T H E S I S.

A BIOLOGIC STUDY OF MACROSPORIUM AND RELATED GENERA

WITH AN ACCOUNT OF SALTATION.

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

George B. Wallace, B.Sc.

October, 1925.

Ph. D. Oct 1925

CONTENTS.

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 mat-
:erial 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(ii)was received.

It was found in the main that the present investigation could
only confirm a number of Bolle's conclusions, but as several modifi-
:vations in these were found advisable, the present work was contin-
:ued.   The necessity for careful technique and critical study be-
:came 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 miscroscopic 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 isoltated 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 cultured 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 inter-;vals 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 auto claving 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 pre-;pared 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 be-
;tween 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 miscroscope.   By means of a clean needle it is sometimes possi-
:ble 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 pick-
:ed 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 in-
:to the drop of water the spore of spores will probably be drawn up
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 seperate fold or part of a fold should be used each time the needle is cleaned. The manipulation of the needle under the miscroscope soon becomes quick and easy. Two needles are not so manageable, but by their use it has been possi-:ble 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 miscroscope slide.   The main 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 show-;ing 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, fluccose,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:-

Matted: 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 def-:inition:-

(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

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 wall-:ed and lighter in colour.

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

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 condiiophores. This author uses the term "Rosenkranzformigen Hyphae" to such con-:idiophores.

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 (Plowr.) 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 alternariaceum 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 for-:merly 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 Phaedodictyae 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.

B.   Origin of Forms now dealt with.

Macrosporium A   Dutch tomato obtained in   March 1924.

    "   B   "   "   "   "   April   "

    "   H   Midlothian, on dead leaves
                   of Echium vulgare.   July   "

    "   Td Teneriffe tomato obtained
                           in   November"

Alternaria   D   Dutch tomato obtained in   September 1924.

    "   R   Teneriffe tomato obtained
                           in   October   "

    "   Ta Teneriffe tomato obtained
                           in   November   "

Stemphylium E   Dutch tomato obtained in   September   "

     All the above forms were isolated from tomato fruits show-
;ing rots, except H, 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 iso-
:lated 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
F. 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 Tll., and Pleospora
herbarum (Pers.) Rbh., 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 mycel-
:ium 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 ap-
:pearance 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 in-
:cubator 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 I . Rate of Growth of Macrosporium Forms
in terms of diameter in m.m.'s per
day.

Macrosporium form		Elliott's Agar	Dox's Agar	Tomato Agar	Prune Agar
			Medium		
Pleospora herbarum					
	Normal	9.4	9.4	10.3	9.7
	Saltant a	5.5			
	b	4.1			
A	Normal	8.4	8.3	9.3	10.2
	Saltant a	6.9			
	b	5.1			
	c	9.0			
	d	8.0			
B	Normal	8.8	9.0	10.0	10.2
	Saltant a	3.0			
Td	Normal	7.5	8.4	9.0	9.3
	Saltant a	7.2			
H	(Normal)	8.0	8.3	7.5	7.1

on each of four media.   The microscopic characters are only record-
:ed for each cultured form on Elliott's agar, since sufficient dis-
:tinction 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", "Td", "H", and
Pleospora herbarum (P.h.)

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, indifinite and adpressed; the edge becomes more def-:inite 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 consid-:erable 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 6μ. the maximum width observed 11μ.

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 in-:variably forms a second spore on the scar left by the first conidium when it is pushed off. Cases have been seen (figs. 21,22)/

21,22) where instead of pushing off the conidium, the conidio-
:phore 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 conidio-
:phore, or to form several cells of hyphae with a terminal con-
:idiophore. 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 infrequent-
:ly. 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 div-
:ided muriformly. The conidium may be pushed off as a second
one is formed in its place, or it may remain and bud off a fur-
:ther 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 inter-
:mediate. In this case older spores are pushed apart. There
may be several buds on a spore so that a branching chain is form-
:ed. 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 ex-:amined, 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 Macrosporium. 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 Macrosporium 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 $12{-}16_\mu$, (Table VI).   The perithecial stage is discussed separately (page 33).

Macrosporium A. (Pl.VI, fig. 43).

Growth is pulvinate, low, falling from $1\frac{1}{2}$ m.m. near the centre to the edge; mostly $\frac{3}{4}$ - 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 constric-:tion at the septa.   The average width is 4.5_μ, the maximum ob-:served/

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 ap-
:pears 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 Macrosporium 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 interest-
:ing 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 -30μ. The size of 100 ascospores was 27-36 x 12-18μ.
Macrosporium B.

The growth is raised to $2\frac{1}{2}$ m.m., but the remainder is low, up
to $\frac{1}{2}$ 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 dis-
:tinguished. No comparisons can be made with the other strain
from the hyphae apart from their width. The average width is 4μ,
the maximum observed 7.5μ, i.e. they are rather finer than those of
Pleospora herbarum , or Macrosporium H (Table II).

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 Macrosporium
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.25-9). The average width was 7.5μ, the maximum observed 8μ.

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/

16

of the very dark outer wall.   There are many conidia.   Verru-
: sosity 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 132-198 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 woolly-
subfelty, 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-
:gular, 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).   Conidiophores 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 hypha , 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 x 11-23μ.   The verrucosity resembles that of strain
B being fine.   The size of 50 asci was 135-204 x 24-33μ.   The
size of 100 ascospores was 26-33 x 10-14μ.

Macrosporium H.

The growth is $\frac{1}{2}$-1m.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 pro-
:bably 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 consider-
:ably in width;   some are so wide as 15.5μ throughout a consid-
:erable length.   The cells in figure 27 of plate III are 13μ
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 Macrosporium isolations, being 6.5μ.   The hyphae
are fairly clear and of a pale green brown colour: a small
proportion are colourless.   There is a marked tendency to con-
:striction 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 graduations from
normal but dark, or thick-walled hyphae to long very dark multi-
septate, thick-walled structures 10μ wide.   The usual conidio-
:phores (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 8μ.   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 strik-
:ing 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 Macrosporium 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 later-:ally. 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 ter-:minal 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 con-:idia. 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/

Dox's Agar.

