Studies in the Genera Cytosporina, Phomopsis, and Diaporthe.[1]

II. On the Occurrence of Saltation in Cytosporina and Diaporthe.

BY

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With Plates XIX and XX and nine Figures in the Text.

CONTENTS.

I. INTRODUCTION.

IN the first paper (20) of this series an account was given of an 'ever-saltating' strain of *Diaporthe perniciosa*. In the present paper it is proposed to deal chiefly with the occurrence of saltation and saltating strains in *Cytosporina ludibunda*; and to a less extent with saltation in *Diaporthe perniciosa*; to give a short description of the saltants and to indicate the bearing of this work on the inter-relationships of the genera *Cytosporina*, *Phomopsis*, and *Diaporthe*.

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II. Saltation in *Cytosporina ludibunda*.

The species of *Cytosporina* under investigation was originally isolated by Dr. Horne from decayed Lane's Prince Albert apples, held in storage in this Laboratory in 1920, and the same fungus was obtained from fifty different apples. When grown in potato mush agar the fungus produced somewhat irregular stromata which upon maturity discharged slender hook-shaped spores ('B' pycnospores of Diedicke) of the kind described for the form genus *Cytosporina*. Since the dimensions of the spores agreed very closely with those recorded for *C. ludibunda* in systematic works, the identity of the fungus appeared to be satisfactorily established.

From 1920 onwards this fungus has been utilized in connexion with investigations carried out in this Laboratory, into the internal resistance shown by apples to fungal invasion (15, 21), and showed no marked sign of instability until 1926, when it was found that fungal growths obtained by re-isolation from the diseased tissues present in apples previously inoculated with *C. ludibunda* showed many unusual features. Although 271 re-isolations were made out of 1,500 inoculated apples, none of the growths obtained exactly resembled the fungus originally introduced into the apple. These growths showed great diversity under standard experimental conditions but possessed one character in common, namely the presence of two types of pycnospores, the 'A' and 'B' pycnospores of Diedicke (12). Although numerous attempts were made to classify them it was found impossible to do so on an exact basis. They were ultimately and provisionally grouped in seven classes (CR_1–CR_7), each class comprising forms which varied within certain limits. This preliminary work which has only been briefly dealt with here, indicated that the fungus was in a state of 'flux' and led to the detailed investigation of saltation in *C. ludibunda* described below.

Of the four stock cultures of *C. ludibunda* available for experiment, three, C′, C″, and C‴, showed well-developed stromata and spore-masses. Before using these cultures the spore-masses were thoroughly examined and the spores in every case proved to be of the filamentous type ('B' spores). From these stock cultures eighteen monohyphal cultures were made, using for each the branched or unbranched end of a single hypha. After a few days, fragments of mycelium from the monohyphal cultures were transferred to plates. The growths formed in plate cultures were of a variable nature but could be arranged in four fairly distinct groups (C, C_1, C_2, C_3). The cultures grouped together in C_3 exhibited a combination of the characters of those placed in the three remaining groups. All the cultures produced pycnospores of the 'A' and 'B' types. The growth characters of C, C_1, and C_2 in standard medium cultures are given below.

C—Aerial mycelium brown, forming a thin felt. Substratum brown. Zonation wide, distinct, or in some cases absent, stromata small, dark brown, scattered, fairly numerous.

C_1—Mycelium superficial, yellowish-brown. Substratum brown. Zonation wide, brown and clear zones alternating. Stromata or pycnidia dark brown, small, restricted to the central region of the culture.

C_2—Mycelium superficial, grey with central band of raised mycelium. Substratum grey. Zonation feeble, wide. Stromata dark brown, few, variable in size.

Three cultures, one from each of the groups C, C_1, and C_2 were selected for more detailed study. The strains were repeatedly subcultured, using mycelial inocula in the standard medium, and the characters shown on each occasion were critically compared. Care was taken to keep the line of descent from each monohyphal parent separate. As a result, in some cases variants were obtained which remained true to type, in others variants which proved to be more or less unstable. In Table I, the relation between the variants derived from the monohyphal parents C, C_1, and C_2 and the groups of the re-isolated strains (CR_1–CR_7) is shown. In each case the positive sign indicates that a particular monohyphal variant falls into one or other of the seven groups. The correspondence between the two sets is remarkable. It is further seen from the table that a strain, CC_2, has been obtained which did not fall into these groups. Similar results were obtained when small masses of pycnospores taken from individual stromata were used instead of mycelium for the inocula.

TABLE I.

Relation between the Monohyphal Variants and the Groups of the Re-isolated Strains of Cytosporina ludibunda.

Strains (Monohyphal).	Nature of the Cultures derived from Monohyphal Strains.							
	CR_1.	CR_2.	CR_3.	CR_4.	CR_5.	CR_6.	CR_7.	CC_2.
C	+	+	+		+			+
C_1	+		+	+	+	+		+
C_2	+	+			+	+		+

The method of plating has, however, yielded the greatest number of variants. Twelve spore-masses taken at random from the monohyphal cultures C, C_1, and C_2 were plated separately, each spore-mass being divided among a number of plates. While the majority of the colonies arising in plates conformed to the parent type, at least four other types were present which were distinctly different. Almost all the plates showed the presence of one or more of these variants, one particular plate, however, prepared

from a spore-mass of C showed all of them. The variants differed in the following respects:

(*a*) Drab-white growth with thick mycelium.
(*b*) White growth with thin mycelium.
(*c*) Grey mycelial growth.
(*d*) Dark brown growth with thick mycelium.

The marked dissimilarity of these variants is evident from the photograph of one of the platings given in Pl. XIX, Fig. 1.

From these variants the following strains were obtained:

CA—derived from variants (*a*) and (*b*). Thin drab-white mycelial felt with moderately numerous stromata of variable size. Zonation wide, (Pl. XIX, Fig. 2.)

CB—derived from variant (*c*). Dusky brown, showing distinct zonation. This strain proved to be quite sterile.

CC—derived from variant (*d*). Thin brown mycelial felt and dark grey to grey substratum. Large stromata disposed in zones. Zonation wide. (Pl. XIX, Fig. 3).

The plating experiment was repeated several times but only one additional strain was obtained—namely CC_2 (Pl. XX, Fig. 12), also obtained from mycelium.

Similar experiments were then carried out with the saltant strain CA. Platings of spores were made from time to time. At first the growths obtained were fairly uniform and remained true to type, but later interesting features were observed. Three dilution plates had been prepared, using spores taken from a single spore-head. In one of these plates two variants were obtained which contrasted sharply with the growths representing the parent type, by reason of their colour and relatively small size (see Pl. XIX, Fig. 4). The first variant (*a*) which showed a dark brown leathery mycelium proved on subculturing somewhat similar to the monohyphal parent C. The second variant (*b*), which was grey in colour and with few stromata, gave rise to a new strain termed CA_1. CA_1 is characterized by the colour of the mycelium ranging from grey to pinkish vinaceous, wide zonation, the frequent presence of a central band of tufted mycelium, and by the paucity of the stromata (Pl. XIX, Fig. 5).

The strain CA_1 was next subjected to plating experiment. The colonies obtained during the earlier platings were apparently all alike, nevertheless, on various occasions subcultures made from some of the colonies gave rise to a strain CA_2 (Pl. XIX, Fig. 6), indistinguishable, as a rule, from CA_1 in mycelial characters, but differing from it in its inability to form fruit bodies. During the course of later experiments an entirely different variant appeared. It was first observed as a small black colony in sharp contrast with the grey colonies of CA_1; later the contrast became

more pronounced owing to the development of pycnidia where its margin came into contact with the CA_1 colonies. Subcultures from the black colony showed a black mycelium and substratum, in which as time went on innumerable small fruit bodies were formed, generally disposed in zones. Several plates were made, using mycelial inocula from the black sporing cultures, the resulting growths showing the presence of grey and black mycelium side by side, and from which eventually two very distinct strains were separated, namely CA_4 (Pl. XIX, Fig. 8), an infertile black strain, and CA_3 (Pl. XIX, Fig. 7), a fertile strain, smoke-grey in colour, differing from CA_1 mainly in its more prolific sporing.

Platings from the spores of CA_3 yielded besides the parent, colonies of CA_1 and CA_2, both grey, and colonies of CA_4 the black strain (Pl. XIX, Fig. 9). The production of the black strain by CA_3 proved to be of particular interest, and has been separately dealt with in p. 356.

Attention was then re-directed to the second sporing strain CC. originally derived from the monohyphal parent C.

Series of plates were made, using different spore-heads, and, in every plate but one, brown colonies showing the character of the parent were obtained. In the remaining case, besides the parent colony CC, two variants were present, showing (*a*) light amber-brown, and (*b*) pale orange-yellow growths respectively (Pl. XIX, Fig. 10). Upon subculturing, both of these colonies yielded the same saltant (CC_1), which is light amber-brown in colour with numerous small pycnidia generally distributed throughout the culture (Pl. XX, Fig. 11), in contrast to CC which shows dark grey mycelium and large stromata in zones (Pl. XIX, Fig. 3). Further, while CC produced both 'A' and 'B' spores, the saltant CC_1 produced only the 'B' type. The platings from the spore-heads of CC_1 yielded only a few colonies of two slightly different types, both of which were pale orange-yellow in colour, but whereas in one of the types the colour was uniform, the other showed an amber-brown marginal zone. Both on subculturing produced the same saltant, which appeared to be completely sterile and identical with CC_2 (Pl. XX, Fig. 12). This strain was also obtained by direct plating from the monohyphal parent C.

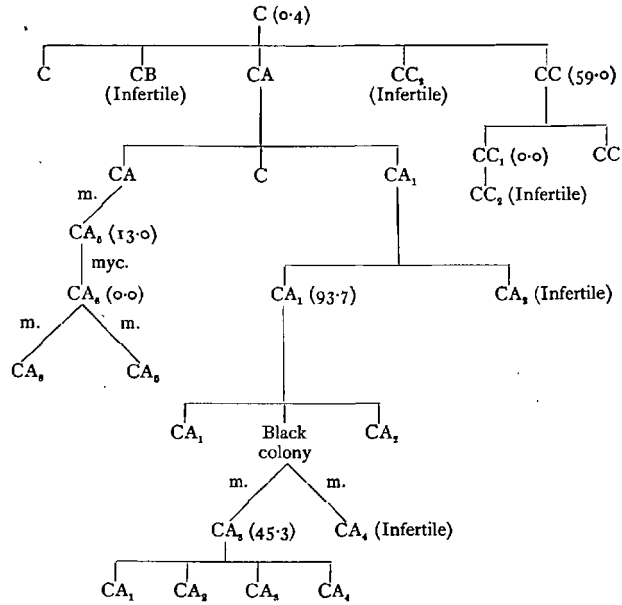
By this method of plating no less than nine distinct strains had been obtained which were derived from a single culture of monohyphal origin. The origin of these saltants is shown diagrammatically in Text-fig. 1.

