THESIS.

A BIOLOGIC STUDY OF MACROSPORIUM AND RELATED GENERA

WITH AN ACCOUNT OF SALTATION.

(From the Mycological Department of the University of Edinburgh).

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DESCRIPTION OF PLATES.
INTRODUCTION.

A. Scope of Investigation.

The present investigation was undertaken in the first place to determine the identification of certain fungi which were being isolated from tomatoes showing rots. They appeared to belong to the group of fungi including Macrosporium, Alternaria and Stemphylium. It was early recognised that this group was very confused in its classification, and that an effort should first of all be made to determine as far as possible the limits of the genera with the material at hand. The first isolations were made in the Spring of 1924. In the Summer a paper by Elliott (37) on the taxonomy of the Phaeodictyae came into the writer's hands, and in October a useful paper on the same growth by Fr. Bolle was received.

It was found in the main that the present investigation could only confirm a number of Bolle's conclusions, but as several modifications in these were found advisable, the present work was continued. The necessity for careful technique and critical study became more and more obvious as the work progressed.

Named species of the genera being studied in culture were given the same treatment and were used as controls. The macroscopic and microscopic cultural characters were observed and comparisons drawn up. Distinctions of generic importance have been observed as this was of first importance. The limits of species have also been considered so far as the material at hand would allow.

Many points of interest in the morphology of the forms in culture have been observed, chiefly in connection with the form of reproductive parts.

Saltation has been found to occur in a number of forms; and as the occurrence is considered of great importance, it has been studied.

B. Methods and Technique.

1. Cultural conditions. The sources of the fungi isolated were rotting/
rotting tomatoes of various origin and obtained at various dates, and also a decayed Echium plant. Scrapings were taken of the spores in each case, and after examination on a slide, typical spores were isolated from each and used to inoculate culture media in Petri dishes. On the resulting growths in each lot proving similar one was cultured from.

The original inoculum in each case was a single spore, and throughout the culture work single spores have at frequent intervals been used in making subcultures. The cultural conditions have been standardised as far as possible. The cultures have been grown on media in Petri dishes and in test tubes. All the dishes used were 3½" in diameter and just over ½" deep inside; the lids were well fitting. 30 c.c. of medium were poured into each plate to give sufficient depth and to prevent drying up.

Various culture media were used; the solidifying substance in each was agar-agar. This was added to tomato extract, pH 4.2 (pH; Clark, 22); prune juice, pH 4.2; potato extract, pH 4.6; ground Quaker Oats, pH unknown (56); and the synthetic media used by Dox and Elliott; the two latter media and referred to here as Dox's agar, pH 6.5, and Elliott's agar pH 7.8. Potato agar was early discarded as being unfavourable for growth, and produced early staling of the cultures. Oat agar was found to give a massive growth of mycelium in most cases. The synthetic media were the most favourable for spore productions; tomato agar was intermediate between oat agar and the synthetic agars as regards the formation of spores, while prune agar more resembles the oat agar.

The preparation and autoclaving of each medium has always been the same. The pH value has been obtained for the extract before the addition of agar and autoclaving. Dox's, Elliott's and the oat agar were prepared according to the methods given in the literature (35, 37, and 56 respectively). Tomato agar was prepared from 8 lb. of tomatoes; these were boiled in 500 c.c. of water for half an hour then strained; 5% agar was added; the pH is 4.2.
4.2. Prune agar, pH 4.2, was prepared by first boiling 100 gm. of stoned prunes in 500 c.c. of water for two hours.

Cultures for comparison were incubated at 27° C. in the dark. Plates were inverted to allow moisture to condense on the lid. They were described on the tenth day after growth was observed; they were then kept at room temperature for further development, e.g. of perithecia.

2. The Method of Isolating Single Spores, whether conidia or ascospores has proved quick and efficient. The method is much like that found to have been used by Hanna (43). In the present case the method is as follows. Round needles kept for the purpose are used which have been finely ground on the oil stone. The extreme point must not be very acute or a spore may be punctured, but should be finely rounded. Oil is removed from the needle by means of a clean cloth, and immediately before use the needle is polished between folds of sterile silk cloth.

On to a flamed and cooled slide are placed in line several small drops of water; these drops are progressively smaller; in the present work tap water was used as the bacterial content was found to be extremely small. Into the first and largest drop a small scraping of the spores, from which one is to be isolated, is placed by means of a sterile platinum needle. The drop is observed under the microscope. By means of a clean needle it is sometimes possible to remove a spore which is separate from the rest from the edge of the drop without disturbing any others. If this cannot be done the drop is allowed to dry up, and one or a number of spores are picked up on the dry needle to which they readily adhere. In any case the spore or spores are placed in the second drop by touching the edge of the latter with the point of the needle; the needle is thrust into the drop of water the spore of spores will probably be drawn up by surface tension. In the latter case the needle must be carefully cleaned on the clean cloth to remove them.

When a single spore or a few spores have been transferred to the/
the second drop, it is fairly easy to remove one by drawing it out and away from the edge; if it does not adhere to the needle at first it will do so on drying up. Having isolated a single spore in the second drop, it is only necessary to make certain that no tiny young spores are adhered to it. The spore is transferred to another drop or through several drops to be quite sure of its being only one individual. It is very important at this stage that the needle be quite clean. All that is now necessary is to transfer the spore to the culture medium by drawing the point of the needle with a rotating motion against the surface of the medium towards the operator. The inoculum can then be observed through the lid of the culture dish or through the medium; in the case of a tube culture, the inoculum is best placed near the side at the centre of the medium, where it can be observed and where the medium will not dry up readily.

In using the needle to pick up a spore, it must be borne in mind that the needle may become contaminated with other spores from a part of the drop not in the field of observation. Until the point of the needle is directly over the spore to be removed it should be kept well above the surface.

To avoid squashing or puncturing a spore, the needle should first touch the slide and then the spore with the side of the tip. In using a cloth for cleaning the needle, it should be sterile and be folded a number of times; a separate fold or part of a fold should be used each time the needle is cleaned. The manipulation of the needle under the microscope soon becomes quick and easy. Two needles are not so manageable, but by their use it has been possible to isolate the eight spores from one ascus.

The ascus is held steady with one needle, and with the other it is cut and the eight ascospores removed. These are picked up in succession, transferred to another drop of water and lastly transferred one at a time to a long agar slope. In the present case they developed to give eight prove growths. The operation of transferring the eight spores from the ascus, through a drop of/
of water, to the slope need only take a few minutes.

Measurement of spores. Using the camera lucida the length and breadth of 100 spores were indicated on a sheet of paper, 1 m.m. on the paper representing 1 on the microscope slide. The mean and standard deviation were found for length and breadth, (21). In *Stemphylium* two kinds of conidia were measured separately; in *Alternaria* and *Stemphylium* the number of septa was noted at the same time.

Colour Scheme. Describing the colours of cultured growths, it was necessary to use a recognised colour scheme; that of the Société Française des Chrysanthémistes (69) was used. The first figure in brackets after the name of a colour refers to the illustration showing the colour and the second figure to the tint of that colour.

Terminology. So far as possible well known terms were used. In the description of cultures, however, certain terms used by Long and Harsch (49), in their description of wood-rotting fungi have been used here. The special meaning of the following terms will be found in the paper by these authors:-

- Adpressed, arachnoid, cottony, downy, felty, floccose, plumose.
- sodden, subfelty and woolly. Hyphenated compound words have the meaning ascribed by these authors, i.e. they indicate a condition between the two names.

It has been found advisable to give a special definition to the following terms:-

- Mattéd: a condition between woolly and felty.
- Superficial: aerial mycelium; the upper looser layer of aerial mycelium, when the latter shows layers.
- Dense layer: The compact lower layer of aerial mycelium, generally with many spores, when two layers can be observed.

The following terms applied by Chester (26, pp.20-25) in describing bacterial colonies have been used here with the same definition:-

(General characters of the surface as a whole) flat, raised, pulvinate, convex.

(Detailed/
(Detailed characters of surface) smooth, squamose, 
(Edges of colonies) entire, ciliate.

In the description of spore formation and germination the following terms have the following significance:

Conidiophore: The use of this term is limited to the hyphal cells, the terminal of which bears a spore; the term implies here some character or characters distinguishing such cell or cells from normal hyphae cells, for example thick walls, darker colour, or greater width. The term is not applied to the connecting neck between spores of a chain; such a neck was called a conidiophore by Wallroth.

Primary spore: The first spore formed by a conidiophore when a chain or buds are present.

Chain: A series of spores, formed one from another.

Buds: A spore formed from, and sessile on, another spore. An Intercalated bud or spore is a spore formed between two others.

Beak: Has the usual meaning applied to conidia of Alternaria, i.e. it is the attenuated distal end of the spore, and is generally marked off from the spore proper in being thinner walled and lighter in colour.

Secondary spore: A spore attached to the primary spore by a germ-tube or hyphae which is still unbranched.

Nodulose conidiophores or germ-tubes: conidiophores on germ-tubes of the type seen on plate IV, figs.32-34. Wallroth (79) describing Stemphylium used the words "Hyphae simplicissime, brevis, articulatae, nodulosae", which Bolle (11), understood it is considered quite correctly, to refer to the conidiophores. This author uses the term "Rosenkranzformigen Hyphae" to such conidiophores.
II. FUNGI ASSOCIATED WITH TOMATO ROTS.

A. General.

A considerable number of causes are known for rots of tomato. Several of these have been found during the present investigation, caused by the following fungi: - Botrytis sp. (9,44), Cladosporium fulvum, (44), Colletotrichum phomoides (Sacc.) Chester (16,24,25.), Phytophthora parasitica (9), Rhizopus sp.(9), Phoma destructiva (Flower.) C.O. Jamieson (16). Blossom end rot was also found (9,44,71). Other causes, not seen during the present work, are Phytophthora infestans (44), Gloeosporium sp. (9), Diplodina lycopersici (Cooke), Hollos, emend. Brooks and Searle, (16). Phoma alterniaceum Brooks and Searle (16). Oospora lactis (57), Bac. caratovorus, (9); (nor has"Leak" (44) been seen now.)

Fungi belonging to Macrosporium and related genera causing tomato rots.

Of these the most important is Alternaria Solani (E. & M.) J. & Gr. (45) which has been observed by a number of workers, (11,27,28,29,37,39,44,59,71). The species was formerly called Macrosporium Solani (E. & M.), but the change in the name of the genus is justified as a result of the work of recent workers, (45,61,11).

The statements in the literature dealing with other Phacodictyae are so confused that there is great difficulty in recognising the fungi that are indicated.

Rosenbaum (62) gives the cause of "Nail head spot" of tomato as M. tomato Cooke.
Origin of Forms now dealt with.

<table>
<thead>
<tr>
<th>Origin</th>
<th>Species</th>
<th>Location</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macrosorium</td>
<td>A</td>
<td>Dutch tomato obtained in</td>
<td>March 1924</td>
</tr>
<tr>
<td>&quot;</td>
<td>B</td>
<td>Midlothian, on dead leaves of Echium vulgare</td>
<td>April</td>
</tr>
<tr>
<td>&quot;</td>
<td>H</td>
<td>Teneriffe tomato obtained in</td>
<td>July</td>
</tr>
<tr>
<td>&quot;</td>
<td>D</td>
<td>Dutch tomato obtained in</td>
<td>September 1924</td>
</tr>
<tr>
<td>&quot;</td>
<td>R</td>
<td>Teneriffe tomato obtained in</td>
<td>October</td>
</tr>
<tr>
<td>&quot;</td>
<td>Ta</td>
<td>Teneriffe tomato obtained in</td>
<td>November</td>
</tr>
<tr>
<td>Stemphylium</td>
<td>E</td>
<td>Dutch tomato obtained in</td>
<td>September</td>
</tr>
</tbody>
</table>

All the above forms were isolated from tomato fruits showing rots, except E, which was isolated from drying decayed leaves of Echium vulgare. Besides the above, other material was obtained, but was sooner or later discarded, as the fungi isolated could not be distinguished from forms being worked with. From potato leaves obtained in August in Midlothian and showing dry brown areas, and from two other lots of tomatoes, Alternariae were isolated, which could not be distinguished in culture from A. tenuis. Also from another lot of tomatoes a Stemphylium was obtained, which showed in culture the same characters as E above.

Other fungi found in tomatoes are referred to later (p. 7).

In order to have named species to culture as controls, several 1-spore cultures were obtained, through the kindness of G. Westerdyk, from Holland.

Of these, Alternaria solani (E. & M.) J. & Gr., A. Cheiranthi (Fr.) Bolle, and A. crassa Rands were not cultured, as their small production of conidia at once separated them from the forms isolated. The species cultured as controls were: Alternaria tenuis Nees, Stemphylium ilicis T11., and Pleospora herbarum (Pers.) Rhb., all isolated and identified by Bolle. A. fasciculata C. & E., isolated by Jensen, was received with the other species, and was cultured to confirm Bolle's conclusions that it was identical with A. tenuis.
C. Morphology in Culture of Forms of Macrosporium.

The various forms were grown simultaneously on several media. The cultural conditions were the same for all; these conditions have been described under "Methods". Small scrapings were used as inocula, and growth started in all cultures within two days.

The first measurement of diameter of the growths were taken when the growth could be observed by the naked eye, and were noted daily for the next ten days. On the tenth day after growth was first observed, notes were taken on the appearance, macroscopic and microscopic. As all forms have passed through a number of generations, the descriptions are in many cases the results of observations on several similar cultures.

The macroscopic cultural characters observed are: surface elevation, which has been estimated roughly, form of growth, type of edge, colour and optical character, relative amounts of mycelium and spores, changes in the appearance in some cases, rate of growth, presence of perithecia, colouration of medium, presence of moisture on the surface.

As regards colouration of the medium, the only definite instance was in a saltant of Macrosporium A. The chief change in the appearance is the collapsing of the mycelium after a week or two. The rate of growth (Table I) was rather faster for the first day or two, and then remained constant. Most cultures continued their growth till they reached the edge of the Petri dish. The presence and number of perithecia could only be known by observing the cultures for some weeks after these were removed from the incubator on the tenth day from the start of growth.

All cultures are 1-spore lines; in the case of saltants, these also are 1-spore lines of the saltants, except in the case of saltant a of Macrosporium Td, and saltant d of Macrosporium A, which were isolated by taking a scraping-transfer, as no spores were found till later.

Macroscopic characters of cultures are given for each form on/
Table 1. Rate of Growth of Macrophomorium Forms in terms of diameter in m.m.'s per day.

<table>
<thead>
<tr>
<th>Macrophomorium Form</th>
<th>Medium</th>
<th>Elliott's Agar</th>
<th>Dox's Agar</th>
<th>Tomato Agar</th>
<th>Prune Agar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pleospora herbarum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td>9.4</td>
<td>9.4</td>
<td>10.3</td>
<td>9.7</td>
</tr>
<tr>
<td>Saltant a</td>
<td></td>
<td>5.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saltant b</td>
<td></td>
<td>4.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td></td>
<td>8.4</td>
<td>8.3</td>
<td>9.3</td>
<td>10.2</td>
</tr>
<tr>
<td>Saltant a</td>
<td></td>
<td>6.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saltant b</td>
<td></td>
<td>5.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saltant c</td>
<td></td>
<td>9.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saltant d</td>
<td></td>
<td>8.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
<td>8.8</td>
<td>9.0</td>
<td>10.0</td>
<td>10.2</td>
</tr>
<tr>
<td>Saltant a</td>
<td></td>
<td>3.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Td</td>
<td></td>
<td>7.5</td>
<td>8.4</td>
<td>9.0</td>
<td>9.3</td>
</tr>
<tr>
<td>Saltant a</td>
<td></td>
<td>7.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>(Normal)</td>
<td>8.0</td>
<td>8.3</td>
<td>7.5</td>
<td>7.1</td>
</tr>
</tbody>
</table>
on each of four media. The microscopic characters are only recorded for each cultured form on Elliott's agar, since sufficient distinction has been observed on this one medium, and being a synthetic medium this agar should not vary in batches prepared separately. This agar has also been chosen when dealing with the subject of saltation, since it is favourable for spore formation. A mycelial saltant lacking spores, or producing them in small numbers is thus given full opportunity to show its capability in that direction.

Fig. 1. Macrosporium: Rate of Growth of forms 'A', 'B', 'Tl', 'H', and Pleospora herbarum (Ph).
**MACROSPORIUM CULTURES.**

**Pleospora herbarum Tub:**

_Elliott's Agar._

Surface elevation low, up to half or three quarters m.m. near centre, becoming lower and straggling towards the edge. The form of growth is a thin powdery mycelium, with scarcely any superficial aerial mycelium. The edge is circular as a whole, but uneven, indefinite and adpressed; the edge becomes more definite later. The growth is densest and darkest towards the centre. The colour is grey, dusty with no white; the growth is dull and slightly transparent. It is a sporing growth with very little mycelium. Perithecia are present, large, and chiefly located on the central half of the culture for a number of weeks. The rate of growth is 9.4 m.m. per day.

The hyphae vary greatly in width, many are very wide. They are straight, thick or thin walled, septate, with considerable variations in the amount of constriction at the septa. There may be no constriction at the septa, or the constriction may be very deep with the ends of the cells quite rounded. As a rule it is slight. Branching is for the most part at right angles, the base of a branch being more or less narrowed. The colour of the hyphae is rather dark so that they are clearly seen unstained; colourless hyphae are present however. The average width is 6, the maximum width observed 11.

The conidiophores are found terminally on the hyphae or side branches. They are darker than the hyphae, and are wider than those from which they arise. They are swollen just below the apex. After bearing a spore, the conidiophore almost invariably forms a second spore on the scar left by the first conidium when it is pushed off. Cases have been seen (figs. 21, 22)
where instead of pushing off the conidium, the conidiophore grows out at the side of the old scar, so that the old conidium may not even be unseated. A conidiophore instead of producing a second spore may grow out to form a second conidiophore, or to form several cells of hyphae with a terminal conidiophore. Further, a conidiophore may occasionally be formed at the end of a conidium. Examples are figured (figs. 19, 20), where the basal spore of a chain grows through the remaining spore or spores and gives rise to a conidiophore. Such penetration of a spore or spores has been seen in this species not infrequently. Where a spore has been borne, a hyaline sometimes sunken scar is left on the conidiophore. The average width is 8μ, and the maximum observed 9.5μ.

The conidia are borne terminally on the conidiophores. They are at first round, becoming oval on the formation of the first, a transverse, septum, and finally more or less oblong. In the larger conidia there is conspicuous constriction round the septum first formed, here called the main septum; but little or no constriction round later walls. The main septum remains quite distinct throughout the development of the spore. After the formation of the first or main septum, the two cells become divided muriformly. The conidium may be pushed off as a second one is formed in its place, or it may remain and bud off a further spore; this spore may also bud, so that a chain is formed. The buds are usually but not always terminal, and may be found, though less often, borne laterally. Intercalary budding occurs resulting in a chain with the youngest spore or spores intermediate. In this case older spores are pushed apart. There may be several buds on a spore so that a branching chain is formed. Thus the elements of a chain often vary considerably in size/
size from end to end. A spore of a chain instead of budding off another spore may form a germ tube and this usually forms a terminal conidium.