Pleospora herbarum.

A pulvinate growth sloping gradually to the surface of the medium; $1\frac{1}{2}$-2m.m. high at centre. Central half is subfelty and opaque, changing gradually on the outer half to downy and trans- :parent. The edge is fairly regular, but indefinite; the mar- :ginal 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.

Macrosporium A.

A pulvinate growth,2 m.m. high at centre, falling away to- :wards 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 curcular, in- :definite 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.

Macrosporium B.

The central three quarters of the growth is evenly raised to $1\frac{1}{2}$ 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 pro- :portion of mycelium, the margin is almost entirely conidia. There are very many scattered perithecia. Rate of growth is 9.0 m.m. per day.

Macrosporium Td./

Macrosporium Td.

The growth is a thin powdery layer consisting of a little
white mycelium and many spores. The edge is even but indef-
:inite; 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 pulvin-
:ate 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 moist-
:ture 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 $2\frac{1}{2}$ 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 lay-:er. There are a number of perithecia. The rate of growth is 9.3 m.m. per day.

Macrosporium 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.

Macrosporium 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 to1 m.m. of loose subfelty mycelium. The edge is even but not definite. The mycelium on the margin resemble the aerial mycel-:ium; it is not adpressed, and instead of being radiate is loose, very open and sligtly 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 show-:ing 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 pres-:ent.   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 $2\frac{1}{2}$ m.m. high at the centre and rounding off evenly to the adpressed margin.   The growth is a felty layer of extremely close-:ly 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, $2\frac{1}{2}$ 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 margine. 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 10.2 m.m. per/

per day·

Macrosporium B.

Pulvinate, 2-2½ m.m. high.   It is deep, opaque felty be-
:low, with 1-1½ m.m. of woolly, sunfelty hyphae above.   The edge is
definite, regular and abrupt.   The colour is at first white, chang-
:ing later to"Leaden grey" (353:4) most of the growth consists of
mycelium.   The superficial mycelium collapses after that of Macros-
:porium 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 col-
:lapsing 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 ₍because₎ 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 abruptt.   There
is some variation in colour in the cultures.   Only Pleospora hebarum
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 unfav-;ourable for the formation or observation of perithecia.

Table II. Width of mycelium (in Microns) of Macrosporium Forms.

Macrosporium Form			Width (in Microns) of hyphae		Width (in Microns) of Conidiophores	
			Average	Maximum	Average	Maximum
"P.h."	Normal		6	II	8	9.5
	Saltant	'a'	5	8	7	8
	"	'b'	5	8.5	7.5	8
"A"	Normal		4.5	7	7	8.5
	Saltant	'a'	4	7	6.5	8
	"	'b'	5	IO	7.5	8
	"	'c'	4	8.5	7	7.5
	"	'd'	3.5	8	-	-
"B"	Normal		4	7.5	7.5	8
	Saltant	'a'	4	6	7.5	8
"Td"	Normal		4	6	7	9
	Saltant	'a'	4	6.5	-	-
"H"	(Normal)		6.5	I5.5	8	IO

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 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 forma-:tion 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 dame as on Elliott's agar but is faster growing, like A and Td. Tomato agar/

Tomato agar.

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

as

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 rates 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 ascopores are larger than in the other forms, which agree among themselves fairly closely; the time taken to produce mature asco-:phores 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 pro-:duced 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, how-:ever, 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 varia-:tion 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

Macrosporium Form			Length (100 Spores)		Width (100 Spores)	
			Mean	σ	Mean	σ
'A'	Normal		18.53 ± .17 (12-26)	2.46	15.01 ± .15 (10-20)	2.18
	Saltant	'a'	15.99 ± .11 (13-19)	1.55	13.42 ± .08 (10-17)	1.20
	"	'b'	17.49 ± .17 (13-23)	2.45	14.10 ± .13 (10-19)	1.87
	"	'c'	15.33 ± .17 (11-23)	2.48	12.69 ± .13 (9 -19)	1.91
	"	'd'	Conidia rare			
'B'	Normal		21.38 ± .23 (14-30)	3.36	17.57 ± .19 (11-25)	2.75
	Saltant	'a'	19.53 ± .22 (13-30)	3.45	13.42 ± .16 (9-19)	2.29
"Td"	Normal		19.04 ± .22 (13-28)	3.28	16.20 ± .18 (11-23)	2.63
	Saltant	'a'	Conidia absent			
"H"	Normal		18.59 ± .18 (14-26)	2.63	15.10 ± .15 (11-22)	2.22
"P.h."	Normal		23.63 ± .28 (15-37)	4.16	16.14 ± .24 (8-23)	3.51
	Saltant	'a'	18.98 ± .21 (12-28)	2.99	11.70 ± .14 (7-16)	2.07
	"	'b'	Few conidia			

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

Macrosporium Form	Normal or Saltant				
	Normal	Saltant 'a'	Saltant 'b'	Saltant 'c'	Saltant 'd'
P.herbarum	2, 3, 4	2, 3, 4	Few conidia		
"A"	2, 2-3	2, 2-3	2, 2-3	2,2-3	Few conidia
"B"	2	0, 1			
"Td"	2	No conidia			
"H"	0, 1				

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.) Rbh., of which the conidial stage is Macrosporium sarcinula Berk.

With regard to the form of Macrosporium from decaying Echium vul-;gare, known as Macrosporium H during its study, this form is considere 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 Niessl.
> Pleospora massarioides Feltg.
> and Pleospora media Niessl.

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 indis-:tinguishable 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/

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 con-:idiophore. The conidia are very dark; they are oval to oblong, measuring 14-26 x 11-22μ, mean of 100 spores is 18.59 by 15.10μ. 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 ab-:sent.

