Th. Scot

PERITHECIAL DEVELOPMENT IN NECTRIA MAMMOIDEA, PHIL. ET PLOWR.

also

THE STUDY OF NECTRIA MAMMOIDEA, PHIL. ET PLOWR. IN CULTURE, WITH AN ACCOUNT OF THE FACTORS INFLUENCING PERITHECIAL PRODUCTION IN THE GENUS.

and

THE PARASITISM OF NECTRIA CINNABARINA (TODE) FRIES.

by

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Perithecial Development in Nectria mammoidea. Phil. et Plowr.

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I. INTRODUCTION.

(a) Salient Features in the Life Histories of the Ascomycetes.

The conclusion arrived at by De Bary (21) and his pupils, that there existed a distinct sexuality in the Ascomycetes, a conclusion that was made despite its denial by van Tieghem (56) and Brefeld (7), has since been confirmed beyond all doubt by later investigators. Since the application of cytological methods to investigations into the life histories of various Ascomycetes, it has been seen that there exists in that group of fungi, as in higher plants, an alternation of generations.

The ascospore on germination gives rise to a hypha whose further growth forms the gametophyte plant. This latter is the dominant stage in the Ascomycetes, and on its hyphae sexual organs are produced, either free or enveloped in the young sporocarp, or stromatic structure. These sexual organs, the male an antheridium, the female an archicarp or reduced to an ascogonium, produce gametes (nuclei) which are brought together in the ascogonium and fuse there, or remain paired through a varying development of ascogenous hyphae to fuse later in the young ascus. Thus the diploid condition is restored in the ascogonium by the fusing or pairing of sexual nuclei and, as a rule, the ascogenous/

ascogenous hyphae represent the short sporophytic phase, for reduction to the univalent condition takes place in the divisions of the definite ascus nucleus to give the ascospore nuclei. The ascospore germinates to give the gametophyte plant and the cycle is completed.

This is a rough outline of the life cycle of the Ascomycetes, an outline which forms a general frame work for those species investigated.

The exact details, however, vary greatly from species to species, especially as to the nature of the sex organs if present, and the nuclear phenomena seen in the various life cycles.

Both antheridia and ascogonia may be present and these may be uninucleate or multinucleate. Connection between these two organs is established and the sexual nuclei come together and fuse in the ascogonium, fusion taking place in pairs, between presumably male and female nuclei in the case of the multinuclear forms. Such has been seen by, Harper in Sphaerotheca castagnei (38), Erysiphe spp (39) and Phyllactinia corylea (39), Barker in Monascus (2), Blackman & Fraser in Sphaerotheca (4), Harper in Pyronema confluens (39), Claussen in Boudiera (15) and to a certain extent by Tandy in Pyronema domesticum (52). In the absence of an antheridium or when the latter is functionless, female nuclei have been seen to fuse in pairs in the ascogonium. Such has been seen/

seen by Blackman & Fraser in Humaria granulata (5), Fraser in Lachnea stercorea (28), Welsford in Ascobolus furfuraceus (58) and Dale in Aspergillus repens (18). A functionless archicarp alone may be developed or there may be no sexual organs whatsoever. In this case fusion takes place in purely vegetative cells as has been seen by Fraser in Humaria rutilans (29), Carruthers in Helvella crispa (14) and Blackman & Welsford in Polystigma rubrum(6). The nuclear fusion in all these cases was regarded as a sexual one, and it was followed by another fusion in the ascus, the latter being looked upon as a purely vegetative fusion and devoid of sexual significance. The feature here, therefore in the nuclear cycle, is the double nuclear fusion.

Lately there have been investigators who have denied the existence of a double fusion. They maintain that the nuclei merely associate in the ascogonium and divide conjugately, fusion taking place in the young ascus between two derivatives of the original pair. This association of nuclei in the ascogonium with conjugate division in the ascogenous hyphae was seen by Claussen in Pyronema confluens (16), Brown (W.H.) in Pyronema confluens (10) Leotia (11) and Lachnea scutellata (12), Faull in Laboulbenia (23,24), Schikorra in Monascus (50), Nienburg in Polystigma (48), Ramlow in Ascophanus carneus and

Ascobolus/

Ascobolus immersus (49), Brooks in Gnomonia erythrostoma (8), McCubbin in Helvella elastica (46), Brown (H.B.) in Xylaria(9), Fitzpatrick in Rhizina undulata (26), Frey in Venturia inaequalis (33), Jones in Rhytisma acerinum (42) and in part Tandy in Pyronema domesticum (52). The above authors regard the fusion of the nuclei in the young ascus as a delayed sexual fusion and thus differ from the previous school who regarded it as vegetative in nature.

In the case where both antheridium and ascogonium were present as in Pyronema and Polystigma, connection between the two was established and nuclei passed from the antheridium to the ascogonium and paired with nuclei there. In Nienburg's investigations on Polystigma (48), he found only one female nucleus present in the ascogonium and one nucleus from a multinucleate antheridium passed in and associated with it. Where the male branch is absent, pairing of female nuclei takes place in the multicellular archicarps for the most part by the breaking down of cross septae and migration of nuclei into a definite ascogonial cell.

This association of nuclei and conjugate division with delayed fusion, brings the Ascomycetes in line with the Basidiomycetes through the Uredineae, where such a process has been commonly seen by various workers. In the latter group however, the paired nuclei are contained in cells, whereas in the Ascomycetes they are free in the ascogenous hyphae. This phenomenon/

phenomenon of the occurrence of conjugate nuclei was referred to by Maire (45) as synkaryon and pushed far by himself and later by Claussen (16), Faull (23), and others in homologising the Ascomycetes and the Rusts. The fusion in the ascus was considered homologous to that in the teleutospore.

Just as there exists conflicting opinions on the fusion which brings about the short intercalation of a diploid or sporophyte phase, so different views are held as to the exact nature of gametophyte restoration which takes place in the ascus. Some of those authors who got a double fusion in the life history, obtained a double reduction of the definite ascus nucleus, the first corresponding to the fusion of the sexual nuclei, the second to the fusion in the ascus. Miss Fraser found such a phenomenon in Humaria rutilans (29) and proposed for it the term brachymeiosis. Such had already been seen by Maire in Morchella esculenta (45) and Dangeard in Ascobolus furfuraceus and Pyromena confluens (19), and since then recprded for Otidea aurantia and Peziza vesiculosa by Fraser & Welsford (32), for Lachnea stercorea, Ascobolus furfuraceus and Humaria granulata by Fraser and Brooks (30), and for Helvella crispa by Carruthers (14).

On the contrary investigators such as Faull on Laboulbenia spp. (24), Claussen on Pyronema confluens/

confluens (16), Guilliermond (35, 36), Brown (W.H.) on Pyronema confluens (10), and Leotia (11), Bagchee on Pustularia bolarioides (1), and Schultz on Peziza domiciliana (51), have obtained only one reduction in the ascus. Tandy, from observations on Pyronema domesticum (52), found that there were both tetraploid and diploid definite ascus nuclei, (derived from double and single fusions), yet the nuclei at the end of the third division were always haploid, so he concluded that there must be in this species both a single and a double reduction division. In respect of fusions and reductions therefore, Tandy's scheme fits in as a liason mechanism between the two views held.

Finally, in regard to the question of chromosome reduction, there are fungi said to exist in which no apparent chromosome reduction takes place. This is due to the intimate association of the homologous chromosomes of the fusing nuclei so that their bivalent and perhaps tetravalent nature cannot be observed. As a consequence, the chromosome number appears constant in the divisions of the ascus nucleus, although the valencey changes. Such was stated by Harper to be the case in Phyllactinia Corylea (40).

(b) Perithecial Development in the Pyrenomycetineae.

Although the study of the life histories of members/

members of the Ascomycetes in general has been fairly extensive, the Pyrenomycetes[1] have not been investigated to any great extent. The earlier investigators studied the perithecial development of this order fairly extensively, but their work lacked cytological detail. Many later investigators, moreover, through various causes, experienced difficulty in following out the full developmental history of the species they were working on, and as a result, many accounts only dealt with a certain phase in the development of the ascocarp.

In Pleospora, Bauke (3), did not find any sexual organs.

In Sordaria fimicola and Melanospora parasitica de Bary (21), got a coiled archicarp consisting of a row of cells, one or more of which gave rise to ascogenous hyphae.

In Polystigma rubrum Fisch (25) described the occurrence of more than one multicellular ascogonium. A definite trichogyne was present and he saw outgrowths from the large ascogonial cells which he described as ascogenous hyphae. Frank (27), working on the same species, obtained the multicellular ascogonium and the trichogyne and got fusion between spermatia and the latter, and so concluded there/

1 The order here is used, in the sense of Gwynne Vaughan (37), to include the four groups Hypocreales, Dothidiales, Sphaeriales and Laboulbeniales. The Laboulbeniales which have been investigated at great length by Thaxter (53, 54), and Faull (23.24), seem to be a distinct group from the others and will not be dealt with here.

there was a normal sexual process like that occurring in various Lichens. These workers of course, merely investigated the morphology of the species, but since then, two cytological investigations have been made on it. Blackman and Welsford (6), got one, seldom two ascogonia. The ascogonium was multinucleate in the earliest stages seen. It later became multicellular and coiled. There was no trichogyne present, although the earlier investigators had got one. The number of cells in the ascogonium and the number of nuclei per cell varied. The ascogonium disintegrated with its content in situ and the asci arose from ascogenous hyphae of vegetative origin. A few cases of fusion in the penultimate cell of the young ascogenous hyphae were seen, while there was some evidence for an earlier nuclear fusion in the ascogenous hyphae at the time of their differentiation. Nienburg (48), opposes these conclusions. He got an archicarp with a coiled base and a chain of multinucleate cells forming a trichogyne. One of the multinucleate cells, the antheridium, lay above the ascogonium. The separating wall dissolved and the nuclei from the antheridium migrated into the ascogonium where one of the antheridial nuclei enlarged, and became the male nucleus, which associated but did not fuse with the single female nucleus present. The ascogenous hyphae arose from the ascogonium and contained paired nuclei but the origin of the ascus could not be observed. He believes/

believes the only fusion takes place in the ascus.

In Claviceps, according to Fisch (25), no ascogonia were present at all, while the same investigator found ascogonia in Xylaria but these disintegrated, and there was no connection between them and the ascogenous hyphae. In Xylaria, Brown (H.B.) (9), got a coiled multicellular archicarp whose cells were at first uninucleate but later multinucleate. The cells became separate and lay in the perithecial cavity. Later some of these cells, derived from separation, sent out branches which gave rise to the ascogenous hyphae. Nuclei passed into these branches and it was thought that the asci arose from the latter. Nuclear fusion was not seen at any time and no crosier formation was seen.

Nichols (47) found in Teichospora aspersa and Teichosporella sp. that the perithecium arose from the continued division of a hyphal cell and that the asci were formed from central cells in the perithecium. In Ceratostoma brevirostre the same investigator found that the perithecium arose in relation to a coiled archicarp with which an antheridial branch was often in contact. These became connected, a portion of the wall between them dissolved and although each organ contained many nuclei no fusion was observed in the archicarp.

In Poronia punctata Dawson (20), found a coiled multicellular archicarp with evidence of a trichogyne/

trichogyne,which later disappeared. The cells of the coil came apart and the ascogenous hyphae arose from stout, deeply-staining hyphae, which occupied the position of the coiled archicarp in the early stages. It is doubtful, however, if these hyphae which gave rise to the ascogenous hyphae were really portions of the archicarp seen earlier on.

In Sordaria fimicola, Faull (22), found the ascus did not always rise from the penultimate cell, but in many cases from the ultimate, while in Sordaria humana and Podospora acerina the asci arose from the terminal cells of the ascogenous hyphae.

Brooks (8), described the development of Gnomonia erythrostoma. He found functionless spermatia in that species. There were usually more than one ascogonium in a perithecium. They were coiled, multicellular and multinucleate. They disintegrated and the ascogenous hyphae arose de novo from the vegetative cells at the base of the perithecium. There was only one fusion, namely in the young ascus. Reduction occurred only in the first division of the ascus nucleus.

In Venturia inaequalis Killian (43), found that a single coiled hypha enlarged in the perithecium to form a multicellular ascogonium. An antheridium was present outside the perithecium and fused with a trichogyne which was produced by the archicarp and protruded through the perithecium. Male nuclei passed into the trichogyne and into the ascogonium. The

walls/

walls of the latter dissolved and the male nuclei paired with the female nuclei and lay in the basal cells of the ascogonium. Further development could not be followed. Frey (33) has worked on the same species. He found that there was a single archicarp extending into a trichogyne and both were non-septate. An antheridium was present outside the perithecium and it applied itself to the trichogyne. A pore was formed and nuclei passed into the trichogyne. The now multinucleate unicellular ascogonium was thought to become septate as a result of the passage of male nuclei into it. Sometimes, however, septation occurred without any evident fertilisation. The nuclei associated in pairs in the multicellular structure, usually two nuclei in each cell, but no fusion was seen. It was thought that these ascogonial cells branched and that the branches became septate and formed the asci. No nuclear fusions were seen nor was there observed any breaking down of the cross walls in the ascogonium as the previous worker on the same species found.

In Nectria, Hartig (41) conjectured that special ascogenous initial organs were present in the very young stroma.

Vincens (57), working with Nectria ribis found the perithecium to arise within a stroma. It arose as a filament of the stroma which possessed the characters of a ascogonium. This latter grew and anastomosed/

anastomosed, the cells increasing in number. Some of the latter disintegrated and by so doing formed a cavity which was enlarged by the outward growth of the perithecial walls. The cells of the walls bordering on the cavity at first gave rise to paraphyses, then later to asci. The cells of the ascogonia disintegrated entirely. Some of the cells of the basal and lateral walls ceased to give rise to paraphyses and formed ascogenous hyphae. These latter were composed of cells mostly binucleate. There was no "crotchet" formation seen, nor were the nuclei seen to fuse.

Cook (17), investigated the life history of Nectria ipomeae. He did not get any nuclear fusions preceding the initiation of the perithecia, nor did he find the perithecium to arise around any archicarp-like structure. He did not find structures in the young perithecial "knot" comparable to the ascogonia which Miss Caley found in the young perithecia of Nectria galligena (q.v.).

The last named worker found that in Nectria galligena (13), several ascogonia arose in one perithecium. When these were first seen they were found to be multicellular and multinucleate and the writer appeared to think that they probably arose as unicellular structures and later became septate. These ascogonia filled the perithecial cavity. The presence of any trichogynes was doubtful. No nuclear fusions were/

were observed in the ascogonia, although the nuclei associated in pairs. There was a single evidence for pore formation between two cells in one of the ascogonial coils and the passage of a nucleus through this opening. Hyphae were produced from the ascogonia and nuclei passed into them. Both ascogonia and their derivatives disintegrated, however, and the asci originated from cells at the base of the perithecial cavity whose origin could not be traced. No nuclear fusion was seen in the latter.

It is seen then, that in these Pyrenomycetes studied, an antheridium may be present or absent. In some cases no sex organs are found in the perithecium while, again, a single or many archicarps may be present in each perithecium. These archicarps are probably at first unicellular and later become multicellular. They may or may not possess a trichogyne. They appear to be uninucleate or multinucleate at their inception. Connection between the cells of the ascogonia by pores as seen in many Discomycetes does not here seem to be general. Nuclear fusion in the ascogonia does not appear to have been seen. The ascogonia may function, i.e. give rise to ascogenous hyphae, or they may disintegrate before doing so. The asci arise from these ascogenous hyphae produced from ascogonia or, where the latter or their hyphae disintegrate, from vegetative cells in the perithecium. The ascus appears to arise from the ultimate or penultimate ascogenous hyphal/

hyphal cell. Very few cases of fusion in the young ascus have been observed, and only Brooks (8) appears to have got satisfactory divisions in the ascus. A double fusion in Polystigma rubrum is suggested by Blackman and Welsford (6).

It appeared then, that in view of the variable nature in the details of the sex organs, of the behaviour of their nuclei, the origin of the ascogenous hyphae and the asci, and the scanty knowledge concerning nuclear division in the ascus in this group, that an investigation into the perithecial development of one of their members would not be superfluous.

II. MATERIAL and METHODS.

Nectria mammoidea produced perithecia abundantly on most of the media used for its culture, viz., on oat agar, French bean agar, 2% and 5% potato dextrose agar and on two year old Alnus twigs (Plate I. Fig.1.). The perithecia developed fully and contained asci which matured, and then ejected normal ascospores. Since the perithecia in culture appeared to follow the normal course of growth as pursued under natural conditions, it was thought that here was an opportunity to study the morphology and cytology of their development. Moreover it was easier to obtain all stages of development on artificial media, and the latter was more amenable to fixing and cutting than the natural woody substratum.

Perithecia in all stages of development were obtained from cultures on oat agar and bean agar. Perithecia were produced freely on both these media irrespective of the source of innoculum; in cultures derived from mono-ascospore, mono-conidial or mycelial plantings. The theoretical importance of this will be discussed elsewhere. Pieces of the media bearing the fungus fruits were then fixed in Flemming's solutions, both strong and weak, and Carnoy's fluid, (Chamberlain. Methods in Plant Histology 4th ed. 1924), but it was discovered that the weak Flemming's was by far the most suitable and it alone was used in further work. The strong Flemming's tended to blacken/

blacken the material, while the Carnoy's fluid rendered it brittle. The air pump was used in order to extract the air and hasten penetration. The material was washed, dehydrated in alcohol, transferred to xylol, and embedded in 52° C. paraffin. Many concentrations of the agents were used in order to make the progress of the material through them gradual, while the same slow process was adopted in transferring from one agent to the other. Sections were cut at a width varying from 2μ-6μ. Heidenhain's Iron Alum Haematoxylin was used as a general stain, with a clove oil solution of either eosin, erythrosin or light green as a counter stain. The method adopted for using this stain was that of Yamanouchi's Schedule. (Chamberlain, l.c. p.45). These various combinations, however, did not bring out cell outline, due to the weakness of the counter stain and so, in preparations treated with them, there was difficulty in tracing various structures to their sources. For this purpose polychrome methylen blue with orange tannin as a mordant was suitable, at the same time acting as a fairly good nuclear stain and though never so brilliant as the haematoxylin, yet was always sure and helped to corroborate conclusions derived from a study of preparations made with the latter stain. Grams method of staining with gentian violet (Lee. Microtomist's Vade Mecum 8th ed. 1924, p. 167) was used, and found to be good for staining nuclei in the vegetative cells and the young cells in the perithecial initial. In the former the haematoxylin stained many cell inclusions and/

and led to confusion in spotting and counting nuclei. Moreover it stained the nucleoli heavily and did not bring out nuclear detail whereas the gentian violet did very well for this purpose.

III. THE VEGETATIVE HYPHAE.

All gradations in the size of the vegetative hyphae are seen from a large thick walled type about 6.5μ wide to a narrower thin walled from 1μ - 2μ thick (Plate I. Fig. 3.). The hyphae comprising the plectenchymic stroma on which sporodochia are borne, and from which perithecia arise, tend to be of the larger type, while the hyphae comprising the growth within the media varies between both extremes. The hyphae are in all cases multicellular, and each cell is multinucleate, as many as twelve nuclei being counted in one cell. This multinucleate condition of the vegetative cells has been seen in Gnomonia erythrostoma (26), Humaria granulata (10), Aspergillus herbariorum (54), Ascobolus furfuraceus (12), Lachnea stercorea (11), and by Vincens in many Pyrenomycetes (52), but it is not necessarily general in the Ascomycetes since uninucleate cells have been seen in the Erysiphales (3,4), in Venturia inaequalis (30), and Chaetomium spirale (55). The nuclei in the vegetative cells of Nectria mammoidea have a well defined nuclear area and nucleolus, and measure on an average about 1.5μ in diameter. The nucleoli stain heavily with gentian violet while the nuclear area stains lighter. Scattered in the nucleus are more heavily staining granules, probably of a chromatin nature. No nuclear divisions were seen in the hyphae.

IV. PERITHECIAL DEVELOPMENT.

(a) The Perithecial Initial.

The thread-like plectenchymic stromata are dispersed generally over the surface of such media as bean agar, oat agar and Alnus stems. They are composed of intertwining hyphae whose cells are elongated in the same direction. On such stromata the perithecial initials arise as little spheres or "knots" of pseudo-tissue (Plate I. Fig. 2.). These latter are at first composed of cells which are shorter, narrower and not so vacuolate as those of the underlying stromatic hyphae. Moreover the number of nuclei per cell is reduced to one, more rarely two or three (Plate II. Fig.1.). These nuclei are slightly larger than the nuclei of the hyphal cells, measuring about 1.9μ and contain an appreciably larger nucleolus. The nuclear area is represented by a clear patch round the deeply staining nucleolus. The cells of the perithecial initial are thus probably in a state of rapid growth and division. For a considerable period of development the cells in the initial "knot" show no differentiation. The perithecium cannot therefore be said to arise round any archicarp-like structure. It is so far an aggregate of vegetative cells.

(b) The Origin, Development and Disintegration of the "Interpolated" Cells.

These cells continue to grow and divide

and/

differentiation appears in them when the "knot" attains an approximate diameter of 40μ. About the middle region of the "knot" several cells become much more protoplasmic, and as a consequence stain more heavily than the remaining "knot" cells (Plate II. Fig.2.). On a superficial examination they might be thought to be the components of a coil but this is not the case, as later development shows. These cells originate separately and simultaneously and are prolongations of cells in the "knot". This is seen in the micro-photograph which shows three such cells with distinct origins (Plate II. Fig. 3.). The developing perithecium by growth and division of its cells expands and a cavity is formed in the interior. This cavity formation does not seem to be due to any lysigenetic action, as Vincens (57) found in Nectria, but is merely an outcome of the rapid outward expansion of the perithecium without replacement in the centre by new cells. This method of cavity formation seems to take place in Gnomonia, Xylaria, and to a certain extent in Poronia. The differentiated cells situated on, or a little in from the surface of the cavity so formed send in tapering prolongations into its interior (Plate III. Figs. 1 & 2.). These cells were never seen to contain more than one nucleus and the latter did not differ appreciably in size from the nuclei of the other perithecial cells. There are thus at this stage in development two distinct cell groups/

groups, the one represented by the heavily protoplasmic cells projecting into the cavity, and the other by the remaining cells of the developing perithecium. Of the latter, these lying near the periphery are becoming thick-walled and very vacuolate.

The perithecium increases in size in all directions, and still retains a roughly spherical shape. The cavity is seen to be enlarged and the number of cell layers increased, (Plate IV. Fig. 1). The central cells are now seen as straight, finger-like structures originating at the cavity surface and extending into it. They are closely packed together and probably owe their straight nature to a mutual support derived from this close arrangement. They extend in from all round the cavity, except for a small gap in the continuity at that point furthest from the substratum, which may for convenience be called the top of the cavity. Those arising from the foot of the cavity are longest (Plate IV. Fig. 3.). They are unicellular, unbranched and for the most part uninucleate. A few, however, reveal a binucleate condition (Plate IV.Fig. 3 at a.). This state is in all probability due to the division of the original nucleus present in these cells. Although such division was never seen, it is suggested by the nearness of the two nuclei to one another and the failure to observe the migration of a nucleus from the vegetative mother cells into the binucleate central cells which arise from them. The remaining perithecial cells, which might/

might be said to form a protective covering to the above, are now becoming less compact at the foot of the perithecium, where they are now no longer oval-oblong but roughly spherical and considerably vacuolate (Plate IV.Fig. 1, at a.). It would appear that these latter cells are giving rise to the better developed central cells. So far there is no indication of an ostiole.

A little later the cells extending into the cavity (i.e. the central cells) show a differentiation (Plate IV.Figs. 2 & 3.). Those occupying the upper half of the cavity and arising from the compact outer perithecial cells stain more deeply and are seen to be becoming multicellular through formation of cross septae. Each cell so derived is short and squarish in outline and contains one nucleus (Plate IV.Fig. 2.). These are in marked contrast to the long narrow cells arising from the loose perithecial cells at the base of the cavity (Plate V. Fig. 2.). Some of the former are beginning to show a septum (Plate V. Fig. 2, at a.), while the others remain unicellular (Plate V. Fig. 2, at b.). This septum arises in the lower half or about the middle of the cell. It arises in those cells which have become binucleate, (as seen earlier) and each of the daughter cells receives a nucleus. It is important to note at this time that there is no difference in general characteristics between the resulting two cells or their nuclei (Plate V. Fig. 2, at a.). An increasing number of cells in this area which remain non septate are becoming binucleate, (Plate V. Fig. 2, at c.) while/

while at this early stage one such cell was seen to be producing a branch (Plate V. Fig. 2 at d.). This early differentiation of the central cells, which at first might seem slight has a distinct importance, since the upper hyphæ of short, squarish cells ultimately form the paraphyses, while the lower are developing cells which may for convenience be referred to as "interpolated" cells. The nature of these cells will be discussed later. These two types of central cells, at first contiguous about the middle of the cavity, (as seen in Plate V. Fig. 2), are later separated in the following way. The cells comprising about three rows in the upper half of the cavity, the inner of which borders on the cavity, commence to elongate in the direction of the top of the perithecium (i.e. the point furthest from the substratum). By so doing, they carry up the central cells which arise from them, so that these latter (i.e. the young paraphyses) carried up and across from either side (Plate V. Fig. 1.) come together along the top of the cavity where they form a structure resembling a comb hanging down in a horizontal fashion. Thus, (Plate VI. Fig. 1, at a.) the paraphyses cells are now separated from the "interpolated" cells which have retained their position at the lower half of the cavity (Plate VI. Fig. 1 at b.). The elongation of the cells above referred to is extended further upwards until the pressure caused by it forces apart the outer layers of the covering. Thus, (Plate VI. Fig. 2.) the ostiole is formed, the action in its formation being apparently mechanical.

There/

There is therefore created in the perithecial covering an opening which is only prevented from being continuous with the cavity, on account of the paraphyses lying across the top of the latter.

Differentiation is now rapid. The outer protective tissue which previously was fairly homogenous now differentiates into three. The first forms the wall proper of the perithecium, the next is a narrow pseudo tissue of elongated thin walled cells, while the third and innermost (excluding the central cells) is a sort of pseudo-parenchymatous "ground" tissue within which are the paraphyses and "interpolated" cells, both of which border on the cavity (Plate VII. Fig. 1.).

These cells of the "ground" tissue lying above the paraphyses and under the ostiole dissolve, (the action being lysigenetic), and a cavity is formed, the ostiolar cavity. Thus we get a smaller cavity (the ostiolar) lying above a larger (the perithecial), the one being separated from the other by the developing paraphyses (Plate VII. Fig. 1.). The cells bordering on the ostiolar cavity so formed protrude little whip-like extensions into it. These are the periphyses, which, along with the various pseudo tissues shortly referred to above will be described more fully later (Plate VII. Fig. 1 at a.).

Meanwhile the progress of the paraphyses and "interpolated" cells will be discussed. The paraphyses by growth and septation have extended in a compact mass of almost parallel hyphae further down/

down into the cavity, and resemble at this stage a descending curtain (Plate VII.Figs.1&2 at b.). Their cells are still regularly uninucleate, except where on occasion, the daughter nuclei of a dividing nucleus have not been separated by a new wall. The lower and at the same time younger cells of the paraphyses (i.e. those nearer the base of the cavity) are narrower and more protoplasmic than those higher up, the latter tending to become broader and more vacuolate as the distance from the advancing tips increases. The region of growth is in all probability at the tips.

The "interpolated" cells by now have developed considerably. By a lateral extension of the hyphae at the base of the cavity the "interpolated" cells arising from them are no longer mutually supported but are pulled apart and so, being delicate structures, drop over and lie irregularly at the foot of the cavity (Plate VII.Figs. 1&2 at c.). When the development of these "interpolated" cells was last discussed, it was seen that several were at that time unicellular, uninucleate or binucleate structures, while septation had occurred in some of the binucleate ones to give a long, thin bicellular structure (Plate V. Figure 2.). It is now seen that these septate and non septate types still exist, but each has enlarged greatly. The unicellular type is now seen to increase in width higher up (Plate VIII.Fig.1 at l). This is probably due/

due to greater room for lateral expansion there, where the perithecial cavity widens. They become bent and coiled in all directions and are exceedingly difficult to follow. They give rise to branches one,(Plate VIII Fig.1, at 10.) or more,(Plate VIII Fig.1, at 12, being seen arising from each. These side branches did not seem to develop much prior to disintegration. The number of nuclei has increased to give in every case a multinucleate structure. These nuclei have increased greatly in size, but this is not due to fusion alone, (although fusion is seen), since nuclei about to fuse, or which have fused and whose nucleoli still remain apart, show an area or in the latter case a nucleolus which is much larger than those seen in the initial "interpolated" cells. The nuclei are definitely paired (Plate VIII. Fig. 1, at 3 and 12.) and have an area which shows as a clear space round a relatively large and deeply staining nucleolus (Plate VIII. Fig. 1.). Fusion between paired nuclei was seen on two occasions. In both these instances the nuclear areas had fused but the nucleoli still remained apart (Plate VIII.Fig. 1, at 5 & 6.). Instances appeared quite common where it seemed that two nucleoli in the one nuclear area were in the late stages of fusion but it was concluded, after lengthy examination, that these "fusing" nucleoli were ~~each~~ the result of in reality a single nucleolus beginning to fragment (see later). Nuclei are seen to pass into the branches arising from the "interpolated" cells (Plate VIII. Fig. 1, at 9 & 12), but it was difficult to determine whether these were/

were derived from a previous fusion or no, since nuclei and nucleoli of varying sizes were seen, due to their gradual growth above referred to. Moreover the size of fusing nucleoli themselves differed.

Disintegration set in in these structures. This was first apparent by the nucleoli fragmenting Plate VIII. Fig. 1, at 7.). Later the protoplasm disorganised and stained various dark shades with iron alum haematoxylin (Plate VIII. Fig. 1, at 7 & 8.). Plate VIII. Fig. 1, at 8. shows a completely dead structure while Plate VIII. Fig. 1, at 4 shows the commencement of general disintegration and Plate VIII. Fig. 1, at 7 shows in the top half the nucleoli breaking up, while in the lower half death of the protoplasm has already resulted. Plate VIII. Fig. 1, at 4 shows blackening to take place at first round the vacuoles, and would suggest that the deeply staining material was a product of interaction between cell protoplasm and cell sap; a mixing of the two latter being in all probability due to the death of the living membrane between them and their consequent association. The fact that the structure such as figured in Plate VIII. Fig. 1, at 8 lay alongside a perfectly staining one as seen in Plate VIII. Fig. 1, at 10 would surely confirm that blackening was not due to improper fixation. Mingling of those well stained and black cells took place freely, and certainly suggests disorganisation of the latter as the cause of blackening. It is interesting to note that disorganisation took place at various stages in the development of these cells, viz., while unbranched or after/

after they had given off side branches.

The septate "interpolated" cells which were seen earlier, have been reinforced in numbers by others which have become multicellular, and would probably be added to by later septation of the above unicellular type. The number of septa varies, four being the greatest seen. Probably more septa exist but it is difficult to follow the structures through serial sections, since they are so intertwined one with the other. They bend, some giving the impression of "crochets" formed in ascogenous hyphae (Plate VIII. Fig. 2, at 14, 15, & 16). In these septate structures no pore-like connections were found between the contiguous cells. Outgrowths may arise from one or more cells (Plate VIII. Fig. 2, at 18-21) but these do not seem to develop far. Plate VIII. Fig. 2 at 24 represents the greatest development seen in these offshoots. The nuclei have increased in size exactly as seen in the unicellular type (q.v.). They are very definitely paired as most of the figures show, and each cell contains as a rule usually one or sometimes more pairs. Fusion was observed between such paired nuclei on one occasion only (Plate VIII. Fig. 2, at 22). Disintegration sets in in a manner described for the unicellular types, the breaking down taking place cell by cell (Plate VIII. Fig. 2, at 19, 22 and 23.).

