

(i) THE ASSOCIATION OF GONATORRHODIELLA HIGHLEY A.L. SMITH

WITH SPECIES OF NECTRIA.

(ii) STUDIES ON HAPALOSPHAERIA DEFORMANS SYDOW.

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INTRODUCTION

The fungus which forms the subject of the present thesis belongs to the genus Gonatorrhodiella of the Fungi Imperfecti. The genus is rare in nature, and is composed of two valid species. In addition to the unique morphological characteristics displayed by both species, they are further distinctive in that they are found in association with other fungi, particularly with members of the Ascomycetous Hypocreales.

Records so far available have indicated that the genus occurs both in North America and Europe, and it was from the former continent that the original type species was collected. Thaxter(51) created the genus Gonatorrhodiella in order to accommodate a fungus which he discovered in Connecticut, and of which he stated in 1891:-

"This species has been met with in several localities about New Haven always growing directly upon, or running a short distance from certain species of Hypocrea and Hypomyces on which it appears to be parasitic."

Accordingly he named the fungus Gonatorrhodiella parasitica, and Ayers(1b) considers the same species to have been collected by von Hoehnel(16), who observed the fungus growing on Tremella lutescens in Wiener Wald, and recorded it in 1907 as G. eximia. Davidson(9) also mentioned the occurrence of G. parasitica in association with Trichoderma lignorum on a sporophore of Polyporus sp., and on Betula nigra decayed by a species of Imbes.

Petch/

Petch(23) recorded the fungus growing on Hymomyces aurantius at Totnes during the foray of the British Mycological Society in 1935.

The original description of the structure and occurrence of the other species Gonatorrhodiella Highlei (Brown Mould), is the only one in which no other fungus is mentioned as an associate. The species was found growing on onion bulbs at Kew by Smith and Rea (29) in 1907. All subsequent recordings of the fungus have been made in North America. The collections on which the major proportion of the reports are based are described by Ayers(1b) in a paper which deals with the persistent association of G. Highlei with Nectria coccinea (Pers.). The latter fungus, together with the scale insect Cryptococcus fagi Baer., is responsible for the bark disease of the beech Fagus grandifolia Everhardt, in North America, and so constant is the occurrence of G. Highlei with the Nectria species that it is possible to detect beech bark disease by the conspicuous clay coloured patches to which the Gonatorrhodiella gives rise when in a freely sporing condition on the surface of the bark. It may therefore be regarded as an 'indicator plant'.

General accounts of the beech bark disease have been given by Ehrlich(13) and Spaulding(30). The fatal effects of the disease are considered to be the outcome of the joint activity of the scale insect and the Nectria species, a full account of which is given by/

by Lohman and Watson⁽²⁰⁾ under the name Nectria coccinea var. faginata Lohman, Watson and Ayers.

The beech scale, Cryptococcus fagi, a member of the Coccidae, commences the disease by infesting the surface of apparently smooth, healthy beech bark in the form of colonies. The insects feed by stylets through which food materials are sucked from the plant tissues, and which are inserted as far as the phloem. The areas of the bark which die as a result of feeding activities become sunken, and cracks form at the edges of the killed areas. Frequently slime-flux appears during the killing of the bark.

Infection by the Nectria species is brought about by its entry through bark cracks and the stylet tracks made by the scale insects. Ehrlich⁽¹³⁾ regards the infection by the Nectria as following within three years of the presence of the scale insects, and the subsequent death of the trees so infected as occurring one or two years later in some cases. Death is the outcome of the invasion of the cortex, phloem, cambium and sap-wood by Nectria hyphae. Infections usually occur at closely situated points on the tree, the functional activity of the vascular tissues is reduced or destroyed, and zones of infected bark peel away, the upper branches of the tree showing defoliation and general symptoms of die-back.

Spaulding⁽³⁰⁾ mentions the prevalence of G. Highleyi in association with the Nectria species since 1929 in/
in/

in Nova Scotia and New Brunswick, and also includes references to various towns in the United States from which the fungus has been recorded. He states that:—"observations show it to be widely distributed in the colonies of the Nectria old enough to have developed abundant fruiting which seems to be necessary for this dependent fungus successfully to establish itself. It has been found only on Nectria coccinea var. faginata and is believed to be parasitic on it." The successional nature of the beech bark disease is also emphasised in his paper, and he remarks that:—"The association of an insect and two fungi in a serious disease of forest trees is believed to be unique in forest pathological literature."

Attempts have been made to grow both G. parasitica and G. Highlei on artificial substrates under laboratory conditions. Davidson(9) found it impossible to culture G. parasitica in the absence of Trichoderma lignorum with which it occurred in its natural habitat, and concluded that a type of parasitism was involved. Ayers(1b) succeeded in growing G. Highlei in pure culture on an oatmeal mush, and in the presence of Nectria coccinea, N. galligena and N. cucurbitula. Negative results were gained with N. cinnabarina, and N. coryli.

Although both species of Gonatorrhodiella have been regarded and frequently referred to as parasites, no direct evidence of the relationships which they bear to their fungal associates has been put forward.

The present work deals with a Gonatorrhodiella which was found at the Royal Botanic Garden, Edinburgh, growing on the bark surfaces of a beech and a poplar log which had been stored in a wood pile for some months. An investigation of this fungus, identified as Gonatorrhodiella Highlei, was made with special reference to its general structure, its relationships to fungal associates and its behaviour in culture.

THE NATURAL OCCURRENCE OF GONATORRHODIELLA HIGHLEI

In its natural surroundings, the fungus is conspicuous. The globular, sporiferous heads of the conidiophores, which are given off from a basal mycelium in great profusion, are cinnamon buff to xanthine orange or clay (Ridgway, 26) in colour in the mass, and patches of them form at the surface of the bark of the trees on which they occur. (Fig. 1.)

The distribution of the fungus on the two logs which were examined in Edinburgh, differed appreciably. On the beech log, the fungus was found to be growing directly upon, and conforming in extent with, the perithecial stromata of a colony of Nectria cinnabarina (Tode) Eries., a species of Nectria with which it has not been previously known to associate. The conidiophores of the Gonatorrhodiella formed patches of distinctive colour some six inches square. The surrounding areas of the bark which were free of Nectria stromata and were not colonised by the Gonatorrhodiella, supported fructifications of Didymium nigripes.

On the poplar log, the conidiophores of the Gonatorrhodiella occurred exclusively in the cracks of the bark surface, forming an irregular pattern, and no other fungal fructifications were observable macroscopically.

OBSERVATIONS ON THE MORPHOLOGY AND CYTOLOGY OF THE
FUNGUS

The vegetative mycelium of Gonatorrhodiella Highlei is composed of hyaline, septate, branched hyphae, 5-8 μ in width. The protoplasmic content of the hyphae is variable. Those cells nearest the growing tips contain abundant granular protoplasm, whilst those in the older parts of the mycelium become progressively more vacuolate in appearance, and finally lose all functional contents. In material obtained both from natural sources and from culture, irregularity in septation was also observed. Groups of two to five septa may be laid down at various points in the hyphae, so that cells 16-25 μ in length are frequently separated by short cells which may be 3-6 μ long. (Fig. 6a.)

Each conidiophore arises as a lateral protuberance from a cell of the mycelium. The protuberance increases steadily in length, becomes obtusely pointed, and is eventually cut off from the parent cell by a transverse septum. In most instances, the width of the conidiophore initial varies from 8 μ to 10 μ , but occasionally conidiophore initials 5-6 μ wide have been observed. In conidiophores derived from the latter, dilation takes place a short distance from the parent cell, so that when maturity is reached, the base of the conidiophore appears to be constricted.

By continued growth and development, the initial cell gives rise to a simple, cylindrical conidiophore, which/

which is 10-15 μ in diameter, and possesses a variable number of cells(3-7) before the production of conidia occurs. When the conidiophore has attained the height of 200-250 μ , the terminal cell undergoes swelling at its distal end, becoming club-shaped. Terminal bulbs which are produced in this way may be ellipsoidal or subspherical in shape when mature(23 x 42 μ), and from their surfaces, primary conidia or nodal cells are given off in a regular pattern.

The primary conidia are obconical in shape, and may produce two to five secondary conidia from their distal ends. The primary conidia vary in length from 14-20 μ , and at their widest points are 13-16 μ across. (Fig. 6b) The secondary conidia agree in shape with the tertiary and quaternary conidia, which are later produced, in being elliptical. General measurements of the last three series of conidia are 13-15 x 7-10 μ .

The arrangement of conidia given off from the conidiophore is therefore in chains of four, where the first branching of each chain is initiated from the primary conidia. Subsequent branching may also occur from the distal ends of the secondary and tertiary conidia, each of which may produce two conidia. The mature sporiferous head of the conidiophore assumes the form of a ball composed of closely approximated conidial chains. (Figs. 2, 3 and 4)

Conidiophores display proliferation by which continued sporing is facilitated. The method involved incorporates/

incorporates further apical growth from the sporing terminal bulb. In most cases a single apical protuberance commences this growth, although two may be formed. By continued development of the outgrowth, a short extension of the conidiophore is produced which gives rise to a further sporiferous bulb, which in turn is capable of proliferation. In the material examined, proliferations rarely exceeded four in number. Fig 5b shows an example of proliferation in which the original outgrowth from the first formed sporiferous head has introduced branching in the conidiophore.

Branching is most commonly brought about in this way, but in rare instances has been observed to occur in the young conidiophore. (Fig. 5a.)

Owing to proliferation and branching, conidiophores may attain the height of 350-400 μ , and when fully mature are yellowish brown in colour by transmitted light.

Coloration is due in part to the thick cell walls, and in part to the protoplasm.

In order to examine the processes attendant on the formation of conidia and the general details of the structure of the fungus, conidiophores and mycelium were stained by the following method. The material was transferred to slide plates of plain agar, to which it adhered sufficiently to allow it to be passed through fixing and staining fluids. Fixation was carried out in Alcohol-Formalin-Acetic (Weak) for two hours, and successful staining was then accomplished by immersion in/

in Iron Alum Haematoxylin or Basic Fuchsin. The latter stain was used as a 2% solution in distilled water. Prior to examination, slides were mounted in Lactophenol.

The cells of the mycelium and conidiophores of the fungus are multinucleate, the nuclei being dispersed in the cytoplasm, within which globular inclusions, presumably of a fatty nature, are also present. The conidia are also multinucleate, and all four series of conidia are produced from their parent structures in a similar way.

Each primary conidium arises as a sac-like outgrowth of the cell wall of the terminal bulb of the conidiophore. As it increases in size, it receives an inclusion of cytoplasm and nuclei from the conidiophore. (Fig. 6c.), and by the growth of the cell wall inwards at the point of origin of the young conidium, a transverse septum with a distinct central pore is laid down. (Fig. 6d.) The pore closes when the conidium is nearing maturity, so that the conidium is borne on a short sterigma. Secondary conidia arise from the primary, tertiary from the secondary, and quaternary from the tertiary by a similar process of development. In all types of conidia, which are released by the eventual fracture of sterigmata, the point of their attachment to the structures from which they were derived is shown by the presence of a hyaline papilla.

Both in the mycelium of the fungus and in the conidiophores, the transverse septa commonly show a distinct,

distinct septal pore. The pore is set in the centre of the septum in a thin zone of the cell wall, and may be easily seen in those hyphae which are partially plasmolysed. A fine thread of protoplasm passes through the pore to connect the protoplasts of adjacent cells. (Fig. 6e.)

NOTES ON TAXONOMY

The general morphology of the fungus is in agreement with that described for other representatives of the species by Smith(29) and Ayers(1b). In his technical account of the fungus in which he amends the original description of the species, Ayers(1b) states that: - "The conidia, ... are elliptical and borne in complex trigeminous chains on obovate, nodal cells."

The number of secondary conidia produced by the primaries (or nodal cells) varied in the specimen examined in Edinburgh from two to five. The most usual number recorded was four, and it is interesting to note that Smith(29), in her original illustrations of the fungus, shows a primary conidium bearing four secondaries, presumably a condition representative of the fungus she examined.

The European members of the species may therefore differ from the American with regard to this character, and it is suggested that the description put forward by Ayers(1b) should be amended.

The distinction between the two species of Gonatorrhodiella is based upon the nature of the primary conidia and the number of spore chains produced from them. /

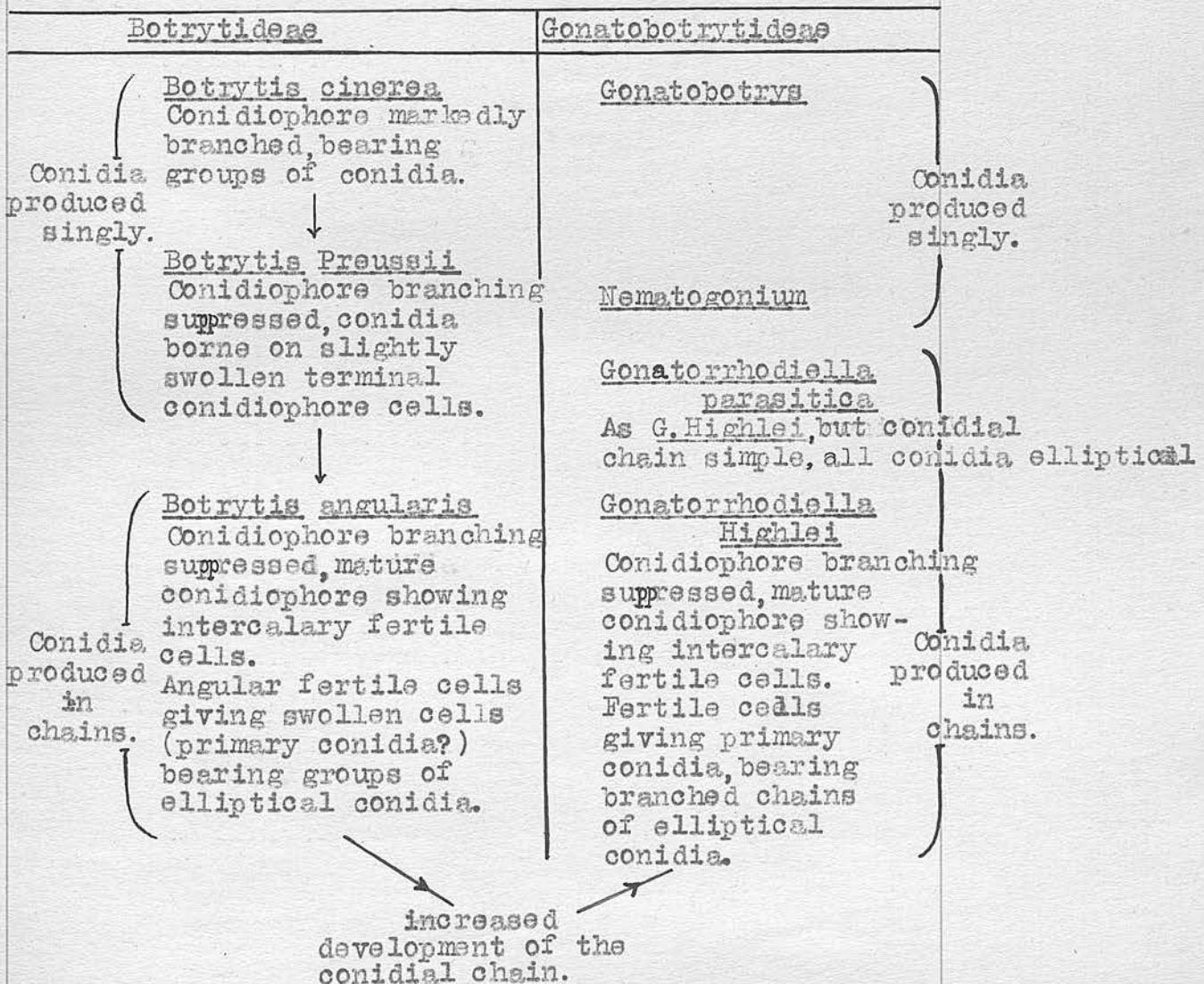
them. In G. parasitica, the primary conidia are elliptical, and each gives rise to a single row of conidia, whilst in G. Highlei, the primary conidia are obovate bearing a variable number of conidial chains. From a study of the paper by Petch(22), in which he describes and illustrates the entomogenous fungus, Gonatorrhodiella coccorum, the writer is in agreement with Ayers(1b) in excluding this fungus from the genus Gonatorrhodiella, with which it shows little morphological affinity.

In his original description of the genus, Thaxter(31) considered it to be Mucedinous, and commented on the resemblance which it bore to Gonatorrhodum Corda., regarded by Saccardo as a member of the Dematiaceae. The genus is grouped by Engler and Prantl(14) in the Gonatobotrytidea of the Mucedinaceae-Hyalosporae. The three genera, Gonatobotrys Corda, Nematogonium Desm. and Gonatorrhodiella Thaxt. which are placed in the Gonatobotrytidea, agree in possessing a conidiophore showing intercalary, fertile, swollen cells bearing conidia. In the first two named genera, however, conidia are produced singly, the presence of conidial chains serving to distinguish Gonatorrhodiella.

It is suggested that features in the morphology of Gonatorrhodiella Highlei are analagous to those found in a species of the genus Botrytis, which is placed in the nearly related Mucedinous Botrytidea. Botrytis angularis A.L. Sm., a fungus described and illustrated by Smith(28), shows the development of terminal, angular, bladder-like cells of the conidiophore which bear obovate/

obovate, swollen cells similar in appearance to the primary conidia of G. Highlei. These swollen cells produce groups of elliptical conidia (10 x 6 μ) borne on sterigmata. The resemblance is further substantiated by the fact that the conidiophore of Botrytis angularis may continue its growth and form other sporiferous heads at a higher level. The tendency to produce a conidiophore, in which the inflated fertile cells assume an intercalary position, suggests affinity with the Gonatobotrytidae.

The following series shows a possible connection between the Botrytidae and the Gonatobotrytidae with Botrytis angularis as the morphological intermediate form.



THE RELATIONSHIP BETWEEN GONATORRHODIELLA HIGHLEI
AND OTHER FUNGI IN NATURE.

In order to investigate the relationship between Gonatorrhodiella Highlei and other fungi in nature, a general investigation of the areas of the beech and poplar bark which showed freely sporing colonies of the fungus was undertaken.

A study of the fungus on the beech bark was made by cutting hand sections through the perithecial stromata of the colony of Nectria cinnabarina, above which a rich growth of conidiophores was to be found. The sections showed that the basal mycelium of the Gonatorrhodiella occurred principally in pockets, variably situated between the stroma proper and the erumpent Nectria perithecia, and also at the base of the stroma, sometimes encroaching into the pseudoparenchyma. (Figs. 7 and 8)

From these localised areas of concentration of the mycelium, ramifying hyphae entered partly into the outer tissues of the bark, and also passed upwards to form loose patches of aerial mycelium lying between the Nectria perithecia. From the hyaline to cinnamon-buff patches of mycelium, stolon-like hyphae bearing conidiophores at intervals along their lengths were freely given off.

The precise relationship between the pseudoparenchyma of the Nectria stromata and the basal mycelium of the Gonatorrhodiella could not be discovered from an examination of the sections. The close connection between/

between the two fungi in a mechanical sense was, however, implied by the observation of the ramification of the hyphae of the Gonatorrhodiella mycelium within the pseudoparenchyma of the Nectria stromata in a number of cases. The hyphae could easily be distinguished in unstained preparations owing to their light coloration, which contrasted with the deeper brownish pigmentation of the cells of the stroma tissue.

An examination of the Gonatorrhodiella colony on the poplar log showed that the conidiophores, which arose from a basal mycelium situated in the cracks of the bark, were loosely invested by other fungal hyphae. (Figs. 9 and 10)

The latter pursued a longitudinal growth. Arising from the substratum where they freely intermingled with the hyphae of the basal mycelium of the Gonatorrhodiella, they ran in close contact with the conidiophores, the terminal bulbs of which were frequently encased by hyphal branches. The presence of such hyphae did not appear to have any detrimental effect on the sporing capacity of the conidiophores, the cells of which were normal in their development.

In preparations mounted in water, slight pressure on the cover glass served to dislodge investing hyphae, showing that the connection between them and the conidiophores was limited to a surface attachment.

STUDIES ON THE FUNGUS IN CULTURE--I. STUDIES IN MIXED CULTURE

An investigation of the fungus flora of the areas of poplar and beech bark which supported colonies of Gonatorrhodiella Highlei was carried out with particular reference to the poplar sample.

It has already been stated that the basal, loose plectenchyme from which the conidiophores arose on poplar bark contained a variety of hyphae which belonged to other fungi, and that in many instances, the conidiophores were invested in such hyphal growths. The fungi were therefore brought into culture by two methods. By the first of these it was hoped to establish the identity of the hyphae which invested the conidiophores. Mature conidiophores were removed aseptically from their situation at the surface of the bark by means of sharp-pointed forceps, sterilised by flaming. The conidiophores were then either transferred directly to agar plates, or first shaken in sterile water and the resulting suspension of conidia and hyphal fragments plated out. The plates were poured in Petri dishes or on slides (Noble, 21). The media used at this stage of the investigation were potato-dextrose, malt and plain 2.5% agars, and cultures were incubated at 18°-20°C.

The colonies which resulted from these isolations proved to be of a fungus provisionally identified as a species of Nectria, and, in rare cases, Trichoderma lignorum (Tode) Harz. was also obtained.

By the second method of isolation, the basal mycelium of the fungus was subjected to the technique described above in order to obtain a general picture of the total fungus flora at the seat of origin of the conidiophores. From the plates which were obtained, the dominant fungus was found to be the Nectria sp., with Trichothecium roseum Link, sub-dominant. A small number of Trichoderma lignorum colonies were also recorded.

Similar investigations carried out with the beech bark which supported a colony of Gonatorrhodiella, showed that the dominant fungal species was Nectria cinnabarina(Tode) Eries., and that Trichoderma lignorum and Trichothecium roseum were also present.

Several plates, each inoculated with a number of conidiophores aseptically removed from poplar bark, and which yielded colonies of the Nectria species were kept for four months in the hope of recovering the Gonatorrhodiella. No aerial growth of the mycelium of the latter fungus could be discerned. From the many plates examined microscopically, however, four in which the medium employed was potato-dextrose agar showed the presence of the large hyphae of the Gonatorrhodiella, interwoven with the finer Nectria hyphae at the surface of the agar. In several cases there was evidence of the degeneration of the Gonatorrhodiella hyphae. Many breakages were observed at the transverse septa, accompanied by extreme vacuolisation of the cell contents, indicating parasitism by the Nectria sp. No growth of the Gonatorrhodiella was ever observed in malt/

malt or plain agar cultures, and similar results were obtained when the fungus was plated in the presence of Nectria cinnabarina.

The transference of mature conidiophores from poplar bark to oatmeal mush, a medium reported by Ayers (1b) as suitable for promoting the growth of Gonatorrhodiella Highlei, gave freely sporing colonies of the Nectria sp. which filled Petri dish plates within fourteen days of inoculation. The appearance of the Gonatorrhodiella in such colonies derived from mixed inoculum was retarded, and under the conditions of incubation used throughout the investigation, the production of aerial hyphae by the fungus rarely occurred before one month.

On oatmeal mush, the Nectria sp. gives rise to a colony which is loose and cottony in appearance and white in colour during the early stages of growth. The white coloration gives way eventually to shades of yellow and then brown. As the Gonatorrhodiella develops, the hyaline aerial hyphae to which it gives rise form a sparse, loose network within the surface layers of the Nectria colony. The conidiophores are produced from these aerial hyphae and appear in scattered groups, which are at first cinnamon-buff to xanthine orange in colour. The groups eventually coalesce, and the colony assumes a conspicuous clay or rufous pigmentation owing to the massed conidiophores. During this development, the Nectria colony continues to spore without detriment. Microconidial fructifications which develop within two to five days' growth/

growth are eventually mingled with white to cream macroconidial sporodochia, which are clearly visible at various points amongst the conidiophores of the Gonatorrhodiella.

Technical Description of the Nectria species.

Mycelium at first white, soon turning to shades of buff yellow, and felting down to become sayal brown and finally chestnut with age.

Microconidia borne on the aerial mycelium in false heads on simple or branched conidiophores, mostly unicellular occasionally 1-septate, hyaline, elongate-ovate. 0-septate 4.2 - 8.7 x 2.1 - 3.6 μ .

Macroconidia borne on white to cream sporodochia, and in older cultures in columns 1-5mm. in height, which become split and recurved with age, hyaline, 3-5 septate, elongate subcylindric, unequal sided with obtusely rounded ends.

3-septate: -30 - 46 x 5.9 - 6.4 μ . 4-septate: -50-66 x 6.3-7.0 μ .

5-septate (rare) 62 - 74 x 6.6 - 7.2 μ .

Perithecia not produced in culture.

Owing to the absence of a perithecial stage in culture, the fungus cannot be fully identified. In the possession of a Cylindrocarpon macroconidial stage which is of a columnar form, in the general type of growth of the mycelium, and in the coloration described above for cultures grown on potato dextrose agar, the fungus resembles varieties of Nectria coccinea Pers. described by Lohman and Watson (20).

The oatmeal mush was used throughout the investigation for the maintenance of sufficient quantities of Gonatorrhodiella Highlei, but the disadvantage of such substrates lies in their opacity, which constitutes a barrier to direct microscopic vision of the fungi in situ.

In order to study the relationship between the Gonatorrhodiella and the Nectria species in culture, experiments were conducted with a view to finding a medium which combined the property of transparency with the ability to supply the substances necessary for the germination of the conidia of the Gonatorrhodiella and the subsequent full development of mycelium and conidiphores. The medium which fulfilled these requirements was produced by boiling a one month old culture of the Nectria species with ten grammes of beech bark for ten minutes in 100cc. distilled water. The filtrate from the decoction was then made up to 250cc. with distilled water and agar incorporated to make a 2.5% medium.

Culture media of this type were also made up from one month old cultures of Trichothecium roseum and Trichoderma lignorum. These media were also found to be effective in promoting conidial germination and further development of the fungus. All fungi grown on extract agars were found to maintain a regular growth at the surface of the medium, which did not encourage abundant aerial development of mycelium.

CONIDIAL GERMINATION IN GONATORRHODIELLA HIGHLEYI

Conidial suspensions were made up by shaking conidiophores of the fungus, obtained from mixed stock cultures grown on oatmeal mush, in sterile water. The suspensions were plated on Nectria, Trichothecium and Trichoderma extract agars on slides which were incubated in darkness at 18°-20°C. The method used was similar to that used by Noble(21). Slides were removed from the incubator at daily intervals during a period of a fortnight, and after a preliminary examination, were fixed in Alcohol-Formalin-Acetic, prior to staining in Iron Alum Haematoxylin or Basic Fuchsin.

Germination of the conidia of the Gonatorrhodiella occurred within four to five days of the inoculation of the slide plates, the proportion of germinations being greatest on the Nectria extract agar. Young colonies of the Nectria species, which had developed from conidia introduced with the spore suspensions, were also observable at this time.

Primary, secondary, tertiary and quaternary conidia of the Gonatorrhodiella were all observed to germinate. The primary conidia were easily distinguished owing to their characteristic obconical shape, but the other conidia were determined on the basis of measurement. Germ tubes, which are hyaline or tinged with a faint yellowish coloration in unstained preparations, are usually produced singly from each conidium. The place of their origin may be proximal, distal or lateral. In cases where two germ/

germ tubes occur, they are diametrically opposite.

The germ tube makes its first appearance as a rounded sac, which becomes swollen at the upper end. (Fig. 11a) Growth then proceeds from the apex of the sac to give rise to a hypha which is filled with densely granular cytoplasm containing several nuclei. The hypha varies in width from 1.5-2.5 μ .

The shape of the original sac is retained, in most cases, as a slightly dilated zone a short distance from the point of origin of the germ tube. (Figs. 11b, c, d and e.) An abnormal case of germination in a tertiary conidium is shown in Fig. 11f. Prior to the transfer to agar, the conidium had already given rise to the initials of two quaternary conidia at its distal end. Apart from the production of a germ tube at the proximal end of the tertiary conidium, a germ tube has also been formed from a quaternary conidium initial.

As growth of the germ tube proceeds, the protoplasmic content becomes vacuolate, and septa, which are difficult to distinguish during their early development, are laid down.

Several examples of regeneration of the mycelium of the fungus by a process of self penetration were also observed in occasional mycelial fragments which had entered the slide cultures. An example is shown in Fig. 33a. The subject of intrahyphal growth is dealt with later (p. 41).

THE RELATIONSHIP BETWEEN GONATORRHODIELLA HIGHLEI
AND THE NECTRIA SPECIES IN CULTURE

Plates of Nectria, Trichothecium and Trichoderma-extract agars were flooded with mixed conidial suspensions of Gonatorrhodiella Highlei and the Nectria species.

The conidia of both fungi germinated readily, those of the Nectria species within two days, and those of the Gonatorrhodiella within four days of incubation at 18°-20°C. The rapid growth of the Nectria species, which follows germination of the conidia, causes the appearance of small fungal colonies bearing microconidial fructifications of the Cephalosporium type, before the germ tubes of the Gonatorrhodiella are much greater than 300 μ in length. The germ tubes of the latter fungus grow much more quickly in the presence of the young Nectria hyphae than when incubated in pure culture. The increase in rate of growth is not, however, accompanied by a corresponding increase in diameter, germ tubes rarely exceeding 2.5 μ in width.