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, 8μwide on the average. The conidiophores are light brown; they are the slight-:ly modified continuations of the hyphae. They are less than twice the width of the hyphae from which they arise; their average width is 6.5μ. 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 12.58μ; long, 56% measuring 17-43 x 8-15μ, mean of 100 spores was 25.17 x 11.59μ. The verrucosity of/

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 Macrosporium 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 Macrosporium: A, B, and Td are natural strains.

The stages in the life history of P. herbarum have been very con-:fused in the past. Miyabe (52,pp. 15-16) refers to the confusion pro :duced by Tulasne (76), Fuckel (38b.), and Hallier (42), who worked with contaminated material. Gibelli and Griffini[41] divided P. herbarum into two distinct species, P. Sarcinula and P. Alternariae, the former of which produced larger ascospores and Sarcinula comidia; . theother producing smaller less septate ascospores and Alternaria conidia. These authors also disproved some of the conclusions of earlier workers. Bauke'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 perithecium 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.

Berlese (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 Macrosporium spores produced only Macrosporium 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 pro-
:cess, 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 to-
:gether, 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 found perithecia. The latter form has been
cultured for a considerable number of generations. It has been is-
:olated 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 with-
:out 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, Macrosporium H,whether a saltant or not, has been
isolated from nature lacking perithecia, the statement that the genus
Macrosporium always has a perithecial stage should not stand.

As regards the relationship of the genera of Macrosporium,
Bolle refers to the results of Miyabe and of Gentner.   These work-
:ers, 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
Macrosporium 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 Macrosporium 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 Macrosporium sarcinula can continue for the general conidial
form P. herbarum, fof which the conidial form of distinct morpholog-
:ical 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
Macrosporium 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
Macrosporium 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 suffi-
:cient to unite forms of Macrosporium together.

As regards the study of the perithecial stage of Macrosporium
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 asci amd of 100 ascospores of each of these forms are given in
tables $\underline{V}$ and $\underline{VI}$; the cultures were two months old, and therefore
comparable. Asci vary considerably in size, $\not{A}$ according as the
ascospores lie in one or two rows in the ascus. Considerable uni-
:formity is however to be observed in the size of asci in the forms
A, B, and Td. The 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 onsidered for differentiation. The measurements of the asco-
:spores 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/

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 germina-:tion in culture; also on keeping two mature perithecia in a moist chamber over night, 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 Macrosporium. Bolle has stated; "These perithecia take about two months to ripen; one can hasten this process by keeping the cultures cold ($\pm 0°$) 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 how-:ever formed ascospores, which were able to germinate in water, twenty days after growth was first observed, and form Td has pro-:duced 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 found 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, taling 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 chlamydospores which are Alternaria-like.

A culture of Phoma alternariaceum was received by the present writer through the kindness of Mr. Brooks; the chlamydospores are at first sight like Alternaria, but are too irregular to conform to the description of the spores of that genus.

No/

No pycnidia have been encountered in any of 1-spore forms of the <u>Macrosporium</u>, <u>Alternaria</u>, or <u>Stemphylium</u> in the present work, and Bolle's conclusion must still hold: that the "occurrence of pycnidia in the development of the <u>Phaeodictyae</u> is quite improbable."

Table V. <u>Measurements of Asci</u> (in microns)

Macrosporium form	Average Size (50 Asci)
P.herbarum	232 × 29 (204-306 × 24-33)
"A"	159 × 27 (132-216 × 21-30)
"B"	165 × 26 (132-198 × 21-30)
"Td"	159 × 28 (135-204 × 24-33)

Table VI. <u>Measurements of Ascospores</u> (in microns)

Macrosporium form	Length (100 Spores) Mean	σ	Width (100 Spores) Mean	σ
P.herbarum	34.03 ± .15 (29-39)	2.22	14.31 ± .06 (12-16)	.87
"A"	31.87 ± .13 (27-36)	1.97	13.43 ± .06 (12-16)	.94
"B"	30.42 ± .18 (22-39)	2.73	13.48 ± .06 (11-16)	.95
"Td"	29.46 ± .10 (26-33)	1.49	12.09 ± .05 (10-14)	.69

ALTERNARIA CULTURES.

Elliott's agar.

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

Surface elevation is very low - less than ½ 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 practially 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 shew up clearly unstained. There are, however, very hyaline hyphae preset - there is more irregularity in width of these, some being as wide as 10μ; the average width was found to be 4.5μ.

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 con-:tinue 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 con-:idiophore, but are very easily detached, leaving scars. The result is a "nodulose" conidiosphore, though this is much less marked than in Stemphylium.

The conidia are very dark. The size of 100 spores was 17-52 x 8-15μ. The verrucosity varies considerably from fine or moderately/

moderately rough in most spores to rough in a small proportion. Rougher verrucosity has been found here than in the other forms of Alternaria.

Figure 40, though it does not show a typical conidiosphore, 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 ter-:minally 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 sec-:ond 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 ad-:pressed. 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 4μ, the maximum observed 6μ,

The conidiophores are only the 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 forma-
:tion 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 $\frac{1}{2}$-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 re-
:gular 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 quanti-
:ties;   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 ter-
:minal 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 ratherfaster
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, 4μ, is the same as in the other strains, but the maximum observed was 5.5μ. The conidiophores are distinct from the hyphae from which they arise, being darker,though only slightly wider;   the average width of 5μ 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 x 7-15μ.

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 $\frac{1}{3}$ 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). Ex-
:cept 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" (286:4). 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 sporing 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./

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 col- :lapsing, 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", "Ta", "D" and A.tenuis ("A.t.")

Rate of Growth in Millimetres per day

Elliott's Agar   Dax's Agar   Tomato Agar   Prune Agar

superficial mycelium than <u>A.tenuis</u> though less than R; it is al-
most confined to the central half. The colour of the superficial
mycelium is different from that in the first two forms of <u>Alternaria</u> ;
it changes from white to "Pale grey green" (247: 4). There is a
slight ringed appearance, but not so marked as in <u>A.tenuis</u>. 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 <u>A.tenuis</u> more than do the other two forms.
It differs from <u>A. tenuis</u> 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.
in terms of diameter in m.m's per
day.