In order to test the constancy of these saltant strains, further cultural work was carried out using only the mycelial inocula. The result is briefly dealt with below:

CA—Constant for a number of cultural generations, subsequently gave rise to an 'ever-saltating' strain termed CA_5 (Pl. XX, Fig. 13), from a part of the culture where no sectoring was evident. CA_5 will be described in greater detail below.

CB—After three cultural generations reverted to a variant type already obtained.

CC—Variation was observed from time to time. The colour varied from dark brown to grey; the stromata were always large but varied greatly in number.



TEXT-FIG. 1. Diagram showing the origin of certain strains of *Cytosporina ludibunda* from a single monohyphal parent.
The origin of strains is from spores except where mycelium (m) is indicated.
Figures against the strains show the average percentage of 'A' spores present in each case.

CA_1—This fertile strain usually bred true to type. Occasionally, however, it produced the sterile saltant CA_2 though no sectoring could be observed in the culture.

CA_2—This infertile strain remained true to type.

CA_3—This strain usually bred true to type. Occasionally sectoring cultures were obtained, the sector showing the characters of CA_2 (Pl. XIX, Fig. 7). CA_2 was also isolated from regions where no sectoring was evident.

An interesting phenomenon was observed while plating the spores of

CA_3 which was not met with during the mycelial subcultures. It has been described in some detail on p. 356.

CA_4—This infertile strain remained true to type.

CC_1—Remained true to type for a few generations, and then it was found that from whatever part of the mycelium the subcultures were made, infertile growths were obtained which resembled CC_2.

CC_2—This apparently infertile strain proved somewhat variable in colour. In exceptional cases subcultures were obtained in which pycnidia with 'B' spores were formed, in this respect resembling CC_1, but differing from the latter in all other characters.

It will be seen that only one new strain CA_5, was obtained in the course of the tests mentioned above. This strain proved to be particularly interesting and therefore deserves more detailed consideration.

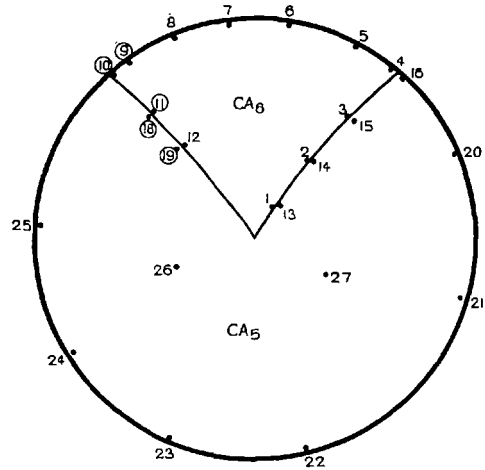
CA_5 differs from CA in producing fewer stromata and in its 'ever-saltating' character. In almost every subculture CA_5 produces sectors, generally one, sometimes more than one, which are quite unlike the parent. In CA_5 the colour of the mycelium is drab-white, and stromata with 'A' and 'B' spores are present; in the sector (CA_6), on the other hand, the colour is brown and numerous small pycnidia with 'B' spores are formed (Pl. XX, Fig. 13). Several preliminary attempts were made to isolate the saltant representing the sector, but without success. The sectoring cultures were then studied in a more systematic manner. A sectoring plate culture of CA_5 grown at room temperature was chosen, and a number of mycelial inocula were taken from different regions, namely from the youngest portion of the culture and also from the regions lying on either side of the line demarcating the saltant from the parent. The particular places from which the inocula were taken is shown in Text-fig. 2, the numbers referring to particular inocula. Each inoculum was transferred to a separate standard medium plate, and these were kept under the experimental conditions employed for the parent cultures. As a result it was found that the growths originating from inocula 9, 10, 11, 18, 19, showed the characteristics of CA_6, the remainder proved to be a mixture of CA_5 and CA_6 in various proportions.

The pure saltant CA_6 was obtained from both the sector (CA_6) and the parental region of the culture (CA_5). It was obtained from both young (Nos. 9, 10) and old (No. 11) portions of the sector, and from old portions (Nos. 18, 19) of the parental region, but it was never obtained from the youngest parental mycelium.

The experiment was then repeated, using the sectoring culture of CA_5 arising from inoculum No. 20, and the pure saltant culture (CA_6) obtained from inoculum No. 9. The results, in both cases, were similar to those obtained in the first experiment. When cultures of CA_5 and CA_6 selected from the second series of plates were in turn subjected to similar experimental

treatment, it was found that none of the inocula, whether taken from CA_5 or CA_6, produced the pure saltant CA_6.

It will be at once evident from the results described above that CA_6 usually does not breed true to type but shows reversion to parental characters.



TEXT-FIG. 2. Diagrammatic representation of sectoring culture CA_5 showing positions 1–27 from which the inocula were taken. The numbers within the circle indicate the positions from which the saltant CA_6 was obtained. No. 17 has been accidentally omitted; its position was in CA_5 close to No. 10.

It is interesting to note that at higher temperatures, viz. 25°C. and 30°C. sectoring was not observed in CA_4. This strain is still under investigation.

During the course of the spore plating already described (see p. 353), it was found that the relative proportion of grey (CA_1, CA_2, CA_3) and black (CA_4) colonies derived from the spores plated from individual stromata of the strain CA_3 varied within narrow limits. In platings made from the first cultural generation of CA_3, the percentage of black colonies varied from 4·1 to 8·0, while the average percentage for the stromata examined was 5·95. When a similar experiment was made with the same strain, using the third cultural generation (CA_{3-8}) arising from spores, not only did the proportion vary within wider limits, but also the average percentage of black colonies was much higher (38·9), and it was also higher for all the individual stromata examined (13·6 to 63·4). The results are given in detail in Table II.

TABLE II.

Percentage of Black Colonies derived from Different Stromata of CA_3.

$CA_{3-(1)}$. First Cultural Generation.

Stromata.	Percentage of Black Colonies in each Plate.					Average percentage of Black Colonies.
	1	2	3	4	5	
1	10·5	10·0	7·5	6·0	5·0	8·0
2	10·0	9·0	7·5	3·5	4·0	6·8
3	7·5	5·5	3·5	3·0	—	4·9
4	7·0	4·0	3·5	3·0	3	4·1
	$CA_{3-(3)}$. Third Cultural Generation.					
1	69·5	63·0	60·0	61·0	—	63·4
2	52·0	49·0	48·5	47·0	46,5	48·6
3	50·0	49·5	39·5	—	—	44·6
4	40·0	39·5	—	—	—	39·3
5	44·5	37·5	36·0	34·5	32·5	37·0
6	44·5	37·5	30·5	23·5	—	34·0
7	33·5	32·5	32·0	31·0	23·0	30·4
8	17·0	16·5	13·5	13·5	7·5	13·6

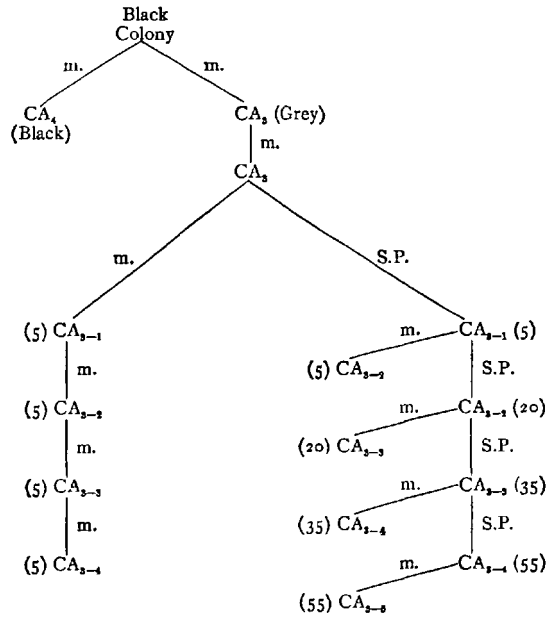
For a further elucidation of the point, investigations were carried out in greater detail by plating spores from different spore-heads and calculating the proportion of 'Blacks' and 'Greys' in each case. During plating every care was taken to standardize the experimental conditions.

The first dilution plate cultures made from different spore-heads of the original culture of CA_3 produced colonies the majority of which were grey (CA_1, CA_2, and CA_3), a few being infertile black (CA_4). The average percentage of 'Blacks' for this particular plating experiment was 4·5. The colonies arising from the first plating were then allowed to grow until some of the CA_3 colonies had sporulated. Platings were then made from a number of spore-heads of one of these colonies (2nd plating). The result was the similar appearance of 'Blacks' and 'Greys'. On this occasion the average percentage of 'Blacks' increased to twenty. Platings were again made, using spore-heads from a colony of CA_3 obtained from the 2nd plating. The average percentage of 'Blacks' in this instance was still higher, viz. 35. Similar platings were made for a few more generations, always using spore-heads from one of the CA_3 colonies obtained as a result of the previous plating. It was found that in the 4th plating generation the percentage of the 'Blacks' had reached its highest, viz. 55, beyond which no further definite increase was observed.

Experiments were made to determine whether the occurrence of black colonies was related to the time of development of spores. Platings were made using the first spores of a stroma, and some weeks later further platings were made from the later discharge of the same stroma. In both cases a certain percentage of black colonies was obtained.

In order to determine whether similar changes in the relative proportion of 'Greys' and 'Blacks' occurred, when the inocula used were of mycelial nature, subcultures were made from some of the CA_3 derivatives obtained from pycnospores using mycelial inocula. When spore-discharge was observed in these cultures they were plated in the usual way. As a result it was found that the cultures developing from mycelial inocula showed approximately the same proportions of 'Blacks' and 'Greys' as those recorded for the parent cultures from which they were derived. For example,the culture derived from a colony which produced 5 per cent.'Blacks' also produced 5 per cent. 'Blacks', that derived from one producing 20 per cent. 'Blacks' also produced 20 per cent. 'Blacks' and so on.

The descent of CA_3 through mycelium and pycnospore respectively, and the percentage of 'Blacks' shown by various descendants are represented diagrammatically in Text-fig. 3.



TEXT-FIG. 3. Diagrammatic representation of the descent of CA_3 through mycelium and pycnospores respectively. The approximate percentage of black colonies shown by various descendants is given in brackets. m. = mycelium; S.P. = spore plated.

III. CHARACTERISTICS OF THE SALTANTS (Standard Medium Cultures).

Since the majority of the strains from the stock culture of *Cystosporina ludibunda* proved to be unstable, a detailed description of all the variants would serve no useful purpose. It is proposed instead to give, first of all, a short general account of the more important characters shown by the variants, and then to compare the characteristics of some selected saltants which have been specially studied in the paper, devoting special attention to spore characteristics, concerning which very little information has been given in the preceding pages.