The subsequent development of the fungus in the presence of colonies of the Nectria species may be considered to fall into three principal stages. Of these, the first is the formation of an appressorium by the germ tube when direct contact is made with the hypha of a developing Nectria colony, and the later growth of the tip of the germ tube to form a hook which encircles the Nectria hypha. A further series of hooks may then arise from the appressorium.

The second stage, which follows directly upon the first, involves the formation of a prolific branch system of distended hyphae, which has its origin in the appressorium and hooks.

The third stage comprises the growth and development of the branch system. This gives rise to a sparse, ramifying mycelium, the hyphae of which form secondary appressoria and hooks on other hyphae of the parasitised Nectria species.

Conidiophores of the Gonatorrhodiella are developed either directly from the first-formed branch system, or later from the hyphae of the mycelium.

As considerable variation occurs in the three stages of development of the fungus, each stage will be described in detail.

I. The Formation of Appressoria and Hook Cells

At the point of contact with a Nectria hypha, the germ tube of the Gonatorrhodiella becomes dilated immediately behind the growing tip, and the dilation adheres closely to the host hypha in the form of an appressorium. The hyphal tip then continues its growth, finally assuming the form of a hook which rings the Nectria hypha. (Figs 12 and 13)

The onset of the process appears likely to be conditioned by the amount of food reserve which the conidium releases to the developing germ tube. As is shown in Fig. 12, the formation of an appressorium and hooks is not necessarily/

necessarily undertaken at the first Nectria hypha which occurs in the path of growth, even though a near contact is operative. In this instance the second hypha of the Nectria colony is parasitised. The width of the germ tube has been observed to become increased a short time after the formation of appressoria and hooks, the enlargement proceeding backwards towards the spore.

In the earliest stages of development, the appressorium and hook are both products of the terminal cell of the germ tube. Eventually the hook becomes cut off from the appressorium by the formation of a transverse septum situated at its base. Subsidiary hook cells may then arise from the dilation so that the host hypha is encircled at several closely approximated points. (Fig. 25) An early stage in the development of multiple hook formation is shown in Fig. 14, where two hook cells clasp the parasitised Nectria hypha.

The degree to which the original appressorium develops is variable. In many cases, no appreciable dilation of the germ tube at the point of contact with a Nectria hypha may be observed. The tip of the germ tube normally gives rise to a hook cell which ceases to grow further when the Nectria hypha with which it is in contact is only half encircled. A similar hook cell then arises from the zone of the germ tube at the base of the original hook, so that the host hypha becomes clasped between a pair of pincer-like hooks. (Figs 15 and 16) Paired/

Paired hook cells have also been observed to arise laterally from a cell of the germ tube. (Fig.17)

Irrespective of the origin of hook cells, it is clear that they are to be regarded as special structures to effect the removal of substances from the host. They show no further growth in length, and in many cases undergo no further development of any kind. The protoplasm which they contain is granular and fills the cell cavity. The repeated examination of stained preparations has failed to demonstrate the occurrence of direct penetrations of Nectria hyphae, either by appressoria or hook cells. That the connection between the two fungi is a close one is nevertheless borne out by the fact that it has not been possible to separate them, even by the use of needles. There is also some evidence to show that the free ends of the hook cells may become connected with the appressorium from which they are given off, by fusion at a later date, and may thus form closed rings about the Nectria hyphae.

Over a period of some six weeks, no change is visible in the parasitised Nectria hyphae, which continue to give rise to microconidia in great profusion. In cultures eight to ten weeks old, however, several parasitised hyphae have been observed to show constriction at the zone to which the germ tube hook cells are attached. No vacuolisation or other manifestations of degeneracy were involved.

II. The Formation of the Branch System

A weak form of branching of the germ tube has already been described from studies made on pure culture, but the stimulus for prolific branching, which precedes the development of the mycelium of the fungus, appears to be provided only by parasitism of a Nectria hypha.

The appressorium produced by the germ tube at the point of contact with a Nectria hypha undergoes increase in size within twelve hours of its first development of a single hook cell or series of hook cells. Septation is absent, and the appressorium is filled with a granular mass of protoplasm which is richly provided with vacuoles. From an examination of stained preparations, it has been found to be multinucleate, the nuclei being dispersed within the cytoplasm. After a further twelve to twenty four hours' incubation at 18°-20°C, the initials of branch hyphae arise from the periphery of the appressorium. (Figs. 17, 18, 19, 20, 21 and 25)

Branch hyphae may be two to five in number and from their first development are 5-6 μ in diameter. As the growth of the hyphae proceeds, they become septate and highly vacuolate. (Fig. 20)

In those instances where no dilation of the Gonatorrhodiella germ tube precedes the development of hook cells, the branch system arises as hyphal initials from the lateral cell walls of the hooks (Fig. 15)

III. The Development of the Mycelium.

The branch hyphae enter a phase of rapid growth. Side branching is rare for several days, and further hook cells are formed when contact is made with other hyphae of the Nectria colony. Hook cells arise, either as lateral outgrowths from hyphal cells which may or may not become previously distended, or may develop, as did the first hook cells of the germ tubes earlier described, from the growing hyphal tip. Examples of secondary hook cells are shown in Figs. 20, 21, 22 and 23, and an early stage of development of a lateral appressorium is shown in Fig. 16. These secondary hook cells also give rise to lateral branches, although their own growth in length is curtailed after the host hypha has been encircled.

In cultures which have been incubated for about a month, some hyphae of the Gonatorrhodiella colony become free at the surface of the agar to form a loose network within the upper zones of the surrounding Nectria mycelium. Hook cells are rarely developed by these aerial hyphae when the fungus is grown on fungal extract agars, but have been observed on rare occasions in cultures grown on oatmeal mash. (Fig. 24.)

It appears, therefore, that the occurrence of appressoria in culture is principally confined to the surface or sub-surface areas of the substratum, and that the aerial hyphae derive sufficient food products from these contacts to remain independent of the hyphae which constitute the aerial mycelium of the Nectria colony.

IV. The Development of Conidiophores and Variations in
their Morphology

Conidiophores may arise from the branch hyphal system which follows the formation of the first appressorium by the germ tube. Each conidiophore is the product of the lateral extension of a hyphal cell.

From its first appearance, it is greater in width than the hypha from which it is derived, being some 8-10 μ in diameter during the earlier stages of its growth.

The terminal sporiferous bulb frequently develops when the conidiophore is only two or three cells in length; on fungal extract agars, the total height of the conidiophores may be 50-150 μ , before the production of conidia occurs. (Figs. 25 and 26)

The development of the fungus from the germination of the conidium to the production of a young conidiophore is shown in Fig. 25. In this case, the Nectria hypha shows encirclement by three hook cells, and the conidiophore arises directly from an immediate hyphal branch of the prolific growth system which has been the outcome of parasitism.

Variation in the development of the conidiophore particularly affects the degree to which the terminal bulb is developed, and the number and arrangement of the primary conidia. In the most extreme cases, the terminal bulb is scarcely delimited from the remainder of the conidiophore, and bears a single apical primary conidium. (Fig. 26.) In more normal conidiophores, the primary conidia/

conidia are grouped around the bulb in a symmetrical arrangement. (Fig.25). It is worthy of mention that the obconical shape of the primary conidia, an important specific character of the fungus, remains constant, and they are always larger than the secondary and tertiary conidia which follow.

Extreme variation, concomitant with the production of submerged conidia, has been observed twice in culture. An example is shown in Fig.27a, where a lateral hypha of the ramifying sub-surface mycelium has become swollen into a bulbous structure bearing small, hyaline ovate conidia $2 \times 3 \mu$ in size, irregularly at the upper end. These structures are considered anomalous as they appeared in four month old cultures. Observations suggest that they play no significant part in the normal life-history of the fungus either in culture or in nature.

STUDIES ON THE FUNGUS IN CULTURE. -II STUDIES IN PURE CULTURE.

The fungus was brought into pure culture by removing conidia and hyphal fragments from slide cultures on which mixed suspensions of Nectria and Gonatorrhodiella had been poured, to fresh plates. Removal was effected by means of a dummy objective, and transferences were made to oatmeal mush, and to potato-dextrose, malt, fungal extract and plain agars.

The conidia of the fungus were found to germinate readily on fungal extract agars, germination being identical with that already described from observations made on mixed cultures. The growth of the germ tube, which remained 1.5-2.5 μ in diameter, was extremely slow, and ceased after a period of two months. During growth, branches derived from lateral extensions of a few cells of the original germ tube were observed to occur after some three weeks in culture. These hyphal branches were produced at an obtuse angle to the axis of the germ tube, and appeared reflexed. An example of a colony of the fungus after two months' growth is given in Fig. 27b, where reflexing of lateral hyphae is well shown. Limited growth of hyphal fragments was also achieved on fungal extract agars.

Neither conidial germination nor mycelial regeneration was recorded on malt, potato-dextrose and plain agars, and conidia pregerminated on fungal extract agars showed no further growth. Oatmeal mush gave a certain growth of mycelium after planting with pregerminated conidia. Hyphal growth was sparse, the colony assuming a white colour, and growth ceased after a month, before the production of conidiophores.

DISCUSSION

That Gonatorrhodiella Highlei is definitely parasitic has been shown by observations made on its behaviour in culture and under natural conditions. When in pure culture, the growth of the fungus which follows germination of the conidia is poor, and the curious reflexed nature of the lateral branches of the hyphae has been described. The behaviour of the fungus in the presence of the Nectria species presents a marked contrast. The fungal extract agars used during the investigation provide the stimulus necessary for conidial germination, and foster a subsequent slow growth of the germ tube. It is not until the germ tube has contacted a living Nectria hypha that a prolific growth of the fungus is initiated.

The comparatively few papers which have dealt with the parasitism of one fungus on another describe types of parasitism which vary, both in the mechanism adopted by the parasite, and ⁱⁿ the effect which is produced in the host. It is, therefore, proposed to describe examples of these types of parasitism, and to discuss the parasitism of G. Highlei in the light of the generalisations which may be drawn from them.

Parasites which effect a complete and relatively rapid degeneration of their hosts frequently adopt an eventual penetration of their host cells, and may actively produce toxic substances by which degeneration is instigated and accelerated. A fungal association incorporating these/

these principles was reported by Weindling(35), who gave an account of the parasitism of Rhizoctonia solani, Sclerotium rolfsii and species of Pythium, Phytophthora and Rhizopus by Trichoderma lignorum. In these associations, parasitic activity is developed by the lateral hyphae of the Trichoderma which coil tightly round the host hyphae, the protoplasm of which undergoes coagulation and loss of vacuolar structure. The host hyphae also suffer breakages at the transverse cell septa, and bursting in those instances where the hyphae of the parasite and host approach under submerged conditions in culture. The active toxic principle elaborated by the hyphae of Trichoderma was later made the subject for researches. (Weindling, 36, 37, 38.) Warren(34) records a similar form of parasitism of Rhizoctonia solani by Papulospora stoveri, the lateral hyphae of which form coils about the hyphae of the host. The protoplasm of the host hyphae becomes separated into two parts at the point where a lateral branch of Papulospora develops, and a general degeneration sets in. Direct penetration of the host is a feature of Trichoderma and Papulospora, unmodified hyphae entering moribund parts of the host. The external method of attack is, however, more prevalent than the internal.

Reinhardt(25) has provided instances of host degeneration which is brought about by the parasitic activity of Peziza(Sclerotinia) species, in which no internal penetration is operative. The Peziza species described showed active parasitism towards Mucor mucedo, Mucor racemosus, Rhizopus nigricans, Phycomyces nitens,

Dematium(Pullularia) pullulans, Acrostalagmus cinnabarinus, Trichothecium roseum and Fumago salicina. On Phycomyces, Rhizopus and the Mucor species, the lateral hyphae of the Peziza either form coils about the host hyphae, finally forming a dense felt, or are more limited in growth, developing a tuft of short peg-like branches when contact is established with the hyphal wall of the host. The vegetative hyphae of the host are eventually destroyed, and ascending fertile hyphae suffer a similar degeneration.

In the above examples, no complete differentiation of specialised hyphal organs has been in evidence. The parasites concerned are all organisms which attain the destruction of their hosts by means of lateral hyphal branches, which show little evidence of modification. When penetration does occur, the penetrant hyphae are morphologically similar to the remaining hyphae of the mycelium.

Parasitism which is effected through the agency of specialised haustorial organs is seen in Dispira cornuta when parasitic on Sporodinia grandis. (Ayers, 1921) In this fungus, the germ tube which is produced on germination of the conidium becomes septate and branched, and some of the branches parasitise the vegetative hyphae of the host. The appressoria which are developed assume a knob-like appearance and give rise to slender penetration tubes which enter the host hyphae, and then develop finger-like haustorial branches. Haustorial development has also been shown to exist in Syncephalis.

Syncephalis(van Tieghem,32) and Piptocephalis(Brefeld,4a), and morphologically and functionally emulates that in Dispira. Under aquatic conditions, Rhizidiomyces apophysatus(Zopf,39) demonstrates a similar type of parasitism. The fungus, which is parasitic on the oogonia of various members of the Saprolegniae, gives rise to a sac-like structure within the oogonium from which rhizoidal hyphae are developed. Chaetocladium and Parasitella(Burgeff,6) show a curious form of association with members of the Mucorales, in which the fusion of the host hyphae with those of the parasite, and nuclear exchange, are the outstanding features.

It is clear, therefore, that as far as fungal associations are concerned, the morphological specialisation of the parasite, except in the unusual examples, Chaetocladium and Parasitella, where sexuality may at one time have been the dominant factor in the development of parasitism, is confined to the formation of appressoria and haustoria. In those parts of the fungus initiating parasitism, there is a general trend from systems of unmodified lateral hyphae such as are seen in Trichoderma and Papulospora, through the stunted peg-like branches of Peziza, which may be regarded as weakly differentiated appressorial developments, to the more strongly evinced haustorial organs of Dispira, Syncephalis, Piptocephalis and Rhizidiomyces.

The parasitism shown by Gonatorrhodiella Highleyi, described in the present work, presents features which diverge/

diverge from those generally observable in the parasitism of one fungus on another. The development of an appressorium, which is undertaken by the terminal cell of the germ tube when contact is made with a Nectria hypha, parallels the condition described in Peziza and in Dispira. The development of hook cells is, however, unique. Their specialised nature is emphasised by their cessation of apical growth, and by the prolific sympodial growth of the mycelium of the fungus which follows from the lateral walls of the hook cells and from the appressoria or undilated hyphae at their bases. A further point in corroboration is provided by the fusion which may occur between the tip of the hook cell and the appressorium, so that the hook forms a closed collar about the parasitised hypha. G. Highley, therefore, appears to develop an unusual degree of specialisation external to the host in addition to the development of an appressorium, a condition which has not so far been encountered elsewhere.

The development of specialised organs of penetration by fungal parasites appears to be correlated with the lessened effect of parasitism in the host. Where morphological specialisation is absent, the host is frequently destroyed quickly. Trichoderma and Panulospora are more rapid and complete in their effects on their hosts than is Peziza, in which a certain, though weak specialisation is developed. In Dispira, Syncephalis, Piptocephalis and Rhizidiomyces, the host does not undergo rapid degeneration, but a much controlled relationship/

relationship between host and parasite exists. The correlation between specialisation of the parasite and diminishing effect on the host is also of general application to the fungal parasitism of higher plants, where effects brought about by non-specialised hyphae of Rhizoctonia, Botrytis and Pythium may be contrasted with those produced by the haustoria-producing, obligate parasites included in the Erysiphales and Uredinales. (Brown, Brooks and Bawden, 5)

The controlled host/parasite relationship which accompanies specialisation is reflected in Gonatorrhodiella Highlei, but is not apparently correlated with the development of haustoria. In effect, the external morphological specialisation shown by the fungus seems to have replaced the internal form, whilst bringing with it a type of reaction in the host which is usually the outcome of penetration by haustorial hyphae in other cases of fungal parasitism. Parasitised Nectria hyphae maintain growth and reproductive activity when encumbered with a series of appressoria and hook cells. Under natural conditions in the field, the parasitised Nectria species develop abundant perithecial stromata.

The investigation has served to show that fruiting of the Nectria species is not a prerequisite of the development of the parasite, as is suggested by Spaulding(30), quoted in the introductory section of the present work, but that parasitism may be established both in the laboratory and in the field on mycelia possessing at most/

most, only asexual reproductive stages. The association between Gonatorrhodiella Highlei and Nectria cinnabarina is one which has not been previously recorded. The parasitism in culture of Nectria cinnabarina by the Gonatorrhodiella, which was found in association with the other Nectria species on poplar bark, could not be brought about on any media, but the natural association is to be upheld by virtue of the close connection between the pseudoparenchyma of the N. cinnabarina stroma and the Gonatorrhodiella mycelium, and by the absence of any other Nectria species within the bark of the beech log which could have represented an associate.

In his experimental work with G. Highlei, Ayers (1b) succeeded in bringing the fungus into pure culture on synthetic malt, potato, potato-dextrose and malt agars, a scanty mycelial growth occurring on all media, and development of conidiophores only on synthetic malt. On oatmeal mush, he obtained freely sporing, vigorous cultures. With this range of media, he also succeeded in growing the fungus in the presence of Nectria galligena, N. coccinea and N. cucurbitula, but failed with N. Coryli and N. cinnabarina. In the present work, oatmeal mush and fungal extract agars were the only media which gave any mycelial development in pure culture, and conidiophores were never observed. In mixed culture, parasitism was found to be reversible on potato-dextrose agar, and could not be obtained on malt and plain agars, although it was easily established on oatmeal mush.

It seems, therefore, that the capacities of Gonatorrhodiella isolates for the development of parasitism on agar media may vary, as it is apparent that the Gonatorrhodiella cultured in Edinburgh more nearly approaches the obligate state than the North American isolates of the fungus described by Ayers(1b). The relationship between G. Highlei and species of Nectria is suggested as one which is dependent on the substratum, in that changes in the nutrient component of the substratum may either inhibit or reverse parasitic activity. The difficulty of growing G. Highlei on agar substrates was particularly stressed by Ayers(1b), who cited several parallel cases of fungi parasitic on insects and on other fungi. It is not clear, however, whether the difficulty of culture is to be considered purely as the outcome of the inclusion of agar in the medium, or is due to the absence of various biochemical substances.

Regarding the germination of conidia, Ayers(1b) stated: -"To secure pure cultures of Gonatorrhodiella Highlei, single-spore isolations were used at first. However, these conidia failed to germinate on the different media used and even to date the conditions necessary for their germination have not been discovered." It has been shown in the present work that extracts prepared from Nectria cinnabarina, a second Nectria sp., Trichothecium roseum and Trichoderma lignorum isolates which were growing in close proximity to the Gonatorrhodiella in nature would, when incorporated singly into agar media, provide/

provide substances which were capable of stimulating both conidial germination and a limited hyphal growth of the Gonatorrhodiella. Although it has not been within the scope of the investigation to define the nature of the substances promoting conidial germination, it is of interest that they are heat stable, and it seems likely that they are easily diffusible growth products. The results obtained with Trichothecium roseum and Trichoderma lignorum extracts are in one sense paradoxical, in view of the antifungal properties of their extracts reported by Freeman and Morrison(15) and Weindling(36,37,38) respectively.

The behaviour of Gonatorrhodiella Highleyi under natural conditions may well be governed by the diffusion of substances from surrounding fungi, the point of greatest interest being that the substances do not appear to be necessarily produced only by the fungal host of the Gonatorrhodiella.

THE FORMATION OF INTRAHYPHAL MYCELIUM

Evidence of the formation of intrahyphal mycelium in Gonatorrhodiella Highleyi was gained in cultures where the media used were fungal extract agars, and in nature from the colony of the fungus which occurred on poplar bark.

Endogenously developed hyphae were observed in both old and young cultures. In cultures six to ten weeks old, they were confined to the upper three cells of young actively growing conidiophores, which were still producing conidia from their terminal bulbs. In the vegetative mycelium of the fungus, intrahyphal growths were observed in cultures which had been incubated for three months at 18°-20°C, and also occurred during regeneration of the fungus within hyphal fragments sown on extract agars. Under natural conditions, limited intrahyphal growth took place during the proliferation of the conidiophores.

All intrahyphal growth forms have their origin in the upper transverse septum of a cell, which may become entirely evaginated, or only partly evaginated, during the course of development, to produce a sac which enters the cell above. They obey the polarity of growth shown by the hypha in which they are produced.

Fig. 28, a, shows an example of localised septal evagination at an early stage of development, in which the growth has originated from the central area of the transverse septum, the area which most commonly gives rise/

rise to intrahyphal mycelia. The occurrence of a distinct septal pore in most septa of conidiophores has been mentioned earlier (p.10). Intrahyphal growths, which have their origin in the area of the septal pore, appear to develop by the assumption of meristematic activity by the zone of the cell wall which defines the limits of the pore. A diagrammatic representation of a transverse septum showing this zone is given in Fig.28b. In that intrahyphal growths are closed at the upper end from their first appearance, closure of the pore must be presumed to occur before or during the earliest stages of septal evagination. It has not been possible to observe pore closure, but in the very early stages of the process of evagination, the continuity of the septum has been clearly defined in many cases.

In the most simple examples, the continued growth of the apex of the first formed sac causes the appearance of a thin-walled, septate hypha, which grows longitudinally within the enclosing cell, gradually increasing in width to fill the cell. Increase in width occurs a short distance from the base in the first instance (Fig.29), and moves progressively upwards.

Intrahyphal growths have been observed to pass through conidiophore septa and invade cells higher in the conidiophore. (Fig.30 a) During the passage of an intrahyphal growth through the upper transverse septum of an enclosing cell, the septum suffers no immediate disintegration. It appears that the tip of the growth effects/

effects a fusion with the septum, and that the circular fusion zone continues active growth, whilst the remaining area of the septum, which is connected to the longitudinal cell wall of the hypha, forms a collar round the intrahyphal growth (Fig. 30b).

Fusion has been seen to occur between endogenously developed hyphae produced within the same enclosing cell. Several prostrate branches were observed to grow from the hyphae of a Gonatorrhodiella colony which lay at the surface of Nectria/extract agar. They arose laterally from hyphal cells, and simulated the appearance of young conidiophores during the earlier stages of their growth. In one of these structures, an intrahyphal growth occurred in the apical cell. (Figs 31 and 32) The intrahyphal growth was joined to the subterminal cell of the parent hypha at two separate points, but formed a continuous loop at the upper end of the enclosing cell. This suggests the growth, in the first instance, of two separate hyphae from the upper septum of the subterminal cell, and the final union of the two by fusion at a higher level in the enclosing cell.

The intrahyphal growths, which are formed within cells in contact with agar, finally become free from the cells by the formation of lateral branches. This is also shown in Fig. 32, where each outgrowth is given off from a short, slightly distended cell, and emerges from the enclosing cell wall to become free to the surrounding agar medium.

During the regeneration of the mycelium of the fungus from hyphal fragments sown on fungal extract agars, intrahyphal growth forms which have originated in localised septal evaginations are comparatively common. An example is shown in Fig.33a. The investigation has made it clear that localised septal evaginations, whether centrally or more peripherally placed, are the most usual means by which the formation of intrahyphal mycelium is attained.

In conidiophores obtained from extract agar cultures and from the natural habitat of the fungus on poplar bark, however, intrahyphal growth forms originating in the total evagination of a transverse septum have been found to bring about a type of conidiophore proliferation. In the earliest stages of development, a transverse septum forms at a variable distance from the apex of the terminal bulb of a conidiophore which is still in a sporing condition. (Fig. 33b,c) By the upward growth of this septum, the original upper wall of the bulb immediately above it is ruptured and forms a collar to the new outgrowth. (Figs 34, 35, 36)

It cannot be clearly ascertained whether intrahyphal growth by means of total septal evagination is the only method involved in some proliferations, which at a final stage of their development, show only short collars at the point of union with the former apical bulb, such as are shown in Figs. 34, 35. Examination of conidiophores showing early stages in proliferation has/

has suggested that some proliferations may have their origin in a tangential splitting of the wall at the apex of the bulb, so that the collar represents the outer of the two walls which are so formed, after the outgrowth of the inner wall has taken place.

The proportion of conidiophores in which intrahyphal growth of tangential wall splitting has been instrumental in effecting proliferation, is small. The majority of conidiophores show no evidence of these processes, and proliferation is apparently brought about by direct growth from a localised area of the wall of the terminal bulb. Some conidiophores, in which several proliferations have occurred, have been observed to show both segments bearing collars and those devoid of them.

Cells, within which intrahyphal growth forms develop, may or may not possess cell contents. In Fig. 29 the enclosing cell contains a considerable quantity of protoplasm which appears to be in a state of dissolution, whereas the evaginating cell is almost empty, and has passed some of its content to the intrahyphal growth. The enclosing cell in Fig. 32 is ^{almost} devoid of protoplasm, and very little cell content remains in the cell from which the intrahyphal growth originated. The occurrence of intrahyphal growth has never been recorded prior to the elaboration of conidia, but is usually observable in conidiophores which have already shed conidia, or are still in the process of sporangium. This point, taken together/

together with the fact that the lower cells of conidiphores have frequently been observed to lose their protoplasmic content during sporing and shedding of the conidia, seems to imply that drain on the protoplasm occasioned by sexual reproduction may provide the stimulus for the development of intrahyphal mycelium.

In the vegetative mycelium of the fungus growing in old cultures, and during regeneration of the mycelium from hyphal fragments, however, intrahyphal growths invariably appear in cells which are empty. Whether the protoplasm was absent before septal evagination, or whether it was lost in participation in the development of the intrahyphal growth, is not clear.

DISCUSSION

From the various observations which have been made of self-penetration, or formation of intrahyphal mycelia, in Gonatorrhodiella Highlei, it has been established that two distinct methods of development are involved. The two differ in the extent to which the transverse septum of the cell which gives rise to the intrahyphal growth, participates during the early stages of evagination.

In the prostrate basal hyphae of the fungal colony, and in the lower cells of the conidiophores, only a limited, central or peripheral zone of a transverse septum is evaginated to initiate an intrahyphal growth, and the position of the septum can be easily distinguished after development is complete. In the subterminal cells of freely sporing conidiophores, however, the entire upper transverse septum may be evaginated to form a hypha, by which the eventual proliferation of the conidiophore is attained. Where this occurs, the transverse septum is wholly incorporated in the new growth, and thus loses its identity.

The distinction between localised and total evagination was mentioned by Lindner(19) in a valuable review of a number of plants which demonstrate the phenomenon of self-penetration. Although the number of fungi which show the phenomenon is small, various examples of intrahyphal growth from localised evagination have appeared in the literature from time to time.

From these references, the following fungi may be mentioned: -Sclerotium hydrophilum(27), Alternaria(19), Botrytis cinerea(2), Rhizoctonia solani(12), Pullularia pullulans(17), Ascobolus magnificus(11), Gymnosporangium spp. (10), Ascoidea rubescens(33), Chaetomium Kunzeanum(40), and Saprolegnia ferax var. hypogyna(24).

In their method of development and appearance, intrahyphal growths which have originated from localised septal evaginations in the above fungi are similar to those which have been described for Gonatorrhodiella during the present investigation. In certain cases, notably, Pullularia pullulans(17), Botrytis cinerea(2) and Ascoidea rubescens(33), intrahyphal growths have been observed to abstrict conidia within the cells containing them. The formation of endoconidia has not, however, been seen in Gonatorrhodiella. The regeneration of hyphal fragments by self penetration, which has been described in the latter fungus, was mentioned by Duggar and Stewart(12) in their researches on Rhizoctonia solani.

As a result of his work on Botrytis cinerea, Epicoccum purpurascens and Alternaria sp., Lindner(19) commented on the frequency of the occurrence of intrahyphal mycelia in old cultures, and their rarity in young fungal colonies. Dodge(11) added a similar observation for Ascobolus magnificus, and Walker(33) for Ascoidea rubescens. The degeneration or death of hyphal cells in fungal colonies, which have been growing on media for some time, is common, and may be attributed in part/

in part to the depletion of nutrients and moisture from the substrate. In those fungi which display the phenomenon of self-penetration, intrahyphal developments have often been directly associated with dead or degenerate cells. Both Lindner(19) and Dodge(11) have viewed many of these growth forms as 'Bridging hyphae', which by their passage through senescent or dead areas of the hypha, connect successive healthy segments.

'Bridging' involves the formation of a cell outgrowth, which effects fusion with a further cell at some distance from its place of origin, intervening cells thus losing their significance as functional units. Many instances of the phenomenon have been figured for Ascobolus magnificus(11) and Alternaria sp.(19). Lewis(18) has also recorded 'bridging' in the Red Alga, Griffithsia Bernetiana, in which he describes and illustrates the continuity of the algal filament which is brought about by the formation of intrafilamental outgrowths, which enter a dead cell from the cells on either side, and finally fuse with each other. Fusions between intrahyphal mycelia which have separate points of origin are also known to occur in the fungi. Lindner(19) has recorded several fusions in Alternaria sp. which have occurred both within enclosing cells, and outside the 'parent' hypha. An example of fusion within a single enclosing cell has been described in Gonatorrhodiella, but no instances of fusion between branches of intrahyphal mycelia, which have subsequently become free by outgrowth/

outgrowth from the enclosing hypha, have been forthcoming.

