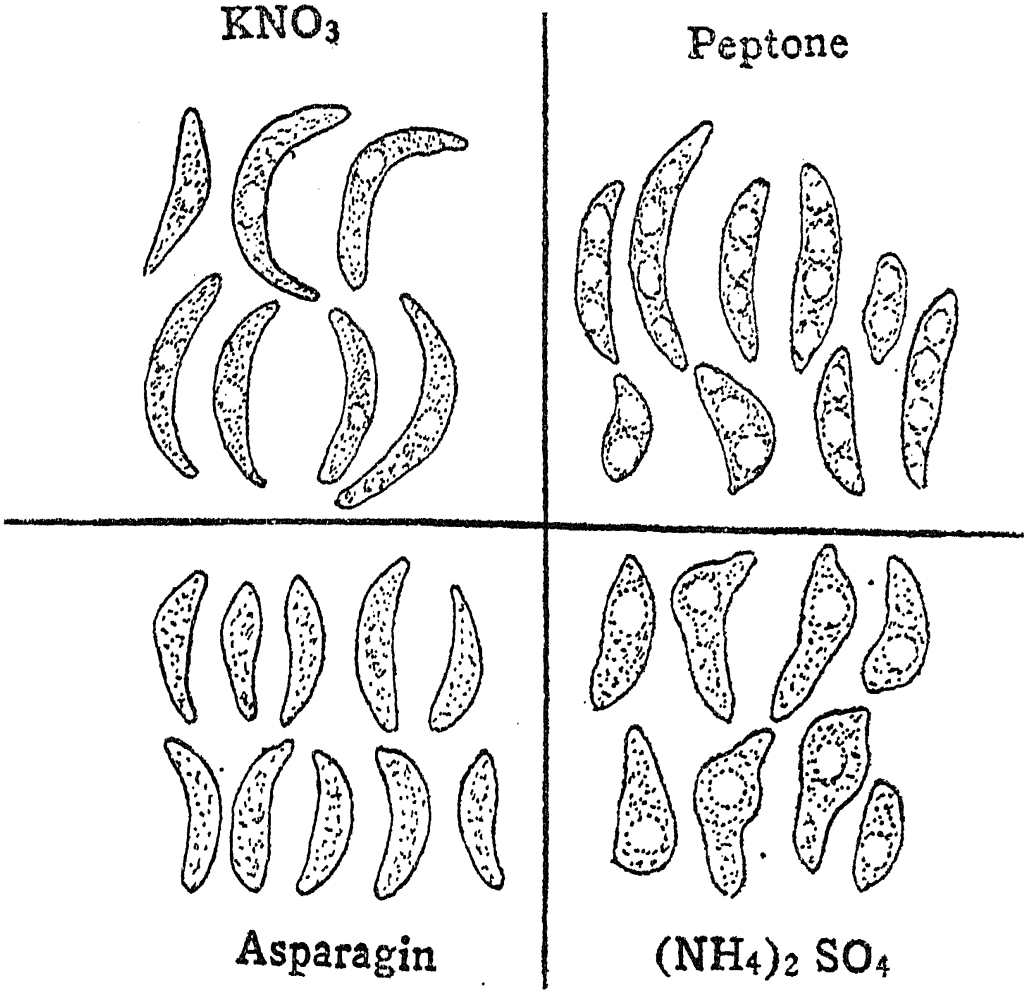
TABLE X

Different Sources of Nitrogen and Growth

Source of nitrogen	Diameter in 7 days in cm.	Mean length of spore in μ	REMARKS
KNO_3	2·2	31·4	Aerial growth pale gull grey, central portion thick; numerous shell pink spore masses forming patches.
Asparagin	4·6	24·4	Best growth, thick mineral grey to light mineral grey, irregular in outline; orange pink spore masses in plenty.
Peptone	7·8	27·6	Thinner growth, sparsely aerial; pearl grey with numerous shell pink spore masses.
$(NH_4)_2SO_4$	0·9	22·4	Extends little, pale olive grey and white; spores not many and abnormal.
Urea	0·9	..	Very thin growth, mostly submerged; no spore masses.
KNO_2	Nil	..	No growth.