The general morphological characters of the variants as observed in the standard medium cultures were as follows:

Colour of the mycelium and substratum. Pure white to various shades of grey, yellow, brown, and black: cultures show one or more of these colours.

Nature of the mycelium. Aerial mycelium may be present or absent. When present forming a shallow or moderately dense felt, in some cases loose, more or less scattered tufts are present. Some strains show torch-like outgrowths, these may be erect or depressed and variously coloured, namely, white, white and yellow, yellow and brown, &c. Zonation may be distinct, indistinct, or absent; the zones may be wide or narrow.

Asexual reproductive organs. All stages between stromata and pycnidia are found, and one or other or both may be produced by the same strain. Stromata are subspherical or irregular, smooth or covered with a mycelial felt, and show various colours, namely, shades of grey, brown, and black. Stromata and pycnidia are few to extremely numerous, scattered or disposed in zones. Spores are discharged in cream, pink, or yellow spherical masses, or in similarly coloured tendrils. The same strain may show both kinds of spore discharge. The spores are of 'A' and 'B' types.

For the sake of convenience the comparison of the selected saltants is given below in tabular form (Table III).

It will be seen that the difference between CA_1, CA_2, and CA_3 lies only in the degree of fertility. Again, the strains CA_6 and CC_1, although very similar in morphological characters, have been considered as distinct for some reasons. Thus CA_6 on subculture almost always reverts to CA_5, whereas CC_1 on subculture gives rise to CC_2, the products in the two cases being entirely different.

The sporing saltants with two exceptions produce both 'A' and 'B' spores, the exceptional strains producing only the 'B' type. The characteristics of these two types of spores are given below:

'A' spores. Hyaline, short, sub-elliptical, sub-ovoid, or sub-oblong. Usually with two distinct oil-drops, sometimes more than two; exceptionally oil-drops are absent (Text-figs. 5, 6, 7, 8).

TABLE III.

Characters of Some Selected Strains in Standard Medium Cultures.

Saltants.	Colour.	Substratum.	Mycelial Character.	Zonation.	Reproductive Character.	Nature of Reproductive Organs.	Spores.
CA	Drab-white.	Grey.	Thin felt.	Wide.	Fertile	Stromata: dark brown, variable in size. Fairly numerous, disposed in zones.	'A' and 'B'
CC	Brown	Brown.	Thin felt.	Wide.	Fertile	Stromata: dark brown, usually large disposed in zones.	'A' and 'B'
CA_1	Grey to pinkish-vinaceous.	Grey.	Thick felt, with a central band of tufted mycelium.	Wide.	Fertile.	Stromata: dark brown, variable in size. Very few.	'A' and 'B'
CA_2	Do.	Do.	Do.	Do.	Infertile.	—	—
CA_3	Do.	Do.	Do.	Do.	Fertile.	Stromata: dark brown, variable in size. Numerous, disposed in zones.	'A' and 'B'
CA_4	Black.	Black.	Thick felt, sometimes fluffy.	Absent.	Infertile	—	—
CA_5	Drab-white.	Grey.	Thin felt, 'ever-saltating'.	Wide.	Fertile.	Stromata: brown, very few; somewhat large.	'A' and 'B'
CA_6	Brown.	Brown.	Thin felt	Narrow.	Fertile.	Pycnidia: brown, numerous, small, scattered.	'B'
CC_1	Light amber-brown.	Do.	Do.	Do.	Do.	Do.	Do.
CC_2	Pale orange-yellow.	Do.	Do.	Do.	Infertile.	—	—

'B' spores. Hyaline, filiform, straight or bent. All intermediate forms are found between straight and strongly bent spores. The spores of different strains vary with regard to the average degree of bending. They are not of uniform width, the straight end being slightly wider; the bent end is more or less pointed (Text-figs. 5, 6, 7, 8).

Taking all the strains into account the length of the 'B' spores varies from 18–40 μ; the variation in width is comparatively small, viz. 1–2 μ. The 'A' spores are shorter, varying in length from 4–16 μ, and in the mean length from 9–10 μ. The variation in width (2·5–5 μ) is greater than that

shown by the 'B' spores. With regard to the difference between strain and strain in spore length, some strains vary within relatively narrow limits, with others the range is wider. As to the mean length the strains show all gradations between the extremes given. These features are shown more markedly by the 'B' spores. In Table IV the limiting and mean lengths in μ for the 'A' and 'B' spores of certain selected strains are given, arranging the strains in order of increasing spore-length.

TABLE IV.

Length of 'A' and 'B' Spores in Certain Selected Strains.

Strain.	'B Spores'.		'A Spores'.	
	Range in μ.	Mean in μ.	Range in μ.	Mean in μ.
CC	21–32	26	4–12	9
CA_8	20–32	29	—	—
C	24–36	30	6–16	9·5
CC_1	22–38	30	—	—
CA_5	18–40	32	6–12	9·5
CA_6	21–40	34	8–14	10

The following points are at once evident: (1) The gradation in length mentioned above; (2) an increase in the mean length of 'A' spores is associated with an increase in the length of 'B' spores; (3) the variation in the mean length of 'A' spores from strain to strain is slight, although in each case the variation covers a wide range.

Brefeld (2) and Wehmeyer (29) found a certain relation between the colour of the spore-mass and the kind of spores in certain species of *Diaporthe*. Thus pink or creamy spore-horns were made almost entirely of 'A' spores; yellowish or white spore-horns, on the other hand, contained usually spores of the 'B' type. A similar correspondence in colour and spore-type is shown by the strains derived from *Cytosporina ludibunda*. Cream or yellow spore-masses or tendrils consist almost entirely of 'A' spores, whereas the white ones are almost exclusively 'B' in character.

With regard to the appearance of 'A' and 'B' spores, Brefeld (2) (*Diaporthe inaequalis* Curr. and *D. spina* Fkl.) and Harter (18) (*D. phaseolorum*) found that the 'A' spores make their appearance in the stroma first, the 'B' spores later. On the other hand, Wehmeyer (29) (*D. oncostoma*), Archer (1) (*Phomopsis arctii*), Cayley (9) (*P. perniciosa*), and Kidd and Beaumont (23) (*P. mali*) record that the 'B' spores develop first, the 'A' spores later. The writer's observations were confined to the periodic examination of the spore-heads of certain *Cytosporina* derivative strains, selecting for the purpose those which produced both 'A' and 'B' spores. Some time prior to the discharge of spores a drop of water is exuded at the tip of the ostiole of the stroma, into which the spores are subsequently discharged.

Liquid was taken from the drop from time to time, using a very fine capillary tube, and examined microscopically.

This method, of course, indicates only the order of the discharge of spores; however, it is thought not unlikely that a certain relation exists between the order of development and discharge. It was found that no general rule could be laid down as to the order of appearance of 'A' and 'B' spores. The stromata discharging 'A' spores may sooner or later discharge 'B' spores in addition and vice versa, so that the spore-heads ultimately contain a mixture of 'A' and 'B' spores. Sometimes, however, some of the stromata producing only one kind of spores may not produce the other kind at all, so that a stroma with pure 'A' or pure 'B' spores results.

The 'A' and 'B' spores are present in different proportions in the stromata or pycnidia. With some strains, for example, CA_1 and C, the two kinds of spores are formed nearly in the same proportions in all the stromata in a given culture, with others (CA_5, CA_3, CC) the ratio varies. The variation reaches an extreme in CC, where stromata containing 'A' spores only, various proportions of 'A' and 'B' spores, and 'B' spores only, are found in the same culture. In standard medium culture, the strains themselves show almost every gradation between the extreme types (1) with 'B' spores only, (2) with 'A' spores only. The point is clearly shown in Table V, where the range of variation in the percentage and the mean percentage of 'A' spores shown by certain stromata are given for various selected strains. In each case the number of stromata examined and the number of spores under observation are stated.

TABLE V.

Occurrence of 'A' Spores in Certain Selected Strains.

Strain.	Number of Stromata.	Number of Spores.	Variation in Percentage.	Average. Percentage.
CC_1	20	20,000	0	0·0
C	8	40,166	0–5	0·4
CA_5	8	3,900	0–21	13·0
CA_3	15	13,712	0–59	45·3
CC	10	8,289	1·6–100	59·0
CA_1	5	9,000	90–95·6	93·7

Cayley (9) has recorded that in *Diaporthe perniciosa* the 'B' spores disintegrate with age, leaving only 'A' spores in the stroma. No definite evidence of such disintegration of 'B' spores has been observed in the case of *Cytosporina*. In some strains the 'A' spores undergo a certain amount of disintegration, as is evident from their size and shape.

IV. Variation in General Morphological Characters as observed in different Nutrient Media.

These experiments were undertaken to find out to what extent the general morphological characters shown by any given strain varied when different nutritive media were employed. For example, whether in certain media stromata were formed, in others pycnidia, or in certain media spores of one type only, in others both 'A' and 'B' types.

The media were chosen to show some marked differences from the standard medium in one or other of the constituents, i.e. the sugar contents, nitrogen contents, &c. The composition of the media selected is given below for reference, each constituent being given in grammes per litre of water.

Table VI.

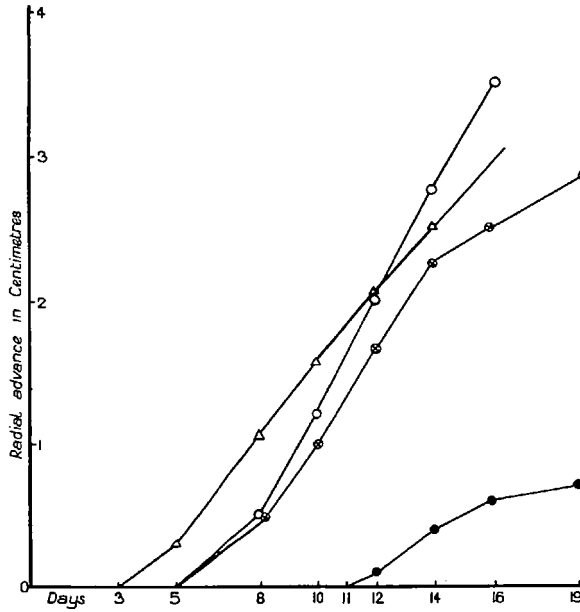
	Standard.	Coons'.	Malt.	Richards'.
KNO_3	—	2·00	—	10·00
$MgSO_4$	0·75	1·20	—	0·25
KH_2PO_4	—	2·70	—	5·00
K_2PO_4	1·25	—	—	—
$FeCl_3$	—	—	—	trace
Asparagin	2·00	—	—	—
Cane sugar	—	—	—	50·00
Maltose	—	7·20	—	—
Glucose	2·00	—	—	—
Malt extract (commercial)	—	—	20·00	—
Potato starch	10·00	10·00	10·00	10·00
Agar	15·00	15·00	15·00	20·00.