The degeneration of cells, prior to their penetration by intrahyphal growths, is further illustrated by Dodge(10) in his work on Gymnosporangium clavariiforme, G. nidus-avis, G. macropus, and G. globosum. In these fungi, the teleutospores originate from the subterminal cells of the tissue which composes the teleutospore sorus. Before the evagination of the upper transverse septum of a subterminal cell, the terminal cell undergoes nuclear and cytoplasmic degeneration, so that in many cases the outgrowth enters an empty cell, within which it differentiates to give a teleutospore.

This frequent occurrence of the penetration of degenerate or dead cells by intrahyphal growths prompted Lindner(19) to forward the suggestion that the formation of septal evaginations may be facilitated by the reduction in osmotic pressure attendant upon degeneration. Klöcker and Schiönning(17), in their account of self-penetration in Pullularia pullulans, preferred to consider the phenomenon as one incorporating a form of parasitism, where 'strong' cells could be regarded as parasitising adjacent 'weak' cells.

During the present work on Gonatorrhodiella Highlei, the variation in the content of enclosing cells has been mentioned, and doubt expressed as to the quantity of protoplasm present within them prior to the development of intrahyphal growth forms. It may be the case that where a certain amount of protoplasm exists, it acts/

acts as a source of nutrients for the developing growth. No evidence has been forthcoming of the contraction of the protoplasm of an enclosing cell, and the subsequent development of a wall around it, as was shown in Botrytis cinerea by Beauverie and Guilliermond(2).

Whether or not osmotic pressure relationships are involved in the process of intrahyphal development, it appears that the occurrence of the latter is always correlated with a physiological drain, which is occasioned either by sporing in the case of conidiophores, or by depletion of nutrients and moisture from the substrate in the case of the vegetative mycelium of the fungus in old cultures. The rather limited type of growth which the fungus shows on fungal extract agars is indicative of the fact that these substrates do not provide the full nutrient requirement of the fungus, a condition which may add to the physiological drain set up in the conidiophores by sporing.

Irrespective of the method by which intrahyphal growth forms are developed, they all tend to bring about an increase in the total surface area of the fungus. This might be viewed as a plant response to adverse conditions, by which a greater absorptive surface is developed in the substrate in those instances where intrahyphal growths become free from their enclosing cells. In young, actively sporing conidiophores, partially empty cells frequently develop intrahyphal growths which enter cells in which a greater protoplasmic content/



content is present. This may also be regarded as a cell response, bringing about an increase in absorptive surface at a remaining source of food materials.

The power to develop self-penetration is only known to be possessed by a limited number of plants. The majority of fungi do not show the phenomenon even when growing under extremely disadvantageous conditions in culture. It is likely, therefore, that the expression of self-penetration is dependent on a genetic basis.

Fungi which display self-penetration in which total septal evagination is involved are rare. Lindner(19) discovered an example during his work on Epicoccum purpurascens. In this fungus, short lateral hyphal branches are cut off by septa from the cells in which they have their origin. Backwardly directed evaginations of these septa have been shown to give intrahyphal growths within the parent hypha. A curious form of total evagination is also to be found in Inzengaea. Borzi(3) records the formation of bladder-like cells in the hyphae which arise from the outer layers of the perithecial wall, and to which he ascribes a protective function. By the total evagination of the upper transverse septum of the subterminal cell of such a hypha, a short intracellular growth appears in the bladder cell.

Although instances of the kind mentioned above have rarely been recorded, total septal evagination is known to operate under somewhat different circumstances, and is characteristic of the genus Saprolegnia(8).

It/

It results in the process of sporangial proliferation. The sporangium sheds its contents, and by the total evagination of the upper transverse septum of the subsporangial cell, the cavity of the sporangium receives an outgrowth from which a further sporangium is later derived. The process may be repeated many times in the same sporangiophore. It is of interest that localised septal evagination from the subsporangial cell may replace the total form. Coker(8) figures both types of development in Saprolegnia monoica var. glomerata. Normal sporangial proliferation occurs together with abnormal proliferations in which localised septal evaginations bear terminal oogonia and antheridia. The phenomenon of sporangial proliferation, attained by a method similar to that described in Saprolegnia, has also been recorded by Butler(7) in Pythium proliferum.

A somewhat more complex form of proliferation is shown by the 'hemiascus' of Ascoidea rubescens. The precise nature of the 'hemiascus' of this fungus is not entirely clear, but it has been stated by Walker(33) to be an apogamously developed ascus. In general, proliferation which is identical in mechanism to that in Saprolegnia follows upon the evacuation of the ascus. The new outgrowth, which arises by the total evagination of the upper transverse septum of the subascal cell, enters an almost empty ascus. A certain amount of variation does, however, occur in the process.

Brefeld/

Brefeld(4b) recorded instances in which the evagination of the septum proceeded whilst the major proportion of the ascus contents still remained. He therefore considered that proliferation assisted the function of ascospore dispersal by the pressure to which it gave rise. From her researches, Walker(33) concluded that such examples were abnormal, and claimed that osmotic pressure relationships were more clearly involved in bringing about spore dispersal.

In some of the proliferations of the conidiophores in Gonatorrhodiella Highlei, the new hyphal growth may also arise from the upper transverse septum of the subterminal cell, and penetrate a terminal cell which still contains protoplasmic contents. Saprolegnia spp., Ascoidea rubescens and Gonatorrhodiella Highlei may therefore be considered to present a series in which the adoption of true intrahyphal growth by a form of total septal evagination is developed. In the sporangia of Saprolegnia spp., and in the normal asci of Ascoidea rubescens, the growth which arises from the septum of the evaginating cell enters a cell cavity usually devoid of contents and open at the upper end. In the strictest sense, true intrahyphal development is not present, although the method of growth adopted is mechanically the same. In the abnormal cases of ascus discharge in Ascoidea, the hyphal growth enters the ascus whilst ascospores and epiplasm are present, but the apex of the ascus is still open. In Gonatorrhodiella, intrahyphal growth of a true type is shown by the entry of

a septal evagination into a closed and active cell. As far as the author is aware, the type of proliferation which results from the development of intrahyphal growth in Gonatorrhodiella is unknown elsewhere in the Hyphomycetes. The phenomenon of tangential splitting of the cell wall which is also concerned in some of the conidiophore proliferations of the latter fungus is reminiscent of the mechanism adopted in Thielavia basicola during the production of conidia. (41)

SUMMARY

Gonatorrhodiella Highlei A.L.Sm., a rare member of the Fungi Imperfecti, only once recorded in Great Britain before, was found on the bark surfaces of stored beech and poplar logs at the Royal Botanic Garden, Edinburgh. On the beech bark, the fungus was found to be associated with the perithecial stromata of Nectria cinnabarina (Tode) Eries., a fungus on which it has not been previously recorded, and on the poplar bark with a fungus provisionally identified as a species of Nectria, possessing a Cylindrocarpon macroconidial stage.

The taxonomic relationships of the fungus are discussed, and amendments to the technical description are proposed.

In culture, conidial germination of G. Highlei and its development of parasitism on contact with the Nectria sp. were observed on agars incorporating extracts obtained from Trichoderma lignorum (Tode) Harz., Trichothecium roseum Link and the Nectria sp. The Gonatorrhodiella also grew well in the presence of the Nectria sp. on oatmeal mush, but no development of the fungus was observed on potato-dextrose, malt and plain agars.

The parasitism shown by the fungus involves the production of appressoria and an elaboration of hook-cells of limited growth which encircle the hyphae of the Nectria sp. The effect of parasitism on the Nectria sp. is slight, and results in a thinning of the hyphae at points/

points where hook cells of the Gonatorrhodiella are present. From observations made on mixed cultures on potato-dextrose agar, certain evidence for the reversibility of parasitism was obtained. Parasitism of Nectria cinnabarina by G. Highlei was never observed in culture.

A limited growth of G. Highlei occurred in pure culture on oatmeal mush and on fungal extract agars. No growth was recorded on potato-dextrose, malt and plain agars.

The type and development of parasitism shown by the fungus, and the nature of its association with other fungal species are discussed.

The occurrence of intrahyphal mycelium was noted in the conidiophores and mycelium of G. Highlei. Two methods of development are distinguished. In the first, intrahyphal growth forms arise by the localised evagination of a cell septum, and in the second by total evagination. Localised evagination is the more usual, and has been recorded in both conidiophores and mycelium, whilst total evagination brings about a specialised form of proliferation in the conidiophores. Proliferation brought about by this means is believed to be unknown elsewhere in the Fungi Imperfecti.

The conditions under which self-penetration occurs, and the mechanism involved in the process are discussed, and the view that physiological drain is the dominant factor concerned is put forward.

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EXPLANATION OF FIGURES.

Fig. 1.

Conidiophores of Gonatorrhodiella Highlei on poplar bark, seen in surface view. x 60.

Fig. 2.

The sporiferous head of a young conidiophore of G. Highlei showing the terminal bulb with primary conidia(p), each of which bears a group of secondary conidia(s). x 320.

Figs 3. , and 4.

Mature conidiophores of G. Highlei showing complex, branched chains of conidia.

Fig. 3. x 320.

Fig. 4. x 580.



Fig. 1.

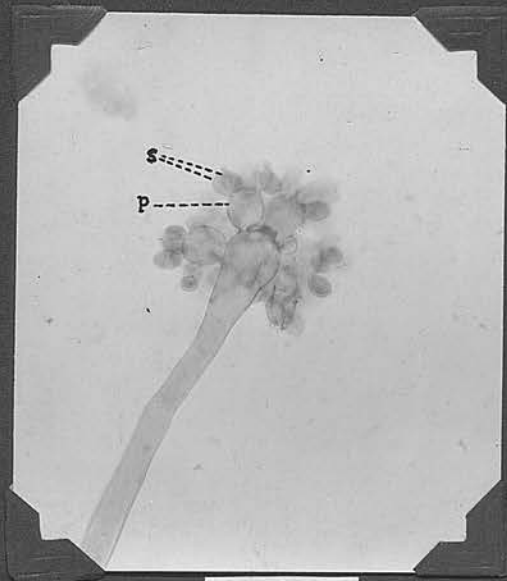


Fig. 2.



Fig. 3.

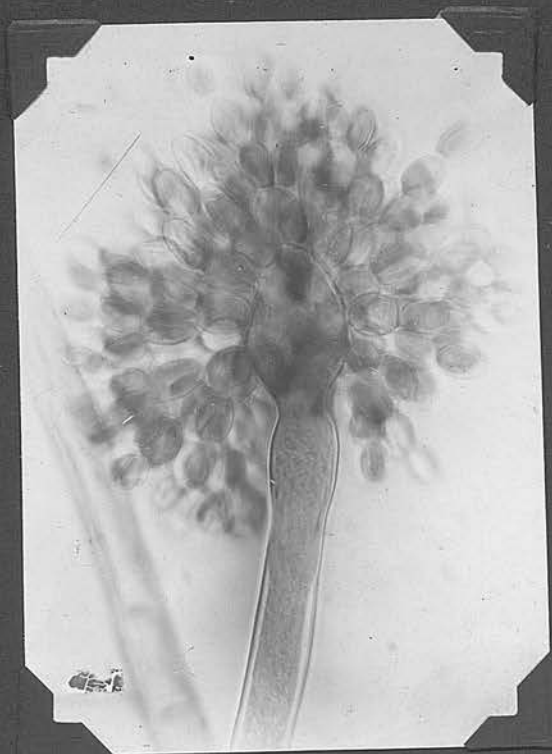


Fig. 4.

Fig. 5.

Branched conidiophores of Gonatorrhodiella

Highlei .

- a) Conidiophore showing branching from the base. x 220.
- b) Conidiophore showing proliferation of the first-formed sporiferous bulb, and subsequent branching. x 220

Fig. 6.

- a) Part of the basal mycelium of G. Highlei obtained from culture, illustrating irregularity of septation. x 680.
- b) A primary conidium bearing a terminal group of secondary conidia. x 1000.
- c) The terminal bulb of a young conidiophore showing early stages in the development of primary conidia. x 1600.
- d) Part of the terminal bulb of a young conidiophore showing a later stage in the development of two primary conidia, one of which shows a basal septum with a distinct central pore. x 750.
- e) Part of a mature conidiophore illustrating the presence of a central pore in the transverse septum. x 1500.

Fig. 5.

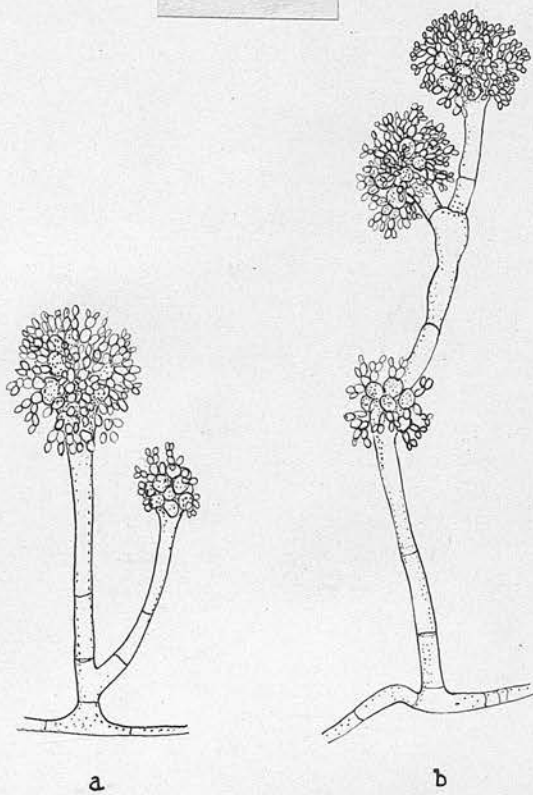


Fig. 6.

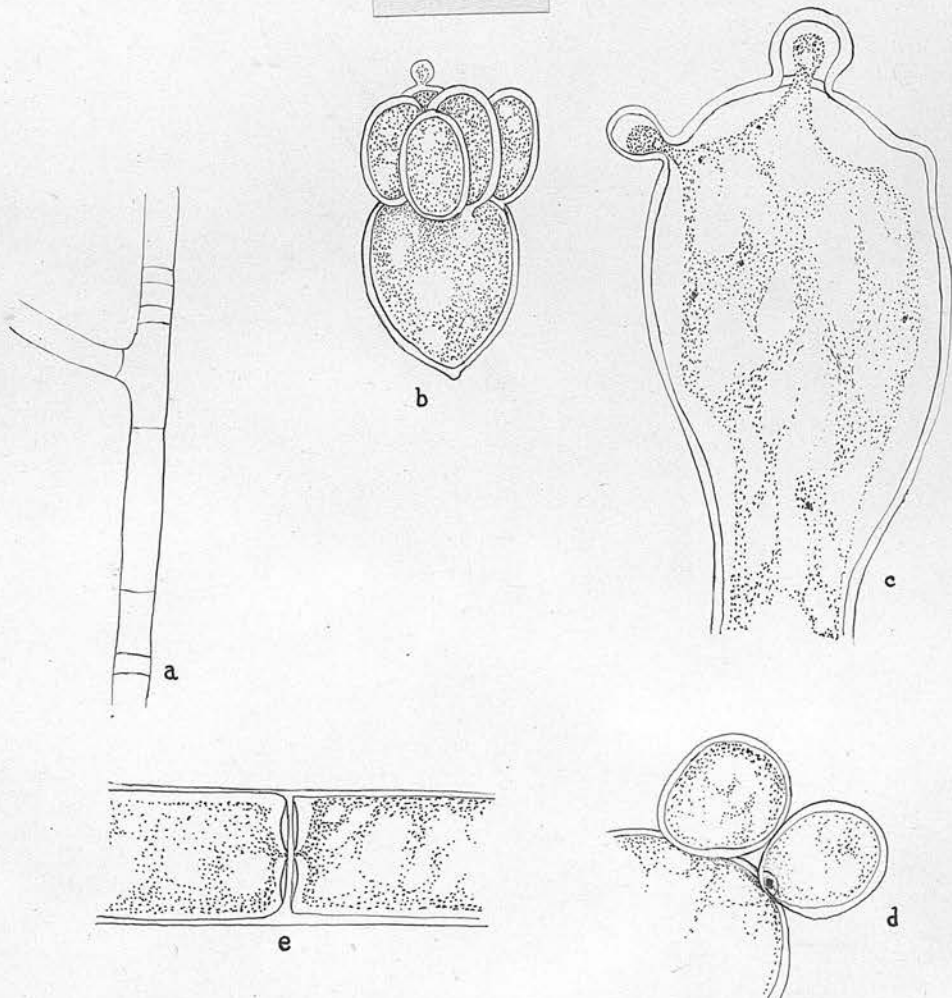


Fig. 7.

A longitudinal section through the perithecial stroma of Nectria cinnabarina on beech, showing the mycelium of G. Highleyi undercutting the pseudoparenchyma of the stroma at (a), and lying between the perithecia at (b). Conidiophores are seen at (c). x 150.

Fig. 8.

As above. x 150.

Figs. 9 & 10.

Conidiophores of G. Highleyi obtained from poplar bark showing investment by the hyphae of the Nectria sp. x 680.

Fig. 11.

- a, b and d) Stages in the development of germ tubes produced by tertiary conidia.
 - c) The same in a secondary conidium.
 - e) A primary conidium showing two germ tubes.
 - f) Abnormal germination of a tertiary conidium showing the development of a distal germ tube from the initial of a quaternary conidium.
- x 970.

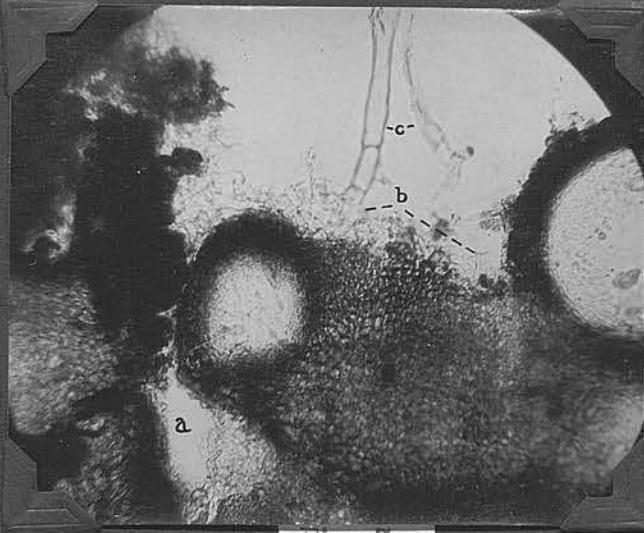


Fig. 7.

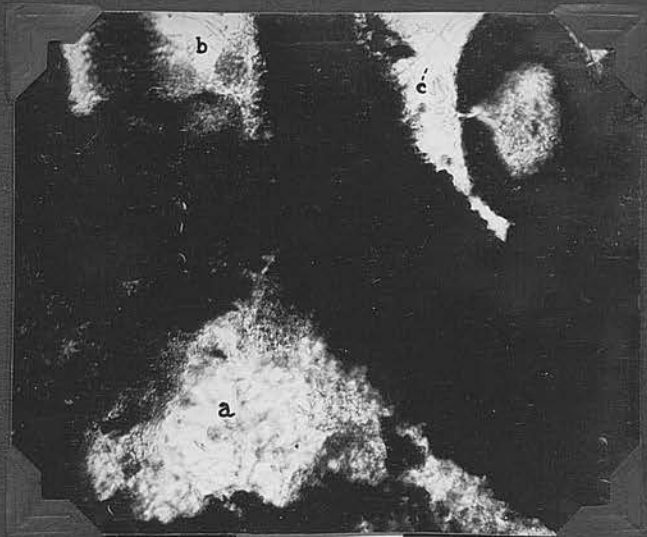


Fig. 8.



Fig. 9.

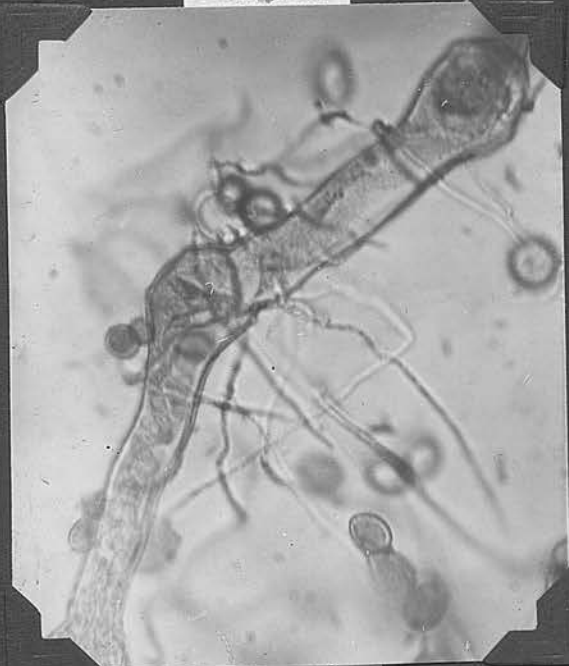


Fig. 10.

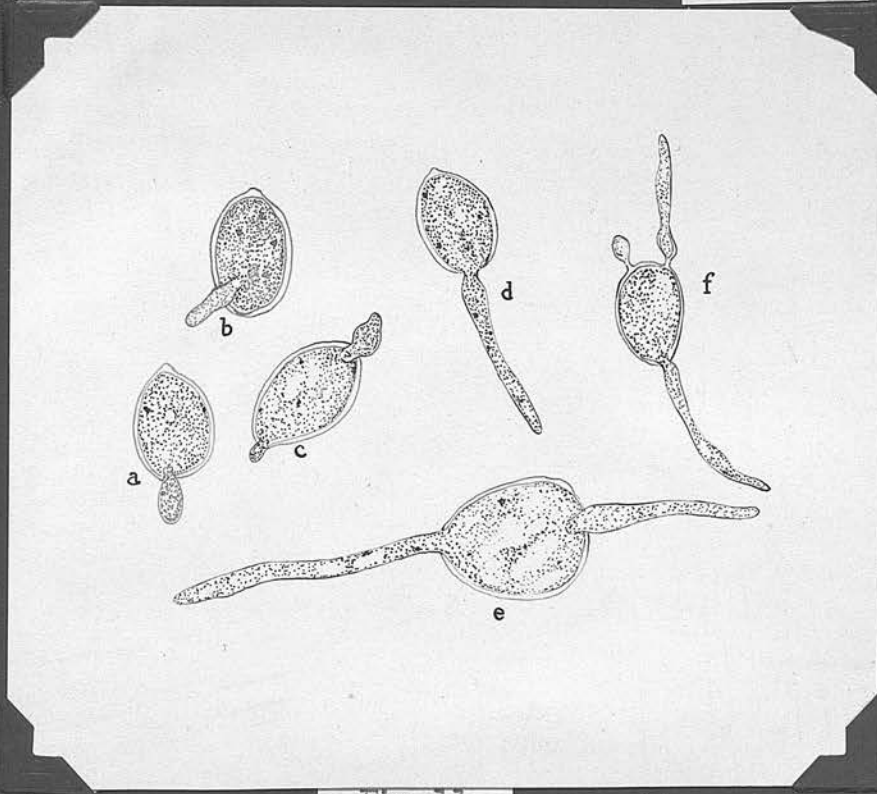


Fig. 11.

Fig. 12.

Parasitism of the Nectria sp. (n) by G. Highlei.

An early stage in the development of an appressorium(a) and hook(c) by the germ tube when contact is made with a Nectria hypha. x 700.

Fig. 13.

The parasitism of the hyphae of a Nectria colony by the germ tubes produced by two conidia of G. Highlei. On the left, the germ tube has produced a hook cell at (c) and has developed an appressorium and hook at (a). On the right, increase in size is seen in the appressorium at (d). x 600.

Fig. 14.

The parasitism of a Nectria hypha (n) by a germ tube of G. Highlei, showing the development of two hook cells(c). Branch hyphae(b) have originated from the zone of the primary appressorium. x 1600.

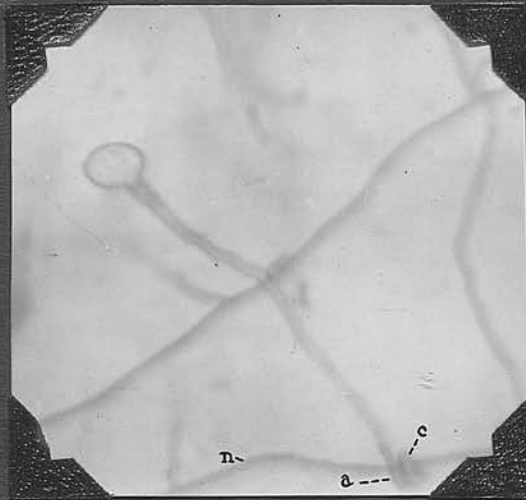


Fig. 12.

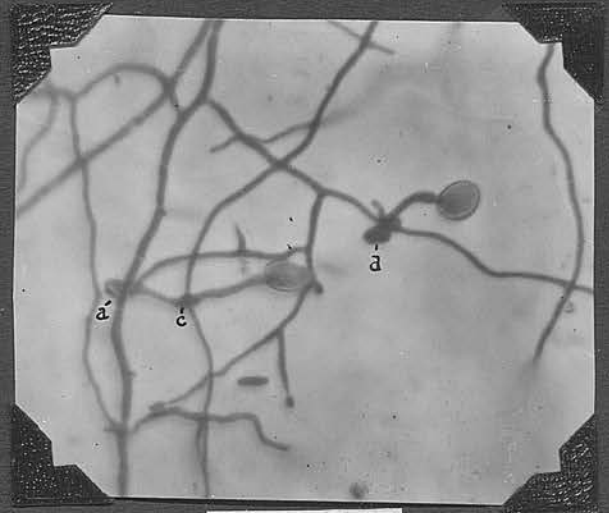


Fig. 13.

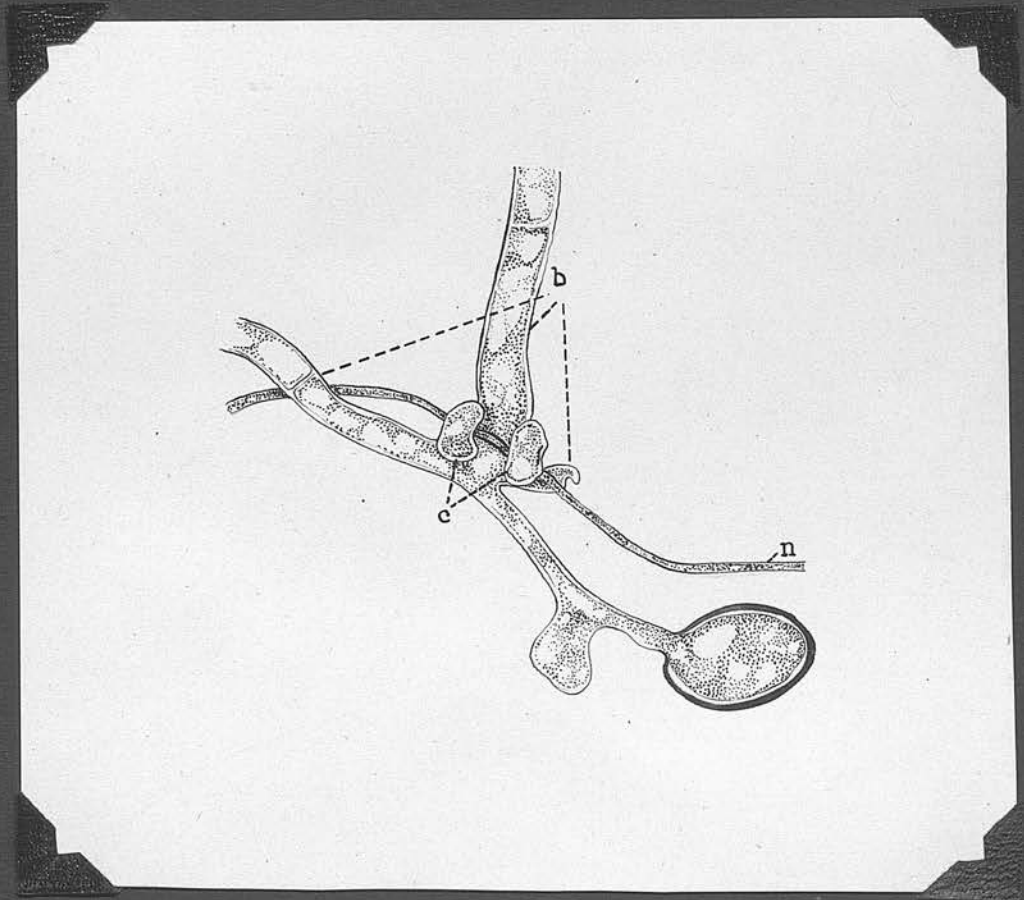


Fig. 14.