Asparagin, peptone and KNO_3 are good sources of nitrogen. In ammonium sulphate there is an initial growth of the fungus which is quickly inhibited. Urea also forms a poor source. KNO_2 does not favour growth at all (Pl. XX, Fig. D). The nitrogen source influences the size of the spore and the intensity of sporulation. In $(NH_4)_2SO_4$ sporulation is limited and the spores are of peculiar shapes being bulged in the middle or otherwise malformed. In asparagin the spores are shorter than in potassium nitrate. Spores produced on peptone are more vacuolated than those of the same age formed on KNO_3 and asparagin (Fig. 1).

C/N ratio and growth.—Different proportions of carbon and nitrogen in Richards' solution and Richards' agar were used keeping the total weight of sugar and KNO_3 at 60 gm. per 1000 c.c. of medium. The different C/N ratios tried were 5/1, 2/1, 1/1, 1/2 and 1/5. On solid media, the best growth was on 5/1 closely followed by 2/1. The growth in the others were in the descending order with the least growth in 1/5. The medium in 1/2 and 1/5 was not completely solidified. In 5/1 and 2/1 a saltant developed. This was almost white, and was the predominant form in 5/1. While the olive



TEXT-FIG. 1

Spores from media with different nitrogen sources (× 500)

grey parental form was predominant in 2/1 (Pl. XX, Fig. E), in the other dishes the growth was olive grey without saltation. The growth was aerial and fluffy in all and spore formation was profuse. But the white saltant showed less of sporulation than the grey parent. At the junction of the two growths blackish stromatoid bodies were numerous. The two forms were isolated separately and in the following pages the white one is called light strain and the grey one, dark strain.

Liquid cultures of the different C/N ratios were also set up and the following table gives the dry weights of growths in these.

TABLE XI

C/N Ratio and Growth

C/N ratio	Initial pH	pH of control after 23 days	pH of inoculated after 23 days	Dry weight of fungus growth in 23 days in gm.
5/1	4·3	4·3	6·7	0·5276
2/1	4·4	4·3	6·3	0·4344
1/1	4·2	4·2	6·5	0·2644
1/2	2·9	2·8	6·5	0·1543
1/5	2·9	2·9	6·0	0·0946

The dry weight decreases as the C/N ratio decreases. The best growth is in 5/1.

Saltation.—Abbott (1938) mentions the occurrence of two distinct races of *C. falcatum*—light and dark—which may be distinguished by the colour and texture of the growth. He has also stated that *C. falcatum* is more or less stable and does not produce mutations. The Indian cultures of the fungus which he examined were morphologically similar to the dark race type of America. The present culture was started from a single spore. For over 10 months it maintained its original character and produced a saltant for the first time on Richards' medium as mentioned above. The production of the white strain as a saltant from the original dark stain throws light on how the light strains might have developed in nature as mutants from the dark one.

Comparative studies were made of the two forms. They were grown on Richards' and quaker oats agars and sterilised bits of cane. On both the agar media the distinction in colour is maintained. The dark form produces more spores than the light one. On sterilised cane bits the difference is more evident. The light strain develops a white growth at first which in course of time turns light grey. The other is dark grey from the start and maintains the colour.

The two forms were grown in liquid cultures kept at different temperatures to note whether there is any difference in their reaction to temperature.

TABLE XII

Temperature and Growth of the Two Strains

Temperature in °C.	Light strain Weight of 17 days' growth in gm.	Dark strain Weight of 17 days' growth in gm.
15	0·0802	0·0529
20	0·1292	0·1380
26·5	0·1965	0·4106
32·5	0·2146	0·4235
37	0·0055	0·0020

Both the forms thrive best at 32°·5 C. The dark form produces more growth at the optimum temperature.

The thermal death point of the spores of the two strains was determined. Fine capillary tubes 2″ in length and closed at one end were placed in spore suspensions and kept under an air pump. On removal they were filled with the liquid containing spores. Six tubes were fastened to the stem of a thermometer with rubber rings and then immersed in sterile water, kept at a constant

temperature for 5 minutes or ten minutes (Thomas, 1934). After removal from the water, the tubes were placed in Petri dishes containing media and crushed by means of sterilised forceps to liberate the spores. Observations were made on the germination of spores after 24 hours.

TABLE XIII

Thermal Death Point

Temperature in °C.	Time in minutes	Light strain	Dark strain
52	5	No germination	No germination
51	10	do.	do.
51	5	do.	do.
50	10	do.	do.
50	5	Germination	Germination
49	10	do.	do.
49	5	do.	do.

The spores are killed in both the strains by 5 minutes exposure at about 51° C. But chlamydospores are able to germinate after 5 minutes exposure at this temperature.