From the following considerations it would appear that those septate and non septate types are homologous/

homologous. Development has shown that they both arise by differentiation from vegetative hyphae at the base of the cavity. Both give rise to branches; in the case of the unicellular type one or more from each cell, in the case of the septate type one from one or more cells. Nuclear fusion occurs in both. That one is probably not the derivative of the other is concluded by the failure to observe both types connected. Both seem to represent end points in development. The only difference between them appears to be in the presence of septa in the one and their absence from the other. Disintegration of the entire mass of "interpolated" cells is practically complete by the time the paraphyses reach the base of the cavity; in fact by that time only a few blackened remains are seen (Plate IX. Fig. 1.). There is now a distinct pause in developmental activity at the base of the cavity while the paraphyses continue to grow down towards it.

When their tips just touch the foot of the cavity the perithecium is beginning to assume a mature appearance (Plate IX.Fig. 2.). It has a beautifully globular outline with a slight suggestion of a flattening at the top and measures approximately 374μ x 385μ. There are four main pseudo-tissues in the fruit body. The first and outermost is the wall of the perithecium, which is composed of two lesser pseudo tissues; an outer and an inner. The inner comprises a layer of three to five rows of cells elongated in the direction of the circumference. They are thick-walled and encircle the entire/

entire perithecium except at the ostiole, where their circular continuity is broken. Here, by their upward growth already referred to, they have pushed back the outer layer of the wall, and their ends protrude on the upper external surface of the perithecium, where they form the disc or platform characteristic of this species. The outer portion of the perithecial wall encloses the other tissues except where, as above referred to, its inner portion has pushed it aside at the disc. This outer portion of the wall is composed of thick-walled cells, whose walls are black, probably through interaction between some contents of a fatty nature, and the osmic acid in the fixitive. This blackening may, however, be an oxidation process. These cells are distributed rather irregularly, not forming well defined layers as the above described inner wall/pseudo tissue. Near the "shoulder" of the perithecium, however, they tend to run in a parallel manner in an outward direction. The layer at this point (i.e. at the "shoulder") is fairly deep and gradually narrows towards the lower half, while across the foot it diminishes greatly in width, and is represented by a few cells scattered irregularly at the line of contact between the perithecium and the underlying plectenchymic stroma.

Within the wall the next pseudo-tissue is a layer composed of about three rows of thin-walled elongated cells whichextend round the perithecium and run almost to a point at the ostiole, where the cells coming/

coming from opposite sides just leave sufficient space to keep the ostiole channel open. At the ostiole region, several of the cells of this layer bordering on the channel are protruded as periphyses.

The third pseudo-tissue has been already referred to as the "ground" tissue. It comprises the greater mass of the central region of the perithecium, and encloses the paraphyses. The cells of this tissue are large, thin-walled and roughly spherical, and form, below the paraphyses, a large pseudo-parenchyma, which narrows at the sides till it disappears at a point where the paraphyses touch the second layer. However, it reappears again above the paraphyses where it forms a further pseudo-parenchyma. The cells of this portion of the "ground" tissue dissolve to form an oval to spindle-shaped cavity extending from the ostiole to the paraphyses. From the surface of the cells bordering this cavity, elongated whip-like cells, the periphyses, are produced. Thus, it is seen that the periphyses are produced almost entirely from that part of the "ground" tissue lying above the paraphyses, while a few are formed from those cells of the second layer bordering on the cavity immediately below the ostiole. A fourth pseudo-tissue is represented by a layer of cells lying above the "ground" tissue at the base of the perithecial cavity, and its cells differ from the cells of that tissue by their smaller size, the irregular outline and extreme delicacy of their walls and by their staining more heavily with protoplasmic stains. This layer is probably derived from "ground" tissue cells/

cells which differentiate. It might be called the "fertile layer" since from its cells arise the true ascogenous hyphae.

The paraphyses now, by downward growth, reach the"fertile layer"and the tips of their cells become intertwined with the "fertile" cells. Actual fusion between those cells was never seen. It is worthy of observation at this stage, that the paraphyses are beginning to disintegrate below the ostiolar cavity. This is well seen in the micro-photograph/ (Plate IX. Fig. 2). Interspersed among the tips of the paraphyses can be seen the last disintegrating remants of the few "interpolated"cells and their hyphae not yet fully broken up.

(c) The Ascogenous Hyphae.

It is at this stage when the descending paraphyses touch the"fertile"cell layer, that the cells of the latter begin to produce the ascogenous hyphae. / (Plate IX. Fig. 3 at a.). It may be that the cells in this layer derive nourishment from the disintegration products of the "interpolated" cells, and that the contact of the paraphyses serves as a stimulus to produce the ascogenous hyphae. The latter arise as outgrowths from the cells of the "fertile" layer. No cell wall is formed to separate the outgrowths and their mother cells (Plate X. Figure 3.). These outgrowths become more densely protoplasmic at their upper/

upper end,and contain at first one nucleus which has a large nucleolus and a clear nuclear area (Plate X. Fig.2, at a.). Later a binucleate condition appears, due to the migration of a nucleus from the lower part of the cell into the upper protoplasmic portion. These nuclei are larger than the nuclei of the vegetative hyphae. This binucleate stage of the ascogenous hyphae is extremely common (Plate X.Fig.1,at a.). In spite of long searching no "crotchets" could be found, nor was there seen,at any time,septation in the young ascogenous hypha to give a multicellular structure. The young ascogenous hyphae are of the simplest type, their cells being prolongations of the hyphae from which they were derived,and containing two nuclei which fuse directly without any preliminary divisions. All stages in their fusion can be seen (Plate X. Fig.3). The nuclear areas fuse first of all to give one area containing both nucleoli (Plate X.Fig. 2 at b.), while the latter fuse considerably later (Plate X.Fig. 3 at 9.).

(d) The Asci.

The nucleus derived from this fusion is the definite ascus nucleus./ (Plate X.Fig.2, at c.). It has an area much greater than that of the nuclei which fuse to form it, and its nucleolus is about twice the diameter of the nucleoli of the fusing nuclei. With the formation of the definite ascus nucleus, the area of hypha surrounding each, along with the nucleus becomes automatically the young/

young ascus. There is no distinct line of demarcation between each ascogenous hypha and the ascus arising from it. They merge imperceptably into one another. This nuclear fusion would appear to stimulate growth in the young ascus, which grows rapidly, pushing its way up among the paraphyses. The latter are now at their highest developmental point (Plate IX. Fig. 3.),and their cells are swollen and highly vacuolate right down to where they intermingle with the "fertile" layer. As the asci develop,the paraphyses disintegrate downwards from a point just below the ostiolar cavity (Plate XII.Figs. 1 & 2.). There is thus no longer any barrier between the asci and the ostiole. A preparation made at this time from a squashed perithecium shows asci at various stages in development,and paraphyses apparently rising from the same layer. This gives the impression that the paraphyses really originate,as do the asci,from the cells lining the base of the perithecium,and has led to the belief that they represent structures which have developed from cells which at one time were probably fertile but have become sterilised in descent. The course of development shows that this is not the case in this species.

(e) Nuclear Divsion in the Ascus

First Division.

Prophase. The period elapsing between the nuclear fusion which forms the definite ascus nucleus and/

and the appearance of the prophase in the latter, is of short duration. This feature has been observed by Fraser (29), Bagchee (1), Faull (24), and others. After the formation of the definite ascus nucleus, the ascus enlarges greatly as if stimulated to rapid growth by the act of fusion. At the appearance of the prophase, the ascus is a plump, almost oval structure, with a homogenous, granular, non-vacuolate protoplasm (Plate XI. Fig. 1,A). The nucleus is large, and the nucleolus especially so. The latter lies at the foot of the nuclear cavity. The chromatic figure appears at the top, and does not seem to be in any way connected with the nucleolus. It is composed of a dense linin in which darker chromatin beads stand out. The maximum number of the latter seen is ten. This is the first contraction stage (Plate XI Fig. 1, A). The hollow spireme stage is initiated by the chromatic figure commencing to send into the cavity towards the nucleolus, loops of linin, in which appear definite chromatin beads. This process goes on, the number of loops increasing until many loops are seen hanging from a common point (Plate XI. Fig. 1, B-E). No central body could, however, be distinguished at this point. The number of beads now seen in the loops has greatly increased over that observed in first contraction, and is probably due to the splitting of those seen in the latter stage. The ascus grows and vacuoles appear at the upper end above the nucleus (Plate XI. Fig. 1, E). The loops seem to detach themselves/

themselves from the common point observed, and distribute themselves freely in the nuclear cavity, where they appear to form an endless spireme along whose linin the beads are evenly strung (Plate XI. Fig.1,F). Vacuolisation of the ascus now appears below the nucleus. The loops in the spireme become twisted and contract and pairing takes place between beads in corresponding threads (Plate XI. Fig. 1,G.H.). Further development shows that these beads in the loops are of a half univalent nature. The loops now appear attached to the nucleolus (Plate XI. Fig.1,J.). The advanced hollow spireme stage is seen in Plate XI, Fig.1, K. & L. . The nuclear area at this time increases to its maximum, elongating in the direction of the ascus. It loses its definite outline and it would almost appear as if the nuclear membrane had ruptured. The loops lengthen greatly and at their distal ends appear to invade the ascus cytoplasm. The beads are distinctly paired and fusion seems to take place betwen the half univalents where the threads cross (Plate XI. Fig.1, L.). The arms of the loop now approach one another in this stretched position, and fuse along a considerable portion of their lengths (Plate XI.Fig. 1, L.). This stage in the hollow spireme is of considerable duration. The nucleus contracts and once again the membrane becomes distinct and the threads which have fused appear to come apart again (Plate XI.Fig.1,M.). A definite condensation of the linin now sets in. Many nuclei are seen in which the spireme is massed as/

as a darkly staining ball round the nucleolus, which occupies a central or a medium lateral position. In such nuclei, (which were especially common in the preparations showing division in the ascus), the beads could not be distinctly seen, nor could any idea be obtained as to the nature of the threads. In stages immediately preceding or succeeding this condensation stage, however, it could be seen that the thread containing the half univalent beads were fusing entirely, while the homologous beads in each thread also fused on the fusion of the latter (Plate IX. Fig. 1,N & O.). At the height of this condensation the ascus is highly vacuolate both above and below the nucleus. For a period the details in the behaviour of the spireme are very difficult to follow. This is due to the linin becoming very thready and staining faintly, while the beads also are extremely difficult to make out. As a consequence nothing definite could be made out until such a stage appeared as figured in Plate XI.Fig. 1,P. Here the beads are once again darkly staining although the linin still appears thready. There is a definite fission in the latter and tetrads are now beginning to appear. The corresponding half univalents unite imperfectly and form a univalent which slightly joins up with a near by similarly imperfectly united pair of half univalents on the same threads (Plate XI. Fig. 1, P.). These tetrads were fairly common in stages from now onwards until they ultimately condense as bivalent chromosomes.

The/

The second contraction seen by so many authors in the heterotyte division, was not seen with certainty here. This may have been due to its exceedingly short duration. A nucleus revealing a stage suggestive of this one is shown in Plate XI. Fig. 1, Q. The nucleolus has been severed from that part of the nucleus figured, but there can be seen large deeply staining beads which stand out clearly from their supporting linin. Tetrads reappear later in increased numbers and still on their linin supports, (Plate XI Fig. 1, R). The fission closes and the linin now segments (Plate XI. Fig. 1, S.), and the tetrads or their condensation products lie free in the nuclear cavity. In Plate XI. Fig. 1, U, two distinct tetrads are seen in the lower right and left quadrants, while two V-shaped intermediate chromosomes are seen in the upper right and left quadrants. The arms of the latter probably represent univalent chromosomes which have fused telosynaptically at one end (the point of the V) while the others still remain free. In the tetrads showing in this figure, the threads attached to the lower half univalents are still apparent. This would appear to settle definitely the true make-up of the tetrads as being as suggested earlier on. From the mode of their formation, and from the appearance of the definite split in the tetrad at right angles to the line of union between the two threads, it is difficult to recognise this fusion as a weak parasynapsis between two homologous univalent chromosomes. It would appear/

appear that the scheme followed out in this prophase is that announced originally by Farmer & Moore in Britain and Montgomery in America, and later seen by Miss Digby, Miss Fraser and others in many plants. (For full account and literature dealing with this phenomenon see scheme B in Sharp's Introduction to Cytology, 2nd ed. 1926, p. 265).

Diakenesis was not seen.

Metaphase. The origin of the achromatic spindle was not observed in any of the divisions, though the mature form was seen in them all. In the metaphase, five large bivalent chromosomes can be counted at the equator of the spindle (Plate XI. Fig. 2, 1 & 1a). It would appear that each arranges itself on the spindle at right angles to the line of union between the univalents, and the split takes place along the latter line (Plate XI. Fig. 2, 1 at a). At this stage the fission between the half univalents has entirely disappeared.

Anaphase. The halves derived from the split move to the opposite poles (Plate XI. Fig. 2, at 2, 2a, 2b.). Ten univalents can be counted as the maximum number. The late anaphase was not seen so that one could not determine if a split took place then. Meanwhile the nucleolus diminishes greatly in size until it disappears entirely at the late telophase.

Telophase. The figure was seen at the late telophase, but the number of chromosomes could not be definitely ascertained, as the latter were already/

already commencing to fuse into one mass at the poles. One preparation, however, showed four chromosomes at one of the poles (Plate XI. Fig. 2 at 3). A feature of interest was the elongation of the spindle at this stage, thus separating the daughter chromosomes far apart. Such a phenomenon was observed and remarked upon by Faull (24), working on species of Laboulbenia, and it seems to be general in the first division of the ascus nucleus. The two daughter nuclei are formed and there is a definite interphase, (Plate XI. Fig. 2 at 4). The nuclei are smaller and contain smaller nucleoli than the definite ascus nucleus.

Second Division.

Prophase. The beads again become apparent on the linin framework (Plate XI. Fig. 2 at 5). The linin threads are on occasion seen to be arranged in parallel (Plate XI. Fig. 2 at 5a). A definite contraction takes place, the parallel threads and their beads uniting (Plate XI. Fig. 2 at 6). This phase was seen by Bagchee (1), in Pustularia bolarioides, who interprets the parallel threads as splits derived from univalent chromosomes, and the contraction as the stage bringing about reunion between these threads.

Metaphase - Anaphase. The entire metaphase of this division was not seen, but only a stage where it enters on the anaphase (Plate XI. Fig. 2 at 7). In Plate XI. Fig. 2 at 8, the lower nucleus shows some chromosomes already split and travelling to the poles, while/

while others are still at the metaphase stage. The upper nucleus has entered on the early anaphase, and nine chromosomes can be counted. It is interesting to note how the chromosomes do not divide simultaneously at the metaphase, so that while some may have divided and almost reached the region of the telophase, others are still undivided at the equator of the spindle. This has been observed generally in the dividing ascus nuclei.

Telophase. At this stage four or five chromosomes can be counted at the poles (Plate XI. Fig. 2 at 9). The spindle here, which forms a rope-like structure, as it does in all divisions, does not seem to elongate as in the first division. Indeed the spindle of this and the third divisions is much shorter at the meta-ana- and telo-phases than in the first division. The chromosomes again run together and "dissolve" at the poles, and again a definite interphase ensues (Plate XI. Fig. 3 at 10; lower two nuclei).

It is interesting to note that the ascus, although highly vacuolate at the first division, becomes less so at the second and third divisions, especially in the region of the nuclei.

Third Division.

Prophase. This repeats almost in detail the second division. The nucleus emerges from a resting interphase and the linin and beads become apparent. The threads again show a parallel arrangement (Plate XI. Fig. 3 at 10; upper two nuclei), and the beads show/

show a tendency to pair (Plate XI. Fig. 3 at 10: top nucleus). Again there occurs a definite contraction (Plate XI. Fig. 3 at 11) when the linin tends to form a dark staining ball around the nucleolus. During the contraction the threads appear to fuse and probably those beads which were associated in the parallel stage fuse also. Although this was never actually seen, evidence would point to this as being the case.

Metaphase. This stage of the third division was not seen.

Anaphase. (Plate XI. Fig. 3 at 12-15). This stage is figured frequently since it has such a bearing on the question of brachymeiosis, as mentioned in the introduction. Probably the most convincing figure is that nucleus showing the anaphase of the third division (Plate XI Fig. 3 at 15: lowest nucleus). Here ten chromosomes are clearly seen. All the other anaphase nuclei figured, give in the region of that number. The top nucleus in Plate XI. Fig. 3 at 15, shows a nucleus at the commencing anaphase. There are four smaller and three larger chromosomes, and it is probable that the three latter have not yet split but are still in the metaphase.

Telophase. (Plate XI/ Fig. 3 at 16). The section here has passed medianly through the dividing figures in the lower nuclei, while polar views are obtained of those in the upper nuclei. It seems to be quite clear from these figures that the number of chromosomes at the poles is five. Eight nuclei form themselves/

themselves round the daughter chromosomes, and enter a definite resting phase (Plate XI.Fig. 3 at 17). The ascus by this time tends to bulge in the region of these nuclei,while it narrows at the top and the foot, The protoplasm is very granular and non-vacuolate around those nuclei.

(f) Spore Formation.

Plate XI.Fig. 3 at 18 shows the spore, which is uninucleate, clearly cut out from the surrounding ascus cytoplasm (now the epiplasm). The spore is without a wall and is formed by a process of free cell formation; the ascus cytoplasm surrounding the spore being clearly delimited from the spore cytoplasm. The mechanism by which this was attained was not seen. The spore cytoplasm is highly vacuolate. The division of the nucleus in the spore was, unfortunately, not seen. The mature spore is a bicellular structure, the septum being medianly placed,and each cell contains one nucleus. The epiplasm is very vacuolate. (Plate XI.Fig. 3 at 19).

V. PYCNIDIA.

Pycnidia were not seen in material on the natural substratum nor in culture. In the latter, sections revealed the presence of large empty, perithecial-like structures which were slightly smaller than mature perithecia. They possessed an outer "shell" composed of several layers of cells, the outer of which were thick-walled. There was an imperfect ostiole present. The "shell" bounded a cavity, at the lower side of which and around the sides, were deeply staining amorphous granules. No spore layer or spores of any sort could be seen, nor were paraphyses present. These structures were present on such few occasions that their development could not be followed. They would appear to represent sterile perithecia in which no asci are developed. The reason for this is obscure. These probably approximate to the sclerotia-like bodies seen by Miss Cayley (13), in Nectria galligena on bark and in culture, and which she calls sterile perithecia. This writer found pycnidia in Nectria galligena on bark but not in culture, but she considers that when they do appear they are probably abortive.

VI. DISCUSSION.

From the manner in which the perithecium in Nectria mammoidea is borne clear of the underlying stroma, the initial development of the former can be distinctly seen. It is evident that in this species the "knot" of vegetative hyphae comprising the perithecial initial does not arise in relation to any archicarp. In the species here investigated, at no time in the process of perithecial development was there the appearance of one or more structures which resembled an archicarp. The "knot" of hyphae in the initial perithecium is composed of vegetative filaments, and until the appearance of the more densely protoplasmic "interpolated" cells, there does not appear to be any specialised cell or cells which might be said to possess the nature of female sex organs (archicarps). Is there therefore in this species any archicarps, or are these structures absent from the life history? This leads us to consider the nature of the so called "interpolated" cells. Are they homologous with any structures seen in the ascocarp development of studied species? Can we regard each one of these as an archicarp in itself and so consider this species to have numerous female organs in the one fruit body?

There is no doubt that these "interpolated" cells arise comparatively late in perithecial development, when we consider the time of appearance of archicarps in the ascocarp development of various Ascomycetes. Whereas in the vast majority of this group of fungi upon/

upon whose development research has been carried out, the appearance of the archicarps precedes the formation of the ascocarp initials, in the species here studied the "interpolated" cells arise a considerable time after the first appearance of the perithecial initial. Such a delay in the time of appearance in perithecial development of these "interpolated" cells does not seem to have been recorded for any archicarps in the various Ascomycetes studied. In Pyrenomycetes fairly closely allied to the genus Nectria the archicarp has been seen early on in perithecial development, but whether it arose prior to or after the appearance of the perithecial initial, is rather obscure. This is probably due to the most of the forms studied, viz., Gnomonia (8), Polystigma (6,25,27, & 48), Xylaria (9), and Poronia (20), having their perithecia sunk in a stroma or a host tissue.

The archicarp seen in the perithecia of Gnomonia and many other Pyrenomycetes, was a coiled structure, composed of usually several cells which were as a rule multinucleate, and the nuclei of these cells were larger and contained larger nucleoli than those of the vegetative cells. The so called Woronin hyphae, seen by many investigators, including the de Bary school, represented in all probability one or more coiled archicarps. These Woronin hyphae usually disintegrated early on or segmented into their component cells. It is very doubtful if ever they/

they or their segments gave rise to ascogenous hyphae. In several fungi, e.g. Claviceps, investigators have failed to observe any archicarp or Woronin hyphae whatsoever in the perithecium.

The number of archicarps usually found in the Pyrenomycete perithecium was one, more seldom two or more, and the archicarp or archicarps, when present, were usually larger than the vegetative hyphae of the perithecium in which they lay. They were distinct organs with distinctive nuclei.

In Nectria ipomeae, Cook (17), saw no signs of an archicarp, while in Nectria galligena, which has been studied by Miss Cayley (13), the perithecial initial was devoid of any archicarp-like structure, and organs called by that writer ascogonia arose later in the "knot". Vincens (57), found the perithecium in Nectria ribis to have its origin in a filament of the stroma, which filament resembled very much an ascogonium. In the three Nectria species investigated we obtain very conflicting results on the question of the presence of archicarps. While the two last named authors have found ascogonia present in the perithecium, Cook did not get any. The time of appearance, the nature and number of these structures in the perithecia of the fungi investigated by Cayley and Vincens differs, and one doubts very much if the structure or structures denominated ascogonia by these authors were in reality ascogonia in the true sense of the term. As a result of the foregoing one hesitates/

hesitates before believing that true ascogonia have been seen in the genus Nectria.

Referring back now to the nature of the "interpolated" cells, we find that they originate comparatively late in perithecial development, when compared to the time of appearance of archicarps in other Pyrenomycetes. Moreover for a considerable period of their development they differ very little in size, and in the size of their nuclei from the rest of the perithecial hyphae. The number of nuclei they contain at first, and for a considerable period of their development, is small. The great number of such "interpolated" cells in each perithecium is a fact which would greatly mitigate against their being considered homologous with true archicarps, as seen in other species. Moreover the irregularity in the nature and extent of development of each of these "interpolated" cells in a perithecium, does not suggest their being of an archicarp nature. One would expect a regularity between several archicarps occurring in the same perithecium. Moreover there is every indication that the "interpolated" cells are homologous with the paraphyses in this species. Both these groups of organs arise simultaneously from the vegetative hyphae in the perithecium. They are indistinguishable at their inception, althoug they soon begin to differentiate.

When one reviews the development and structure of these "interpolated" cells, it would appear that/

that they do not resemble those called archicarps in other Ascomycetes; each is not homologous with that Ascomycetous female sex organ, and cannot, in the present state of our knowledge of ascocarp development in that group, be called an archicarp. It would appear that in this species, the sex organs (both antheridia and archicarps) are entirely absent; that they have been eliminated from the life history.

The "interpolated" cells have been provisionally so called. They are special hyphae whose development probably takes place between a hypothetical archicarp forerunner in perithecial development, which has now disappeared, and the true ascogenous hyphae as seen now. Their function may have been at one time to produce ascogenous hyphae, thus carrying out what was previously the duty of the archicarps. This production of hyphae is still seen in them, and these hyphal branches may represent imperfectly developed ascogenous hyphae. It is impossible to say whether these hyphal outgrowths from the "interpolated" cells (as seen now), ever produced asci during the evolutionary history of the species. Certainly at the present stage in the evolutionary development of this species, they no longer function but disintegrate along with their parent "interpolated" cells. The species now no longer depends on them for the production of asci, for these latter are developed from ascogenous hyphae which a rise de novo from the cells at the foot of the perithecial cavity.

The growth of the nuclei in the "interpolated" cells/

cells, and the occasional fusion between two such nuclei, is interesting. This occasional fusion may be a relic of what was at one time a regular event in these structures - a fusion giving rise to a definite ascus nucleus. The asci may have arisen direct from the cells in which such a fusion had occurred. This fusion in the "interpolated" cells may,however, be an abnormality, and not a relic of an at one time established event in the life history. The presence of closely associated nuclei in a confined space might encourage this fusion. Fusions seen by Claussen (16), in the oogonial region of Pyronema and by Ramlow (49), in Ascophanus carneus and Ascobolus immersus, have been considered by these authors as pathological phenomena.

The association of nuclei in pairs in these "interpolated" cells, may not mean anything important, but may be merely the association of two nuclei resulting from a division, without their moving far apart. Such associations are common in the vegetative hyphae of many fungi.

There does not appear to be any parallel to these "interpolated" cells in the various species investigated. Their production in those representatives of the species now existing would appear to be a very wasteful process.

Having failed to homologise these "interpolated" cells with any known existing ones, it is difficult to assign to them their true nature and function/

function.

There seems to be no doubt that the "interpolated"cells in this species disintegrate, and certainly their appearance before this takes place would have forecasted such an event. The irregularity of their structure, of the size of the nuclei,of nuclear fusions, might all point to them as being structures which were either not established in the life history, or were decadent cells which had failed in their purpose,and whose function was perhaps now being performed by other structures.

The origin of the true ascogenous hyphae (i.e. those giving rise to asci) is in this species, from the vegetative hyphae in the perithecium. The simple nature of the former is interesting. There is no signs of proliferation of the ascogenous hyphae as seen in Gnomonia (8), Laboulbenia (23), Rhytisma (42), Humaria (29), Helvella elastica (46), Helvella crispa (14), and Pyronema (39), nor any nuclear fusion in the penultimate cell, which has been frequently described. This fusion in the ultimate cell is not however unique, cases having been recorded among others in Gnomonia (8), Sordaria fimicola, S. Humaria and Podospora acerina (22),and probably Nectria ribis (57).

As to the question of nuclear fusion, two fusions were undoubtedly seen in the course of perithecial development in this species, viz., in the "interpolated"cells and in the young ascus, but can we say/

say that two fusions occur in the nuclear cycle. The nuclei resulting from fusion in the "interpolated" cells disintegrate with the latter so that, whether we regard the fusions in the "interpolated" cells as normal or abnormal, in the direct nuclear cycle in the life history there is only one fusion, namely in the young ascus.

The mode of origin and development of the paraphyses in this species has not been recorded (as far as the writer knows) in any other species described. At the early stage of their differentiation, they do not appear to differ from the contiguous initial "interpolated" cells. They originate from a tissue comprised of more compact and thicker walled cells than those from which the "interpolated" cells arise. Otherwise, in the younger stages, they resemble these cells completely. They might almost be said to be homologous, later differentiation being due to a question of nutrition; the cells arising from the more compact and thicker cells receiving less nourishment. From the nature of the course of their development and their position in the perithecium, their function is difficult to determine. They do not appear to function in the formation of the ostiole and they disintegrate by the time the asci have fully matured their ascospores. It may be they serve to protect the developing asci and perhaps their disorganisation provides good material to the young asci.

The divisions in the ascus show that only

one/

one reduction takes place. There was no signs of a brachymeiosis, nor was there any reason to believe that a chromosome association existed. The course of events is almost exactly that seen by Bagchee (1), in Pustularia bolarioides. Definite contractions before the second and third divisions, as seen by him in that fungus, were observed in the species studied here, but as with that author, they are not followed by any reduction in chromosome number, and admit of the same explanation as given them by that investigator. This one reduction division would correspond to the fusion observed in the ascus and is as would be expected, since only this one fusion was seen in a direct nulcear cycle in the life history.

In the genus Nectria there was probably a gradual elimination of the sexual organs and of fusion or association between nuclei produced in them, and a substitution for the latter by a fusion of nuclei occurring in the same hypha - a form of pseudo-apogamy comparable in some measure to that seen in other groups of plants. What isthenecessity for this fusion? It has been suggested that in those cases where the ascus nucleus is produced from a fusion of nuclei in vegetative hyphae and not from a fusion of nuclei produced in sex organs, it would be necessary to compensate for the reduction division in the ascus (established in descent) by this fusion of nuclei occurring in the ascogenous hyphae of vegetative nature. In the case of Nectria mammoidea, therefore, the fusion between nuclei in similar ascogenous hyphae of vegetative/

vegetative origin,is substituted for the fusion of nuclei derived from sex organs, and so a definite chromosome number is retained in the nuclear cycle from generation to generation. Wager in his Presidential address to the British Mycological Society, referring to endokaryogamy in fungi, says that its function is probably "to provide for the nuclear reorganisation and re-invigoration of the individual reproductive cells just at the time when large numbers of spores are about to be formed". It appears that this may have been the function of the sexual fusion, and the latter has given way to a simplier method - endokaryogamy.

There is in this fungus an alternation of generations, but it is evident that the sporophyte phase is almost entirely eliminated. The diploid condition obtains for a very short period in the fusion nucleus in the ascus, for the prophase of the first division is soon entered upon. It is in fact scarcely possible here to recognise a sporophyte plant, and indeed, it would be difficult to define its limits. It is not cut off from its gametophyte forebear nor the gametophyte tissue of the ascus and its nuclei. In fact the alternation in the life cycle is purely a nuclear one.

An investigation into the life history of this species and the review of the life histories of the Ascomycetes in general,would lead to the conclusion that, while the ascocarp developments of the species nearly related in our systematic tables would appear/

appear to resemble one another in a general way, the more particular points seem to vary greatly in nearly allied forms. At the same time we get striking resemblances in developmental stages between distantly related forms.

In the light of present day knowledge on the development of the ascocarp of these organisms, it would appear to be premature to attempt any system of classification on that basis. It is only, however, when the entire life histories of the species of the group have been worked out, that we can attempt to classify them according to their natural affinities.

VII. SUMMARY.

The paper deals with the development of the perithecium in Nectria mammoidea. Phil. et Plowr.

The cells of the vegetative hyphae are multinucleate.

The perithecial initial is a knot of vegetative hyphae, and at its inception and throughout its development no trace of a structure or structures resembling archicarps can be seen.

The appearance and development of structures provisionally termed "interpolated" is described and it has been found impossible to homologise them with known existing structures in the ascocarps of other Ascomycetes. These structures disintegrate prior to the appearance of the ascogenous hyphae.

These latter arise de novo from the cells lining the base of the perithecial cavity. They are simple binucleate structures and are not separated by a cell wall from their parent hyphae, but are merely prolongations of the latter. Without proliferation of these hyphae or division of their two nuclei, the latter fuse to give the definite ascus nucleus.

The ascus grows and is a prolongation of the parent ascogenous hyphae.

The definite ascus nucleus divides three times/

times, one reduction division only taking place.

The spores are formed from the ascus cytoplasm by a free cell formation.

The spore nucleus divides and a wall separates the two daughter nuclei which thus each occupy a cell in the spore. The latter becomes invested by a spore wall.