The conidia are verrucose over their entire surface. The verrucosity is not rough as in the Stemphylium forms examined, and is more regular in degree on all the spores. Verrucosity varies from fine to moderately rough but is generally rough (Table IV), - more so than in other forms of Macrosorium. The conidia especially the older ones are very dark. Chain formation may take place very rapidly, so that a chain can be formed entirely of unicellular conidia. Spores of a chain may not show distinct terminal walls until a number of spores have been formed. The ascospore and ascus of strain Td in figs. 28 and 29 of plate IV are typical of this and other Macrosorium strains. Old ascospores become very irregular (Pl. IV, fig.30). The size of 50 asci was 204 - 306 x 24 - 33 μ, (Table V). The size of 100 ascospores was 29 - 39 x 12-16 μ, (Table VI). The perithecial stage is discussed separately (page 33).

**Macrosorium A.** (Pl. VI, fig. 43).

Growth is pulvinate, low, falling from 1½ m.m. near the centre to the edge; mostly ½ - 1 m.m. it is low, loose, powdery mycelium. The edge is not quite even, nor is it definite as it shades off in loose adpressed strands. The colour is grey; it is dull and fairly translucent, chiefly conidia. Perithecia are present, and distributed all over in ten days. In the figure some of the perithecia show here and there as black dots. The rate of growth is 8.4 m.m. per day.

The hyphae (Pl. 1 fig. 1) vary from hyaline to rather dark. Most are slightly colourless. They are straight, branching at right angles. They are very regular and show little constriction at the septa. The average width is 4.5μ, the maximum observed/
observed 7μ, (Table II). The conidiophores (figs. 1,2) are darker and wider than the hyphae from which they arise. They may be formed as ends to hyphae or as branches. As in *Pleospora herbarum* they swell just below the apex. One conidiophore may give rise to another terminally, but there is less irregularity in this form A than there is in *Pleospora herbarum*. The average width is 7μ, the maximum observed 8.5μ. Fig.10 of Pl. 2 shows an unusually small conidiophore commencing to grow on after the fall of a spore.

In distinction from *Pleospora herbarum* this strain has rather less tendency to catenulation of the conidia so that under the microscope the spores appear more regular in size. Chains occur but to a small extent, as in plate 2, fig.11, where a young spore "a" has just been formed, and another is forming at "b", by intercalary budding. On tomato agar chain formation is much more common (figs.2, 4). The conidia (fig.1) are very dark, as in *Pleospora herbarum*, but there appears to be less constriction at the main septum; also there is a much smaller percentage of irregularly shaped conidia. The size of 100 spores was 12-26 x 10-20μ. Verrucosity of the conidia is fine or almost moderately-rough. Fig.1 shows a typical spore formed apically on the conidiophore. A smaller 1-celled spore is shown borne laterally, which is unusual. An interesting feature of the conidiophore shown, and uncommon in the genus *Macresporium* is that it has grown on after bearing a spore, and it has formed a second conidiophore from which a spore has fallen. Fig.2, apart from showing a short chain, shows a spore of unusual shape and septation in this genus. The similar spore in fig. 3 is also interesting in showing germination on the medium from the subtermin- al cell, to form a new conidiophore. While it is usual for spores germinating in water to throw out long germ tubes a few cases have been observed of short germ tubes bearing secondary/
secondary conidia (Pl. IV, fig. 31). The size of 50 asci was 132 - 216 x 21 -30 μ. The size of 100 ascospores was 27-36 x 12-18 μ.

**Macrosorium B.**

The growth is raised to 2½ m.m., but the remainder is low, up to ½ m.m. It is a thin subfelty powdery growth; the edges slightly uneven, and not very definite, thinning out in adpressed ragged threads. The colour is "Mouse colour" (360:1-4). It is dull, translucent on outer half, transparent on the inner half. The growth is chiefly spores. Perithecia are present in considerable numbers eight days after growth was first observed, scattered all over. Rate of growth is 8.8 m.m. per day.

The hyphae are not dark; they are hyaline, but can be distinguished. No comparisons can be made with the other strain from the hyphae apart from their width. The average width is 4 μ, the maximum observed 7.5 μ, i.e. they are rather finer than those of Pleospora herbarum, or Macrosorium H (Table III).

The conidiophores are dark and therefore distinct from the rest of the mycelium. Chains of conidiophores are common. The conidiophores are half as wide again as the hyphae from which they arise. A conidiophore was observed growing through a spore, and forming another (figs 13, 14). It is unusual in the Macrosorium forms examined for more than one conidiophore to be found from one point on a hyphae, but fig. 9 shows five arising near together. The strain also has exhibited elongation of a conidiophore through the spore it has formed (figs. 13, 14). There are no short stout conidiophores, but they resemble those of strain Td which are figured (fig. 25, 26). The average width was 7.5 μ, the maximum observed 8 μ.

The conidia are very dark; there is not so much tendency to formation of chains, so that the conidia appear very regular. The main septum is not conspicuous here; the other septa are formed in fair numbers, but cannot be so well seen in this strain because of/
of the very dark outer wall. There are many conidia. Verru-
caosity is fine, even, and very close. The size of 100 spores
was 14 - 30 x 11-25μ. There is extremely little germination of
conidia on the medium. The size of 50 asci was 132-196 x 31-30μ.
The size of 100 ascospores was 135-204 x 34-33μ.

Macrosporium Td: (Pl. VII, fig. 48).

Pulvinate growth, 2m.m. high at centre, falling very little
till near the margin. The form of growth is at first woody-
subfeltly, with a fair amount of mycelium and not very powdery.
When this growth is 30 days old it shows mycelium, collapsed, only
on the central half. The edge is circular, not perfectly re-
regular, and is indefinite; it shows adpressed but not radiating
hyphae and is rather powdery. The colour apart from some dirty
white superficial mycelium is dull "Mouse colour" (360:1 changing
to 360:4). The central half is opaque, the remainder is trans-
lucent. A sporing growth; the production of conidia goes on
more quickly after one or two weeks. The chief change apart from
collapse of mycelium is the darkening of the growth through the
formation of conidia. There are a fair number of perithecia
scattered on the central half at ten days; there are a large
number, chiefly buried, are found after thirty days. Mature
ascospores have been observed earlier in this strain than in the
others. The rate of growth is 7.5 m.m. per day.

The hyphae are very dark, and fairly thick. The average
width is 4μ, the maximum observed was 6μ. The conidiophores
are rather thicker than the hyphae and are considerably darker.
The average width is 7μ, the maximum observed 9μ. The are often
one-celled, short and thick, but characteristically they are two-
celled and fairly long (fig. 25). Ascospores may branch (fig. 26)
but this is not often seen. The conidia have a great tendency
to form chains, so all sizes are present. There are a great
number of conidia. A conidium has been seen to arise directly
from/
from a hyphae, with no conidiophore or branch below, (fig.24). The cell from which the conidium arose in this case was not at all darkened and only slightly swollen on the side below the spore. Other cases were observed where a spore was borne sessile at the side of a conidiophore. The size of 100 spores was $13-28 \times 11-23\mu$. The verrucosity resembles that of strain B being fine. The size of 50 asci was $135-204 \times 24-33\mu$. The size of 100 ascospores was $26-33 \times 10-14\mu$.

**Macrosorium H.**

The growth is $\frac{1}{2}$-lm.m. high over the greater part, but falls gradually to the edge on the outer one-fifth. It is subfelty, with little superficial aerial mycelium as a rule. An occasional culture bears white superficial mycelium, as in the darker growths of figure 52 on plate VII, but this is probably a saltant appearing: it is discussed later (page 72-73). The edge is circular and even, though indefinite. The colour is "Iron grey" (357:2) below, in the central half, becoming lighter outwards. It is dull and opaque but transparent at the margin. It chiefly consists of dark mycelium. There are no perithecia. The rate of growth is 8.0 m.m. per day.

The hyphae are long and straight. They vary considerably in width; some are so wide as $15.5\mu$ throughout a considerable length. The cells in figure 27 of plate III are $13\mu$ at their widest part. The maximum width is greater than in any other form dealt with. The average width is greater than in the other Macrosorium isolations, being $6.5\mu$. The hyphae are fairly clear and of a pale green brown colour; a small proportion are colourless. There is a marked tendency to constriction at the septa; adjacent cells are frequently only contiguous across half the diameter, and have rounded ends. It, thus frequently happens that many hyphae break up into short/
short lengths. Many of these short pieces of mycelium are very wide and thick-walled (Pl.III. fig.27), and such cells may be seen still attached to narrow thin-walled mycelium. These thick-walled pieces of hyphae are never verrucose. Branching is, for the most part, at right angles but is also very often at a very small angle to the main hyphae. There is a small or large constriction at the base of the branch. It is quite unusual for more than one branch to develop at one point in a hypha, but two from one point have been observed.

The conidiophores in this *Macrosporium* are quite unlike the normal club-shaped forms - which are almost exceptional in this form. Figure 16 shows a club-shaped conidiophore of this form. Figure 16 shows a club-shaped conidiophore of this form, also unusual in having continued growth near the old spore. They are very variable, and show all gradations from normal but dark, or thick-walled hyphae to long very dark multi-septate, thick-walled structures 10 μ wide. The usual conidiophores (figs. 17, 18) here are very long, and are often two to four times as wide as the hyphae from which they arise. The apex is not thicker than the rest. The average width was found to be 8 μ. They are thick-walled and very dark in colour throughout their length. They can often become detached from their hyphae without much difficulty. Sometimes they are seen to be so constricted at some point in their length that the terminal cells are ready to fall apart (fig. 17). More striking examples have been seen than that figured. The cells of the conidiophore are short, often shorter than wide, and these may be as many as 12 or more.

Short and very stout dark branches acting as conidiophores are seen here (fig.19) as in some other *Macrosporium* isolations. It is very usual for these short conidiophores to arise at the base of the usual long conidiophore. After bearing a spore the conidiophore/
conidiophore may grow out terminally to form a new conidiophore, and this process may be repeated; the method is not so common however as in other *Macrosorium* isolations. Many conidiophores throw out secondary conidiophores laterally; spores also may be borne laterally. A few conidiophores have been observed showing an unusual occurrence. In fig. 18 for example a conidiophore is shown with a hyaline terminal cell, and hyaline spore borne laterally. Both the terminal cell and the spore appear empty. The point of interest lies in the bulge which takes place from the subapical cell into the terminal one, and the similar bulge from the hyphae laterally into the spore. The reason for these bulges is not clear; they may be new buds taking the place of the terminal cell and the spore respectively on these becoming empty through rupture or other cause. Or they may be simply due to osmotic pressure, in the cells from which they arise, against the thin dividing walls.

The conidia are very dark in colour when multicellular. Chain formation is very abundant, so that conidia are found in all stages of development. There are considerable number of conidia present, but mycelium is also present in quantity. The verrucosity of the conidia on which it can be made out is exceedingly fine; it is apparently absent on many. The size of 100 spores was 14-26 x 11-22 /µ. Rapid chain formation may occur, so that a chain consists of a number of small equalised thin-walled 1-celled conidia. The spores in a chain are not always found end to end, though that is by far the most usual method; a few cases have been seen where a spore a chain has budded from the side. Germination of conidia occurs to a small extent in the culture; the germ tubes are usually rather thick; secondary conidia may be seen (Pl. 5, fig 42).

Dox's Agar/
DDx's Agar.
Pleospora herbarum.

A pulvinate growth sloping gradually to the surface of the medium; 1½-2m.m. high at centre. Central half is subfelty and opaque, changing gradually on the outer half to downy and transparent. The edge is fairly regular, but indefinite; the marginal mycelium is not visible to the naked eye, but the border is indicated by the thickly scattered perithecia. The colour is "Iron grey" (357;1-4). The growth is dull. Perithecia are present. Moisture appears on the mycelium part. The rate of growth is 9.4 m.m. per day.

Macrosorium A.

A pulvinate growth, 2 m.m. high at centre, falling away towards the edge. The greater part of the growth is thin, dull, subfelty, and opaque, with some superficial aerial mycelium. The outer quarter thins out to a transparent dusty film, through which the perithecia show distinct. The edge is circular, indefinite and adpressed. The superficial aerial mycelium is white, turning darker; the lower growth is "Iron grey" (357;1-4). The growth is chiefly spores. Perithecia are distinct on the tenth day, and on ripe asci on the thirteenth day. Has exuded moisture. The rate of growth is 8.3 m.m. per day.

Macrosorium B.

The central three quarters of the growth is evenly raised to 1½ m.m. the marginal quarter falling away to the edge. The greater part of the growth is downy - subfelty; the outer part is thinly powdery. The edge is circular, regular but indefinite and powdery. The colour is "Iron grey" (357;1-4); most of the growth is opaque. The large central area consists of a large proportion of mycelium, the margin is almost entirely conidia. There are very many scattered perithecia. Rate of growth is 9.0 m.m. per day.

Macrosorium Td./
Macrosporium Td.

The growth is a thin powdery layer consisting of a little white mycelium and many spores. The edge is even but indefinite; it is however defined by the presence of many perithecia. Most of the area is translucent or transparent. The colour is "Iron grey" (357;2). Rate of growth is 8.4 m.m. per day.

Macrosporium H.

Resembles the culture on Elliott's Agar, except for rather faster rate of growth - 8.3 m.m. per day.

Tomato Agar.

Pleospora herbarum. In surface elevation the growth is pulvinate 3 m.m. high, soon collapsing on the central half. The central half is felty below, and woolly above; the outer half is felty below sometimes with small tufts arising all over - almost "plumose". The edge is circular definite and regular finally adpressed. The colour is white above on the central half; the remainder varying from "Pearl grey" (355) to pale "Greenish grey" (352;1). Most of the growth is opaque, but margin is translucent. There is a fair amount of mycelium, and many spores. There are many perithecia; as is usual, these form on and below the surface. A culture has, however been observed having aerial perithecia found well above the surface of the medium, on the tops of the many white tufts of mycelium which arise all over the surface. There is very little moisture on the culture. The growth is deeper and denser than the other Macrosporium forms. The rate of growth is 10.3 m.m. per day.

Macrosporium A.

Pulvinate, but the greater part is of an even height of 3 m.m.; falling away at the edge. There are 2 layers; the upper or superficial layer is 'matted'; being fairly closely intertwined/
intertwined, but translucent and showing the lower dense opaque layer as a felty mass 1 m.m. high. The marginal 2½ m.m. are even and definite. The marginal hyphae are radiate and adpressed below, but there are some loose hyphae above. This margin is translucent and therefore distinct from the inner layer. The superficial aerial mycelium and marginal mycelium is white. The lower, dense layer is "Grey green" (254:1) having indefinite rings of whiter and greener tint. There are not many spores in the superficial mycelium, but a large number in the lower dense layer. There are a number of perithecia. The rate of growth is 9.3 m.m. per day.

Macrosporum B.

In surface elevation the growth is pulvinate 2 m.m. high at centre, sloping straight to the edge. The central two thirds is subfelty changing upwards to a woolly superficial mycelium. The outer third has less superficial mycelium outwards, and is powdery at the edge. The central area is mostly composed of mycelium, the outer part of spores. The mycelium collapses in the second week. Perithecia are present after two weeks. Moisture is present all over the mycelial growth. Rate of growth is 10.0 m.m. per day.

Macrosporum Td:-

The growth is evenly raised to 2-3 m.m. There are not very distinct layers of dense and loose aerial mycelium here. but the lower mass 2 m.m. high, which is closer and felty, changes upwards to 1 m.m. of loose subfelty mycelium. The edge is even but not definite. The mycelium on the margin resemble the aerial mycelium; it is not adpressed, and instead of being radiate is loose, very open and slightly tangled. The colour of the superficial aerial mycelium, which varies from the lightest to the darkest "Sage tint" (4: 1-4) is only a thin layer, though fairly close, and does not prevent the colour of the felty layer below from showing through, especially on the outer centimetre of growth, as some tint/
tint of "Iron grey" (357:1-4). The whole surface is dull and opaque, except on the marginal ring of 2 m.m. of very loose hyphae. There are a considerable number of spores. Perithecia are present. The rate of growth is 9.0 m.m. per day.

**Macrosporium H.**

The growth except for the adpressed outer 2 m.m. is pulvinate, being 2½ m.m. high at the centre and rounding off evenly to the adpressed margin. The growth is a felty layer of extremely closely woven hyphae. There is an extremely thin band of adpressed mycelium on the extreme margin, seen by reflected light. The colour of the growth is "Iron grey" (357:4). Only the margin is not opaque. This culture is chiefly mycelium. Perithecia are absent. The rate of growth is 7.5 m.m. per day.

**Prune Agar.**

**Pleospora herbarum.**

Pulvinate opaque growth, about 2 m.m. high at centre, with a definite regular and abrupt edge. The form of the growth is deep felty below and woolly and subfelty above, not collapsing for three weeks. The colour is white changing to "Leaden grey" (353:4). Growth is chiefly mycelium. Perithecia present in small numbers. Rate of growth is 9.7 m.m. per day.

**Macrosporium A.**

Pulvinate, 2½ m.m. high at centre, falling off gradually to edge. Felty below, changing upwards to subfelty and finally woolly. There are no very definite layers. The outer 3 m.m. are low adpressed on the extreme margin, which is even and definite. The colour is at first white, but soon turns green. When ten days old the colour varies from "Pale grey green" (247: all tints) to "Grey green" (245:1). There is a considerable amount of mycelium; but not many spores in the lower part. Perithecia are present ten days after growth starts. The rate of growth is 10 to 10.2 m.m. per/
Macроспориум B.

Pulvinate, 2-2½ m.m. high. It is deep, opaque felty below, with 1-1½ m.m. of woolly, sunfelty hyphae above. The edge is definite, regular and abrupt. The colour is at first white, changing later to "Leaden grey" (353:4) most of the growth consists of mycelium. The superficial mycelium collapses after that of Macrosporium strains A and Td, but before Pleospora herbarum and H. Perithecia are present, but are observed by the deep growth above. There is no moisture. Rate of growth is 10.2 m.m. per day.

Macrosporium Td.

Pulvinate, 1½ - 2 m.m. high with an abrupt edge. It is deep felty and opaque below, with 1 m.m. of woolly hyphae above, soon collapsing and forming a thin skin over the felty layer. The edge is definite and regular. The colour is at first white, changing to "Ashy grey" (358:2). Most of the growth is mycelium. Perithecia are present, but no easily observed of the mycelium above. There is no moisture. The rate of growth is 9.3 m.m. per day.

Macrosporium H.

Pulvinate, 2-2½ m.m. high but falls away to the edge. The growth below is felty with 1-2 m.m. of subfelty mycelium above, not collapsing for three weeks. Most of the growth is "Leaden grey" (353:4) below. The extreme margin is almost black. The growth is largely mycelium. There are no perithecia. There is no moisture on surface. The rate of growth is 7.1 m.m. per day.

Notes on growth of Macrosporium forms on prune.

All have a dense felty opaque lower layer of aerial growth, which is pulvinate. There is also a thinner looser layer above. Form A and H fall away at the edge; the others are abrupt. There is some variation in colour in the cultures. Only Pleospora herbarum and Macrosporium form A. have moisture on the surface. All are dull and/
and opaque, and have a considerable growth of mycelium. The medium is a favourable one for encouraging mycelial growth; spores are not found in such numbers as on synthetic media. This medium is unfavourable for the formation or observation of perithecia.