Fig. 15.

The development of paired hook cells (p)
by the germ tube of Gonatorrhodiella.
The origins of two hyphal initials are
shown at (h). x 1600.
n- Nectria hypha.

Fig. 16.

The development of paired hook cells (p)
by the germ tube of Gonatorrhodiella.
A lateral appressorium is shown at (a).
x 1600. n-Nectria hypha

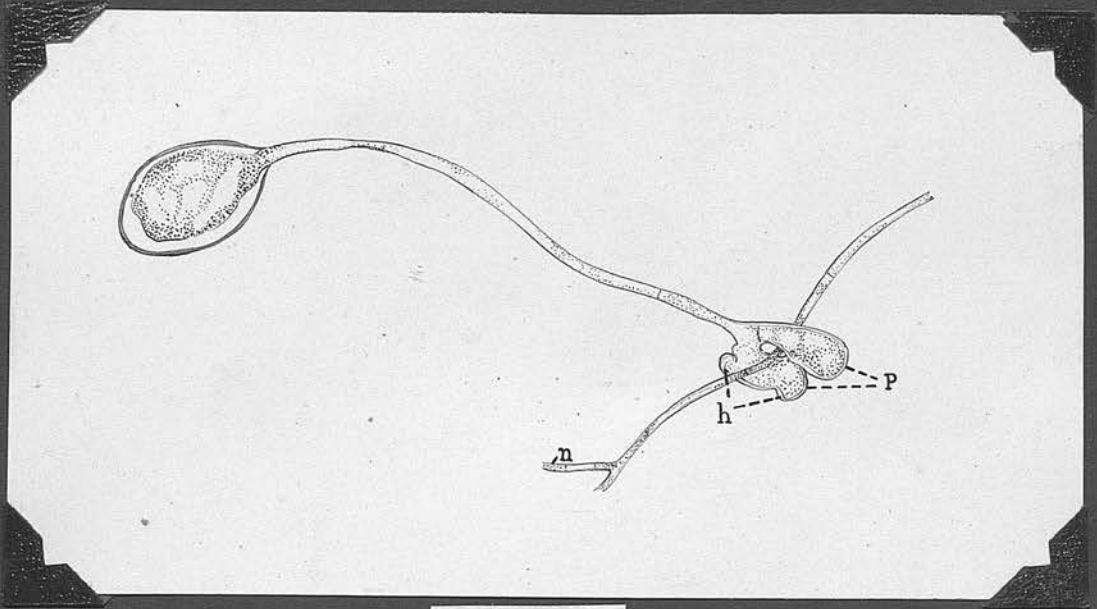


Fig. 15.

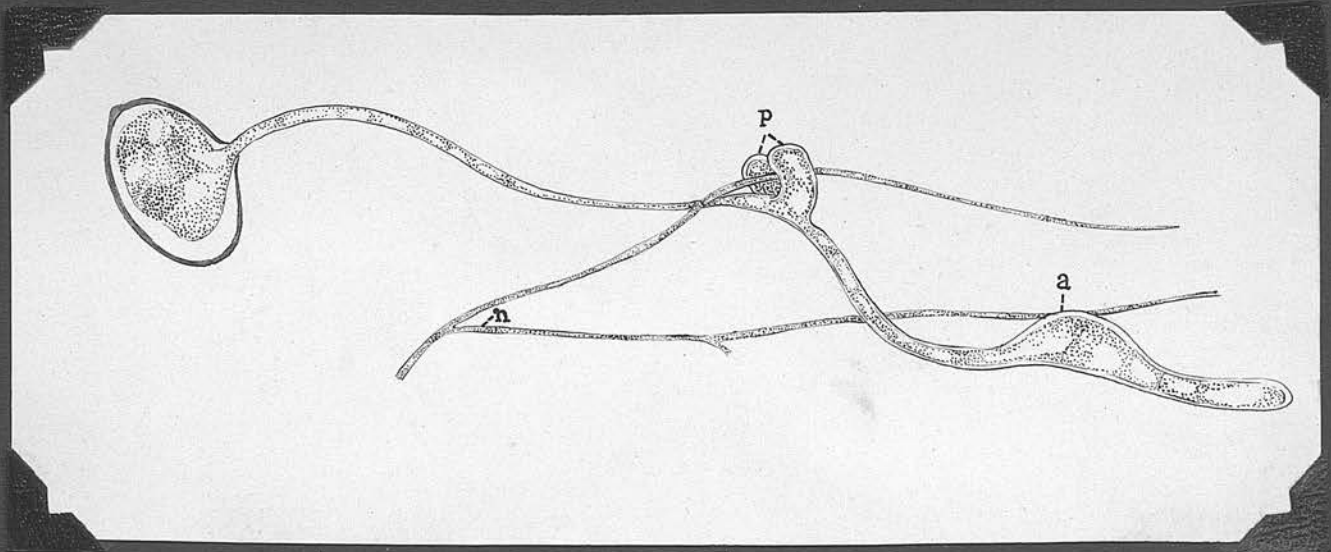


Fig. 16.

Fig. 17.

Paired hook cells(p) arising laterally from the germ tube of G. Highleyi. A young branch hyphal system derived from an appressorium (a) is also shown.

x 600.

Fig. 18.

An early stage in the development of a branch hyphal system derived from an appressorium (a) of G. Highleyi.

x 1600.



Fig. 17.

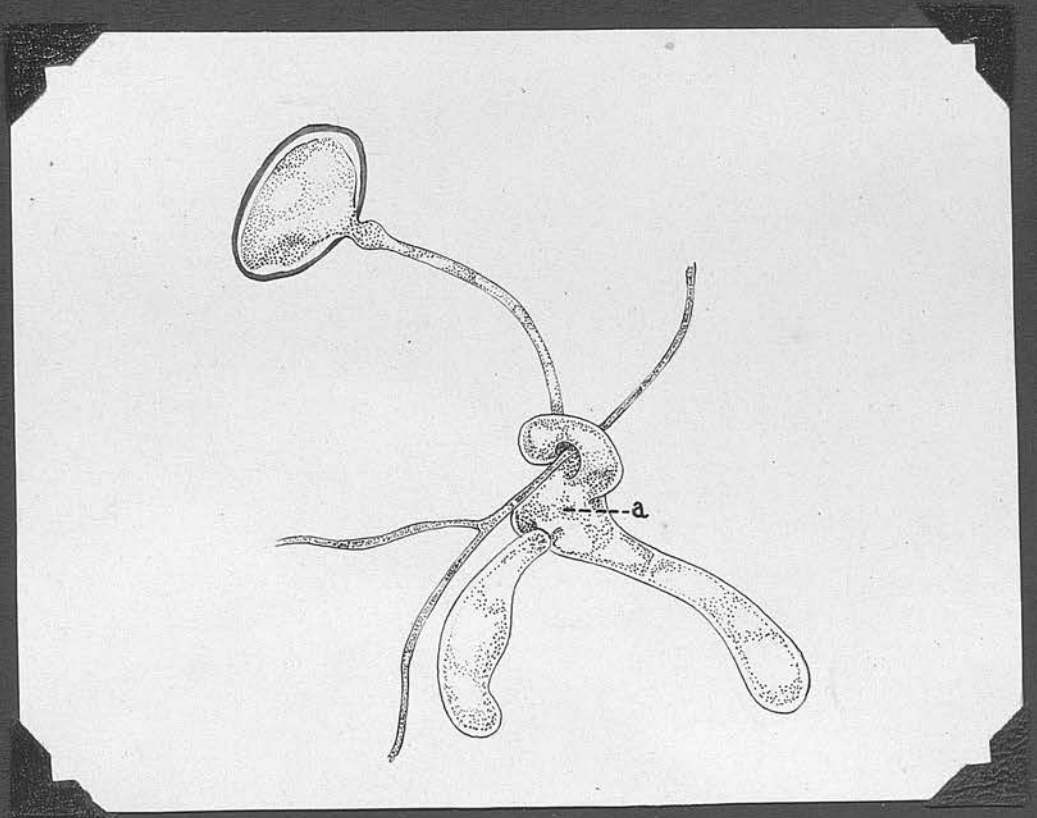


Fig. 18.

Fig. 19.

A stage in the development of a branch
hyphal system of G. Highleyi. Distended
hyphae(h) arise from the zone of the
primary appressorium(a). x 1650.



Fig. 19.

Fig. 20.

A late stage in the development of a branch hyphal system of G. Hichlei. The hyphae produced from the zone of the primary appressorium(a) have produced secondary hook cells(c) at various points on the surrounding Nectria hyphae(n). x 1650.



Fig. 20.

Fig. 21.

A late stage in the development of a branch hyphal system of G. Highlei. The hyphae produced from the zone of the primary appressorium(a) have given rise to secondary hook cells(c) at various points on the surrounding Nectria hyphae(n). x 1600.

Fig. 22.

A secondary appressorium of G. Highlei is shown at (a). Hook cells(c) have given rise to lateral hyphae(h). x 1600.

Fig. 23.

A similar stage to that shown in Fig. 22, showing secondary hook cells(c), and a secondary appressorium(a) giving rise to three hyphae. x1600.

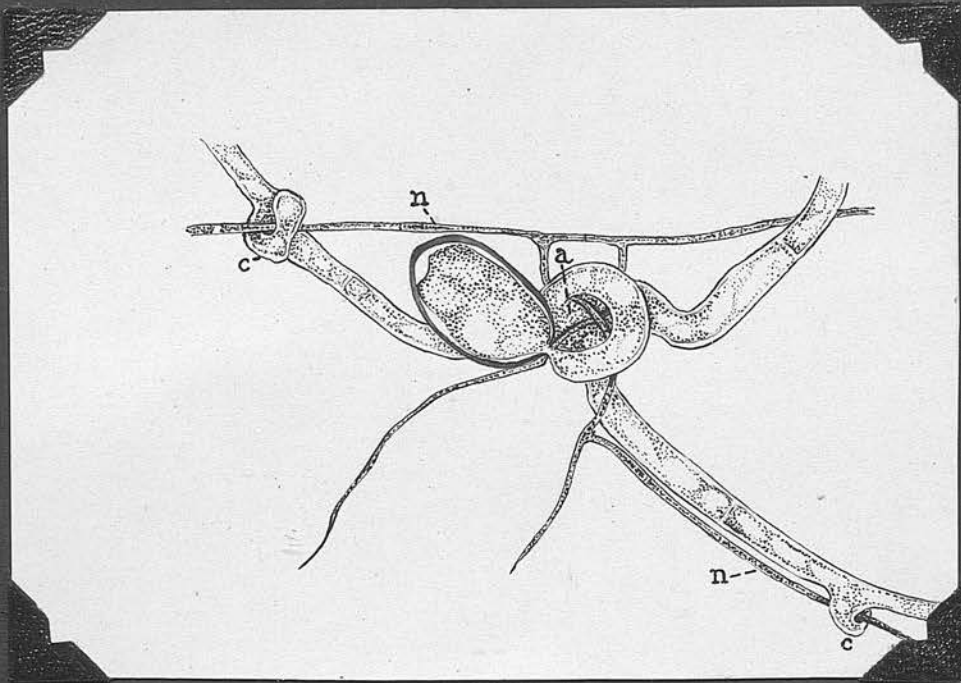


Fig. 21.

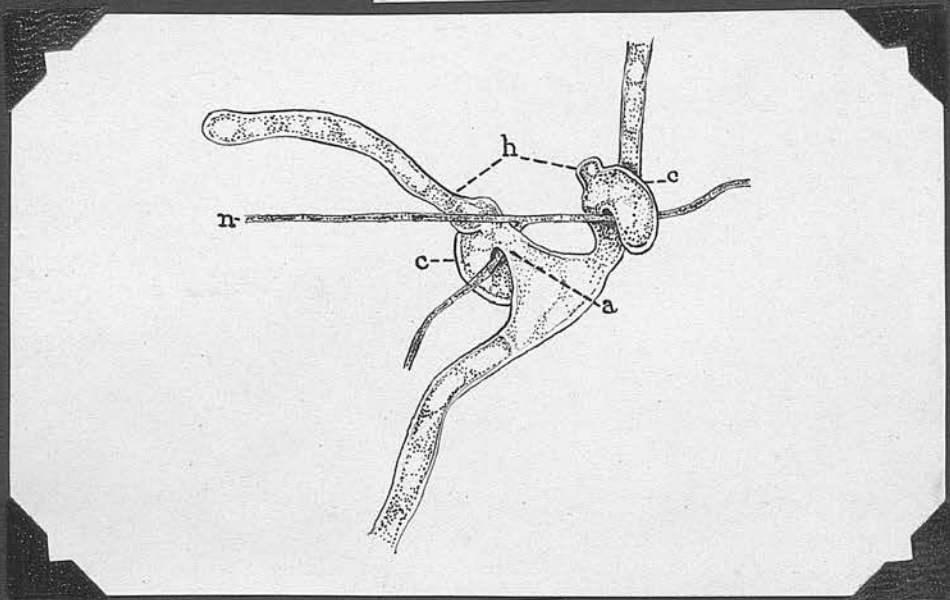


Fig. 22.

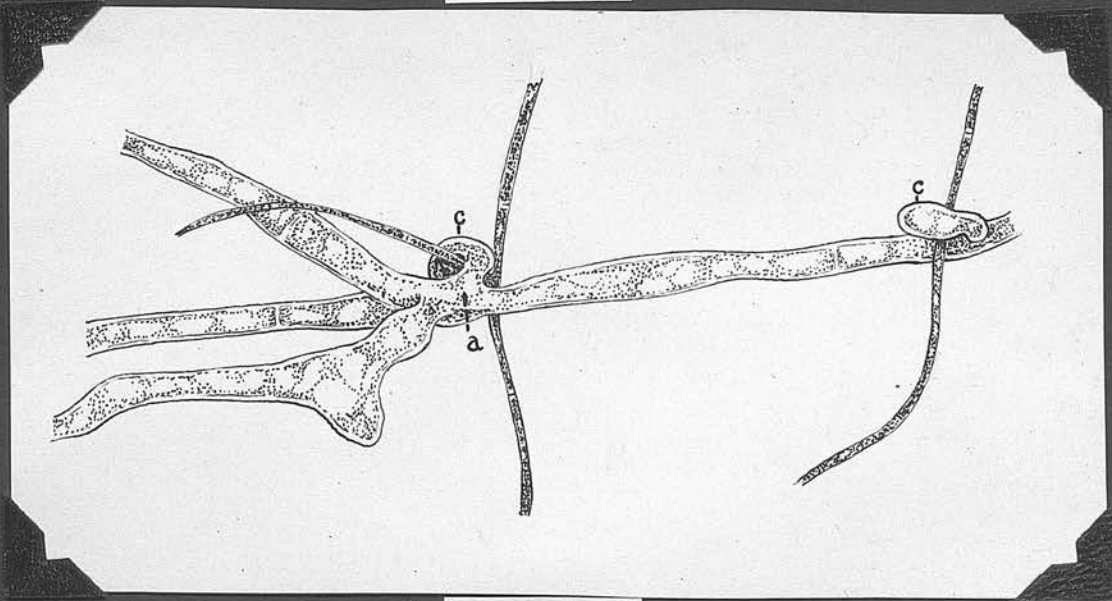


Fig. 23.

Fig. 24.

The parasitism of a Nectria hypha(n) by a hypha of the aerial mycelium of G. Highley. a) dilated zone of the appressorium, c) hook cell, d) the base of a conidiophore. x 550.

Fig. 25.

An example of the parasitism of a Nectria hypha(n) by G. Highley, showing the development of a conidiophore from a hypha of the branch system. a) extended area of the appressorium, c) hook cells, d) conidiophore bearing primary conidia, h) hyphae of the branch system, o) conidium of G. Highley, t) germ tube. x 420.

Fig. 26.

Variations in the morphology of the conidiophore of G. Highley. The two conidiophores show limited development of the terminal bulb, and the production of a single primary conidium (p). x 420.

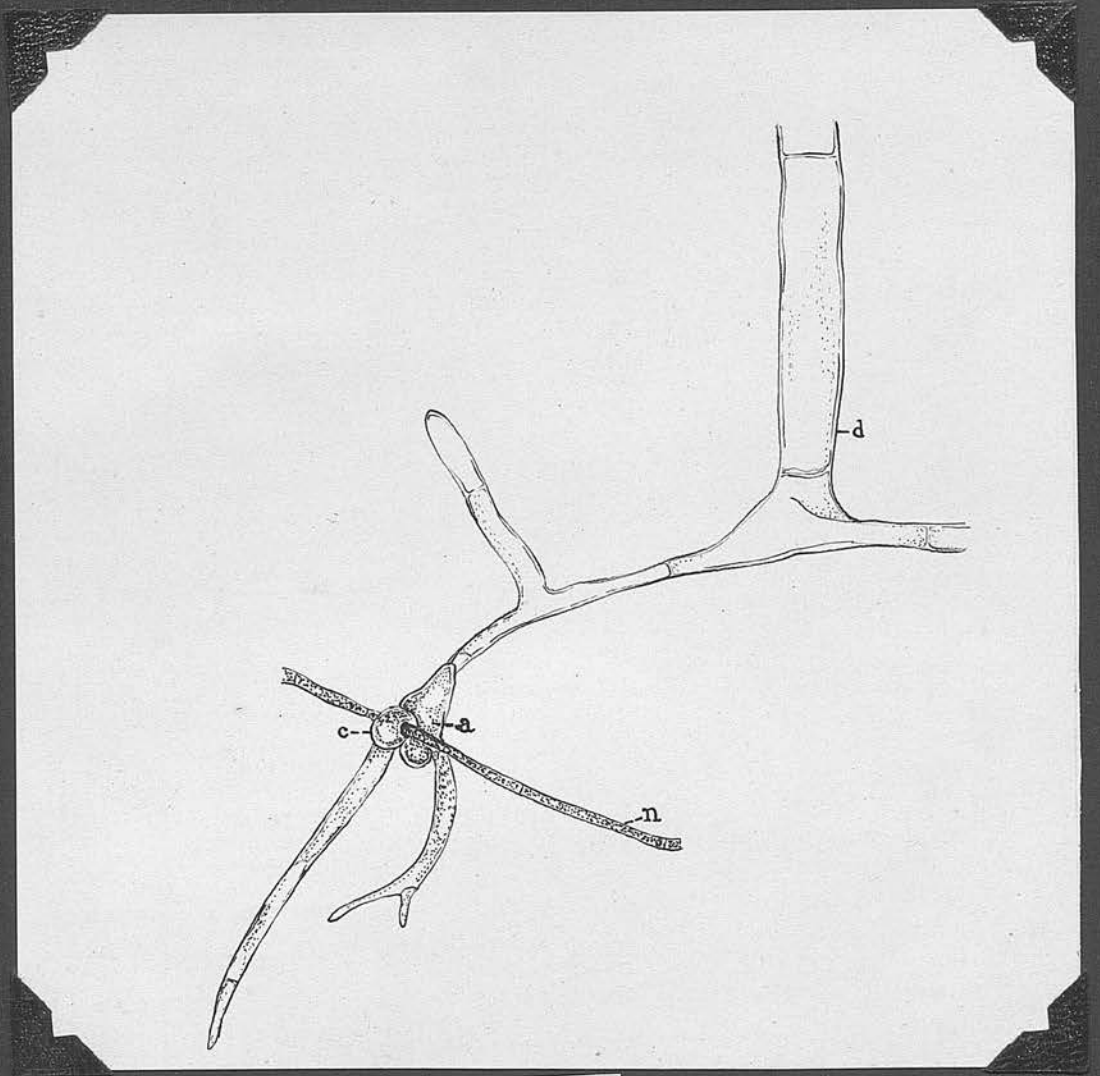


Fig. 24.

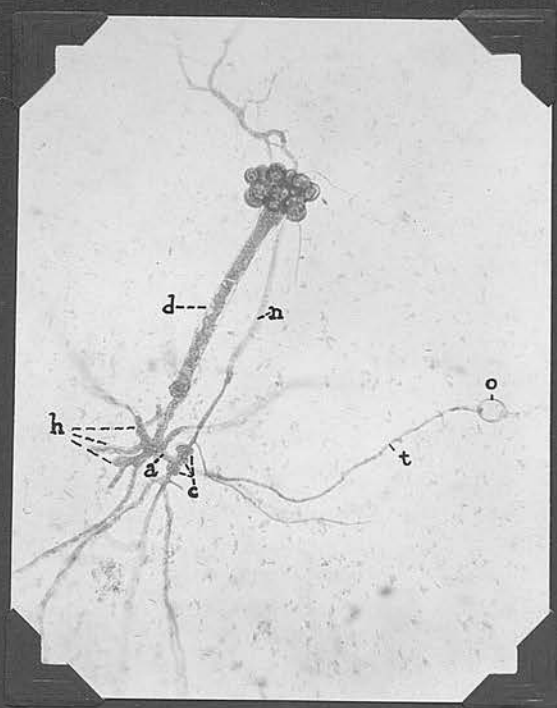


Fig. 25.

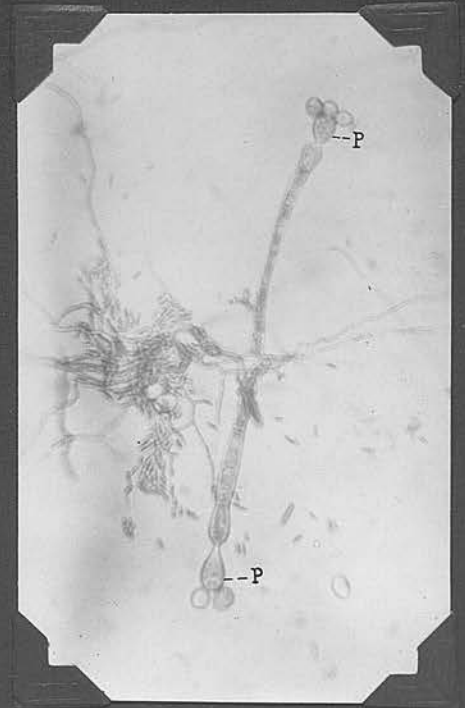


Fig. 26.

Fig. 27.

- a) The production of conidia from a swelling set at the base of a lateral hypha of the submerged mycelium of G. Highleyi. x 660.
- b) The extent of the development of the germ tube of G. Highleyi in pure culture on Nectria extract agar after two months' growth. x 800.

Fig. 28.

- a) An early stage in the development of an intrahyphal growth in the cell of a conidiophore of G. Highleyi, showing localised evagination of the transverse septum. x 1600.
- b) The diagrammatic representation of a transverse septum showing a central pore, and the area (a) from which intrahyphal growth proceeds.

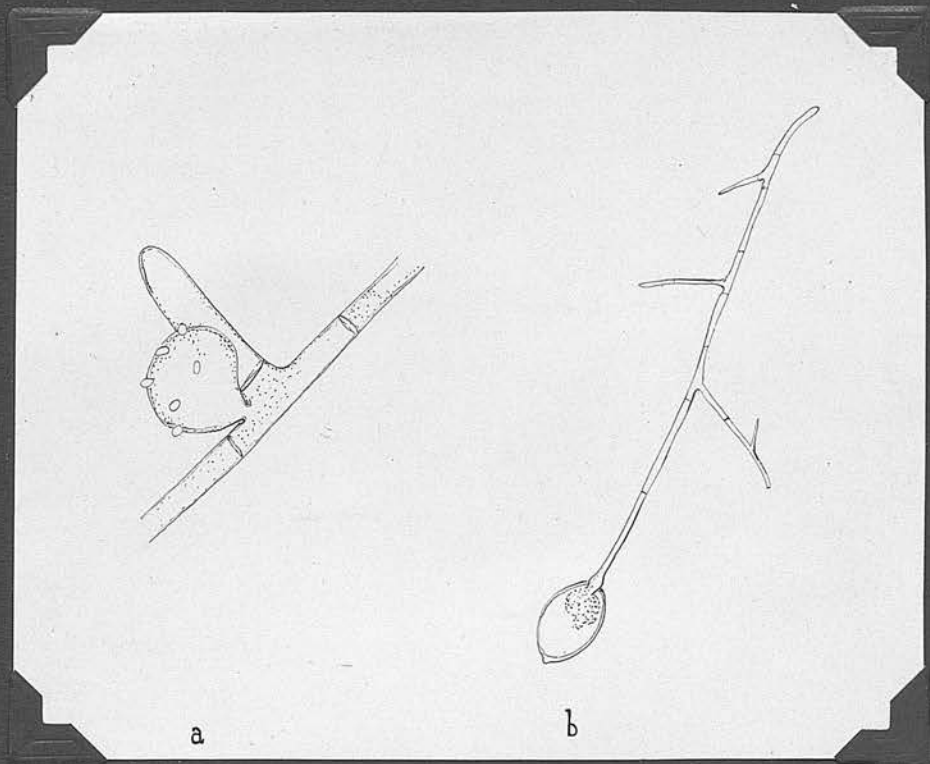


Fig. 27.

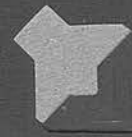


Fig. 28.

Fig. 29.

A subterminal cell of a conidiophore of G. Highleyi showing the development of intrahyphal growth. x 580.

Fig. 30.

- a) Part of a conidiophore of G. Highleyi. Septal evagination has given rise to an intrahyphal growth passing through a transverse septum higher in the conidiophore. x 560.
- b) A diagrammatic representation of the passage of an intrahyphal growth through a transverse septum. The zone of fusion (d) between the intrahyphal growth (i) and the transverse septum (s) continues growth (g) into the next cell of the conidiophore.

Figs. 31 & 32.

A lateral prostrate hypha of the mycelium of G. Highleyi showing the presence of an intrahyphal growth in the terminal cell. The growth becomes free from the 'parent' cell at (a).

Fig. 31. x 500. Fig. 32. x 750.

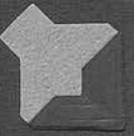
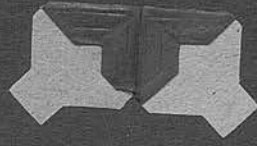


Fig. 29.

Fig. 30.

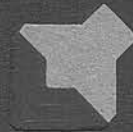
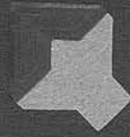


Fig. 31.

Fig. 32.

Fig. 33.

- a) The regeneration of a fragment of the mycelium of G. Highleyi by means of intrahyphal growth. x 600.
- b) The formation of a transverse septum at the apex of the terminal bulb of a conidiophore of G. Highleyi. x 600.
- c) Total evagination of the upper transverse septum of the subterminal cell of a conidiophore of G. Highleyi. x 600.

Figs 34, 35 & 36.

Proliferation of conidiophores of G. Highleyi brought about by total septal evagination, showing the presence of collars at the bases of the outgrowths.
Fig. 34 x800. Fig. 35 x600. Fig. 36 x580.



Fig. 33.

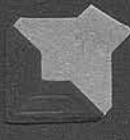
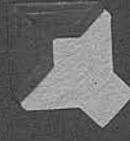


Fig. 34.



Fig. 35.

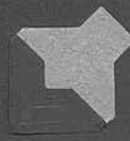


Fig. 36.



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STUDIES

ON

HAPALOSPHAERIA DEFORMANS SYDOW.

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INTRODUCTION

The discovery of a fungus parasitic upon the flowers of Rubus dumetorum Weihe (syn. R. caesius L.) was first intimated in 1907 by H. and P. Sydow (12), who described material which had been observed by Diedicke and Reinecke in Thuringia, Germany. From an examination of infected flowers, H. and P. Sydow noted the occurrence of white, powdery masses of globose, hyaline conidia at the surfaces of the anthers, within which the mycelium of the fungus was scarcely visible. No indication of the method by which the conidia were produced from the mycelium could be obtained, and the fungus was provisionally placed in the genus Paepalopsis, as a new species, Paepalopsis deformans Syd. In the account of the fungus, a quotation from a communication by Diedicke, which gave a report of the latter's observations on the disease, was included. The report was later enlarged and published by Diedicke and Sydow (2) in 1908.

This paper deals principally with a detailed account of the work of Diedicke, who stated that the disease was first noticed by him in a bush of R. dumetorum Weihe near Horba in 1900, and later in a bush of the same species near Erfurt in 1907. He recognised a series of symptoms which he associated with the presence of the fungus. Diseased plants showed the presence of Witches' Brooms in the inflorescences, which were found to be excessively branched, and gave rise to flower buds showing abnormally rapid development combined with deformation.

The/

The deformation was brought about by continued apical growth of the sepals, one or more of which curved over the bud in the earliest stages of development, and later gave rise to elongated apical segments.

From sections which he made from fresh and alcohol preserved material, Diedicke undertook an examination of the tissues of the diseased inflorescences, but could find no trace of the fungus in the axis of the inflorescence, in the pedicels of the flowers, or in any of the flower parts with the exception of the anthers. The mycelium did, however, occur between the anthers and within their intercellular spaces. In the wall of the anther, pycnidia containing globose, hyaline pycnospores were observed, and the fungus was, therefore, placed in a new genus of the Sphaeropsidales, Hapalosphaeria Syd., as H. deformans Syd.