The relation between temperature and the rate of spread of infection as shown by the reddening of the internal tissue was studied with setts of Co. 419. After inoculation the setts were placed in basins along with moist cotton wool and kept inside incubators at different temperatures. Top setts and bottom setts were inoculated. The following table gives the virulence index (Abbott, 1938) in 14 days.

TABLE XIV

Virulence Index at Different Temperatures

Temperature in °C.	Light strain		Dark strain	
	Bottom setts	Top setts	Bottom setts	Top setts
15	0·19	0·86	0·03	0·11
20	0·29	1·00	0·18	0·34
26	0·39	1·00	0·30	1·00
30	0·46	1·00	0·39	1·00
34	0·80	1·00	0·23	0·56

In the controls the red colour was confined to the immediate neighbourhood of the bores and there was no spread. Infection spreads quicker in the top setts. The maximum infection by the dark strain is at about 30° C. and by the light strain at about 34° C.

Three varieties of canes—Co. 213, Co. 413 and Co. 421—were inoculated with the two strains for comparing their pathogenic capacities. Below are given the virulence indices at the end of 18 days.

TABLE XV

Virulence Index

Variety	Dark strain	Light strain
Co. 213 ..	0·67	0·54
Co. 421 ..	0·62	0·78
Co. 413 ..	0·61	0·62

The pathogenecity of the two strains varies in certain varieties.

Antagonism.—During the course of studies it was found that when the cultures were contaminated by a dark green *Aspergillus*, the growth of *C. falcatum* was arrested. This was common to both strains. It was presumed that this may be due to formation of some staling products by *Aspergillus* which inhibited the growth of *Colletotrichum*. To confirm this the dark strain was grown on media containing filtrates of *C. falcatum*—both strains, *C. indicum* and the *Aspergillus* sp. These fungi were first grown in Richards' solution for 16 days. The media were then filtered and the filtrates mixed with equal quantities of fresh Richards' solution. In the control equal quantities of water and Richards' solution were used. After sterilisation the media were inoculated with the dark strain. The dry weight of the fungus was determined after 17 days.

TABLE XVI

Growth on Filtrates of Other Fungi

Medium	Dry weight in gm.
Filtrate of *C. falcatum* (dark)	0·2615
Do. (white)	0·2768
Filtrate of *C. indicum*	0·2537
Do. *Aspergillus*	0·0203
Control	0·1147

The filtrates of *Colletotrichum* have no inhibitory effect on growth. But the filtrate of *Aspergillus* reduces growth to a large extent, the effect being persistent even after autoclaving.

Trichoderma lignorum was grown along with the two strains of *Colletotrichum falcatum*. *T. lignorum* and strains of *Colletotrichum* were inoculated

in two places in the same dish. In five days *T. lignorum* had grown over the growth of the dark strain. The light strain was surrounded and was just beginning to be overgrown (Pl. XX, Fig. A). In 10 days the light strain also was overgrown. In both cases the *Colletotrichum* hyphæ had disintegrated in the portion overgrown by *T. lignorum* though there was no evidence of penetration of the hyphal cells by *Trichoderma* (Pl. XX, Figs. F & G). The dark strain is more easily affected than the light strain.

The growth of *Colletotrichum* in the filtrate of *Trichoderma* was tested. *T. lignorum* was grown in Richards' solution for 15 days. The solution was then filtered. Three series of media were prepared, on containing equal quantities of the filtrate and Richards' solution, another Richards' solution alone and a third with equal quantities of distilled water and Richards' solution. The dry weights of the fungi were determined after 17 days' growth.

TABLE XVII

Trichoderma Filtrate and Growth

Medium	Light strain in gm.	Dark strain in gm.
Trichoderma filtrate *plus* Richards' solution	0·2049	0·2669
Richards' solution	0·2780	0·3843
Richards' solution *plus* water ..	0·1720	0·2216

The autoclaved filtrate does not appreciably inhibit the growth of the two strains of *C. falcatum*.

Enzymes.—Some work on enzyme production by *C. falcatum* has been carried out by Lewton Brain (1908) in Hawaii. The production of invertase and very restricted quantities of cytase have been noted. Experiments were conducted with the Madras strain of *C. falcatum* and its saltant to observe the different kinds of enzymes produced.

Invertase.—This was investigated by growing the fungus in autoclaved sugarcane juice for one week and estimating the relative amounts of sucrose and glucose in the filtrate.