<table>
<thead>
<tr>
<th>Macrosporium Form</th>
<th>Width (in Microns) of hyphae</th>
<th>Width (in Microns) of Conidiophores</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>Maximum</td>
</tr>
<tr>
<td>&quot;F.h.&quot; Normal</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>Saltant 'a' Normal</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Saltant 'b' Normal</td>
<td>5</td>
<td>8.5</td>
</tr>
<tr>
<td>&quot;A&quot; Normal</td>
<td>4.5</td>
<td>7</td>
</tr>
<tr>
<td>Saltant 'a' 'b'</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Saltant 'c'</td>
<td>4</td>
<td>8.5</td>
</tr>
<tr>
<td>Saltant 'd'</td>
<td>3.5</td>
<td>8</td>
</tr>
<tr>
<td>&quot;B&quot; Normal</td>
<td>4</td>
<td>7.5</td>
</tr>
<tr>
<td>Saltant 'a'</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Normal &quot;Td&quot;</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Saltant 'a'</td>
<td>4</td>
<td>6.5</td>
</tr>
<tr>
<td>&quot;H&quot; (Normal)</td>
<td>6.5</td>
<td>15.5</td>
</tr>
</tbody>
</table>
Comparison of Macroscopic Characters of Macrosporium Forms.

Elliott's agar.

Pleospora herbarum and A are distinguished by relatively larger numbers of conidia in A; the perithecia in A are of larger size than in the other. The rate of growth of P. herbarum is greater than in A.

B differs from P. herbarum and A in having a constant ringed appearance. There are more conidia especially towards the outer part of the growth than in A. A plate of B seen from below always shows a slightly radiate appearance which is absent in the first two; this is very much less marked than in saltant a of A which is discussed later. The perithecia are not formed in such numbers as in the first two, and there is a constant formation of submerged perithecia round the margin of this form. B is slower growing than P. herbarum but slightly faster than A.

Td differs from these in having a more scanty growth with many fewer conidia and thus is more translucent. Perithecia are formed in fair numbers on the central half, but scarcely at all on the outer half. The medium is discoloured round the centre. This is the slowest growing form on this medium.

H is distinguished by its thin growth, consisting chiefly of mycelium, and having no perithecia.

Dox's agar.

A lacks the downy appearance of P. herbarum at the margin, and a powder of spores renders that part less translucent and slightly more defined. A is a slower growing form.

B differs from the last two in having the greater part of the growth comparatively higher, and consists of relatively more mycelium than the other forms. The growth is downy-subfelty, i.e. not so dense as the others. B again is slower growing than P. herbarum but faster than A.

Td is a very thin growth, almost entirely conidia. The rate of growth resembles than of A.

H is the same as on Elliott's agar but is faster growing, like A and Td.
**Tomato agar.**

*P. herbarum* is distinguished by having a denser growth than the other forms. Again it is the fastest growing form.

A is deep and fairly dense throughout. The upper mycelium layer is close and matted. It is slower growing than both *P. herbarum* and B.

B also has two layers in the growth, which is less high here. The upper layer is woolly, the lower is only subfelty. It is faster growing than A and practically the same as *P. herbarum*.

In Td the layers in the growth are indistinct, as the lower felty layer changes gradually into subfelty mycelium. Only slightly slower growing than A.

In H there is only one layer, which is felty. It is chiefly mycelium, there being fewer spores. It has no perithecia. Has very slow growth.

**Prune agar.**

In *P. herbarum* the whole growth is dense and opaque. The margin is very abrupt. The rate of this and the three following forms are all very similar.

In A the margin is very low and adpressed. The colour is greener than in *P. herbarum*.

B is a dense growth with abrupt edge and in *P. herbarum*. The superficial aerial mycelium, which is less dense than in *P. herbarum* collapses sooner.

Td has lower growth, dense below, but the superficial growth is loose.

H is high near the centre but rapidly falls away to the edge. The growth is dense throughout; it has very dark mycelium at the margin. There are no perithecia. The rate of growth has on tomato agar is very slow.
Comparison of Microscopic Characters of Macrosporium Forms on Elliott's agar.

*Pleospora herbarum.*

The widths of the hyphae and conidiophores are slightly greater than in the other forms, except H. The hyphae are dark. Compared with strain A, the average size of the conidia is sufficiently greater to be significant, but the other forms B, Td and H are intermediate. The ratios of length to width is greater than in the other forms. The verrucosity is rougher than in any of the other forms, particularly B, Td and H. Both asci and ascospores are larger than in the other forms, which agree among themselves fairly closely; the time taken to produce mature ascospores is greater.

*Macrosporium A.*

The conidia are the smallest. The colour of the hyphae resemble *P. herbarum,* but the verrucosity is intermediate. There is less tendency to chain formation than in *P. herbarum,* and there is much less variation in microscopic characters than in that form. Mature ascospores are produced very early.

*Macrosporium B.*

The hyphae are very light coloured. The conidia are the widest. B differs markedly from the two previous forms in the degree of verrucosity, which is fine; in this respect it resembles Td; it is more marked, however, than in H. There is little constriction round the main septum in this strain.

*Macrosporium Td.*

The hyphae are very dark. The verrucosity resembles that of B. There is a great tendency to chain formation, and a corresponding variation in spore size. The ascospores in this strain are the smallest. As in A ascospores are mature very early.

*Macrosporium H.*

Differs from the others in having a greater average width of hyphae, and in having many hyphae extremely wide. The colour of the hyphae is very light. The conidiophores differ in not being club-shaped; they are long, and wide and sometimes become detached. It has the largest conidia and
and the verrucosity of these is absent or extremely fine. Perithecia do not occur.

Table III. Size of Conidia (in Microns) of Macrosporium Forms

<table>
<thead>
<tr>
<th>Macrosporium Form</th>
<th>Length (100 Spores)</th>
<th>Width (100 Spores)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>σ</td>
</tr>
<tr>
<td>Normal</td>
<td>18.53 ± .17</td>
<td>2.46</td>
</tr>
<tr>
<td>Saltant 'a'</td>
<td>15.99 ± .11</td>
<td>1.55</td>
</tr>
<tr>
<td>'A'</td>
<td>17.49 ± .17</td>
<td>2.45</td>
</tr>
<tr>
<td>'b'</td>
<td>15.53 ± .17</td>
<td>2.48</td>
</tr>
<tr>
<td>'c'</td>
<td>21.38 ± .23</td>
<td>3.36</td>
</tr>
<tr>
<td>'d'</td>
<td>19.53 ± .22</td>
<td>3.45</td>
</tr>
<tr>
<td>'Tad'</td>
<td>19.44 ± .22</td>
<td>3.28</td>
</tr>
<tr>
<td>'U'</td>
<td>18.59 ± .18</td>
<td>2.63</td>
</tr>
<tr>
<td>'H'</td>
<td>23.65 ± .28</td>
<td>4.16</td>
</tr>
<tr>
<td>'Rb'</td>
<td>18.98 ± .21</td>
<td>2.99</td>
</tr>
<tr>
<td>Few conidia</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table IV. Degree of Verrucosity of Conidia in Macrosporium Forms.

<table>
<thead>
<tr>
<th>Macrosporium Form</th>
<th>Normal</th>
<th>Saltant 'a'</th>
<th>Saltant 'b'</th>
<th>Saltant 'c'</th>
<th>Saltant 'd'</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. herbbarum</td>
<td>2, 3, 4</td>
<td>2, 3, 4</td>
<td>Few conidia</td>
<td>2, 2-3</td>
<td>Few conidia</td>
</tr>
<tr>
<td>'A'</td>
<td>2, 2-3</td>
<td>2, 2-3</td>
<td></td>
<td>2, 2-3</td>
<td></td>
</tr>
<tr>
<td>'B'</td>
<td>2</td>
<td>0, 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>'Tad'</td>
<td>2</td>
<td>No conidia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>'H'</td>
<td>0, 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The figures 0 to 5 have the following significance:

0 No verrucosity; 1 Extremely fine;
2 Fine; 3 Moderately rough;
4 Rough; 5 Very rough (warty).
Conclusions as to identity of Macrosporium forms.

As a result of the comparative work on the Macrosporium forms A, B, and Td, isolated from rotting tomatoes, it is found that these forms differ in various small features among themselves and from Pleospora herbarum.

It is concluded that the cumulative effects of these differences are not sufficient to justify the consideration of the forms as species. They are considered to be related strains or varieties of Pleospora herbarum (Pers.) Rh., of which the conidial stage is Macrosporium sarcinula Berk.

With regard to the form of Macrosporium from decaying Echium vulgare, known as Macrosporium H during its study, this form is considered distinct from other species of the genus. On Echium vulgare three Pleospora species have been recorded in the "Sylloge Fungorum" of Saccardo (63, Vol. II, p. 251; XVII, p. 752, II, p. 244); these species are:

- Pleospora dura Niesl.
- Pleospora massarinoides Feltg.
- Pleospora media Niesl.

The present form of Macrosporium isolated from Echium has shown no perithecia stage. The descriptions of these three species of Pleospora only refer to their perithecial stages; since the conidia are not mentioned, there is nothing on which to found a comparison. Should the conidial stage of any one be a Macrosporium and indistinguishable from the present form, the latter could be considered as a possible saltant in nature, lacking perithecia.

No records of other Pleospora or Macrosporium species on Echium vulgare appear to exist. The present form, since it differs from known species of the latter genus, is considered a new species, and is named Macrosporium Wilsoni n.sp., with the following description:

The hyphae are long and straight, varying greatly in width up to a maximum of 15.5μ; the average width is 6.5μ; they are generally almost hyaline, and pale green-brown in colour. Septa are frequently deeply constricted, and the hyphae may break up into short lengths.
Branching is mostly at right angles. The conidiophores are modified ends of hyphae or their branches; they are usually long often two four times the width of the hyphae from which they arise; they are wide throughout their length, thick-walled and dark in colour. Club-shaped conidiophores rarely occur. A conidiophore may grow out after bearing a spore, to form a new conidiophore. The conidia are very dark; they are oval to oblong, measuring 14-26 x 11-22 µ, mean of 100 spores is 13.69 by 15.10 µ. There is a main septum, otherwise the septation is muriform. Verrucosity is exceedingly fine, or it is absent. The conidia very frequently occur in chains. Perithecia are apparently absent.

Conclusions as to Identity of Alternaria Forms.

The results of the comparative work on the Alternaria forms R. Ta and D, isolated from rotting tomatoes, indicate these to differ in small particulars. They are not sufficiently different from A. tenuis to be separated, and may therefore be considered to be strains of that species.

Conclusions as to Identity of Stemphylium Forms.

As a result of the study on the Stemphylium form referred to as E, from tomato it has been concluded that it is distinct from other recorded species. It is therefore considered a new species and the name Stemphylium Lycopersici n.sp. is proposed, with the following description:

The hyphae are hyaline or slightly coloured, 3 µ wide on the average. The conidiophores are light brown; they are the slightly modified continuations of the hyphae. They are less than twice the width of the hyphae from which they arise; their average width is 6.5 µ. Spores are borne terminally; after the formation of each spore the conidiophore continues its growth near the apex. There are conidia of two kinds; round 44%, measuring 13-22 x 8-18 mean of 100 spores was 17.02 by 12.58 µ; long, 56% measuring 17-43 x 8-15 µ, mean of 100 spores was 25.17 x 11.59 µ. The verrucosity
of the conidia varies from fine to rough. The base of the long spores is frequently attenuated. The round spores are divided by a transverse and a longitudinal wall into four cells, the long spores have as an average in 100 spores, 3.27 transverse septa, and 1.19 longitudinal septa. Chains of conidia do not often occur. No perithecia have been found.
PERITHECIA IN MACROSPORIUM.

A perithecial stage has only been observed in the genus the
Macrosorium of the three genera treated; Alternaria and Stemphylium
have not given this stage under any conditions. The perithecia belong
to the genus Pleospora and, it has been concluded, to the species P.
herbarum, of which the forms of Macrosorium: A, B, and Td are natural
strains.

The stages in the life history of P. herbarum have been very con-
fused in the past. Myabe (52, pp. 15-16) refers to the confusion pro-
duced by Tulasne (76), Fuckel (38b), and Hallier (48), who
worked with contaminated material. Gibelli and Griffini divided P.
herbarum into two distinct species, P. Sarcinula and P. Alternariae,
the former of which produced larger ascospores and Sarcinula comidia;
the other producing smaller less septate ascospores and Alternaria
comidia. These authors also disproved some of the conclusions of
earlier workers. Bauld's (4) conclusions on the pleomorphism of
P. herbarum still require confirmation. He obtained pycnidia on two
occasions in cultures made from ascospores; on cultivating pycnospores
he obtained, besides pycnidia, Alternaria spores, but these were never
associated with perithecia or Sarcinula spores in the same cultures.
He found that some ascospores from one perithegium would produce
Sarcinula spores and perithecia, while others produced only the
Alternaria spores alone, or, in two cases only, with the pycnidia also.
He found a micro-conidial form in both cultures regularly. He con-
cluded that mycelia of two different characters belong to the same
species.

Kohl (46) as a result of his culture work on the fungus has
much the same conclusions as Gibelli and Griffini, and separated the
Sarcinula and Alternaria forms into two different species.

Berleece (8) places P. Sarcinula of Gibelli and Griffini under P.
herbarum, and their P. Alternariae under P. infectoria Fuckel.

Miyabe (52) worked with 1-spore cultures; his results agree
with those of Kohl. His Macrosorium spores produced only
Macrosorium spores and perithecia in a nutrient fluid; he did not
obtain/
obtain pycnidia in his ascospore cultures. From *Alternaria* conidia which he worked with he obtained *Alternaria* conidia, and no perithecia nor pycnidia.

Bauke, and Miyabe were unable to observe any sexual process connected with the formation of the perithecia. The latter author stated: "The formation of the perithecium is entirely a vegetative process, which resembles essentially the formation of pycnidia." Both Bauke and Miyabe describe "resting hyphae." Such are figured as spirally-coiled clusters of hyphae by the latter author, who states that whatever their function they recall some of the sexual organs represented in the *Ascomycetes*. Such "resting hyphae" have not been seen in the present work, and their significance is doubtful.

Gentner (40) working with 1-spore cultures, found *P. herbarum* to be the perithecial stage of *Macrosporium* sarcinaeforme.

Bolle (11) in 1-spore cultures of *P. herbarum* did not find *Alternaria* conidia, while her *Macrosporium* cultures formed perithecia abundantly. Recognising the possibility of sex-differentiation, this worker made every possible mixture with the forms she dealt with, but as no perithecia resulted, she has no doubt that *Alternaria* has no ascus-form.

Results of the present investigation indicate the conclusion arrived at by Miyabe and by Bolle that *Alternaria* has no perithecial stage. The forms dealt with in this genus have been cultured together, without perithecial production; there is thus no evidence of sex-heterothallism. *Stemphylium E* and *S. ilicis* in culture have never formed perithecia.

Bolle's statement (11, P. 49) that *Macrosporium* always forms perithecia requires modification. In the forms now dealt with, all except *Macrosporium* H formed perithecia. The latter form has been cultured for a considerable number of generations. It has been isolated by taking single spores from its original host, decaying *Echium vulgare*, on different occasions during the summer of 1924, and all isolations agree in having no perithecia. Nor were such observed/
observed on the host itself. Some sixty cultures have been made of this form, and subjected to many treatments without any culture being induced to form perithecia. Temperature from zero to 28°C, moisture, aeration, and food supply have been greatly varied without success. The possibility of this form being a saltant in nature is discussed later, but it may be said here that saltation accounts for considerable change in perithecia formation in other forms, where a saltant may almost lose the power to produce that stage. Since it is probable that saltants occur in nature, and since this form, *Macrosporum h,* whether a saltant or not, has been isolated from nature lacking perithecia, the statement that the genus *Macrosporum* always has a perithecial stage should not stand.

As regards the relationship of the genera of *Macrosporum,* Bolle refers to the results of Miyabe and of Gentner. These workers, dealing with *M. parasiticum* Thum, and *M. sarcinaeforme* Cav. respectively, found *P. herbarum* to be the perithecial stage. Bolle’s work goes much further; dealing with these and many other forms of *Macrosporum* and with *P. herbarum* itself, this author concludes that in culture the conidia of all these forms cannot be distinguished from one another. By comparing the size of 1400 ascospores of each of the two *Macrosporum* species mentioned above, Bolle was able to state that they are two distinct forms, but that a smaller number of measurements would not always differentiate them. *M. parasiticum* is shown to be a saprophyte, or at all events not a severe parasite, and *M. sarcinaeforme* is shown to be a parasite; Bolle concludes that they are not distinct species, but that they represent the highest biologically distinct conidial forms of distinct races of the botanic species *Pleospora herbarum.* It is also stated that the name *Macrosporum sarcinula* can continue for the general conidial form *P. herbarum,* for which the conidial form of distinct morphological races cannot be distinguished.

Bolle’s species, apart from *P. herbarum,* have not been dealt with in the present investigation, but a cultural study of the *Macrosporum* forms isolated from tomato has shown that these may each/
each be distinguished in general cultural characters, macroscopic and microscopic. The conidia themselves, though 100 of each were carefully measured cannot be distinguished in size. The Macros porium H from Echium vulgare agrees very closely with the other forms of this genus in the size of its conidia, though the degree of verrucosity is very different. In other characters also this form is quite distinct. Size of conidia, considered alone, is not sufficient to unite forms of Macros porium together.

As regards the study of the perithecial stage of Macros porium in the present work, this stage was found in the forms A, B and Td; Pleospora herbarum was cultured as a control. The measurements of 50 asc i and of 100 ascospores of each of these forms are given in tables V. and VI; the cultures were two months old, and therefore comparable. Asc i vary considerably in size, according as the ascospores lie in one or two rows in the ascus. Considerable uniformity is however to be observed in the size of asci in the forms A, B, and Td. They are considerably shorter than the P. herbarum asci, but of almost the same width. The ascospore measurements of the four forms measured vary to a small extent, but insufficiently, it is considered for differentiation. The measurements of the ascospores of P. herbarum are smaller than those given by Bolle; the figures obtained by that author are probably nearer a true average, as much larger numbers of ascospores were measured. The influence of substrate may account for the discrepancy, as precautions were taken in both studies to consider few and only mature perithecia, grown under controlled conditions. Strain Td, Strain Td, though it forms its perithecia earlier than strain B, and slightly earlier than strain A, has the smallest ascospores.

The result of these measurements would therefore indicate that by using a larger number of ascospores the strains could be differentiated on size of these alone.
An ascus of typical shape and transparency has been figured on plate IV fig.29, and a typical sole-shaped ascospore in fig.28. Eight is the normal number of spores in the ascus, but it has been quite usual to find a smaller number. The latter figure shows the ascospore germinating through the wall of the ascus after three and a half hours in water. Such germination through the ascus wall may not however occur in nature. Miyabe (52) found such germination in culture; also on keeping two mature perithecia in a moist chamber overnight, he found many ascospores ejected, and that all had germinated.

Ascospores in old dried-up cultures are found lying free, the asci having disappeared; the shape of such spores is frequently very irregular and all trace of the chief septa lost. Such an ascospore, four months old is shown in fig.30 of plate IV. The septation of normal ascospores is distinct. In the forms dealt with there are three very marked transverse septa and other four less marked; a slight constriction occurs round the septa. There is no verrucosity.

No germination of ascospores has been observed in the cultures where they have been formed. In water-cultures they have not been observed to produce secondary spores.