In Coons' and in malt extract agar the strains utilized usually show distinctive characters, but in Richards' medium the growths formed by certain strains so closely resemble one another that it was difficult to distinguish between strain and strain. As a rule the cultures in Coons' medium do not markedly differ from those obtained in standard medium. The zonation is, however, less marked, and the colour more strongly developed. The strain CC_2 proved to be exceptional, the characters of the cultures being entirely changed, showing superficial mycelium without zonation and dark green colour instead of the mycelial zones and orange-yellow colour characteristic of standard medium culture. Cultures of CC_2 grown in the two media differed to such an extent that they might easily be mistaken for different fungi.

Malt agar cultures usually show more aerial mycelium than that developed in Coons', and the zonation is indistinct. The colouring is more strongly developed than in the standard medium. The colour shown by CC_2 varies from dark grey to dark green.

Growths in Richards' medium usually show an irregular outline, and the aerial mycelium forms a thick compact felt. Colour of the mycelium

is at first white or almost white, subsequently changing into brown; ultimately the aerial mycelium becomes tough and wrinkled, forming a crust on the surface of the medium. The strain CA_4 alone retains its main



TEXT-FIG. 4. Graph showing the rate of radia advance in cm. of the strain CC_2 for four different media at room temperature.
△ = standard synthetic medium. ○ = Coons' agar.
⊗ = malt agar. ● = Richards' agar.

characters observed in the standard medium cultures. C, CC, and CA_5 are indistinguishable from one another. CA_2 and CA_3 resemble one another, and differ only slightly from C, CC, and CA_5. In the case of CC_2 the radial growth is greatly retarded, but the surface of the culture is covered with dense white tufts of aerial mycelium. This point is illustrated in Text-fig. 4, where the rate of radial growth in centimetres is given for four different media.

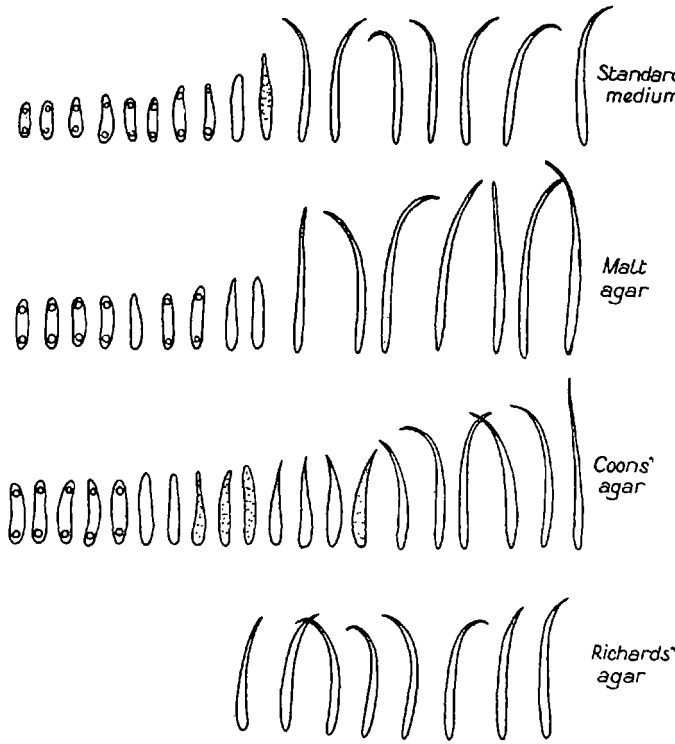
The change of medium was reflected in a very striking manner in the reproductive characters. Strains which proved infertile in the standard medium were also infertile in Richards' medium. In the case of sporing strains it was found that instead of the large stromata formed by certain strains in the standard medium, the same strains in Richards' medium

produced only relatively small stromata or pycnidia. Among the strains used for these experiments there were four, viz. C, CA_3, CA_5, and CC, which in standard medium cultures invariably produced 'A' and 'B' spores in varying proportions. All these strains in Richards' medium *produced only 'B' spores*. In each case, culture in one medium conformed in sporing character to the form genus *Phomopsis*; in the other medium to the form genus *Cytosporina*. In some of these media, again, the spores showed a great variability in their morphological characters which is worthy of special consideration.

Van Höhnel (28) and Bubák (7, 8) found all transitional stages between 'A' and 'B' spores in some species of *Phomopsis*. Diedicke (12), on the other hand, in 'Die Gattung *Phomopsis*', denied the occurrence of such intergradation in any of the species investigated by him. Later on, finding intergradation in *P. arctii*, he stated (13) that the culture was 'abnormal'. The observations of these authors were confined to the behaviour of the fungi growing under natural conditions. That intermediate stages are produced in artificial cultural media has been noted by Brefeld (3) for some species of *Diaporthe*. The observed variability in the dimensions of the spores shown by certain strains in culture suggested the occurrence of intergradation in *Cytosporina* also, and the question was followed in some detail. The four strains C, CA_3, CA_5, and CC were cultured in the four media already mentioned. When the cultures, sixteen in all, were in a sporing condition, microscopical examination of all the cultures was made, choosing spores taken at random from various stromata or pycnidia. Next, slides were prepared to represent quite fairly the nature of the sporing shown by each strain in each of the media used. Finally a representative 'field' was selected in each case, and the spores were measured and drawn. The result is illustrated in Text-figs. 5–8, the spores in each case have been arranged in serial order of length.

It will be seen that a complete intergradation between 'A' and 'B' spores occurs in the strain C and CA_5 when grown in Coons' medium (Text-figs. 5, 6). The 'A' spores in the former strain are comparatively large, but in both the strains the smaller 'A' spores contain two oil-drops. In the larger 'A' spores, however, the oil-drops disappear and the spores become either granular or hyaline. The transitional forms are very abundant in C (Text-fig. 5), and gradually taper to a point at one end, but this attenuation is not so prominent in CA_5 (Text-fig. 6). The strain CC shows incomplete intergradation in the standard and malt media, but none at all in Coons' (Text-fig. 7). The intermediate forms are generally of the same type. In CA_3 no intergradation was observed (Text-fig. 8). Grove (16, 17) has observed the occurrence of a third type of spores ('C' spores) in some species of *Phomopsis*. It seems that these spores are in many respects similar to the intermediate spores just mentioned.

Owing to the occurrence of spores which show transitional stages between typical 'A' and 'B' spores, it is clearly difficult to use the



TEXT-FIG. 5. Illustrates the spore characters of the strain C in various media.

character of spore dimensions for comparative purposes. The difference between strain and strain is often more adequately expressed by the extent to which the spores vary within a given range of experimental conditions. The question of spore dimensions will therefore be considered very briefly here. In Table VII the limiting range in length and width in μ for the 'A' and 'B' spores only is given for the strains C, CC, CA_3, and CA_6 as recorded for different media. The measurements in each case are for spores showing typical characters.

TABLE VII.

Dimensions of 'A' and 'B' Spores in μ for Certain Saltants of Cytosporina ludibunda.

Strain.		Standard Medium.	Malt Medium,	Coons' Medium.	Richards' Medium.
		'A' Spores.			
C	Length	6–16	6–13	12–16	—
	Width	2·5–3·3	2·5–3·3	2·5–4	
CC	Length	4–12	8–10	8–16	—
	Width	3–4·5	2–3·5	2·5–3·5	
CA_3	Length	6–12	8–12	8–12	—
	Width	3–4	3–5	2·3–3·5	
CA_5	Length	8–14	9–16	9–14	—
	Width	2·5–3·5	3–4	2·5–3·5	
		'B' Spores.			
C	Length	24–36	30–40	20–30	20–34
CC	Length	21–32	22–50	28–50	24–34
CA_3	Length	18–40	28–36	20–34	23–35
CA_5	Length	22–40	28–40	24–36	20–35

N.B. Width generally varies between $1\mu - 2\mu$.

The incomplete intergradation among 'A' and 'B' spores in standard and malt media, the complete intergradation in Coons', finally the absence of 'A' spores in the Richards' medium, indicated some influence of the nutritive conditions on the production of the two types of spores. Experimental work which has been started by varying the concentration and constituents of the media, in order to elucidate the factors concerned, has however, entered only upon an initial phase. The information available relates primarily to the mycelial and growth characteristics. It is hoped to give a more detailed account in a later paper.

V. COMPARISON OF THE SALTANT STRAINS OF *CYTOSPORINA* WITH AUTHENTIC SPECIES OF *PHOMOPSIS*.

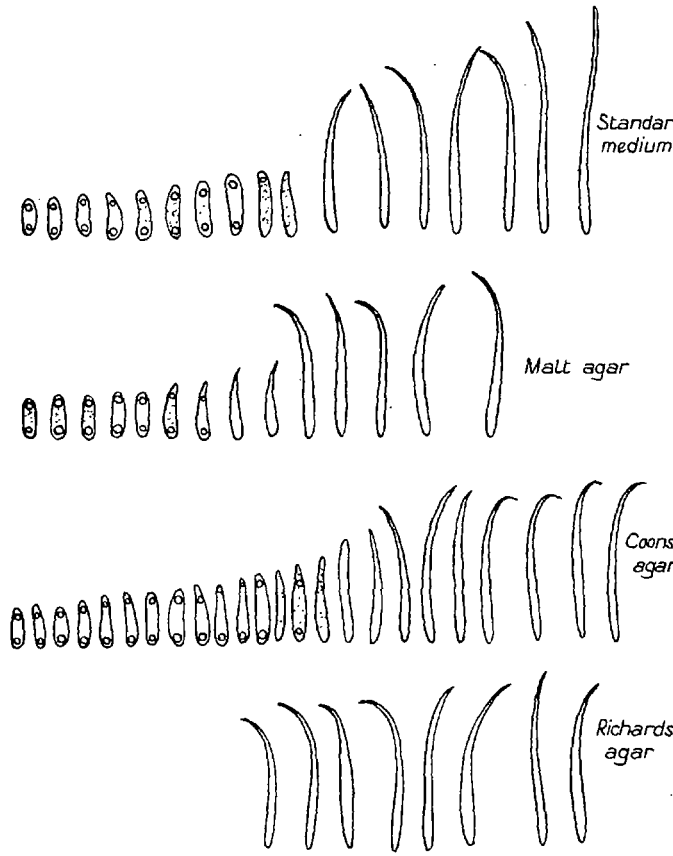
The species of *Phomopsis* available for comparative purposes were as follows :

Phomopsis coneglanensis Trav. Isolated by Archer from branch of *Aesculus hippocastanum*. (Baarn, Holland.)

Phomopsis californica. Fawcett. Isolated by Fawcett from lemon fruit and bark of lemon tree. (Baarn, Holland.)

Phomopsis citri. Fawcett. Isolated by Fawcett from Citrus. (Baarn, Holland.)

Phomopsis vexans. (Sacc. & Syd.) Harter. Isolated by Harter from *Solanum melongana*. (Baarn, Holland.)