Alternaria form.	Medium.			
	Elliott's Agar	Dox's Agar	Tomato Agar	Prune Agar.
A. tenuis				
Normal Saltant a	9.4 9.6	10.5	9.7	10.6
R	9.7	11.7	10.7	12.0
Ta	9.5	10.9	9.2	11.7
D	8.9	10.9	9.0	11.3

Comparison of Macroscopic Characters of Alternaria forms.

Elliott's agar.

A. tenuis is a low sporing growth, with no visable 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 def-:inite; 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 D.

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 an adpressed 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 A. tenuis; it is a dense growth and has more superficial aerial mycelium and this has a different colour.

Ta has more mycelium than A. tenuis though less than 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 A. tenuis.

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

Thus R can be told on Elliott's agar by the deep mycelial growth. It is the fastest growing on all media.

A. tenuis is so far distinguished from the others by its continually throwing a white saltant. It is a low sporing growth, distinct from R on Elliott's agar, and from Ta and D on Prune agar.

Ta can be told on Elliott's agar by its mass production of spores giving a dense opaque growth.

D can be distinguished from Ta on Elliott's agar and from R and A. tenuis on Prune agar.

Table VIII. Width of Mycelium (in Microns) of Alternaria Forms.

Alternaria Form	Width (in Microns) of Hyphae		Width (in Microns) of Conidiophores	
	Average	Maximum	Average	Maximum
"A.t." { Normal	4.5	IO	5	6.5
"A.t." { 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

Table IX. <u>Size of Conidia (in Microns) of Alternaria Forms.</u>

Alternaria Form	Length (I00 Spores)		Width (I00 Spores)		Septa	
	Mean	σ	Mean	σ	Trans.	Long.
"At." {Normal	27.8I ± .37 (I7-52)	5.45	I0.8I ± .II (8-I5)	I.57	3.57	.94
{Saltant 'a'	26.33 ± .38 (I7-44)	5.72	9.57 ± .08 (8 -I5)	I.24	3.67	.25
"R" (Normal)	30.22 ± .39 (I8-50)	5.85	II.26 ± .II (7-I7)	I.65	3.83	.96
"Ta" (Normal)	29.4I ± .45 (I9-53)	6.62	I0.66 ± .II (7-I4)	I.64	3.76	I.03
"D" (Normal)	30.I2 ± .40 (2I-46)	5.9I	I0.30 ± .II (7-I5)	I.60	3.87	.73

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 conidio-
:phores, 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.

Alternaria	Normal or Saltant	
Form	Normal	Saltant 'a'
A. tenuis	2-3, 3, 4	2-3, 3
"R"	3, 3-4	
"Ta"	2-3, 3, 3-4	
"D"	2-3, 3, 4	

The figures 0 to 5 have the same sig-
:nificance as in Table IV.

STEMPHYLIUM CULTURES.

Elliott's agar.

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

The growth is very low, only $\frac{1}{2}$ m.m. high at the centre and falling away to the edge. It is subfelty-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 m.m. 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 3.5μ, the maximum observed was 5μ. 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 conidio-:phores 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 al-:ternately 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, id 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 (II), who considered these conidiophores to be referred to in the term used by Wallroth (79): "Hyphae/

"**Hy**phae ...... 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 prodominating type in S.ilicis; 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 condiiophores the germ-tubes are under these culture conditions are nodulose (figs.33, 34).

Stemphylium E.

The macroscopic features are similar to those of S.ilicis 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.ilicis 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/

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 6.5μ, the maximum 9.5μ, both figures being greater than in S.ilicis. They are light brown and therefore distincy from the hyaline hyphae.

Generally conidia fall in making a miscroscopic 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 pro-:portion of the long condiia. In fact, among 100 measureable conidia which came into a few scattered fields of the miscroscope 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 rhe 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 <u>Alternaria tenuis</u> on the same medium, but rate of growth is different in each - <u>S.ilicis</u> 8.6 m.m. per day, and in <u>Stemphylium E</u> 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 $1\frac{1}{2}$ 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 super-:ficial 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 $1\frac{1}{2}$ m.m. high, falling abruptly at the margin which is even and definite, with 1 m.m. of adpressed trans-:parent 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 <u>S. ilicis</u>. There is no exudation of moisture. Rate
of growth is 9.4 m.m. per day.

Table XI. Rate of Growth of Stemphylium Forms
in terms of diameter in m.m's per
day.

Stemphylium Form.	Medium.			
	Elliott's Agar.	Dox's Agar.	Tomato Agar.	Prune Agar.
S. ilicis Normal Saltant a	8.3 8.8	8.6	8.3	8.0
E	9.4	9.5	10.0	9.4

Fig. 3. Stemphylium : Rate of Growth of
form "E" and S.ilicis (S.i.)

superficial aerial mycelium is thin and "greyish white". The
height of the growth does not fall away abruptly at the edge

Table **XII.** Width of Mycelium (in Microns) of Stemphylium Forms..

Stemphylium Form	Width (in Microns) of Hyphae		Width (in Microns) of Conidiophores	
	Average	Maximum	Average	Maximum
"S.i." Normal	3.5	5	5.5	6
Saltant 'a'	3.5	5	5.5	5.5
"E" (Normal)	5	8	6.5	7.5

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

Stemphylium Form	Length (100 Spores)		σ	Width (100 Spores)		σ	Septation	
	Mean			Mean			Trans.	Long
"Si." Normal Round Conidia	17.75 (13-22)	.11	1.70	15.25 (12-19)	.09	1.36		
Long Conidia	24.68 (19-35)	.19	2.86	12.93 (9-16)	.12	1.74	3.08	1.4
"Si." Saltant 'a'	Few conidia							
"E" (Normal) Round Conidia (44%)	17.02 (13-22)	.11	1.70	12.58 (8-18)	.10	1.41		
Long Conidia (56%)	25.17 (17-43)	.32	4.76	11.59 (8-15)	.09	1.28	3.27	1.1

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.