The grosser features of the perithecial development are described throughout the account in the sequence of their occurrence in development.

Pycnidia were not found in culture but structures which may be sterile perithecia were seen on a few occasions.

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IX. PHOTOGRAPHS,

Illustrating the paper on Perithecial Development in Nectria mammoidea, Phil. et Plowr.

All drawings were made with the aid of a Reichert camera lucida, under a 2 mm. apochr. oil imm. Zeiss objective, and with varying comp. oc., and then photographed.

PLATE I.

Fig. 1. Piece of bean agar medium showing all stages in the development of the perithecia. Thirty-six days in culture. x c.3.

Fig. 2. Semi-diagrammatic figure, showing the origin of the perithecium from the thread-like stroma. A young stage is shown on the right. From bean agar, thirty days. x c. 60.

Fig. 3. Cells of the vegetative hyphae. The upper belongs to a hypha in the stroma, while the lower is a constituent of a hypha immersed in the medium. From bean agar, thirty days. Gentian violet. x c. 1300.

PLATE. I.

Fig. 1.

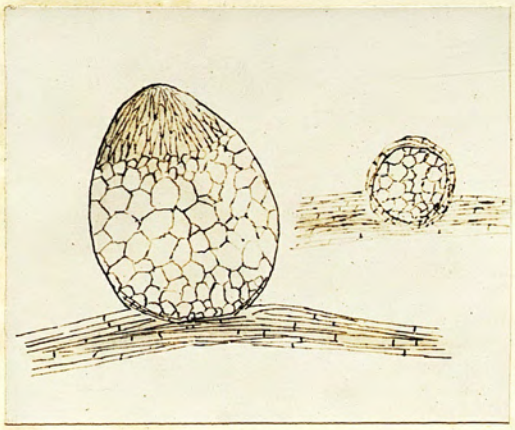

Fig. 2.

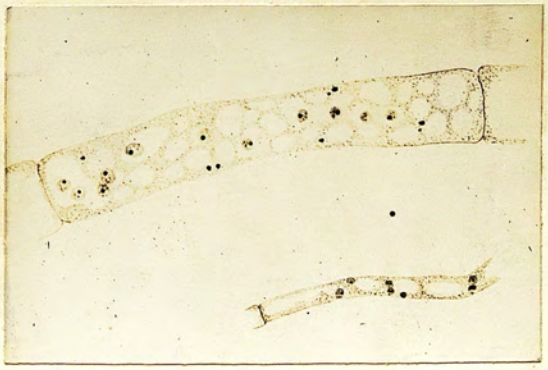

Fig. 3.

PLATE II.

Fig. 1. Photograph of a drawing of the perithecial initial. Note the presence of numerous uni- and bi-nucleate cells. So far there is no differentiation in those cells. From bean agar thirty days. Gentian violet. x c. 1600.

Fig. 2. Photograph of a drawing of the young perithecium, showing the origins of the central cells. From a thirty days' old culture on bean agar. Gentian violet. x c. 1000.

Fig. 3. Microphotograph of the young perithecium showing three central cells with distinct origins. From a thirty days' old culture on bean agar. Heidenhain's iron alum haematoxylin and eosin in clove oil. x c. 1000.

PLATE. II

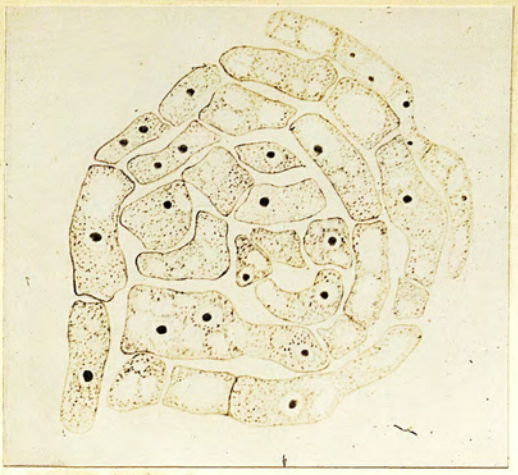

Fig. 1.

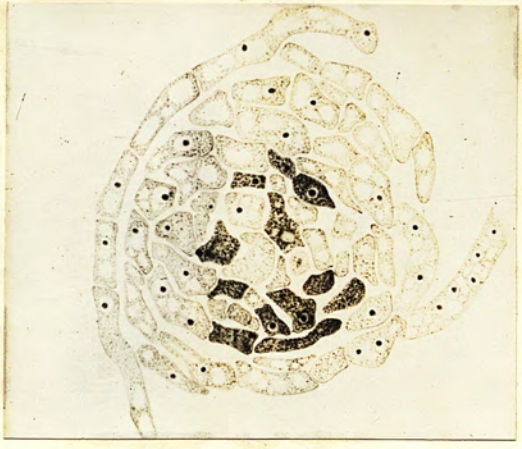

Fig. 2.

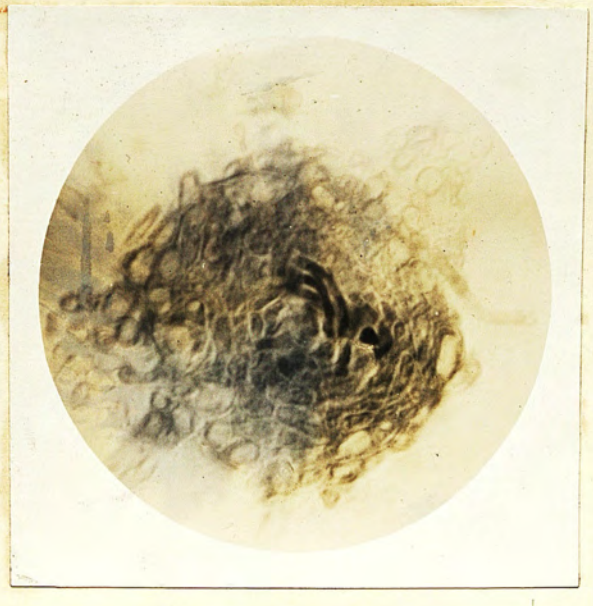

Fig. 3.

PLATE III.

Fig. 1. Photograph of a drawing showing an enlargement of the central cells as seen in Fig. 2 below, illustrating the uninucleate condition of these cells at their inception. From a thirty days' old culture on bean agar. Heidenhain's iron alum haematoxylin and eosin in clove oil. x c. 1800.

Fig. 2. Microphotograph of the young perithecium at the time of the appearance of a cavity. Note how the young central cells are staining more heavily with the haematoxylin, and are sending tapering prolongations into the cavity. From a thirty days' old culture on bean agar. Heidenhain's iron alum haematoxy and eosin in clove oil. x c. 1000.

PLATE. III.

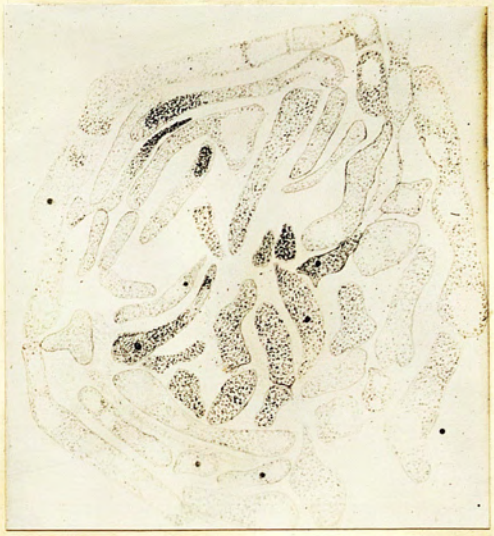

Fig. 1.

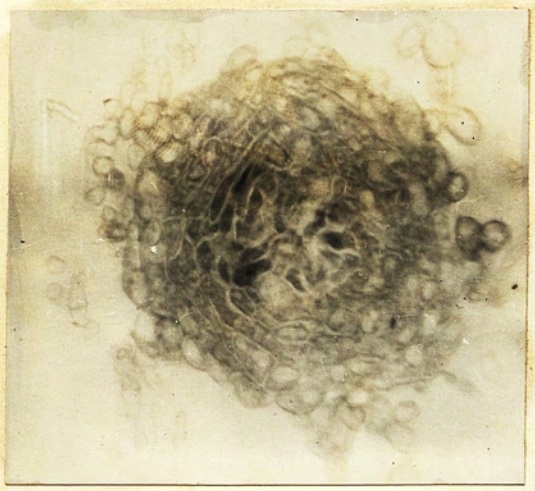

Fig. 2.

PLATE IV.

Fig. 1. Photograph of the young perithecium at the stage when the central cells are beginning to differentiate. Note at a. the more loosely arranged perithecial cells which give rise to the "interpolated" cells. From a thirty days' old culture on bean agar. Heidenhain's iron alum haematoxylin and eosin in clove oil. x c. 300.

Fig. 2. Photograph of a drawing illustrating the young paraphyses. Note the uni-nucleate condition and squarish outline of their cells. From a thirty days' old culture on bean agar. Heidenhain's iron alum haematoxylin and eosin in clove oil. x c. 2000.

Fig. 3. Photograph of a drawing showing the young "interpolated" cells arising from the loose cells at the base of the perithecial cavity. Note how/at a. some are beginning to become bi-nucleate. From a 30 days' old culture on bean agar. Heidenhain's iron alum haematoxylin and eosin in clove oil. x c. 1800.

PLATE. IV.

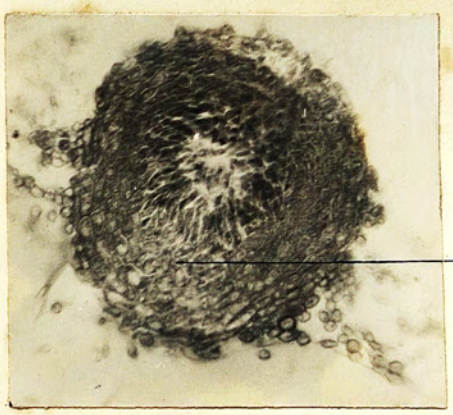

Fig. 1.

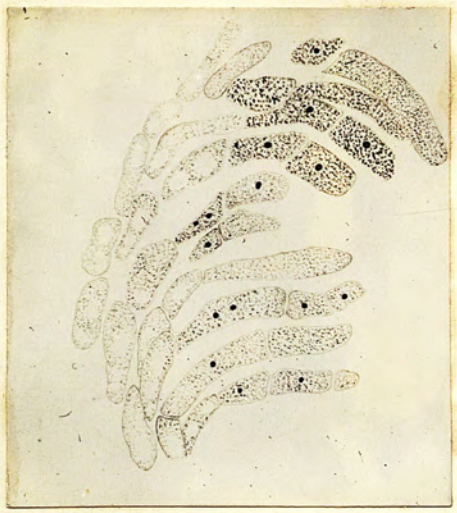

Fig. 2.

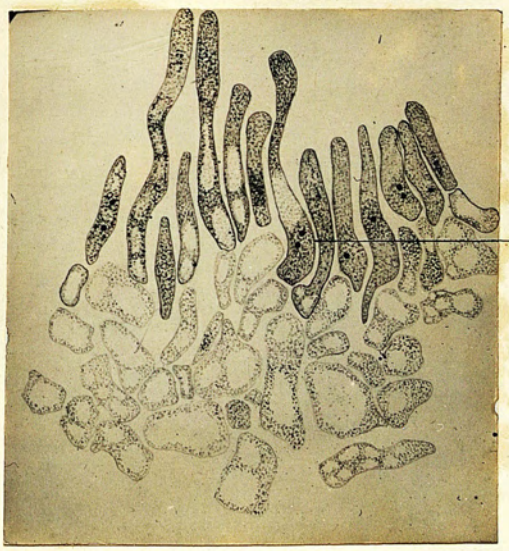

Fig. 3.

PLATE V.

Fig. 1. Microphotograph showing a further stage in perithecial development. Note how the upper central cells i.e. the paraphyses are staining more heavily than the lower ones i.e. the "interpolated" cells. From a thirty days' old culture on bean agar. Heidenhain's iron alum haematoxylin and eosin in clove oil. x c. 300.

Fig. 2. Photograph of a drawing showing the lower right hand quadrant of the perithecial cavity seen in Fig. 1. The paraphyses are seen to be contiguous with the "interpolated" cells about the transverse median line of the cavity. The cells of the young paraphyses stain more heavily and are not so vacuolate as the "interpolated" cells. Note, in the latter the presence of a septum at a., the bi-nucleate condition at c. and the production of a branch at d. From a thirty days' old culture on bean agar. Heidenhain's iron alum haematoxylin and eosin in clove oil. x c. 1800.

PLATE. V.

Fig. 1.

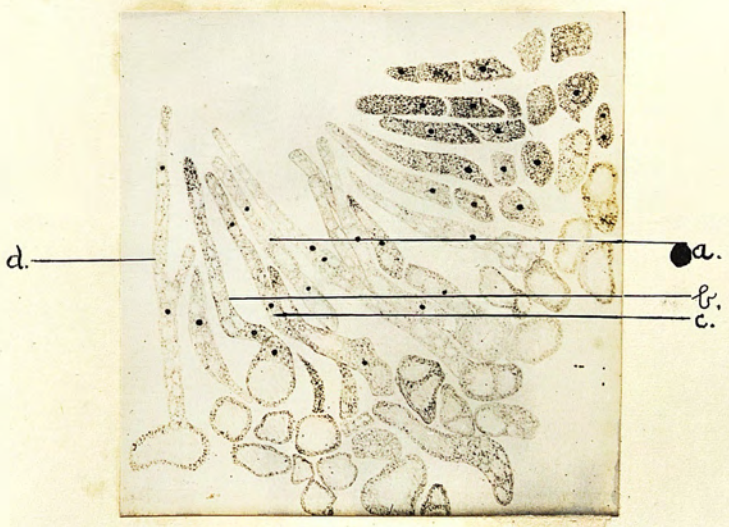

Fig. 2.

PLATE VI.

Fig. 1. Microphotograph of a further stage in perithecial development. The paraphyses have been separated from the "interpolated" cells, by being carried up from the sides of the perithecial cavity and along the top. They are now seen at a. hanging down into the cavity. At b. the "interpolated" cells. From a twenty days' old oat agar culture. Polychrome methylen blue and orange tannin. x c. 300.

Fig. 2. Microphotograph showing the extension of the hyphae bordering on the upper half of the perithecial cavity. This extension is responsible for the change of position of the paraphyses and is, moreover, carried on, as shown at a., until it forces back the outer perithecial covering, and forms the ostiole. From a twenty days' old oat agar culture. Polychrome methylen blue and orange tannin. x c. 350.

PLATE. VI.

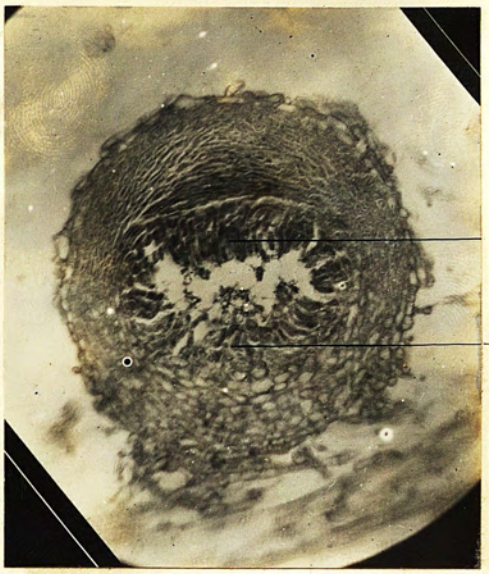

Fig. 1.

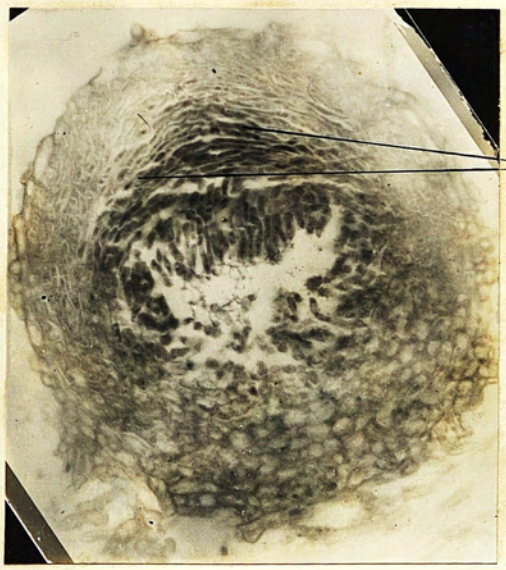

Fig. 2.

PLATE VII.

Fig. 1. Microphotograph showing the perithecium beginning to assume a mature form. The ostiole is seen to be formed at a., and periphyses are projecting into it. The various layers of cells comprising the perithecial covering are now quite distinct. From a thirty days' old culture on bean agar. Heidenhain's iron alum haematoxylin and eosin in clove oil. x c. 280.

Fig. 2. Microphotograph showing an enlargement of the centre of the perithecium as shown in figure 1. At b. the paraphyses are seen to be extending down into the cavity in a compact mass. Note how the tips of these hyphae stain heavily with the protoplasmic stain. At c. the "interpolated" cells are lying loosely at the base of the perithecial cavity. x c. 560.

PLATE. VII.

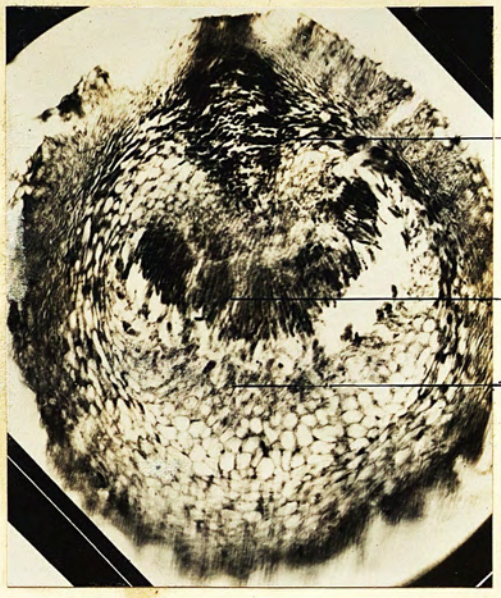

Fig. 1.

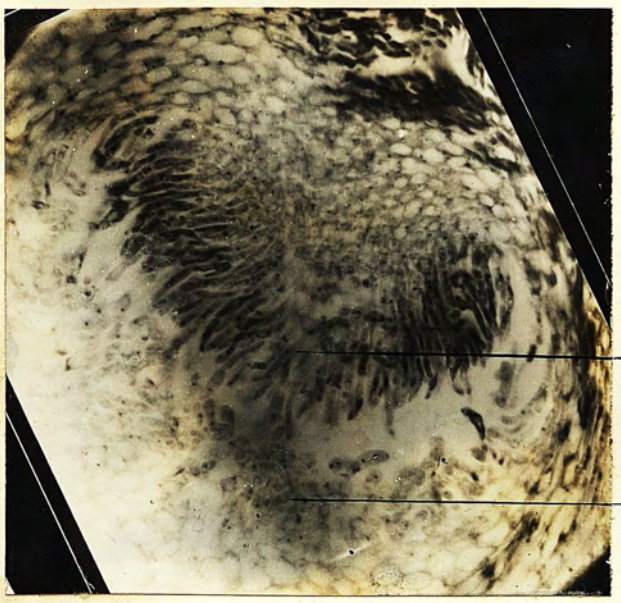

Fig. 2.

PLATE VIII.

Fig. 1. Photograph of drawings revealing the appearance of the various stages in development of the non-septate type of "interpolated" cells. The nuclei are seen to have relatively large nucleoli. The nuclei are definitely paired as seen at 3 and 12. Fusion is seen at 5 and 6. Branches are seen to arise from the cells at 9-12 and nuclei are seen to pass into them at 9,11, and 12. Various stages in disintegration of these cells are seen in 4,7 and 8.

Fig. 2. Photograph of drawings showing the septate type of "interpolated" cells. Structures resembling very much "crotchets" in ascogenous hyphae are seen at 14-16. Branches are seen to arise from the cells at 17-20 and 24. The nuclei are definitely associated in pairs and fusion is seen at 22. Disintegration is seen at 12, 15, 22 and 23.

The "interpolated" cells figured in those drawings were from perithecia from a thirty days' old bean agar culture. They were stained with Heidenhain's iron alum haematoxylin and eosin in clove oil. All are x c. 1500.

PLATE. VIII.

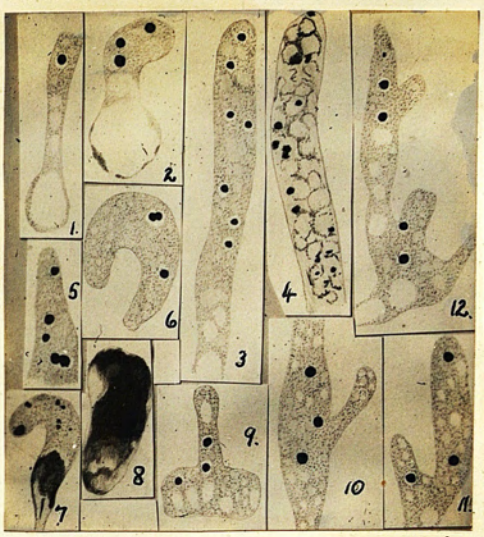

Fig. 1.

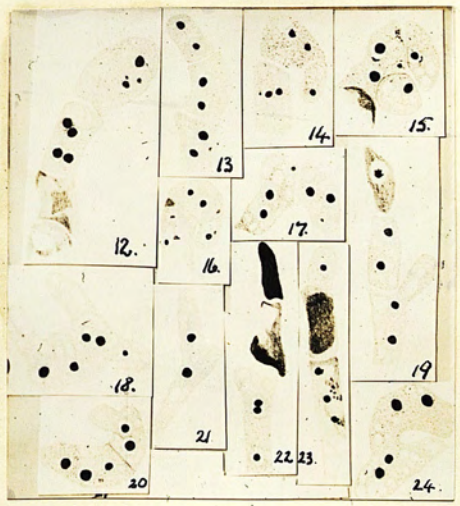

Fig. 2.

PLATE IX.

Fig. 1. Microphotograph of a perithecium showing the almost complete disappearance of the "interpolated" cells at the base of the perithecial cavity. Note the disintegrated remnant of one such cell at the left hand side of the cavity. From a twenty days' old culture on oat agar. Heidenhain's iron alum haematoxylin and light green. x c. 200.

Fig. 2. Microphotograph of the perithecium just before the appearance of the ascogenous hyphae. The various layers comprising the perithecium are very distinct at this stage. Note how the paraphyses have reached the cells at the base of the perithecial cavity, and are now beginning to disintegrate under the ostiolar cavity. From a fifteen day old culture on bean agar. Polychrome methylen blue and orange tannin. x c. 140.

Fig. 3. Microphotograph of the perithecium at the time of origin of the ascogenous hyphae. These latter arise from the "fertile" cell layer along the foot of the perithecial cavity. The young ascogenous hyphae are seen as darkly staining structures as at a. From a thirty days' old bean agar culture. Heidenhain's iron alum haematoxylin and light green. x c. 180.

PLATE. IX.

Fig. 1.

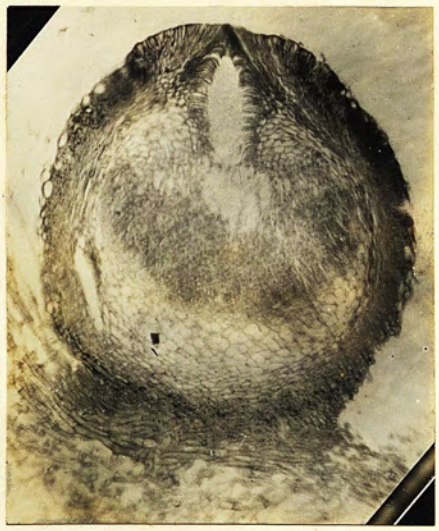

Fig. 2.

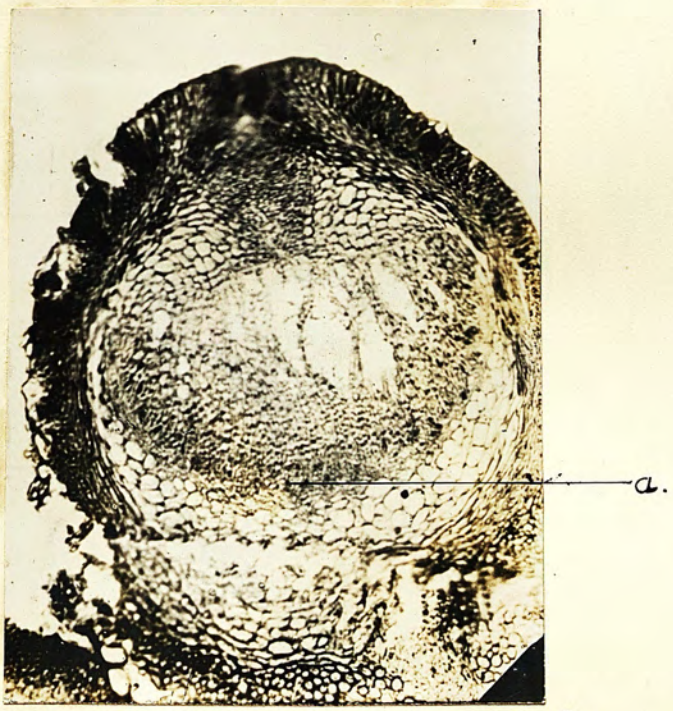

Fig. 3.

PLATE X.

Fig. 1. Microphotograph showing an ascogenous hypha in the bi-nucleate stage at a. Polychrome methylen blue and orange tannin. x c. 1000.

Fig. 2. Microphotograph showing various stages in ascus formation from the ascogenous hyphae at the base of the perithecial cavity. At a. an ascogenous hypha is seen in the uni-nucleate condition. At b. the two nuclei normally present in the young ascogenous hypha are seen to have fused. Their nucleoli, however, are still apart. At c. is seen the definite ascus nucleus during the prophase of the first division. Heidenhain's iron alum haematoxylin and light green. x c. 600.

Fig. 3. Photograph of drawings showing the various stages in the fusion of the nuclei in the ascogenous hyphae to form a definite ascus nucleus. Note how the young ascus is not cut off from its parent ascogenous hypha by a cell wall. 1 and 7 were stained with polychrome methylen blue and orange tannin while the remainder were stained with Heidenhain iron alum haematoxylin and light green. All are x c. 800.

PLATE. X.

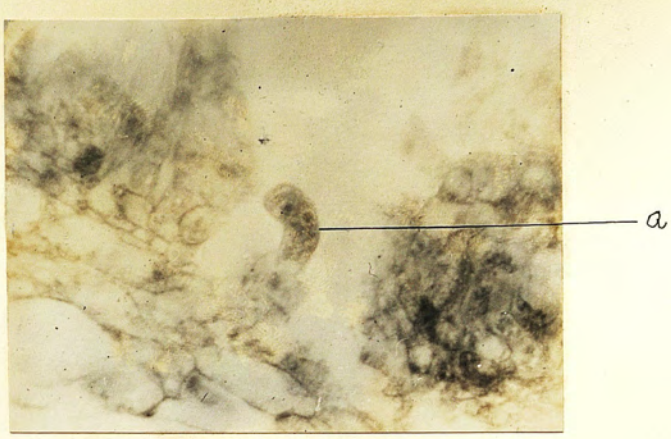

Fig. 1.

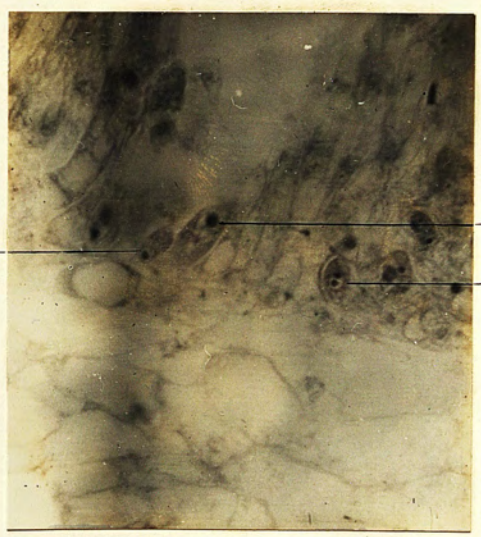

Fig. 2.

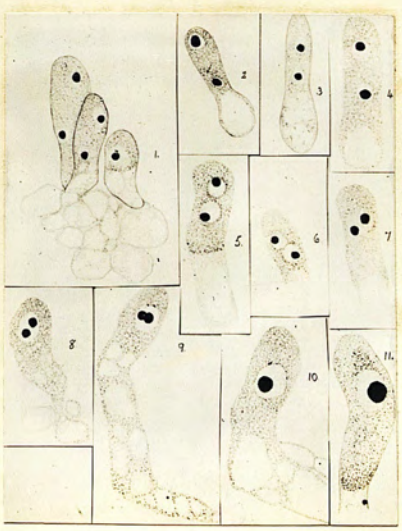

Fig. 3.

PLATE XI.

Nuclear Division and Spore Formation in the Ascus.

All drawings were made from preparations stained with Heidenhain's iron alum haematoxylin and counterstained with eosin or light green in clove oil.

Fig. 1. Prophase of the first division of the ascus nucleus. The figures reveal the heterotype nature of this phase. For full explanation of the various drawings, see text. x c. 850.

Fig. 2. Various stages in the nuclear divisions in the ascus. The stages shown here extend from the metaphase of the first division to the telophase of the second division. For a full account of these stages see text. x c. 850.

Fig. 3. The third division is shown at 10-16. The uni-nucleate ascospores are seen at 18 cut out from the ascus cytoplasm (now the epiplasm). The mature bi-cellular ascospores are seen at 19. x c. 850.

PLATE. XI.

Fig. 1.

Fig. 2.

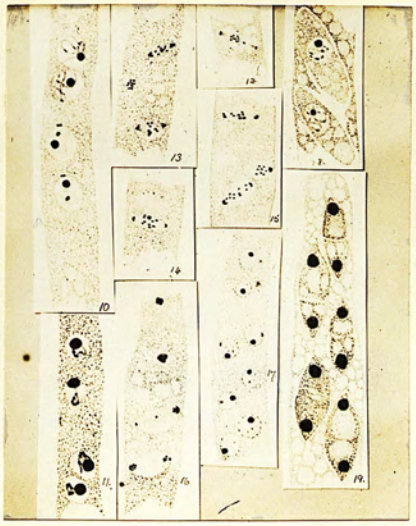

Fig. 3.

PLATE XII.

Fig. 1. Microphotograph of the central region of a perithecium showing various stages in the development of the asci. Note how the paraphyses have almost completely disintegrated, thereby leaving a free passage for the exit of the ascospores, when mature, through the ostiole. From a thirty days' old culture of bean agar. Heidenhain's iron alum haematoxylin and eosin in clove oil. x c. 280.

Fig. 2. Microphotograph of an almost mature perithecium. From a thrity days' old culture of bean agar. Heidenhain's iron alum haematoxylin and eosin in clove oil. x c. 180.

PLATE. XII.