TEXT-FIG. 6. Illustrates the spore characters of the strain CA_8 in various media.

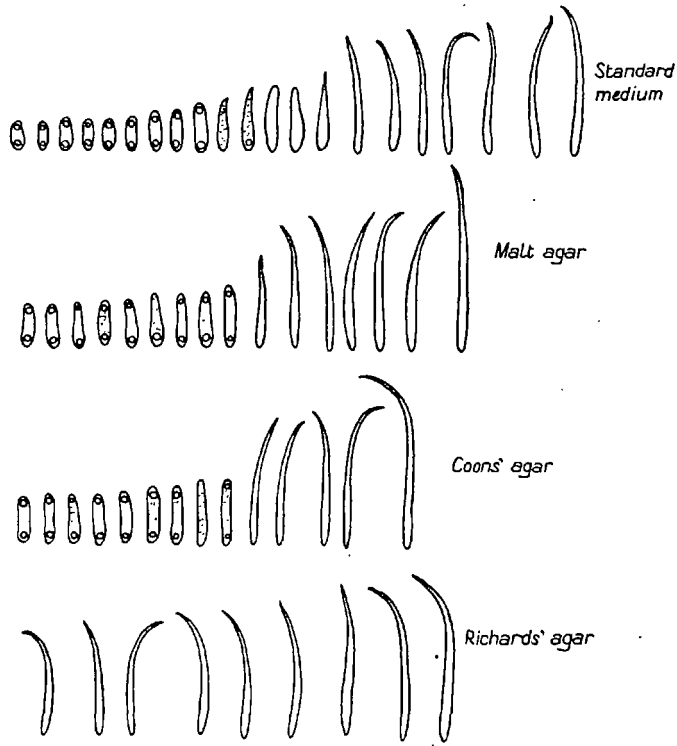
Phomopsis quercina (Sacc.). Died. Isolated by Dulfer from branch of *Tenellis quercinus*. (Baarn, Holland.)

Phomopsis mali. Roberts. Isolated by M. N. Kidd from the apple fruit. (Received from Mr. F. T. Brooks, Cambridge.)

In addition to the above, a culture of *Cytosporina ludibunda*, referred to as CK (isolated by M. N. Kidd from the apple fruit) was sent by Mr. F. T. Brooks, Cambridge. In the first subculture of CK in standard

medium, stromata were produced which yielded both 'A' and 'B' spores.

The general characters shown by the above-mentioned strains in standard medium cultures are given below.



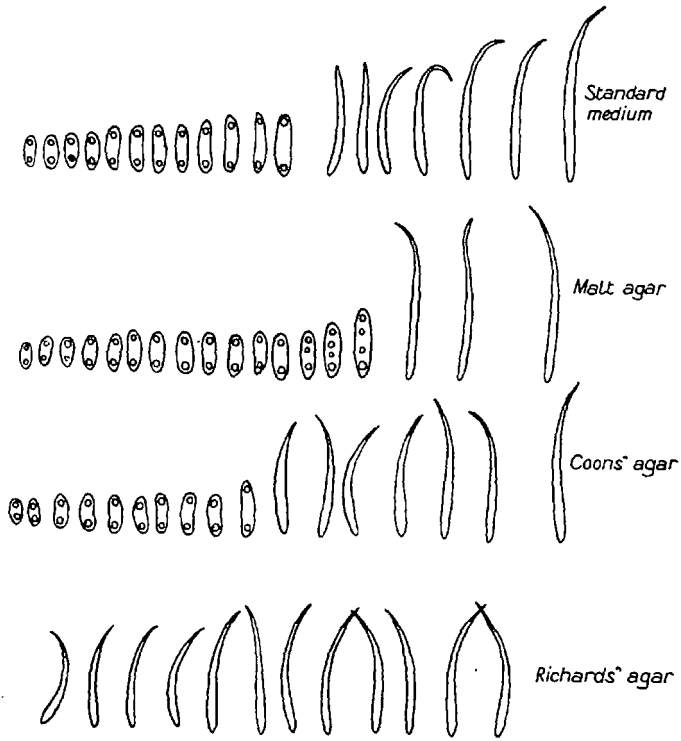
TEXT-FIG. 7. Illustrates the spore characters of the strain CC in various media.

Phomopsis coneglanensis. Aerial mycelium, forming a thick felt, white to pale yellow, darkening with age. Substratum dark brown. Zonation wide, but a narrow zone of tufted aerial mycelium occurs in the centre of the culture. Stromata numerous, dark brown, disposed in interrupted zones, and spore-heads cream containing both 'A' and 'B' spores.

Phomopsis californica. Aerial mycelium fine, silky white, turning grey with age. Substratum brown. Zonation wide, distinct. Stromata

somewhat large, dark brown, scattered, spore-heads white to cream-yellow, containing both 'A' and 'B' spores.

Phomopsis citri. Mycelium superficial, white, showing regular wide



TEXT-FIG. 8. Illustrates the spore characters of the strain CA_3 in various media.

zonation. Substratum brown. The central region of the culture pale yellow, the peripheral region white. Stromata absent.

Phomopsis vexans. Aerial mycelium thin, hirsute, brown. Substratum very brown. Zonation indistinct. Stromata absent.

Phomopsis quercina. Mycelium mostly submerged, yellowish; growth extremely retarded. Zonation absent. Substratum yellowish. Stromata absent.

Phomopsis mali. Aerial mycelium silky, white, becoming brown with

age. Substratum yellowish-brown. Zonation wide, distinct. Stromata large, numerous, dark brown, disposed in zones. Spore-heads cream, containing both 'A' and 'B' spores.

Cytosporina ludibunda (CK). Aerial mycelium copious, silky, white, procumbent with age. Substratum yellow-brown. Stromata numerous dark brown, disposed in zones. Spore-heads dull white, containing both 'A' and 'B' spores.

As far as the general features are concerned the various *Phomopsis* species, with the solitary exception of *P. quercina*, do not show any characters which separate them sharply from the *Cytosporina* derivatives. In the latter case, however, the mycelium is more or less coloured in shades of yellow, brown, grey, and black, never pure white, whereas the *Phomopsis* species form white mycelium. The distinction is merely one of degree, since in *P. coneglanensis* and *P. californica* colour develops as the cultures become older; moreover, the strain of *Cytosporina ludibunda* obtained from Cambridge does produce white mycelium. *P. quercina* differs from all the species in its slower rate of growth and absence of aerial mycelium in all the media employed.

Only *P. coneglanensis*, *P. californica*, *P. mali*, and *Cytosporina ludibunda* (Kidd) produced stromata in the standard medium, and in each case both 'A' and 'B' spores were formed. On examination of a large number of stromata it was found that in each case stromata contained both kinds of spores, but the proportion varied from strain to strain. In the case of *P. coneglanensis* only a few 'B' spores were present in the stromata. The 'A' and 'B' spores were usually present as distinct types, transitional forms being rarely observed in standard medium cultures.

The dimensions of 'A' and 'B' spores are given in Table VIII, where the limiting range in length and width and the mean dimensions are given.

TABLE VIII.

Dimensions of 'A' and 'B' Spores in Certain Species of Phomopsis.

Fungus.	'B' Spores.		'A' Spores.	
	Limiting Range in μ.	Mean Dimension in μ.	Limiting Range in μ.	Mean Length in μ.
P. coneglanensis . . .	15–18	17	6–9 × 3·5–4·0	7·5 × 3·5
P. californica	25–32	27	7–12 × 2·5–4·0	9·5 × 3·5
Cytosporina ludibunda (Kidd)	24–32	30	8–11 × 2·0–3·5	9·5 × 3·5
Phomopsis mali (Kidd) . .	25–40	31	6·6–9·8 × 3·0–3·3	7·5 × 3·2

A comparison of Table VIII with Table IV shows:

(1) With regard to 'A' spores, the limiting range varies from 6–12 μ as opposed to the range 4–16 μ in the saltant strains. That is to say, the

range shown by the species is more restricted, this being due to the absence of transitional types.

(2) With regard to the 'B' spores, the range of variation shown by *P. coneglanensis* is very narrow (15–18 μ), which is below that shown by any of the saltant strains of *Cytosporina*. But in the case of the remaining species it was within the limit.

It will be interesting to note that in the limiting range of 'B' spores and in the mean length of 'A' spores *Cytosporina ludibunda* obtained from Cambridge shows the same variation as *P. californica*.

From the observations recorded above it will be seen that in the standard medium cultures the species have distinct characteristics of their own, but a comparison with the saltant strains shows that the difference between species and species is often less marked than that between one saltant and another. Indeed, the variations shown by the saltants cover such a wide range that the species in question may be easily incorporated within the series.

VI. Saltation in *Diaporthe perniciosa*.

The cultures obtained by re-isolation from diseased apples examined late in the storage season included, in addition to strains of the *Phomopsis* type, certain strains which possessed the following characters in common in standard medium cultures. The cultures were white, the mycelium formed a dense felt; zonation present, the substratum brown, and after a time the surface of the mycelium was studded with a few, scattered, irregular bodies of a stromatic nature. At the end of six months some of the cultures showed no further development, others on the contrary had produced one or more well-developed perithecial stromata with short necks. On crushing the stromata well-developed asci were found, each of which contained eight 2-celled ascospores with two prominent oil-drops in each. The fungus was subsequently identified as *Diaporthe perniciosa*. Experiments were then made in order to ascertain the most suitable medium for the production of perithecial stromata. The best result was obtained with the oatmeal medium used by Wehmeyer (29) for the study of the imperfect stage of some of the higher Pyrenomycetes. The composition of this medium is as follows:

$CaNO_3$	0·2 gm.
$MgSO_4$	0·2 „
KH_2PO_4	0·2 „
NaCl	0·1 „
Levulose	0·1 „
Ground Quaker oats	60·0 „
Agar	15·0 „
Water	1 litre.

In this medium the perithecial stromata were large and numerous, and developed larger necks than those found in standard medium cultures.

All the original standard medium cultures were examined for the presence of the imperfect stage, but pycnidial stromata were observed in only one culture in which perithecial stromata were also present. These stromata discharged 'A' spores. Subsequently a few cultures derived from the apparently infertile ones developed pycnidial stromata under certain experimental conditions. These stromata also discharged only 'A' spores.

Experiments were next made to discover whether the derivatives from the parent cultures showing perithecial and non-perithecial characters remained true to type. Subcultures in the standard medium were made from selected cultures belonging to each group, using mycelial inocula. In the case of the fertile group the majority of the subcultures were fertile, but a few remained infertile; in the case of the infertile group the opposite result was obtained—the majority proved infertile, a few fertile. The infertility of certain cultures was at first assumed to be due to accidental circumstances, such as drying up when the perithecia were immature, &c. This assumption, however, did not very adequately explain the variation recorded above; instead, the variation seemed related to some characteristics of the strain itself. In order to pursue the subject further work was started on new lines, using monohyphal and mono-ascospore cultures.