With regard to the other flower parts, Diedicke stated that the petals were often of a greater number than usual, and were larger, whilst the fruit was normally developed. Although no evidence of the presence of the fungus in the tissues of the axes of the inflorescence could be obtained, the view was expressed that infection probably took place in the axillary bud, giving rise to the inflorescence during late winter. Diedicke remarked that he intended to make a further study of the disease in order to determine the time and method of infection, but no further publication appeared.

The occurrence of the fungus in Great Britain was first recognised by Borthwick, who collected infected material of/

of Rubus fruticosus L. near Aberlady, Haddingtonshire, Scotland, in 1904. The material was described by Wilson (13) in 1922. He regarded it as showing features generally similar to those possessed by diseased specimens described in Germany. Sepal deformation and anther blight were present, but no evidence of Witches' Brooms was forthcoming.

The first recording of Hapalosphaeria deformans Syd. outside Europe was made by Dearness and Foster (1), who, in 1933, described the anther and stigma blight of the Loganberry in British Columbia. The inclusion of stigma blight in the title of their communication was, however, somewhat misleading in that it did not refer to a direct infection of the gynoecium, but to the incidental effect of fungal attack on the development of the fruit. The latter showed abnormality owing to the lack of pollination which followed the emasculation of the anthers by the fungus. The same effect and its consequences upon fruit production, by rendering berries unsuitable for canning, were mentioned in a further two communications from North America. The first of these was intimated by Zeller (14), an abstract of whose paper, given at the Annual Meeting of the Pacific Division of the American Phytopathological Society, was published in 1939. The substance of the paper, together with an account of further work, formed the basis of the second publication, which was made by Zeller and Braun (15) in 1943.

In describing the disease attributable to H. deformans, which is stated to be widespread throughout the Pacific North/

North West, in particular, west of the Cascade Mountains, Zeller and Braun included the following plants as hosts of the fungus; -the Wild Trailing Blackberry (Rubus macropetalus), the Evergreen Blackberry (Rubus laciniatus Willd.), and the Young and Boysen Dewberries.

The emasculation of the anthers is put forward as the major characteristic of the disease, and the lack of pollination is stated to result in abnormal fruit development. Deformed berries result from bee pollinated flowers. Apart from R. macropetalus, the infected plants did not show the presence of Witches' Brooms, and no mention is made of deformation of the calyx. Histological work showed no trace of the fungus in any floral organs other than the anthers, penetration of the walls of which was observed to take place from appressoria developed by the hyphae of the fungus. The invariably total infection of the flowers of the fruiting lateral was taken to indicate the infection of the unfolded axillary bud, and this was borne out by infection experiments on commercial plantings of Young Dewberries. It was shown that a great increase in the incidence of the disease took place when the axillary buds of the canes were dusted with pycnospores obtained from infected flowers. Sections showed the mycelium to be present both between and within the flower buds in the axillary bud, and it was assumed that the infection of the axillary buds by pycnospores was the normal method of infection under natural conditions. A considerable control of the disease was achieved by the/

the application of lime sulphur to the axillary buds, and outstanding proof that the axillary buds of the canes of the current season were directly infected from flowers in their immediate vicinity was obtained by excising all the canes at the height of the flowering period. The new canes which were produced late in the same season, when no source of fungal inoculum existed, gave rise to fruiting laterals, which were entirely healthy, the following year.

Fluctuations in the aerial environment were cited as the principal factors in influencing the occurrence of the disease, which could cause seasonal epidemics varying in intensity from 15% to 70% infection in commercial plantings of Young Dewberry in Oregon.

Attempts to germinate the pycnospores of the fungus were made by Diedicke (2), but proved to be unsuccessful.

Cultures were, however, obtained by Zeller and Braun (15) from anthers dissected aseptically from closed buds. The fungus grew slowly on agar, to which it imparted shades varying from yellow to deep Burgundy, the coloration of any single isolate remaining constant. A whiter strain was also isolated, but no spores or sclerotia were ever produced by any isolate in culture.

From a review of the literature, it is evident that the disease caused by Hapalosphaeria, as far as it is known, involves the infection of the anthers of the flower, and the appearance of Witches' Brooms in those cases where the hosts of the fungus are wild species of Rubus.

The present work was undertaken in order to make an investigation of the disease of Rubus fruticosus L caused by H. deformans, with a view to obtaining information concerning the full effect of fungal attack on the plant, the life history of the fungus, and the behaviour of the fungus in culture. It was also thought desirable to investigate the possibility of the transference of infection from the Bramble to cultivated Raspberries.

The opportunity to study the disease was afforded in the first place by the receipt of diseased material from Dr. M. Noble, who discovered the fungus in East Lothian, Mid Lothian, Perthshire and the suburbs of Edinburgh in 1947. (7). The investigation, which is still incomplete, was made from this material, and from diseased plants collected by the author in the Edinburgh district. Slide preparations made by Dr. Malcolm Wilson from the material originally collected by Borthwick (13) have also been examined.

SYMPTOMS OF THE DISEASE.

I. EFFECTS PRODUCED IN THE INFLORESCENCE.

The inflorescence of the healthy Bramble is in the form of a panicle, which arises by the growth and development of an axillary bud of a straggling cane produced from the rootstock of the plant during the previous season, or earlier. The majority of inflorescences, which bear flowers showing the characteristic 'blight' symptoms of the anthers, are normal in appearance. The panicle varies in length from eighteen to twenty-four inches, and is terminated by a single flower, below which axillary cymes of two or three flowers are produced laterally. (Fig. 1).

The disposition of the lateral cymes in relation to the terminal flower may, however, be considerably altered in diseased specimens, and result in the appearance of Witches' Brooms. In certain cases, which may be taken as representative of only a small proportion of diseased inflorescences, the development of the axillary cymes proceeds beyond normal limits, and the inflorescence more nearly approaches a corymbose form, in which the flowers of the lateral cymes lie on a level with the terminal flower. An example of this type of growth form is shown in Fig. 3, in which the 'bushy' nature of the whole inflorescence is evident. The compact grouping of the flowers at the apex of the inflorescences reduces the straggling habit of the plant in those cases in which Witches' Brooms arise in groups.

During early stages in development, Witches' Broom inflorescences show unusual features. The appearance of the terminal flower cluster may take place when the young flower axis is less than two inches in length. The normal, healthy axis, however, may be as much as a foot long before buds become evident. An example of a short axis bearing a terminal flower bud cluster is seen in Fig. 4. Growth and development in this structure are at first confined to the terminal bud, but later become transferred to the lateral cymes, which undergo abnormal elongation. This apparent transference of growth brings about the condition which is seen in the mature Witches' Broom. Such diseased inflorescences are also characterised by a general shrubby appearance during early stages of growth, and their axes are frequently of greater girth than those produced by healthy panicles. In some cases, external fluting of the axis is clearly marked, and is well shown in Fig. 3.

In the early spring of 1948, a mild season in South Scotland, abnormal growth forms were observed to occur from basal axillary buds of panicles bearing withered flowers of the previous season. An example is shown in Fig. 5, from material which was collected in early March, and which showed the most extreme form of Witches' Broom encountered. In this specimen, the lower of the two outgrowths produced is normal in appearance, with regular leaf development and thin axis. The upper growth, however, shows reduced leaf development which is confined to the production/

production of a single leaf of limited size. The rest of the outgrowth gives rise to a panicle of the same general plan as that seen in a healthy plant, but confined to the space of some three inches. The bracts are of a reduced type, and the flower buds of the lateral cymes are in some cases at a higher level than the terminal bud.

The occurrence of all Witches' Brooms is essentially sporadic. They may be dispersed singly within a bush or may occur in scattered groups, and appear to mature earlier than the healthy or less severely affected inflorescences. A single straggling cane may bear one or more Witches' Brooms in the axils of its leaves, interspersed with infected though more normal inflorescences. The infection of flowers in Witches' Brooms is total, but in the case of other inflorescences, lateral cymes of healthy flowers have been observed amongst the diseased ones in the same panicle, and on several occasions, a single healthy flower was observed in an otherwise diseased lateral cyme.

There is also a seemingly random distribution of infection as regards the natural occurrence of the disease in the field. Many bushes show a high percentage of diseased panicles, whilst others produce a few, most frequently located at scattered points towards the periphery of the bush, which is otherwise free of infection.

II. EFFECTS PRODUCED IN THE FLOWER.

The occurrence of disease attributable to Hapalosphaeria deformans is correlated with the introduction of radical changes in the calyx, corolla, androecium and gynoecium of the flower. Change is greatest in the calyx, androecium and gynoecium, all of which show some degree of abnormality, but only rarely appears to occur in the corolla.

(a) Calyx.

Although infection of the flower may not be evident until the time of opening, the presence of disease is frequently indicated during the early development of flower buds, particularly of those which occur terminally in the panicles, by the appearance of deformation in the calyx. Where the inflorescence is apparently normal in its general pattern, the amount of deformation is small or negligible, but where Witches' Brooms are developed, deformation is always clearly present in varying degrees of intensity.

The globular shape of the normal bud gives way to an irregular pouching which is brought about by the appearance of longitudinal folds or distended zones at the surfaces of individual sepals, the tips of which are often swollen and blunt. Buds of this type can be seen in the panicle shown in Fig. 1, and in the more highly magnified apex of the same inflorescence presented in Fig. 2. As the extent of sepal deformation is slight, and the aspect of the inflorescence as a whole is normal, the/

the specimens showing this form of the disease may easily remain undetected in the field before flower opening. Apart from the limited pouching of flower buds shown by examples of this kind, and which may be attributed to the irregular increase in width of one or more sepals, deformed buds may show further abnormality. This is confined principally to the unusual elongation of the bud as a whole, and to the differential growth and development of the individual sepals.

Early stages in the production of these effects are shown in Fig. 5, where pouching is accompanied by an elongation and consequent flattening of the buds, the apices of which are irregular in appearance owing to the separation and partial twisting of the tips of the sepals. (Fig. 6) In the apical cluster of flower buds in the panicle, the terminal bud is normally found to be the more obviously affected, and in Fig. 7, a series of terminal buds removed at various stages of their development illustrates the method by which abnormal growth is brought about. In the young bud shown at (a), pouching is slight, but apical growth of the tips of the sepals is well under way, and they are separated one from the other. The bud at (b), which shows a slightly later stage in development, possesses longer sepal tips, and abnormal growth of the sepal on the right is beginning. Similar development of a single sepal is shown in the bud at (c). From this point, the abnormal apical growth of the individual sepals is accompanied by an increase in

in their lateral growth. As this increase is restricted to one or more of the sepals in each flower and varies in speed, the pocked effect produced in the bud is greatly emphasised. The remainder of the series of terminal buds, (d-j), show varying degrees of sepal development, in which the sepals of the more obviously malformed examples display a striking tendency to the formation of leaf-like extensions. In many cases, sepals show the development of apical, strap-shaped segments, which may number from two to five, and it is from these structures that leaf-like form is produced.

Leaf-like segments are variable in size and shape, but show marked reticulate venation and marginal serration. A good example is shown in Fig. 8, in which the terminal flower is again the most clearly deformed. Although the flower buds borne in the lateral cymes never appear to show such marked abnormalities as those which terminate the growth of the inflorescence, they show similar features. In the example shown in Fig. 9, the differential lateral and apical development of the calyx segments has brought about the effect of pouching, very strongly in evidence in this case, and has resulted in the arching of one, or at most, two sepal tips over each bud apex in the form of a thin spine-like projection. A certain increase in the length of the lateral cymes in this particular inflorescence gives it a semblance of a weakly developed Witches' Broom.

In more definite Witches' Brooms, however, the deformation/

deformation of buds borne in lateral cymes is much greater, as can be seen in Fig. 3 . The overall effect of abnormal growth of the calyx in flowers infected by Hapalosphaeria deformans leads, therefore, to considerable increase in both the length and width of the sepals, and it is not unusual to find that they are up to 2.5 cms. in length, as compared with the sepals of unaffected flowers which rarely exceed 1.4 cms.

In the healthy plant, the sepals become reflexed when the flower is open, but this character is frequently absent in infected flowers. Severely deformed sepals remain upright, whilst those free of abnormal growth become reflexed, and it is usual to find flowers in which sepals are set in both positions. Examples may be seen in Figs. 3 & 8.

A further effect of fungal attack is shown in the variation which occurs in the number of sepals which make up the calyx. The normal number of five may be replaced by numbers ranging from four to nine. In some cases, however, the disturbance to meristic precision is misleading owing to differential growth, and sepal segments, which are free to the bases of the sepals in which they had their origin, can be erroneously regarded as separate elements of the calyx.

(b) Corolla

The majority of infected flowers deviate little from the normal with regard to the size and shape of the petals. A few of the flowers examined did, however, show/

show a considerable increase in the width and length of the petals, and this was accompanied by a prolonged retention of the crumpled appearance due originally to package in the bud. In the normal flower, petals are usually smooth and unfurrowed when maturity is reached. The crumpled petals of infected flowers may be as long as 2.3 cms., and may number as many as eight. They also show a tendency to irregular doubling, which is brought into being by the development of small outgrowths from the base of the petal. Several may occur on a single petal, and when the petal is removed from the flower, are removed with it. Some are tubular and open at the upper end, whilst others are strap-shaped.

The effect of increase in the length of the petals is to produce a larger flower, which is given a ragged appearance owing to the retention of crumpling. An example is shown in Figs. 1 & 2, and a series of doubled petals is seen in Fig. 10.

(c) Androecium

At the opening of the normal flower, the smooth walled anthers are at first a lightish green colour, and as they reach maturity and release pollen by introrse dehiscence, ^{they} become cream coloured. The colour change is due in part to the wall of the anther, and in part to the pollen which is released. In the older flower, dehiscid anthers eventually turn brownish, prior to their loss.

Flowers which are attacked by Hapalosphaeria deformans show anther development which differs considerably from/

from the normal. The terminal buds obtained from the more deformed inflorescences, and which show pouching and a tendency to leaf-like form in the sepals, are completely brown inside when cut longitudinally, and the anthers are covered with white spore masses derived from the pycnidia of the fungus which are formed within them. The surface of the anther is irregularly swollen, showing bulges where the pycnidia occur below the surface, and when the flower opens, the anthers, which eventually become almost black in colour, show numerous white tendrils of pycnospores at their surfaces. Examples of the general appearance of such anthers are seen in Figs. 2 & 8, which show the typical anther-blight symptom, and also emphasise the absence of anther dehiscence, a function which is entirely inhibited by fungal attack.

In flower buds which are borne in inflorescences of an almost normal appearance, the early browning of the anthers may not occur, although the presence of the fungus is indicated at the opening of the flower, when spore masses are seen in great profusion on the anther surfaces. Such anthers are brown, and fail to dehisce in the normal manner.

(d) Gynoecium

During early development, the carpels of severely affected terminal flower buds may also be brownish in colour, and softish in consistency, but this symptom is ^{comparatively} rare. In the normal course of events, the young carpels remain green, and in no way deviate from those which are produced by/

by healthy flowers. The compound fruit does not, however, always develop normally, and in many cases, individual drupelets remain permanently small and green, whilst others show various stages of enlargement and pigmentation. The final effect is often that in which the entire shows an unbalanced appearance owing to the disproportionate development of the drupelets, and in more extreme cases is a small, stunted structure which is green throughout.

MICROTECHNIQUE.

An examination of the Witches' Brooms, of less severely infected inflorescences and of the straggling canes which bore them was made from hand sections of fresh material and microtome sections of preserved material, after freezing or embedding in paraffin wax.

Hand sections made from fresh material were stained with either cotton blue in lactophenol, or picro-nigrosin in lactophenol, both stains being suitable for picking out the free mycelium of the fungus associated with the plant parts.

Material for sectioning by means of the microtome was fixed in either Karpechenko's solution, or Alcohol-formalin-acetic solution No. 1, both fixatives being made up according to Rawlins(9). The fixatives caused little or no distortion of the tissues. After fixation, a number of plant parts were cut by means of a freezing microtome, and were embedded in blocks of plain agar prior to sectioning. The use of the freezing microtome proved successful even in the case of the flowers, and was found to be particularly valuable for studying the mycelium of the fungus in the tissues of the anthers. Each flower section cut was removed, by means of a paint brush, to a drop of water placed on a slide, and individual anthers were transferred to further slides by the use of a fine pipette, before staining.

For the embedding of fixed material in paraffin wax, a Cedar oil schedule was found to be the most suitable,

as it caused less hardening of the tissues than one incorporating xylol. The material was gradually brought to absolute alcohol, and was placed in a 50% absolute alcohol/50% Cedar oil mixture for three hours, from which it was removed to pure Cedar oil for a further three hours before embedding in paraffin wax in the usual way. Sections were cut from 4μ to 10μ in thickness.

Staining to differentiate between the hyphae of the fungus and the tissues of the host was found to be difficult to achieve, as the fungus exists in all forms ranging from thin-walled, narrow hyphae to thick-walled pseudoparenchyma, and at different stages in its life history may vary in its reaction to any single stain or combination of stains. The following staining techniques were tried:-

Carbol-thionin and Orange G. (Stoughton 11.)

Basic Fuchsin as a 2% aqueous solution, followed by a saturated solution of Light green in clove oil.

Triple stain (Rawlins 9).

Iron Alum-Haematoxylin (Rawlins 9).

Picro-nigrosin as a 2% aqueous solution, followed by a 0.5% solution of Orange G in Absolute alcohol.

Magdala red followed by Light green (Rawlins 9).

Although some success was gained in the case of the flower with the Picro-nigrosin/Orange G combination, the best results were obtained by dividing each batch of sections representing any one plant part into three groups, and staining each group with a different stain combination. This allowed/

allowed for the variability shown by the fungus in accepting stains at the various stages in its development. The three staining techniques most frequently used were:- Iron Alum-Haematoxylin, Basic Fuchsin/Light green, and Triple.

MICROSCOPIC EXAMINATION OF INFECTED FLOWERS OF RUBUSFRUTICOSUS L.

The great variation noted in the case of diseased inflorescences was also found to apply in the flower, with regard to the amount of fungus present and its effect on the plant tissues. The following account is largely devoted to the more extreme form of the disease, in which deformation of the calyx is taken as the index of severe infection. The effect of the fungus on infected flowers, almost normal in appearance, is also described.

I. INFECTED FLOWERS FROM WITCHES' BROOMS

In the earliest stages of its development, the flower arises in the axil of a bract as a cushion-shaped mass of undifferentiated tissue, which, as it develops, assumes the form of a cup, the rim being represented by the primordia of the sepals. The growth of the calyx, as is normal in most flowers, proceeds rapidly, so that the sepals enclose and protect the primordia of the other flower parts. The stamens, which are laid down from the tissues of the receptacle, are carried outwards with the primordia of the sepals and petals by the lateral growth of the receptacle, which, in the centre, becomes raised as a conical projection, giving rise peripherally to spirally arranged carpel primordia. The flower, therefore, assumes a perigynous form, in which the receptacle is made up of a central swollen area surrounded by a circular flange, with the stamens, petals and sepals borne at the rim.

Before/

Before the inclusion of the remaining flower parts by the sepals, the fungus is present as a sparse network of fine hyphae, which ramify over the surface of the young flower primordium and lie between it and the subtending bract. The hyphae of the fungus are hyaline, septate, 2μ to 4μ in width, and have a uniform, densely granular content. No evidence of the penetration of the young primordium has been obtained. The tissues appear to be normal in their development, and the cells composing them entirely healthy.

The further development of the fungus is apparently largely influenced by the overgrowth of the sepals, which causes part of the mycelium to become included within the bud, the remainder lying on its outside surface. Whereas the outer mycelium remains comparatively sparse, that within the bud increases greatly in amount and eventually shows considerable differentiation, presumably as a response to the increase in humidity which may be regarded as following upon the overgrowth of the sepals.

The hyphae become numerous at the flange of the receptacle, surround the primordia of the stamens and petals, and show a longitudinal distribution on the inner surfaces of the sepals. They seem to be comparatively scarce on the raised part of the receptacle which bears the carpel primordia. The hyphae undergo modification, becoming aggregated into cell complexes, which may have their origin in the close approximation of several hyphae or in the lateral branching of a single hypha. The cells of/

of the complexes undergo increase in size, and the complexes either assume the form of flat plates, one cell in thickness, or appear as irregularly shaped knot-like masses of pseudoparenchyma. In the latter structures, which are more common in occurrence than the flat plates, the fungal cells are squarish to globular in shape, being some 4μ to 8μ in width, and are thick walled, the walls adopting a brownish pigmentation. The content of the cells becomes progressively more vacuolate, so that in older complexes, the brown walled cells appear to be entirely empty. Both plate-like and knot-like cell complexes of the fungus which are attached to the host tissues may be extensive in their development, measuring $75-80\mu \times 30\mu$, and give rise to fine, hyaline hyphae from their peripheries. Such hyphae may also develop cell complexes at some distance from the original, so that a certain continuity is maintained between otherwise isolated zones of fungal pseudoparenchyma.

Cell complexes have been observed in the young flower bud on the flange (Fig. 11) and, more rarely, ^{on} the raised central part of the receptacle, on the inner surfaces of the sepals (Fig. 12), and on the stamen primordia. (Figs 13, & 14) In older buds, they may occur on both outer and inner surfaces of the petals (Fig. 15). The development of cell complexes outside the bud also takes place to some extent, and they have been seen on the outer surfaces of the sepals near the base. (Fig. 16). The relationship between the fungal cell complexes and the tissues of the /

the host is not entirely clear. As may be seen from the figures mentioned above, they invariably present a sunken appearance, and a certain, though limited amount of intercellular penetration occurs from their bases. The hyphae which effect penetration are thin walled and hyaline, and the host cells in their immediate vicinity frequently show the presence of deeply staining products of disintegration. (Fig. 14).

The extent to which the cell complexes are embedded in the host tissues varies little. Most frequently, the dilated, parenchyma-like fungal cells are observable between the cells of the epidermis, and only on rare occasions occur in the intercellular spaces of the hypodermal layer, although thin walled penetrant hyphae may be seen in the intercellular spaces below. Apart from this localised penetration, no other evidence of the presence of the fungus within the tissues of the young flower bud has been obtained.

As the effect which is produced by the fungus on the separate whorls of floral organs differs considerably, the calyx, corolla, androecium and gynoecium will be dealt with individually.

i) Calyx

In young flower buds, one millimetre or so in length, the cell complexes of the fungus may be seen at various points on the inner and outer surfaces of the sepals. At this early stage in the development of the bud, signs of irregular growth are already present in the calyx/

calyx as a whole, some sepals being longer than others. The structure of the elongate sepals is, for the most part, normal, but in some cases, the growth increase and irregularity may be directly due to the formation of zones or bands of undifferentiated tissue, which is usually developed laterally near the sepal apex. (Fig. 17) Such tissues are clearly produced at parts of the sepals which bear cell complexes of the fungus, the complex lying opposite to the undifferentiated tissue. Renewed activity of the sepal is not, however, always undertaken when fungal cell complexes are present, and the latter are sporadically distributed towards the sepal apices. It is likely that the cell complexes of the fungus which occur at these points owe their position to passive carriage during the growth of the host tissues.

The abnormal sepals, which show an irregular package in the bud, frequently possess a large number of cells containing hypertrophies of the cell wall. The cells are situated principally in the epidermis, but may also occur in the lower parenchyma. In rarer instances they have been observed around the vascular traces, and it is possible that the formation of hypertrophies is due to the early attack of the sepal primordia by the fungus.

In deformed buds at a stage of development just prior to opening, the sepals show unusual increase in thickness at the base, although no mycelium of the fungus appears to be present within their tissues. The abnormal apical/

apical outgrowths which occur in the most severely deformed sepals are provided with a richly branched vascular system.

ii) Corolla

The overall effect of the fungus on the petals of the flower is small. Only in rare cases do they deviate in general structure and appearance from those which are formed in normal unaffected flower buds. Doubling may, however, arise by the irregular growth and development of the petal primordium. (Fig. 13). The primordium gives rise to a normally developed petal, which bears one or more small outgrowths produced on its inner surface near the base. In the bud, these outgrowths are surrounded by cell complexes and hyphae of the fungus, and penetrations of the tissues are numerous. Where doubled petals arise, the tissues of the torus immediately at the point of their insertion contain a great number of cells which are in a state of disorganisation and show the development of cell wall hypertrophies.

iii) Androecium

The cell complexes of the fungus which occur on the young anther primordia bring about local infection by giving rise to thin walled, hyaline hyphae which enter the intercellular spaces, the cells around which become irregular in shape and contain deeply staining products of disintegration. (Fig. 14). The infection does not, however, produce a marked disorganisation, and the primordia appear to be capable of further growth and development/

development. The proportion of stamen primordia which are directly attacked seems to be small, and the majority remain free from infection.

Although it is difficult to trace the final effect of the early penetration of the stamen primordium, it is likely that it results in the formation of the intercellular mycelium which has been observed in a small number of anthers in the early stages of their development. Such anthers are characterised by inequality in the development of sporogenous tissue, with which the intercellular mycelium becomes directly associated and may be seen to ramify between the cells of the tapetal layers. In the transverse sections of some anthers, the sporogenous tissue of one or both lobes may be destroyed, and this leads to the unequal development of the anther as a whole. In extreme cases, one lobe may suffer complete atrophy.

As a rule, however, the development of the anther proceeds more normally. The sporogenous tissue is well defined, and the fungus is situated round the periphery of the anther in the form of a fine mycelial felt, in which cell complexes are numerous. The latter are often aggregated in the cleft between the anther lobes as knot-like masses of brown walled pseudoparenchyma, which are also distributed on the anther surface. Flat plates of fungal cells are also commonly developed at the anther surface. (Fig. 19.) On rare occasions, cell complexes have been observed on the filaments of the stamens, but these structures remain remarkably free of fungal mycelium.

The hyphae of the fungus give rise to appressoria at the surface of the anther cuticle. The appressoria may arise terminally or laterally from a single hypha, and the intercellular penetration of the epidermis is brought about by hyphae developed from their bases. (Fig. 20.). It seems likely that cell complexes are developed from such appressoria, or from groups of approximated appressoria. As may be seen in Fig. 21., early stages in the development of cell complexes are closely linked with the formation of appressoria. It seems that at least a number of complexes may result after an initial penetration of the host tissues, and well developed cell complexes with penetrating hyphae issuing from their bases are seen at the epidermis of the anther. (Figs. 22 & 23.) If this is the correct interpretation of the formation of cell complexes, it would explain their sunken position with regard to the host tissues, provided that some of the penetrating hyphae eventually assumed the form of pseudoparenchyma.

In the early stages of fungal attack, the hyphae within the intercellular spaces of the anther are 2μ - 3μ in width, and do not exert any marked effect on the surrounding cells. They may run for some time below the epidermis, but finally become generally distributed in the intercellular spaces of the parenchyma of the anther. (Fig. 25.) As development proceeds, the hyphae present a beaded appearance, owing to the increase in size of individual cells.

The influence of the fungus on the further development of the sporogenous tissue is fairly constant. The hyphae of the fungus, which enter by passing between the cells of the tapetum, ramify between the spore mother cells, which produce pollen tetrads apparently unimpeded. The pollen grains, however, become completely encased in fungal hyphae, the cells of which undergo increase in size. This general increase in size, whereby the fungal cells become dilated and rounded, brings about the formation of pseudoparenchyma, which occurs generally throughout the intercellular spaces of the parenchyma of the anther and fills the pollen sacs. (Fig. 24) During its development, the fibrous layer of the anther is produced normally.

The rounded cells of the fungal pseudoparenchyma are multinucleate and the cytoplasm is granular. The cell walls are thick and hyaline. The pycnidia of the fungus are produced from the peripheral zone of the pseudoparenchyma lying immediately below the fibrous layer, and arise as rounded groups of cells, which gradually increase in size, pushing intercellularly towards the surface of the anther. The upper part of each young pycnidium gives rise to a short neck which is blunt at the apex. As development proceeds, the pycnidium becomes hollow, and the wall is made up of three to five rows of cells, the cells of the outer row being thick walled. The cell layer nearest the cavity of the pycnidium becomes modified to consist of a series of club-shaped cells/

cells projecting into the cavity of the pycnidium, and from the apices of these cells, the pycnospores are budded off.

The longitudinal growth of the pycnidium leads to the stretching and eventual rupture of the epidermal cells of the anther, so that the tissue which forms the apical part of the neck of the pycnidium projects beyond the anther surface prior to the liberation of the spores. The mechanism of liberation appears to be dependent on the breakage of the cells at the tip of the neck, so that the pycnidium becomes ostiolate and releases the pycnospores in the form of a tendril at the anther surface. Mature pycnidia are globose to ovate in shape, measuring $116-75 \times 116-70 \mu$. On rare occasions, multiloculate pycnidia have also been observed, in which ingrowths of the wall of the pycnidium enter the central cavity. Typical pycnidia are shown in Figs. 24, 26 and 27.

The pycnospores are variable in shape and size, whilst still in the pycnidium or free, and may be ovate, subglobose or globose, with the subglobose form the most usual. They are hyaline, measuring $7.5-3.6 \times 5.5-3 \mu$, when lying free between the flower parts.