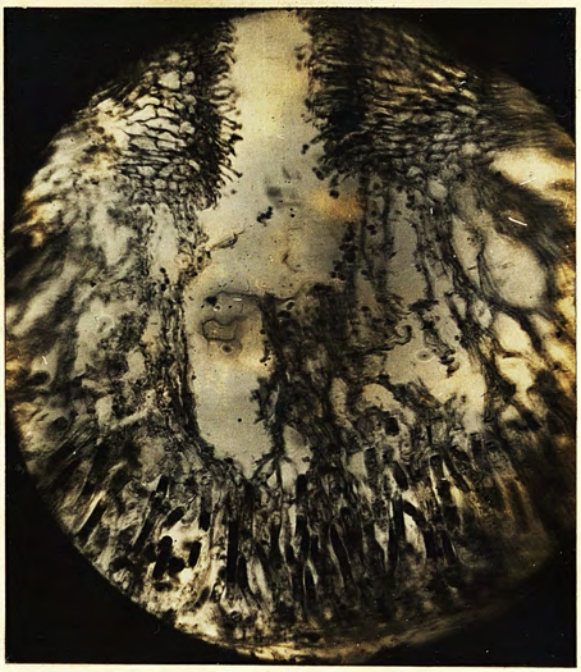

Fig. 1.

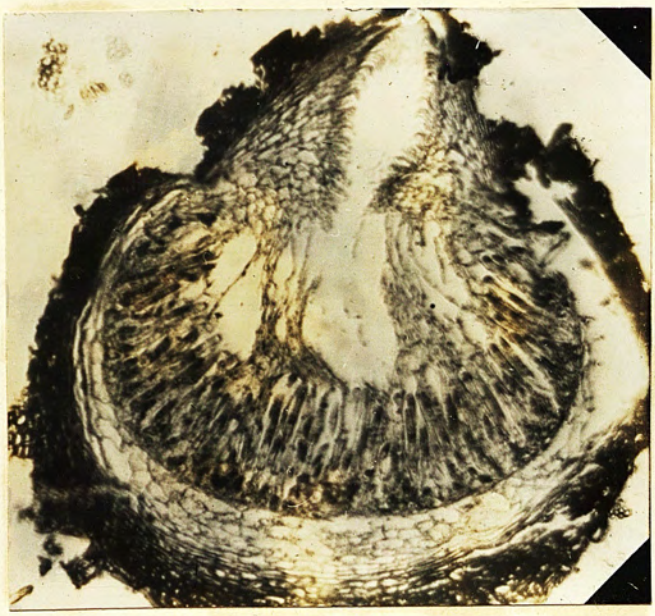

Fig. 2.

The Study of Nectria mammoidea, Phil. et Plowr. in Culture, with an Account of the Factors influencing Perithecial Production in the Genus.

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A. The Study of Nectria mammoidea, Phil. et Plowr. in Culture.

I. INTRODUCTION.

The cultural study of numerous species of the genus Nectria, has allowed Wollenweber (46,47,51, 52) to obtain a large proportion of, or on many occasions, the full life history of many species in the genus. By this method, stages in the life history which are normally or frequently absent in the development of the fungus in nature, are obtained. With a good knowledge of the life history, obtained by this means, Wollenweber (51,52,) has carried out extensive comparisons between the species involved, and has originated a scheme of classification on a many character basis. Such a classification tends to group the species in the genus more according to their natural affinities. The system has, however, obvious difficulties, chief among which is, perhaps, the inconvenience of the probable necessity for culturing a species as a means of identifying it. The system, however, has much to recommend it, in that it is much more accurate than the older systems. These latter subdivided the genus according to the size of the stroma, a character which, as has lately been seen, is in itself not a constant. Further emphasis were laid on the nature of the perithecial envelope, its colour, on the presence or absence of hairs on the perithecium,

on/

on the paraphyses, on the shape and size of the asci and ascospores and/on the nature of the walls of the latter. Most of these characters vary appreciably according to the stage of perithecial maturity reached, and to the nature of the environment. The size of the spores alone seems a fairly constant feature in the various species, but spore measurements overlap from species to species and cannot alone lead to the determination of a species. It appears that perithecial characteristics alone, in this genus, are not always quite enough to enable one to determine the species. Wesse (42) lately has laid great emphasis on the microscopic appearance of the perithecial wall in vertical sections. The number and nature of the layers comprising the wall seem to offer a fairly stable and consequently reliable guide to the systematist. Westerdijk and van Luijk (44) have found, however, that this feature is in itself variable, and can be altered by changes in the relative amount of crowding of the fruiting bodies on the substratum. Wesse (41), on his system, called the Nectria rubi of Osterwalder (31), Nectria mammoidea var. rubi but Wollenweber (52), on cultural grounds, has regarded it has a distinct species, thus according with Osterwalder's view. The action of Wollenweber appears to the author (who has compared both fungi in culture and in nature) to be justified. Wesse judged the two fungi on their perithecia only; Wollenweber on a many character basis/

basis; the characters for comparison being obtained for the most part in artificial culture. In a genus which contains numerous species which are not easily distinguished one from the other, a system, such as Wollenweber has adopted, is surely justifiable.

It appears that the original description of Nectria mammoidea given by Phillips and Plowright (32), is,for present day purposes, not extensive enough. With the ever increasing number of species, the description of a new species becomes a more and more involved undertaking, in order that by it, the fungus may be recognised by workers in the future, and may not be redescribed under a new name.

It was thought that a cultural study of the interesting species Nectria mammoidea, with a revision of the species description, would not be superfluous. A cultural study is, moreover, valuable, in that the effect of changing the conditions of the environment (which is so easily done in artificial culture) on the numerous characteristics of the fungus, can be observed. This allows one to determine which features are variable and which are constant, or comparatively so, in a multiplicity of external conditions. This knowledge is invaluable for a system of classification, no matter on what basis the latter is founded. At the same time, features of interest appearing during the culture of the fungus were noted and commented on in the conclusions. On account of the species forming its perfect stage so freely in culture/

culture, experiments were carried out in order to try to elucidate the factors influencing this phenomenon.

II. (a) The Fungus on the Natural Substratum.

Nectria mammoidea does not appear to be a fungus of common occurrence. It was first recorded and described as a new species by Phillips and Plowright (32) in 1874. These collectors found it on stumps of Ulex in North Wotton and on Birch bark at Ercall. In 1890 Trail (40) included it in his Report on the Fungi of the East of Scotland. He found the fungus on the dead stumps of Ulex europaeus near Aberdeen. The fungus was found during the British Mycological Society's Excursion to Tintern in 1925. Apart from these reports of its occurrence the fungus appears to be fairly uncommon in this country. It is not recorded by Winter (45) in Rabenhorst's Kryptogamen-Flora, nor in Lind's (30) Danish Fungi, and Saccardo (34) only gives the reference to the original description of Phillips and Plowright. Dr. Wollenweber, in a letter to the author dated November, 1927, states, that he cannot give any reliable record of its occurrence on the continent. Seaver (35) does not list it in his contribution to the North American Flora, so it is highly probable that the species is not represented in that continent. It has at no time been reported as a parasite, but leads a saprophytic existence on the stumps and fallen branches of various trees and shrubs.

The material obtained for this work was found in Fifeshire, on the dead stumps of a tree member/

member of the Rosaceae, whose name it was not possible to determine. The fungus appeared on the natural substratum in its fruiting stage only. The perithecia were densely aggregated and slightly sunken in a well defined stroma, which appeared at various places through cracks in the bark. The perithecia were all at a fairly uniform stage in evelopment, and, as a consequence, resembled one another fairly closely. They were spherical, except for, at their tops, a very prominent disc, in the centre of which was a definite papillate ostiole (see Wollenweber (52 Tafel IV, Abb. 37) for illustration). They measured on an average 540μ in width and 527μ deep. The perithecial wall was smooth. Owing to the crowded nature of these fruiting bodies, the younger stages could not be seen, but the various developmental stages present showed the perithecium to go through several colour changes. At the youngest stages seen, the perithecium was a dull carmine lake[x], the surface of the disc, and a slight distance down the sides from its margin, being a darker shade. The disc tended to reflect the light, and as a consequence, appeared lighter than it really was. This made the short darker portion extending down the sides, show up in strong contrast, and gave the perithecium a very typical appearance. Older/

[x] The various colours seen during the study of the species and noted in this account, were determined by matching with colour plates in the reference (33).

Older perithecia were a mars orange, and on ageing, a reddish apricot. At this period, the ring round the disc was a garnet colour, and showed up very prominently. The perithecia then became, throughout their covering, a blood red colour. The ascospores were ejcted now through the ostiole in a dirty white tendril. Paraphyses were abundant at the stage where the perithecia were coloured mars orange. Later they seemed to disappear. This agreed with what was seen of their development (see paper on "Perithecial Development, etc."). The paraphyses were unbranched, multicellular hyphae, whose cells were much swollen and vacuolate, and they resembled very much the paraphyses of the well known Nectria galligena, Bres. The asci were cylindrical with a blunt apex, and measured 122μ (102μ - 140μ) x 7.8μ (7.5μ - 8.5μ). The ascospores were oval to spindle-shaped, straight, or slightly curved, bicellular, with a slight constriction at the septum. The mature spore had a wall covered with minute papillate projections and measured 17.8μ (17. 0μ - 22.5μ) x 7.6μ - (7.1μ - 8.4μ).

(b) The Fungus in Culture.

(i) Germination of the ascospores in water.

Ascospores were obtained from the dehiscing perithecia and placed in a drop of distilled water on a slide, and the latter placed in a petri dish. Germination occurred within twenty-four hours. Germinating cells swelled up, but the septum in each ascospore/

ascospore remained fixed in length, so that the latter appeared like a balloon with a thread round its middle (Plate I. Figs. 1 and 2). Each ascospore usually produced one germ tube from one of its cells (Plate I. Fig. 1 at c.), more rarely one tube from either cell (Plate I. Fig. 2 at b. and d.), and still more rarely, two germ tubes from one cell (Plate I. Fig. 1 at e.). The germ tube was produced from the side and practically never from the terminal point of the cell; a common point being midway between the point and the septum (Plate I. Fig. 1 at c.). On germination, oil drops, which were originally contained in each ascospore cell, broke up into smaller globules which crowded the protoplasmic cell. Septation of the germ tubes occurred sooner or later and fusions between them were common. Branches from the germ tube often returned and fused with the parent hypha, so forming a sort of clamp connection (Plate I. Fig. 2 at a.). Hyphae from spores, on approaching germ tubes from other spores, often gave rise to branches which approached one another and fused, so forming a bridge or connection between the two hyphae (plate I. Fig. 2 at c.). A hypha might be linked up thus with another hypha, or these connections might link up the one hypha with more than one other hypha (Plate I. Fig. 2 at e.). These bridges were formed by the branch hypha growing out at almost right angles to the parent, and going all the way and fusing with a neighbouring hypha (Plate I. Fig. 2 at e.), or the latter/

latter might send out a branch just opposite the place of origin of the branch hypha from its neighbour, and the two resultant branches fuse about midway(Plate I. Fig. 2 at c.). Such fusions were extremely common between the hyphae arising from the ascospores. This fusion is looked on as being of a purely vegetative nature. The oil globules in the germinating cells gradually disappear, being probably used up in the production of the germ tubes. The germinating cells ultimately became emptied of their contents, and nothing of each remained but a thin wall.

Several spores did not germinate, nor even swell, but remained elliptical with little or no constriction (Plate I. Fig. 1 at b.). These had in each cell, a large oil drop, and moreover, such a spore had a violet purplish tinge. They possibly represented dead cells.

In fourteen days macroconidia were produced directly from germ tubes from ascospores; some quite near the parent ascospores (Plate I. Fig. 1 d. and e.). These were produced on simple conidiophores, and were cut off from the latter by a septum. They were straight, even when mature (Plate I. Fig. 1 at a), and thus differed from those obtained in media (see later). Moreover, they were longer and narrower, and resembled very much the macroconidia of Nectria galligena, Bres.

Description. - Conidia long, narrow, cylindrical, or perhaps/

perhaps very slightly narrowing to each end, rounded at ends, straight or very slightly curved, hyaline, or very lightly tinged green 94μ (75μ - 108μ) x 5.5μ (5.1μ - 6.4μ).

Various swellings occurred in the hyphae in water, but no true chlamydospores were seen.

(ii) Growth in solid media.

Single ascospore isolations were made by the dilution method as used in bacteriological technique. Platings of spores were made in a thin sheet of prune agar in petri dishes, and a single ascospore observed under the low power of the microscope, and marked by means of a drop of ink on the back of the petri dish. The media immediately surrounding the spores was removed aseptically, and placed in a petri dish containing a thin layer of the same agar. These pieces of media were again examined under the low power of the microscope, by inverting the petri dish containing them on the stage of the microscope. By this means it was definitely ascertained that each piece of medium contained one spore only (see this method in Fusaria of Potatoes, Sherbakoff, C.D., N.Y. Cornell Agr. Exp. Sta. Mem. 6, p. 102). Cultures were made on the various media recommended by the committee that formulated the fundamentals for a taxonomic study of Fusarium (50). All cultures were kept at ordinary room temperature, and subjected to the normal alternation of day and night. The terms used for describing the various types of growth in culture are those used by/

by Sherbakoff (38) in his studies on the Fusaria of Potatoes.

Oat Agar.

Growth radiates out from the point of inoculation. Aerial mycelium is only developed at the advancing growth margins. It is here loose, radiate, and pure white. The remaining mycelium is confined to the medium surface or immersed. The surface growth is composed of stromata made up of plectenchymic strands of thick-walled hyphae, on whose surface are enormous numbers of dark violet crystals, giving the stromatic crust this colour. The hyphae are thick-walled, and their cells heavily charged with oil drops. Conidia are produced from/the stromata conidiophores on forming this crust-like growth, and by their massing together on the surface of the medium form slimy pionnotes. These latter are at first of a fleshy white colour, but later become a reddish violet hue. The latter colour is due to the conidia forming those pionnotes being mixed with crystals which have become detached from the stromatic hyphae. The medium is at first coloured a naples yellow, and later a vinous mauve. This is a non-staling medium; growth in area being unstricted.

Conidiophores arise from the stromatic mycelium. They are much branched. The basal cell is usually short and stout, and gives rise to numerous branches, which latter may again branch so that the ultimate conidiophores are of the most complex type. Conidia/

Conidia are produced in great numbers from them and form the pionnotes.

Conidia (Plate II. Fig. 2 at d.) apedicellate. slightly curved, cylindrical tapering slightly to the ends which are rounded.

Septation. (Average of 200 conidia).

Septae.	%age occurring.
3.	2.
4.	22.
5.	70.
6.	6.

Size. (Average of 200 measurements.)

78μ (68μ - 89μ) x 6.3μ(5.5μ- 7.0μ).

Perithecia are freely produced and arise from the plectenchymic stromata. Ascospores are matured and ejected in the usual tendril.

Asci. 148μ (133μ - 165μ) x 7.9μ (7.5μ - 8.5μ).

Ascospores.17.4μ (15.7μ - 18.8μ) x 7.5μ (6.8μ- 7.9μ).

No chlamydospores or sclerotia were seen on this medium.

Bean Agar.[x]

Growth on bean agar is chiefly within the medium. Surface hyphae and aerial mycelium are very little developed. The former forms thread-like strands of honey yellow hyphae. The latter is loose radiate, white, with a distinct tendency to zoning. This zoning is not a result of the alternating light of night and day, but is probably a phenomenon induced by a reaction between the fungus, during its metabolism, and the medium, as seen and described by Brown/

x For preparation of this medium see Duggar (18).

Brown (7) in his Fusarium studies. This is a non-staling medium.

Conidia are produced on compound (Plate I. Fig. 3 at b. and c.) or simple (Plate I. Fig. 3 at a.) conidiophores arising from the stromatic hyphae. These conidiophores increase in number from the inoculation point. They produce vast numbers of conidia and the latter congregate on the surface and form slimy pionnotes.

(Plate I. Fig. 3 at d.)
Conidia,/apedicellate, slightly sickle-shaped, cylindrical except where they narrow slightly at either end, rounded at the ends, pale maize yellow.

Septation. (Average of 200 conidia).

Septae.	%age occurring.
3.	7.
4.	61.
5.	31.
6.	1.

Size. (Average of 200 measurements.)

68μ (60μ - 73μ) x 6.3μ (5.3μ - 6.9μ).

Perithecia were freely formed and matured in the normal fashion.

Asci. 136μ (120μ - 147μ) x 7.7μ (7.2μ - 8.7μ).

Ascospores. (Plate I. Fig. 3 at e.).

17.6μ (15.2μ - 18.3μ) x 7.5μ (6.3μ - 7.9μ).

The external appearance of the perithecium during its development is easily followed on this medium. It first appears as a little yellowish white knob on the surface of the underlying thread-like stroma. It then assumes a greenish tinge and is circular. A pale/

pale buff colour appears at the top and gradually works down. The top is,from now onwards in development, always deeper in colour than the lower part of the perithecium. The body soon assumes a mars orange colour and the top, which is now appearing slightly flattened, a light morocco red. At maturity the perithecium is globular with a flattened disc bearing a distinct papilla. The body assumes a blood red colour, while the top is a darker shade of the same. The perithecium dehisces now, the ascospores emerging in a yellowish salmon ball.

Chlamydospores and sclerotia were not observed on this medium.

5% Dextrose Potato Agar.

This medium was interesting in that, in several cultures, two types of growth appear, (a) an aerial hyphal type and (b) a non-aerial hyphal type.

In the former there is a rich development of dense, fluffy,erect hyphae along part of the inoculation line. The ends of these hyphae are a rosy white. Lower down the hyphae become a vinous mauve. Pseudo-pionnotes are developed on simple or sparingly branched conidiophores arising from the strands composing this aerial hyphae. These pseudo-pionnotes and the lower hyphae are a vinous mauve, due to grains of that colour present among them. These hyphae vary from a thin-walled fairly septate type with flesh coloured contents, to a thick-walled mycelium of a dark brown colour. This mycelium (Plate I. Fig. 4) in parts becomes/

becomes swollen, thick-walled and dark brown, with numerous septae and oil drops. The cells of these thickened hyphae give off side branches which may terminate soon, or branch and be prolonged into thinner hyphae. The side branches are usually swollen at their bases, and are not so deeply coloured as their parent hyphae. These abnormally appearing structures in the mycelium may possibly be resistant bodies of a sclerotial nature. Chlamydospores were not seen.

In the remaining part of the inoculation line, production of aerial hyphae is very restricted, and only occurs at the edges. It is a pure white to vinous mauve colour. The fungus growth forms a thin, dark purple sheet on the surface of the medium. Dark violet pionnotes are freely produced on this thin surface growth. The hyphae are large, thin-walled, hyaline or with a purplish tinge, and contain a great quantity of oil.

The conidia are the same for both growth types.

Conidia, apedicellate, slightly curved, cylindrical, narrowing at both ends which are rounded.

Septation. (Average of 200 conidia).

Septae.	%age occurring.
2.	2.
3.	40.
4.	52.
5.	6.

Size. (Average of 200 measurements).

65μ (57μ - 77μ) x 6.9μ (6.3μ - 7.7μ).

Perithecia/

Perithecia were formed in great numbers in the aerial hyphal type of growth, but were only sparingly produced in the other growth types.

Asci. 130μ (110μ - 145μ) x 7.8μ (7.0μ - 9.1μ).

Ascospores. 17.2μ (15.9μ - 18.4μ) x 7.6μ(6.1μ-8.4μ).

Remarks.

Staling soon sets in with the aerial hyphal type of growth, while this phenomenon is not so quick in appearing in the other growth type . The only condition which is likely to vary along the line of growth, is the depth of the medium. According to Brown (6), staling is not so quick in appearing in deeper media as on shallow, and yet, in these cultures, staling took place in the aerial growth at the lower half of the medium in the test tube (i.e. the deeper half). The causes leading up to the development of these two types of growth may be as follows. The inoculation was made in a line in the middle of the media in the test tubes. The inocula were conidia from pionnotes. In every case, a greater number of conidia were deposited in the lower half of the inoculation line. The heavy growth resulting at the lower half would cause a vigorous enzyme action between the hyphae and the substratum. Staling products (which appear to be produced in this medium) would be rapidly produced, and so the lateral growth would be checked. In the upper half of the inoculation line staling products would not be produced in such quantities, due to the lesser growth resulting from/

from a smaller number of conidia there. It is quite probable also, that accumulation of a volatile metabolic product such as ammonia, would be greater at the foot of the tube and so tend to increase staling there, as Brown (6) found in Sphaeropsis.

2% Dextrose Potato Agar.

Both types of growth were obtained in this medium as in the 5% Dextrose Potato Agar. (Plate II. Fig. 2 at e.).

Conidia./ These were the same shape as the conidia developed in the 5% Dextrose Potato Agar.

Septation. (Average of 200 conidia).

Septae.	%age occurring.
3.	occasional.
4.	51.
5.	48.
6.	.5.

Size. (Average of 200 measurements).

64μ(55μ - 75μ) x 6.8μ(6.5μ - 7.2μ).

Perithecia. These were formed in few numbers on the non-aerial hyphae producing type of growth, while they were abundant in the aerial hyphal growth type.

Rice.

In eight days there is considerable growth. The aerial mycelium is loose, pure white at the tips, while lower down it gradually deepens to a bluish lilac colour. The hyphae of the tips are thin-walled but get thicker-walled as they near the substratum, where they tend also to connect up by means of cross fusions, and form strands (Plate II. Fig. 1 at d.). Near the base of the strands, the surface of the hyphae are/

are thickly coated with bluish lilac to vinous mauve coloured crystals (Plate II. Fig. 1 at b.). The contents of the hyphae may be hyaline or the cell sap may be tinged with a bluish lilac colour. The crystals, however, are on the surface of both colour types of hyphae. A coloured liquid, which appears to be secreted by the hyphae, is absorbed by the rice grains. The growth extends down the rice and the older growth (i.e. the growth nearer the top) tends to change colour. The change is through a pale lilac rose to pure white, then deep greenish white and finally a lemon yellow. This change of colour is probably an acid modification, due to increased acidity in the medium resulting from active starch (rice grains) fermentation.

Conidia are produced freely on the aerial hyphae and the stromatic hyphae. The latter cover the grains as thread-like strands, each thread representing a strand composed of parallel and connected hyphae. The aerial hyphae produce simple conidiophores or, nearer their bases, compound conidiophores (Plate II. Fig. 1 at c.). The former are hyaline, while the latter are at first vinous mauve and later pale ecru. This coloration is due to an aggregation of crystals on their surface; the colour of the crystals changing in all probability with the reaction of the medium. The compound conidiophores tend to group, and together with the spores produced by them, form aerial sporodochia which can be seen macroscopically. The spore masses on the sporodochia are/

are at first a pale vinous mauve and later a pale ecru. This again is not due to coloration in the conidia, but the presence of granules among them. Pionnotes are produced on the surface of the grains from compound conidiophores arising from the thread-like stromata. In one culture the conidia, cut off from these conidiophores, had remained together (although not connected) and formed beautiful columns.

The stromatic hyphae are thick- walled, and vary in colour according to their age. Bladder-like swellings (Plate II. Fig. 1 at e) occur on them, but never become at any time, chlamydospores. They remain thin-walled. There is a tendency to form sclerotial bodies as seen in the 5% Dextrose Potato Agar.

There is a great accumulation of oil in the young conidia (Plate II. Fig. 1 at a).

Conidia, (Plate II. Fig. 1 at a) apedicellate, slightly sickle-shaped, cylindrical, slightly tapering to the rounded ends, greenish in transmitted light.

Septation. (Average of 200 conidia).

Septae.	%age occurring.
3.	4.
4.	72.
5.	24.

Size. 65μ (60μ - 75μ) x 6.9μ (5.8μ - 7.4μ).

Perithecia are freely produced, singly or in groups, and arise from the thread-like stromata on the surface of the grains.

Asci. 134μ (105μ - 158μ) x 7.9μ (7.5μ -9.1μ).

Ascospores. 17.3μ(15.0μ- 17.8μ) x 6.8μ(6.2μ- 7.5μ).

Potato/

Potato Cylinders.

The growth forms produced on this medium are variable.

The common type of growth is a mycelial one and is fairly sterile. The mycelium forms a thin growth on the surface of the cylinder. Between these thinly distributed mycelial strands are numerous crystals of varying sizes. These crystals are soluble in glacial acetic acid and concentrated hydrochloric acid. The aerial and surface mycelium is a smoke grey colour.

Conidia are very sparingly produced, and never in sufficient numbers to be seen in macroscopic groups. They are, moreover, small. Many are abnormally shaped and possess peculiar swellings, and might suggest the results of development from a poorly or improperly nourished mycelium./ (Plate II. Fig. 2 at c.). Microscopically the mycelium possesses a yellowish tinge, and is thick-walled, sparingly branched, with cells which are vacuolate and often greatly swollen to give vesicles of varying shapes.

From this general type there are gradations to a sporodochial type, the latter being particularly well represented in one culture. The stromata in this case, radiate from the point of inoculation. The marginal hyphae are loose, radiate and pure white. Within the margin there is a great development of sporodochia whose spores are heaped in a milk white mass in the centre, but further out tend to become zonally distributed/

distributed. The conidia here are larger and the septation average is higher than those produced by the non-sporodochial type.

There were intermediate types of growth. In many cases one obtained a mycelial growth on the tuber surface, while below and at the sides the sporodochial type was produced.

Perithecia were scantily produced except for one culture where there was a great crop. The perithecia in the latter, however, were devoid of asci and ascospores. They were merely perithecial walls (see however experiments on perithecial production on this medium).

It is interesting to note that, in the sporodochial type of culture, and in that one where perithecial "shells" were freely formed, the potato cylinders had undergone greater decomposition than in the mycelial growth types. This was concluded from the much darker nature of the potato flesh in the sporodochial and perithecial culture. In these latter the flesh of the tuber became quite black. In the common mycelial type of culture, the colour of the flesh was only slowly turned to a slatey grey and usually no further. These observations would tend to show that in the production of asexual spores (conidia) and ascospores, there is a much greater metabolic activity on the part of the fungus, a greater decomposition of the substratum (the potato slope), which is necessary for increased assimilation to produce propagative/

propagative bodies, or to be used in a greater respiration to give the necessary energy for this increased output. Here, it is possibly for both greater respiration and greater growth.

The reasons for the irregular production of perithecia on this medium will be discussed later. The occasional production of a sporodochial type of culture is interesting. The first cultural studies were made on four tubes of potato cylinders. Three of these were inoculated with conidia from pionnotes on bean agar, while the fourth was an ascospore inoculation. The former produced mycelial growth types, while the latter gave a sporodochial type of growth. It was thought therefore, that the type of inoculum influenced the type of growth obtained. Accordingly, further cultures were started from ascospore inoculations. The same medium was used and all tubes were incubated in the usual conditions. However, in every case a mycelial and not a sporodochial type of growth, resulted. It was then considered that the production of the sporodochial type in the first series of cultures might have been due to a saltation, such as Brown and Horne (8) found in the genus Fusarium. To pursue this possibility further subcultures were made with the conidia of this sporodochial type for inocula, on to potato cylinders. Here, however, in six out of the eight tubes inoculated, the mycelial type of growth was produced. The other two tubes showed the intermediate growth type previously/

previously referred to.

Since this sporadic appearance of the sporodochial type of growth does not appear to be the result of a saltation, nor to be dependent on the type of inoculum, one must look elsewhere for a solution. Later experiments on perithecial production (q.v.), make it apparent that moisture is not a factor influencing the sporodochial production phenomenon on this medium, and since the other physical factors cannot be held to affect it, the only possibility is that there is a variability in the composition of the medium. That this varies is not improbable, when one looks to the distribution of the protein grains in a potato tuber. Brown (7) has shown how, in the genus Fusarium, the nature and concentration of the most important constituents of nutrient media have a very decided influence on growth types. It is suggested that there is, between those potato cylinders, a difference of either the nature, or more probably the concentration, of their constituents, which is sufficient enough to influence the growth type to the extent seen.

Since the conidia in the mycelial type of growth were largely abnormal, they were not included for the purpose of measurement. This latter was made on conidia derived from a sporodochial type of growth.

Conidia. (Plate II. Fig. 2 at a.).

These were apedicellate, slightly sickle-shaped, cylindrical, narrowing slightly to the rounded ends.

Septation./

Septation. (Average of 200 conidia).

Septae.	%age occurring.
1.	occasional.
3.	16.
4.	58.
5.	26.

Size. (Average of 200 measurements).

60μ(53μ- 70μ) x 6.9μ(5.3μ- 7.5μ).

No true chlamydospores were found in this medium, although swellings on the hyphae were common.

On Two year old Alnus Stems.

Growth is poor on this medium, and is practically confined to the upper cut surface where the aerial hyphae are fairly compact, brownish at the foot, and dirty white at the tip. Aerial sporodochia are formed, and are a dirty white. Pionnotes are formed on the cut curface. These are slimy, reddish apricot. On the sides of the stem the growth is poor and thread-like, and by no means covers the bark. Sporodochia are seldom formed, and if so, they appear at the lenticels.

Conidia are here shaped as in the other media. Their size and septation is wonderfully constant. These features were observed for woody media by Sherbakoff (38) in his cultural studies of the various Fusaria from potatoes.

Conidia. (Plate II. Fig. 2 at b.).

Septation. (Average of 200 conidia).

Septae.	%age occurring.
3.	70.
4.	29.
5.	1.

Size./

Size. (Average of 200 measurements).

50μ(47μ- 56μ) x 4.9μ(4.8μ- 5.3μ).

Perithecia are formed freely on the cut surface, but are developed to a lesser extent from the thread-like stromata on the surface of the stem.

Asci.138μ(110μ- 160μ) x 8.0μ(7.5μ- 8.8μ).

Ascospores.16.8μ(14.7μ- 19.2μ) x 7.4μ(6.2μ- 8.1μ).

(iii) Germination of conidia in water.

Conidia from pionnotes on oat agar germinated in less than thirty hours in distilled water on a slide in a petri dish. Germ tubes were usually produced from the extremities of either end cell. Occasionally they were produced by intermediate cells, from a point just below the septum. Fusions took place between germ tubes, and these fusions were as variable in their nature as those seen between the ascospore germ tubes. Conidia were not produced after twenty days from these conidial germ tubes.

(c) Description of the Species.

A more extended account of the species than that of its original description is appended.

Nectria mammoidea. Phil. et Plowr.

Conidial Stage.

The conidiophores arise from the underlying hyphae and are simple or compound, dichotomously branched structures, the branches forming two to four tiers. The conidiophores are hyaline or faintly

tinged/

tinged purple,with, in most of the media employed for the growth of the fungus, an aggregation of reddish purple crystals on the surfaces of the lower cells. They are borne on the stromatic hyphae, or on aerial mycelium, and abstrict conidia to form in media, in the former case, pionnotes, in the latter case, aerial sporodochia.

The conidia are apedicellate, slightly sickle-shaped, cylindrical and tapering slightly to the rounded ends, hyaline or light green. They are, as a rule, four and five septate and measure on an average - 66μ(53μ- 89.6μ) x 6.6μ(5.3μ- 7.5μ).

Perithecial Stage.

The perithecia are borne singly or in groups on a thread-like or a well defined stroma. They are smooth, globular except at the top where there is a definite disc in the middle of which appears a slightly papillate ostiole. They measure approximately .45 mm. x .42 mm. At dehiscence of the ascospores they are blood red, the disc being a darker shade than the body of the perithecium. At dehiscence the perithecial wall is composed of two layers, the outer of about five rows of thick-walled, irregularly shaped cells,while the inner has three rows of thin-walled cells, elongated in the direction of the circumference.

Paraphyses are present in quantity just prior to dehiscence, but are disintegrated before the ascospores are ejected. They are composed of elongated, multicellular/

multicellular hyphae, whose cells are swollen, thin-walled and vacuolate.