The monohyphal cultures were prepared from certain selected infertile cultures. Subcultures in the oatmeal agar were then made, as affording the most favourable opportunity for perithecial development. All the subcultures proved infertile. Additional experiments yielded the same result. Acting on the hypothesis that these monohyphal cultures were of unisexual nature, numerous attempts were made to cross growths derived from different parents and those derived from different parent hyphae of mono-ascospore cultures. All these attempts proved unsuccessful. A result of this kind would have been obtained if the growths were all of the same sex, but in the absence of positive evidence the question of heterothallism must be left open until further work has been carried out. It may be mentioned that Cayley (10) demonstrated that some ascospores are bisexual in certain strains of *D. perniciosa,* but reached no definite conclusion as to the occurrence of heterothallism. In the case under consideration here some of the mono-ascospore cultures produced perithecia, and were therefore homothallic, others on the contrary remained infertile.

The first standard medium cultures derived from growths of monohyphal origin showed considerable similarity, such differences as there were being related to the degree of mycelial colouring, the nature of the sterile stromata, &c. These cultures were termed DH_B. It was from one of these cultures that the 'ever-saltating' strain DH_C described in some detail in an earlier paper (20) was obtained. Later, another culture produced

well-marked sectors in the malt extract medium, the sectors differing from DH_B in mycelial characters, and in the presence of numerous pycnidia which discharged only 'A' spores. Subcultures taken from the parent and sector as a rule reproduced the parent and saltant type respectively, but occasionally inocula taken from the saltant produced infertile growths. The new strain (DH_D) failed to produce pycnidia in standard medium cultures, and was accordingly subcultured from time to time in the malt extract medium. Derivatives from these cultures after a time failed to produce pycnidia.

One definite case of sectoring in mono-ascospore culture was observed. A standard medium culture showed four sectors, the sectoring starting from the centre of the plate (Pl. XX, Fig. 14). One of these sectors was strikingly different from the remainder—the aerial mycelium forming a band round the central region of the culture, and very few stromata were present. Upon subculturing from the sector a new strain DH_M was obtained. Subcultures taken from the other three sectors resembled the parent strain DH_A. These on further subculturing sectored, giving rise to DH_A and DH_M. A strain DH_N, differing in certain respects from both DH_A and DH_M, was obtained from another ascospore.

The chief characteristics shown by the strains derived from ascospores and hyphal tips in standard medium cultures are summarized below.

DH_A. Obtained from a single ascospore. Aerial mycelium dense, silky, white. Substratum brown. Zonation wide, distinct. Dark brown fertile perithecial stromata and infertile stromata present.

DH_B. Of monohyphal origin, chiefly resembles DH_A (20).

DH_C. 'Ever-saltating' strain derived from DH_B (20).

DH_D. Derived from a culture of DH_B and in the standard medium indistinguishable from DH_B. *In malt extract medium*, mycelium superficial, yellowish-white; substratum brown; zonation absent; pycnidia numerous, scattered, brown, discharging only 'A' spores, spores fusoid $5{\cdot}5–9\,\mu \times 2–3\,\mu$, with two prominent oil-drops.

DH_M. Obtained as a sector from DH_A. Mycelium superficial, yellowish-white. Substratum yellow. Zonation feeble or absent. Dark brown fertile perithecial stromata and infertile stromata present.

DH_N. Obtained from another single ascospore. Mycelium superficial, a wide belt of white, loose aerial mycelium round the centre. Substratum yellowish. Zonation absent. Dark brown infertile stromata, scattered, few. Brown pycnidial stromata discharging 'A' spores only.

The variants described above have all originated from the cultures of *Diaporthe perniciosa* isolated from diseased apples by Horne in this

laboratory. All the variants belonging to this group are designated by the symbol DH. The writer had an opportunity of examining strains of *D. perniciosa* obtained from the following sources, and comparing them with the DH.

DC isolated by Cayley, received from Mr. Ashby (Kew), 1927.

DK isolated by Kidd, Cambridge, received from Mr. F. T. Brooks, 1927.

DM sent by Mr. Marsh, Bristol, 1927.

DN sent by Dr. Nattrass, Bristol, 1927.

The more important general characters shown by the strains DC, DK, DH, and DN are recorded in Table IX, observations relating to the perfect stage as developed in original cultures are given in the second column. The general characters shown by the perithecial stromata in the oatmeal medium are recorded in the third; characters relating to the imperfect stage and the mycelial characters as shown in the standard medium are given in columns four and five respectively.

TABLE IX.

Comparison of the Strains of Diaporthe perniciosa *obtained from Different Sources.*

Strain.	Original Culture.	Perfect Stage.	Imperfect Stage.	Mycelial Characters.
DC (Cayley).	Fairly numerous stromata (Twig culture).	Absent.	Stromata numerous. 'A' and 'B' spores equally numerous, spore-heads white to yellow.	*Yellowish.* Aerial mycelium mostly prostrate, feeble zonation. Substratum light brown.
DM (Marsh).	Pycnidial stromata.	Absent.	Stromata, black, larger than in DC. 'A' and 'B' spores, the latter few, more or less straight. Spore-heads creamy yellow.	*Brown.* Aerial mycelium silky, copious, base brown. Zonation wide. Substratum dark brown.
DH_B	Perithecial stromata.	Stromata many fertile and infertile.	Stromata, more prevalent in malt agar. 'A' spores only. Spore-heads pale yellow.	*White.* Aerial mycelium, copious, silky, erect, disposed in one or more zones. Substratum dark brown.
DK (Kidd).	Perithecial stromata.	Numerous stromata.	Stromata large (in malt agar only). 'A' spores.	*Yellowish-white.* Aerial mycelium sparse. Zonation feeble. Substratum light brown.
DN (Nattrass).	Numerous perithecial stromata.	Infertile stromata very few or absent.		*White.* Aerial mycelium white: copious, zonation feeble. Substratum white.

It is clear from the table that the strains are not identical, they differ from one another in various more or less important details. The range of variation in the pycnidial stromata is interesting. DC and DM form pycnidial stromata with 'A' and 'B' spores, but in the former 'A' and 'B' spores are almost equally numerous, and the 'B' spores are usually hook shaped, whereas in the latter 'B' spores form only a small proportion of the total spores, and they are usually straight. DH_B and DK produce pycnidial stromata, which form only 'A' spores. These have usually been obtained on malt agar. Lastly, DN formed no fertile pycnidial stromata in the medium used.

The dimensions of the pycnospores in *Diaporthe* strains, where such spores are formed, are given in Table X.

TABLE X.

Variation in Dimensions of the Pycnospores in Strains of D. perniciosa *obtained from Different Sources,*

Strain.	'A' Spores.		'B' Spores.	
	Variation in length in μ.	Variation in width in μ.	Variation in length in μ.	Variation in width in μ.
DC	7·0–10	2·0–3·3	25–40	1–1·5
DM	6·6–10	2·5–3·5	20–35	1–1·5
DH_B	5·5–9	2–3	—	—
DK	6–10	2·5–4	—	—

As regards the spore length, there is hardly any difference between one strain and another, and a comparison of this Table with Table IV will show, that in length these spores wholly lie within the limits shown by the spores of the saltant strains of *Cytosporina ludibunda.*

VII. DISCUSSION.

The widespread occurrence of saltation in various species of fungi has in recent years been recorded by a number of investigators. In almost all the cases mentioned, however, the changes occur in the mycelium and are manifested in the form of sectors, differing from the rest of the culture in colour, nature of the mycelium, the reproductive organs, &c. In *Cytosporina ludibunda*, on the other hand, typical sectors are of rare occurrence and when present they are usually ill-defined. Such sectors do not usually breed true but on subculturing give rise to forms which differ from the sector. Very occasionally distinct strains have been obtained from regions

of cultures which show only the parental character, for example, the strain CC_2.

While saltation in the mycelium has been followed in some detail by various investigators very little work has been done on saltation in relation to spores. Stevens and Hall (27), in 1909, while investigating the effect of environment on fungi obtained two types of colonies, namely 'a type with few pycnidia' and 'a type with many pycnidia' from the plated spores of *Ascochyta chrysanthemi.* stev. These colonies, each of which could be traced back to a single spore, subsequently proved to be distinct strains. Horne (19) described the origin of several distinct strains from individual spores of a certain species of *Fusarium.* Edgerton (14) during platings from the ascospores of some species of *Gloeosporium* found that 'Instead of one kind of colony in the plate there were two and they were very distinct'. The parent fungi, in these cases, however, were not of monosporous or monohyphal origin. Therefore, while it is quite conceivable that the appearance of colonies other than normal was due to the actual saltation in the fungus, the probability of original mixture of two fungal strains and their subsequent separation during the dilution culture could not be overlooked.

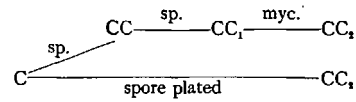
During the course of the present work on saltation in *Cytosporina ludibunda* the origin of saltants direct from the spores has been followed in greater detail. The method consisted in plating the pycnospores, which were always taken from individual spore-heads of cultures originally derived from a single hypha, and noting the different types of colonies obtained. By this way usually one, two, and occasionally as many as four variants have been obtained. In some cases the variants have proved to be distinct strains, in others, the variations observed in plates were not of a permanent nature.

This evident difference among the spores showed that the changes leading to the variations in pycnospores must have occurred at a certain stage during the development of the culture. It is quite obvious from what is known about saltation in mycelium, that such a change might occur at any time between the earliest growth stage and the formation of the mature stromata. Indeed, it is quite conceivable that in certain cases stromata may be of composite character, that is to say, compounded of the mycelia of two or more saltant strains. Very little direct evidence on this question has been obtained, but in one case the evidence is in favour of saltation during the development of spores, viz. the strain CA_8, where the colonies obtained from plating of spores, show the occurrence of a saltant strain CA_4, whereas no saltation is evident in cultures arising from the mycelium.

Usually with the saltating strains of *Cytosporina* the saltants may be obtained both from the mycelium and the pycnospores. The strains obtained by the two methods show a certain correspondence, but generally

those obtained from pycnospores vary within wider limits than do those derived direct from the mycelium.

Stevens (26) and Mahendra (24) found instances of reversion in some saltant strains of *Helminthosporium* and *Alternaria* respectively. Brown (5) working with *Fusarium* states that 'No case has been seen where, say a strain I saltated to a strain II and the latter subsequently saltated back to I'. A definite case of reversion has been encountered in *Cytosporina ludibunda*. The strain CA_5 produced as a result of sectoring the saltant CA_6. On subculturing CA_6 from different regions of the mycelium it was found that a few of the subcultures bred true to type, but the majority have reverted back to CA_5. In this case reversion in CA_6 does not take place uniformly throughout the mycelium, nor does it bear any relation to the age of the hyphae nor to any other outward characteristic.