TABLE XVIII

Inversion of Sucrose

Medium	pH	Glucose %	Sucrose %
Control	4·7	1·64	16·27
Inoculated and one week old	6·1	6·52	11·21

The increase in the percentage of glucose with a decrease in sucrose contents in the inoculated juice shows the activity of the invertase produced by the fungus. One c.c. of the crude enzyme extract was mixed with 9 c.c. of 2% sucrose solution. Toluene was added. The solution was tested after 6 hours with Fehling's solution. There was no precipitate in the control while a deep red precipitate was formed in the tubes to which the extracts had been added. Both the strains produce invertase.

Diastase.—Two per cent. soluble starch was added to the stock medium. After 8 days of fungus growth the dish was flooded with 1% iodine solution. A small area one inch in diameter in the centre of the growth remained unaffected while the rest of the medium turned dark blue. This indicates that small quantities of diastate are formed.

One c.c. of the crude enzyme extract was added to 9 c.c. of 0·5% solution of soluble starch and tested with Fehling's solution after 24 hours. Both the strains showed the formation of precipitate, but the dark strain produced a deeper coloured precipitate showing greater enzyme activity. There was no change in the control.

Pectinase.—Potato discs 1 mm. in thickness were kept in the enzyme extracts of both the strains, boiled extracts and in sterilised water. In three hours, the discs kept in the unboiled extract of the dark strain became limp and could be easily torn while it took 4 hours to reach this condition in the extract of the light strain. In water and the boiled extracts the discs remained turgid. The limpness and the quick tearing of the potato discs show the presence of a comparatively higher pectinase content in the extract of the dark strain (Brown, 1915).

Oxidase.—Bavendam's method (Venkatarayan, 1936) was used for the determination of oxidase by growing the fungus in potato dextrose agar with 0·25% and 0·5% tannic acid. In the tannin agars, a dark brown halo developed round the growths. This was absent in the controls (Pl. XX, Fig. C). The halo increased with the time and was in advance of the mycelial growths. The growth of the fungus was much less in tannin agars compared to the control. The growth decreased with the increase in tannin contents. The tannin is utilised by the fungus by the production of oxidase.

Trypsin.—Egg albumen agar (Uppal and Kulkarni, 1937) was used. The light strain produced a thin growth and the medium cleared to some extent under the growth showing the utilisation of the coagulated albumen. The dark strain produced a poorer growth and the clearance of the medium was much less. The presence of small amounts of trypsin in the mycelium is indicated by the partial clearance of the medium.

Amidase.—This was determined by growing the fungus on asparagin-rosolic-acid agar. The white strain produced poor aerial growth but the medium was reddened on the 4th day. The dark strain produced less growth and changed the colour of the medium to red only on the 8th day. The white strain produces more amidase than the dark one.

Lipase.—Litmus cream agar was used to test the presence of this enzyme. Both the strains produced medium aerial growth and the colour of the agar was changed into red. This reddening must be due to the formation of fatty acids produced by the action of lipase on fats.

Erepsin.—The two strains were grown on casein agar. It was found that in the dishes containing the light strain the medium became clear under the growth due to the digestion of casein, by the production of erepsin. The medium in which the dark strain was growing was cleared to a slight extent.

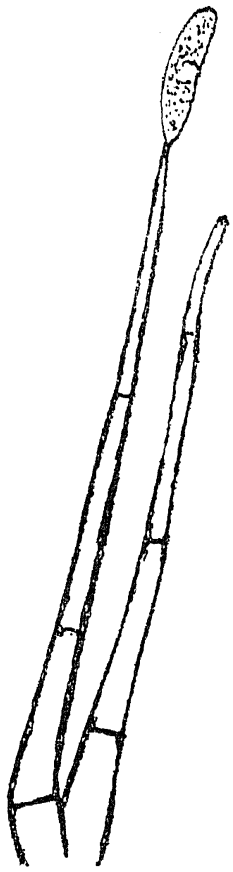
Emulsin.—Salicin agar was inoculated with both the strains. The light strain produced a thin growth 2 cm. in diameter in 12 days, while the dark strain did not grow at all. Hence it is concluded that the light strain produces very little emulsin and the dark one none at all.

Inulase.—Inulin agar was used to test the presence of inulase in the mycelium. Both the strains produced thin aerial growth showing that inulase is produced by them and hence they are able to digest inulin.