As regards the time required for ripe ascospores to be formed in a culture, this has been found to vary to some extent between the forms of *Máculosporium*. Bolle has stated; "These perithecia take about two months to ripen; one can hasten this process by keeping the cultures cold (≈0°) in the second and third week". The time necessary varies to a certain extent even within one form; *P. herbarum* in the present work required about two months, but that time may be considered about the maximum for the form A, B, and Td; generally about four to six weeks are necessary. Form A has however formed ascospores, which were able to germinate in water, twenty days after growth was first observed, and form Td has produced ripe ascospores in eighteen days.

The effect of the medium on the time necessary for ascospore formation/
formation was found to be that on oat agar ascospores were ripe in
the shortest time; on the artificial media (Dox's agar and Elliott's
agar) they took rather longer; tomato agar was less stimulating
and prune agar the least so.

As regards position of the perithecia in culture, generally they
are formed at the surface of the medium. A number are however formed
at various depths below the surface. This is clearly seen in a
translucent medium like Dox's or Elliott's artificial agars.

An exceptional position was however in the case of Pleospora
herbarum on a plate of tomato agar, kept to 27° for ten days after
growth started, and then maintained at room temperature. Thirteen
days after growth started it was observed that there arose in great
numbers over the whole surface of the agar, small tufts of mycelium
about 1 m.m. high. Some of these bore small round black bodies,
similar to the perithecia on the surface of the medium below the
mycelium. They were found to be perithecia. A subculture was made
under identical conditions and gave a similar appearance. Such
aerial perithecia have not been observed in any other cultures in the
present investigation; they are not however constant even in P.
herbarum under uniform conditions.

Pycnidia. Among the earlier writers mentioned above in discussing
perithecia several found pycnidia in their cultures, but later workers,
taking precautions to deal with pure culture have not recorded this
stage in the life history of the genera under consideration. Within
recent years Alternaria-like spores have been found in association
with Pycnidia. Reference need only be made to Phoma Richardiae
described by Mercer (51); Phoma alternariaceum described by Brooks
and Searle (16); and Phyllosticta pirina Sacc., all of which Bolle
has examined. In each occur chlamydoospores which are Alternaria-like.

A culture of Phoma alternariaceum was received by the present
writer through the kindness of Mr. Brooks; the chlamydoospores are
at first sight like Alternaria, but are too irregular to conform to the
description of the spores of that genus.
No pycnidia have been encountered in any of 1-spore forms of the Macrosporium, Alternaria, or Stemphylium in the present work, and Bolle's conclusion must still hold: that the "occurrence of pycnidia in the development of the Phaeodictyae is quite improbable."

Table V. Measurements of Asci (in microns)

<table>
<thead>
<tr>
<th>Macrosporium form</th>
<th>Average Size (50 Asci)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>P. herbarum</strong></td>
<td>232 x 29 (204-306 x 24-33)</td>
</tr>
<tr>
<td>&quot;A&quot;</td>
<td>159 x 27 (132-216 x 21-30)</td>
</tr>
<tr>
<td>&quot;B&quot;</td>
<td>165 x 26 (132-198 x 21-30)</td>
</tr>
<tr>
<td>&quot;Td&quot;</td>
<td>159 x 28 (135-204 x 24-33)</td>
</tr>
</tbody>
</table>

Table VI. Measurements of Ascospores (in microns)

<table>
<thead>
<tr>
<th>Macrosporium form</th>
<th>Length (100 Spores)</th>
<th>Width (100 Spores)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± σ</td>
<td>Mean ± σ</td>
</tr>
<tr>
<td><strong>P. herbarum</strong></td>
<td>34.03 ± .15 (29-39)</td>
<td>14.31 ± .06 (12-16)</td>
</tr>
<tr>
<td>&quot;A&quot;</td>
<td>31.67 ± .13 (27-36)</td>
<td>13.43 ± .06 (12-16)</td>
</tr>
<tr>
<td>&quot;B&quot;</td>
<td>30.42 ± .18 (22-39)</td>
<td>13.46 ± .06 (11-16)</td>
</tr>
<tr>
<td>&quot;Td&quot;</td>
<td>29.46 ± .10 (28-33)</td>
<td>12.05 ± .05 (10-14)</td>
</tr>
</tbody>
</table>
Alternaria cultures.

**Elliott’s agar.**

*Alternaria tenuis* (pl. VII, fig. 50).

Surface elevation is very low - less than $\frac{1}{2}$ m.m. all over; thins out at the edge to a powder of spores. The growth is very compact and powdery, densest as a ring within the margin, where it thins out. Scattered over the surface are flecks of white hyphae (not the saltant). The edge is even, but indefinite. The colour is "Greenish black" (351:3); the growth is dull and opaque all over. Three is practically no mycelium visible to the naked eye.

Rate of growth is 9.4 m.m. per day.

The hyphae, conidiophores and conidia are all much darker and more defined than in any of the strains R, Ta, or D.

Hyphae are dark and show up clearly unstained. There are, however, very hyaline hyphae present - there is more irregularity in width of these, some being as wide as 10 $\mu$; the average width was found to be 4.5 $\mu$.

The conidiophores are also very dark. They are darker and slightly wider than the hyphae from which they arise. Generally they are formed terminally on the hyphae. The first spore is borne terminally on the conidiophore. Usually further spores are produced distally, and a chain results (Pl. 5 fig. 40). It is not unusual to find small spores budding from the side of a spore. After the first spore is borne, the conidiophore may continue its growth near the point of attachment, and at a slight angle, as in plate 4, fig. 35 of strain Ta. A second spore is borne terminally, then the conidiophore again grows out. This process may be continued until a number of spores are produced. The spores appear more or less alternately on the zig-zagged conidiophore, but are very easily detached, leaving scars. The result is a "nodulose" conidiophore, though this is much less marked than in *Staphylium*.

The conidia are very dark. The size of 100 spores was 17-52 x 8-15 $\mu$. The verrucosity varies considerably from fine or moderately/
moderately rough in most spores to rough in a small proportion. Rougher verrucoity has been found here than in the other forms of *Alternaria*.

Figure 40, though it does not show a typical conidiosphere, is interesting in that it brings out a number of other points: the largest spore is the oldest, it is typical and has been borne terminally on the conidiophore; the latter has continued its growth near the apex then branched, the branches showing a spore on one and the scar left by a fallen spore on the other. Meantime the large spore has become the first of a chain, and also has sent out a lateral and an almost terminal germ-tube, with a single secondary spore on one, a chain of secondary spores on the other. The second spore of the large chain has also sent out a germ-tube which has formed a secondary spore.

*Alternaria R* is a pulvinate growth less than 2 m.m. high, low at the centre. Has superficial aerial mycelium, a thin subfelty layer 1 m.m. high, which extends all over and to the edge. The edge is even and fairly definite. Marginal mycelium is not adpressed. The colour of the superficial mycelium is "Grey" (359:2); the layer below is "Greenish black" (351:2). The whole growth is opaque and dull. There is a fair amount of mycelium in this form. The rate of growth is 9.7 m.m. per day.

There is a certain amount of mycelium. The hyphae are straight, varying to some extent in width. The colour varies from hyaline to light brown. There are many septa and where these occur the hyphae are often contracted. Irregular swellings in the hyphae are unusual. Branching is almost at right angles, and the ultimate branches resemble the general mycelium. They are frequently contracted at the base but above their base may be wider than the hyphae from which they arise. Only seldom do two branches appear at one point in the length of a hyphae. The average width is 4 μ, the maximum observed 6 μ.

The conidiophores are only slightly modified ends of hyphae or their branches. They differ from the normal hyphae in being darker.
darker, though not so dark as the conidia. They are only slightly wider than the hyphae from which they arise, being 4.5μ. The maximum width observed was 5μ. The development of the conidiophore and formation of the spores resemble A. tenuis.

The conidia resemble those of A. tenuis in appearance. The size of 100 spores was 18-50 x 7-17μ. The verrucosity is moderately-rough, and varies within narrower limits than in A. tenuis. The spores are very regular in appearance; an unusual one is figured growing out at two points of the distal end (Pl. 5 fig. 41).

*Alternaria Ta.*

The growth is evenly raised to ½-1 M.M. Powdery at the edge. There is no superficial aerial mycelium as a definite layer, but simply a sprinkling of grey dusty mycelium, with here and there a tuft of white mycelium. The colour is "Greenish black" (351: 2-3); the edge is regular but indefinite because powdery. The greater proportion of the growth is spore. The rate of growth is 9.5 m.m. per day.

The hyphae resemble those of strain R, but are not in such quantities; the average width is the same, i.e. 4μ, the maximum observed was greater being 8μ. Compared with strain R the conidiophores are not so clearly differentiated from the hyphae, being scarcely darked. In both strains the conidiophores are only slightly wider than the hyphae from which they arise, the average width being 4.5μ, the maximum observed 5μ. The conidia resemble those of A. tenuis and of strain R. The verrucosity however, is more variable than in the latter strains, having many spores finally verrucose, others almost as black as in A. tenuis. The size of 100 spores was 19-53 x 7-14μ. A short chain of two conidia is figured (Pl. IV, fig.35), the terminal of which has thrown out a terminal germ-tube, and on this are formed two secondary spores; such a germ-tube would become nodulose with further development and spore formation.

*Alternaria D.*

This strain resembles Ta on Elliott's agar, which showed rather fast growth, 8.9 m.m. per day.

The hyphae are dark in much of the mycelium, but there are also present/
present other quite hyaline hyphae. The average width, $4\mu$, is the same as in the other strains, but the maximum observed was $5.5\mu$. The conidiophores are distinct from the hyphae from which they arise, being darker, though only slightly wider; the average width of $5\mu$ is practically the same as in the other strains. The conidia are darker than in the strains R or Ta, but resemble closely the conidia of *A. tenuis*. The verrucosity is the same as in that form, varying from fine or moderately-rough to rough. The size of 100 spores was $21-46 \times 7-15\mu$.

Dox's agar.

*Alternaria tenuis.*

Shows a *low* growth less than $\frac{1}{2}$ m.m. high all over. The form of growth is dense and powdery, there being no aerial mycelium to the naked eye. The colour is "Greenish black" (351: 4); by transmitted light the growth is translucent. The edge is regular but indefinite. There is no exuded moisture. The rate of growth is 10.5 m.m. per day.

*Alternaria* R.

This culture resembles *A. tenuis* on the same medium in most characters. It differs in having a downy-floccose aerial mycelium over the surface of the dark spore layer. The rate of growth is distinctly greater than in the other forms of *Alternaria*, 11.7 m.m. per day.

*Alternaria* Ta. and D.

The growth of these forms on Dox's agar cannot be distinguished from that of *A. tenuis*. Both do have moisture on the surface, which does not occur in the other two forms, but no significance can be attached to one difference of so small a nature. The rates of growth in each case is 10.9 m.m. per day.

Tomato agar.

*Alternaria tenuis.*

This growth is very flat, with a lower even dense layer of less than $\frac{1}{2}$ m.m. high, and falls away abruptly at the edge to the surface of the medium. There is some aerial mycelium in many small tufts rising/
rising erect straight and like scales on the central \( \frac{3}{4} \) of the growth and a little within the border. At the edge of the growth there is border lm.m. wide of dull white ciliate hyphae, which are adpressed and give the growth and even definite edge. The colour of the dense layer, which is by far the greater part of the growth, is "Pure black" (349:3). The superficial aerial mycelium is "Grey" (359:1). Except for the marginal lm.m. which is translucent, the growth is dull and almost opaque. The rate of growth is 9.7 m.m. per day.

**Alternaria R.**

The growth is evenly raised except at the edge and at 2 rings of subfelty mycelium on the surface. The lower growth, 1 m.m. high, is very close and dense due to masses of spores and felty mycelium. The aerial mycelium is a thin layer 1 m.m. high over the dense layer. On the marginal one quarter of the growth the superficial mycelium is not produced, and the lower spore layer thins outwards to the edge, which is even and fairly definite. The dense lower layer is seen at the edge is "Ivy green" (236:4). The aerial mycelium is dull and "Grey" (359:4). Except at the edge the growth is opaque. The dense layer forms the greater part of the growth. The rate of growth is 10.7 m.m. per day.

**Alternaria Ta.**

The growth is evenly raised to lm.m. all over, except where rings of scanty superficial mycelium rise. The dense lower sporing growth forms the greater part, and no mycelium can be made out in it. The superficial mycelium is not conspicuous at appears as a central disc and as subfelty rings 1 m.m. high between the centre and margin. The edge is abrupt, rather irregular, but definite, and without a border of mycelium. The colour of the sporulating layer changes from "Dark drab green" (237:4) to "Greenish black" (351:3). The scanty superficial mycelium is "Pearl grey" (355:2). The growth is dense and opaque, except at the margin, where for 1 m.m. which is powdery it is translucent. Most of the growth is spores. The rate of growth is 9.2 m.m. per day.
Alternaria D.

This culture very much resembles *A. tenuis*. The edge is perfectly even and quite definite, and formed of short, 1 m.m., very close and adpressed white radiate hyphae. This marginal ring is very sharply marked off from the dense area within. The greater part of the growth is 2 m.m. high, but on the central third, the superficial mycelium rises 2 m.m. or more above that before collapsing, otherwise it has the appearance of *A. tenuis*. The rate of growth is 9 m.m. per day.

Prune agar.

*Alternaria tenuis.*

Has a pulvinate growth 2 m.m. high at the centre, gradually sloping to the edge. There is a dense opaque layer consisting chiefly of spores, and a thin layer of superficial mycelium above. The edge is even and definite, and rather adpressed. The colour of the spore layer is "Greenish black" (351:4). The superficial mycelium changes from white to a very definite "Grey" (359:4). There is no moisture on the surface. With successive days' growth there is a marked ringed effect, except on the marginal 1/5th of the area. The rate of growth is 10.6 m.m. per day.

Alternaria R.

This growth differs from *A. tenuis* in several characters: the pulvinate growth is 3 m.m. high at centre, and the whole growth is dense and fairly deep. There is more superficial aerial mycelium and this is closer, felty in the central half, and subfelty on the outer half of the area. The colour of this superficial mycelium is a plain white at first, changing later from the margin inwards to "Putty colour", (311:4). The lower growth is "Greenish black" (351:4). Though the lower layer is again the deeper, the mycelial layer is fairly thick especially towards the centre. The rate of growth is 12.0 m.m. per day.

Alternaria Ta.

The growth is 2½ m.m. high at centre and has rather more superficial/
Fig. 2. Alternaria: Rate of Growth of forms R, T, D and A. tenella (At.)
superficial mycelium than *A. tenuis* though less than *R*; it is almost confined to the central half. The colour of the superficial mycelium is different from that in the first two forms of *Alternaria*; it changes from white to "Pale grey green" (247: 4). There is a slight ringed appearance, but not so marked as in *A. tenuis*. There is moisture present on the surface. This form resembles *R* more than any of the others. The rate of growth is 11.7 m.m. per day.

*Alternaria D.*

This form resembles *A. tenuis* more than do the other two forms. It differs from *A. tenuis* chiefly in the larger amount of superficial aerial mycelium, which here almost covers the surface; also here the colour of this mycelium darkens very much slower, after seventeen days growth, it was almost all still white. It differs also in the presence of moisture over most of the surface of the mycelium. There is a ringed appearance, but this is almost confined to the central two-thirds of the area. The rate of growth is 11.3 m.m. per day.

**Table VII. Rate of Growth of Alternaria Forms.**

<table>
<thead>
<tr>
<th>Alternaria form.</th>
<th>Medium.</th>
<th>Elliott’s Agar</th>
<th>Dox’s Agar</th>
<th>Tomato Agar</th>
<th>Prune Agar</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. tenuis (Normal Saltant a)</td>
<td>9.4</td>
<td>10.5</td>
<td>9.7</td>
<td>10.6</td>
<td></td>
</tr>
<tr>
<td><em>R</em></td>
<td>9.7</td>
<td>11.7</td>
<td>10.7</td>
<td>12.0</td>
<td></td>
</tr>
<tr>
<td><em>Ta</em></td>
<td>9.5</td>
<td>10.9</td>
<td>9.2</td>
<td>11.7</td>
<td></td>
</tr>
<tr>
<td><em>D</em></td>
<td>8.9</td>
<td>10.9</td>
<td>9.0</td>
<td>11.3</td>
<td></td>
</tr>
</tbody>
</table>
Comparison of Macroscopic Characters of Alternaria forms.

Elliott's agar.

*A. tenuis* is a low sporing growth, with no visible mycelium; there are so few characters for observation here that this and the forms Ta and D are alike. *A. tenuis* and R are slightly faster growing than D.

R. is a mycelial growth, deeper than the others which are thin and which consist chiefly of spores. The edge is fairly definite; this is not the case in the other forms.

Ta is a much denser sporing growth than *A. tenuis* or D. It is also more opaque. It is faster growing than B.

D has more superficial grey mycelium than Ta or *A. tenuis*, though the mycelium of the latter must not be mistaken with its white saltant. It has slightly slower growth than the other two.

Dox's agar.

*A. tenuis* is a low powdery growth.

R differs from the other forms in having a downy floccose aerial mycelium and is faster growing.

Ta and D resemble *A. tenuis* to the naked eye; but both are very slightly faster growing.

Tomato agar.

*A. tenuis* and D appear the same to the naked eye, but the former is faster growing.

R and Ta are much alike. They differ from the other two on this medium in having an abrupt and not ad pressed margin. The abrupt margin is most marked in R, and was the first character observed in this form. *A. tenuis* is the faster growing form here of these two.

Prune agar.

R is most like *A. tenuis*, and D is most like Ta. *A. tenuis* has two layers. The superficial aerial mycelium is ultimately grey. There is a ringed appearance over most of the/
the area.

R is deeper than \textit{A. tenuis}; it is a dense growth and has more superficial aerial mycelium and this has a different colour.

\textit{Ta} has more mycelium than \textit{A. tenuis} though less than \textit{R}. The aerial mycelium differs in colour from the other forms, and is confined to the central part. A ringed appearance here is less marked than in \textit{A. tenuis}.

\textit{D} is more like \textit{Ta} than the others. It differs from \textit{A. tenuis} in the presence of more superficial aerial mycelium, and this darkens slowly.

Thus \textit{R} can be told on Elliott's agar by the deep mycelial growth. It is the fastest growing on all media. \textit{A. tenuis} is so far distinguished from the others by its continually throwing a white saltant. It is a low sporing growth, distinct from \textit{R} on Elliott's agar, and from \textit{Ta} and \textit{D} on Prune agar. \textit{Ta} can be told on Elliott's agar by its mass production of spores giving a dense opaque growth. \textit{D} can be distinguished from \textit{Ta} on Elliott's agar and from \textit{R} and \textit{A. tenuis} on Prune agar.

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|c|c|}
\hline
Alternaria Form & Width (in Microns) of Hyphae & & Width (in Microns) of Conidiophores & \\
 & Average & Maximum & Average & Maximum \\
\hline
"A.t." (Normal) & 4.5 & 10 & 5 & 6.5 \\
"Saltant 'a'" (Saltant 'a') & 5 & 7 & 5 & 5 \\
"R" (Normal) & 4 & 6 & 4.5 & 5 \\
"Ta" (Normal) & 4 & 8 & 4.5 & 5 \\
"D" (Normal) & 4 & 5.5 & 5 & 5.5 \\
\hline
\end{tabular}
\caption{Width of Mycelium (in Microns) of Alternaria Forms.}
\end{table}
Table X. Size of Conidia (in Microns) of Alternaria Forms.