Before the opening of the bud, spore discharge may be well advanced, and masses of pycnospores are to be found as a white powder on the expanded flange of the receptacle between the filaments of the stamens. In severely diseased buds, the infection of the androecium is total, and great numbers of pycnidia are produced in each/

each anther lobe. (Fig. 28.)

The pseudoparenchyma of the fungus in the inter-cellular spaces of the anther becomes progressively more vacuolate with age, and in old anthers is devoid of contents, the cell walls becoming golden brown in colour. The cells of the anther also undergo degeneration. The presence of the fungus within the anther inhibits dehiscence, and pollen is never liberated. The effect is to be regarded as a mechanical one, as the fungus causes no interference to the differentiation of the fibrous layer, which plays the major part in the normal process of dehiscence. Bands of pseudoparenchyma which occasionally pass between the cells of the fibrous layer, tend to bind it to the mass of pseudoparenchyma which occupies the pollen sac below, and the retraction of the fibrous layer which would automatically follow dehydration is prevented. In a similar way, the development of pycnidia, which cause intercellular penetration as they pass towards the anther surface, tends to inhibit the functioning of the fibrous layer. Partial dehiscence may apparently take place on some occasions, and the pseudoparenchyma of the fungus has been observed to become free to the exterior of the anther at the thin slit formed between the pollen sacs. No pollen is shed, however.

iv) Gynoeceium

Although it is usual to find that the hyphae of the fungus are less numerous on the raised part of the receptacle from which the primordia of the carpels are developed, than on the other flower parts at an early stage in the development of the bud, the carpels eventually become attacked, and a complete destruction of the seed frequently results.

The infection of the carpel may occur in two distinct ways. In rarer instances, direct intercellular penetration may be effected, cell complexes being in evidence at the points from which entry is made.

The hyphae, which branch freely within the invaded intercellular spaces, usually become massed into strands and ramify within the tissues of the carpel, finally entering the loculus. Other hyphal strands pass into the outer tissues of the receptacle, and occasionally become associated with the vascular traces to the carpels, where they have been observed to separate the elements of the xylem. In transverse sections of flower buds which are affected in this way, numerous intercellular channels of infection may be seen to pass from the carpels to the peripheral tissue of the receptacle, which may also be infected by direct penetration. (Figs. 29 & 30)

Taken as a whole, the cells of the carpels and receptacle show little sign of degeneration. Some crushing does, however, take place, especially in the cells adjacent to the fungal strands, and disintegration products may/

may be seen in the epidermal cells which are nearest the points of original penetration at the surface of the host.

In most flower buds infected by the fungus, however, the infection of the carpel is brought about without intercellular penetration, and is dependent for its achievement upon the normal stages of growth and development which result in the formation of the mature carpel. In the earliest stages of development, the carpel arises from the receptacle as a horse-shoe shaped projection of undifferentiated tissue, which, by further growth, forms a tubular structure open on the ventral side, the opening passing in most examples well into the style developed at the apex. The carpel becomes closed by the progressive upward fusion of the two free edges of the tube, giving rise to a ventral suture.

In the earliest stages of the development of the carpel, the hyphae of the fungus enter the ventral opening (Fig 31), and as the formation of the ventral suture proceeds, come to lie within the loculus of the carpel. The progressive upward closure of the ventral opening tends to separate the mycelium on the outer surface of the carpel from that within the loculus, and the hyphae become aggregated in the space which remains. In many examples, the normal closure of the ventral opening is retarded owing to the formation of a wedge of fungal hyphae.

The final effect of this parallel growth and development of fungus and carpel is one in which the fungus becomes included within the style and loculus of the/

the carpel. (Fig. 34). Within the style, the hyphae increase steadily in width and form a cylinder of pseudoparenchyma which is situated centrally in the host tissues. (Figs. 32 & 33.) The base of the cylinder occupies the zone of the ventral aperture situated between the two placentae, from each of which a pendulous ovule is developed. (Fig. 34.).

At an early stage, the pseudoparenchyma is hyaline and composed of thick walled cells possessing granular, multinucleate contents. Later, the walls of the cells situated at the periphery become dark in colour, so that the fungus may be readily distinguished from the tissues of the host by the presence of a marginal brown line, which is clearly perceptible in unstained preparations. (Fig. 32)

The darkening of the cell walls proceeds inwards, and in sections of carpels made from older flowers, the pseudoparenchyma is uniformly empty and brown walled. (Fig. 35)

The mycelium in the loculus of the carpel is continuous with the pseudoparenchyma, and is composed of hyphae which ramify over the surfaces of the developing ovules and form a loose network on the inner surface of the loculus of the carpel. (Fig. 36) As development proceeds, the network becomes organised to form knot, and plate-like cell complexes, assuming a brownish coloration with age. The complexes are often connected together by hyaline thin walled hyphae, which show irregularly swollen intercalary cells, and between which anastomoses appear to be common.

The primordia of the ovules appear as rounded structures/

structures which arise before closure of the ventral opening from the margins of the ventral lips of the carpel near the apex of the loculus. By the growth of the primordia, two pendulous, anatropous ovules are formed in each carpel. (According to Pechoutre(8), the ovule is provided with two fused integuments, which are difficult to distinguish from each other when the ovule approaches maturity, but can be recognised in the earliest stages of the development of the ovule, and later in the testa of the seed. During the present investigation, no clear distinction could be drawn at any of the stages in the development of the ovule, and it is proposed to refer to a single integument throughout the following account.

Owing to the assumption of anatropy, the micropyle formed by the apical rim of the integument frequently lies immediately below the pseudoparenchyma of the fungus which occupies the central tissue at the base of the style. Hyphae, continuous with the pseudoparenchyma, enter the micropyle, forming a loose plug which surrounds the apex of the nucellus. (Fig. 37). The direct intercellular penetration of the latter tissue, which is frequently composed of a single layer of cells above the embryo sac, has been observed to take place. The hyphae finally occupy a position at the side of the embryo sac. (Fig. 38). Intercellular penetration appears to be comparatively rare at this stage, however, and in most cases the hyphae of the fungus lie between the integument and the nucellar tissue.

The/

The development of the embryo rarely takes place. Sections made at later stages show that most carpels contain a single ovule, the integument being largely free of fungal hyphae. Within the integument, however, the tissue of the nucellus is destroyed by hyphae, which are both inter- and intra-cellular. Carpels which are subject to this form of fungal attack do not undergo further development, and the internal differentiation of the wall of the carpel, which would normally take place during the formation of the druplet, cannot be observed.

In other diseased specimens, the druplets may be well developed, showing a clear differentiation of epicarp, mesocarp and endocarp. The seed may also attain a considerable size. It is clear from a study of such examples, that the ovule has undergone changes which would normally be the outcome of fertilization. The testa of the seed is well developed, containing groups of intercellular hyphae, and the endosperm is represented by three or four layers of cells. (Fig. 39) Hyphae which lie between the cells of the endosperm ramify inwards, completely permeating the remnants of the nucellar tissue, and fill the centre of the seed. At this point, the hyphae may become swollen, measuring about 6μ in width, and amongst them disorganised cells of the embryo may be observed.

From the fungal hyphae which occupy the zone of the embryo, pycnidia are commonly developed. Three or four pycnidia may be found in any one seed. They are similar in shape and size to those which occur in the anthers, and/

and are fully functional. (Fig. 39).

The cell complexes formed at the inner surface of the loculus of the carpel have been observed to show penetrant hyphae bringing about a localised infection of the host tissues. The infection occurs at an early stage in the development of the carpel, and remains localised as the fruit is formed, and hyphae may be seen within the stony endocarp of the druplet. Pycnidia are also formed at the surface of the endocarp, and project into the loculus of the druplet. (Fig. 40 .) The mesocarp appears to be devoid of fungal hyphae.

Pycnidia occur on the styles of the carpels in older flowers. They occur laterally and are partly embedded in the tissues, frequently those adjoining the centrally placed fungal pseudoparenchyma. (Fig. 41) Occasionally, pycnidia are to be found on the expanded lobes of the stigma (Fig. 42.), and are again closely associated with pseudoparenchyma. It is not always possible to determine whether the latter is merely a continuation of the fungal tissue placed lower in the style, or is derived from a separate infection of the stigma during the early development of the flower.

In many of the infected flowers which had suffered complete emasculation, pollen was observed on the stigmatic lobes of diseased carpels. The pollen had, presumably, been brought from surrounding, healthy flowers by insects.

v) The flower at a late stage in its history.

The anthers and carpels show thorough infection by the time the flower has reached a late stage in its history. In these older flowers, the mycelium of the fungus may become generally systemic in the tissues of the floral whorls. The hyphae are principally intercellular, and are evenly distributed in the receptacle, sepals, petals and the filaments of the stamens, although the compound fruit appears to remain surprisingly free from a general attack, the mesocarp of the drupelets being devoid of hyphae.

In some parts of the flower, particularly the flange and raised central portion of the receptacle, the hyphae become aggregated together, and by increase in the size of their cells, give rise to pseudoparenchyma in the intercellular spaces. ^{Fig. 43.} Some intracellular penetration occurs in the receptacle, but the cells of all floral parts are usually free from direct invasion by the hyphae of the fungus.

Brown walled pseudoparenchyma, which is made up of empty cells, may be frequently observed embedded in the inner surfaces of the sepals. These pockets of the fungus are probably derived from the cell complexes, which, in the early stages of the development of the flower, are laid down at the surface of the host. Indications that the systemic invasion of the tissues is effected from such sources have been suggested by a study of younger flowers.

The tissues of the pedicel also contain fungal hyphae, which, for the most part, pursue a longitudinal course in the intercellular spaces of the pith and cortex, occasionally running between the xylem vessels.

This widespread infection of the tissues of the flower which occurs at a late stage, is characterised by the production of pycnidia on both surfaces of the sepals and petals, on the flange and raised part of the receptacle, and, in rare cases, at the bases of the filaments of the stamens. Examples are shown in Figs. 44 & 45.

The pycnidia arise from pseudoparenchyma situated in the intercellular spaces of the host tissue, and finally break through the epidermis before the pycnospores are shed. The pycnidia are usually much larger than those which occur in the anthers and within the druplets, being 130μ to 200μ in width, and are sub-globose to globose in shape. The necks of the pycnidia may be well developed, and are composed of short cones of cells, 12μ to 17μ in length.

II. INFECTED FLOWERS FROM NORMAL INFLORESCENCES.

In young flower buds, which show no abnormal growth of the calyx, the mycelium of the fungus is sparsely distributed around the primordia of the flower parts in the form of thin-walled, fine hyphae. No penetration of the primordia has been observed to take place, and cell complexes appear to be entirely absent, although at a later stage they may be seen on the surfaces of the anthers.

When the flower has reached maturity, the sepals which remain short and possess undivided tips, show fewer cell complexes than may be observed on abnormally elongated sepals from flowers more severely affected by disease. The infection of the anthers and carpels, however, occurs with great frequency. The infection of the androecium is usually complete, but flower sections have shown examples in which approximately a half of the anthers was occupied by fungal hyphae, the other half dehiscing normally to shed pollen. The extent of the fungal attack on the anther may also vary, one lobe becoming parasitised, the other undergoing dehiscence and showing no sign of infection. In the anthers diseased in this way, the mycelium rarely forms extensive masses of pseudoparenchyma within the pollen sacs, but remains as fine, branched hyphae, although small areas of pseudoparenchyma bearing pycnidia are formed at widely separated points towards the periphery of the sacs. The infection of the carpel is apparently always undertaken by the entry of hyphae into the ventral opening during early development, and no direct penetration of the lateral wall of the carpel has been observed.

THE FORMATION OF CONIDIA DIRECTLY FROM THE MYCELIUM OF
THE FUNGUS

In a number of the thoroughly infected, mature flowers which were examined, the hyphae of the mycelium, which was abundant between the floral whorls and on the expanded flange of the receptacle, showed the development of conidia.

The hyphae which bear conidia are about 5μ in width, and show the occurrence of dilated intercalary cells situated sporadically. The conidia arise laterally from both the dilated and undilated cells, or terminally from the ends of hyphal branches, but no differentiation into morphologically recognisable conidiophores appears to take place. The conidia may be developed from any point on the lateral surface of the hyphal cell (Fig. 59.), although they are also to be found in clusters of three to five near to a transverse septum, and are produced from a slightly swollen area of the cell. The part of the hyphal cell from which the conidia arise may also become dilated to form a globose, lateral extension, about 4μ across, attached to the remainder of the cell by a short stalk-like base. The conidia are borne on sterigmata from the dilated apex of the capitate structure. (Fig. 59).

The conidium is at first globose, about 1μ in diameter, the protoplasmic contents being in direct communication with the hyphal cell through the centre of the sterigma. As development proceeds, the conidium becomes elongate ovate, and when mature, measures $6.2\mu - 4\mu \times 3.3\mu - 2\mu$. It is released by the fracture of the sterigma.

MICROSCOPIC EXAMINATION OF THE STRAGGLING CANES AND
INFECTED INFLORESCENCES OF RUBUS FRUTICOSUS L.

In the young Witches' Broom type of inflorescence such as that shown in Fig. 5, no trace of the fungus could be found within the pith, vascular system or inner cortex of the main axis and the peduncles and pedicels of the lateral cymes. An examination of the lower leaves (or foliose bracts) and the entire upper bracts of such inflorescences also yielded a negative result, and no anatomical abnormality could be recognised in any of the plant tissues.

The fungus is present, however, on the main axis, and on the peduncles and pedicels of the lateral cymes in the form of cell complexes, essentially similar in construction and appearance to those which have been described in the flower. The complexes, which are distributed sparsely over the whole of the axial system of the inflorescence, appear to be most numerous on the pedicels of the flowers, where they may attain a considerable development, becoming some 80 μ wide. They become progressively smaller in size towards the base of the main axis of the inflorescence, and may be composed of three or four cells.

The larger complexes which occur on the pedicels are cushion-shaped and closely appressed to the surface of the epidermis, and each complex is embedded in the epidermis by means of an intercellular plug of pseudoparenchyma from which thin, hyaline hyphae penetrate the intercellular spaces of the outer cortex to a depth of two or three cell layers/

layers. From the upper cells of the fungal complexes, thin hyphae are given off to ramify for a short distance over the surface of the host, and become intermingled with the simple and multicellular hairs which are situated there.

The cells of the fungal complexes which occur on young Witches' Brooms are rounded, possessing a thick, hyaline wall and granular protoplasmic contents. The cells of the host, in the immediate vicinity of the fungal pseudoparenchyma and fine intercellular hyphae, show no symptoms of degeneration. Although the cell complexes of the fungus appear to be generally distributed over the various axes of the inflorescence, they do not seem to develop to any extent on the bracts, and have only been observed on these structures on rare occasions.

In the mature Witches' Broom, cell complexes are easily distinguishable in longitudinal and transverse sections owing to their highly vacuolated or empty cells, the walls of which are deep brown in colour. Fungal complexes have been found on the peduncles and pedicels of the lateral cymes, and have been traced on the main axis of the panicle to a point some ten inches from the terminal flower. The complexes are frequently seen on the ridges or flutings which occasionally become exaggerated on the axes of severely infected specimens (Fig. 46), and are particularly associated with the multicellular hairs and prickles.

They may occur towards the base of the multicellular hair, where union is effected with the subtending axis, (Fig. 47),
or/

or may lie along the dilated apex of the hair. On the prickles, the development of the pseudoparenchymatous complexes is frequently great, and they may form masses of cells some $90\ \mu$ across at the surface of the epidermis. (Fig. 48) The amount of penetration, which occurs from the bases of the cell complexes situated on the multicellular hairs and prickles, is slight. The effect of intercellular penetration is seen in a thickening of the cell walls of the host, but the cell contents as a whole remain healthy, and only rarely have products of disintegration been recorded.

No evidence of the presence of internal hyphae has been obtained in the case of the main axis and peduncles of the mature Witches' Broom. Towards the end of the flowering period, however, hyphae are present in the pedicels of severely diseased flowers, in some of the entire bracts borne on the main axis of the inflorescence, and in the bud scales which are situated at the base of the axis.

In the infected bracts and scales, the hyphae of the fungus are largely intercellular, and owing to swelling of intercalary cells, present a beaded appearance similar to that which has been described in the case of the anthers. Aggregates of swollen cells give rise to pseudoparenchyma, from which pycnidia are frequently developed. Pycnidia have been observed on the bracts, bud scales and the prickles of the main axis of the inflorescence, late in the flowering season, and in every case were found to arise from/

from pseudoparenchyma. Examples of pycnidia are shown in Figs. 49 & 50.

The pycnidia agree in shape and size with those which occur on the sepals and petals of older flowers, and are provided with short conical or rounded necks. At maturity, each pycnidium is partially embedded in the tissues of the host, which are partly crushed during its development.

The development of cell complexes on infected inflorescences, which show no abnormal growth, is much less than in Witches' Brooms. In most specimens studied, no fungal pseudoparenchyma could be found, and where it did occur, was confined to the pedicels of the flowers. The bracts, bud scales and axes of the inflorescences were entirely free of internal fungal hyphae.

A study of the straggling canes, which gave rise to infected inflorescences, produced no evidence of the presence of the fungus, either within the tissues, or at the surface of the epidermis. The canes were comparable in structure and appearance to those obtained from uninfected bushes.

MICROSCOPIC EXAMINATION OF THE AXILLARY INFLORESCENCEBUDS

The inflorescences of the Bramble arise by the growth and development of axillary buds of straggling canes, which were produced from the rootstock of the plant during the previous year. The cane may often give rise to inflorescences over a number of subsequent years, the inflorescences of following seasons being derived from accessory buds, which are situated on the cane at the bases of old inflorescences. In some cases, as many as three inflorescences arise from closely situated accessory buds in the same season.

New straggling canes are developed from the rootstock of the plant in February and March, although the time of their appearance is largely influenced by the situation of the plant in the field, and the weather conditions which prevail. The canes continue growth throughout the summer when older canes are bearing inflorescences, and the buds in the axils of their leaves are well developed by September. The whole growth and development of the new straggling cane therefore takes place when an abundance of fungal spores is being produced in the inflorescences, and a general examination of infected bushes has sufficed to show that the spores, presumably disseminated by wind and insects, are present on most of the aerial parts of the plant by the end of summer. The buds of the new canes usually 'break' about the middle of April, and by May, the bushes show the presence of young inflorescences.

The buds, both of canes due to produce inflorescences
in/

in the following summer, and of older canes which had already borne inflorescences, were examined in January and February of the present year (1949), by means of dissection and microtome sectioning. The typical bud is set on a short stalk, and is sheathed by a number of scales, the upper ones eventually developing into foliage leaves (or foliose bracts), and the lower ones retaining scale form throughout the subsequent development of the inflorescence. The bud scales are loosely folded at the apex of the bud.

The apical parts of the lower scales were usually found to be composed of dead cells, and the tissues showed the presence of inter- and intra-cellular hyphae. That some of these hyphae could be regarded as belonging to Hapalosphaeria deformans was suggested firstly by their shape, as they assumed a pseudoparenchyma-like form in the intercellular spaces, and showed swollen intercalary cells when they became intracellular, and secondly by the fact that the pycnidia of the fungus are known to occur in the basal scales late in the season. (p. 43).

From the upper, dead areas of the scales, hyphae often pass downwards into the intercellular spaces of the lower, healthy tissues. The hyphae are thin walled, being some 2.5 μ wide. (Fig. 51.) In all the buds examined, no evidence of the presence of hyphae other than those found in the scales was obtained.

The axis of the inflorescence bud gives rise to the primordia of the various plant parts from its periphery.

In/

In sections made from buds collected in February, thin-walled septate hyphae were found at various points at the surfaces of the primordia. Positive proof that these hyphae were of Hapalosphaeria deformans was gained by the observation of the germinating pycnospores of the fungus enclosed within the young developing leaves. (Fig. 52.)

Under natural conditions, the pycnospores become globose and about 6μ in diameter before germination occurs. Each pycnospore gives rise to a single, thin-walled, hyaline germ-tube, some 2μ wide. Direct intercellular penetration of the tissues of the host has been observed to occur in the case of the developing leaf base, and is probably undertaken at several points. A hypha running closely appressed to the young tissues showed the development of a terminal dilated appressorium, from the base of which a thin-walled penetrant hypha passed between two cells of the epidermis. (Fig. 53.)

Cell complexes are also present within the bud by late February, and have been observed to adopt the characteristic sunken position with regard to the host. (Fig. 54.) Although it is often difficult to identify the primordia which arise from the short axis of the bud, the cell complex shown in the above figure is on a bud primordium placed in the axil of a foliose bract. The primordium is little affected by the presence of the fungus, and the only signs of degeneration are seen in the cells adjoining the axis of the axillary bud.

A small proportion of the buds examined showed that/

that a different form of fungal attack may occur. The inflorescence buds were found to be completely permeated by fungal hyphae. The latter were most numerous in the bud scales, where they occurred both inter- and intracellularly. They showed the usual characteristics of the hyphae of Hapalosphaeria deformans, intercalary cells being swollen, and localised pockets of brown walled pseudoparenchyma occurred within the intercellular spaces. Cell complexes were also observed on the inner (adaxial) surfaces of the bud scales, where thin-walled, septate hyphae, about 3μ in width, were abundant. The main axis and meristematic apex of the buds surrounded by such scales were destroyed by hyphae, pockets of brown walled pseudoparenchyma being numerous in the intercellular spaces. In general appearance, such buds are brownish in colour, and in section the tissues are much disorganised. The cells are often warped and devoid of contents.

In order to study the development of new straggling canes and make an investigation of their tissues, several plants were removed from a bush known to have shown a thorough infection during the previous summer, and were grown under greenhouse conditions. The plants were removed from the bush in January, and at the time of their transplanting, possessed a number of buds arising from the rootstock near the bases of canes which had produced inflorescences during the previous summer. It was not known at what time the rootstock buds first made their appearance. Under greenhouse conditions, the buds underwent rapid growth and/

and development, and young canes were evident in February. Longitudinal and transverse sections of the canes were made at various stages in development, and showed that fungal hyphae were present between the bud scales, and were carried upwards by the elongation of the internodes of the cane. In one particular cane growth, removed from the plant when about one inch in length, the scales were found to enclose tracts of fungal pseudoparenchyma. At this early stage in development, the scales, which later become foliage leaves by progressive apical modification, possess buds in their axils. The pseudoparenchyma also lay between the scales of the axillary buds, and could be seen to invest the bud axis. (Fig. 55.)

As can be seen from the above figure, the pseudoparenchyma bears a considerable resemblance to that which has been earlier described as lying within the styles of infected carpels. The fungal cells are provided with hyaline walls, and their shape varies from squarish to globular. In size, the globular ones are some 8μ in diameter, and the protoplasmic content is granular. The disorganisation of the tissues of the apices of a number of the bud scales in contact with the fungal pseudoparenchyma was observed to have taken place, but the details of penetration could not be clearly seen. Apart from the presence of well developed fungal pseudoparenchyma, thin walled narrow hyphae were present at several points between the scales of the axillary buds.

CULTURE TECHNIQUE

The fungus was readily brought into culture from the pycnospores which occurred on the surfaces of diseased anthers. Stamens were removed from the infected flowers by means of heat-sterilised, fine-pointed forceps, and shaken in sterile water. The resulting suspension of pycnospores was transferred to Petri dish plates, or slide plates (Noble, 6) of malt extract agar, and these were incubated in darkness at 23°-25°C. On this medium, germination took place within twenty four hours, and agar discs containing single germinating pycnospores were cut out by means of a sterilised dummy objective and removed to agar slopes. The mono-pycnospore cultures were grown on malt extract, potato-dextrose, oat and Bramble leaf extract agars. The leaf extract medium was prepared by filtering a decoction of boiled leaves, and adding sufficient agar to the filtrate to make a 2.5% medium.

The pycnospores also germinated easily in water within van Tieghem cells. Groups of germinating spores were removed from the cells by means of a pipette, and fixed in Karpechenko's solution before incorporating in egg-albumen smears on glass slides. The smears were allowed to dry and were stained with Kleinenberg's Haematoxylin or Triple stain. Stained slides were passed through a graded series of alcohols and mounted in Canada balsam.

Fungi were isolated from the remaining parts of of the flower and young unopened flower buds, the axes and/

and bracts of the inflorescence, and the straggling canes by the following method. The part of the plant to be examined in culture was firstly surface-sterilised in either a 1/1000 aqueous solution of Mercuric Chloride, or in a 1/14 suspension of bleaching powder, and then washed in two changes of sterile water. Thin slices of the surface-sterilised material were cut by means of a scalpel, sterilised by flaming, and transferred to agar medium in Petri dishes. The fungi which resulted from plant material incubated in darkness at 23°-25°C, were brought into pure culture on agar slopes.

THE GERMINATION OF THE PYCNOSPORES IN WATER.

Prior to germination, the pycnospore becomes swollen and assumes a broadly ovate shape, being about 7 μ in length, and 6 μ in width. Two oil globules may occur within the spore at this time, and are polar in position. In some cases a single globule develops in the centre of the spore, and very rarely a central transverse septum is laid down.

The germ tube arises as a short, rounded papilla from one pole, or, rarely, from both poles of the spore. The papilla is soon cut off by a basal transverse septum and increases in size, finally assuming an ovate shape. At this stage it resembles a secondary conidium produced by budding of the first. The apex of the secondary/

secondary cell also gives rise to a papilla, and the process of enlargement is repeated. Eventually, however, a thin-walled, septate germ-tube is developed, and the dilated cells first produced in early germination are seen at its base. The germ tube grows for a considerable time before branching occurs, and in the early stages of its development is about 2μ wide.

Anastomoses have been observed to occur between germ tubes growing in close contact in the same drop of water. Narrow conjugation tubes may arise laterally or terminally from the germ tube. The protoplasm of the cell from which a conjugation tube arises becomes aggregated at the tip of the tube. The tip of the tube fuses with a cell of an adjoining germ tube, and protoplasmic union is established. Spore germination and anastomoses of germ tubes are shown in Fig. 56.

THE GROWTH AND DEVELOPMENT OF THE FUNGUS IN CULTURE.

1) On malt extract, potato dextrose and oat agars.

The growth of the fungus on these media is slow, the colony forming an appressed mat about three quarters of an inch in diameter after eighteen days' incubation in darkness at 23° - 25° C.

In young cultures, the colony is at first white, but soon changes towards the centre to shades of pinkish buff. (Colour nomenclature is that outlined by Ridgway 10). The centre of the colony then assumes a pale grey coloration, which becomes mouse grey or deep mouse grey with age.

The/

The advancing hyphae at the circumference of the colony remain white, or are tinged with buff, and the development of aerial mycelium is sparse. Increase in the amount of aerial mycelium takes place after some eighteen days' incubation, when localised white patches appear at the surface of the colony.

After a period of three weeks or a month, drops of a straw-coloured exudate occur at the surface of the mycelium, and the agar on which the fungal colony is growing becomes pigmented morocco red to garnet brown. The reverse of the colony is black. The coloration of the agar is more rapidly and completely effected when the fungus is grown on potato dextrose and oat agars, than when it is grown on malt extract agars.

In three month old cultures, the aerial mycelium is in the form of an appressed mat of a sea shell pink or pinkish buff colour, and patches of light buff, mars brown and mouse grey are observable at various points.

The reverse of the colony is black, and the agar supporting the colony is garnet brown in colour.

The hyphae of the aerial mycelium are thin-walled, hyaline and septate, measuring about 2μ in width. The basal and submerged mycelia are composed of hyphae which become thick-walled and brown, 5μ wide, and which contain swollen intercalary and terminal cells. The latter, which frequently become globose, are about 9μ in diameter. The aggregation of the hyphae of the basal and submerged mycelia brings about the formation of a layer of pseudoparenchyma about/

about 130μ in thickness. This layer of fungal cells accounts for the mouse grey coloration of the colony.

The production of pycnidia in culture is variable. The pycnidia are more profusely developed on oat and potato dextrose agars, than on malt extract agar, and on the latter medium, spore production is often small. No free conidial form has been observed in culture, and no perithecial development took place in cultures subjected to periods of freezing during the winter months.

The Development of Pycnidia in Culture.

Pycnidia most frequently arise directly from, or within the layer of fungal pseudoparenchyma which forms at the surface and subsurface of the agar. ^{Fig. 57.} More rarely, they appear in the aerial mycelium, just below the surface. In the earliest stages of development, the pycnidium is in the form of a globular mass of dilated brown-walled cells, which originate directly from the layer of pseudoparenchyma, or from one or more hyphae of the lower zone of the aerial mycelium. As development proceeds, the cell mass becomes differentiated into an outer layer, composed of two or three rows of empty, brown-walled cells, and an inner, central group of smaller cells. The latter are squarish in outline and possess hyaline walls. The cell content is granular, and fills the cavity of the cell.

The central cell group eventually shows tracts of disintegration, the cell walls becoming almost indistinguishable, and within these tracts, which finally coalesce, the pycnospores first make their appearance. The pycnospores appear to be/

be derived, by budding, from thin-walled, ovate or club-shaped sporophores, some 1.5 μ in length. When the central cavity of the pycnidium is completely filled with pycnospores, the wall is from three to five cells in thickness.