The enzyme studies show that the light and the dark strains produce plenty of invertase. Inulase, lipase and oxidase are formed in equal amounts, in both. The dark strain produces more of diastase and pectinase and the light strain more of trypsin, amidase and erepsin. Emulsin is not produced by the dark strain while the light strain forms small quantities of this.

General.—Abbott's (1938) observations from a study of numerous isolates showed that *C. falcatum* remained stable morphologically and that 'sectoring' did not appear in the cultures. In the course of the experiments described in this paper the cultures were true to the original isolate except on one occasion when sectoring developed in Richards' agar giving rise to a dark and a light form. In the beginning there was difference in spore production between the two strains, the dark one producing more spores than the light one. But spore formation after a number of sub-cultures is not of the same original intensity. The pink masses are very rare after 26 months in culture. Atkinson (1938) has observed the cessation of sporulation in culture after some time. The spores are in the earlier cultures of the isolate in pink masses produced from hyphal branches. Some times stromata are formed and these bear one or two setæ. These may rarely bear spores at

their apices (Fig. 2). Appressoria develop in large numbers in both the strains being more profuse in the dark strain. Sporulation in the old cultures of the dark strain can be improved by growing the fungus on sterilised sugarcane bits.



TEXT-FIG. 2
Seta producing spore

Acknowledgments

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Summary

C. falcatum grows well on a number of media. The best growth is formed between pH 4·5 and 5. About 32° C. is the optimum temperature for growth. The pores are killed by an exposure of 5 minutes at about 51° C. The spore length is affected by the temperature at which the fungus grows.

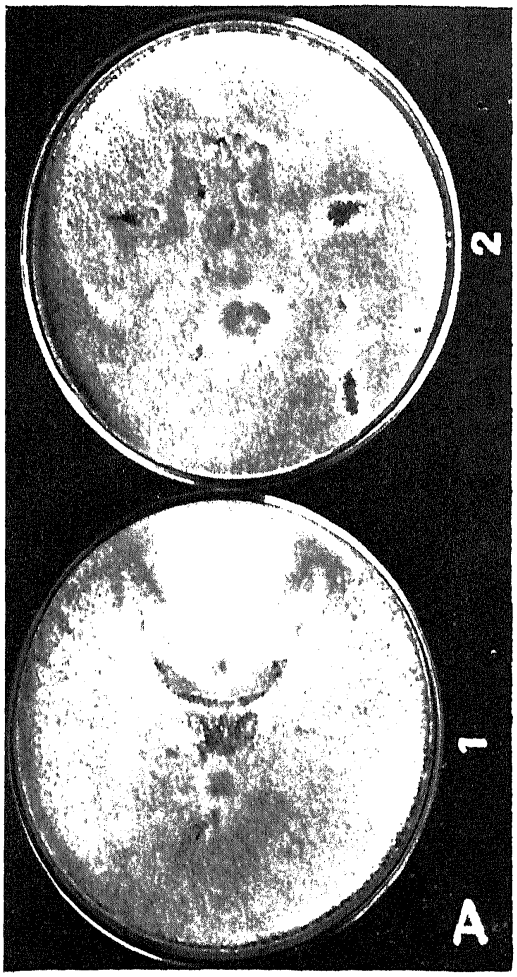
Sucrose is the best source of carbon. The source of carbon has no influence on spore length. But the intensity of sporulation is affected.

Asparagin, KNO_3 and peptone are readily utilised by the fungus. The growth is poor on $(NH_4)_2SO_4$ and urea. There is no growth in KNO_2. The spore length and intensity of sporulation are influenced by the particular nitrogen compounds used. The best growth occurs when C/N ratio is 5/1.

A light coloured saltant was formed. The two strains have similar temperature ranges and thermal death points. But they exhibit differences in their sporulation, pathogenicity, amount of growth and colour.

Growth is inhibited by *Trichoderma lignorum* in paired cultures, but the autoclaved extract has no inhibitory influence. An *Aspergillus* sp. inhibits the fungus in culture and its autoclaved filtrate has also a similar effect.