TEXT-FIG. 9. Diagrammatic representation of saltation in 'jump' as shown by the production of the strain CC_2 direct from C, and again through the intermediate saltants CC and CC_1.

The production of a saltant on one occasion by way of intermediate stages, and on another occasion in one 'jump' as noted by Brown (5) and Mahendra (24) has also been found in *Cytosporina*. Text-fig. 9 will elucidate the point.

The production of two types of colonies, 'Greys' and 'Blacks', parent and saltant types respectively—by the spores of the strain CA_3 and the gradual numerical increase of the 'Blacks' in successive sporal generations up to about 55 per cent., are features in some respects analogous to the 'Ever-sporting five-leaved clover' investigated by De Vries (11). Increase in the percentage of the five-leaved clover plants, however, was due to selection. In the case of CA_3 the selection must have been due to chance, since there is at present no means of ascertaining from the appearance of pycnospores the nature of the colonies that are likely to be produced.

A comparison of the saltant strains considered in relation to the order of their origin, reveals, in certain instances, a gradual simplification of the reproductive organs, ending generally in the total loss of fruiting character. For example, the strain CC which is characterized by the presence of large stromata gives rise to a saltant strain CC_1 which produces numerous small pycnidia; the strain CC_1 in its turn gives rise to the saltant CC_2 which does not produce any fruit bodies at all. Simplification of similar nature is also observed along another line of descent ($CA \rightarrow CA_5 \rightarrow CA_6$). But in this case no infertile saltant has been produced. It must be noted, however,

that all infertile strains do not reach that stage by gradual reduction, but that a stromatic strain may suddenly throw off a saltant which is apparently infertile.

Associated with the simplification in the reproductive organs, there is the reduction in sporulation. For example, the strain CC which produced stromata yields both 'A' and 'B' spores, while the saltant CC_1 which produced small pycnidia yields only 'B' spores. The case is exactly similar with another stromatic strain CA_6, and the pycnidial saltant CA_6 derived from it.

In general, the smaller the fruit body the fewer 'A' spores it tends to produce.

Some saltant strains (CC_1, CA_6) do not produce 'A' spores in a medium where their parents have been producing them more or less freely. When grown on other media these strains either produce only 'B' spores or remain completely infertile. From this it was naturally inferred that these strains have lost the 'A' spore-character, and can produce only 'B' spores which according to most of the investigators (4, 9) do not germinate. These strains, therefore, undoubtedly show a trend in the direction of sterility. Such sterile condition has been reached in more than one instance.

Brown and Horne (6) and Horne and Mitter (22) have found that in *Fusarium* the shape and septation of the spores can be altered by changing the composition or the concentration of the medium. In *Cytosporina*, however, the media affect the sporing in a somewhat different manner. In standard medium cultures the majority of the strains show typical 'A' and 'B' spores. In Coons' medium the strains C and CA_5 show all intermediate stages between 'A' and 'B' spores (Intergradation). Finally in Richards' medium all the *Phomopsis* type of saltants (i.e. those with 'A' and 'B' spores) produce only 'B' spores.

There is a difference of opinion as to the occurrence of intergradation between 'A' and 'B' spores. It has been shown in the course of the present work that the complete intergradation occurs only for certain strains in certain media, the partial intergradation being of more general occurrence. Although intergradation has been recorded by several investigators, its significance has not been fully realized. It finally disposes of the theory that the so-called 'B' spores are not actual spores. It is of some interest from the point of view of germination. Since according to various investigators (4, 9) the 'A' spores germinate very readily and 'B' spores do not, it is perhaps possible to discover a gradual loss of germinal power in each transitional form denoting advance towards 'B' spores.

Brown (5) found that in *Fusarium* the saltation is influenced by the medium. He states that 'the tendency of the *Fusarium* strain to saltate is a function of the cultural medium'. Mitter (25) found that a saltant strain

of *Fusarium* which had shown remarkable stability in various media for several years saltated when grown in modified standard medium with 16 and 18 per cent. glucose, and continued to do so even after its return back to original normal standard medium. In the case of *Cytosporina ludibunda*, however, the saltation does not seem to be conditioned by the medium, although it has been observed that in certain media the saltation remains masked.

The original fungus in its reproductive organs and bent filiform pycnospores—the 'B' spores of Diedicke—conformed to the genus *Cytosporina*. The saltant derived from *Cytosporina* yielded in addition to the filiform spores, spores of a fusoid type—the 'A' spores of Diedicke. These saltants might quite fairly be classified under *Phomopsis*. These *Phomopsis* strains differed among themselves in such characters as colour, texture and form of aerial mycelium, zonation, and also in relative proportion of 'A' and 'B' spores present in the stromata or pycnidia. Indeed, the range is so wide that two authentic species of *Phomopsis*, namely *P. coneglanensis* and *P. californica* may be easily incorporated within it. In some cases a saltant has been derived from the *Phomopsis* strains which produces the filiform ('B') spores alone, but differs from the parent *Cytosporina* in forming pycnidia instead of stromata. Such strains would in all probability find a place in the neighbourhood of *Phoma*. The most striking feature of saltation, however, is the origin of certain sterile strains, which if their origin were unknown could not be placed under any genus. That this sterility might be due to the loss of the fruiting character has been already pointed out. In a similar way might be explained the presence of various strains of *Diaporthe perniciosa*, which behave very differently in the same medium. Firstly, those that have only the perfect stage and no pycnidial stage (*D. perniciosa* isolated by Nattrass). Secondly, those that have pycnidial stage only (a) with 'A' and 'B' spores (*D. perniciosa* isolated by Marsh, Cayley), (b) with 'A' spores only (*D. perniciosa* isolated by Horne, Kidd). The first of these might have arisen by loss of the pycnidial character, a case which has often been encountered during the course of the present investigation, others by the loss of the sexual character due to saltation. Although saltation has been observed in mono-ascospore cultures of DH and some of the saltants have as yet failed to produce fertile perithecia, the writer is not in a position to definitely state the loss of sexual character in culture. Edgerton (14), however, had observed a partial loss of sexual character in a species of *Gloeosporium*. This fungus, which normally produced fertile perithecia, gave rise to a 'mutant' in which the perithecia never matured. A complete loss of sexual character has been observed by Mahendra (24) in *Neocosmospora vasinfecta*—where a saltant arising from a perithecial culture as a sector has ever since failed to produce perithecia although cultured in a number of media. Pushing this analogy further, it

may be remarked that in *Fungi imperfecti*, while some strains cannot produce a perfect stage due to the lack of suitable medium and others on acccount of unisexual nature, it is not improbable that there are some which have lost their sexual character by saltation, and are therefore constitutionally incapable of producing the perfect stage.

VIII. Summary.

A detailed description is given of saltation in a strain of *C. ludibunda* originally isolated from diseased apples. Saltation takes place on an extensive scale—more than ten saltants have been obtained from a single monohyphal parent culture. Saltants originate from pycnospores, from sectoring cultures, or from cultures showing no sign of sectoring. The origin of saltants from pycnospores, as shown by the appearance of different colonies from different spores of one and the same pycnidia is a distinctive feature in this strain of *Cytosporina*, and by far the greater number of saltants has been obtained in this way. The majority of the saltants are unstable, and nearly all of those which show the constant character are infertile. The unstable saltants comprise a number of strains of 'ever-saltating' types. For example, CA_3 which produces both black and grey colonies from the same pycnidium, and CA_5 which almost always produces a sector of CA_6. In some cases saltants show reversion to parental characters.

In course of saltation the *Cytosporina* has given rise to saltants which are of *Phomopsis* type (i. e. having 'A' and 'B' spores) and some of these by further saltation have produced strains comparable to *Cytosporina* in spore character (only 'B' spores). In some cases again, a number of infertile saltants have been produced which, if their origin were unknown, could not be placed under any genus.

The variation in general morphological characters shown by the saltants in standard medium cultures covers a wide range. The majority of the saltants are of *Phomopsis* type forming both 'A' and 'B' spores. The relative proportions in which these spores are found vary from stromata to stromata as well as from strain to strain. The strains range from those with 'B' spores only to those in which such spores are rare. Some of the strains form pycnidia instead of stromata.

The character of the sporing may be changed by altering the nutrient medium. In standard medium cultures the 'A' and 'B' spores are usually present as distinct types; in Coons' certain strains show all transitional stages between these types; in Richards' medium the same strains fail to produce 'A' spores.

The saltant strains have been compared with some authentic species of *Phomopsis—P. coneglanensis*, *P. californica*, &c.—and it has been found that these species do not show characters which sufficiently distinguish them from the *Cytosporina* saltants.

A description is also given of saltation in monohyphal and mono-ascospore cultures of a strain of *Diaporthe perniciosa* (DH) obtained from diseased apples. Saltants mainly differ in mycelial characters. Some of the mono-ascospore cultures produce perithecia, hence they are homothallic; others do not produce perithecia, but it is not yet established whether they are heterothallic. One monohyphal culture produced pycnidia but not perithecia.

Strains of *D. perniciosa* obtained from different sources are compared. No two strains are identical, but they differ mainly in their capacity to form the perfect and imperfect stages with regard to which the following gradation is observed. (*a*) Perithecia and stromata forming 'A' and 'B' spores. (*b*) Perithecia and stromata forming 'A' spores only. (*c*) Perithecia only, (*d*) Stromata only forming 'A' and 'B' spores. The pycnospores of *D. perniciosa* resemble those of *Cytosporina* in shape and size.

It is suggested that these *Diaporthe* strains might have originated from a common parental form by loss of sexual or asexual characters as a result of saltation.

I wish to express my indebtedness to Dr. A. S. Horne for his invaluable help and criticism throughout the work. My thanks are also due to Professor V. H. Blackman for the great interest he has taken in this investigation. I take this opportunity of thanking those who have kindly sent me cultures, I also thank Mr. H. Tooley for taking the photographs of the saltant strains.

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EXPLANATION OF PLATES XIX AND XX.

Illustrating Dr. Das Gupta's paper on Studies in the Genera *Cytosporina*, *Phomopsis*, and *Diaporthe*. II. On the Occurrence of Saltation in *Cytosporina* and *Diaporthe*.

PLATE XIX

Fig. 1. The four types of variant colonies *a*, *b*, *c*, *d*, besides the parent obtained on plating an individual spore-mass of the monohyphal parent culture C.

Fig. 2. Illustrates the characters of the saltant CA derived from variants *a* and *b* of Fig. 1.

Fig. 3. Illustrates the characters of the saltant CC derived from the variant *c* of Fig. 1.

Fig. 4. Two additional types of variant colonies *a* and *b*, besides the parent obtained on plating an individual spore-mass of CA.

Fig. 5. Illustrates the characters of CA_1 produced from the variant colony *b* of Fig. 4.

Fig. 6. Illustrates the characters of CA_3 derived from CA_1.