The asci are cylindrical, blunt at the upper ends, and measure on an average - 134μ (102μ- 160μ) x 7.9μ(7.0μ- 9.1μ).

The ascospores are oval to spindle shaped, bi-cellular, with a faint constriction at the septum, arranged obliquely in one row in the ascus, At maturity they are rough walled and measure - 17.4μ(14.7μ- 22.5μ) x 7.5μ(6.1μ- 10.1μ).

The fungus has been found in Britain as a saprophyte on the stumps, decaying wood and bark of Ulex and Betula. It does not appear to have been recorded from the continents of Europe and North America.

III. CONCLUSIONS derived from a CULTURAL STUDY.

Staling in Culture.

Staling only took place in the dextrose potato agars and to a slight extent on potato cylinders. Staling would not necessarily therefore appear to be an inherent tendency in this fungus as Brown (6) suggests for some fungi. That author, however, finds that staling also depends to a great extent on the medium, and it is probable that the products of the fungus metabolism in these specific media are sufficient in amount and toxicity to prove inimical to a further spread of the fungus. In Brown's work, potato agar is said to a staling medium. No attempt was made to dilute these agars in order to avoid staling.

The Stroma.

In culture this never assumes the size that it attains on the natural substratum. The perithecia are grouped and partly sunken in a large stroma in the latter, while they develop singly, or in small groups, on thread-like plectenchymic stromata in culture. This is only another instance of showing the unreliability of using the stroma as a taxonomic character. This fact was recognised by Wollenweber (46) in 1913. He says - " the variation in formation of a stroma on different substrata prove this character to be unreliable for a classification of the Ascomycetes".

Colour Formation./

Colour Formation.

In this fungus colour in the cultures was chiefly produced by numerous crystals covering the hyphae, or lying free on the medium. It appears probable that these crystals are the results of crystallisation of some cell secretion. This liquid secretion seems to be absorbed by the substrata and colours the latter (oat agar, dextrose potato agars, bean agar and rice). The secretion which is not absorbed by the substrata appears to crystallise. The hyphae and their contents which secrete such a liquid, are not necessarily coloured like the resulting crystals, but may be of a different colour, or even quite hyaline. These crystals are greenish in bean agar and potato cylinders; in the remaining media they tend to be of various purple hues. This colour difference may be due to the reaction of the medium, since in rice we get a change on ageing from a vinous mauve to a lemon yellow, which change is thought to be correlated with a change to a more acid substratum. This change from blue to yellow and orange colours has been seen by Appel and Wollenweber (1) and Sherbakoff (38) in their cultural studies of Fusaria, and is accounted for, by these authors, by a change of the substratum from an alkaline or neutral to an acid reaction.

These crystals vary in size and in shape. They are especially large on potato cylinders.

Conidia.

Microconidia/

Conidia.

Microconidia were never seen, and would not appear to be produced by this fungus.

Macroconidia. Below are the sizes and septation of the conidia on various media -

TABLE I.

The septation and size of the macroconidia of Nectria mammoidea in various media.

Medium.	Septation. No. of septae & %age occuring. 1. 2. 3. 4. 5. 6.	Size. Length. Breadth.
Bean Agar	- - 7 61 31 1	68μ(60μ-73μ) x 6.3μ(5.3μ-6.9μ).
Oat "	- - 2 22 70 6	78μ(68μ-89μ) x 6.3μ(5.5μ-7.0μ).
5% Dext.Pot.	- 2 40 52 6 -	65μ(57μ-77μ) x 6.9μ(6.3μ-7.7μ).
2% " "	- - - 51 48 -	64μ(55μ-75μ) x 6.8μ(6.5μ-7.0μ).
Rice	- - 4 72 24 -	65μ(60μ-75μ) x 6.9μ(5.8μ-7.4μ).
Potato	- - 16 58 26 -	60μ(53μ-70μ) x 6.9μ(5.3μ-7.5μ).
Average for all media.	- .3 11.5 53 34 1	66.6μ(59μ-76μ) x 6.6μ(5.8μ-7.3μ).

It is common knowledge that the size of the conidia vary more or less as the substratum varies.

In Nectria mammoidea the length of the conidia varies from medium to medium, so that if frequency curves were drawn for the spore lengths on various media, the maxima for each would differ. Except for range of conidial lengths on oat agar the average range for the conidia on all media lies within the length ranges in each of the other media. In the case of oat agar the lengths of the shorter spores come within the compass of the spore length ranges for the other media. Thus a general curve drawn on a basis/

basis of the spore lengths/numbers for all media would include to a greater or less extent the individual curves made for the spores on the same basis, in each of the various media employed.

The comparative constancy in size between these vegetative propagative units from medium to medium is rather striking. Although the average spore size variesin the different media, the spore shape is a constant, and would indicate its usefulness as a systematic character in some,at least, of the Fungi Imperfecti. Indeed Sherbakoff (38) has said - " The most important character in the classi-
"fication of these fungi (Fusaria) - the type and
" shape of the conidia - is after all sufficiently
" stable to be used safely in a morphological treat-
" ment. Even the size of the conidia, when a suffi-
" cient number of measurements is made and averaged,
" and when only conidia of the same type are compared,
" is of rather surprising uniformity". Archer,(2), after extensive studies of various members of the Sphaeropsidales, has come to a similar conclusion. He says - "It is clear that spore characters are,
"on the whole, quite reliable, and therefore should
"be employed as far as possible in any scheme of
"classification".

Conidial Bridges.

Zeller (53) found in Nectria sanguinea (Sibth.) Fries, that, in macroconidia lodged in masses in the crevices of bark, an outgrowth proceeded from one/

one cell in a conidium and established communication with an adjacent cell on the same conidium or with a cell of a neighbouring conidium. Nuclear migration took place from one of the cells connected thus to the other, and association of the two nuclei resulted, but there was no case of a fusion recorded between them. Cayley (11) has found a similar phenomenon in the macroconidia of Nectria galligena, Bres.

Numerous preparations of conidia of Nectria mammoidea, derived from pionnotes and sporodochia on various media, failed to reveal the presence of those bridges. The cells of the conidia were uninucleate, and in no case was a bi-nucleate cell seen.

The Perithecial Structure.

This feature, which Wesse (42) has used so much in classifying the members of the Hypocreaceae was closely watched in this species. Wesse laid great emphasis on the structure of the perithecial envelope, in conjunction with the content of the perithecium. If the former is a constant characteristic, then it would appear to be a sound feature to use in a systematic key for the Hypocreaceae.

In Nectria mammoidea the wall of the perithecium on the natural substratum and on various artificial media was an essentially similar structure. The wall of the mature fruit body was composed of two fairly distinct pseudo-tissues. The number of cell layers in each was also fairly constant, and the breadth of each pseudo-tissue was found to vary only slightly/

slightly. This feature in Nectria mammoidea at least, would be a very useful character on account of its constancy under varying conditions. It must be borne in mind, however, that Westerdijk and van Luijk (44) found that in Nectria coccinea, Pers. and Nectria galligena Bres.this feature was variable and uncertain, so that the constancy seen in Nectria mammoidea does not appear to hold good throughout the genus.

Colour of the Perithecium.

The colour of the fruiting body of Nectria mammoidea when grown on media, varies greatly during the course of development. This would not matter so much, as far as a specific description is concerned, if the perithecium on nearing, and at maturity (to which period in development descriptions usually refer), was of a constant colour. In this species this is not the case. The colours shown just before,and at dehiscence, are widely different, so that a description of the fruit body which does not pay full regard to this is apt to be misleading. It would be safer in such a case to state in a description - the colour of the perithecium at dehiscence is -. In the species here studied the colour of the perithecium at this period in development was the same. Such a procedure might help to make perithecial colour a useful systematic character, instead of, as it sometimes is in this genus, a rather useless one.

In a large genus such as the genus Nectria, where/

where the divisions between the species are often very nice, it would be of advantage in descriptions to give a more exact colour, and not to treat this feature lightly by using a general colour term, such as "red". Many Nectria perithecia could come under this colour denomination, and the mention of it does not get one further ahead in running down a species.

Asci and Ascospores.

It is interesting to compare the measurements of the asci and ascospores on the natural substratum and on artificial media. (See Table II page 100).

The asci in culture appear to be longer than those on the natural substratum. This may be due to better nutrition in the artificial substrata. The size of the asci vary a great deal when compared with the ascospores. The measurements of the latter show a wonderful constancy, even on the various substrata, and ascospore measurement in this species, at least, forms a very reliable systematic character. The shape of the asci and the one rowed nature of the ascospores in them were very constant features. The shape of the ascospores also remained comparatively constant, but it is interesting to note that the rough nature of the ascospore wall (a feature upon which stress is laid) did not appear until the end point in ascospore development. Indeed numerous ascospores which appeared mature, had not yet acquired this character.

TABLE II.

Size of asci and ascospores of Nectria mammoidea from the natural substratum and from artificial media.

Substratum.	Asci.			Ascospores.		
	Number measured.	Length.	Breadth.	Number measured.	Length.	Breadth.
Natural	20	122μ(102μ-140μ).	7.8μ(7.5μ-8.5μ).	100.	17.8μ(17.0μ-22.5μ).	7.9μ(7.5μ-10.1μ).
Oat Agar	20	148μ(133μ-165μ).	7.9μ(7.5μ-8.5μ).	50	17.4μ(15.7μ-18.8μ).	7.5μ(6.8μ-7. 9μ).
Bean Agar	20	136μ(120μ-147μ).	7.7μ(7.2μ-8.7μ).	50	17.6μ(15.2μ-18.3μ).	7.5μ(6.3μ-7. 9μ).
5% Dext. Pot.	10	130μ(110μ-145μ).	7.8μ(7.0μ-9.1μ).	50	17.2μ(15.9μ-18.4μ).	7.6μ(6.1μ-8.4μ).
Rice	20	134μ(105μ-158μ).	7.9μ(7.5μ-9.1μ).	50	17.3μ(15.0μ-17.8μ).	6.8μ(6.2μ-7.5 μ).
Alnus Stems	20	138μ(110μ-160μ).	8.0μ(7.5μ-8.8μ).	50	16.8μ(14.7μ-19.2μ).	7.4μ(6.2μ-8.1μ).

Chlamydospores.

In 1913 Wollenweber (47) published results of his extensive cultural studies on Ramularia, Mycosphaerella, Nectria and Calonectria. As the result of these studies, that author has used, until quite recently, the presence or absence of chlamydospores in the life history, as a characteristic to separate species belonging to Hypomyces and to Nectria. Those species with chlamydospores and possessing Hypomyces or Nectria characteristics, were definitely placed in the genus Hypomyces, and conversely, those without those bodies were placed in the genus Nectria. As a consequence, great importance was attached to the presence or absence of these structures. It appears, from a study of this species and others in the genus Nectria, and from consulting various figures of so called chlamydospores pictured by several authors, that such a procedure as adopted by Wollenweber was not entirely justifiable, and for several reasons. It is difficult to state exactly what is a chlamydospore. Such bodies vary in shape, size and appearance, and a structure called a chlamydospore by one investigator, may not be so considered by another. Several illustrations of chlamydospores seen by the author, appear to be only swellings in the hyphae, and are not of the nature of true, thick-walled, resting spores - chlamydospores. The very uncertainty of the ideas prevailing in the minds of investigators as to what is a chlamydospore and what is/

is not, would be sufficient to condemn the use of their appearance or absence in a fungus, to determine the genus to which it belongs. Another objection which may be raised is that, in some species, chlamydospores may be formed only under very particular conditions of the environment. As a result, possession of these bodies by a fungus may go by unnoted by an investigator simply because these particular conditions necessary to favour their production had not obtained during the growth of the fungus.

On this basis of presence or absence of chlamydospores many species in the genus Nectria e.g. Nectria rubi, Osterwalder, had been shifted by Wollenweber and placed in the genus Hypomyces, owing to their possessing chlamydospores. The author, who has cultured Nectria rubi, has never seen anything approximating to chlamydospores in this fungus. Nectria rubi would, however, appear to be, in all its other characteristics, a good example of a species of Nectria, and yet, on the very occasional appearance of chlamydospores in its life history, it is removed to the genus Hypomyces. Recently, however, Wollenweber (52) has given up this basis for classification, and has reinstated those Nectrias, which he transferred to the genus Hypomyces, Those species in the genus Nectria possessing chlamydospores, are now included by Wollenweber in the section Chlamydospora in the subgenus Coryneconnectria (52, p. 179). On growing a suspension of conidia of Nectria/

Nectria mammoidea in a tube of sterilised distilled water for six weeks, a good growth of mycelium was at first obtained, but later slowed off and finally ceased, probably due to lack of air or lack of nutrient. Swellings were formed freely on the hyphae, but these never assumed the appearance of true chlamydospores. At no time in the cultural life history of the species were these bodies seen, and it is difficult to see how one could otherwise favour their production. The presence or absence of these bodies in this genus, seems to the author to be a not too sound character on which to base any classification.

B. The Factors influencing Perithecial Production in the Genus Nectria.

I. EXPERIMENTAL.

Six species of the genus Nectria, viz., N. mammoidea, N. coccinea, N. rubi, N. galligena, N. cucurbitula, and N. cinnabarina were obtained in culture. Various experiments were carried out to observe the effects of the various factors in the environment on the production of the perfect stage. The environment of a fungus can be roughly divided into (a) the substratum on which it grows, (b) the physical factors which surround it, It is realised that this division is not strictly accurate, but it is, however, convenient.

(a) the influence of the substratum.

Various media were used as substrata for the growth of these fungi . The oat agar, 2% and 5% dextrose potato agars, potato cylinders, two year old Alnus twigs and rice were used as recommended in the account given of the Fundamentals for the Taxonomic Study of the Genus Fusarium (50). The bean and prune agars were made according to Duggar's (18) formulae, while the glycerine carrot, potato and raspberry roots were portions of these substances with the addition of 6 cc.s. of 1% glycerine.

The following table (Table I. page 105), shows the extent of perithecial formation by the various species on these media.

TABLE I./

TABLE I.

Showing the extent of perithecial formation by six species of Nectria on various media.

Medium.	Fungus.					
	Nectria mammoidea.	Nectria coccinea	Nectria rubi.	Nectria galligena.	Nectria cucurbitula.	Nectria cinnabarina.
Oat agar	+	-	+x	-	-	-
Bean agar	+	-	-	-	-	-
Prune agar	+	-	-		-	-
5% dext. pot.	+		+x			
2% " "	+		+x	-		
Pot. cylinders	+		+x	-		
1% glyc. pot.	+	+	-	-		-
1% " carrot	+	+				
Alnus twigs	+		-			-
1% glyc. rasp. root			+x	-		
Rice						-

x denotes sterile perithecia.

Observations on these results will be reserved till the discussion.

In a species, such a Nectria mammoidea, which produces perithecia on all media, it is interesting to observe the comparative numbers produced on the different media. This is indicated in the following table. In every case the average number of perithecia produced on a single type of medium, was taken. Each culture was derived from a conidial inoculation, the latter being obtained from the same source, and incubated under equal laboratory conditions. The surface area for growth in each of the various types of media was taken as being approximately equal.

TABLE II.

Relative number of perithecia produced by Nectria mammoidea on various media.

Medium,	Number Produced.
Oat agar	100.[x]
Bean agar	92.
Alnus twigs	86.
Rice	60.
5% dext. pot.	40.
2% " "	36.
Prune agar	30.

[x] This figure is purely an arbitrary one, and served as a standard with which to compare the others.

(b) the influence of the physical factors.

In order to test the influence of these factors on perithecial production, a fungus forming these bodies freely on artificial media, viz., Nectria mammoidea, was used.

(i) Moisture. The normal Alnus stem medium contains 4 cc.s. sterilised distilled water. Two series of parallel cultures were run. In one series the medium used was the normal Alnus stems plus water, while in the other series, Alnus stems without the addition of water were used. Two series of potato cylinders were also used; one of these series having 4 cc.s. of sterilised distilled water added to each tube, while the other series was used without the addition of this moisture. The results are tabulated below.

TABLE III.

The effect of varying moisture contents on perithecial production in Nectria mammoidea grown on Alnus twigs and potato cylinders.

Moisture Condition.	Medium.	No. of tubes.	Source and type of inoculum.	Presence or absence of perithecia.
Dry.	Alnus.	6.	Conidia from pionnotes on bean agar.	-
Moist.	"	10.	do.	+
Dry	Potato.	6.	do.	(5 - (1 + x
Moist.	"	4.	do.	(3 - (1 +

x Denotes "sterile" perithecia.

It must be added that the growth in the dry Alnus stems was poor and tended to be of a mycelial type/

type. The moist Alnus stems produced less aerial mycelium, but conidia were freely formed and appeared in slimy pionnotes at the cut ends and in erect columns on the stem surfaces. The moist culture looked much healthier than those on the dry stems. As a general rule presence or absence of moisture did not affect the type of growth on the potato cylinders.

(ii) Temperature. The effect of this factor on perithecial production was studied, and the results are tabulated below. (Table IV). Incubators were used for the higher temperatures, while, for lower temperatures tubes were placed outside during the winter months, and the daily temperatures ascertained by readings of the maximum and minumum thermometers each morning during the experiment. Other factors were kept equal. At first the inoculated tubes were placed at the varying temperatures in the dark, but since light was found to be necessary for perithecial production, the effect of varying temperatures had to be studied in cultures in the ordinary alternating light of night and day. For this reason the number of different temperatures obtainable was restricted, since it was not possible in many cases to expose several of the incubators to light and retain the correct temperature.

TABLE IV./

TABLE IV.

Effect of temperature on perithecial production in Nectria mammoidea.

Temperature of incubation.	Number of tubes.	Type of inoculum.	Source of inoculum.	Medium,	Light condition.	Presence or absence of perithecia
30° C.	6	conidial.	pionnotes on oat agar.	bean agar.	day and night.	-
18° C.	10	"	do.	do.	do.	+
Outside[x]	6	"	do.	do.	do.	+

[x]

	minimum	maximum
average	.6° C.	8.4° C.
extreme	- 6.5° C.	12.0° C.

At 30° C. no fungus growth took place whatsoever. Perithecia were formed abundantly at 18° C., while they were produced in lesser numbers and were slower in appearing at the temperatures obtaining outside. Temperature appears only to affect perithecial production in so far as it affects the growth of the fungus. Thus the latter is retarded by the lower temperature and, as an apparent result, perithecia appear later and in fewer numbers than at 18° C.

(iii) Light. The effect of the presence or absence of this factor was studied at two temperatures. At 18 C. an incubator was used as a dark chamber, while at outside temperatures the light was excluded by inserting the tubes in a box among sawdust. Two media were used, viz., bean agar and oat agar. The results are appended below.

TABLE V./

TABLE V.

The effect of light on perithecial production in Nectria mammoidea on media.

Light condition.	Temperature.	Kind and source of inocula.	Number of tubes.	Medium.	Presence or absence of perithecia.
Darkness.	18° C.	conidia ex pycnotes on oat agar	10	8 bean agar	-
				2 oat "	-
Night and day.	"	do.	10	8 bean agar	+
				2 oat "	+
Darkness.	Outside[x]	do.	4	bean agar	-
				2 " "	+
Night and day.	"	do.	4	2 oat "	+

[x]

	minimum	maximum
average	.6° C.	8.4° C.
extreme	- 6.5° C.	12.0° C.

The time allowed for perithecia to appear was ten weeks. In a species such as Nectria mammoidea which, under ordinary laboratory conditions produces its perfect stage so freely within two weeks, the absence of perithecial development in the dark, under the conditions of the experiment, can only be attributed to absence of light.

II. DISCUSSION.

In the growth and reproduction of any organism there are two main factors which influence its course of development, viz., (a) the organism itself and (b) its environment.

We are here especially dealing with that stage in development - reproduction by means of perithecia and their contents - and must consider the parts played by the above two main factors in this phenomenon.

In how far is the organism itself responsible for the production of its perfect stage? Is the latter dependent on any sexual process, or can we eliminate this as one of the factors? Sexuality in the Ascomycetes is, according to numerous investigations, a very variable thing (for summary of this see Introduction to the paper on Perithecial Development in Nectria mammoidea). It is true that in many Ascomycetes, e.g. in the Discomycetes, the Erysiphales and the Plectascineae, there may occur a normal sexual process between fully developed sexual organs around which the ascocarp originates. When we come to the higher members of the group, however, such as those in the order Pyrenomycetineae, the existence of a true sexual process is doubtful, and moreover, the occurrence of sex organs themselves is a very variable feature from species to species. There seems to be a trend in the development of these Pyrenomycetous fungi, whereby sexuality is entirely eliminated in the life/

life history and ascus production is from purely vegetative hyphae in the fruit body. In Pyrenomycetes closely related to the genus Nectria, a sexual process is probably for the most part non-existant, while sex organs, if present, do not appear to function, but degenerate sooner or later. The entire absence of sex organs associated with the perithecium in its development as noted by Cook (13) in Nectria ipomeae and by the author in Nectria mammoidea (see paper on Perithecial Development) would suggest that, in those members of the genus at least, the perithecium did not arise as the result of the stimulus of a sexual act, or in association with sexual organs. In these species the origin of the perithecium would appear to be a purely vegetative phenomenon. The ascogonia seen by Miss Caley (11) in Nectria galligena arose in the young perithecial "knot" of hyphae, and disintegrated before the origin of the true ascogenous hyphae which arose de novo from vegetative hyphae. Here again therefore, the existence of these degenerate female sex organs does not seem to affect either the origin of the perithecium or the development of its essential contents.

It is true that, from cultural evidence, it has been seen that in various Ascomycetes there exists the necessity of a union between two strains in order to produce normal ascocarps. The existence of so called plus and minus strains has been reported for other groups; by Blakeslee (5) in certain forms of Mucorine fungi, by Burgeff (10) in Phycomyces nitens/

nitens, while in the Basidiomycetes, Bensaude (3) has shown the necessity for the mixture of strains derived from single spore cultures in order to give normal fruit bodies in Armillaria mucida, Tricholoma nudum and Coprinus fimetarius, while Kniep (27), Buller (9) and Hanna (21) have shown the existence of sexual strains in various Basidiomycetes.

This phenomenon of heterothallism has been seen also in the Ascomycetes. Derx (54) has proved the necessity of the mixture of plus and minus strains for the formation of normal perithecia in Penicillium luteum, while Dodge (16) showed the necessity for such a union in Ascobolus magnificus. Betts (4) has proved conclusively that Ascobolus carbonarius is heterothallic and that the genetic characters of the plus and minus strains are segregated in that species at the time of ascospore formation. Shear and Dodge (36) have proved satisfactorily that Neurospora sitophila and N. crassa are both heterothallic, while a nearly allied species, Neurospora tetrasperma is normally homothallic. Quite recently Dodge (17) has published the results of his cytological investigations of these species, Neurospora tetrasperma, which is normally homothallic has four ascospores in the ascus. Cytological evidence shows that each of these spores is formed around two of the nuclei usually resulting from the divisions of the definite ascus nucleus. Dodge (lit. cit.) shows that the sex factor so segregates in nuclear divisions

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in the ascus that two nuclei of different sexes are borne in one spore. On occasion one nucleus only may be included in an ascospore, and so only one sex is represented. In this case there is a necessity for a union of thalli produced from such nuclei of different sex. On an occasion such as this the species is said to show "pseudo-heterothallism". So in species normally heterothallic e.g. N. crassa and N. sitophila it is possible on occasion to have a large abnormal spore developed containing two or more nuclei which are representative of different sexes. Thus in these species, normally heterothallic, a homothallism is displayed by the mycelium arising from such a spore. Dodge says -

> "Clearly heterothallism and homothallism in the "species of Neurospora are not absolutely fixed "specific characters, although the sexual nature "of a individual haplont is definitely determined "by the time the spore is cut out."

Kirkby (23) found that two strains of the "Take-All" fungus, Ophiobolus cariceti, when grown apart in culture, did not form the perfect stage, but did so when mixed. Edgerton (20) got interesting results in the genus Glomerella. He observed two strains which he called plus and minus. The minus mycelium formed many perithecial initials, but these required the stimulus of a special medium to produce mature asci. The plus strain formed perithecia in sporadic groups. Edgerton is of the opinion that here the sexual strains are not completely isolated. He suggests that the plus strain is the antheridial strain, and on few occasions/

occasions gives oogonia, while the negative strain is the oogonial which produces numerous perithecial initials which do not develop further on account of the lack of antheridia to fertilise them. On mixing these two strains a good production of mature perithecia was obtained, due presumably to the sexual act at the junction of these two strains causing perithecial production. Until something is known of the ascocarp development here, the above theory is only of a speculative nature.

It might almost be questioned if the production of the perfect stage through the union of two strains is always a sexual phenomenon. In cases, such as Edgerton's or of Kirkby's, must we regard the two strains whose mixture was necessary for a normal production of perithecia as being sexually distinct? If we postulate for a moment, that in these fungi there are no sexual organs, or if present, only degenerate and non-functional (as we have seen to be the case in nearly related fungi), and that the asci arise from vegetative hyphae in the perithecium, how are we to regard both these strains whose union is a necessity? It may be that in these fungi, these strains are physiologically, yet not of necessity sexually, distinct, and that one in a medium, during its metabolism releases a substance or substances which stimulate perithecial production in the other. Such is not impossible when one has in view the experiments which have shown the dependence of perithecial/

perithecial production on the presence of specific stimuli. In such a hypothetical case of this physiological hyphal differentiation between two strains, this difference need not be sexual and the so-called heterothallism displayed is not necessarily a sexual phenomenon.

At this point it is of interest to note that Davis (15) repeated Kirkby's cultural work with the same New York strain of the fungus. Davis found it to be quite homothallic, and showed, moreover, that perithecia were formed freely along the line of union between the mycelial tips of two colonies in a petri dish each derived from inocula from / the same single monospore culture.

It is quite probable that established cases of heterothallism in the Ascomycetes has only been demonstrated in Penicillium luteum by Derx (54), in Ascobolus magnificus by Dodge (16), in Ascobolus carbonarius by Betts (4), by Shear and Dodge (36) in Neurospora and by Thaxter/(55) in several species of the Laboulbeniaceae. It is of interest to note that, with the exception of Neurospora, sexual organs function in the other fungi mentioned.

On the other hand many cases are known in the culture of Pyrenomycetes, where the perfect stages were formed in single ascospore cultures, and were not dependent for their formation on the mixture of two strains. Davis (15) obtained perithecia of Ophiobolus graminis in single spore cultures.

Wehmeyer/

Wehmeyer (43) obtained perithecia in single ascospore cultures of several Valsaceous fungi, while Miss Cayley (12) found the same to obtain in the culture of Diaporthe perniciosa. The author obtained perithecia freely from single ascospore cultures of Nectria mammoidea and N. coccinea. Although in these cases the ascospores are bi-nucleate, yet both nuclei are the result of an equational division of the parent nucleus so that each are genetically alike. The ascospores are formed around one nucleus so that complications such as Dodge (17) found in Neurospora do not normally concern us here.

It would appear that in several species at least in the genus Nectria, sexuality plays no part, nor is the union of sexual strains necessary for the production of perithecia.

Is there anything in the nature of the fungus which gives it a tendency to produce perithecia? Is a great or little predisposition to form fruiting bodies an inherited tendency, and does this feature vary from species to species, or is high and frequent perithecial production only due to suitable environmental conditions during development?

On Table I is shown the extent of perithecial production of six Nectria species on various media. Of these six, four failed to form mature perithecia while one formed them on two of the media used. The remaining one, Nectria mammoidea, formed its fruiting bodies freely on all media. It may be that some

fungi/

fungi are more particular in their nutritional demands necessary to stimulate perithecial production. Some fungi have been found to be very particular in their food requirements for perithecial production, and a special stimulus is necessary. Miss Cayley (11) found such to be the case in Nectria galligena, but even the stimulus (the addition of a 1% glycerine solution) used by her to that fungus on potato medium, did not excite perithecial formation in the same species during this experiment. Leonian (28) found, that the addition of sodium chloride or 2 - 12% sugar to oat-meal agar stimulated perithecial formation in Valsa leucostoma. It is strange that in the range of medium type used in this experiment, several of the fungus species grown did not get their requirements for perithecial production. This stimulus must be of a very special nature and must exist in the case of parasitic forms on the host plant. Thus we see the customary use of host plant tissues and decotions as media, in order to encourage the production of fruiting bodies. Many strongly parasitic forms have failed to produce their fruits in culture. Klebahn (25) got the perfect stage of several Mycosphaerellas and Didymella lycopersici (26) by over-wintering infected leaves, but did not get such bodies in culture. Stoneman (39) got perithecia of Mycosphaerella ontarioensis and M. pinoides on dead host leaves, but did not get the perfect stages in culture. The perfect stage of the notorious Chestnut Blight fungus, Endothia/

Endothia parasitica has never been obtained in culture. Wehmeyer (43), in his cultural studies on the Valsaceae, got four out of the thirty forms studied by him to form their perfect stage in culture. These cases show, how in the case of the parasitic species, a specific stimulus in all probability present in the host, is lacking in culture. It would appear as a general rule (subject of course to exception), that the formation of perithecia by a fungus in culture is inversely proportionate to the virulence of its parasitism. Saprophytic species appear to form their perithecia more freely and on a wider range of culture media than the parasites. This is as might be expected, since saprophytism does not require a fine adjustment betwen the saprophyte and its substratum. The saprophyte is wide in its tastes and not particular in its requirements. Hence, the common artificial media used are more likely to contain the necessary ingredients to allow the saprophyte to flourish and fruit as it does in its natural substratum. In the case of the parasitic species, it is in all likelihood a chance if one grows it on a medium which contains the nourishment or stimulus necessary for its perithecial production. Nectria galligena,N. cucurbitula, N. coccinea and N. cinnabarina are all potential parasites, while Nectria mammoidea and probably N. rubi are saprophytic. These facts are more or less reflected in their perithecial production on the various media.

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It is interesting to note that some groups of fungi are more prone to form perithecia in culture than others. Klebahn (25) found many species of Gnomonia formed their perfect stage freely in agar culture while Shear and Wood (37), Miss Stoneman (39) and Edgerton (19) obtained the perfect stage of various Anthracnose fungi in culture. Within species also it would appear as if there existed perithecial and non-perithecial producing strains. Nattrass, in a letter to the author dated July 13, 1926, states that he got mature perithecia of **Nectria** rubi in about eight weeks on sterilised raspberry roots. The author has repeated his work but without success as regards obtaining perithecia. The same result was obtained by repeating Miss Caley's treatment of Nectria galligena in order to obtain perithecia. Harter and Field (22) discovered two strains in Diaporthe batatatis, one of which produced perithecia in culture while the other did not. The morphology of the pycno - and stylospores of the two strains was the same when compared on various media. The authors, however, did not appear to be working with single spore cultures, so that one cannot entirely dismiss the possibility here for the necessity of the union of two thalli for perithecial production. These two thalli, in [illegible] cases of multi-ascospore inoculations, may have been present in the perithecial strain, but one of them only in the non-perithecial strain. Davis (15), however, worked with three strains of Ophiobolus graminis, Sacc. While one of these/

these, obtained from New York, formed perithecia freely in culture the others refused to do so even when the media and the various physical factors were varied. The writer was inclinded to think that the latter two strains would fruit if only the right environmental conditions could be found. The New York strain was homothallic, and various experiments with the other two strains whereby they were mixed with themselves and with the New York strain, failed to produce perithecia from their own mycelium. It would appear that in this species, under the varying conditions of the experiment, the author was dealing with a perithecial and two non-perithecial producing strains. The author attributed the failure of the latter two strains to produce their fruit bodies as being due to a lack of the reproductive ability consequent on their long continued growth on artificial media with mycelium used for transfer. It is possible that an acquired character such as this non-production of the fruiting stage might persist throughout several generations until vigour was restored. We may thus have in a species a definite division into temporary perithecial and non-perithecial strains. It may be therefore that there is in groups and in species an inherent tendency to perithecial production, and that even within a species we may get perithecial and non-perithecial producing strains.