The outer layer of cells is brown in colour, giving rise on its outer surface to short hyphae, so that the contour of the pycnidium is rough in appearance. The cells of the inner layers of the wall are smaller, and the cell wall is almost colourless. The sporophores are almost indistinguishable when the pycnidium reaches maturity.

Young pycnidia which are filled with spores may often show the development of a neck, about 45 μ in length, possessing a rounded apex beset with short, brown-walled hyphae. (Fig. 58.) Most of the neck tissue appears to disintegrate well before the pycnospores are shed. In older cultures, it is usual to find that the neck is of three or four cells in length, and may show a ragged apex. In surface view, such pycnidia show a distinct ostiole (Fig. 60.) In a number of cases, pycnidia with two or more necks have been observed.

The mature pycnidium is globose, or globose depressed in shape, measuring some 200 μ in diameter. Where several pycnidia are developed in close proximity to one another from the layer of pseudoparenchyma, however, great irregularity in shape usually occurs. Pycnidia are light, or golden brown in colour by transmitted light, and the wall is soft in consistency.

The discharge of pycnospores in a tendril only rarely occurs in culture, and has been observed from pycnidia formed by mycelia grown from spore suspensions plated on potato dextrose agar. The tendril is five to ten spores in width, and contains mucilaginous material in which oil globules are numerous. The pycnospores, when shed from the pycnidium are elliptical, or, less commonly, rod-shaped, the ends being obtusely rounded. They measure $8\mu - 5.5\mu \times 4\mu - 3\mu$.

In older cultures, spores which have been shed, and lie between the hyphae of the aerial mycelium of the fungus, are sub-globose or globose in shape, averaging 4.6μ in diameter. It appears that the shape of the pycnospore, whether derived from pycnidia produced under natural conditions, or produced in culture, is dependent on the state of maturity reached.

ii) On leaf extract agar.

Cultures grown on leaf extract agar differ considerably from those described above on nutrient agars. The amount of mycelium produced is small, but growth is more rapid. Pseudoparenchyma is not produced in quantity, and the colony is white in colour, imparting shades of morocco red and garnet brown to the medium. Pycnidia arise at scattered points after a month's growth in darkness at $23^{\circ} - 25^{\circ}C$, but are few in number.

PARTS OF THE PLANT FROM WHICH TYPICAL CULTURES OF
HAPALOSPHAERIA HAVE BEEN OBTAINED.

The fungus has been isolated from the sepals, petals, stamens, carpels and pedicels of infected flowers, and from the main axis of the inflorescence to a distance of a foot from the terminal flower. It also cultured readily from the young, unopened flower buds obtained from Witches' Brooms. The fungus was obtained in January from axillary buds due to give rise to inflorescences in the coming season, but has not been isolated from the young axillary buds of straggling canes in process of growth and development from the rootstock.

The isolation of the fungus from the axes of infected inflorescences is not always successful, and ^{this} is probably due to the use of chemical surface sterilisation before plating on agar. The external nature of the fungal cell complexes renders them liable to destruction by the sterilising agent employed, and successful isolation is presumably achieved because of the incomplete action of the agent on the cells of the complexes, or on the fungal hyphae situated in the intercellular spaces of the outer cortex of the host.

OTHER FUNGI ASSOCIATED WITH DISEASED INFLORESCENCES.

The older, infected inflorescences are normally colonised by a number of other fungi towards the end of the year. The main colonists are species of Fusarium.

The axes of young inflorescences bearing unopened flower buds, showing the symptoms of deformation attendant on/

on the presence of Hapalosphaeria deformans, occasionally yielded another fungus when plated on agar after surface sterilising. The same fungus was obtained from the axes of mature inflorescences bearing thoroughly infected flowers, but has never been cultured directly from the the flower itself.

On potato dextrose and malt agars, the aerial mycelium of the young colony is white, appressed to the substratum, and remains white to cartridge buff in colour until the colony becomes some three months old, when patches of pinkish buff or cream buff appear. In two month old cultures, the hyphae of the aerial mycelium are usually grouped into strands, and measure about 3μ in width, being hyaline and septate. The basal mycelium is at first of a chestnut, or burnt sienna colour, but in cultures six weeks old, becomes black. The mycelium at the surface and sub-surface of the agar is made up of hyphae, which are brown-walled, septate, containing swollen intercalary and terminal cells, which by aggregation give rise to a mat of pseudoparenchyma.

In cultures two months old, cushion-shaped sclerotia, measuring as much as a centimetre across, arise from the pseudoparenchyma. Each sclerotium is at first black in colour, and is composed of hyaline, septate hyphae compacted to give a white medulla, around which two or more layers of brown-walled hyphae are laid down to form a cortex. The hyphae may become irregularly warted on the upper surface, from which a white, or cartridge buff mycelium is produced in cultures three months old. The production of spores has not been recorded.

INFECTION EXPERIMENTS

A series of infection experiments was made in the summer of 1948, with a view to investigating the possibility of directly infecting the flower of the Bramble. The experiments were conducted under field conditions, using bushes which were known to have shown no infection the previous summer.

Fungal inoculum was used either in the form of suspensions of pycnospores, derived from the anthers of naturally infected flowers and freely sporing cultures of the fungus, or in the form of agar discs containing fungal mycelium.

Drops of the spore suspensions were added to the young buds which would give rise to axillary cymes, and to the terminal flower cluster during an early stage of its development.

In other experiments, the suspensions were introduced into flower buds which were two millimetres in length and over, by means of a hypodermic syringe. By using the syringe, it was found that a considerable quantity of inoculum could be introduced directly into the cavity of the young bud, by piercing the sepals with the needle at a point near the apex of the bud.

An additional set of experiments was set up using agar discs instead of the suspensions of pycnospores. The discs were cut from cultures of the fungus grown on potato dextrose agar, and they ~~discs~~ were placed within the buds through slits cut in the sepals by means of a scalpel. The results of all the above experiments proved to be negative/

negative, no symptoms of anther blight being observed either macroscopically, or from sections of inoculated flowers cut by means of the freezing microtome.

In order to study the effect of the fungus on the straggling canes of the plant, agar discs containing the mycelium of the fungus were placed at the surface of the canes, and the inoculation points were bound with cotton wool which was kept moist with sterile water. No penetration was observed even after periods of two months. Infection did, however, occur if the discs were partly introduced into cuts made in the surface of the canes by means of a scalpel, and the fungus was successfully reisolated from the plant tissues after surface sterilisation.

Where the incision is shallow, simply breaking the epidermis and the outer cells of the cortex, lateral spread of the hyphae of the fungus may be seen to take place between the cortical cells, and also within them for a short distance on either side of the place inoculated after two months. Where the incision is deeper, injuring the vascular system, hyphae will penetrate as far as the first two or three layers of cells in the pith after a similar period of time. The hyphae are both inter- and intracellular, and remain about 3μ in width. They pass from cell to cell in the pith, through the pits set in the cell walls, and no disintegration of the cell walls takes place. Hypertrophy of the cell wall does, however, occur in some cases, but is thought to be due to the mechanical injury sustained by the plant through wounding, as the cell cavities in which the/

the hypertrophies lie are frequently empty of hyphae, and no hyphae can be seen in the surrounding intercellular spaces.

To ascertain the effect of inoculating the axillary inflorescence bud, a number of inoculations was made in December, 1948, on plants which were kept under greenhouse conditions. Suspensions of pycnospores in sterile water were dropped from a hypodermic syringe into the apex of the bud after the bud scales had been parted with a dissecting needle. Agar disc inoculum was also used in a series of buds. The results of these experiments are not yet available, as it has not been found possible to induce early flowering under greenhouse conditions.

Experiments of the above kind have been made with the following varieties of Raspberry which are grown commercially in this country:- Mitchell, Lloyd George, and Sir Walfred.

In the Raspberry, it is possible to obtain flowering twice in the same year, when the plants are grown in the greenhouse. An early flowering occurs in March from canes produced by the rootstock in the previous season, and a late flowering occurs towards the end of the year from new canes. Direct inoculation of the flowers and uninjured canes gave negative results. In inflorescence bud inoculation, although it did not bring about anther blight, produced complete withering of the bud in several cases. The outer bud scales and the developing axis of the inflorescence were permeated by fungal hyphae which could be brought into culture. The result may be due to adverse cultural conditions imposed in ensuring flowering.

DISCUSSION

From the evidence which has been presented, it is possible to propose a description of the life cycle of Hapalosphaeria deformans, in so far as the fungus is concerned in bringing about a disease of Rubus fruticosus under natural conditions in the field.

It has been shown that the pycnospores of the fungus are included between the young primordial tissues of the inflorescence whilst the latter is still enclosed by the scales of the bud. During the early spring months, germination of the pycnospores takes place, and intercellular penetration of the tissues of the host occurs from appressoria developed by the hyphae of the fungus. Cell complexes of the fungus are also observable within the inflorescence bud by February. The cell complex, a structure which is characteristic of the fungus, can be regarded as a development from the division of an appressorium, or series of approximated appressoria, once penetration of the host has been effected. This conclusion is supported by the fact that the cell complex is embedded within the tissues of the host, at a much later stage in development, by means of a plug of pseudoparenchyma, which usually cannot be seen to penetrate deeper than the epidermal layer, and is presumably the outcome of the growth and division of the cells of the hypha or hyphae bringing about original penetration. From the bases of the complexes, the intercellular spaces of the host tissues receive thin walled hyphae, which pass to a depth of three or four layers of cells. The /

The young tissues of the inflorescence have been observed to suffer direct penetration some considerable time before further growth and development takes place. A certain amount of variation in the severity of the infection is operative at this stage. A proportion of buds becomes completely destroyed by hyphae which permeate the tissues both intra- and inter-cellularly.

During further growth and development of the panicle, the points at which the fungus enters the tissues become increasingly widely separated by the elongation of the internodes of the axis of the inflorescence, and at a much later stage, can be seen to bear embedded cell complexes. The distribution of the cell complexes on the mature inflorescence is therefore a general one. The complexes appear to be most dense on the pedicels of the flowers, and become progressively fewer as the base of the main axis of the panicle is reached. The distribution may have a certain significance, as it indicates that early penetration of the host must have been greater in the upper parts of the young inflorescence. This might be expected, as pycnosporos which enter the inflorescence bud through the comparatively loosely folded apices of the bud scales, would aggregate around the apex of the inflorescence axis, and would rarely find their way to a lower level owing to the developing bracts of the axis.

This restriction of the pycnosporos, and the mycelium of the fungus produced by their germination, to the apex of the inflorescence ensures that a considerable quantity of fungal inoculum is present around the primordia of the/
the/

the flowers. In the first instance, the inoculum appears to be in the form of fine hyphae, which show great increase in amount as they come to be included within the developing bud by the overgrowth of the sepals. Although the early penetration of the flower bud primordium has not been observed, the longitudinal distribution of the cell complexes of the fungus on both the adaxial and abaxial surfaces of the sepals in older buds implies that the sepal primordia probably suffered direct penetration during their early development. The petals may also show a considerable number of cell complexes, and the latter are particularly well developed around the young anthers. The intercellular spaces of the anthers become invaded by fungal hyphae, which assume the form of pseudoparenchyma. The pycnidia of the fungus are produced in great numbers within the anthers, and the pycnospores are shed even before the flower buds open.

The infection of the gynoecium of the flower presents several unusual features. Direct penetration of the wall of the carpel is rare, and the fungus appears to effect no penetration of the style within which it becomes included during the formation of the carpel, although a certain amount of penetration of the epidermal tissue which lines the loculus of the carpel takes place. Active parasitism apparently occurs at a late stage, and is largely confined to the ovule. The fact that infected carpels may contain seeds in which an obvious development of embryonic tissue has taken place, raises the question of the origin of this tissue/

tissue. It has been shown that pollen, undoubtedly carried by insects from surrounding uninfected flowers may be found on the stigmatic lobes of thoroughly diseased carpels. The extensively developed fungal pseudoparenchyma which lies within the style would presumably constitute a mechanical barrier to the passage of a pollen tube. It may, therefore, be the case that the formation of embryonic tissue is the outcome of the penetration of the embryo sac by nucellar tissue, the process of apomixis being known to occur in some species of Bramble. (5). Pycnidia of the fungus occur within the tissues of infected seeds, and may be produced at the inner surface of the stony endocarp of the druplet. The infection of the gynoecium results in its abnormal development as a whole, mesocarp tissues rarely forming in severely diseased carpels.

In the future course of the disease, a major feature is the eventual permeation of the tissues of the flower by the fungal hyphae, which occur principally in the intercellular spaces. This adoption of a systemic position is also found to occur in the pedicels of the flowers, the upper entire bracts and the basal scales of the inflorescence, and to a more limited extent in the prickles, with which the axis of the panicle is clothed. The hyphae become irregularly swollen, and aggregate to form masses of pseudoparenchyma from which pycnidia are developed. The occurrence of a general infection of the tissues at a late stage in their history indicates an added power of/

of colonisation which is possessed by the fungus when the host tissues show incipient senescence. The colonisation is thought to be brought about by the further growth and development of the intercellular hyphae connected with the cell complexes of the fungus.

Great quantities of spores produced by the pycnidia situated in the flowers and in the other parts of infected inflorescences must be easily blown by wind or washed by water to the young axillary inflorescence buds of the newly developed straggling canes, which arise from the rootstock of the plant. The life cycle of the fungus therefore becomes completed by the entry of pycnospores between the open tips of the scales of the young inflorescence buds. There is no reason to believe that a sexual phase occurs in the life history of the fungus.

A general survey of the effects of the disease has shown that it is possible to arrange infected inflorescences in a series, ranging from extremely deformed specimens on the one hand to almost normal ones on the other. Where disease of the inflorescence brings about a structure, which, owing to its early development in time and excessive deformation, may be conveniently referred to as a Witches' Broom, it is found that cell complexes are numerous, whereas in the infected inflorescences which approximate most nearly to normality, the cell complexes are few, or may be absent. The same condition is seen in the flower, where the deformed flower buds are richly provided with cell complexes, whilst flower buds showing no abnormal sepal development are almost devoid of them. There is, therefore, a strong positive/

positive correlation between the amount of deformation produced in the host, and the amount of development of cell complexes by the fungus.

From infection experiments, it is clear that the direct infection of the flower bud cannot be attained either by introducing pycnospores or by introducing fungal mycelium. This suggests that the important factor in the infection of the host is the early establishment of the fungus in a parasitic role. Accepting this hypothesis, it follows that the appearance of abnormal inflorescences is dependent on the early establishment of the fungus in quantity, whilst the inflorescences are within the bud. This may probably depend on the time at which the pycnospores germinate, and the number that are present. As the pycnospores have been shown to germinate readily in water, there is no reason to suppose that a resting phase is necessary before germination takes place under natural conditions. This implies a possible early infection of the inflorescence buds. The infection may occur late in summer, and would account for the presence of cell complexes within the bud by February of the following year. No satisfactory explanation can be forwarded for the occurrence of the tracts of pseudoparenchyma known to occur between the scales of the axillary buds borne by straggling canes newly produced from the rootstock of the plant. The assumption that pseudoparenchyma of this kind is of Hapalosphaeria deformans, can only be based upon morphological similarities between it and the pseudoparenchyma which occurs in the styles/

styles of infected carpels, and the buds in which it occurs have not yielded cultures of H. deformans.

No evidence has been gained which would indicate that the hyphae of the fungus are generally systemic within the plant. No internally placed mycelium, apart from that which definitely has its origin in a cell complex, has been observed, and from experiments in culture, and observations in the field, the impression that it does not exist has been confirmed. Infected inflorescences are sporadic in occurrence on any one straggling cane, and the fungus cannot be cultured from canes which have been surface sterilised. It, therefore, appears that the cause of abnormal growth of the host must be due entirely to a multiple infection of its tissues when the latter are at an early stage in development, within the axillary bud.

It is proposed, that where the infection occurs early, and where penetrations are numerous, the affected apical parts of the inflorescence, which give rise to the terminal flower cluster, undergo unduly early development, and this development continues irregularly, resulting in the formation of a Witches' Broom. Where the penetration of the inflorescence is not severe in the bud stage, due perhaps to the late germination of the pycnospores, combined with their comparative scarcity, then the inflorescence develops almost normally, although flower infection takes place from mycelium which is included within the buds. The deformation of the calyces and corollas of infected flowers/

flowers may be explained on the assumption that the quantity of penetrations, and the time at which they occurred, followed a random distribution in any one flower. The sepals and petals showing abnormal growth were therefore the earliest and most severely attacked, whilst of the remainder, some escaped infection entirely and developed normally in consequence. This proposed behaviour of the fungus would account for the extreme variation which is known to be a feature of infected inflorescences and individual flowers.

It is probable that inflorescences, which are originally attacked in the bud, suffer a number of additional penetrations as their growth and development proceeds. Experimental infections indicate that older, mature tissues cannot be attacked unless they are first wounded, and this can be taken to mean that the cuticle withstands penetration when fully mature. The cell complexes of the fungus which occur on the upper parts of the inflorescence give rise to thin-walled hyphae, which ramify over the surface of the host. Many of these hyphae are in direct contact with tissues which are still meristematic, and over which the cuticle is thin. Penetrations of the host may, therefore, be brought about over the greater part of the growth period.

The irregular growth and development of tissues which are invaded by fungal hyphae which maintain a localised distribution, are known to occur in other plant diseases, particularly those caused by species of Taphrina and species of Exobasidium. The original infection of the/

the host by Hapalosphaeria deformans is similar in its operation to that which has been described in the case of the peach, when attacked by Taphrina deformans (3). The ascospores of the Taphrina become enclosed within the open leaf buds, and germinate in spring, eventually effecting a localised intercellular penetration of the leaf. The cells of the latter undergo an enlargement, to which the symptom of leaf curl may be attributed. The malformation in the case of the peach, as in the case of the Bramble, is confined to the growth of the current year, and the perennating mycelium of the fungus cannot be found in the twigs. The diseases caused by Hapalosphaeria deformans and Taphrina deformans are also similar in that they are subject to seasonal fluctuations in intensity.

The formation of Witches' Brooms is also known to occur in the hosts of various species of Exobasidium. The hyphae of Exobasidium are largely intercellular, but are perennial in many cases. A good example is provided by E. parvifolii, the life history of which is described by Holston (4), but apart from the development of Witches' Brooms by the host, Vaccinium parvifolium, little comparison can be drawn with the disease caused by Hapalosphaeria.

The present investigation has shown that the symptoms of the disease of Rubus fruticosus are similar to those which have been described in the case of Rubus caesius by Diedicke and Sydow (2). Although no detailed information is available concerning the Witches' Brooms produced in Rubus/

Rubus macropetalus, (15), it is likely that they are similar to those which occur in the other two species.

In their paper on the disease of R. caesius, Diedicke and Sydow (2) recognised the presence of cell complexes within the flower, but did not appreciate their significance as marking isolated penetrations of the host tissues.

Mention was made of the deformations which occur in the sepals and petals, but the development of the gynoecium was regarded as normal.

The investigation has confirmed that infection takes place in the axillary inflorescence buds as suggested by Diedicke and Sydow (2) and Zeller and Braun (15).

Evidence has been presented to show that the disease of the flower in Rubus fruticosus is much more severe than has hitherto been reported in the case of other Rubus species, and that the mycelium and pycnidia of the fungus are not confined to the tissues of the anthers, but occur on other floral organs and on the prickles, bracts and bud scales of the inflorescence.

The formation of appressoria by the mycelium of the fungus at the surface of the anther in the Bramble also occurs in the Dewberry (15), but the subsequent infection of the anther differs in detail. In the anthers of the Dewberry, the pseudoparenchyma which forms in the loculi is reported as being two to five cells in thickness, whereas in the Bramble, the loculi of the anthers in severely infected flowers are completely filled with fungal pseudoparenchyma. The disease of the Loganberry (1), and of the Dewberry is reminiscent of that which has been found to/

to occur in the almost normal, infected inflorescences of the Bramble. No mention has been made of the presence of Witches' Brooms or fungal cell complexes in the Loganberry and Dewberry. It appears, therefore, that the cultivated hosts of the fungus show a form of disease, which, in the case of the Bramble, would be considered mild.

The fact that no infections have been obtained in the case of cultivated Raspberries, using pycnospores and isolates of the fungus obtained from the Bramble, suggests that free transference of infection from the Bramble to the Raspberry would not occur in the field.

The isolates of Hapalosphaeria deformans made by Zeller and Braun(15) did not spore in cultures as did those obtained during the present work, and the above authors mention that a whiter strain of the fungus may be obtained from the flower. It is not known whether the whiter strain bears any resemblance to the fungus found to be associated with the axes of diseased inflorescences in the Bramble. The investigation of the latter fungus is still in progress, and it cannot be stated whether it is concerned in the disease.

SUMMARY

The disease of Rubus fruticosus L. caused by Hapalosphaeria deformans Syd. has been investigated, using infected material obtained from a variety of sources in Scotland.

The disease affects the inflorescences, which may be normal in pattern, or show varying degrees of abnormality giving rise to structures which can be regarded as Witches' Brooms. The flowers borne in Witches' Brooms are subject to deformation, which affects all the floral whorls. The sepals and petals show irregular development, the anthers fail to dehisce, and the compound fruit does not develop normally.

Both abnormal and normal infected inflorescences have been examined at various stages of their development. The fungus has been shown to effect a local penetration of the tissues of the flower, and the tissues of the axes of the inflorescences. The points of penetration are marked by knots of pseudoparenchyma (or cell complexes), from the bases of which, thin-walled hyphae penetrate the intercellular spaces of the underlying tissues. No internal mycelium other than that derived from cell complexes has been discovered in any of the tissues of the plant at an early stage.

Cell complexes are numerous where deformation of the host is extreme, and may be entirely absent where no abnormal growth of the host occurs.

The effect of the fungus on the various floral structures is described in detail, and it has been found that/

that the tissues of the floral whorls and the pedicels become invaded by fungal hyphae at a late stage in development. Pycnidia of the fungus have been observed in all parts of the flower, in the seed, and in the upper, entire bracts, basal bud scales and prickles of mature, severely infected inflorescences.

The mycelium of the fungus situated between the flower parts has been observed to give rise, terminally or laterally, to conidia.

Germinating pycnospores and cell complexes of the fungus have been found in the inflorescence buds of the plant in February, and penetration of the tissues of the inflorescence has been observed.

The results of the experimental inoculation of the inflorescence buds of the Bramble are not yet available, but no infection occurred from the inoculation of the inflorescence buds of Lloyd George, Mitchell and Sir Walfred Raspberries. Direct inoculation of the flower buds and straggling canes of the Bramble gave negative results, but the fungus became established in the tissues of wounded canes.

The fungus has been grown in culture, and has produced pycnidia. Another fungus, characterised by the production of sclerotia in culture, has been obtained from diseased inflorescences.

The results of the investigation are discussed, and a life cycle of the fungus is proposed.

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EXPLANATION OF FIGURES.

Fig. 1.

An infected inflorescence of Rubus fruticosus. The panicle is almost normal in appearance, but shows an enlarged terminal flower. Slightly deformed flower buds are borne in lateral cymes. $x \frac{1}{2}$

Fig. 2.

The apical part of the panicle shown in Fig. 1.. The petals of the terminal flower are greatly increased in size, and retain the crumpled appearance due originally to package in the bud. Pycnospores are shown at the surfaces of the diseased anthers. Approx. $x 2$.

Fig. 3.

A diseased panicle which has adopted the form of a Witches' Broom owing to the extensive development of the lateral cymes. The individual flowers show extreme sepal deformation. $x \frac{1}{2}$.

Fig. 4.

A young, infected inflorescence showing abnormally early development of the terminal flower cluster.

Fig. 5.

An example of an extreme form of Witches' Broom at an early stage in development. The panicle shows early maturation of flower buds, and suppression of leaf development. $x \frac{1}{2}$.

Fig. 6.

An enlargement of the apex of the panicle shown in Fig. 5., demonstrating the elongation and pouching which occurs in the flower buds. $x 3$.

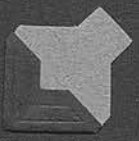


Fig. 1.



Fig. 2.

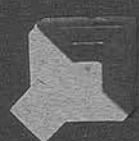
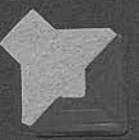


Fig. 4.



Fig. 3.



Fig. 6.



Fig. 5.

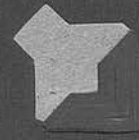


Fig. 7.

A series of terminal flower buds removed from Witches' Brooms, illustrating the differential growth of the sepals, and the effect of pouching. Slightly enlarged.

Fig. 8.

The apex of a Witches' Broom, showing a severely deformed terminal flower, in which the sepals have adopted a leaf-like form. Slightly enlarged.

Fig. 9.

A panicle which can be classified as a weakly developed Witches' Broom. The tips of the pouched flower buds are overarched by spine-like projections of the deformed sepals. $x \frac{1}{2}$.

Fig. 10.

A group of petals, which show increase in size and irregular basal doubling. Approximately natural size.

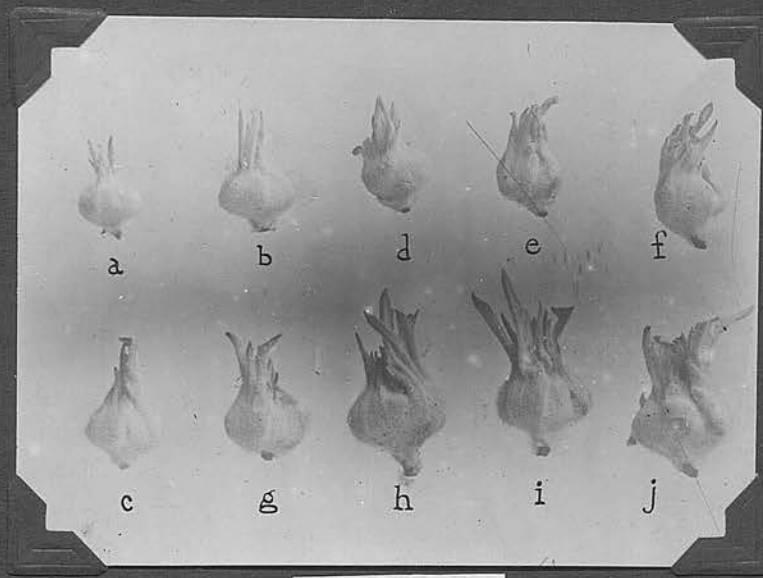


Fig. 7.



Fig. 8.

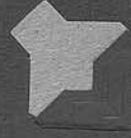


Fig. 9.

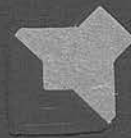
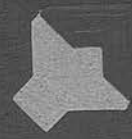


Fig. 10.

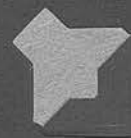


Fig. 11.

Part of a longitudinal section of an infected flower, showing a fungal cell complex set in the tissues of the flange of the receptacle near the base of the filament of a stamen. x 475.

Fig. 12.

Part of a longitudinal section of a sepal, with a cell complex of the fungus embedded between the epidermal cells at the adaxial surface. Intercellular hyphae are also shown below the complex. x 1000.

Fig. 13.

Part of a longitudinal section of a young flower bud, showing a cell complex of the fungus which has formed at the surface of a stamen primordium. The tissue of the primordium can be seen below the cell complex. x 300.

Fig 14.

Part of a longitudinal section of a young flower bud, showing a fungal cell complex(c), embedded in the epidermal layer of a stamen primordium. The cells of the primordium are distorted, and stain deeply. Fine hyphae of the fungus are shown at(h). x 300.

Fig. 15.

Part of a longitudinal section of a petal, which shows the presence of a cell complex of the fungus embedded between the epidermal cells at the abaxial surface. x 475.

Fig. 16.

Part of a longitudinal section of the base of a sepal.

A fungal cell complex is embedded between the epidermal cells at the abaxial surface. Intercellular hyphae occur below the epidermis. x 1000.

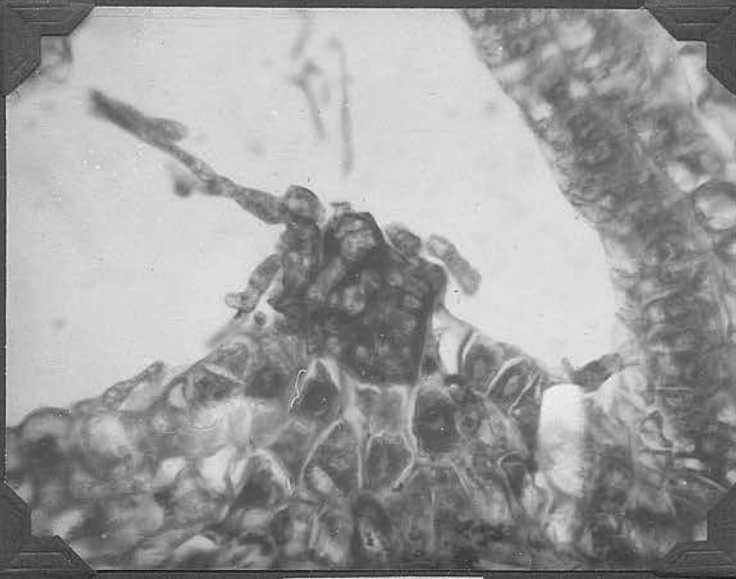


Fig. 11.