<table>
<thead>
<tr>
<th>Alternaria Form</th>
<th>Length (100 Spores)</th>
<th>Width (100 Spores)</th>
<th>Septa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± σ</td>
<td>Mean ± σ</td>
<td>Trans</td>
</tr>
<tr>
<td>Normal</td>
<td>27.81 ± 3.7</td>
<td>10.81 ± 1.1</td>
<td>1.57</td>
</tr>
<tr>
<td>&quot;At.&quot; Saltant</td>
<td>26.55 ± 3.8</td>
<td>9.57 ± 0.8</td>
<td>1.24</td>
</tr>
<tr>
<td>'R' (Normal)</td>
<td>30.22 ± 3.9</td>
<td>11.26 ± 1.2</td>
<td>1.65</td>
</tr>
<tr>
<td>&quot;Ta&quot; (Normal)</td>
<td>29.41 ± 4.5</td>
<td>10.66 ± 1.2</td>
<td>1.64</td>
</tr>
<tr>
<td>&quot;D&quot; (Normal)</td>
<td>30.12 ± 4.0</td>
<td>10.30 ± 1.2</td>
<td>1.60</td>
</tr>
</tbody>
</table>
Comparison of Microscopic Characters of Alternaria Forms on Elliott's agar.

A. tenuis.

The hyphae, conidiophores and conidia are all much darker and more definite than in any of the strains R, Ta, or D. Also more variety has been found in the width of hyphae and of conidiophores, and rougher verrucosity has been found on the conidia. Size and septation of conidia cannot be used in differentiating the forms.

Alternaria R.

There is a constant and distinctly greater proportion of mycelium in this strain.

Alternaria Ta.

The conidiophores and hyphae are more alike on this strain than in any of the other forms.

Alternaria D.

The colour of the spores is darker than those of Ta or D; they resemble more closely those of A. tenuis. The ratio of spore length to spore width is greater here.

Table X. Degree of Verrucosity of Conidia in Alternaria Forms.

<table>
<thead>
<tr>
<th>Alternaria Form</th>
<th>Normal or Saltant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>A. tenuis</td>
<td>2-3, 3, 4</td>
</tr>
<tr>
<td>&quot;R.&quot;</td>
<td>3, 3-4</td>
</tr>
<tr>
<td>&quot;Ta&quot;</td>
<td>2-3, 3, 3-4</td>
</tr>
<tr>
<td>&quot;D&quot;</td>
<td>2-3, 3, 4</td>
</tr>
</tbody>
</table>

The figures 0 to 5 have the same significance as in Table IV.
STEMPHYLlUM CULTURES.

Elliott's agar.

Stemphylium ilicis. (Pl. 6, fig. 47, the two dark growths 4 days old).

The growth is very low, only 2 mm. high at the centre and falling away to the edge. It is subfetly-powdery, opaque in the central half, with scarcely any superficial mycelium, and this soon collapses. The edge is regular, rather indefinite though powdery and adpressed, with no marginal radiating threads. The colour is dusty "Ivy green" (286;2). The growth is almost entirely conidia, but there is some mycelium. The rate of growth is 8.3 mm. per day.

The hyphae are very hyaline. Branching is practically at right angles, and there is very little constriction at the septa. A certain amount of constriction occurs at the base of the branches. The average width of the branches was found to be 3.5 μ, the maximum observed was 5 μ. The conidiophores (Pl. IV, fig. 32) are much more sharply defined than in any of the Alternaria strains. Here a conidiophore is much wider than the hyphae from which it arises, being two to three, sometimes four times the width. The conidiophores are also darker than the hyphae; they occasionally branch as seen in fig. 32.

The first spore is produced terminally, then the conidiophore grows out near the point of attachment, and at a slight angle. A second spore is borne at the new apex, then the conidiophore again grows out. This process may be repeated until a considerable number of spore are produced. The spores are produced more or less alternately on the zig-zagged conidiophore; they are very easily detached when disturbed, and leave scars, as shown in fig. 32. The bunching appearance of the conidia, seen under the microscope in a culture, is due to their very close formation. When spores have been borne the conidiophores show small projections, each of which has a small ledge where the spore has been seated. The writer is in full agreement with Bolle (11), who considered these conidiophores to be referred to in the term used by Wallroth (79): "Hyphae/
"Hyphae... nodulosae," in his description of the genus. Stevens (74) has figured an essentially similar nodulose conidiophore in *Helminthosporium sativum*.

This type of conidiophore is less common in the *Alternaria* forms. It has been seen, but only exceptionally, in the *Macrosporium* forms.

There are two forms of conidia, round, (fig.34) and long (fig.33). The former are by far the predominating type in *S. silicis*; the long spores were found only to the extent of 2-3% of the total number of spores. The long spores are more *Alternaria*-like in their septation, but as already pointed out by Elliott (37) and by Bolle (11) the pointed end if present is the base in this case. That the apex may be the pointed end is shown by fig. 37 on Pl.5, of *Stemphylium E*. The long spores have rather fewer transverse septa, and rather more longitudinal septa than in *A. tenuis*.

The verrucosity of the round conidia is very rough or warty, and is more pronounced than in any other of the forms being dealt with in the three genera. The long spores are less verrucose. The average size of the round conidia is 13-22 x 12-19μ, and of the long conidia 19-35 by 9-16μ. Chain formation is quite unusual but has been observed. Germination of the spores occurs to a great extent in the parent culture, and like the conidiophores the germ-tubes are under these culture conditions are nodulose (figs.33, 34).

*Stemphylium E.*

The macroscopic features are similar to those of *S. silicis* on this medium, but there is faster growth, 9.4 m.m. per day.

The hyphae are hyaline to slightly coloured; they vary greatly in width. In distinction from *S. silicis* the average width of 5μ, and the maximum of 8μ are greater than in that species.

There is here, as a rule, less distinction in the width
of the conidiophores from the width of the hyphae from which they arise, than in the case of *S. ilicis*. Here a hyphae gives rise to a conidiophore usually less than twice its own width. The conidiophores frequently take the form of short lateral branches to hyphae. The average width is 6.5\(\mu\), the maximum 9.5\(\mu\), both figures being greater than in *S. ilicis*. They are light brown and therefore distinctly from the hyaline hyphae.

Generally conidia fall in making a microscopic preparation, but fig.36 on Pl.5 shows a conidiophore with five spores still attached, a sixth is separated but is held by the spores on either side. The distinction in width of the hyphae and conidiophore is here exceptionally great. Figure 37 shows that a long spore of *Stemphylium* may have the apical end pointed on occasion. The very nodulose germ-tube is however sufficient to distinguish it from *Alternaria*.

In distinction from *S. ilicis* there is here a very large proportion of the long conidia. In fact, among 100 measureable conidia which came into a few scattered fields of the microscope 56\% were put into the long spored group. The remaining 44\% being put into the round spored group. In *S. ilicis* only 2-3\% were found to be long spores. A feature of the round spores is that in a great number the septa are not transverse and longitudinal, but diagonal, and there is a deep constriction at the septa in the outer wall in many of the round spores.

The verrucosity is not so rough on the average as in *S. ilicis* but varies within wider limits; further the verrucosity of the round spore cannot be considered rougher than that of the long.

Several cases of budding have been noted in this form, and short chains have been seen (figs.38, 39). The chain shows in fig.38 was formed in a four day culture in a hanging drop of water: it is typical of a number of short chains which were seen.

*Dox's agar.*

*Stemphylium ilicis* and *Stemphylium E.*

The macroscopic appearance of each of these growths on *Dox's agar/*
agar cannot be distinguished from that of Alternaria tenuis on the same medium, but rate of growth is different in each - S. ilicis 8.5 m.m. per day, and in Stemphylium E 9.5 m.m. per day.

Tomato agar.

**Stemphylium ilicis.**

Pulvinate opaque growth, 2 m.m. high at centre, falling away gradually to the edge. There is some subfelty superficial mycelium. The edge is even, and fairly definite, being bounded by a ring of close straggling adpressed hyphae. The colour of the lower growth is "Greenish black" (351:1). The superficial mycelium is at first white, but turns darker. Rate of growth is 8.3 m.m. per day.

**Stemphylium E.**

The lower flat opaque growth is not more than 1 m.m. in height. Superficial aerial mycelium is present; that on the inner half is loose and straggling, rising 1½ m.m. above the lower growth, but away from the centre it becomes straighter and less tangled and the hyphae rise upwards and outwards. On the outer half of the area the superficial mycelium is downy and scarcely visible. The edge is even, but not definite, being powdery and without mycelium. The colour of the lower layer, as seen on the exposed outer half of the area is "Greenish black" (351:4); the colour of the superficial mycelium on the central half is white inside and "Grey" (359:1) outwards. Rate of growth is 10 m.m. per day.

Prune agar.

**Stemphylium ilicis.**

Evenly raised growth 1½ m.m. high, falling abruptly at the margin which is even and definite, with 1 m.m. of adpressed transparent hyphae. A lower opaque layer of the growth is dense, and "greenish black" (351:2); the superficial aerial mycelium, which forms half the height of the growth is evenly subfelty all over and is "Grey" (359:2-4) in colour. There is no moisture present on the growth. Rate of growth is 8 m.m. per day.

**Stemphylium E.**

Almost the entire growth here forms a dense spore mass; the superficial/
superficial aerial mycelium is thin and greyish white. The height of the growth does not fall away abruptly at the edge as in \textit{S. ilicis}. There is no exudation of moisture. Rate of growth is 9.4 m.m. per day.

Table II. Rate of Growth of \textit{Stemphylium} Forms in terms of diameter in m.m.'s per day.

<table>
<thead>
<tr>
<th>Stemphylium Form</th>
<th>Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{S. ilicis}</td>
<td>8.3</td>
</tr>
<tr>
<td>Normal Saltant a</td>
<td>8.8</td>
</tr>
<tr>
<td>E</td>
<td>9.4</td>
</tr>
</tbody>
</table>

Fig. 3. \textit{Stemphylium}: Rate of Growth of form E and \textit{S. ilicis} (S1.)
### Table XII. Width of Mycelium (in Microns) of Stemphylium Forms.

<table>
<thead>
<tr>
<th>Stemphylium Form</th>
<th>Width (in Microns) of Hyphae</th>
<th>Width (in Microns) of Conidiophores</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>Maximum</td>
</tr>
<tr>
<td>&quot;S.t.&quot; (Normal)</td>
<td>3.5</td>
<td>5</td>
</tr>
<tr>
<td>&quot;S.i.&quot; (Saltant 'a')</td>
<td>3.5</td>
<td>5</td>
</tr>
<tr>
<td>&quot;E&quot; (Normal)</td>
<td>5</td>
<td>8</td>
</tr>
</tbody>
</table>

### Table XIII. Size of Conidia (in Microns) of Stemphylium Forms.

<table>
<thead>
<tr>
<th>Stemphylium Form</th>
<th>Length (100 Spores)</th>
<th>Width (100 Spores)</th>
<th>Septation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>σ</td>
<td>Mean</td>
</tr>
<tr>
<td>&quot;S.t.&quot; Normal</td>
<td>I7.75</td>
<td>.11</td>
<td>I.70</td>
</tr>
<tr>
<td>Round Conidia</td>
<td>(I3-22)</td>
<td></td>
<td>(I2-I9)</td>
</tr>
<tr>
<td>Long Conidia</td>
<td>24.68</td>
<td>.19</td>
<td>2.86</td>
</tr>
<tr>
<td>&quot;S.i.&quot; Saltant 'a'</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Few conidia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot;E&quot; (Normal)</td>
<td>I7.02</td>
<td>.11</td>
<td>I.70</td>
</tr>
<tr>
<td>Round Conidia</td>
<td>(I3-22)</td>
<td></td>
<td>(I2-I9)</td>
</tr>
<tr>
<td>Long Conidia</td>
<td>25.17</td>
<td>.32</td>
<td>4.76</td>
</tr>
<tr>
<td>(55%)</td>
<td>(I7-43)</td>
<td></td>
<td>(8-15)</td>
</tr>
</tbody>
</table>
Comparison of Macroscopic Characters of Stemphylium Forms.

On all four media Stemphylium E is faster growing than *S. ilicis*, otherwise they are very similar.

On Dox's agar the growths of both forms cannot be distinguished from *A. tenuis*, but the rates of growth are slower than in that species.

On Prune agar Stemphylium E has a sporing growth, while *S. ilicis* has, besides a layer of spores, a layer of superficial aerial mycelium.

Comparison of Microscopic Characters of Stemphylium Forms on Elliott's agar.

Stemphylium E.

Differs from *S. ilicis* as follows: both hyphae and conidiophores have a higher average and maximum width; the proportion of long to round spores is very much greater, being 56% compared with 2-3% in *S. ilicis*; the round spores are considerably narrower than those of that species; finer verrucosity is found in this form.

Table XIV. Degree of Verrucosity of Conidia in Stemphylium Forms.

<table>
<thead>
<tr>
<th>Stemphylium Form</th>
<th>Normal or Saltant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td><em>S. ilicis</em></td>
<td>4, 5</td>
</tr>
<tr>
<td>&quot;E&quot;</td>
<td>2, 3, 4, 5</td>
</tr>
</tbody>
</table>

The figures 0 to 5 have the same significance as in Table IV.
Notes on the Genera Macrosporium, Alternaria, and Stemphylium.

The hyphae do not vary sufficiently between the genera, to be of use in diagnosis, but variations may occur in species, as for instance in Macrosporium H. There the hyphae are frequently of great width, also they tend to break up into short lengths. Anastomoses or H-connections have been observed in every form examined, particularly it would seem, where hyphae leave the medium and grow up the glass of the culture dish or tube.

The conidiophores are diagnostic in the genera. They are really only ends of the hyphae or their branches, but are sufficiently defined to be called by the name conidiophores. They are generally wider and darker than the hyphae; the widening may be confined to the region just below the apex as in normal conidiophores of the bulk of Macrosporium species, or the widening may be throughout the length of the conidiophore. The latter applies to Alternaria, still more to Stemphylium and to the greatest extent in the unusual form Macrosporium H. In these cases other characters define the genus, as in Stemphylium where terminal spore formation and continued growth of the conidiophore near the seat of the spore alternate, resulting in a zig-zagged organ, with a nodulose appearance due to the projecting seat of spores. In Macrosporium H, growth of the usual conidiophore here after forming a terminal spore does not frequently occur, so that the organ is not zig-zagged, that is nodulose, but straight or evenly curved.

In culture, when spores form they frequently grow out never so far as seen to give branching hyphae, but short germ-tubes, which may be identical in appearance to the conidiophores. Such germ-tubes may form new spores. In the case of Stemphylium the germ-tube may have the nodulose appearance of a conidiophore, after a few or many secondary spores have been/
been formed.

In **Macrosporium** the conidiophores may grow out, after the fall of a spore, at the scar to form a new conidiophore, and thus a chain of conidiophores is frequently formed. In hanging drop cultures, of tap water, germination takes place in two to three hours in all forms. Almost invariably the germ-tube grown out rapidly, but they may bear a few secondary spores before branching takes place. Fig. 31 on Pl. IV, shows a conidiophore of **Macrosporium** germinated in tap water to give several short germ-tubes bearing secondary conidia; there it is seen growth may continue after a spore is formed, and the latter is pushed to the side, in a **Stemphylium** or **Alternaria** manner.

There is no evidence that either **Alternaria** or **Stemphylium** has an ascus stage. **Macrosporium** H is an exception in that genus in having an ascus stage.

An unusual and interesting manner of continuing growth has been observed in four of the **Macrosporium** forms. Growth there takes place from an intermediate structure, either conidiophore or spore, or a cell of either, and continues through the parts beyond. Examples have been chosen and figured.

Plate I, fig. 3 illustrates the case of continued growth taking place from an intermediate cell of a spore (in this case of abnormal shape and septation), through the remaining part of the spore and bearing a conidiophore-like germ-tube beyond.

Plate II, fig. 18 illustrates a case comparable with the last, but in a conidiophore. The subterminal cell shows an early stage of continued growth into the terminal cell, through which it would probably have grown.

On Plate II, fig. 13 and 14 each show continued growth of a conidiophore terminally through the spore it has already borne. In fig. 18 a cell of the conidiophore is growing into a spore it has just borne (laterally).

Plate III, fig. 19 shows continued growth from a spore through/
through a further spore which the first had borne; in fig. 20 similar growth from the lowest spore of a chain takes place through the two distal spores.

It is noteworthy that there is a conidiophore-like structure at the end of most of the newly developed structures.

The occurrence is probably due to the cell showing continued growth being more turgid than the cells through which it grows. Miyabe (52) in dealing with *Macrosporium parasiticum* Thüm. had observed similar cases. He found conidiophores borne at old scars, as have been seen in the present forms, but he states: "a far more common form ...... was one where a new growth took its origin not from the swollen cells, but from the cell next below"; he illustrates such a case in his fig. 3.
D. Taxonomic Characters of Macrosporium, Alternaria and Stemphylium.

This subject has been studied by Elliott (37) and Bolle; it will therefore only be necessary here briefly to review the literature, and to state the confirmations or modifications in the conclusions of the authors mentioned, which are suggested by the study of the forms now being dealt with. The present results are based on the forms mentioned on page 8, and it will be observed that of these Pleospora herbarum Pers. Rbh., Alternaria tenuis Nees, Stemphylium ilciis T11., were isolated and identified by Bolle, and were received by the present author, through the kindness of Westerdijk, from the Centraalbureau voor Schimmelcultures.

The genus Alternaria dates back to 1817, when Nees (33) described A. tenuis. The species called Macrosporium by Fries belong as has been pointed out by Elliott (37) and by Bolle (II) to the genera Sporidesmium which had previously been described, and to Alternaria; Berkeley's (7) description of Macrosporium sarcinula agrees with our own conception of that genus, and therefore he is the author of the genus Macrosporium as we understand it.

Among the distinctions between the genera Macrosporium and Alternaria, the occurrence of chain-formation of the conidia in the latter genus has been given first importance in the literature. The study of the forms of these genera emphasises the fact that no significance can be attached to the character at all. Bolle's Pleospora herbarum and four forms of Macrosporium have been studied along with the Alternaria tenuis, and three other forms of that genus. In both genera each form has formed chains of conidia perfectly regularly. A chain of conidia was observed by Elliott in one culture of M. sarcinula, Berk. and by Bolle once in a culture of that species and twice in one of M. sarcinaeforme Cav.

The form of the conidia is the best indication of the genera. In Alternaria (Fl. V, fig.40) the conidia are much longer than wide, also the attenuation of the apex is characteristic. The latter feature is not constant in all the spores of a culture, e.g. of A.
A. tenuis, but its occurrence in many of the conidia is diagnostic. The septation in Alternaria is distinctive, since there are a number of definite transverse septa, the number depending on the length of the spore, but few longitudinal septa. The first septum, transverse, loses its identity on others being formed.

In the genus Macrosporium, on the other hand, the shape of the conidia (Pl. I fig. 1) is oval at first, but becomes oblong, and they are not much longer than wide. Also the septation is characteristic; in the great majority of older spores in a culture of this genus the identity of the first formed septum remains distinct, being definite and having the spore wall constricted round it to a greater extent than round other septa. On the first septum being formed, the two cells rapidly become muriformly divided. The degree of verrucosity has no generic importance.