Stemphylium	Normal or Saltant	
Form	Normal	Saltant 'a'
S. ilicis	4, 5	2, 3, 4, 5
"E"	2, 3, 4, 5	

The figures 0 to 5 have the same signifi-
icance 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 fre:quently 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 conidio:phore. 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 nod:ulose 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 germtubes, which may be identical in appearance to the conidio:phores. 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 hang-:ing 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 con-:idium 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 conidio:phore 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 lit-
:erature, 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 Tll., 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 (53)
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 there-
:fore 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 per-
:fectly 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 (Pl..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 hypha become marked out by becoming thicker walled, darker and wider just below the apex. One 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.^{II}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 conidio-:phore 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 Alternarias 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 in-:fluence 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, nodulosae", referred to the char-:acteristic 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 gener-:ally 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 Mystrosporium Cda. with Stemphylium Wallr.; and Sporidesmium Lk. with Clasterosporium v. Schwein.

III. SALTATION.

A. Saltation in Ma/crosporium, 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 allow-:ing for the fact that saltants have on occasion themselves salta-:ted to give still another form. (No particular meaning is at-:tached to the word 'form').

With one exception, no strain was observed to saltate un-:til the fungus had been grown in culture for several generations. Again with one exception, each saltant has been observed and re-:isolated 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 immed-:iate parental form; environmental conditions were thus the same, and the drawing of conclusions as to difference in the growths was thus permissable.

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 con-:sidered 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 condi-:ditions 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 pres-:ent 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 al-:ready 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 re-:mainder 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 Macrosporium 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 hav-:ing 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 ir-:regular 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 Macrosporium, Alternaria, and Stemphylium are con-:sidered separately.

Saltation in Macrosporium.

Of the five forms of Macrosporium investigated earlier, four have saltated; the fifth, a form from Echium vulgare, known as Macrosporium/

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 ob-;tained 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 tranalucent 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 abnor-;mally 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 miscroscope, being darker and wider. The average width is 7μ, the maximum ob-;served 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 conidio-;phores. 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 7-16μ.

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·μ, the maximum observed 8.5μ.

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 7.5μ, and the maximum 8μ. Several conidiophores were seen in series.

Saltants of Macrosporium A.

Saltant/

<u>Saltant a.</u> (Pl Ⅵ fig. 44).

This Saltant is quite distinct from the normal form, The
growth is subfelty, 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 trans-
;lucent 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 be-
;low is the many very short branches acting as conidiophores. The
hyphae are mostly fairly dark, The average width is 4μ, the maximum
observed 7μ.

<u>Conidiophores.</u>

A marked feature in this saltant is the very short and very
thick conidiophores (figs.5,6). It is unusual here for the conidio-
;phores to take on characters of the spores, but cases have been ob-
;served where a conidiophore has become thicker walled, of a darker
colour, and with thicker septa that 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.

<u>Conidia.</u>

Budding/

Conidia.

Budding of the conidia to form chains is very marked, so that in a scraping, spores in all stages of development are seen. Bud-:ding 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 mi$\not$cro-:scope, hav$\not$ing many thick and fairly uniformly dark hyphae; the$\not$y are wider here than in a however. Th average width is 5μ. the max-:imum observed 10μ. Also there are here the same short, wide conidio-:phores 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 max-:imum 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 mi$\not$croscope, 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 sim-:ilar/

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 pre-:vious 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 gradu-:ally. 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. Perithecia 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 $\frac{1}{4}$ 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 d, 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 sal-:tant 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 asso-
:ciated 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
l-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
l-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 mix-
:ture 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 l-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 num-
:ber 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 hyphae which had six such swellings.

Since a relatively very small number of conidia are formed, the formation of these conidiaphore-like swellings must be followed im-:mediately by continued growth as a hyphae 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 hyphae 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/

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 $2\frac{1}{2}$ m.m.) than the normal, and only falls away near the edge. The growth though all mycelium is felty, and begins to collapse com-:paratively early - after eleven days. The edge is irregular in-:definite 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 subsultured from this normal
dark growth it gives a culture resembling its parent;   but with re-
:peated 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 pres-
:ent 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 <u>Alternaria</u> 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 sec-:ondary 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 $2\frac{1}{2}$ 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 produc-
:tion.   The verrucosity 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.

Fig.4.   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 them-
:selves, and no instance has occurred where any saltant resembled
a saltant or the normal form of any other strain.   Further in-
:vestigation/

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 Macrosporium A differ very markedly from the normal form, and in fact agree more closely with Saltant b of Pleospora herbarum, and Sa/ltant a of Macrosporium Td.

Macroscopic Characters of Saltants Compared wiihh 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 like-:wise 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 inconsistant. Conidia are formed late in exceed- :ingly small numbers. They may be absent. Perithecia are also found late in small numbers and are confined to parts of the margin.

Macrosporium B.

Saltant a.

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

Macrosporium 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.

Macrosporium 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 condiia.

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.

Macrosporium 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.

SALTATION.

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 ob-:servable 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 referes 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 Pestal-:ozzia Guepini might be resolved into an indefinite number of de-:monstrably distinct strains, the number depending only upon the precésion of the methods.   In illustration, we have shown a possi-:ble 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 biologic-:al 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 work-ers, notably Eriksson (38), Stakman and his collaborators (70,72,73) and Arthur (2,3); in the Erisiphaceae the subject has been investi-:gated by Neger (54), Salmon (64), and Reed (60).

Again, a number of species has been shown to develop char-:acters 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 sub-:ject 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 satify the pathologist, oecologist 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 def-:ining 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 be 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 accord-:ing/

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 Skull, who speakes 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 ulilitarian principles should be crucial in the establish-:ment 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 o wn require-:ments.