Within a species perithecial production is influenced by the quality of the medium (Tables I and II). Why it is that some media should favour perithecial/

perithecial production and others have no influence is obscure. It is not only a question of general nutrition alone, for in cultures which are apparently well nourished and growing and producing conidia strongly, perithecia are not formed. This was well seen while culturing Nectria cinnabarina and N. galligena, where in both instances growth on many of the media used was very vigorous and conidia were produced in great abundance and yet no perfect stages were obtained. The converse does not appear to hold true, however, for in media supporting starved, poorly growing cultures, perithecia were not produced.

As far as medium is concerned it would appear that the conditions for perithecial production are, (a) a medium encouraging a normal healthy growth and (b) a specific stimulus or stimuli to perithecial production in the medium.

Perithecial production in Nectria mammoidea appeared to be correlated to a certain extent with production of conidia, for in media favouring a large production of pionnotes, perithecia were more abundantly produced.

In the same fungus the kind of inoculum used did not influence perithecial formation as Klebahn (24) found in Gnomonia veneta and Leonian (28) in Valsa leucostoma. Perithecia were equally well produced on suitable agars with either conidial, ascospore or mycelial inoculations.

The literature on the occurrence of perithecia on media would seem to indicate that temperature did not/

not influence the production of the perfect stage to any great extent. Leonian (29) in his Sphaeropsidales studies found that temperature did influence pycnidial formation in culture, and at some optima even substituted for the light factor when the latter was an essential in pycnidial formation under certain conditions. Perithecial production in Nectria mammoidea was affected by temperature only in so far as the latter affected the growth of the fungus. Provided the temperature is such as to admit of a fairly normal growth in media then, other factors being favourable, perithecia are produced.

The exact influence of moisture is difficult to estimate. There is for consideration here the moisture in the substratum and the humidity of the atmosphere surrounding the fungus growth. Wehmeyer (43) found, in his cultural work with the Valsaceae, that in twig cultures, perithecial production was favoured by a comparatively dry medium, and that in tubes where the medium was saturated with water and as a consequence the humidity of the atmosphere within the tube was high, a mycelial growth resulted and an abnormal stromatic formation developed on the surface. It appeared that, according to that investigator's observations, a high atmospheric humidity and lack of circulation led to a superficial mycelial growth, and as a result the food supply was diverted from a possible perithecial formation to supplying this mycelium and the formation of the imperfect stage.

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In Wollenweber's (47, 54) cultural studies, however, it appears that perithecial formation in the genus Nectria and nearly allied genera was encouraged by saturating comparatively dry cultures. This method was frequently attended with success. In the author's own experience, presence of moisture almost to saturation was necessary to bring about the production of the perfect stage in Nectria mammoidea, and rather than encourage mycelial growth, added moisture favoured conidial production. In the moisture experiment where potato cylinders were used, it is interesting to note (Table III) that only when moisture was added to that medium, were mature perithecia produced. The absence of perithecia on several dry and moist potato cylinders may be accounted for, as in the case of sporodochial production, by the absence of certain stimuli from these cylinders which was present in those producing perithecia. Those dry potato cylinders which did not develop perithecia, did not produce asci and ascospores. It is suggested that this was due to the lack of moisture in them.

The author has noticed that in fallen twigs covered with Tubercularia vulgaris pustules, the perithecia developed readily on those pustules on the under and wet side of the twig. In the places where these twigs were found, the twigs were so placed that a free air circulation could not well have taken place, so that the atmosphere about the under surfaces of those twigs must have been well nigh saturated.

It would appear that in the species of the genus Nectria, perithecial production is favoured by a plentiful supply of moisture in the substratum and the surrounding atmosphere.

In regard to the influence of light on perithecial production, it is of interest to read that Davis (15) found, while culturing Ophiobolus graminis, Sacc., that perithecia were not produced in cultures incubated in the dark, whereas they were freely developed in cultures subjected to the normal light of night and day. Coons (14) found also, while investigating the factors involved in pycnidium formation in Plenodomus fuscomaculans, that light was an essential factor. Even if all the other requirements were satisfied, but light was absent, no pycnidium formation would take place. The experiments with Nectria mammoidea on the influence of this factor show that, in the media used, and under the conditions obtaining during the experiment, light is an essential factor in the production of ascocarps in the species.

It is difficult to assign a reason for the necessity of this factor in perithecial production, and to consider what is the nature of its influence.

Reviewing the literature in general and having in view the results of these observations on perithecial production, it would appear that each species has its own requirements and no generalisation can be made for the members of the group as to the factors influencing their perithecial production.

While/

While it would seem that sometimes one factor is all important, yet as rule many factors appear to be concerned, and each species is not affected to the same extent by the same factor or factors. While these statements appear to be true for the Pyrenomycetes as a whole, they appear to apply in no smaller degree to the members of the genus Nectria, and indeed, it seems probable that each species, and perhaps strains in the species, require separate investigations in order to determine the particular factors influencing the production of their perfect stage.

It is only as a result of such investigations into individual cases that the relative importance of the various factors concerned can be estimated. Only then will it be possible to determine which factors have a general influence on perithecial production throughout the group, and those less general ones upon whose presence specific fungi depend for the production of their perfect stage.

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IV. PHOTOGRAPHS.

Illustrating the paper on the Study of Nectria mammoidea, Phil. et Plowr. in Culture.

All preparations were mounted in water, and the drawings were made with the aid of a Reichert camera lucida.

PLATE I.

Fig. 1. Ascospores from dehiscing perithecia on bark germinating in distilled water. Note the production of macroconidia from simple conidiophores. At b. are ascospores which have failed to germinate and are probably dead. 14 days x c. 280.

Fig. 2. Ascospores germinating in distilled water. Observe the varying nature of the connections between the germ tubes. 14 days x c. 280.

Fig. 3. Preparations made from the fungus after 20 days' growth on bean agar. At a. are simple conidiophores while at b. and c. they are compound. At e. are ascospores obtained from dehiscing perithecia on the medium. x c. 280.

Fig. 4. Various swellings on the hyphae from 5% dextrose potato agar. 20 days x c. 280.

PLATE. I.

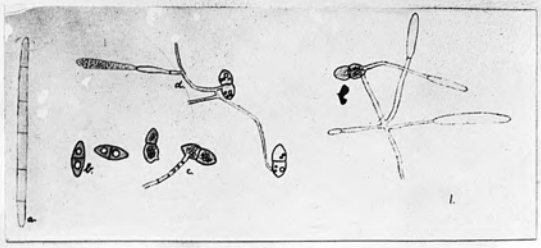

Fig. 1.

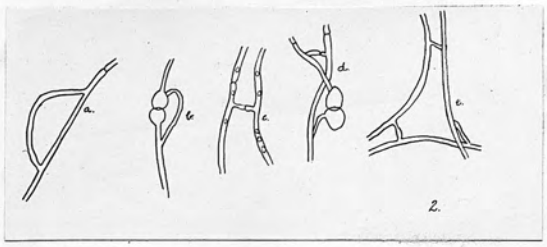

Fig. 2.

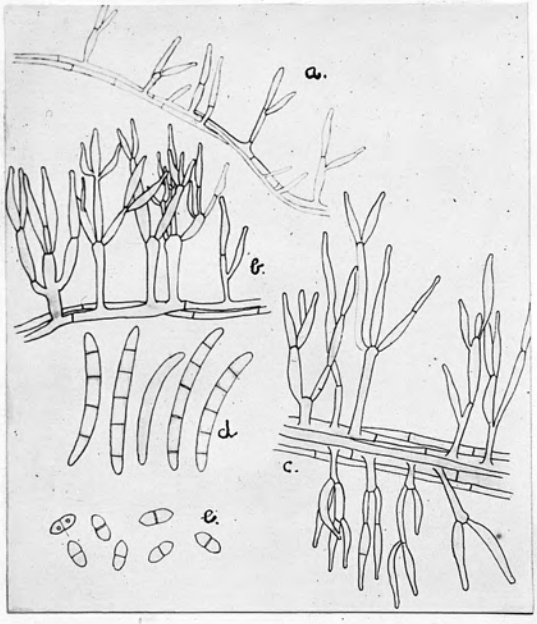

Fig. 3.

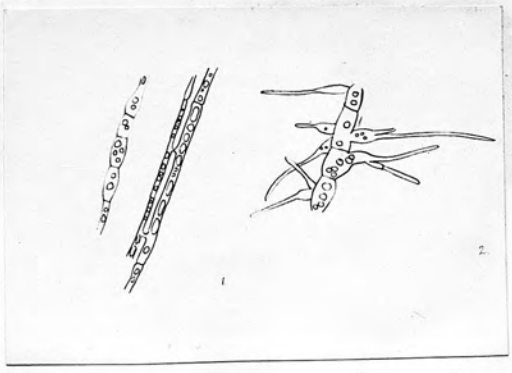

Fig. 4.

PLATE II.

Fig. 1. The fungus from an eight day old culture on rice. At d. are seen various swellings in the hyphae. At b. crystals are seen on the surface of the hyphae. x c. 280.

Fig. 2. Camera lucida drawing of conidia from various media. At a. from potato cylinders (sporodochial growth type). 14 day old culture. Note the abnormal type of conidia on the right hand side. At b. from Alnus stems, 20 days. At c. from potato cylinders, (mycelial growth type). Observe the irregularity in size and the swelling on a conidium. These features seem to be associated with conidial formation on a medium providing poor nourishment. At d. conidia from a 12 days' old oat agar culture. Note their size. At e. from 2% dextrose potatoagar. All are x c. 280.

PLATE. II.

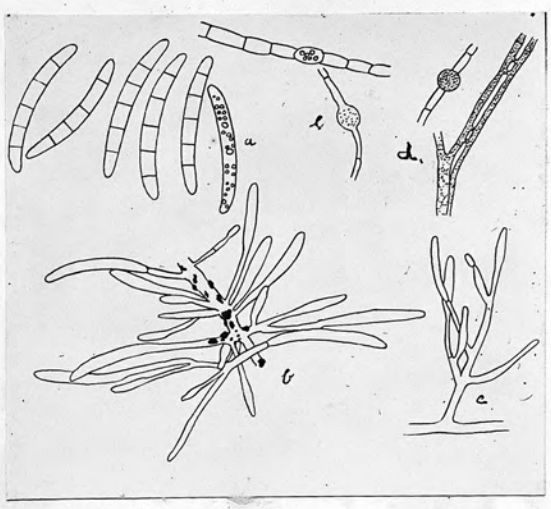

Fig. 1.

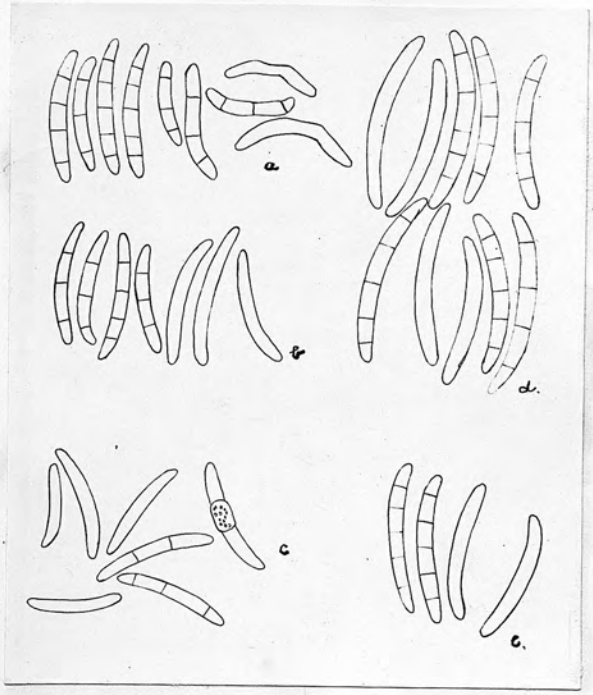

Fig. 2.

The Parasitism of Nectria cinnabarina (Tode) Fries.

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I. INTRODUCTION.

Nectria cinnabarina (Tode) Fries, growing on dead twigs, branches, etc., has for long been observed, and recognised on such substrata as a saprophyte. Its occurrence on various parts of growing shrubs and trees, however, has lead to much investigation and discussion as to its exact mode of action on such plants.

It is fairly well agreed that the fungus in question is a facultative parasite, and that it can, by its action, either direct or indirect, bring about the death of living parts of many plants.

The manner of entry into a living plant is through some dead portion attached to a living part, which latter is subsequently invaded, or through a wound caused by various agencies, both natural and artificial. Wehmer (17) considered it possible that attack might take place through cracks in the leaf scars, as Wiltshire (19) has since found as a means of entry for the Apple Canker fungus Nectria galligena Bres. The results of observations and investigations of various authors would make it apparent that two points in its parasitism, however, seem to vary - firstly - is there a variation in the particular host tissue attacked, and if so, why? Secondly - what are the reactions of the host plant to the fungal invasions? Is there any callus formation?

Can/

Can Nectria cinnabarina be responsible for a canker formation in its host?

Mayer (12) observed infection take place through wounded and dead portions, and also obtained positive results by innoculating the wood on cut surfaces of Acer, Alnus, Aesculus, Robinia and Ulmus. He observed the fungus travel primarily in the wood, causing a discoloration there, and attributed death of the cortex cells to lack of water, due to a stoppage of the vessels below by fungal hyphae. No canker formation was,seen. Brick (2) made practically the same observations. Mangin (11) observed the fungus in the wood, and at the same time noticed the formation of gum tyloses in Tilia, Castanea and Acer. Hartig (7) stressed the importance of frost killed areas as a means of entry, and pointed out the parasitic incidence of the fungus on Acer, Tilia and Aesculus. The fungus, according to him, could not directly injure the living cambium and the cortex, and could only invade them when these tissues were killed by lack of water or by frost. Tubeuf (16) pointed out the necessity of wounds as a means of entry, the fungus being unable to penetrate living bark. The mycelium spread through the wood, the cambium and cortex not being attacked directly, but killed in consequence of the destruction of the wood. Line (10) found that the normal method of the fungus attack on red currant was by spreading through the wood cells from a dead portion into the healthy/

healthy wood, the blocking of the wood cells thus by the fungal hyphae causing a wilt and death of all parts above the point affected, which were then invaded. In the leaflet of the Ministry of Agriculture (13) dealing with this fungus, an entry is said to be obtained into living tissue through adhering dead twigs, or more rarely by means of wounds. The mycelium is at first confined to woody tissues and the death of the cortex above the invaded wood is due to a wilt induced by the consequent stoppage of water. No callus formation is said to take place in Coral Spot disease.

The observations of these authors would make it apparent that the wood was the particular tissue attacked, that canker formation by attacked hosts was absent, and that death was really due to the improper functioning of the wood following on an attack by the fungus.

Other investigations have, however, revealed the parasitic action of the fungus in a different light.

Firstly Wehmer (17) in 1849 in Germany, asserted that the area of attack was the cortex, that the fungus travelled in the region of the cambium, that no canker was formed, and that the mycelium did not invade the wood. The greatest advance of the fungus in the host was made during the resting period of the latter. These observations were made on Tilia, Ulmus, and Acer platanoides. He carried out innoculation experiments and got the same symptoms as he had observed in trees attacked/

attacked in nature. Later, in 1895, he observed the same symptoms in Carpinus and Juglans regia. It is interesting to note that he only found the perithecial stage of the fungus on Carpinus. On the other hosts, only the imperfect stage, viz. Tubercularia vulgaris (Tode) Fries, was present. He does not seem to hesitate, however, in concluding that the latter belonged to Nectria cinnabarina. Behrens (1) found Tubercularia vulgaris causing a disease of the young shoots of Abies balsamea. The bark of the killed shoots was sunk, and the fungus stromata appeared on the dead tissue. At the line separating the diseased from the healthy tissue there was a decided swelling. This was due to continued formation of irregular cambia by the cortical cells at that point. The fungus mycelium was seen to travel in the cortex. Behrens called attention to the similarity in symptoms seen in this case, and in the case of those seen by Wehmer in Tilia (q.v.). He mentioned how his fungus resembled Tubercularia vulgaris morphologically, but must differ biologically from the Nectria cinnabarina of Mayer, for, while his (Behrens) fungus travelled in the cortex, Mayer's travelled in the wood.

In America Duggar (6) describes the fungus as affecting principally the cambium and the soft bast, probably killing the affected twigs so soon as they are girdled. Schrenk and Spaulding (15), working on diseases of various deciduous forest trees, observed the attack of Nectria cinnabarina take place through/

through wounds and the wood was invaded. The stimulus of the presence of the fungus mycelium there, accelerated callus formation at the edge of the wood. The callus of the first year was invaded by the fungus and killed, and a second layer developed, which in turn perhaps suffered attack, and so the process was repeated for a number of years, thus endangering the life of the affected tree. Rankin (14) describes Nectria cinnabarina as a cause of a canker especially on severely wounded or recently pruned trees. In the Maple (Acer platanoides), the twigs and small branches were killed by the girdling action of the fungus. A canker was formed. The mycelium grew most luxuriantly in the sapwood, and the bark adjacent to that affected tissue died, the mycelium then invading the bark. Cook (5) found the Tubercularia stage of a Nectria (presumably Nectria cinnabarina) which was parasitic on Norway Maples in New Jersey. The fungus attacked through wounds, caused by twigs breaking off. The stems were girdled by a canker and the above parts wilted and died.

Cunningham (22) records his observations on the Coral Spot Fungus in New Zealand thus:- "Apparently the fungus cannot attack living bark or cambium, these tissues dying in consequence of the destruction of the sapwood lying immediately beneath. Where entry has been effected in a large branch, it may be several years before the death of that branch occurs/

occurs. In such a case a canker is formed, which gradually enlarges year after year until the branch becomes girdled when it dies." These observations are interesting, since they reveal the capability of the fungus to call forth a variability in host disease symptoms according to the age of the branch attacked. The writer does not mention if the mycelium of the fungus in the second case penetrates the living bark where the canker is formed.

These authors, for the most part, seem to find the fungus capable of travelling in the living cortex cells, and calling forth a formation of cork cambia in the latter.

In view of the conflicting reports of these investigators the following case is of interest.

II. APRICOT CANKER.

A case of Tubercularia vulgaris (Tode) Fries occurring on apricot, and causing a canker thereon, was observed in Midlothian in January of this year. The tree was in a garden situated in a hollow surrounded by trees. Late frosts were common, and the atmosphere was apt to be more moist than that usually obtaining in gardens. The tree affected was of the fan type, trained up a high brick wall with a south-west exposure.

(a) Symptoms.

The portion affected was a branch of three years growth which, for a distance of about three inches on either side of a dead short side shoot, showed a sunken flattish area extending laterally about half the circumference of the branch on the side next the wall (Plate I. Fig. 1). On either side of the sunk portion were swellings which had grown so much as to burst the overlying bark at the junction of the raised and sunken area. The bark on the latter portion was dead, and bore numerous salmon pink coloured pustules of a Tubercularia, which on examination and comparison proved to be morphologically similar with the conidial stage of a specimen of Nectria cinnabarina whose perfect stage was found on conidial stromata on a dead branch of beech. The pustules on the apricot appeared through the dead bark mostly at the lenticels. On examination, no/

no other important fungus could be found on the bark. On cutting the wood near the side shoot above mentioned and examining with a hand lens, it was observed that the woody cylinder was quite brown in one half (that near the side shoot), while the remaining portion was normal (Plate I.Fig.2 at a.). That pith which still remained as a little ring in the centre, showed a browning of its cells in the area touching on the discoloured wood, while its centre remained a greenish white. The part of the woody cylinder highly discoloured, showed two complete annual rings, while the cortex overlying this was quite dead. The cambium touching on that wood which was considered normal, had given rise in the spring of 1926 to spring,but no autumn wood (Plate I.Fig. 2 at A). Its activity must have ceased about half way during the growing season of 1926. There was, moreover, in the area of the cambium and inner cortex overlying this imcomplete annual ring, a decided brown line, which at one part had extended to the epidermis. The cambium had been killed during the summer. In the spring of 1926, at the junction of the yet living and the dead cambium, there was the appearance of a decided callus which was starting to grow over the discoloured wood, but had not progressed very far before its activity had been stopped, and a part of the cortex, originally protected by this callus and overlying apparently sound wood, had been killed, so that only a small portion of normal looking cortex remained (Plate I.Fig.2 at A,i). Even in the/

the latter irregular cork cambia were being formed (Plate I.Fig. 2 at A,ii).

About three inches further down the stem, a cross section showed the area of discoloured wood had decreased to about one-quarter of the woody cylinder, and was a V shape, the arms almost touching at the pith. The bark above the discoloured wood was dead (Plate I. Fig.2,B.). At the point of contact of the dead bark above the brown wood and the cortex above the normal looking wood, there was a callus formation just starting, but it was not so pronounced as the previous one. In the area of cortex here, not yet dead, numerous cambia were formed (Plate I.Fig.2, B,i.).

A cut surface, more remote from the point of origin of the trouble, showed the normal result of a three years growth, with three complete rings of spring and autumn wood, and a sound cortex overlying these (Plate I.Fig. 2, C.). Cuts made in the direction going up the branch showed almost precisely similar symptoms, except that in the cortex not yet fully killed, could be seen an exceptionally large number of cork cambia of various shapes, and running in several directions, yet mostly in the mid cortex and not reaching to the normal cambia.

(b) Morbid Anatomy.

For the detection of fungus hyphae in the plant tissues, cotton blue in lactic acid was used. Transverse/

Transverse, radial and tangential longitudinal sections of the brown discoloured wood revealed the following:- At the outer strip of the gummed wood, the vessels and wood fibres were filled with a gum like substance, which gave the reactions for wound gum. The vessels and fibres of the outside edge of this strip, although filled with gum, showed no hyphae in their lumina, but in from this edge, fungus hyphae appeared, and a little further in were plentiful in vessels and wood fibres with a scanty gum content (Plate VII.Fig. B.). These hyphae varied greatly in diameter, varying from large, stout Rhizopus-like hyphae to finer Verticillium-like threads of fungus filaments. These hyphae of varying breadth were found to be connected, however, by hyphae of intermediate breadths. The thin hyphae represented younger growths and were sparingly septate. The thicker hyphae seemed to be proportionately more heavily septate, and their cells tended to become swollen, more especially at their septae. The hyphae resembled those pictured by various authors for Nectria cinnabarina. It was noticeable in this area of heavy gumming of the vessels and wood fibres, that the medullary rays and xylem parenchyma cells, especially those touching on the gummed elements,were almost devoid of contents, except for a few spherical globules of gum-like substances scattered irregularly in their cells. A further stage, represented by the area lying within the heavily gummed edges, showed the vessels and fibres losing/

losing their gum contents until they were practically empty. The hyphae were present in abundance here, and moreover, had now penetrated the xylem parenchyma and medullary ray cells, which latter cells still contained a few spherical globules. In that part of the wood just outside the area of heavily gummed vessels, sections treated with iodine showed that the starch grains, although plentiful in the medullary ray cells, were not stained the usual blue, but remained a lemon yellow colour. It would appear that the grains were undergoing some decomposition which resolved them into a liquid state: in short, it would appear as if the starch grains in these ray cells were decomposed to form the gum. A little further away from the gummed wood, the grains in the ray and xylem parenchyma cells stained the normal deep blue with iodine. This appears to resemble the sequence of events in, and method of formation of wound gum observed by Brooks and Moore (4) in their examination of the production of wound gum in plum trees. No hyphae were seen in the wood outside the gummed area, yet gumming did not seem to stop the progress of the fungus entirely in the wood, despite the fact that the gum seemed to be produced just ahead of the advancing hyphae.

The first appearance of an abnormality in the cortex was in those parts which superficially appeared sound, but where, on examining sections, the walls of the normally white phloem fibres were seen/

seen to be turning into a honey yellow. The cells of the latter then came apart and were ultimately disintegrated. The cortex cells surrounding these areas became meristematic and formed, round the groups of bast fibres, circles composed of numerous layers of brick shaped cells (Plate II. Fig. 1). Since the fibres were disposed in larger or smaller groups at random in the central region of the cortex, the appearance of these circles was of widespread occurrence, when the fibres in the cortex were generally affected (Plate III. Fig.1.). These symptoms were appearing in cortex which was obviously not, from its position, affected by gumming in the wood. Sections of the cortex nearer the source of the disease and showing signs of further progress of the disease, showed the above circular cambia to have run together (Plate III.Fig.1.). Now also, there was further production of wound cork cells on the inner and outer sides of the phloem fibre belt in the cortex, and these cork tissues, so formed, ran parallel to the epidermis (Plate II.Fig.2). It was fairly evident that these secondary protective tissues were being produced to stem the advance of destruction from the region of the phloem fibres. They were, however, being destroyed in places, and destruction had been carried to the cells in the outer cortex, and to the side of the fibres next the wood. In the former, cells containing chloroplasts with chlorophyll present had the latter turned brown.

In/

In the inner cortex the cells bordering on the medullary rays were first destroyed and crystals of calcium oxalate, present there, were turned brown. Cork cambia produced in the cortex often cut off externally entirely healthy pieces of cortex, which suggests that attack in these cases was proceeding from inside. In fact, many cases were seen where a piece of outer cortex containing healthy chloroplast-containing cells was markedly cut off from a diseased adjacent area, where these chloroplast cells were dead. The contrast was striking, and would go to prove the temporary efficacy of such a protective layer. The callus at the side of the cortex killed during the dormant period of 1925-26, was composed of several layers of cells produced by a cork cambium stretching across the cortex from the exposed normal cambium to the epidermis (Plate III.Fig. 2.). Cells on its surface were protruding large, swollen, bladder-like outgrowths like intumescences. This was probably associated with rapid growth in a tissue without any restraining influence like a thickened epidermis. This callus did not appear to function as a protective tissue, since the disease symptoms were appearing behind it, in an area supposedly protected by it (Plate III. Fig. 2 at A.).

Despite this very evident destruction in the cortex, and the great reaction of the host in forming numerous cork cambia there, no fungus hyphae could be seen in or between these cells, when they were/

were first being destroyed. In fact, not for a considerable time did any hyphae appear, and these certainly appeared in an area whose cells were previously killed.

What then accounted for their death? As a working hypothesis, it might be suggested that the wood was first attacked, gummosis ensuing as a response by the living xylem ray and xylem parenchyma cells to the stimulus of a substance secreted by the fungus hyphae. Now, the cortex cells, overlying the gummed wood, may have been attacked directly by this fungal secretion and so killed, or they may have died as the result of dessication, following on a gummosis of the underlying wood. It is significant that there was no appearance of cork cambia in the dead cortex in the region of first attack. This theory might account for the large area of dead cortex seen near the dead side stub, and in which fungus hyphae were present, but it does not account for the appearance of irregular cambia and the layers of cells formed in cortex which overlies apparently sound wood. It would appear as if the symptoms of host reaction in these areas of cortex, was due to a stimulus or stimuli, which were travelling in the cortex, primarly through the phloem fibres. The yellow appearance of the latter as a primary sign in cortex tissue destruction, and the formation of circular cell layers produced round their groups by secondary cambia would suggest this. The toxic secretion appeared/

appeared to diffuse out from the phloem fibres and destroy the immediately adjacent cortex cells. The browning of healthy chloroplasts in the outer cortex cells would suggest that these latter were being killed. Since no hyphae were apparent in those cells when they were being destroyed, and since cambia were forming cork cells in an effort to avoid an extension of their destruction, and moreover when it does not appear, for various reasons, that these cells are dying as a result (?) of dessication, then the only feasible explanation left is that they are being directly killed by some toxic substance. This latter is in all probability produced direct by the fungus, or it may be some substance resulting from the diseased conditions of the wood or cortex. The callus produced at the edge of the dead cortex was the normal act of a living cambium closing over the wound made in 1925-26. How is the wood in the stem below the entrance point of the fungus affected? Brooks and Storey (3), in their work on Silver Leaf, mention how, in innoculation experiments, the influence of the fungus secretion (here Stereum purpureum) is felt below the discoloured wood as well as above it, either due to static diffusion or downward flow of a toxic secretary substance.

III. THE FUNGUS.

Since the Tubercularia stromata on the dead bark did not contain any perithecia, one could not positively assert that they represented the conidial stage of Nectria cinnabarina (Tode) Fries. Nectria cinnabarina perithecia were found arising on Tubercularia pustules on a dead branch of beech. The conidiophores and conidia from perithecial-containing stromata agreed in morphological detail with those obtained from Tubercularia stromata on the apricot (see Table I).

TABLE I.

Spore measurements of the fungi on the Apricot and the Beech (100 spores measured).

Beech		Apricot
Ascospore	Conidia	Conidia
17.8μ(16.3μ-18.8μ)x 6.0μ(5.2μ-7.2μ).	7.4μ(6.5μ-7.9μ)x 2.6μ(2.5μ-2.8μ).	7.5μ(6.3μ-8.1μ)x 2.8μ(2.5μ-3.2μ).

The measurements given by Wollenweber (21) for Nectria cinnabarina (T) Fr on Sambucus are:- ascospores, 16.6μ x 5.2μ, while for the conidia 7.3μ x 2.6μ. Wollenweber gives two varieties of the Coral Spot Fungus, but both of these have smaller ascospores and conidia than the Nectria cinnabarina on Sambucus given above, and do not concern us here. It is here concluded that the fungus on the beech is/

is Nectria cinnabarina (Tode) Fries, and that the Tubercularia stage on the apricot is the conidial stage of the same fungus.

IV. SCOPE of the EXPERIMENTAL INVESTIGATION.

It would appear that we are dealing with a fungus attacking the apricot and causing a canker there, which morphologically in its non-perithecial stage agrees with Nectria cinnabarina (Tode) Fries. Since no other fungus of importance was found on the diseased apricot branch, and at the same time considering the facts accruing from investigation, it would appear that Nectria cinnabarina was here the active agent in calling forth the symptoms observed. It is also very probable that the fungus, as the result of the secretion of its hyphae, is here capable of killing living cells, and of calling forth a gummosis in the wood and the formation of cambia in the cortex. According to the literature, the latter symptom is evidently not common in plants attacked by this fungus, and indeed this method of host reaction does not appear to have been observed in this country. It is difficult to account for this variability in the nature of the action of the parasite and the reaction of the host. At the same time the variation in the particular tissue primarly attacked is peculiar. The manner of host reaction to an attack of this fungus and the particular host tissue primarly attacked may vary in the same host species, e.g. Acer platanoids (see Wehmer (17), Mayer (12), and Rankin (14)), and one looks to three possible causes for this variation - (1) the host, (2) the fungus/

fungus and (3) the environment.

As regards the host plant, it is not probable (other things being equal) that the nature of the same species of host plant varies in a country, Under equal environmental conditions one expects a definite host to react in the same manner to the attack of members of a morphological fungus species physiologically alike. At the same time, however, the results of Cunningham's findings with the Coral Spot Fungus (q.v.) cannot be ignored. The age of the host part attacked may influence the progress of the fungus and the resultant symptoms.