A number of enzymes are produced by the two strains.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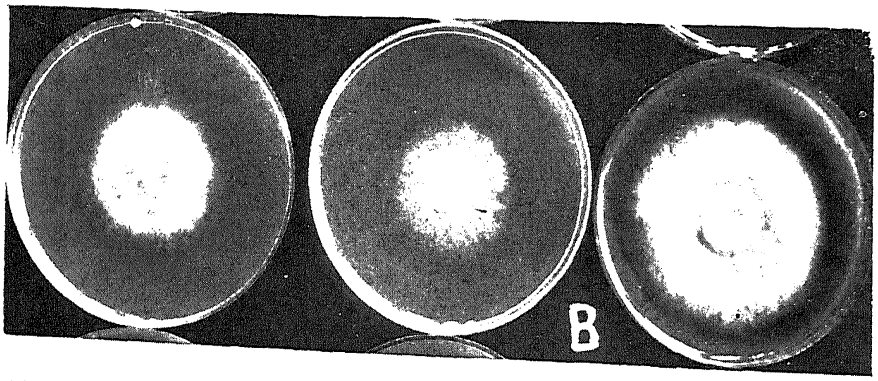
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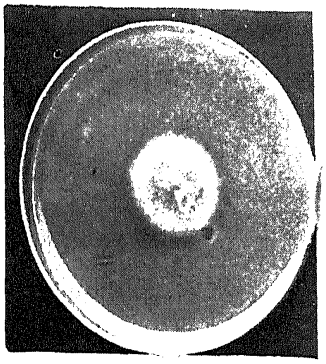