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 them transmitted to subsequent generations. The word does not imply anything con-:cerning the magnitude of the change, its suddenness, or the manner of its acquisition. The term denotes a change in genetic constitu-:tion. All other changes which are impermanent - depending generally upon changes of the environment - and note heriditarily 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/

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.

Stewens (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 Macrosporium, 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 vari-
:ations 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 morpholog
:gically 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 consider-
:able 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
knowvery 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 pro-
:duced 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 ad-
:visable. 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 variationsin 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 re-
:port of mutation - in that author's sense of the term[x].

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 nøt made
from single-conidium isolations it is   possible, though not pro-
:bable, 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, ob-
:tained two types, which he calls plus and minus strains.   The
latter he considers "a øport 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 iso-
:lated 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
with/

[x]Stevens (74) quotes from a personal letter received from
Edgerton:- "All of the cultures that I used in that work were ob-
:tained 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 elem-
:entary species ".

with the normal growth. "Pure cultures of the albino material
were carried on through sixteen consecutive non-sexual genera-
:tions 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 be-
:came sterile, but if these sterile cultures were transferred
to sterilised chilli stems, the conidia forming capacity was re-
:gained.... This process of getting the conidial stage could not
be carried on indefinitely ". The loss of the condiia 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 cultur-
ing 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 races 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 lerge 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- Vermicularia- Volutella group. A single co-;nidium culture developed variant sectors. He obtained two saltants with "1. All conidia only, and 2. Conidia profuse, immature minute 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 ne 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-:eigth to the sixty-ninth and last selection B remained constant". Photographs of three culture plates are refered 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 success-:ion of 15 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 Alter-:naria 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 mycel:ial. 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 isol-:ated 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 florms of the speci
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 par
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 dis-:tinct 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 I, 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 generstions, 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 spor-:angium, 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-Callunae. 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 occurence in the forms being investi-:gated having been observed. Mutual aversion between cultures, as recorded by Cayley (20) in Diaporthe perniciosa has not occured 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 occurence.

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 proble cause of the origin of their strains of Fusarium, which they group under the name F. Blackmanni.

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, Colleto-
trichum, Phoma, Macrosporium 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 so
saltants may themselves saltate and their individual characters
be lost.

IV. CONCLUSIONS.

The study of the relationships of forms of the genera Macrosporium,Alternaria, and Stemphylium, which had been found associated with rots of tomato, has shown that three Macrosporium 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 Macrosporium form isolated from decaying Echium vulgare is now recorded and is named Macrosporium Wilsoni n.sp.

The three genera mentioned above are distinct, but modifica-
:tions in the limits of the genus Macrosporium 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 Macrosporium Berk., three of Alternaria Nees., and one of Stemphylium Wallr., all isolated by single spores from tomato fruits showing rots; also of a Macrosporium from decaying Echium vulgare. Named species have been cultured as controls; Pleospora herbarum, A.tenuis and S.ilicis.

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

3. Strains of Pleospora herbarum (Pers.) Rbh. and A.tenuis, to which the Macrosporium 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, eight-:een days after growth was observed in a culture.

9. Saltation has been observed in P.herbarum 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 physiol:logical 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.

Bibliography.

1. Arcichovskij,V. : Zur Frage über den Einfluss von $ZnSO_4$ auf eine
      Reihe von Gerationen von Aspergillus niger. Autoreferat
      in Centr. f. Bakt. II, 21 : 430, 1908.

2. Arthur, J.C. : Cultures of Uredineae in 1916 and 1917. Mycol. 9,:
      295-312.

3. ————— : North American Flora, VII, 4, 296.

4. Bauke, H. : Zur Entwickelungsgeschichte der Ascomyceten. Vor;
      :laufige Mittheilung. Bot. Ztng. 313-326, 1877.

5. Baur, E. : Einführung in die experimentelle Vererbungslehre.
      Berlin, 1914.

6. Benedict, R.C. : The origin of new varieties of Nephrolepis by
      orthogenetic saltation. Bul. Torr. Bot. Club, 43 : 207-
      234, 1916.

7. Berkeley, M.J. : Notices on British Fungi. Ann. Nat. Hist. I,261,
      1838.

8. Berlese, A.N. : Monographia dei generi Pleospora, Clathrospora,
      e Pyrenophora. Nuovo Giornale Bot. Ital.,XX,1,[91.]1888. Tav.
      V, f. 2-6.

9. Bewley, W. : Tomato Diseases. Jour. Roy. Hort. Soc., XLVII, 2
      and 3, I69-I74, 1923.

10. Blakeslee, A.F. : Mutations in Mucors. Jour. Hered.,II, 278-284,
      1920.

11. Bolle, P.C. : Die durch Schwärzepilze (Phaeodictyae) erzeugten
      Pflanzenkrankheiten. Med. uit het Phytop. Lab. "Willie
      Commelin Scholten",VII, 1924.

12. Bonar, L. : An albino mutation of the Dematiaceous fungus Brachy-
      :sporium trifolii. Science N.S.,LVI, 226-227, 1922.

13. Brierley, W.B. : Experimental studies in the specific value of
      morphological characters in the fungi. Proc. Linn. Soc.
      London, Oct. 1918.

14. ————— : On a form of Botrytis cinerea with colourless
      sclerotia, Phil. Trans. Roy. Soc., London, Ser. B, CCX,
      83-114, 1920.

15. Briton-Jones, H.R. ; Strains of Rhizoctonia Solani Kühn. (Cor-
    ;ticium vagum Berk. and Curt.). Trans. Brit. Myc. Soc., IX,
    4, 200, 1924.

16. Brooks, F.T. and Searle, G.O. : An investigation of some tomato
    diseases. Trans. Brit. Myc. Soc., VII, 3, 173-197, 1921.