The type of conidiophore is of considerable diagnostic value. In Macrosporium the end cells of a fertile hyphae become marked out by becoming thicker walled, darker and wider just below the apex. The important exception is Macrosporium H from Echium vulgare; there the club-shaped conidiophore is present only exceptionally, while a very long dark thick walled structure (Pl. Figs.17,18) takes its place. It must, however, be noted that such structures have been found much modified, and only differing from the hyphae of that Macrosporium form in one character; also short stout conidiophores are frequently seen, (fig.17) resembling similar conidiophores in the saltants of the other forms (e.g. Pl. I, fig. 6).

In both genera, a conidiophore may continue its growth. In Alternaria the new growth takes place near the point of attachment of the first spore. In Macrosporium, elongation of the conidiophore takes place almost invariably from the point of attachment of the first spore, the latter being pushed off in the process; but elongation similar to that in Alternaria has been observed on many occasions - it occurs more frequently than the literature would lead us to expect. Variations in connection with conidiophores, spores, and/
and chains are recorded elsewhere (pp. 56–58).

Bolle has stated it as a distinction that *Macrosporium* always forms perithecia, while in her *Alternaria* cultures no ascus-form appeared. The *Alternariae* now studied agree with those of Bolle in never having formed perithecia; but *Macrosporium* H as stated in the chapter on perithecia (p. 35) never has shown that stage. The influence of saltation on perithecia-production is discussed later (pp. and ).

As regards the genus *Stemphylium*, Elliott (57) combined the forms now known as *Macrosporium* and *Stemphylium* under the latter name, and used the former name to cover the forms coming under the name *Clasterosporium*. The present writer however fully agrees with Bolle that the genus *Stemphylium* is quite distinct from *Macrosporium*.

The distinguishing characters of *Stemphylium* are found in the conidiophores and conidia. Wallroth (79) in his phrase "Hyphae simplicissime, brevis, articulatae, noduloseae", referred to the characteristic conidiophores. The nodulose character is explained by fig. 32 of plate IV, and fig. 36 of plate V. The small elevations are the seats of detached spores. This character has been observed in *Alternaria* and *Macrosporium* also, but to a much less extent, being rare in the latter genus. The conidiophore is darker, thicker walled, and much wider as a rule than the hyphae giving rise to it.

There are two forms of conidia, round and long present in the *Stemphylium*, the relative number of each kind apparent by depending on the species. *S. ilicis* was found to have 2–3% of long conidia in a ten day culture on Elliott's agar while *Stemphylium E* had 64% of long conidia in a similar culture. Intermediate shapes occur, but these are considered to be undeveloped long conidia. The round conidia are distinguished from *Macrosporium* spores in being divided by two septa, which are generally at right angles (Pl. IV. fig. 34). The long conidia can be distinguished from *Alternaria* by being generally rounded at both ends; also where one end is attenuated, it is the basal end. A pointed apex to a long spore can however occur; such a spore is shown in plate V, fig. 36. Bolle has also united *Hyatrosporium* Cda. with *Stemphylium* Wallr.; and *Sporidesmium* Lk. with *Clasterosporium* v. Schwein.
III. SALTATION.

A. Saltation in Macrosorium, Alternaria and Stemphylium.

The term saltant is applied here to variant growths, usually showing in the form of sectors, which have appeared in cultures, and which differ from the remainder of the growth in one or more characters. Such cultures have all been mono-spore cultures, or have been derived from such a few generations previously.

A variant growth has not been called a saltant unless it was subsequently isolated and fixed in its characters - but allowing for the fact that saltants have on occasion themselves saltated to give still another form. (No particular meaning is attached to the word 'form').

With one exception, no strain was observed to saltate until the fungus had been grown in culture for several generations. Again with one exception, each saltant has been observed and reisolated on more than one occasion.

Though variant sectors may appear perfectly distinct from the remainder of the growth, it was repeatedly found, especially at the initial stages of the work on saltation, that transfers made by taking scrapings from the variant could not be relied upon not to contain some of the remainder of the growth. Such cultures from scraping transfers have thrown sectors, with the same type of growth as that from which the transfer was taken. For this reason single growth units, usually spores were used to isolate the saltants themselves; where the saltant has few or no spores, a single hyphae has been used in making the transfer.

In the earlier stages of the study on saltation, the saltant forms were grown in the same Petri dishes as their immediate parental form; environmental conditions were thus the same, and the drawing of conclusions as to difference in the growths was thus permissible.

Pure stock cultures of the normal and saltant forms were retained; as an important precaution where two forms were grown together in one Petri, neither growth in the dish was considered sufficiently pure to be subcultured. The saltants themselves/
themselves have been repeatedly transferred to new culture media by using single spores or single hyphae.

Where a saltant has been itself observed to saltate, both forms have been subcultured from single growth units. The process of sub-culturing from units, was discarded when the study had been completed.

The distinctive characteristics of each saltant have been made from cultures grown along side and under the same environmental conditions as the normal form of the strain.

The necessity for some of the above precautions was not at first apparent, and the earlier part of the work was essentially a refinement of the technique. Reference to some of the literature on saltation makes it very clear that a statement of the methods employed are equally important as the results obtained, if the interpretation of these are to carry conviction.

In the genus Alternaria "Variants considered as due to mutation" has been recorded by Roberts (61), who observed it in single spore cultures of A. mali and of an Alternaria from lilac leaves, and also in an Alternaria from Blackberry; the latter is stated, however, not to have been a single spore culture. Roberts obtained no "mutation" in single spore cultures of A. tenuis, but a case of variation, considered as saltation is recorded now. In the genera Macrosporium and Stemphylium no instances of mutation or saltation are known to the present writer, apart from those now recorded.

The cases of saltation now recorded occurred in some of the strains of the three genera dealt with previously in this paper, and therefore occurred in single spore lines. When the strains concerned had been in culture for some months, variants appeared along with the normal growths. The Macrosporium strain "A" was an exception, in that it threw a saltant in the second generation. The variant growth was, as already stated, generally in the form of a sector, and though it was quite unusual for the sector to commence anywhere near the centre, it has on one or two occasions commenced very close to it. A feature of the sector is that the two straight sides form a more obtuse angle than/
than they would if they lay along radii; that is to say, the variant growth grows out laterally relatively faster than the remainder of the growth. Also a variant sector may show almost an entire absence of growth. A culture may show a number of sectors; also one culture may throw more than one kind of variant, that is more than one saltant; in the case of *Macrosorium A*, two saltants were isolated from a single spore culture of the normal form.

Where a saltant differs from the normal type of growth in having a considerable larger quantity of mycelium, it may appear over the surface generally, as well as in the form of sectors; such "mycelial saltants" have been observed in all three genera.

The above manifestations of the saltant only refer to cultures made from single growth units, such as spores; Where other and irregular methods of manifestation of the saltant have been observed, it has been noted that the transfer from which the culture is made has been a scraping; and it is concluded that saltation has occurred, probably late, in the previous culture, though it was not observable, and that the inoculum has consisted of a mixture of original and saltant types of growth. In such cultures the saltant has been found at any part of the growth, even at the centre. With continued subculturing of growths by scraping transfers, it has been found that the saltant form may become the dominant one, and the normal form may simply show as sectors or even as isolated areas in the saltant form.

The same criteria have been used in investigating the saltants as were used in studying the normal forms though only one medium, Elliott's agar, has been used. The cultural characteristics which follow can thus be compared with those given earlier.

The genera *Macrosorium*, *Alternaria*, and *Stemphylium* are considered separately.

**Saltation in Macrosorium.**

Of the five forms of *Macrosorium* investigated earlier, four have saltated; the fifth, a form from *Echium vulgare*, known as *Macrosorium/*
Macrosporium H showed a variant in culture. This latter case will be discussed later as the variant form was not isolated.

The Macrosporium forms which saltated are Pleospora herbarum, originally isolated by Bolle, and obtained originally from Westerdijk, and three strains of Macrosporium known respectively as A, B, and Td, originally isolated from tomatoes showing rots.

Elliott's Agar.

Pleospora herbarum.

This form threw two saltants a and b; saltant b was also obtained from a.

Saltant a.

On Elliott's agar this agrees with the normal growth of P. herbarum in most characters, but differs in several. The growth is white by reflected light, and is of a slightly grey colour and translucent by transmitted light. The central part is no darker than the rest here. The light colour of the saltant is due to there being few conidia. The margin is not definite till very late, when perithecia appear. The perithecia occur, as in the normal growth on the central half of the area first, but soon appear all over.

The growth is much slower than in the normal form, being 5.5 m.m. per day.

The hyphae as a whole are fairly clear, and not very darkly coloured. The average width is 5μ, the maximum observed 8μ. They are rather narrower than the hyphae of the normal form, and abnormally thick hyphae have not been seen.

Irregularities in the conidiophores have been noticed as in the normal form. They stand out from the hyphae, under the microscope, being darker and wider. The average width is 7μ, the maximum observed 8μ. As usual they are wider than the hyphae giving rise to them; but like the hyphae of this saltant they are narrower than in the normal form.

The conidia are as usual darker than the mycelium and conidiophores. There are a comparatively small number of conidia; they are slow in development, and therefore as a whole appear lighter in colour.
colour than those of the normal form at a similar age. The fully
developed conidia are however as dark as those of the normal culture.
In verrucosity they resemble the normal form, that is, the verrucosity
varies from fine to rough. Many of the abnormalities found in the
normal conidia have been seen in the saltant. A case of a spore
giving rise to a conidiophore, and this to a second conidiophore was
noted (Pl. III, fig.23). The size of 100 spores was found to be
12-28 x 7-16\mu.

Saltant b.

The appearance of this saltant is a loose pulvinate growth of
mycelium, 2m.m. high at the centre. There are scarcely any conidia
present, and the growth is white and transparent, except in parts,
particularly the centre, where the hyphae are massed and darker.
Perithecia are formed, but the lateness of their formation is a dis-
tinguishing character of this saltant. After two weeks 1 culture of
this saltant still showed no perithecia, another showed only four;
whereas in the cultures of the normal forms, and of the Saltant a
perithecia were seen in eight days. After four weeks the Saltant b
had only a few perithecia, while the normal and Saltant a forms had
perithecia in considerable numbers all over the growth. In two months
the perithecia appeared in the Saltant b in large numbers, but as the
medium was beginning to dry up, they were small and immature. The
growth is slower than in the normal or Saltant a forms, being 4.1 m.m.
per day.

The mycelium is light in colour, and resembles that of the Saltant
b in appearance. The average width of the hyphae is 5.5\mu, the maximum
observed 8.5\mu.

The conidiophores are frequently formed in series. There are
not a great many spores and there are few conidiophores, insufficient
in each to give accurate average dimensions. The average width of the
conidiophores seen is 7.5\mu, and the maximum 8\mu. Several conidiophores
were seen in series.

Saltants of Macrosporium A.

Saltant/
Saltant a. (Pl. fig. 44).

This Saltant is quite distinct from the normal form. The growth is subfetly, it is denser, rather deeper and darker than the normal form, but its chief distinguishing character is the constant radiating strands of mycelium, which run from the centre to the margin. The growth is more opaque than the normal, but is translucent on the outer 1 cm. The dark colour is due to the presence of many conidia; the mycelium, indicated by the dark strands is in fair quantity and prevents the powdery appearance seen in the normal. This saltant has a thin layer of superficial mycelium, but it soon collapses. Perithecia produced but in small numbers and they are formed much later than in the normal forms. The rate of growth is 6.9 m.m. per day.

The hyphae are long and straight. A feature mentioned below is the many very short branches acting as conidiophores. The hyphae are mostly fairly dark. The average width is 4µ, the maximum observed 7µ.

Conidiophores.

A marked feature in this saltant is the very short and very thick conidiophores (figs.5,6). It is unusual here for the conidiophores to take on characters of the spores, but cases have been observed where a conidiophore has become thicker walled, of a darker colour, and with thicker septa than in usual in the conidiophores. The average width is 6.5µ, the maximum 8µ. It is very usual for the conidiophore to take the form of very short stout branches to the hyphae (fig.6) in which case the hyphal cell which swells out to form the branch is bounded by close end walls. A normal or a short lateral conidiophore, after bearing a conidium may continue its growth as a hyphae, which accounts for short thick walls, dark, swollen cells occurring in the length of the hyphae. A case was noted where after bearing a spore, the conidiophore grew out not at the scar, but on one side.

Conidia.

Budding/
Budding of the conidia to form chains is very marked, so that in a scraping, spores in all stages of development are seen. Budding is seen in fig.5. Verrucosity is not marked, but most of the more mature conidia bear an even fine verrucosity. The size of 100 spores was 13-19 x 10-17μ.

Saltant b. (Pl. VI fig.45).

This growth has more mycelium than the normal and is not so powdery; it is therefore lighter in colour, but of the same transparency. There are however many spores present. There is no thinning out of the growth from the centre towards the margin as in the normal growth. But the growth here has a blotchy appearance, due to massing of conidia in parts. The margin is very irregular, having wide lobes. The dark sporing area stops abruptly at 1 cm. from the margin which is indefinite and transparent. Perithecia form in considerable numbers but very much later than in the normal growth. Being formed late, they are small. The rate of growth is 5.1 m.m. per day.

This saltant very much resembles Saltant a under the microscope, having many thick and fairly uniformly dark hyphae; they are wider here than in a however. Th average width is 5μ, the maximum observed 10μ. Also there are here the same short, wide conidiophores seen in Saltant a. There are the same methods of forming conidiophores and conidia. Further growth of a conidiophore along side a conidium, without displacing the latter has been seen in this saltant. The average width of the conidiophore is 7.5μ, the maximum observed 8μ. There is the same tendency to budding and chain formation as in Saltant a and there are many conidia.

Though macroscopic differences between this saltant and a are quite apparent, these two saltants cannot evidently be distinguished under the microscope, except by the abundance of very thick hyphae, and the rather larger and less stout conidiophores in Saltant b.

The size of 100 spores was 13-23 x 10-19μ. The verrucosity is similar.
similar to that in the other forms of this strain, being fine in most of the spores.

Saltant c. (Pl. VI, fig. 46).

This is quite distinct from the normal growth of the two previous saltants. Pulvinate, 3 m.m. high at the centre, and 2 m.m. high over the greater part of the area. Very loose woolly growth, which does not collapse so soon as the white saltant of S. ilicis, since it had a loose mycelium for almost three weeks. The edge is even, but not definite, because it is adpressed and thinned out gradually. The colour is white, and the growth is almost sterile for several weeks. Conidia appear after a few weeks, but in comparatively small numbers, and they are confined almost exclusively to the marginal region, which gradually becomes slightly dusty. Perithecium likewise are produced very late; after three weeks they are still absent but after that begin to show in small numbers, and like the conidia, they are found only on the marginal 1/6th to 1/4 of the area. Rate of growth is 9.0 m.m. per day.

This is a mycelial saltant; there are few conidia present, enough were however found to give a fair average. The hyphae are all hyaline and without any colour; the average width is 4μ, the maximum observed 8.5μ.

The few conidiophores which are present are coloured rather darkly like Saltants a and b. They are however mostly rather longer than those of Saltant b, being club-shaped and usually two celled (Pl. II, fig. 12). There are a few with a Saltant a character, short, wide and one celled. The average width is 7μ, the maximum observed 7.5μ. Some of the hyphae show swellings also noted in Saltant 4, and which are taken to be conidiophores, checked in their growth and producing no spores. The verrucosity is fine as in the other forms of this strain A. Budding of the conidia to form chains has been noted.

Saltant d.

There are less marked differences between this and the last saltant than there are between any other two growths (Normal and Saltants) of/
of *Macrosporium A*. These differences are however sufficiently marked and constant for this present form to be considered another saltant. The height, form, and white colour are the same as in the white saltant. A marked character here is the exceedingly small number of conidia.

The first culture of this form was from a septa in the normal growth which showed a pink colour; spores were never seen during several subcultures, so subcultures were made from small scrapings of mycelium. The pink colour appeared in the first subcultures, and with the pink, there was also a blue colour present, and associated with these was a very definite change in the colour of the medium to an orange brown, especially below the central half of the growth. Conidia were however seen in one later culture, so in order to establish that they belonged to this saltant and to get a 1-spore line, several of these were cultured separately. Each growth which resulted had the pink and blue colours present in the growth associated with the colour of the medium. One of these 1-spore cultures has been subcultured through several generations and so far the colours have been almost constant in each. It is thought that previous to finding conidia, the cultures were a mixture of the previous white saltant and of the colour producing form.

Another character of this colour producing form is the very scanty production of perithecia. They may begin to form in two to three weeks, but have only been formed in very small numbers at parts of the margin.

Though a 1-spore line was established, conidia are apparently absent in cultures or are produced in exceedingly small numbers — not sufficient for a fair average of size etc. to be obtained. The rate of growth is 8.0 m.m. per day.

This is a mycelial saltant. No conidia were seen for a number of generations, and were only seen in very small numbers in fertile cultures.

The hyphae are hyaline or pale in colour; their average width/
width is 3.5μ, that is less than in any other form studied, and the maximum observed 8μ. The conidiophores are practically absent. A feature of this saltant, seen to a less extent in Saltant c is the occurrence in a considerable number of the hyphae of club-shaped cells, rather wider than the normal cells and just a little darker. These are considered to be conidiophores, not fully developed; and are more reduced than those of Saltants a and b. Fig.8 on Pl.I shows part of a hypha which had six such swellings.

Since a relatively very small number of conidia are formed, the formation of these conidiophore-like swellings must be followed immediately by continued growth as a hypha which can again swell out in the same manner at the tip. Such swellings when seen in Saltants a and b were generally associated with conidia, that is to say the terminal swelling bore a spore or a distinct scar.

Macrosporium B.
Saltant a.

The growth is close and woolly, even and translucent. The colour is white; the growth is chiefly mycelium; there are not a great number of conidia. The mycelium does not collapse for 19-21 days. Perithecia are produced but not in large numbers, and they are very late in forming, only after three to three and a half weeks. The Rate of growth is very slow, 3 m.m. per day.

This saltant appears in the normal growth as irregularly shaped areas of slow growth at the margin; the normal growth may thus have a very uneven ragged edge.

There is much mycelium; the hyphae are very hyaline accounting in part for the light colour of the culture. The average width is the same as in the normal form, 4μ; the maximum width observed was 6μ, a little less than in the normal form. An unusual swelling in a hypha was seen (Pl.II.fig.15); it was dark in colour, and thick-walled, but not verrucose, the septation is irregular.

The conidiophores are few in number, but resemble those of the normal form. Their width is the same, having an average of 7.5μ,
and a maximum of 8μ. There are also a very small number of conidia, thus the dark colour of the normal culture, which is due to conidia, is absent here. The size of 100 spores was found to be 13-30 x 9-19μ. Verrucosity is absent in many spores, and when present is extremely fine. There is a distinguishing feature of the saltant.

Macrosporium Td.

Saltant a. (Pl. VII, fig. 51).

This saltant was isolated from a single hypha, as up till the present no conidia have been seen. The growth is slightly higher (being 2½ m.m.) than the normal, and only falls away near the edge. The growth though all mycelium is feltly, and begins to collapse comparatively early - after eleven days. The edge is irregular indefinite and adpressed, but not radiate. The colour is at first white all over, but from the second week onwards the mycelium darkens to "grey" (359: 4). The opacity round the centre is due to darkening of the medium; this also occurs in the normal culture, but to a greater extent. Perithecia produced from the third week onwards, that is they are late in forming; they are produced in small numbers, compared to the normal culture, and are found almost entirely on the central half (as in normal). The rate of growth is 7.2 m.m. per day.