The environment may influence the course of a disease in so far as it influences the condition of the host plant, and consequently, its relative susceptibility to fungal attacks and disease.

As regards the fungus, in fungi, the existence of special physiological forms within the morphological species is well known, and it is quite possible that here, in Nectria cinnabarina, we have an example of such. The variability in symptoms produced by an attack of Nectria cinnabarina may be due to the existence of physiological forms within that species. Westerdijk and van Luijk (18) in their work on Nectria galligena Bres. and Nectria coccinea Pers. conclude that there may be for each species morphological and physiological races. Lind (9) suggests that, since the habitus of Nectria cinnabarina differs according

to/

to the host plants it frequents, then it should be investigated if there are not biological forms within the species.

With a view to trying to throw some light on the cause or causes of this variation seen in the host symptoms, two strains of the fungus were obtained, viz. from beech and from apricot. These were at first compared culturally, under equal conditions of environment, in order to see if there existed any physiological difference in these strains. To follow out this possibility further, experiments were made by inoculating strains derived from these two sources on to a similar host, viz. the apricot. Two plants of the same variety of apricot were used and placed in conditions, where it was thought they were subjected to as similar an environment as was conveniently possible. In this manner, the influence of the nature of the host and the environment were eliminated as factors influencing any variation in symptoms of disease, if present, and the latter could only be accounted for by a variation in the nature of the fungus strains used, or as a result of the varying age of the shoot or branch innoculated. Observations were principally made on, the tissue of the host primarly attacked, the method of the attack adopted by the fungus, the rate of fungus progress in the host and lastly, the reaction of the host to attack.

(a) Cultural.

Single ascospore cultures were made from dehiscing/

dehiscing perithecia of Nectria cinnabarina on the beech, and single conidial isolations from the conidial pustules, while at the same time mono-conidial cultures were made from the pustules on the apricot. Various pieces of diseased tissue were taken from the apricot, viz. pieces of gummed wood, pieces of wood containing no gummed portion, pieces containing parts of diseased cortex where the disease was first becoming apparent, and lastly, pieces including cortex which had been killed for a while. These were cut out with the usual aseptic precautions, flamed lightly, and planted on bean agar. In about two days mycelium appeared from pieces containing gummed wood and those including cortex which had been dead for a considerable time. Bacteria were fairly common in the latter, probably acting saprophytically on the dead tissue. No mycelium appeared in pieces of non-gummed wood, nor in pieces with the cortex just going off. In the latter in one case, little bacterial colonies appeared in the region of the cambium, but these were probably acting saprophytically on cells already killed there. They probably travelled up the cortex in the wake of cell destruction.

A series of parallel cultures of the fungi from the two sources of the apricot and the two types of spore on the beech were made, and their cultural characteristics compared. Since there appeared to be no difference between the cultures derived from ascospores and conidia on the beech, they were no longer/

longer compared but considered alike in their cultural characteristics. Likewise with the cultures from the mycelium in the wood and the conidia from the pustules on the apricot.

The growth characteristics of the two strains of fungi on different media will not be described in detail, as it would only lead to much irrelevant information. A type of growth such as occurs on oat agar will be taken as an example of the normal growth. Growth here is characterised by the appearance of a scanty, loose, white aerial mycelium. Sooner or later white pustular growths appear on the surface of the medium (Plate IV. Fig. 1.). These growths are sterile, permanently, or till a time when salmon pink spots appear in them and gradually occupy all their surface area. These pink growths are really due to masses of conidia which are borne on typical Tubercularia-like conidiophores which abstrict conidia of a size and type similar to those of Tubercularia vulgaris. These pink cushions are not so uniform in shape and size as they are on the natural substratum. Irregularly sized conidia were found on the aerial hyphae, but macroconidia were at no time found. Many devices were tried to encourage perithecial production in culture, but these were unsuccessful.

Certain of the above outstanding cultural features of the two strains varied on the different media employed, and the summary of these is put forward in/

in tabular form below (Table II). In each case twelve tubes of each medium were used, and innoculated with the fungi from both sources (six tubes of each). All tubes were incubated at room temperature and in the ordinary alternating light of night and day, The cultures were periodically inspected and their growth characteristics noted, (see Table II page 158).

As a result of this experiment it was possible to distinguish the two strains culturally. While the apricot strain produced a very definite aerial mycelium in all media, this feature was absent in the beech strain (Plates IV. and V. all Figs.). The apricot fungus, moreover, produced sterile pustules considerably earlier than the beech strain, and developed in many instances Tubercularia pustules when the beech strain failed to do so, or only after a long period of growth on the cultural medium (Plate IV. Fig. 1.). These features were not sporadic in any of the six cultures of each strain on each medium (except Tubercularia pustule production on oat agar), but all six tubes of any medium innoculated with one strain showed a very good agreement. As a result of this, one can look upon the features present in them as constant and characteristic and they can well be used for comparison. The two strains, therefore, are definitely separable on a cultural basis.

The similarity in conidial size of the two strains on similar media is very interesting, and/

Table II.

A COMPARISON of the CULTURAL FEATURES of the TWO STRAINS of FUNGI from BEECH and APRICOT.

	Oat Agar		Rice.		Maize.		Alnus.		1% glycerine potato.		1% glycerine carrot.	
	Beech	Apricot	Beech	Apricot	Beech	Apricot	Beech	Apricot	Beech	Apricot	Beech	Apricot
Aerial Mycelium.												
(i). presence or absence.	—	+	—	+	—	+	—	+	—	+	—	+
(ii). possession of conidia or no.	—	+	—	—	—	—	—	+	—	—	—	—
(iii). colour.	—	rosy white.	—	reddish salmon.	—	white.	—	yellowish salmon.	—	light apricot.	—	maize yellow.
Pustules. (sterile).												
(i). Presence or absence.	+	+	+	+	+	+	+	+	+	+	+	+
(ii). Size. (relative.).	X.	·8X.	·7X.	·6X.	·5X.	1·5X.	·5X.	X.	1·5X.	1·5X	1·3X.	1·4X.
(iii). Relative number developed.	60	64	60	30	80	90	20.	10.	100	60	90	80.
(iv). Colour.	Rosy white	Deep rosy white.	Light salmon.	Yellowish salmon.	Light salmon.	white.	Purplish tinted white.	Yellowish salmon	Pale rosy flesh	Putty-chamois.	yellowish flesh — light red.	putty colour.
(v.) Rate of appearance (days).	36	14	40	40	60	42	42	40	36	14	30	12.
Tubercularia Stage.												
(i.). Presence or absence.	+	+	—	+	—	+	+	+	+	+		
(ii.) Relative number developed.	4	40	—	80	—	100	4	60	18	50		
(iii.). Rate of appearance. (days)	90	20	—	150	—	90	90	60	80	36		
(iv.). Size of spores.	7·5μ × 2·7μ	7·4μ × 3·2μ	—	7·1μ × 2·7μ	—	7·5μ × 2·6μ	7·4μ × 3·1μ	7·6μ × 3·0μ	6·8μ × 2·8μ	6·7μ × 2·6μ		

and shows that in this very important feature they are alike. We here have, therefore, a case of morphologically similar imperfect stages (Tubercularia vulgaris) whose members can differ physiologically, if one may judge by their constantly different behaviour on the several media used in the experiment.

(b) Innoculation Experiments.

The parasitism of these two strains was now tested.

For this purpose, two five-year old apricot trees were used as hosts. These were planted in large pots and placed in a greehouse. Two series of innoculations were carried out (1) when the trees were in the dormant state and (2) when in full leaf. Innoculations were made with pieces of culture medium containing the fungi concerned, or by means of conidia from pustules in culture. These were inserted into tangential cuts to the cortex or to the wood of living shoots of various ages, or onto dead side shoots. These innoculations werecarried out with all aseptic precautions. Control cuts were made like those of the innoculated parts, but no fungus was inserted. In all cases the cuts were moistened with sterilised distilled water, wrapped round with raffia, and then enclosed in cotton wool and labelled. The results of these innoculations are put forward in Table III.

(see next page).

Results of inoculating two Strains of Nectria cinnabarina (Tode) fries. on to Apricot.

Strain.	Date of inoculation.	Number of inocula.	Nature of the inoculum.	Inoculum: where inserted.	Result.	Remarks.
Apricot.	16/2/27	6	mycelium on bean agar.	1. dead stub.	+	Penetrated into sound wood, but did not progress far. See Plate IX. Fig 2.
				1. cut to wood.	—	Fungus occluded by a gum barrier, and callus formed over the inoculation cut.
				1. dead stub.	+	Only a little way advanced into sound wood.
				1. cut into cortex only	—	
	18/5/27	3		1. cut to wood	+	Canker formed here. See. Plate. XI. Fig. 1.
				1. cut to wood	—	Fungus occluded by a gum barrier.
			conidia and mycelium on oat agar.	1. cut to wood	—	All occluded by a gum barrier, and wound in every case healed over by a callus. Plate XIII. Fig. 1.
				1. do do	—	
				1. do do	—	
Beech.	16/2/27	6	mycelium on bean agar.	1. dead stub.	+	Fungus penetrated into sound wood, further than Apricot strain in similar circumstances. See Plate IX Fig. 1.
				∓		
				1. cut to cortex only	—	
	18/5/27	3		1. cut to wood	+	All caused a gumming in the wood and a "Die-back" of the inoculated shoot. See Plate VI.
				1. do. do.	+	
				1 do. do.	+	
			conidia and mycelium on oat agar.	1. cut to wood	—	Fungus occluded by a gum barrier
				1 do do	+	A gumming of the wood and a "Die-back" of the inoculated shoots. Plate. X.
				1 do do	+	
					—	Fungus occluded & wound healed over. Plate XIII. Fig 2.
Control	16/2/27	4.	——	3 cuts to wood.	—	Wounds healed over by a callus. See. Plate XIII. Fig 3
				1 cut into a dead stub	—	
	18/5/27	4.	——	4 cuts to wood	—	do. do.

The results would tend to show that for both strains of the fungus, inoculations made during the dormant period of the host were readier in taking than those made in the period of vegetative activity. This is probably accounted for by a more rapid host reaction by gum production in summer than in winter. The tree is likely to react more quickly to a stimulus when it is in an active state of growth, than when it is in a passive condition during its dormant period. It may be also, that the quality of the wound gum is different at the two innoculation periods, and that the summer product was more efficient as a barrier than the winter. No positive proof for this opinion was, however, obtained.

In the case of the beech strain of the fungus, all the inoculations made in the dormant period gave positive results, with the exception of that one made into cortical tissue. The latter healed up rapidly. Those innoculations made in the summer with the same strain gave, in two cases, positive results, while in the third case the fungus was successfully occluded by gum and the cut was healed over by a callus.

In all cases of positive results derived from an innoculation into sound wood in a branch, the progress and symptoms of the disease were essentially alike, and one case may be taken as typical and a detailed account given. Plate VI, Fig. 3. shows such an example. The first external symptoms observed was/

was the collapse of the leaves at the tips of those shoots arising from the inoculated branch and above the point of innoculation of the latter. The leaves even in this collapsed state were quite green, and the petioles were no longer rigid but arched over. The leaves suggested those of a plant which had wilted severely. Ultimately a yellowing appeared between the veins of the leaves, and extended until the leaves yellowed completely, shrivelled and finally fell off. The tips of the shoots began to darken and shrivel, and the unopened buds on them shrivelled and fell off. This shrivelling of the shoots extended back to the inoculation point. In the case where the fungus attack had taken effect before the buds opened, the latter failed to produce shoots, but shrivelled up and died (Plate VI. Fig. 1.). For a short distance below the innoculation point the bark was seen to be depressed, and was separated distinctly from the normal bark below it (Plate VI. Fig. 2 at A.). This depression was due to the drying of the bark, and at its junction with the sound bark, there appeared to be a callus formed in the cortex, which was made evident superficially, by this ridge on the bark, but later examination showed that there were no cambia present there. The area of bark depressed in such a manner, gradually extended to a variable distance down the shoot. During its progress, if it embraced a side shoot, the leaves of the latter wilted and its bark shrivelled, just as was seen above (Plate VI. Fig. 1 at A.).

The/

The extent down the shoot to which the bark was dried up, varied considerably in the various shoots inoculated, as did also the rate of progress of this spread. In three cases it spread rapidly, and for a distance of about six inches, while in another it spread only about one inch and stopped. This stoppage of the downward spread of the dessication of the bark which always occurs sooner or later, and is (as later examination showed) associated with the extent of gumming of the underlying wood.

For the detection of fungus hyphae in the host tissues, pianeze IIIb[x] was found to be a good differential stain. On making an examination of the diseased cortex, no formation of cork cambia was seen in it, nor were any hyphae apparent in it, until it had been dead for a considerable time. In every case this diseased cortex overlay a brown patch of wood (Plate VII.Fig.B.).

The browning of the wood was due to an exudation of gum into its vessels from the living wood cells. The area of greatest gum accumulation in the vessels was always at the edges of this gummed wood. Within this, the vessels were not so heavily charged with gum, and indeed, in parts within the gummed area, but more remote from its edges, the amount of gum in the vessels was negligible. Fungus hyphae were absent from gum-free areas in the wood, but were present in abundance in that part of the gummed/

[x] For preparation and method of using this stain see Ann. Miss. Bot. Garden, Vol. I, p. 241, 1914.

gummed area of wood where gum was not so plentiful, or where it had disappeared almost entirely. No hyphae were seen in the heavily gummed vessels at the edges of the gummed area. The gum in the vessels, lying within these latter vesels, stained differently with the pianeze 111b, and appeared to be undergoing some change. A point worthy of observation was the extent of gumming in the wood, and its evident effect on the cortex. In a vertical section of a diseased shoot (Plate VII.Fig.B), the gumming in the wood was always observed to be ahead of discoloration in the cortex, and the latter appeared to become discoloured only when the wood immediately underlying it became gummed. It was quite apparent, therefore, that the depressed nature of the bark was due, in all probability, to the browning and collapse of the cortex cells as the result of the wood underlying the latter becoming gummed.

The following hypothesis for the progress of the disease and the fungus causing it, would appear to coincide with the observations. The presence of the fungus inserted into the wood, stimulated the living cells there to secrete a gum into the vessels, in all probability to act as a barrier to further fungal advance. This gum, so secreted, appears to have been changed in some way, perhaps through the action of the fungus secretions, so that it no longer inhibits an existence of the fungus/

fungus in it. Its nature appears to be so changed, that the fungus can now penetrate into, and live in it. New gum is, however, produced in the vessels bordering on those where the gum has just been rendered inefficient, and it in turn changes or is changed in nature, so as to admit of the fungus penetration. Thus we get a constant renewal of gum just beyond the line of the advancing hyphal tips, and a constant imparing of its efficiency. The gum is not, therefore, a true barrier, since it continues to recede before the advancing fungus. The extent of this retreat is variable, but in one instance measured nine inches. Brooks (4) and Hoggan (8), in their studies on the parasitism of Stereum purpureum and Plowrightia ribesia respectively, found that this gum or resinous barrier definitely stopped the progress of the fungus, and in neither case did it retreat,as seen here. It is difficult to understand why this retreat should cease, and the barrier finally function as such. It may be due to a weakening of the fungus hyphae, due to a feeble or improper nourishment in the wood, or to an increase in the resistant properties of the gum to some fungus secretion,which normally enfeebles its host-protective properties. It does not appear to depend on the varying food material offered to the fungus in the wood at the different periods in the year, for this retreat occurred equally in winter and in summer. Whatever may be the cause of the retreat/

retreat of the gum, it sooner or later functions as a barrier and the fungus progress is stopped.

It is quite apparent that, once the cross sectional area of the wood is completely gummed, then further water conduction to those parts above is impossible, and this accounts for the sudden collapse in the leaves in the shoots above, and the drying and shrivelling of the shoots and dropping of their buds. Below the inoculation point the cortex dries and dies in all probability as a result of dessication, due to the vessels of the underlying wood being blocked, and no longer being able to conduct water. At the same time one cannot divorce from one's mind, as the cause of death of these cortex cells, the possibility of a toxic secretion from the wood, either produced direct from the fungus, or as the result of some interaction in the gummed wood between the fungus and the wood. The manner in which the cortex destruction lags behind the gumming of the wood, would suggest, however, a drying of the former as the cause of its death.

The wilting and death of side shoots below the inoculation point resulted from the gumming in the wood spreading in the inoculated shoot , below the point of origin of these side shoots from it. Their water supply was cut off as a consequence (Plate IX.Fig. 1 at A).

It is of interest to note that in the inoculated shoot where the fungus did not prosper, but was occluded by gum, there was a production of the latter/

latter in the vessels for a distance of about five inches above the inoculation point and about two inches below it (Plate XIII.Fig. 2). No hyphae were seen in these streaks of gum, and it is suggested that the latter were produced in the host wood cells as the result of the stimulus of a secretion of the fungus hyphae at the inoculation point carried up the vessels in the transpiration current, and downwards by static diffusion.

As regards those inoculations of the beech strain of the fungus into splits made in dead side stubs; in both cases the hyphae had called forth a gumming on reaching sound wood (Plate IX.Fig. 1.). The gumming extended into the shoots from which the stubs arose, and in one instance had proceeded so far as to cause the wilting and death of a large side shoot which originated from, and was supplied with water via the area of wood blocked by this gum.

In older shoots, the progress of the fungus down the wood was slower, but no cork cambia were seen to be formed in the overlying cortex. The latter dried off in the usual manner, behind the advancing gumming in the wood.

As regards the inoculations with the apricot strain, it is seen that none of the three inoculations made during the summer succeeded. In every case gum was formed by the host, encircling the inoculum and apparently preventing further spread of the fungus. In all three cases a callus was closing over/

over the wounds made by the cuts (Plate XIII.Fig.2 at A.).

An inoculum placed in the cortex failed to cause disease, for the latter soon healed up.

Inocula placed, during the dormant period, into dead stubs, showed that the hyphae had progressed in the wood down those stubs and into the shoots from which the latter arose. It is of importance to note that, at the time of examination of the living shoots (nine months after inoculation), no cork cambia had appeared in the cortex overlying the gummed wood. The cortex had dried behind the advancing gum as in the previous observations, and, as in those, so here there was no formation of wound cambia in the cortex. One difference, however, was that, the extent of gumming in similar inoculations of the beech strain was about twice that of the apricot strain. It may be that the beech strain of the fungus is capable of travelling more rapidly in this host than the apricot strain.

During the dormant period, two inoculations with the apricot strain were inserted into cuts to sound wood. While one of these gave a negative result, the result of the other is of very great interest. This concerned an inoculation into the living wood near the foot of a cut side shoot (Plate XI. Fig. 1). By about June, distinct sunken areas were seen in the bark of the parent shoot around the base of the inoculated side shoot. This shoot was the main one of the tree, and was in its fifth year of growth. The sunken area increased during the months following, and/

and the line separating it from the contiguous normal bark became more pronounced, due to the latter swelling along the line of junction. Ultimately at one side a split appeared, and formed an elongated cavity , which gradually widened (Plate XI. Fig.1 at B.). A true canker was suspected here, and on examination it proved to be so.

The surface of a cut made across the middle of the sunken bark area, showed the wood on the side of the latter to be gummed (Plate XI. Fig.2.). The cortex overlying this was dead, and at its edges were distinct cork cambia, presumably attempting to exclude the fungus and heal over the area of dead cortex. These cambia were destroyed at several places, and new cambia had been formed behind them.

Fungal hyphae were detected in the dead cortex. Cuts were made at varied intervals in the bark in the downward direction of the stem, and showed the formation of circular cambia in the mid cortex just opposite the gumming in the wood. (Plate XII. Fig. B.). It appeared that the circular cambia were always produced round the phloem fibres, which latter no longer retained their normal colour, but turned a honey yellow, presumably due to a decomposition of the thick cellulose walls of these fibres. In spite of searching in numerous stained sections, both transverse and longitudinal, no fungus hyphae could be detected within these circular cambia, nor could any connection be traced between the latter and the gummed area/

area of wood lying in the woody cylinder opposite them. The gumming of the wood, in this case, extended about a quarter of an inch only beyond the extreme point of cork cambial formation in the cortex. (Plate XII.Fig. C.). A vertical section of this shoot made down to the extreme point of gumming in the wood shows that, in the cortex, several wound cambia are formed across the cortex, but appear to be insufficient, as the destruction in the cortex re-appears below them, even down to the lowest cambium. (i.e, the cambium formed furthest from the inoculation point).

The following hypothesis is put forward to account for the observed facts. The fungus, once established in the wood of the side shoots, commenced to travel downwards in the wood behind the usual receding gum barrier. It entered the wood at the main shoot, and by its secretions, which were either well ahead or just around its hyphae, killed the cortex cells in those areas of the latter which ultimately collapsed and appeared, from the outside, as sunken parts. The fungus continued to travel slowly down the shoot in the wood, just behind the constantly receding heavy gum barrier. In the meantime, secretions from the hyphae in the dead cortex were carried longitudinally in the cortex in the phloem fibres. Their presence stimulated the formation of cork cambia around these fibres, and so gave rise to the numerous layers of cork cells found. The/

The passage of the secretion through these fibres would account for the inability of the cork cambia, stretching across the cortex, to retard its advance, since these fibre cells are not occluded by cork cambia cells. It is evident that these transverse cambia do not function successfully, since they appear to be continually destroyed, and others are formed at lower levels to take their place. The final collapse and browning of the cortex cells may be due to dessication as the result of their being superimposed on gummed wood.

The symptoms resulting from this inoculation agree closely with those seen in the original specimen of apricot canker.

The fungus was reisolated from those shoots which had been inoculated in the dormant period with the beech strain of the fungus. The cultural characteristics of the isolated fungi agreed with those already seen for the original beech strains. Time did not permit of isolations of the apricot strain, but one can be all but positive that the symptoms observed, in those parts inoculated with it, were due to its presence.

V. CONCLUSIONS.

Owing to the comparatively few numbers of inoculations made, it would be impossible to make any conclusive statements on many of the points raised in the parasitism of this fungus. It is, however, possible to be definite on some things, and to suggest possibilities in others.

It would appear that, in the fungus Nectria cinnabarina (Tode) Fries., there exists physiological races, whose difference in constitution is made evident by constant cultural differences.

The two strains experimented with here showed a difference in their parasitic virulence on the apricot, for while the beech strain created disease in its host in about 78% of its inolculations, and moreover, appeared to move quickly when once established, the apricot strain was successful in 33% of its inoculations only, and moreover, travelled more slowly in the wood.

The reappearance of a definite canker formation by the host as a result of the fungus presence, would show definitely that canker formation can occur as a result of the attack of Nectria cinnabarina. It would have required many more inoculations and positive results in order to conclude that the beech strain was essentially the cause of a Die-Back in the apricot, while the apricot strain/

strain was primarily a canker producing one, but it may be that this is so, and for the following reasons. A strain with a greater virulence, on establishing itself in the wood, would travel quickly there, and as a result, the overlying bark would be dessicated quickly, before it had time to react. In a slower progressing strain, however, the bark would not be killed so quickly, but the dessication would be more gradual, and so the cortex could react to any fungus secretion penetrating it, by forming cork cambia. In the former case, the fungus might be thought to be primarily the cause of disease in the wood; in the latter, it could be held to affect both wood and cortex equally.

It is of interest at this point to consider the results of Wollenweber's (20) inoculation experiments with the apple canker fungus, Nectria galligena, Bres. According to that author Nectria galligena from cankers on the apple, gave cankers in the year of inoculation on the apple. Nectria galligena from the beech also caused canker on the apple. Nectria galligena from cankers on the apple, however, goes on to the beech but does not form open cankers on that host but only slight sunken areas in the bark under which the wood is browned. The author has seen on specimens of beech open cankers which resulted from an attack of Nectria galligena. We have here, therefore, a case parallel to the case of/

of Nectria cinnabarina under discussion, viz - one and the same morphological species of fungus, Nectria galligena, Bres. , causing on the beech a canker in the one case, and not in the other. There is here again the suggestion of two strains. Westerdijk and van Luijk (18) consider that, in this fungus, Nectria galligena, Bres., and also Nectria coccinea, Pers., there may be physiological races. The original observation on the apricot canker and the results of the experimental investigation, both cultural and infection studies, leads the author to suspect the same for the species Nectria cinnabarina (Tode) Fries. This may possibly account for the difference in disease symptoms which appear to be produced by hosts attacked by it.

The beech strain of the fungus affects primarily the wood, and the cortex dies probably as the result of this. The apricot strain appears to move more slowly in the host, and may progress equally in the wood and the cortex, destroying living cells in the latter.

Wilting of parts above the points of inoculation would not appear to be of necessity due to a stoppage in the ascent of sap, as the result of the choking of the host vessels by fungus hyphae, but rather to a stoppage through accumulation of gum in the vessels.

As a result of the foregoing observations and experiments, it would appear that the nature of the parasitic/

parasitic action of Nectria cinnabarina is a variable feature, as the literature dealing with it would suggest. It would appear that several factors appear to contribute to this variability, and that it would be premature to generalise on the behaviour of this fungus in respect to its host. While definitely a wound parasite of apricot and causing a Die-Back on that host, it may at the same time be responsible for a canker formation there. While the experiment showed that this might be due to the existence of strains in the species, yet, the fact that Cunningham found the variability in symptoms to be due to the age of the host part affected, has not been disproved by the experiment.

The result of the foregoing investigations would appear to point out that more conclusive evidence could be brought to bear on the question of this symptom variability, by making more extensive inoculation experiments, keeping in mind the two probable possibilities for this variation, viz:- the presence of physiological strains in the fungus, and the age of the host part affected.

VI. SUMMARY.

The variation in the literature dealing with observations on the parasitism of Nectria cinnabarina (Tode) Fries. is reported in the Introduction.

These variations are concerned chiefly with two points in the parasitism of this species viz:- (a) the part of the host tissue primarily attacked, and (b) the reaction of the host to attack.

The case of this fungus causing a canker of apricot, and the various symptoms observed are dealt with.

With a view to trying to throw some light on the reason or reasons for this symptom variability in the host, cultural studies and inoculation experiments were carried out with two strains of the fungus (1) a strain from the beech, and (2) a strain from the apricot.

The two strains were found to be separable on a cultural basis, while it appeared from inoculation experiments, that the beech strain might be a more virulent parasite of the apricot than the apricot strain. The latter was shown to be the cause of a canker on the apricot.

It was concluded that, as seen in the Introduction, this fungus was capable of causing a variability of symptoms in its host plant. The investigation indicated that this latter might be due to/

to the existence of physiological strains in the species, or be the result of the varying ages of the host parts attacked.

A more extensive series of inoculation experiments than that carried out during this investigation, would probably decide which of these two factors was the causative one. Both, however, may be concerned.

VII. LITERATURE.

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VIII. PHOTOGRAPHS.

Illustrating the paper on the Parasitism of Nectria cinnabarina (Tode) Fries.

PLATE I.

Fig. 1. Branch of apricot from an orchard showing typical canker symptoms. Note the dense covering of the sunken area of bark with pustules of Tubercularia vulgaris. Infection probably took place at the dead side stub at A. x c. $\frac{1}{2}$ natural size.

Fig. 2. Cross cuts of the cankered apricot branch shown in Fig. 1. The cut seen at A. was made near the dead side stub. Note at ii. the formation of a circular cambium in the cortex. The cut seen at B. was made further away from the side stub and shows at i. the formation of numerous cork cambia in the cortex. At C., which shows a cut surface beyon the canker region, the wood and cortex are seen to be sound. Natural size.

PLATE I.

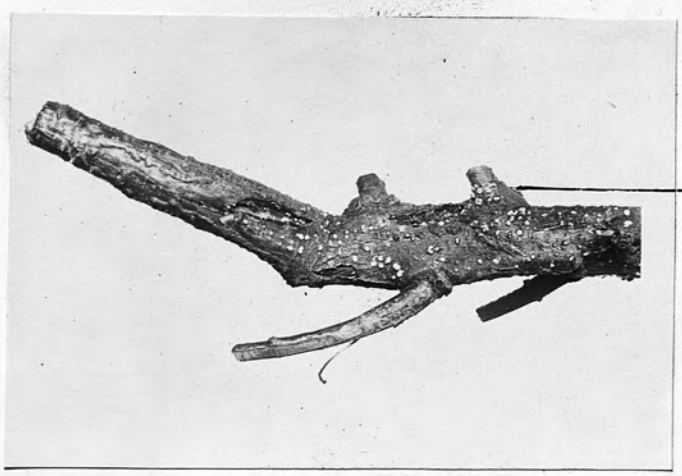

Fig. 1.

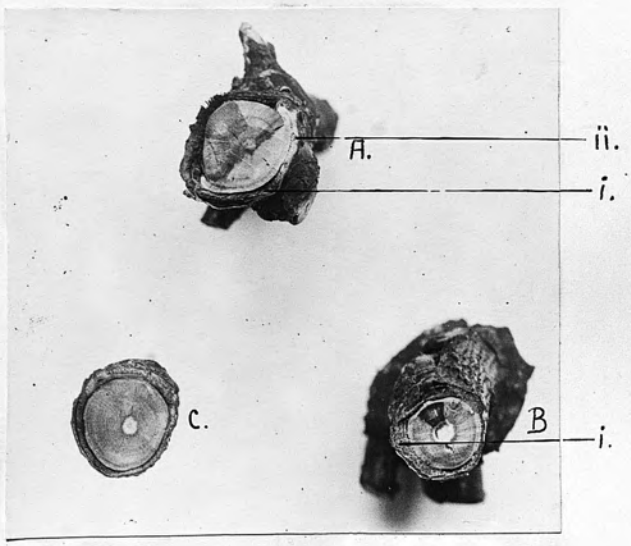

Fig. 2.

PLATE II.

Anatomy of the cankered apricot branch seen in Plate 1.

Fig. 1. Disease symptoms are first apparent in the cortex as seen at A, where a small bundle of bast fibres are surrounded by cells derived from a cork cambium. A larger cork cambial growth is seen in the centre of the photograph. x c. 30.

Fig. 2. A further stage in the formation of cork cambia. These latter are seen to be running parallel with the epidermis. x c. 25.

PLATE II.

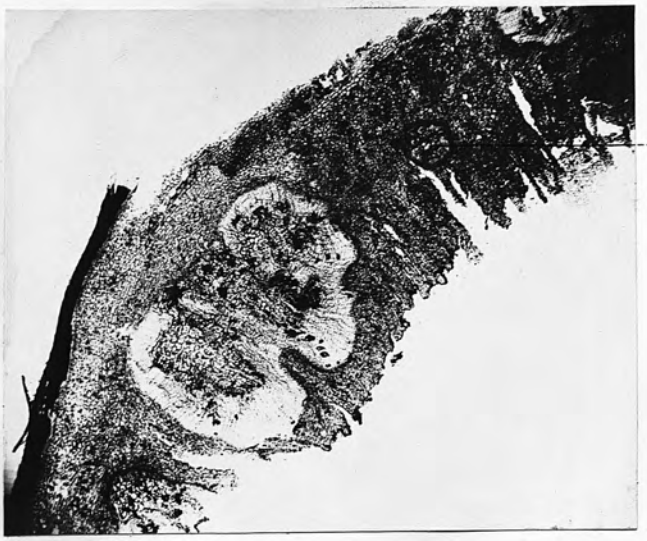

Fig. 1.