Fig. 7. Illustrates the character of CA_2 with a sector of CA_1.

Fig. 8. Illustrates the character of CA_4 derived from CA_1 by plating.

PLATE XX

Fig. 9. The production of two types of colonies 'Blacks' and 'Greys' from the same pycnidium of CA_4 as a result of plating.

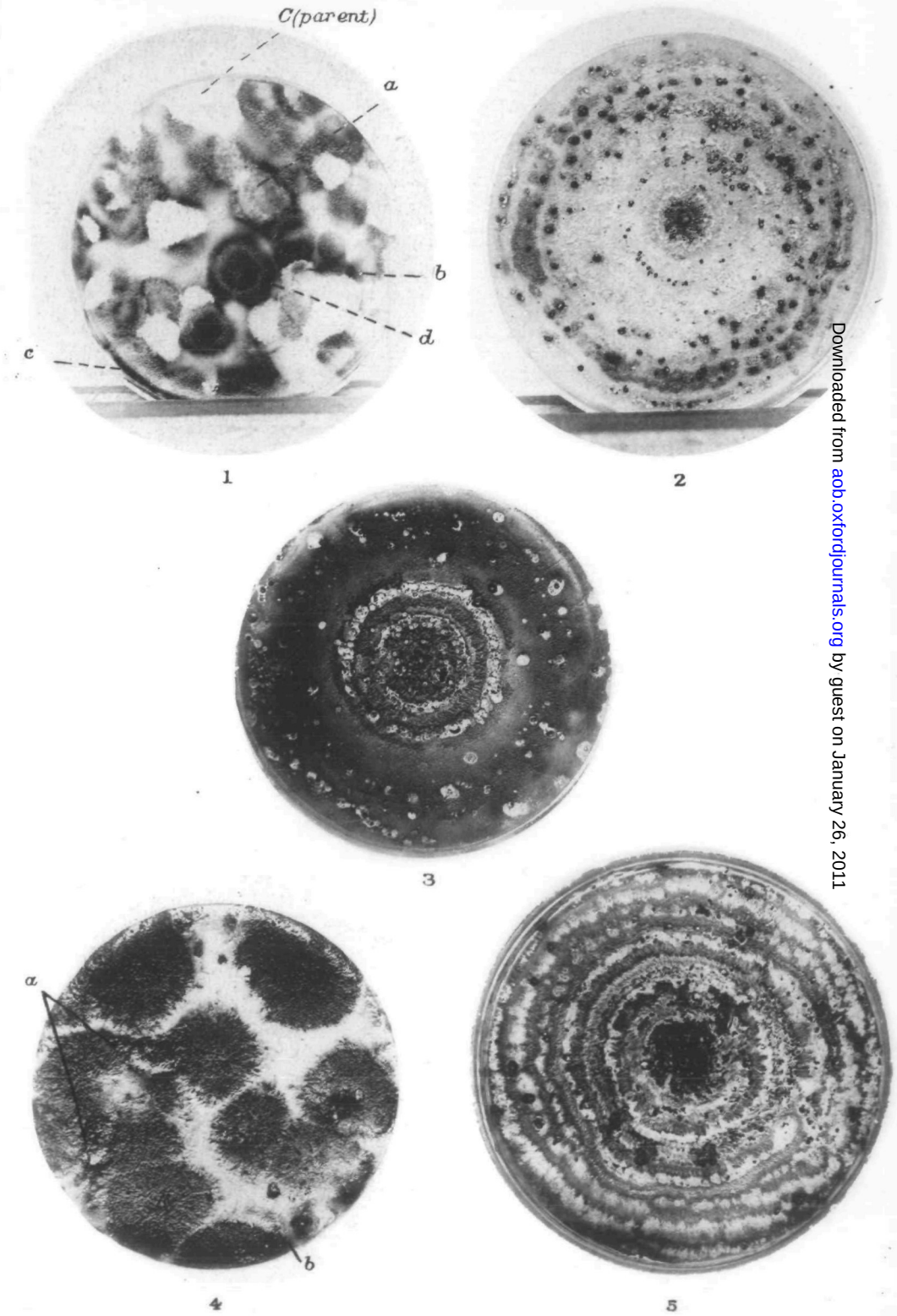
Fig. 10. The two additional types of variants *a* and *b*, besides the parent obtained on plating an individual spore-mass of CC.

Fig. 11. Illustrates the characters of CC_2 derived from the variant colonies *a* and *b* of Fig. 10.

Fig. 12. Illustrates the characters of CC_1 derived from CC_1.

Fig. 13. The sectoring strain CA_5 obtained from CA; the sector being CA_6.

Fig. 14. The monoascospore culture of *Diaporthe perniciosa* sectoring into four; the sectors starting from the centre.



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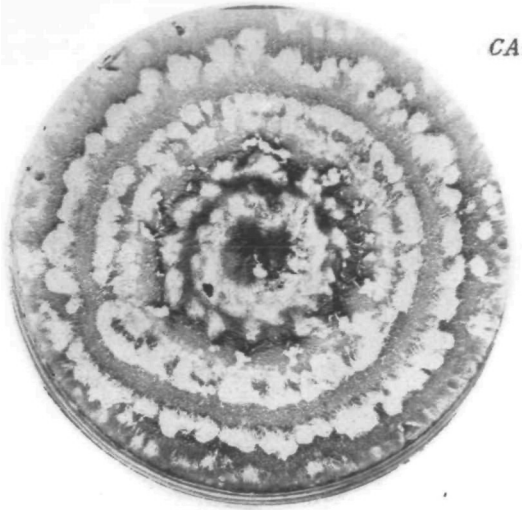

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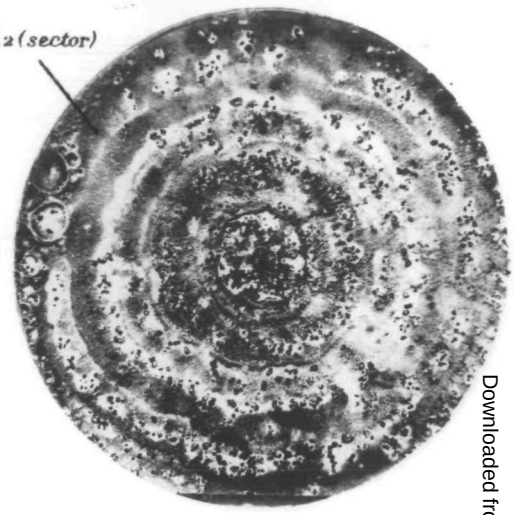

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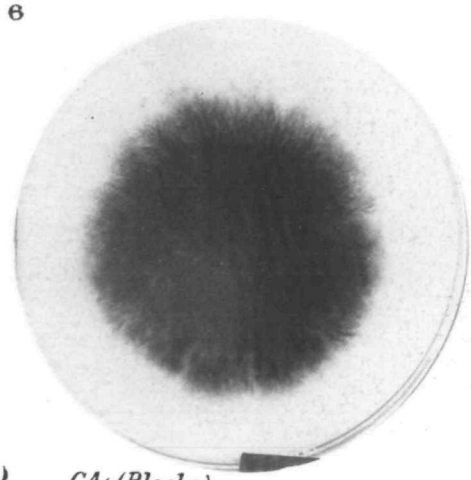

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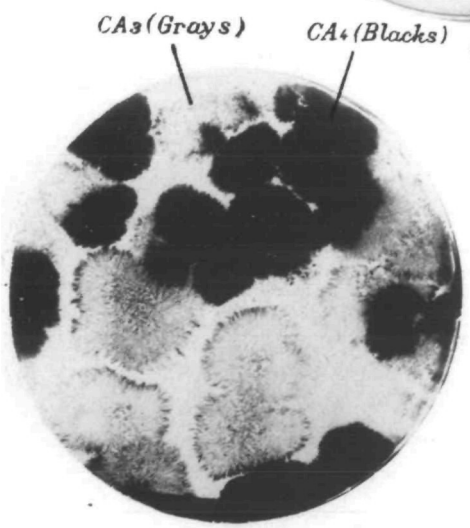

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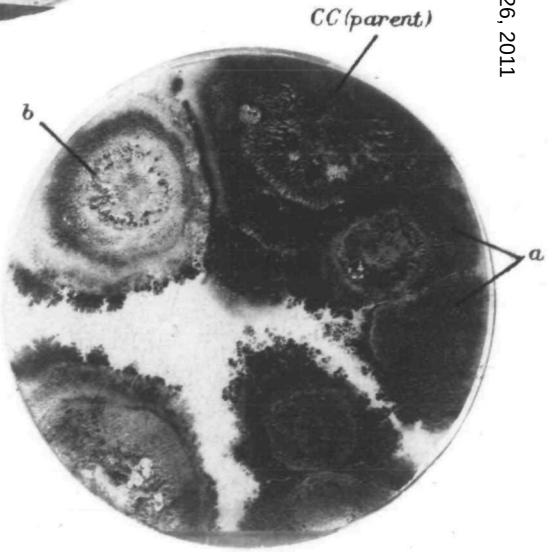

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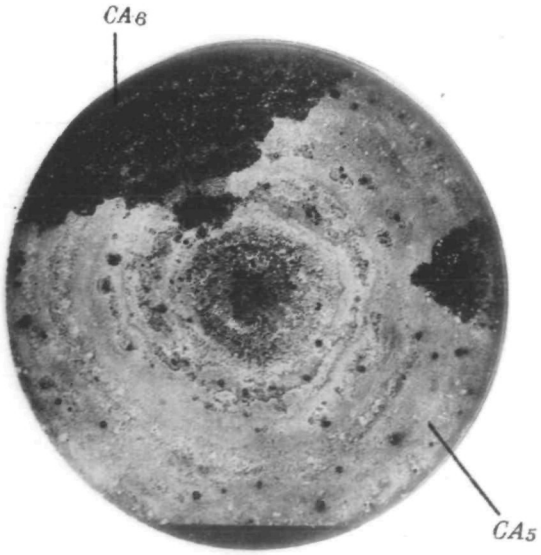

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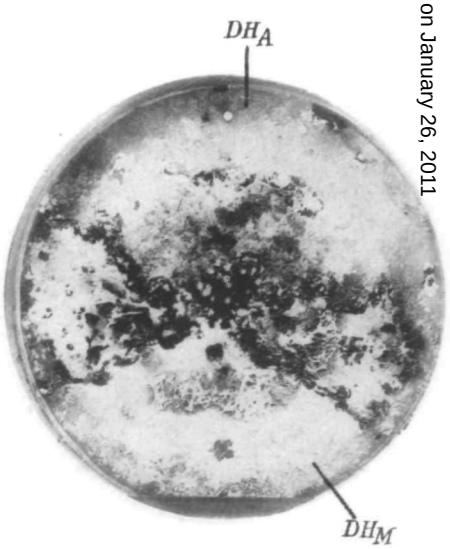

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