The hyphae are very pale coloured; being slightly yellow-brown they can however be made out unstained. They do not vary greatly beyond a certain width, but appear less constant than the normal form. The average width is 4μ, and the maximum observed was 6μ, as in the normal form.

No conidia, nor conidiophores have so far been observed in this saltant. The ability to form conidia may not however be lost, but simply weak or suppressed, judging by Saltant d of strain A, where conidia were only seen after several generations.

Probable Saltation in Macrosporium H.

A variant growth has been repeatedly observed in this form, but the term "Saltant" has not been applied to it since it has not been isolated pure. The normal appearance are tomato agar is, as stated above/
above a close dark felty growth, chiefly mycelium but bearing a number of conidia. When a single spore is subcultured from this normal dark growth it gives a culture resembling its parent; but with repeated transfers of scrapings, a superficial white aerial mycelium appears, as in either of the dark growths seen in fig.52 on Pl.VII. A scraping transfer from the lower dark area of such a growth gives a similar growth, while a scraping transfer from the superficial white mycelium gives a growth with the appearance seen in either of the white growths of fig.52. In the white growth appear dark areas with spores; if one of these spores is subcultured it gives an entirely dark growth; if a scraping is used the result is a similar growth to the parent.

The four growth figured were obtained by taking scrapings from the lower and upper layers of the two types of growth — that is the black superficial with/white mycelium, and white with low dark areas.

The white mycelium apparently has no conidia, or very few. Single hyphae have not been subcultured, and since the white type of growth has not thus been isolated pure, it is not permissible to use the term "saltation" with the significance attached to it in the present investigation.

Saltation in Alternaria.
Saltant a. of Alternaria tenuis. (Pl.VII fig.51).

This is the only saltant observed in this species. This is a deeper growth than the normal, being 2 m.m. high, and only falling off in height near the edge. There are two layers, though both are chiefly mycelium; the lower is subfelty to felty. The superficial layer is extremely loose mycelium present all over the area. The edge is even, abrupt and quite definite; it is not adpressed. The superficial mycelium remains white, but from a few days onwards the lower layer turns dark, "Leaden grey" (353:2), as it becomes denser and forms spores. The central half of the growth is opaque, the remainder translucent. A little moisture forms on the surface. The rate of growth is practically the same as in the normal form, 9.6 m.m. per day.

There appears to be a greater tendency to ramification of the hyphae/
hyphae in this saltant, and the branches are less constantly at right angles than in the normal forms or in the strain R, Ta, or D. The hyphae are very hyaline, more so that in any other *Alternaria* form examined. Their average width is 5μ, the maximum observed 7μ.

The conidiophores, which occur in very small numbers, are also hyaline. Their width is 5μ, but insufficient were seen to give an accurate average.

There are a comparatively small number of conidia. The size of 100 spores was 17-44 x 8-15μ. The verrucosity is moderately rough, and is not so variable as in the spores of the normal form. A number of cases have been observed in this saltant where a germ-tube, after bearing a secondary spore, has grown out at two sides near the point of attachment. This saltant showed a greater tendency of the conidia to germinate in the culture, throwing out germ tubes and forming secondary conidia, than in the normal form.

**Saltation in Stemphylium.**

Saltant a of *Stemphylium ilicis* (Pl.VI fig.47).

This is the only saltant observed in the species. Higher growth than the normal, being 2½ m.m. high. The growth consists of a very loose woolly mycelium, with no differentiation into layers. The edge is even, definite, and adpressed. The colour is white, there being very few conidia. There is a little moisture on the surface of the medium. The rate of growth appears to be slightly faster, 8.8 m.m. per day.

The hyphae are quite hyaline, thus showing up white in culture. Their average width is 3.5μ, and the maximum observed 5μ, as in the normal form.

The conidiophores are coloured like those of the normal forms, and show up distinctly among the colourless hyphae. The distinction here is their very small numbers. The width of those observed did not vary from 5.5μ.

There are a very small number of conidia, but both forms, round and long are seen. They are produced rather late; a gradual change in/
in the colour of the culture from white to light grey is considered due more to a darkening of the mycelium than to conidial production. The verruosity of the spores is moderately-rough, but is not so marked as in some of the spores of the normal form.

General Results on Observations of the present Cases of Saltation.

The saltants differ from the forms from which they have arisen in one or more characters. But no generalisation can be made that saltation always implies a loss of some character or characters, the term "Factor" is not used here, as a significance is attached to the term from a genetic point of view which precludes its use in the fungi.

In the case of Macrosporium A, the saltants a, b, c and d show on the whole increasing differences from the normal form.

![Diagram showing the origin of Saltants a, b, c, and d of Macrosporium A.]

The above diagram shows the origin of the saltants of Macrosporium A. The normal form has given rise to the four Saltants; Saltant a has thrown b and c; Saltant b has thrown c. No difference could be observed in the Saltant b from the normal form and Saltant a; nor in Saltant c from normal, or b or c.

With longer study and more critical methods others saltants might have been isolated, and a series made of saltants, showing a more gradual loss of characters than has been observed.

It is noteworthy that no saltant has thrown any form which occurs before it in the diagram above.

The strains of Macrosporium isolated differed among themselves, and no instance has occurred where any saltant resembled a saltant or the normal form of any other strain. Further investigation/
investigation might have yielded saltants which did so, and if cases were sufficiently numerous, they original strains might have been connected on these grounds.

The Saltants c and d of *Macrosorium A* differ very markedly from the normal form, and in fact agree more closely with Saltant b of *Pleospora herbarum*, and Saltant a of *Macrosorium Td.*
Macroscopic Characters of Saltants Compared with Normal Growths on Elliott's agar.

Pleospora herbarum.

Saltant a.
Has a more even and whiter colour than the normal growth, because of the smaller number of conidia. The growth is much slower.

Saltant b.
A deep clear transparent mycelial growth. There are very few conidia present. Perithecia are formed very late. The rate of growth is thus lowest of the three forms.

Macrosporium A.

Saltant a.
Has rather a deeper growth than the normal; it is denser and much darker in colour and is not so powdery. The mycelium has a very characteristic radiating appearance. Perithecia are produced late and in small numbers. The rate of growth is slower.

Saltant b.
Lighter in colour than normal, due to it having more mycelium; it is not so powdery but the transparency is the same. The density of the growth does not diminish from the centre outwards; apart from a blotchyness it is of an even colour and density. The margin is very irregular. The central dense area ends abruptly and is followed by a transparent indefinite outer 1 cm. Perithecia are found late, but in numbers.

Saltant c.
A high loose woolly white growth. Its conidia form very late and in comparatively small numbers at the margin. Perithecia likewise form late and in small numbers at the margin. There is a slightly faster rate of growth.

Saltant d.
As in Saltant c the growth is high, loose and woolly and mostly white. In some culture there may be, however, a pink and blue colour/
colour present in the mycelium especially at the centre and the medium in such cultures is darkened to an orange brown colour. This characteristic is inconsistent. Conidia are formed late in exceedingly small numbers. They may be absent. Perithecia are also found late in small numbers and are confined to parts of the margin.

**Macrosorium B.**

**Saltant a.**

A much lighter coloured growth, chiefly mycelium with many fewer spores. Perithecia occur later and in small numbers.

**Macrosorium Td.**

**Saltant a.**

Differs in being lighter in colour, and in being a mycelial growth apparently lacking spores. Perithecia are produced later and in smaller numbers.

**Macrosorium H.**

The variant is this form, though not isolated pure, differs in being white in colour and having no or few spores.

**Alternaria tenuis.**

**Saltant a.**

A deeper lighter coloured growth than the normal, and has many fewer conidia.

**Stemphylium ilicis.**

**Saltant a.**

Differs from the normal growth of this species in practically the same manner as does the saltant of *A. tenuis* from the normal growth of that species.
Microscopic Characters of Saltants compared with Normal Growths
(on Elliott's agar).

Pleospora herbarum.

Saltant a.

The hyphae and conidiophores are narrower and not so variable. The conidia are significantly smaller, in length and width; they are fewer in number, and later in developing.

Saltant b.

The hyphae and conidiophores here also are narrower and are not so variable. There are not many conidia.

Macrosporium A.

Saltant a.

The hyphae and conidiophores are scarcely smaller than in the normal form. The conidiophores are generally shorter, and may arise as short branches.

Saltant b.

In microscopic features it resembles Saltant a most closely.

Saltant c.

The hyphae are colourless. There are very few conidia, and they are smaller than in the normal form and Saltants a and b. The conidiophores are two celled.

Saltant d.

Conidia may be absent, or present in exceedingly small numbers. Club-shaped cells in the hyphae is a feature.

Macrosporium B.

Saltant a.

Has more mycelium than the normal form, and the hyphae are more hyaline. The conidia are narrower, and have finer, or no verrucosity.

Macrosporium Td.

Saltant a

Conidia/
conidia are apparently absent. The hyphae are more hyaline.

**Macrosorum H.**

The variant has not been isolated, but apparently differs in having clearer and more mycelium, and few or no conidia.

**Alternaria tenuis.**

**Saltant a.**

The hyphae are more hyaline and tend to ramify more. There is not such rough verrucosity on the conidia and these occur in relatively much smaller numbers.

**Stemphylium ilicis.**

It varies in the same manner as the Saltant of *A. tenuis.*
Discussion:

The results of mycological investigation during past years show clearly that variations in the physiology, morphology, and habits of the fungi are extremely common. This tendency to variation is expressed in various ways. Firstly, in a number of groups of forms fungi are found in nature showing close relationships, yet differing in morphological details which may or may not be considered of specific importance. Such forms if not considered distinct species, are called varieties, strains, races etc.

Briton-Jones (15) isolated Rhizoctonia Solani from various crops obtained from widely separated countries. Of these he states "some of the above isolations are easily distinguishable macroscopically; on the other hand some of these when examined microscopically cannot be separated from each other by any observable character. The microscopic differences observed in some cases are only slight, and it is considered that they do not justify a multiplication of species. This author refers to Matz (50) who separated into different species of Rhizoctonia isolations which differ considerably less than his own two extreme forms.

Sherbakoff (67), working with Fusarium found many forms, a number of which he considered species, others as strains.

Stevens (74), in his study of the Helminthosporium Foot Rot of wheat, found a large number of races or elementary species in H. sativum. 

Next there are the cases of physiological species or biological forms in nature, also known under a considerable number of other terms (see Stevens, pp. 164-5). Such variants in any species, though very similar or identical morphologically differ appreciably in their physiological characters.

Examples in the Uredineae have been studied by several workers, notably Eriksson ( ), Stakman and his collaborators ( )/
species of *H. sativum*.

La Rue and Bartlett (47) state: "it appears that by using a sufficiently refined technique, a nominal species such as *Festaliozzia Guepini* might be resolved into an indefinite number of demonstrably distinct strains, the number depending only upon the precision of the methods. In illustration, we have shown a possible allocation of thirty five strains to fourteen groups, each of which contains one or more strains that cannot be placed in any other group.

Brierley (13) working with single spore cultures of *Botrytis, Penicillium*, and *Stysanus*, showed strains or elementary species to exist.

Burger (19), also referred to later, find that "*Colletotrichum gloeosporiodes* is a polymorphic species made up of a number of strains which give when grown in artificial media distinct cultural characteristics."

Many other cases have been recorded.

Next there are the cases of physiological species or biological forms also known under a considerable number of other terms (see Steven, 74 p. 164-165); such variants in any species though very similar, or identical, morphologically differ appreciably in their physiological characters.

Examples in the *Uredineae* have been studied by several workers, notably Eriksson (38), Stakman and his collaborators (70, 72, 73) and Arthur (2, 3); in the *Erisiphaceae* the subject has been investigated by Neger (34), Salmon (64), and Reed (60).

Again, a number of species has been shown to develop characters in response to their environmental conditions; such may be merely temporary, yet may become fixed if the conditions responsible for the new habit are continued, or repeated sufficiently often. An immediate, permanent and invariable change in the habits of a fungus in response to environmental conditions has not, to the writer's knowledge, been demonstrated.

Lastly/
Lastly there are the recorded variants in a number of fungi to which the terms "Mutant" and "Saltant" have been applied; in these cases the change does not occur throughout the entire organism, though there is the possibility of the new form swamping the normal form, unless the latter is more or less frequently reisolated from the mixture of normal and variant which may result in culture.

In dealing with fungi, as with other organisms, we require in the first place to be able to identify them, and in the next to classify them. Our unit is the "species", but the difficulty of defining our unit to satisfy the requirements of workers in various fields becomes more and more aggravated, firstly as research becomes more critical, and secondly as the field of study widens and the subject is approached from different sides.

It has become impossible for the physiologist invariably to give the same definition to his unit species as does the morphologist, or for either of these to define his unit to satisfy the pathologist, ecologist or geneticist. There must be plasticity in the term species if it is to be used by workers in all these fields; but it is highly advisable that within any one field sound criteria be used in defining the unit.

The variations which occur in the fungi will only be accounted for, and their significance understood, if they are studied from the proper angle, - which may be genetic, physiological, etc. The best line of attack is not certainly at once obvious, and it may easily happen that the significance of variation in an organism will be best understood by its investigation along more than one line.

The morphological criteria in the description of an organism imply definite and stable characters; distinctions between forms must be sufficiently marked to warrant multiplication of species. Studied physiologically, the species of the morphologist may prove capable of being subdivided. The pathologist may find it of practical importance to consider finer units than the morphologist, as for instance, biologic forms, which to the morphologist agree sufficiently according/
according to his criteria to justify their being grouped together. The geneticist working in a newer field and with still finer units is as yet unable to explain in many cases the variations in the species of the morphologist and physiologist, - variations probably due in many cases ultimately to differences in genetic constitution.

Skully, Harper, Reed and Stakman in a symposium (68) on the species concept, describe the nature of the units which each worker requires as a morphologist, physiologist, etc., but each recognises the importance of the units used by the others. To quote Skully, who speaks from the point of view of a geneticist: - "the fact is that although genetical phenomena form the basis of nearly all biological classification, there is no genetical criterion - nor any other criterion - of specific difference, which is found generally applicable or generally accepted". Also; ".... it seems proper to insist that utilitarian principles should be crucial in the establishment of new species and the maintenance of old ones".

Among variations recorded in the literature, some are recognised by the authors as belonging to one other of the types mentioned above (p.81-82). A few are doubtful. As regards mutation, this term has been variously defined by different authors to suit their own requirements.

To quote Steven (74, p. 178-158): - "The term mutant is defined by Dobell (34), following Wolf (81), and Baur (5) as follows: - "By mutation, accordingly, I mean a permanent change - however small it may be - which takes place in a bacterium and is then transmitted to subsequent generations. The word does not imply anything concerning the magnitude of the change, its suddenness, or the manner of its acquisition. The term denotes a change in genetic constitution. All other changes which are impermanent - depending generally upon changes of the environment - and not hereditarily fixed, are called modifications. The word "mutations" has been used with such different meanings by so many bacteriologists and others, that the foregoing statement seems called for ".

A mutation, according to Brierley (14) is a "genotypic change
in a pure line ", and according to Vaughan (77), as "Those changes in form or function which persist through one or more generations after the cause of the alteration has ceased to operate ".

The first two definitions presume more knowledge of the genetic constitution of micro-organisms than can be universally applied. Vaughan's definition of mutation agrees more closely with Dobell's definition of a modification.

Stevens (74) and Brown and Horne (18) used the term "saltation" to describe the phenomena of variation which occurred in their cultures of Helminthosporium and of Fusarium respectively. It is in their sense that the term is used here.

Both Bauke (4) and Miyabe (52) were unable to observe any sexual process connected with the formation of the perithecia of Pleospora; the latter author states "The formation of the perithecium is entirely of a vegetative process, which resembles essentially the formation of pycnidia ".

The importance of the presence of a sexual stage at all in Macrophomina, after all is small, since similar types of variation in culture has been found in this genus and the apparently asexual genera Alternaria and Stemphylium; it is recognised however that the latter two genera may have had a sexual stage in the not distant past. So far as our knowledge goes, Brierley's definition of a mutant cannot hold here, as the requirement of "pure lines", which involves definite knowledge of the homozygosity of the organisms is wanting.

Stevens states of his Helminthosporium variants "Since the variations herein reported occur in structures purely vegetative and result from no intervening sexual act, there are in kind comparable with vegetative variation known elsewhere - bud variation etc. - with the exception that since the mycelium, consisting of a single row of cells, is the seat of origin of the variations the case is morphologically simpler than where tissues are involved, as in bud variation... Many examples of vegetative variation has been studied extensively and reported upon under the terms mutation, saltation, sporting, etc."

Benedict /
Benedict (6) applies the term saltation to variants in the Boston fern as the variations were "discontinuous and of considerable magnitude". He describes his saltation as "orthogenetic" "since these variations occur in definite series along a few limited lines".

As stated, in Alternaria and Stemphylium, no sexual stage has been recorded, but in Macrosporium a sexual stage, of which we know very little, is present in the forms worked with by Bolle (11) and the present writer.

With regard to the variants now recorded in these three genera, they may not be considered mutants, because of the lack of sufficient knowledge concerning their genetic constitution. The term "strain" in any of its various meanings is not used for variants which occurred in culture for the following reasons: the variants are produced suddenly; they are not invariably produced under the conditions which permitted their first production; so far as can be seen the variants are permanent changes, and though they may be influenced to a small extent by other conditions, they at once exhibit their variant characters on being returned to their original conditions. Because of these same characters, these variants are called saltants. It is not considered that these characters will hold invariably in the fungi, for in the light of further research modifications or additions will be found advisable. But a term to cover the changes is necessary, and the explanation of the term given above will explain the author's meaning.

Other records of variations in the fungi are as follows; the terms used below are those given by the respective authors.

Edgerton (36) in 1908, on making a dilution culture of Glomerella rufomaculans obtained two kinds of colonies. He found the new form "considerably different from other known forms". "The typical form produced perithecia in nodules, scattered over the media....; but the new form produced them singly, or occasionally in twos or threes scattered over the plate". They "developed in such/
such abundance that all of the nutrient material was used up before they could mature. "The perithecia however, so far as they could be studied, were identical with the typical form". "Mutations, so far as is known to the writer, has not previously been recorded among fungi, but the form just described seems to be one without question".

This is an interesting record, since it is the first report of mutation - in that author's sense of the term.

Stevens and Hall (75) in 1909 obtained variant sectors in plate cultures of *Ascochyta Chrysanthemi*. Stevens (74) in 1922, referred to this variant; "since the study was not made from single-conidium isolations it is possible, though not probable, that I had merely a segregation of elementary species". The variant was considered a saltant.

Shear and Wood (66) worked in 1913 with *Glomerella*, found that sudden variations which occurred in single ascospore cultures ran true for three generations.

Crabill (31) in 1915 working with *Coniothyrium pirinum*, obtained two types, which he calls plus and minus strains. The latter he considers "a sport or mutant arising from the plus strain at irregular and unprognosticable intervals".

Bonar (12) in 1921 found a variant sector in one of a series of cultures of *Brachysporium trifolii* which he had isolated from a single spore two years previously. The sector completely lacked the usual dark brown colour, although the mycelium and the conidia were identical in all other respects.