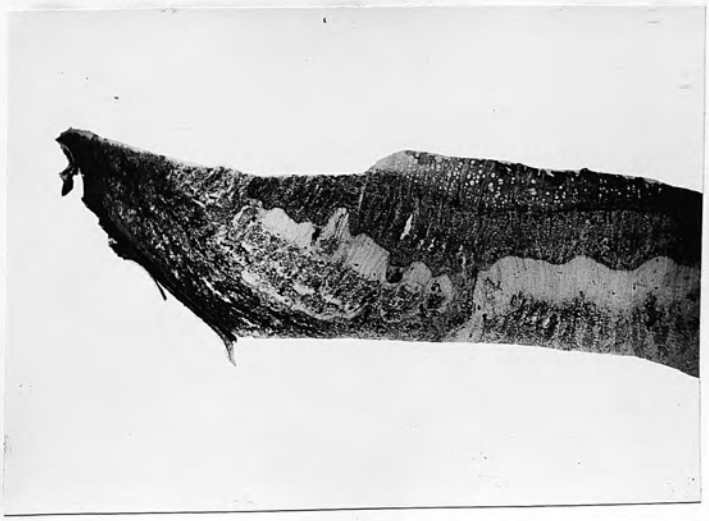

Fig 2.

PLATE III.

Anatomy of the cankered apricot branch/in seen Plate I.

Fig. 1. A transverse section of the cortex illustrating the enormous formation of cork cambia. These are mostly in the region of and surrounding the bast fibre bundles. x c. 30.

Fig. 2. Microphotograph of the callus seen in Plate I, Fig. 2. At A. the disease symptoms are re-appearing in an area of cortex supposedly protected by it. x c. 25.

PLATE III.

Fig. 1.

Fig. 2.

PLATE IV.

Cultural Comparisons of two strains of Nectria cinnabarina, viz., (a) from beech and (b) from apricot.

Fig. 1. The two strains on oat agar. At C. is the beech strain, while A. and B. are cultures of the apricot strain. Observe the relative number of sterile pustules produced by those two strains and the presence of Tubercularia pustules in the cultures of the apricot strain. 50 days' old cultures.

Fig. 2. The two strains cultivated on maize kernels. Observe the production of aerial mycelium by the apricot strain which is represented by tubes C. and D. This aerial hyphal development is absent from tubes A. and B, which are cultures of the beech strain. 50 days' old cultures.

PLATE. IV.

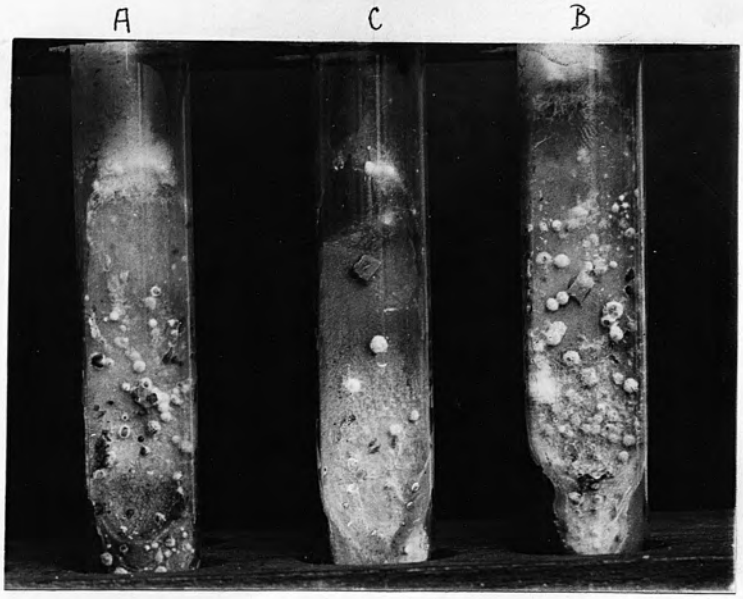

Fig. 1.

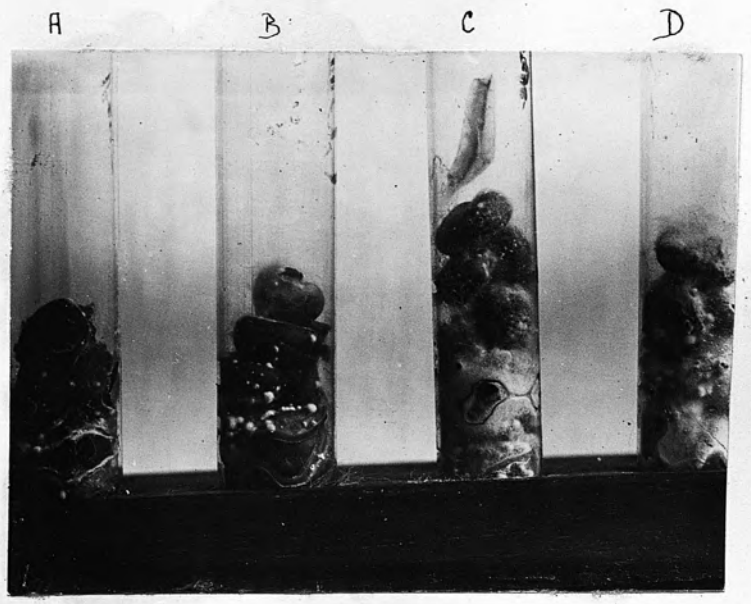

Fig. 2.

PLATE V.

The two strains of Nectria cinnabarina in culture.

Fig. 1. The apricot strain is seen growing in tubes A. and B. on rice medium while the beech strain is seen at C. and D. Observe the difference in the amount of the aerial mycelium produced. 20 days' old cultures.

Fig. 2. The two strains on Alnus twigs. Observe the production of aerial mycelium by the apricot strain at the cut surfaces of the twigs which is lacking from the beech strain culture as seen at C. 30 days' old cultures.

PLATE. V.

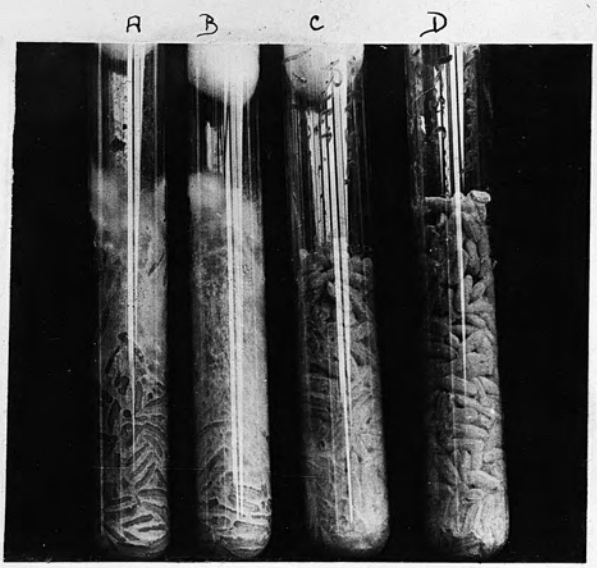

Fig. 1.

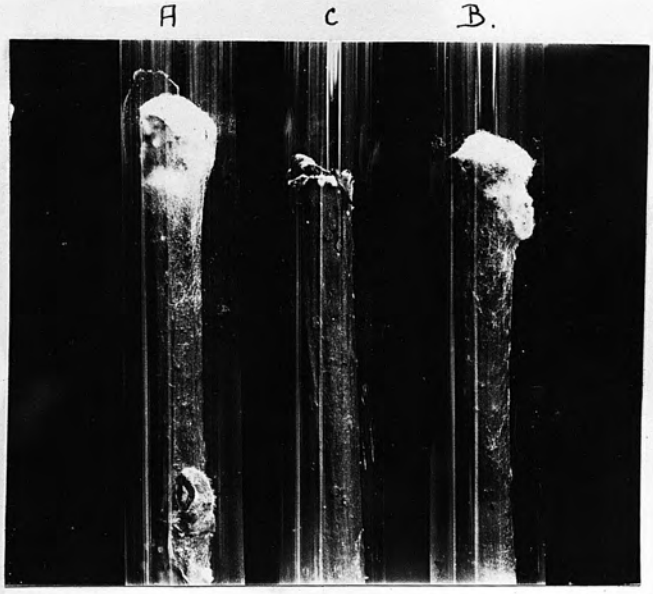

Fig. 2.

PLATE VI.

The inoculation experiments with the two strains.

Fig. 1. A shoot inoculated during the dormant period with the beech strain, by inserting the fungus on bean agar into a cut to the wood. Note the failure of the buds above the inoculation point to develop. Observe how at A. the leaves of the young side shoot have wilted. This is due to the downward progress of the fungus in the wood from the inoculation point until it has affected the wood from which the young shoot receives its water supply. x c. $\frac{1}{6}$ natural size. Four months after inoculati

Fig. 2. An enlargement near the inoculation point of the shoot seen in Fig. 1 of this plate. The inoculation cut is seen at C. The destruction of the cortex has proceeded from this cut down to A. The young shoot figured in Fig. 1 at A. is now seen here at D. to have lost its leaves entirely. Shoots E. and F. are still unaffected. The stromata of the fungus are seen appearing at B. Eighteen weeks after inoculation. x c. $\frac{2}{3}$ natural size.

Fig. 3. The first symptoms of the disease caused by the beech strain of the fungus inoculated into sound wood. Observe how the leaves have wilted and the petioles collapsed in the shoots arising above the point of inoculation. Four months after inoculation. x c. $\frac{1}{8}$ natural size

PLATE VI.

Fig. 1.

Fig. 2.

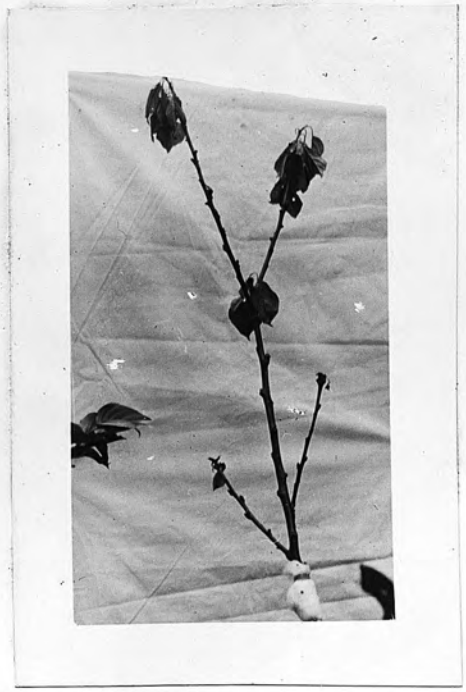

Fig. 3.

PLATE. VII.

Nectria cinnabarina (Tode) Fries (Beech Strain) on Apricot.

Diagram of inoculated shoot shown in Plate VI, Fig 3, illustrating the relative extent of progress of the disease in the cortex and in the wood, and the distribution of the fungal hyphae.

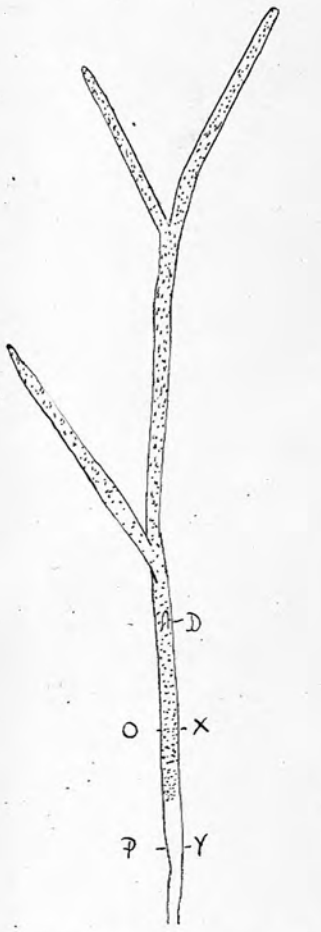

Fig. A. Diagram of the shoot. Point of inoculation at D. X c. $\frac{1}{8}$ natural size.

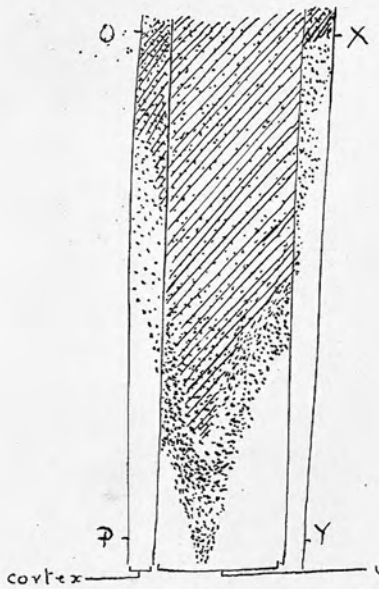

Fig. B. Part of shoot as seen in Fig. A. X approx 8. Vertical cut. Lettering as in Fig. A.

Portions of the cortex and wood stippled thus represent diseased areas.

Portions shaded thus indicate areas where fungal hyphae are present.

PLATE. VIII.

Nectria cinnabarina (Tode) Fries. (Beech Strain) on Apricot.

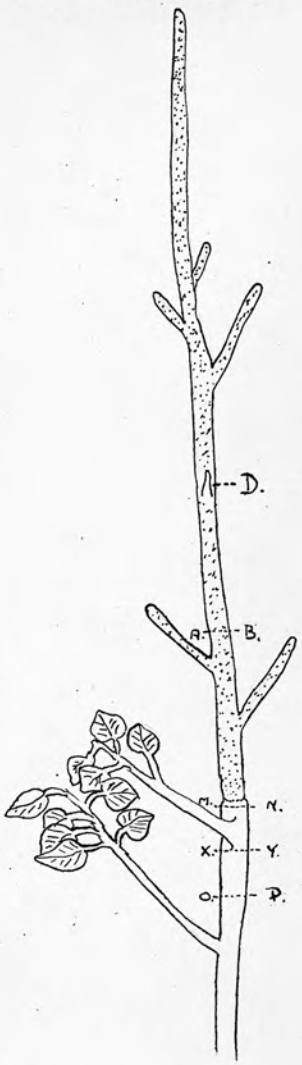

Fig. A. Diagram of shoot showing point of inoculation at D. X 1/6 natural size.

Diagram of shoot shown in Plate VI. Fig. to illustrate the relative extent of progres of the disease in the cortex and in the woo

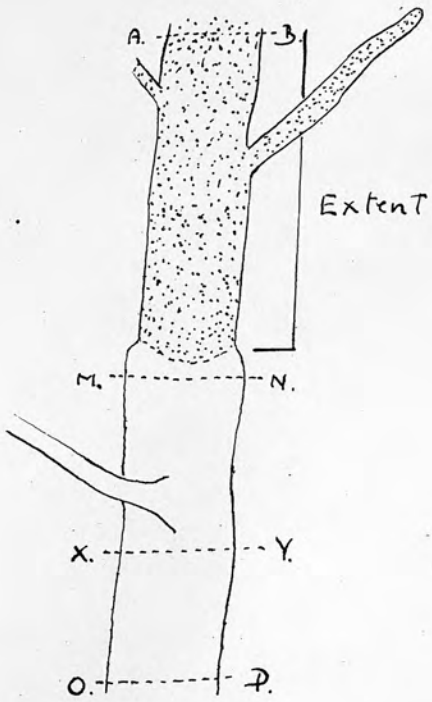

Fig. B. Enlargment of A. Lettering as in A. A X approx. 4.

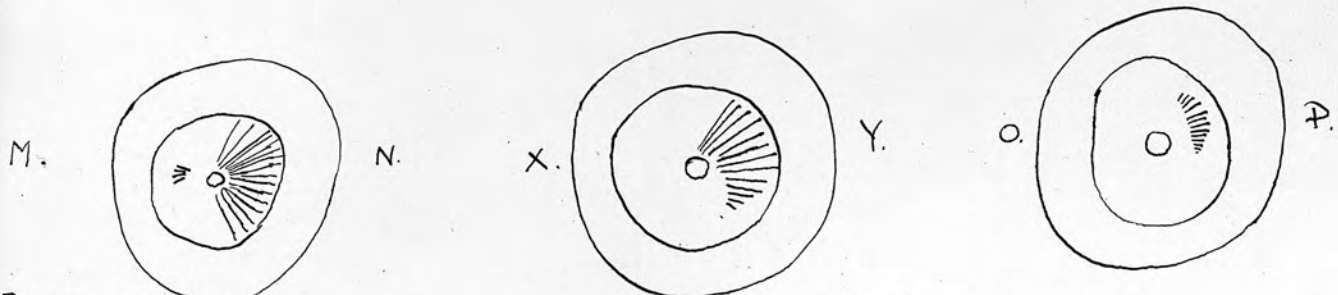

Fig. C. Cross cuts of shoot shown in B. X approx 2. Lettering as in B.

▨ Portions in the wood shaded thus represent areas of gum in the vessels.

▩ Portions stippled thus represent diseased cortex areas.

PLATE IX.

Inoculations with both strains of the fungus on to dead stubs.

Fig. 1. The beech strain of the fungus is here seen to have spread considerably down from the dead stub into the sound wood and caused a gummosis. The latter is seen in the photograph as a dark area in the wood. The shoot at A. has been dessicated as a result of this gummosis. The other shoot seen on the right of the photograph is not entirely cut off by this gummosis from its water supply and is seen to be still alive. Eight months after inoculation. x c. $\frac{3}{4}$ natural size.

Fig. 2. The result of inoculating a dead side stub with the apricot strain of the fungus. Note the lesser amount of gumming in the wood below the stub than that produced by the wood on an attack of the beech strain as seen above in Fig. 1. The extent of gumming also is not so great as seen in the beech strain inoculation. The shoots arising at both sides are still alive. Eight months after inoculation. x c. $\frac{3}{4}$ natural size.

PLATE IX.

Fig. 1.

Fig. 2.

PLATE X.

Fig. 1. An inoculation made in the summer with the beech strain of the fungus. The inoculum was inserted into the side shoot at A. The leaves on the shoot wilted and finally dropped off. The destruction of the wood and the cortex proceeded downwards from the inoculation point to a point just short of the parent shoot (see below Fig. 2). Two months after inoculation. x c. $\frac{1}{6}$ natural size.

Fig. 2. An enlargement of the affected shoot seen above in Fig. 1. The destruction of the cortex and the wood proceeded downwards to B. At this point the darkly printing area represents the gum barrier which seemed to stem the futher progress of the fungus.
x c. $\frac{3}{4}$ natural size.

PLATE. X.

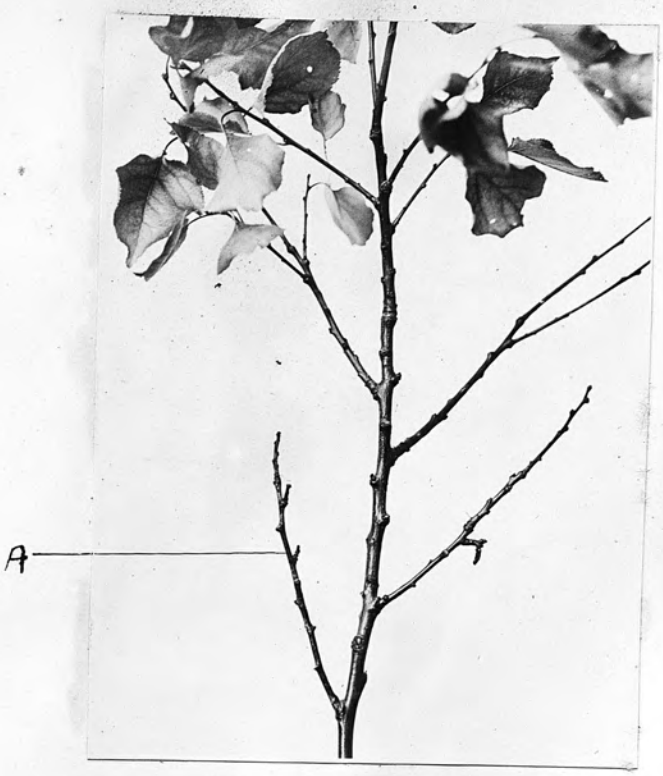

Fig. 1.

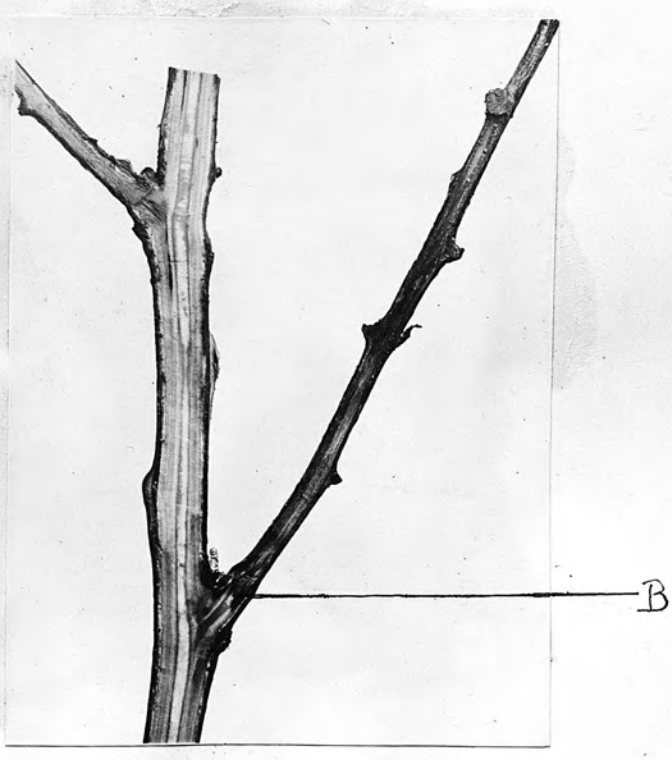

Fig. 2.

PLATE XI.

Fig. 1. Canker symptoms produced in a five year old shoot at B. These resulted from the passage into that shoot of the apricot strain of the fungus from the inoculation point at A. Eight months from time of inoculation. x c. $\frac{3}{4}$ natural size.

Fig. 2. A cross cut of the shoot seen in Fig. 1 above in the region of B. Observe the gumming in the wood in that side of the shoot lying towards the inoculated side shoot. Note the death of the cortex overlying this gummed wood and the formation of cork cambia at the edges of this dead cortex. Destruction of the cortex is seen to be taking place at A. in an area supposedly protected by the callus formation. x c. $1\frac{1}{4}$ natural size.

PLATE XI.

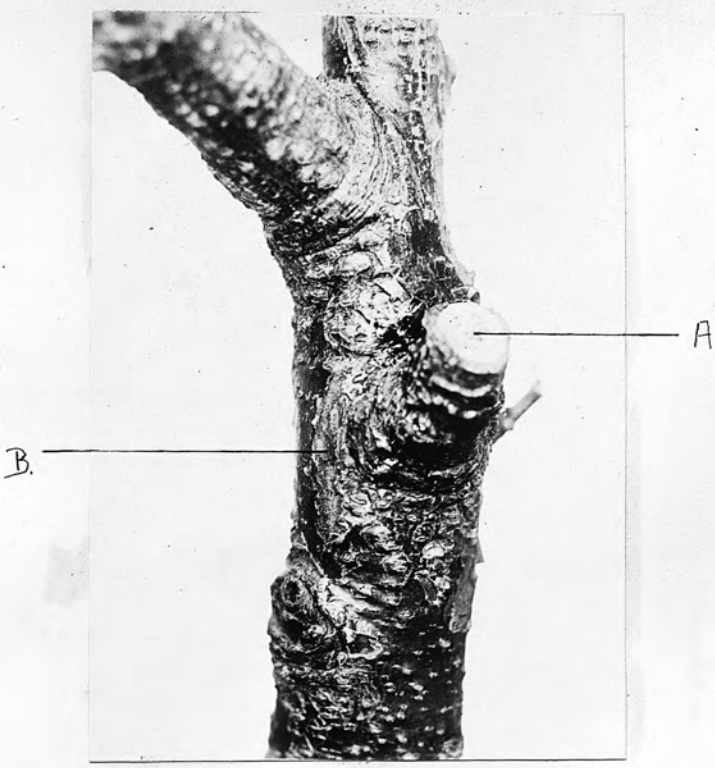

Fig. 1.

Fig. 2

PLATE. XII.

Nectria cinnabarina (Tode) Fries, (Apricot Strain) on Apricot.

Diagram of cankered shoot, shown in Plate XI. Fig. 1, illustrating the relative extent of progress of the disease in wood and in cortex and the nature of cork cambial formation in the latter.

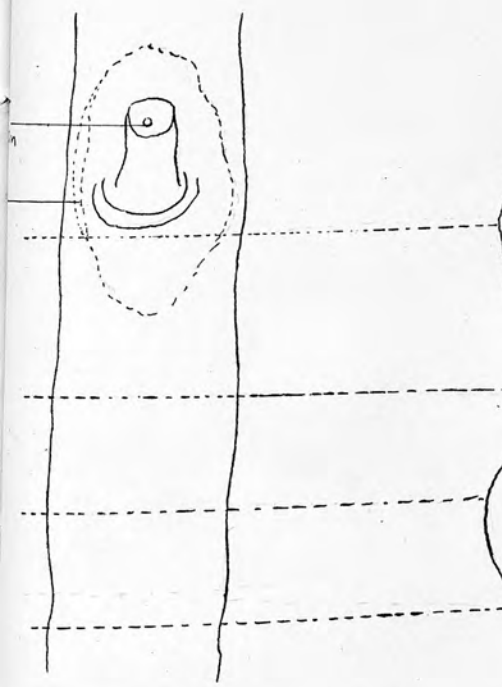

Fig. A. Diagram of cankered shoot. × c. 3/4 natural size.

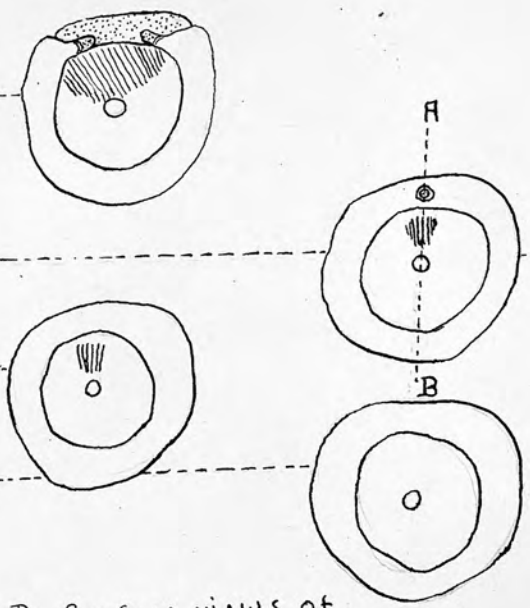

Fig. B. Surface views of various cross sections of shoot in A. × c. 3/4 natural size.

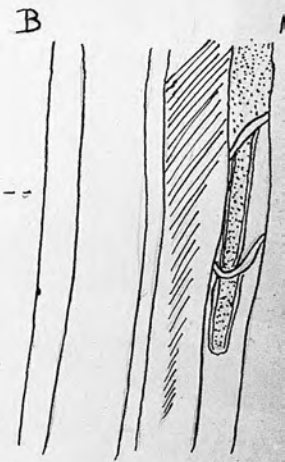

Fig. C. Surface view of vertical section through A--B in Fig B. × c. 1. natural size.

Portions stippled thus represent diseased cortex areas

Double lines in the cortex represent cork cambia

Portions in the wood shaded thus represent areas of gum in the vessels.

PLATE XIII.

Fig. 1. The failure of an inoculation with the apricot strain of the fungus to penetrate into a two year old shoot of the apricot. The inoculation was made at C. The fungus has been occluded by a gum barrier in the wood and the inoculation cut has been healed over by a callus. Five months after date of inoculation. x c. $\frac{3}{4}$ natural size.

Fig. 2. Failure of the beech strain of the fungus to penetrate from the inoculation point at A. into a one year old apricot shoot. A callus is gradually closing over the inoculation cut. Note the production of gum in the wood a considerable distance above and below the inoculation point. No hyphae are present in those gummed areas. Five months after date of inoculation. x c. $\frac{3}{4}$ natural size.

Fig. 3. A control cut made at B. completely healed over by a callus. Three months after date of inoculation. Natural size.

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Fig. 3. A control cut made at B. completely healed over by a callus. Three months after date of inoculation. Natural size.

PLATE. XIII.

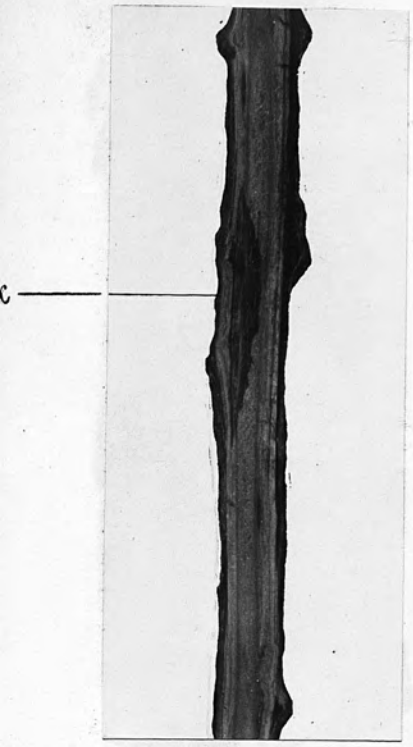

Fig. 1.

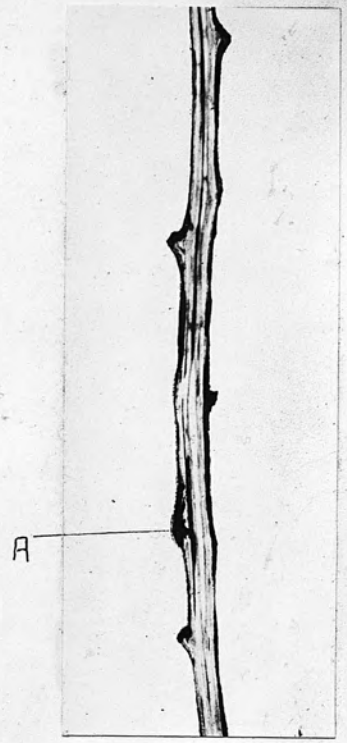

Fig. 2.

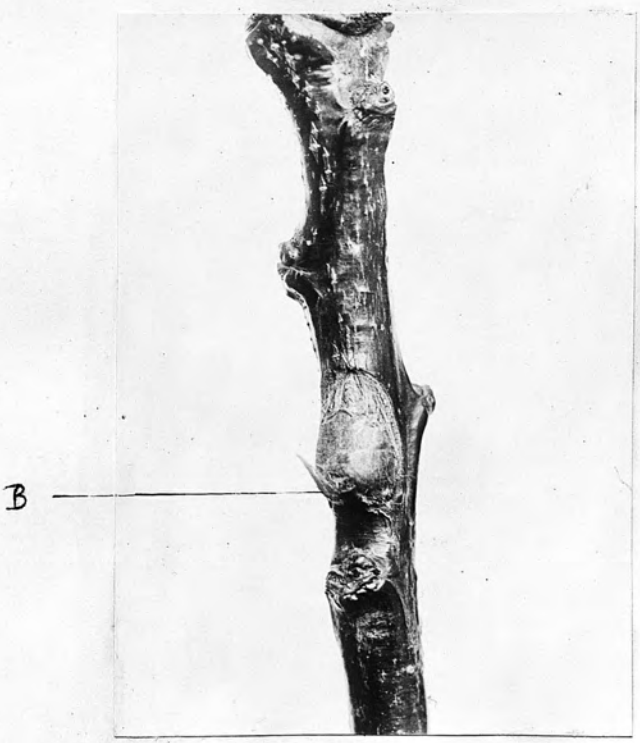

Fig. 3.