17. Brown, W. : Experiments on the growth of fungi on culture media.
    Ann. Bot., XXXVII,   , 1923.

18. ――――― and Horne, A.S. : Studies in the genus Fusarium. I,
    General account. Ann. Bot. XXXVIII, 150, 1924.

19. Burger, O.F. : Variations in Colletotrichum gloeosporioides Perz.
    Jour. Agr. Res., XX, 723-736, 1921.

20. Cayley, D.M. : The phenomenon of mutual aversion between mono-
    spore mycelia of the same fungus (Diaporthe perniciosa
    Marchal). With a discussion of sex-heterothallism in fungi.
    Journ. of Genetics, XIII, 3, 1923.

21. Castle, W.E. ; Genetics and Eugenics. Camb. Harv. Univ. Press,
    91-108, 1924.

22. Chandhuri, H. ; A description of Colletotrichum biologicum n.sp.
    and observations of the occurence of a saltation in the
    species. Ann. Bot. XXXVIII, 152, p. 735, 1924.

23. Clark, W.M. : Determination of hydrogen ions. 1920.

24. Chester, F.D. : A leaf blight of the potato. Del. Agr. Exp.
    Sta. Rept. 4, 56, 1891.

25. ――――――― : The ripe rot or anthracnose of tomato, Colleto-
    trichum phomoides Sacc. 6th Ann. Rept. Del. Coll. Agr.
    Exp. Sta. 1894.

26. Chester, F.D. : A manual of determinative bacteriology, pp.20-
    25. 1909.

27. Clinton, G.P. ; Potato spraying experiments, 3rd report. Conn.
    Agr. Exp. Sta. Rept.,pp. 47o-471, 1915.

28. Cochayne,A.H. ; Note of the facultative saprophytism of Alter-
    ;naria Solani. 13th Ann. Rpt. New Zealand Dept. Aric.
    pp. 428 -430. 1905.

29. Cook, Mel. T: Sunburn and tomato fruit rots. Phytopathology
    XI, 379, 1921.

30. Dastur, J.F.: Glomerella Cingulata (Stoneman) Spauld. and v.
       Sch. and its conidial forms, Gloeosporium piperatum E.and E.,
       and Colletotrichum. nigrum E. and Hals., on chillies and
       Carica papaya. Ann. Appl. Bill. VI, 245-268, 1920.

31. Crabill C.H.: Dimorphism in Coniothyrium pirinum Sheldon.
       Amer. Jour. Bot. II, 449-467, 1915.

32. Dickson,B.T.: Saltation in the organism causing "Black Dot"
       disease of potato in Canada. Trans. Roy.Soc. Canada, 3rd
       Ser., XVII, Section V, 123-127, 1923, Plate III.

33. _____ : Further studies on the saltation of the "Black
       Dot "organism. Report of May meeting 1925 at Ottawa of
       Roy.Soc. of Canada.

34. Dobell.,C: Some recent work on mutation in micro-organisms.
       II, Mutations in bacteria. Jour. of Genetics, II, 325-350,
       1913.

35. Dox, A.W. : U.S.D.A. Bur. Ann. Ind. Bull.-no. 120, 1910.

36. Edgerton,C.W.: The physiology and development of some anth-
       :racnoses. Bot. Gaz. XLV. 367-408, 1908.

37. Elliott, J.A. : Taxonomic characters of the genera Alternaria
       and Macrosporium. Amer.Jour.Bot. IV, 439- 1917.

38. Eriksson, J.: Ueber die Specialisirung des Parasitismus bei
       den Getreiderosteilzen. Ber. d. deut. Bot.Ges,XII:292-331.
38a. Fries, E. : Syst. Myc. III, 373-375, 1829
38b. Fuckel, L. : Symbolae mycologicae, p.130, 1869.
39. Galloway, B.T.: In "Report of the chief of the section of veg.
       path."for 1888. Notes on Black rot on the tomato.
       Washington Govt. printing office, 1889.

40. Gentner, G.: Über durch Macrosporium sarciniformae Cav. hervor-
       gerufene Erkrankungen der Luzerne und des Klees. Prakt.
       Blätter f. Pflanzenbau und Pflanzenschutz, Heft IX and X,
       1918.

41. Gibelli, G. and Griffini, L.: Sul polymorphismo della Pleospora
       herbarum Tul. Arch. Trienn. del Lab. di Bot. crittog.
       Pavia, I, 53-102. 1874. Tav. V -IX.

42. Hallier, C.: Untersuchungen des pflanz. Organismus, welcher die,
       unter d. namen Gattine bekannte Krankheit der Leidenraupen
       erzeugt. Potsdam 1868. Die Mustardine des Kieferspinners,
       in Zeitschrift für Parasitenkunde, Bd. I, 18.

43/

43. Hanna, W.F. ; The Dry-needle method of making Monospore cultures
       of Hymenomycetes and other fungi. Ann. of Bot,
       XXXVIII, 152, 1924.

44. Humbert, J.G.: Tomato disease in Ohio. Ohio Agric.Exp.Sta.Bull.,
       No. 321, 159-196. 1918.

45. Jones, L.R.: and Grout A.J.: Notes on two species of Alternaria.
       Bull. Torr. Bot. Crub, p.254, 1897.

46. Kohl, E.G. : Ueber den Polymorphismus von Ploespora herbarum
       Tul. Bot. Centralb. XVIII, p.23,1883.

47. La Rue, Carl D.C. and Bartlett, H.H.: A demonstration of numerous
       distinct strains within the normal species. Pestalozzia
       Guepini Desm. Amer. Jour. Bot.IX, 2, 79-92, 1922.

48. Leonian, L.H: Physiological studies in the genus Phytophthora.
       Amer.Jour.Bot. XII, 7, 444-498, and plates, 1925.

49. Long,W.H. and Harsch, R.M.: Pure cultures of wood-rotting fungi on
       artificial media. Jour. Agr.Res. XII, 33-82, 1918.