Stevens (74) quotes from a personal letter received from Edgerton: "All of the cultures that I used in that work were obtained by the dilution plate method and presumably came from single spores" and he states "his apparent variations may have been due - though it is highly improbable - to segregation of elementary species".
with the normal growth. "Pure cultures of the albino material were carried on through sixteen consecutive non-sexual generations without any variation in the appearance or nature of the strain". "The phenomenon of albino mutation must therefore" because of the non appearance of a sexual process in the normal or albino strains" be referred to some sudden change, hitherto inexplicable, in the mycelium or conidia of the normal strain".

Blakeslee (10) in 1920 obtained a number of variants in colonies of Mucor geneviensis. Most of these tended to revert to the normal type, but two appeared to be stable.

Brierley (14) in 1920 recorded his albino mutants of Botrytis cinerea, from a study of which he gave his definition, mentioned above, of the term mutation.

Dastur (30), also in 1920, found that his species of Gloeosporium piperatum, in culture, gave marked variations in their development. The following extracts are interesting:-

"By continuous subculturing on glucose-meat-extract-agar the conidia bearing faculty was gradually lost and the cultures became sterile, but if these sterile cultures were transferred to sterilised chilli stems, the conidia forming capacity was regained.... This process of getting the conidial stage could not be carried on indefinitely ". The loss of the conidia bearing faculty was ultimately complete and transfers on sterilised chilli stems gave only sterile aerial growth". "The perithecia-producing faculty does not depend on the nutrient medium on which the fungus is grown but depends on the race or strain....."This faculty is not fixed hereditary characters but is lost by culturing successive generations on the same medium at room temperature." He found variation in the virulence of the form, conidial, sterile and perithecial which he obtained from one culture.

Burger (19), whose strains in Colletotrichum have been referred to already, found that "Variation in the strains were caused by environment; some are considered due to mutation. These/
These mutations have kept their peculiar characters, although grown under the same conditions as the cultures from they arose. In his single spore isolations he obtained a mutant differing from the normal form in having white mycelium instead of black.

Stevens (74) in 1922, working with single spore cultures of race of Helminthosporium found 126 variant sectors, and he states that this number might easily have been doubled or trebled. These variants are considered saltants, possibly mutants. A considerable number of saltant characters are given; "Certain saltants differed so markedly from their parents as to far exceed the usually accepted specific limits". Some interesting correlations and tendencies of characters in saltation were noted, such as the association of slow linear growth with high conidial production, etc. A large proportion of the saltants were permanent in character, though occasional apparent reversions were found to occur.

Dickson (32) in 1923 found saltation in the Black Dot organism of potato, of which his study was not complete, but which he places in the Colletotrichum- Verdealia- Volutella group. A single conidium culture developed variant sectors. He obtained two saltants with "1. All conidia only, and 2. Conidia profuse, immature sclerotia". In 1925 the same author reports (33) that his saltants have remained constant.

Roberts (61) in 1924 applies the term mutation to variant forms he obtained in single spore cultures of Alternaria mali, and in an Alternaria he isolated from Lilac leaves. These appear to be the only cases of the phenomenon hitherto described in the genus. Roberts' cultures have not been seen by the present writer, but as a result of the present work, a modified interpretation is offered to Roberts' results. Roberts' cultures of Alternaria mali were originally made from a single conidium. In one plate culture he obtained a section of the growth, which he calls A differing from the remainder, which he calls B. "Transfers were made weekly from the growths of previous transfers showing the greatest growth of A and B respectively". "From the first selection A showed but little tendency to/
to break up into A and B. After the tenth selection it came true until the cultures were discarded. B broke up into A and B sections with great constancy during the first 57 selections, though the B parts of the growth were usually much the larger. From the fifty-sixth to the sixty-ninth and last selection B remained constant”. Photographs of three culture plates are referred to. His explanation is as follows: “We have thus two races arising from a single conidium, neither of which in culture is exactly like the parent, while B fails to produce the thin carpet like layer bearing characteristic conidia, producing, instead, the long slender spores at or beneath the surface of the culture medium. B grows more rapidly than A. There was no chance that A and B were from a mixed or contaminated culture, for they were not only the progeny of a single conidium, but of a succession of singly selected conidia”. On p. 707 he says “It should be noted that the measurements of race A and the parent are not greatly different”.

In the first place it is not clear if the races mentioned above were subcultured by taking scrapings or single conidia. If the latter were the case then it could only be said here that the phenomena in these cultures are different from those which have come under the observation of the present writer. In each of the two species Alternaria tenuis and Stemphylium ilicis of the present writer, cultures have been found showing two types of growth, each differing from the parent culture in the same way as Roberts’ A and B races differ from their parent culture. In both species the two differing growths have been subcultured both from scrapings and from single conidia. When scrapings are used the resulting growths each show both forms distinctly or they resemble the parent, and just as in the case of A. mali the mycelial form shows less tendency to give the sporing form in part of its area, than does the sporing form to give the mycelial. Roberts found his mycelial form break up into A and B with great constancy during 57 generations, after which 11 generations remained constant. In both A. tenuis and S. ilicis the white forms were isolated in the first subculture made from a single one of the few conidia.
conidia present in this form, and these white growths remained constant.

In A. tenuis and S. ilicis the sporing forms have behaved like race of A. mali, though subcultured through fewer generations. When such sporing forms have been kept a few weeks they have almost invariably thrown the mycelial form, which has been repeatedly isolated, and obtained pure, from single spores; the sporing forms in the present case cannot be relied on to remain constant, i.e. not to throw the other form.

(A difference between the forms obtained from A. mali and those obtained from A. tenuis, is that in the former the conidia of the mycelial form B are larger than the conidia of the sporing form A, whereas in the latter the reverse holds true).

In the case of A. tenuis and S. ilicis, it is concluded that the sporing form is the normal growth of the species, that the white form is a saltant from it, and that cultures showing characters of both are mixtures of the normal and the saltant forms of the species. Without having worked with Roberts' species A. mali it is not possible to infer that the same phenomena are taking place there. On the assumption that Roberts' isolations of the two forms were not individually made from single spores, the present writer is persuaded that scraping transfers have consisted of both forms in making successive subcultures, until, in the case of the mycelial form, a uniform growth was obtained. And on the same assumption the description of these would lead the present writer to suggest that the same phenomena are taking place there as in the two other species, and that the same interpretation may be made as in the present case.

Brown and Horne (18) in 1924 recorded that in six strains of Fusarium isolated from apple, a considerable number of saltants in monospore cultures were obtained. These cultures are placed in four groups according to their type of growth. The authors state: "Typical members of all the groups can be derived from a single parent/
parent. Further, each of the six original parents - which were obtained from nature and of which the relationships are therefore not known - gives an assemblage of forms the members of which inter-digitate with those derived from the others. Hence it is concluded that all the forms now in cultivation are derived from a single original parent."

This is probably one of the most interesting investigations on saltation, as the conclusions arrived at in the case of *Fusarium* may hold in other fungi, and serve to account for the appearance of strains, microspecies, etc, in nature generally.

Leonian (48) in 1925 has reported saltation in *Phytophthora parasitica-rhei*. For nearly seven months after this species had been received it behaved normally. After that colonies showed distinct forms, and four new forms or saltants were obtained. Three saltants were found to remain constant. Another which the author calls type IV was able to give rise to his original form, which he calls type I.

Leonian's text fig. 2 shows the many lines of descent from a single-sporangium culture of his type II, but he does not record whether any subculture figured in the chart has been made by using single-sporangium inocula. His type I continually throws type IV, but he found that the latter could as readily throw the former, and concludes: "A more complete example of reversion could not be pictured.

That may indeed be true, but the criticism applied to Roberts' account of saltation in *Alternaria mali*, must hold here; that is to say, we must know that Leonian's saltant type IV has been itself isolated by taking a single unit, in this case a single sporangium or hypha, before we can be sure that the so-called reversion is not accounted for merely by separating out of the original form from a mixture of the original and the saltant types. The fact that normal type I appeared in a culture of the saltant type IV, which had been apparently pure for twenty generations can be understood in the light of the present author's experience. In dealing with a mixed culture in/
in which a saltant is greatly in excess of the normal type, the latter may be masked for a number of generations. In such cultures the normal type will frequently show up among the saltant growth, if the cultures are kept for some time, which probably explains Leonian's finding traces of his normal type in some cultures of his twenty generations, when he examined them later.

Apart from this alternative interpretation which is offered, if Leonian's saltant type IV was itself isolated from a single sporangium, the record of reversion is of considerable interest. This is the first definite case of saltation among the Phycomycetes.

Rayner has reported, at a meeting of the British Mycological Society, held in London on March 21st, 1925, a case of sectoring in cultures of Phoma radicis-Galliniae. From these cultures a white mycelial saltant was isolated.

Arcichovskij (1), Waterman (80), and Schiemann (65) have recorded mutations, due to influence of environment, in Aspergillus and Penicillium. Brierley (14) shows such changes not to be fixed, and therefore merely modifications due to environment.

The subject of sex-heterothallism has not come into the present study, no indications of its occurrence in the forms being investigated having been observed. Mutual aversion between cultures, as recorded by Cayley (20) in Diaporthe perniciosa has not occurred among the strains of Macrosporium, etc. dealt with.

The Significance of Saltation.

Mutation and saltation in the fungi, as has been shown above, have been repeatedly recorded in the literature, and the phenomena will probably be found of frequent occurrence.

As regards saltation in nature, this has been considered possible by Stevens (74), and it has been stated by Brown and Horne (18) to be a probable cause of the origin of their strains of Fusarium, which they group under the name F. Blackmanii.

It is of considerable interest that saltation has been reported in groups of fungi in which many species or strains exist, and in which/
which variation, to whatever due, seems of natural occurrence. We need only refer to the cases of Fusarium, Helminthosporium, Colletotrichum, Phoma, Macrosporum and Alternaria.

A few cases are recorded (e.g. 30), where saltants of a species have varied in their pathogenicity.

The occurrence of saltation may be a difficulty in retaining fungi in culture, and in the case of type cultures the effect may be exceedingly misleading. This difficulty can only be overcome by discovering a medium on which the fungi will not saltate. Even saltants may themselves saltate and their individual characters be lost.
IV. CONCLUSIONS.

The study of the relationships of forms of the genera *Macrosorium*, *Alternaria*, and *Stemphylium*, which had been found associated with rots of tomato, has shown that three *Macrosorium* forms are closely related to *Pleospora herbarum* of which species they are considered strains; three *Alternaria* forms are considered to be strains of *A. tenuis*; a *Stemphylium* form is apparently new, and is named *Stemphylium Lycopersici* n.sp. All these forms are saprophytes capable of inducing rot in wounded tomatoes; they were incapable of infecting healthy plants.

A *Macrosorium* form isolated from decaying *Echium vulgare* is now recorded and is named *Macrosorium Wilsoni* n.sp.

The three genera mentioned above are distinct, but modifications in the limits of the genus *Macrosorium* are made.

The phenomenon of saltation in the three genera probably accounts for the number of strains which occur in nature.

Saltation must be reckoned with in identifying, describing, or retaining in pure culture, fungi in which it occurs.

V. SUMMARY.

1. A cultural study has been made of three forms of *Macrosorium* Berk., three of *Alternaria* Nees., and one of *Stemphylium* Wallr., all isolated by single spores from tomato fruits showing rots; also of a *Macrosorium* from decaying *Echium vulgare*. Named species have been cultured as controls; *Pleospora herbarum*, *A. tenuis* and *S. silicis*.

2. The cultural characters of these forms have been observed and compared on several media, under controlled environmental conditions.

3. Strains of *Pleospora herbarum* (Pers.) Rhb. and *A. tenuis*, to which the *Macrosorium* and *Alternaria* forms isolated from tomato belong, are distinguished by a consideration of macroscopic and microscopic/
microscopic cultural characters.

4. The genera are to be distinguished by the shape and septation of the conidia, and as a rule by the conidiophores.

5. The formation of conidia in chains is not a differentiating character between *Alternaria* and *Macrosporium*, since the forms of the latter genus studied have shown chain formation constantly.

6. The type of conidiophore is generally an index to the genus but an exception is the *Macrosporium* from *Echium vulgare*, named *M. Wilsoni* n.sp., where instead of being club-shaped the conidiophores are characteristically long, dark, and wide throughout their length.

7. The genera *Alternaria* and *Stemphylium* do not appear to have a perithecial stage; *Macrosporium Wilsoni*, n.sp., is exceptional in this genus in not having that stage.

8. The time taken to form ripe ascospores in the perithecia of the genus *Macrosporium* varies between strains, and even between comparable cultures of one strain. Generally five to seven weeks have been found necessary, but ascospores have been found, capable of germination, eighteen days after growth was observed in a culture.

9. Saltation has been observed in *Pherbarum* and in the three forms of *Macrosporium*, which are considered strains of that species; also in *A. tenuis* and *S. ilicis*. In *Macrosporium Wilsoni*, n.sp., a variant, probably a saltant, was observed but was not isolated pure.

10. Saltant manifestations are found to be chiefly absence or loss of characters which are constant in the normal forms of a species or strain. Thus saltants have been thrown by forms having many perithecia and conidia and little mycelium while the saltant has few, sometimes late formed, perithecia and fewer or no conidia. A physiological effect found in one saltant was an inconstant colouration of the medium and of the culture by a pink, and sometimes blue exudation. Other saltant characters are recorded.

11. Saltation could not be induced artificially.

12. Saltants may themselves saltate.


64. Salmon, E.S.: On specialisation of parasitism in the Erisipheae.


76. Tulasne, C.R. et C.: Selecta Fungorum Carpolologia. Tom.II p.261; Pl. 32, figs.1-14; Pl. 33, figs.11-14, 1863.


**DESCRIPTION OF PLATES.**

Except where stated, the magnification is x 1000.

**PLATE I.** (Figs. 1-9)

Figs. 1-8, **Macrosporium**, strain A. 1-4 normal:

1. Conidiophore bearing lateral spore, and continuing growth from near point of attachment of a terminal spore, to form a second conidiophore from which a spore has now fallen. From 8-day culture in hanging drop of water.

2. Conidiophore bearing chain of two spores. The lower spore has abnormal shape and septation, and has formed a new conidiophore laterally.

3. Spore of abnormal shape and septation germinating from sub-terminal cell. Terminus of germ-tube has form of a conidiophore.

4. Chain formation by intercalary budding.

5. *Saltant a*. Very wide conidiophore, characteristic of this saltant, and beginning of a chain of spores.

6. A terminal and a short wide lateral conidiophore.

7. Very short branch of thick-walled cell acting as conidiophore.

8. *Saltant d*, part of a hyphae showing three conidiophore-like swellings.

Fig. 9. **Macrosporium**, strain B, *normal*. Five conidiophores arising from near one point.

**PLATE II.** (Figs. 10-18).

Figs. 10-12 **Macrosporium**, strain A 10 and 11 *normal*.

10: Conidiophore commencing to continue growth after fall of old spore.

11. Chain formation by intercalary budding. A young spore 'a' is just formed; 'b' is a young spore forming.

12. Saltant *c*, the predominating conidiophore in this saltant, i.e. two-celled and club-shaped.

Figs. 13-15. **Macrosporium** strain B.

13-14: Normal two cases of a conidiophore continuing growth through a spore it has borne, to form a new conidiophore.

15: Saltant *a*. Unusual swelling in a hyphal branch.

Figs. 16-18. **Macrosporium**, B

16: This type of conidiophore is unusual in this **Macrosporium**. The conidia shows continuation of growth without displacing the spore at right side.

17 and 18: Both show the typical long, thick-walled conidiophores of this **Macrosporium**. The conidiophore in 17 is characteristic in being wider than the hypha from which it arises; in becoming constricted, in this case in the subapical cell, and also in bearing at its base a short wide conidiophore. Spores are generally formed terminally.

18: Also shows a hyaline empty spore and a similar apical cell of the conidiophore, into each of which small buds arise from the cell immediately below.
PLATE III. (Figs. 19-27).


19. Chain of two spores, from lower of which a hyphae grows out through the other, and forms a conidiophore.

20. Similar to last, but shows a chain of three spores; the hypha from basal spore grows through the other two, then forms a conidiophore.

21 and 22. Conidiophore growing past spores which had been terminal.

23. Saltant a, A spore giving rise to a conidiophore, and this to a second.

Figs. 24-26. *Macrosorium*, strain Td, 'normal'.

24. Hypha giving rise to a sessile lateral spore.

25. A typical conidiophore. Spore shows first two septa which are disgonal in this specimen.


Fig. 27. *Macrosorium H*. Spore-like cells broken away from a hypha.

PLATE IV. (Figs. 28-35).

Figs. 28 and 29. *Macrosorium*, strain Td, 'normal'.

28. Typical ascospore, germinating through wall of ascus. From 3½ hours culture in hanging-drop of water.

29. Typical ascus. x550.

Figs. 30 and 31. *Macrosorium*, strain A 'normal'.

30. Ascospore, four months old.

31. Spore germinating to give secondary spores. From 8-day culture in hanging-drop of water. x 200.

Figs. 32-34. *Stemphylium ilicis*, 'normal'.

32. Branching conidiophore, showing scars left by fallen spores.

33 and 34. Long and round spores germinating; germ-tubes show scars left by fallen secondary spores.

Fig. 35. *Alternaria*, strain Ta, chain of two conidia, terminal of which has a herm-tube, on which are still attached two secondary conidia.
PLATE V. (Figs. 36-42).

Figs. 36-39, Stemphylium E.
36. Conidiophore, with five spores still attached.
37. Long spore germinating. Two secondary spores are still attached.
38. Chain of two conidia, connected by short neck. Four day culture in hanging drop of water.
39. Chain of four conidia.

Fig. 40. Alternaria tenuis, 'normal'. Shows chain formation and continued growth of conidiophore past oldest spore, which has a lateral germ-tube. The two largest spores have germ-tubes almost terminal being secondary spores.

Fig. 41. Alternaria strain Ra. Spore continuing growth from the two apical cells.

Fig. 42. Macrosporium H, spore with two germ-tubes and secondary conidia.
PLATE VI.  (Figs. 43-47).

Figs. 43-46, *Macrosporium*, strain A, 12-day cultures on Elliott's agar.

43: 'Normal', very dark woolly growth, with many conidia; the transfer has probably contained a little of the saltant c, which has appeared at centre of the culture.

44. Saltant a, a dark spore-forming growth; showing the radiating nature of the mycelium.

45. Saltant b, less dark, has more mycelium but comparatively fewer spores. The edge of the growth is lobed; the rate of growth is slow.

46. Saltant c, a white mycelial growth, in which conidia are almost absent.

47. *Stemphylium ilicis*, cultures on tomato agar, four days after growth was first observed. Shows two dark 'normal' growths, and two of the pale saltant a.
PLATE VII. (Figs. 48-52).

Figs. 48 and 49, Macrosporium, strain Td, 12-day cultures on Elliott's agar.
48: 'Normal', dark in colour and powdery due to presence of many conidia.
49: Saltant a, felty growth due to massed mycelium; light in colour.

Figs. 50 and 51, Alternaria tenuis.
50: 'Normal', very dark due to spore formation.
51: Saltant a, light in colour because of white mycelium and few spores.

Fig. 52. Macrosporium H, Cultures on tomato agar, 6 days after growth was first observed. The dark growths are normal, the white growths are almost certainly a saltant. See description on page...
Plate V.