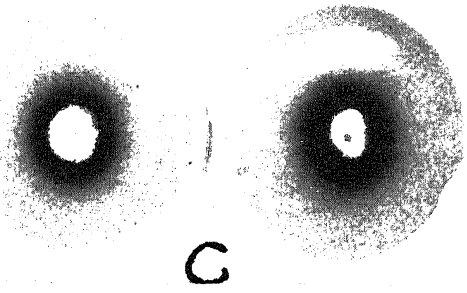
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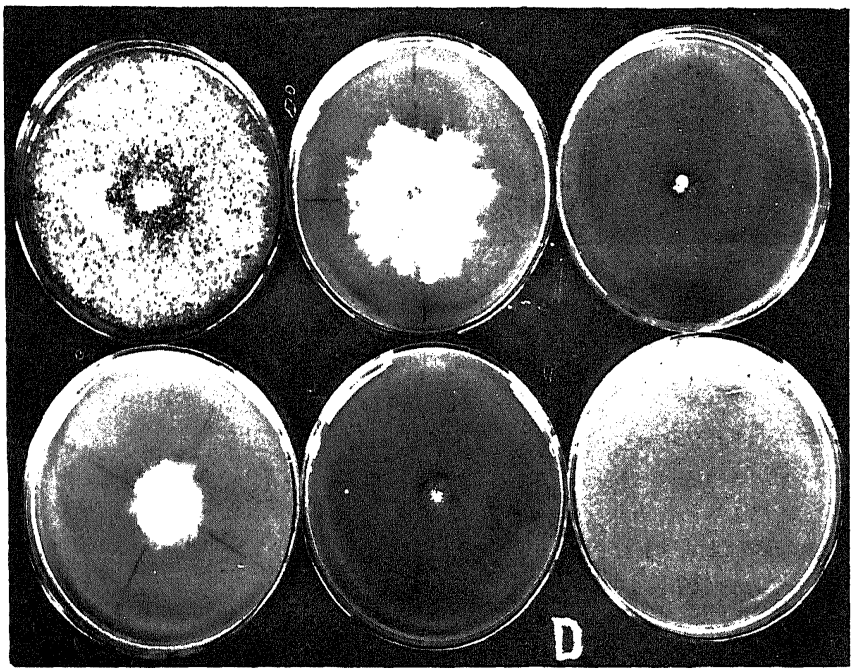
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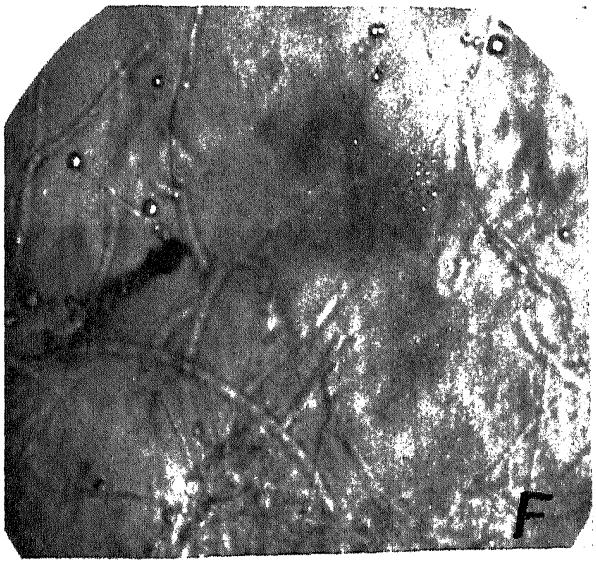
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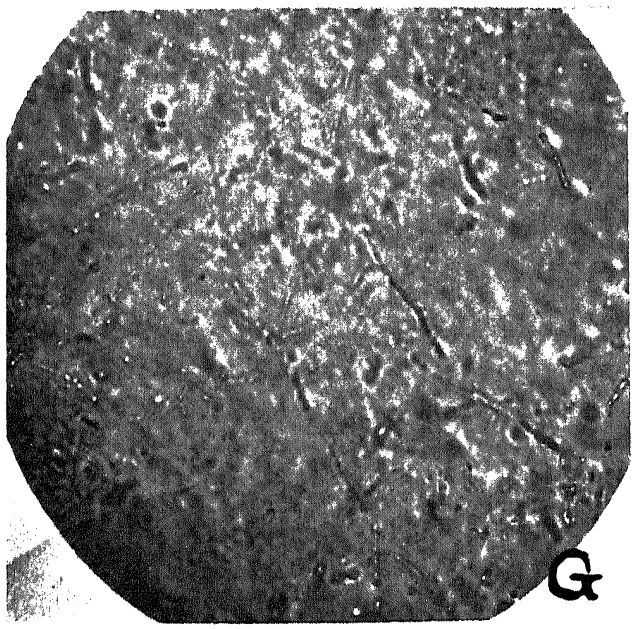
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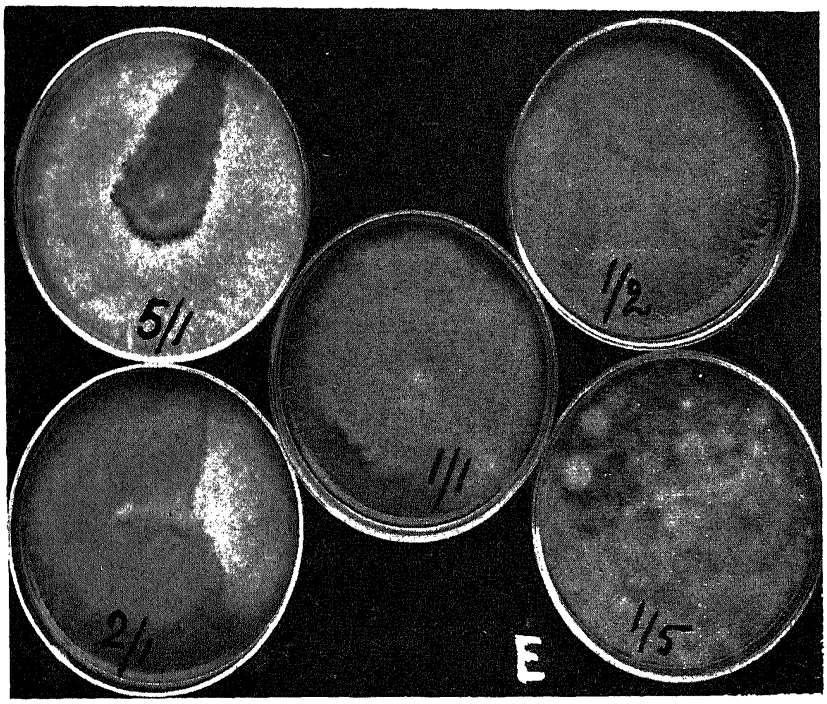
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E

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EXPLANATION OF PLATE XX

A. Paired cultures of *Trichoderma lignorum* and light strain (1) and dark strain (2) of *C. falcatum*.

B. Growth of *C. falcatum* on Richards', Brown and French bean agars (left to right).

C. Growth on potato dextrose agar (extreme left). ·25% Tannin agar (middle) and ·5% tannin agar (right). The dark halo is visible in the tannin agars.

D. Growth on different nitrogen sources (top row, left to right). Peptone, Asparagin and urea (bottom row, left to right). KNO_3 $(NH_4)_2SO_4$ and KNO_2.

E. Growth on different C/N ratios.

F. Hyphæ of *C. falcatum*.

G. Hyphæ of *C. falcatum* disintegrated by *T. lignorum*.