50. Matz, J. : The Rhizoctonias of Porto Rico. Jour.Dept.of Agric.
       Porto Rico, V., 1, 1921.

51. Mercer, W.B.: On the Morphology and development of Phoma Richardiae
       n. sp. Mycol. Centralb. II. 1913, pp.326-331.

52. Miyabe, K.: On the Life history of Macrosporium parasiticum Thüm.
       Ann.of Bot. 1889,III 1-25 and plates 1 and 2.

53. Nees. C.G.: Das Syst. der Pilze und Schwämme,II 72. 1817.

54. Neger, F.W.: Beiträge zur Biologie der Erysipheen. Flora,90,
       221-271, 1902.

55. Niessl. G. von: Notizen vel. neue u. krib. Pyrenomyceten, p.29, tab.
       IV fig. 14. 1876.

56. Pethybridge, G.H. : Sci.Proc.Roy.Dub.Soc,XIII (N.S.), 36, 1913.

57. Poole,R.F.: A new fruit rot of tomatoes. Bot.Gaz.LXXIV, 2, 210-
       214, 1 Pl. 1922.

58. Prillieux,et Delacroix: Bul.Soc.Myc.France, IX, 201, 1893.

59. Rands, R.D.: The production of spores by Alternaria Solani in pure
       culture. Phytopathology VII, 316, 1917.

60. Reed, G.M.: Physiological specialisation of parasitic fungi. Memrs.
       Brooklyn/

Brooklyn Bot.Gard. I, 348-369, 1918.

61. Roberts, J.W.: Morphological characters of Alternaria mali Roberts. Jour.Agr.Res.,XXVII. 9 , p. 699, 1924.

62. Rosenbaum, J.: Studies with Macrosporium from tomatoes. Phyto- :pathology, X, p.9, 1920.

63. Saccardo,P.A.: Sylloge fungorum, II, p.251,; XVII, p.752;II,p.244.

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

65. Schiemann, E.: Mutationen bei Aspergillus niger van Tieghem. Zeit. f. Indukt. Abstamm.- und Vererb. VIII, 35, 1-35, Taf.1 u. 2. 1912.

66. Shear, C.L.; and Wood, A.K.: "Studies of fungus parasites belong- :ing to the genus Glomerella. U.S.B.P.I. Bull.252,1913.

67. Sherbakoff, C.D.: Fusaria of Potatoes, Cornell Univ. Agr.Expt.Sta.Memr, No.6 1915.

68. Skull, G.H., Harper, R.A. Reed, G. and Stakman, E.C. Symposium - The species concept ...... Amer. Jour.Bot. X, 5. 1923.

69. Société Française des Chrysanthémistes. Repertoire de Couleurs pour aider à la determination des couleurs des fruits, des feuillages et des fruits. 1905.

70. Stakman, E.C.: A study in cereal rusts. Physiological races. Minn. Agr. Expt. Sta. Bull, 138, 1914.

71. _____:, Leach, J.E. and Seal, J.L.: Fruit and Vegetable dis- ;eases. Univ. of Minn.Agr.Exp.Sta.Bull. 199, p.69, 1922.

72. _____, Piemeisel, F.J.: Biologic forms of Puccinia graminis on cereals and grasses. Jour.Agr.Res.X, p.429-495, 1917.

73. _____, and Levine M.N.: Plasticity of biologic forms of Puccinia graminis Jour.Agr.Res, XV,p.221-249. 1918.

74. Stevens, F.L.: The Helminthosporium foot rot of wheat, with observa- :tions on the morphology of Helminthosporium and on the occurr :ence of saltation in the genus. Ill.Dept.Reg. and Educ., Div. of Nat. Hist. Survey. XIV, p.76, 1922.

75. Stevens, F.L. and Hall, J.G.: Ann.Rpt.N.C. Agr.Exp.Sta.(1909) 32,p. 47, 1911.

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

77. Vaughan, V.C.: Mutation in bacteria. Jour. Lab. and Clin. Med. I, 145, 1915.

78. Wakefield, F.W.: A biometric study of the condia of Macrosporium and Alternaria. Roy. Soc. of Tasmania; Papers and Proceed- :ings, 27-31, 1922.

79. Wallroth, F.G.: Flora Cryptogamica Germaniae, p.300, 1833.

80. Waterman, H.J.: Mutatie bij Penicillium glaucum en Aspergillus niger onder invloed van bekende factoren. Kon. Akad. Wetensch. Amsterdam, verslagen Natuurkundige, XXI, 33-38, 1912.

81. Wolf, F.: Ueber Modificationen und experimentelle ausgeloste Mutationem von Bacillus prodigiosus und anderen Schizophyten. Zeit. indukt. Abstamm - u. Vererbungslehre II, 90-132, 1909.

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 sec-
    :ond 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 conidio-
    :phore laterally.

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

4. Chain formation by intercalary budding.   x 600

-5,7,saltant a.   5: 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 conidipphore-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, H
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 characteris-
    :tic 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 cells immediately below.

PLATE III.(Figs. 19-27).

Figs. 19-23, Pleospora herbarum. 19-22, 'normal'.

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 frowing past spores which had been terminal.

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

Figs. 24-26, Macrosporium, 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.

26 Branching conidiophore.

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

PLATE IV. (Figs. 28-35).

Figs. 28 and 29. Macrosporium, strain Td,'normal!

28. Typical ascospore, germinating through wall of ascus.   From
    $3\frac{1}{2}$ hours culture in hanging-drop of water.

29. Typical ascus.   x550.

Figs. 30 and 31. Macrosporium, 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, <u>Macrosporium</u>, 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 cul-
:ture.

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

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

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

47.   <u>Stemphylium ilicis</u>, 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 1.

Plate II.

Plate III.

19.

25.

20.

23.

24.

26.

21.

22.

27.

Plate IV.

28.

30.

31.

29.

32.

33.

34.

35.

Plate V.

43.

44.

47.

45.

46.

Plate VI.

48.

49.

52.

50.

51.

Plate VII.