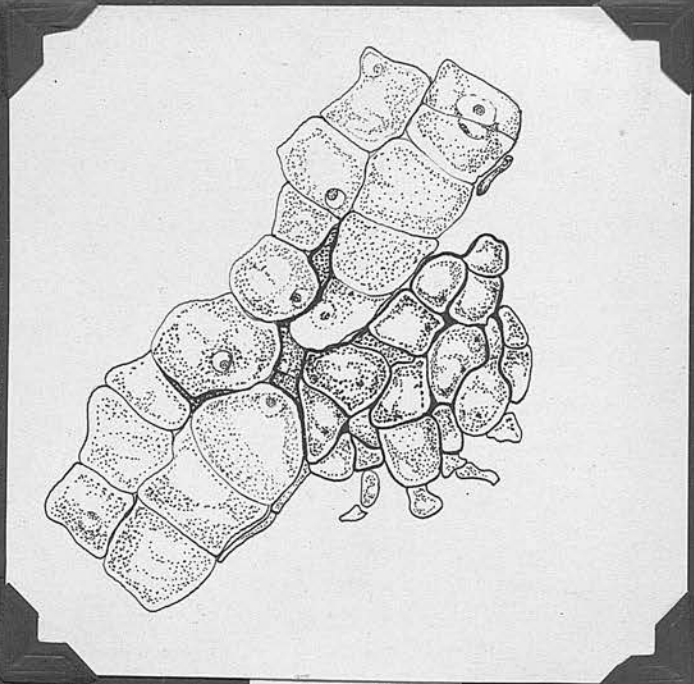


Fig. 12.

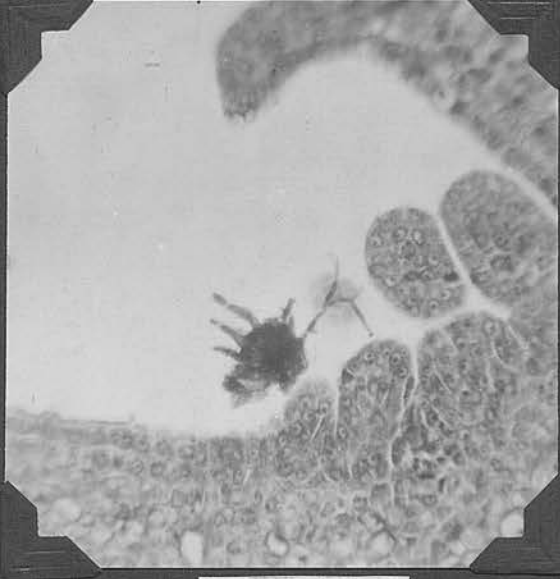


Fig. 13.

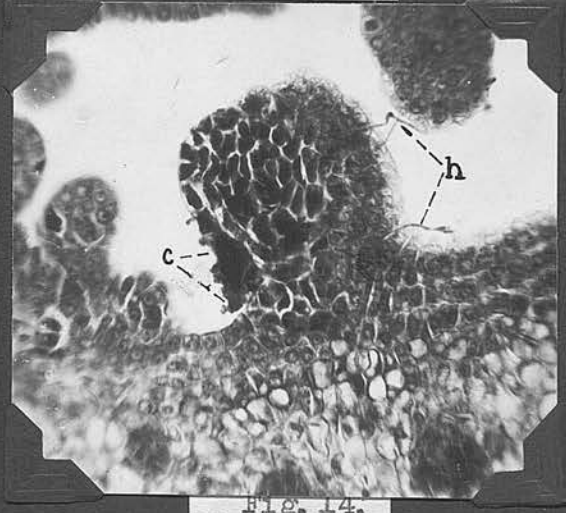


Fig. 14.

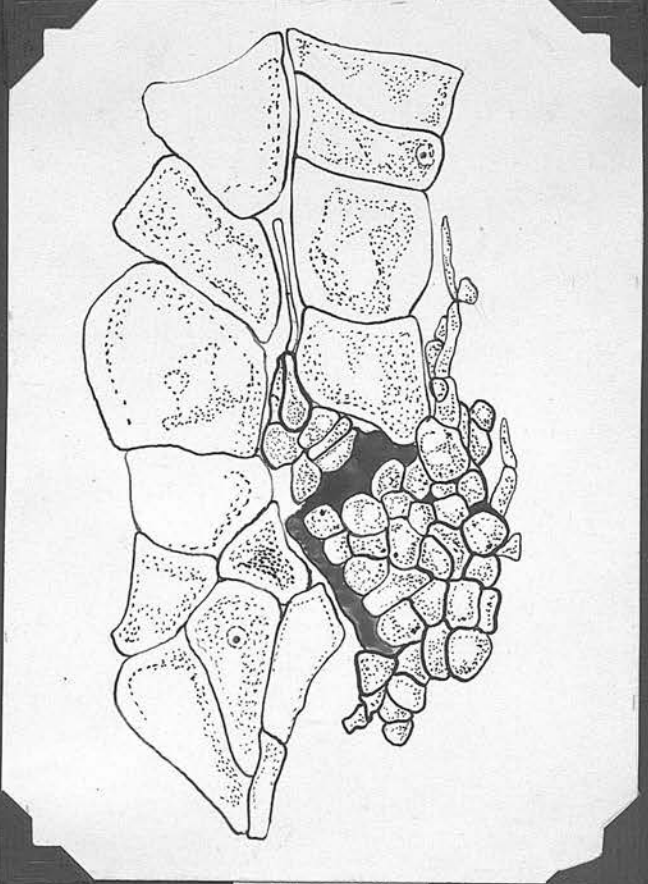


Fig. 16.

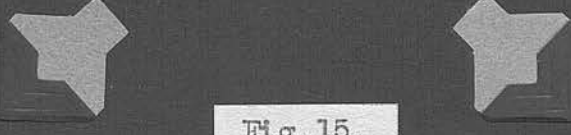


Fig. 15.

Fig. 17.

A longitudinal section of a young flower bud, in which deformation has been initiated by the abnormal lateral growth of the sepal which is shown on the left of the section. x 70.

Fig. 18.

Part of a longitudinal section of an infected flower bud, showing irregular doubling of a petal(p). x 200.

Fig. 19.

The surface of an anther which bears a fungal cell complex. The complex is in the form of a flat plate. x 260.

Fig. 20.

Part of a longitudinal section of an anther: the fungus has given rise to an appressorium at the surface of the epidermis. x 1300.

Fig. 21.

Part of a longitudinal section of a young anther, showing the development of appressoria by the hyphae of the fungus, and young stages in the formation of fungal cell complexes. x 550.

Fig. 22.

Part of a transverse section of a young anther, showing part of a fungal cell complex at the surface of the epidermis, and intercellular hyphae lying between the cells of the parenchyma. x 800.

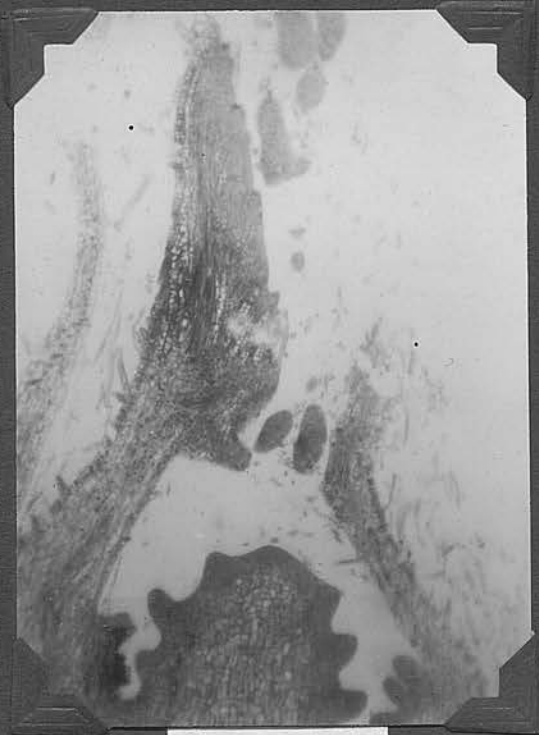


Fig. 17.



Fig. 18.



Fig. 19.



Fig. 20.

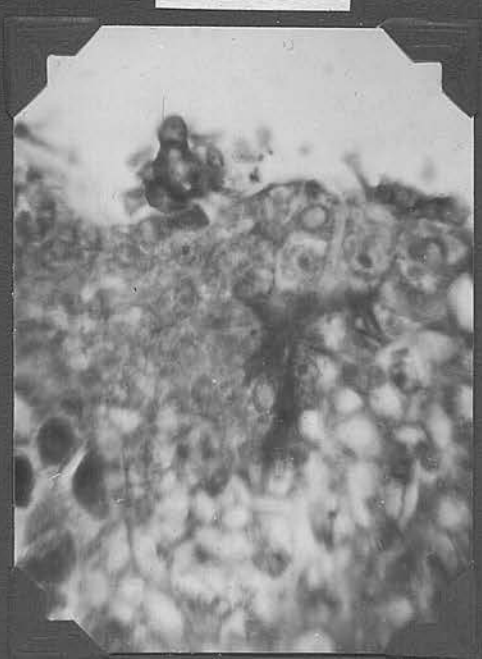
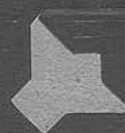


Fig. 21.

Fig. 22.

Fig. 23.

Fungal cell complexes situated at the surfaces of young anthers. Intercellular penetrant hyphae are also shown. x 1000.

Fig. 24.

Part of a transverse section of a mature anther, showing the development of fungal pseudoparenchyma in the intercellular spaces and within the pollen sac of the anther. Pollen is shown at (d), and a pycnidium, in oblique section, occurs at (p). x 500.

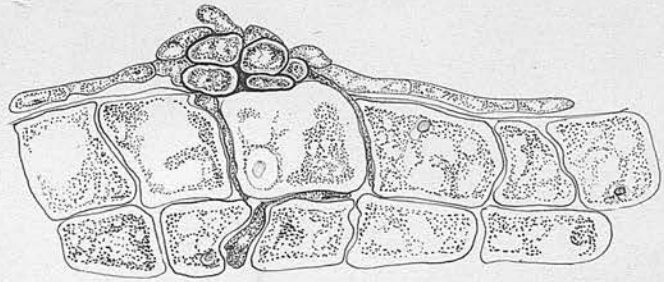
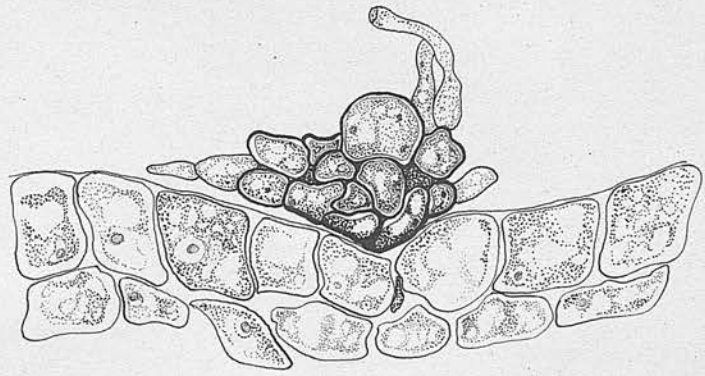


Fig. 23.

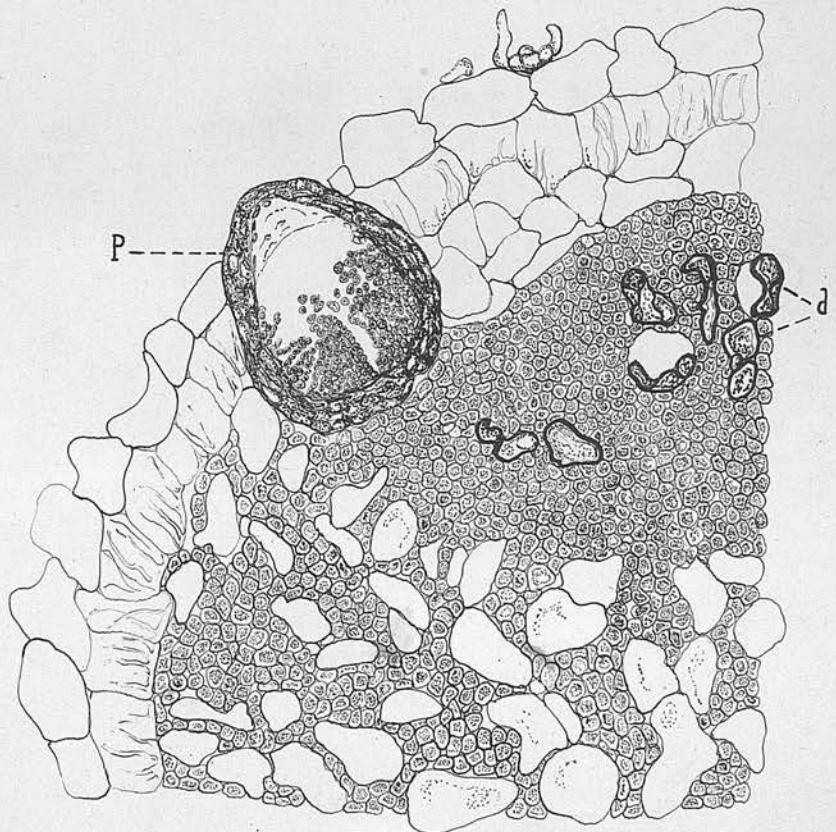


Fig. 24.

Fig. 25.

An oblique section of an anther, showing deeply stained intercellular hyphae of the fungus.

x 220.

Fig. 26. & Fig. 27.

Pycnidia of the fungus arising from the pseudoparenchyma which occupies the pollen sacs of the anthers.

x 450.

x 300.

Fig. 28.

A slightly oblique longitudinal section of an infected anther. Products of disintegration occur in the cells of the host tissues, and the pycnidia of the fungus arise in great numbers from the pseudoparenchyma.

x 175.

Fig. 29.

Part of a transverse section of an infected flower bud.

The section shows the raised central part of the receptacle bearing carpels. Deeply stained hyphae of the fungus occur in the intercellular spaces of the tissues of the receptacle and carpels.

x 150.

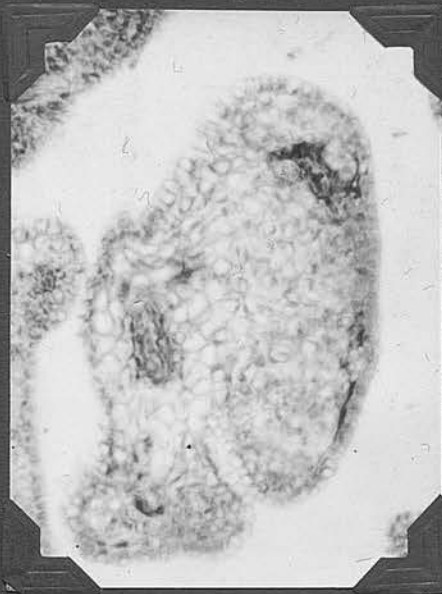


Fig. 25.

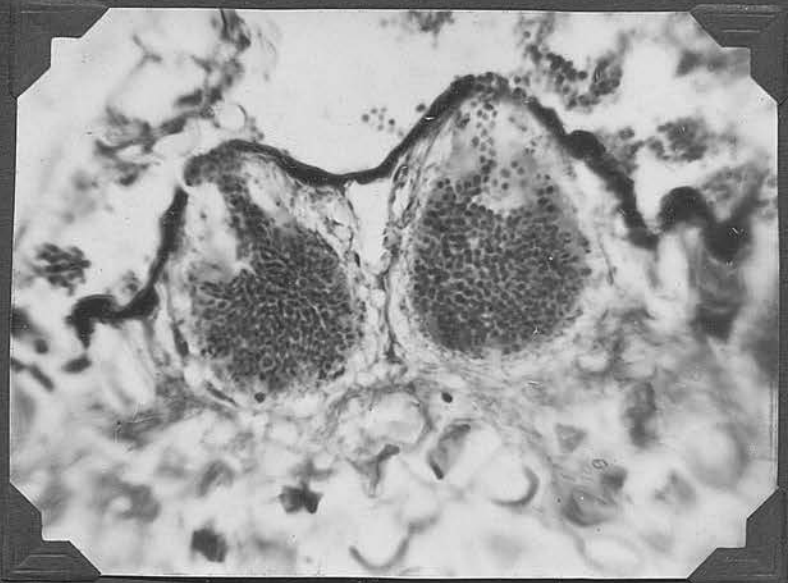


Fig. 26.

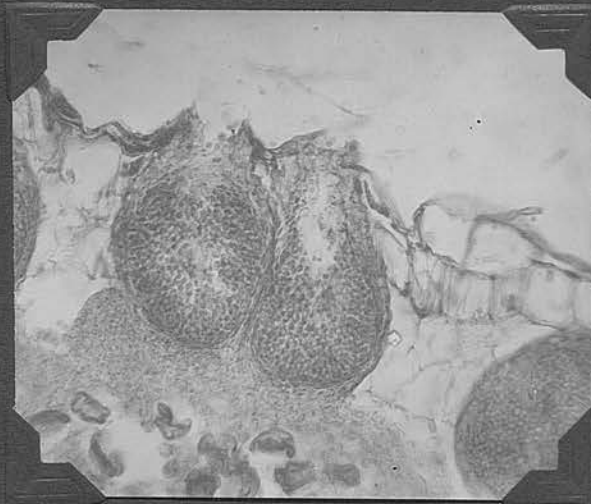


Fig. 27.

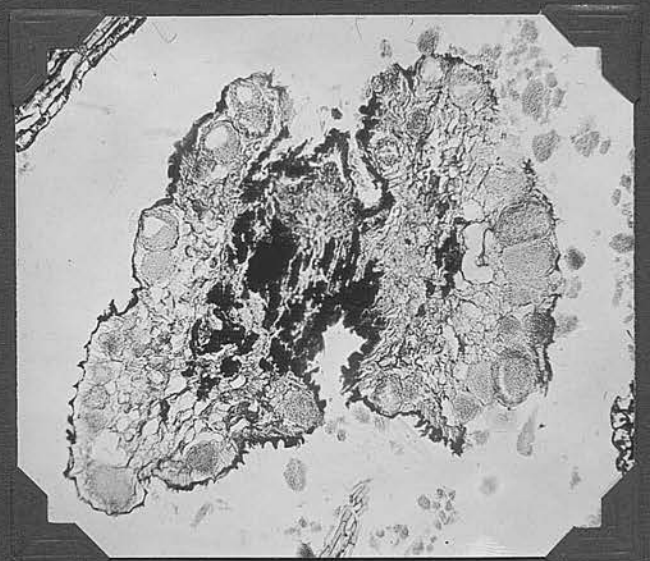


Fig. 28.



Fig. 29.

Fig. 30.

A single carpel from the section shown in Fig. 29. Massed hyphae of the fungus are seen in the intercellular spaces. x 300.

Fig. 31.

An oblique section of a young carpel, showing the hyphae of the fungus lying within the ventral opening and loculus of the carpel. The primordia of the ovules are shown at (o). x 260.

Fig. 30.

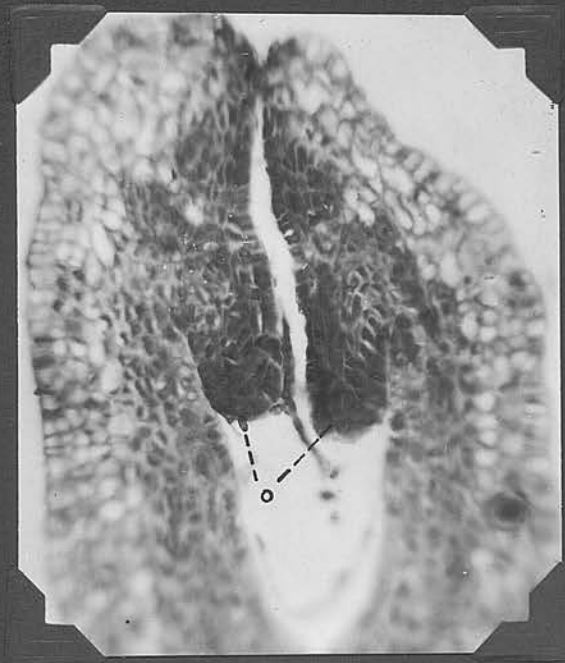


Fig. 31.

Fig. 32. & Fig. 33.

Parts of the styles of infected carpels,
showing the presence of fungal pseudoparen-
chyma within the tissues.

x 600.

x 300.

Fig. 34

A longitudinal section of a young carpel,
showing the pseudoparenchyma of the fungus(s),
lying between the developing ovules. Free
hyphae(h) occur in the loculus of the carpel.

x 300.

Fig. 35.

A longitudinal section of a carpel at a later
stage of development, showing brown-walled
fungal pseudoparenchyma(S). Fungal hyphae(h)
can be seen in the loculus of the carpel.

x100.

Fig. 32.

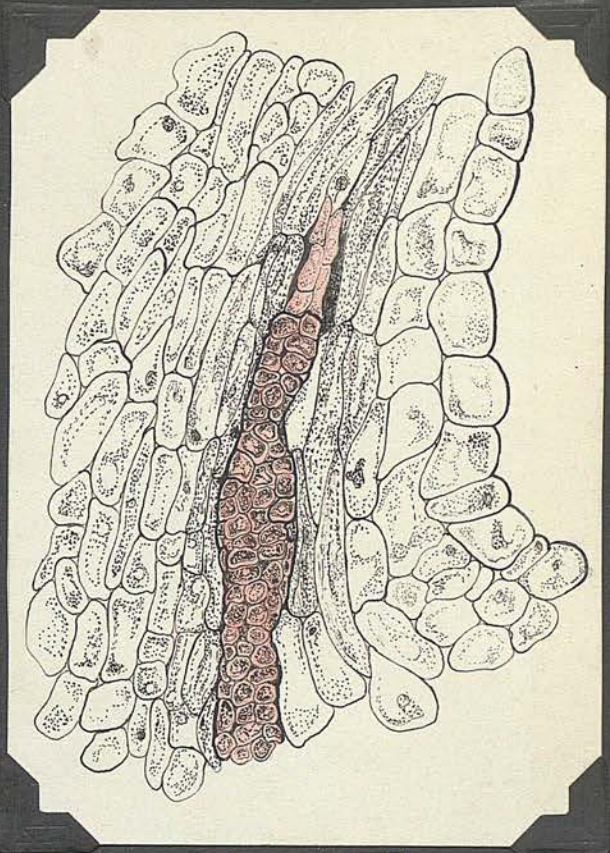


Fig. 33.

Fig. 34.



Fig. 35.

Fig. 36.

Part of a longitudinal section of a carpel, showing fungal pseudoparenchyma(s), and hyphae(h) within the loculus of the carpel. x 300.

Fig. 37.

A longitudinal section of a carpel, showing fungal pseudoparenchyma(s), and hyphae(h) entering the micropyle of the ovule. x 100.

Fig. 38.

A longitudinal section of an ovule, showing hyphae of the fungus penetrating between the cells of the nucellus and lying beside the embryo sac. x 300.

Fig. 39.

Part of a longitudinal section of a druplet, showing fungal hyphae(h) on the inner surface of the endocarp. The seed contains fungal pseudoparenchyma(s), from which a pycnidium (p) has been developed. x 300.

Fig. 40.

A longitudinal section of a druplet, showing the hyphae of the fungus at (h), and a pycnidium(p) at the inner surface of the endocarp. x 300.

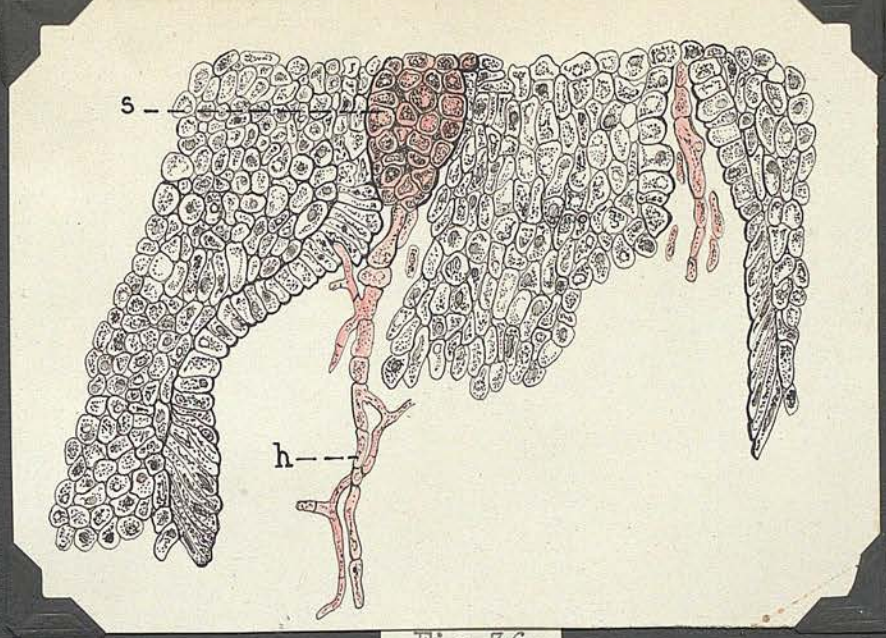


Fig. 36.



Fig. 37.



Fig. 38.

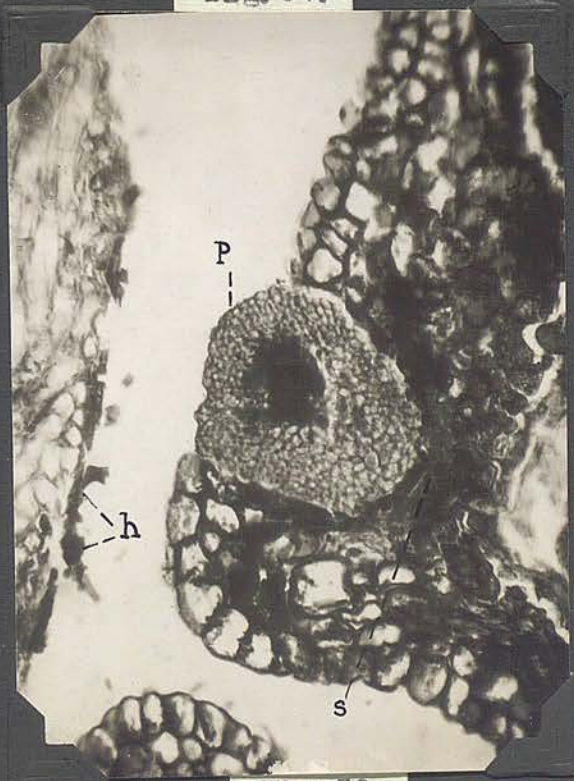


Fig. 39.



Fig. 40.

Fig. 41.

A longitudinal section of an infected carpel, showing fungal pseudoparenchyma(s), which has given rise to a pycnidium(p) situated towards the base of the style. x 65.

Fig. 42.

A longitudinal section of the stigmatic lobes and part of the style of an infected carpel, Fungal pseudoparenchyma occurs centrally in the upper stylar tissue, and a pycnidium has developed on a lobe of the stigma. x 120.

Fig. 43.

A longitudinal section through part of the pith of the raised central part of the receptacle, showing intercellular fungal hyphae massing to form pseudoparenchyma. x 700.

Fig. 44.

Part of a transverse section of a sepal, showing pycnidia situated below the epidermis of the adaxial surface. x 65.

Fig. 45.

A pycnidium of the fungus within the tissues of a petal, which is cut in longitudinal section. x 200.

Fig. 46.

Part of a longitudinal section of the fluted main axis of an infected panicle, showing a cell complex of the fungus. x 275.

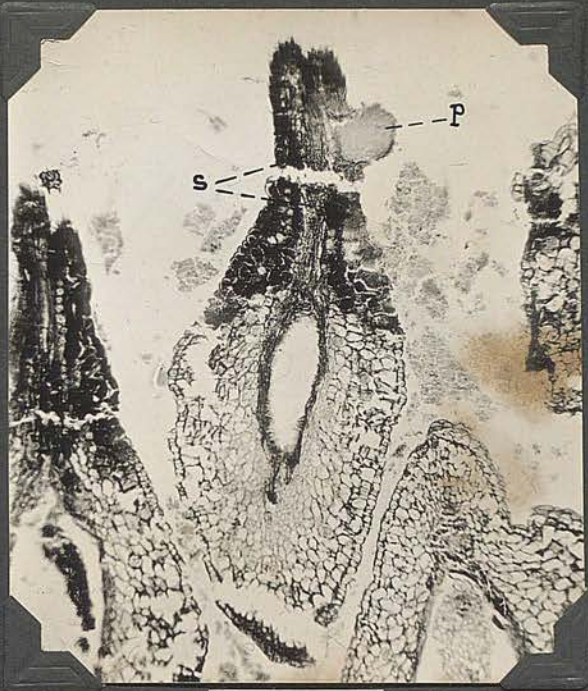


Fig. 41.

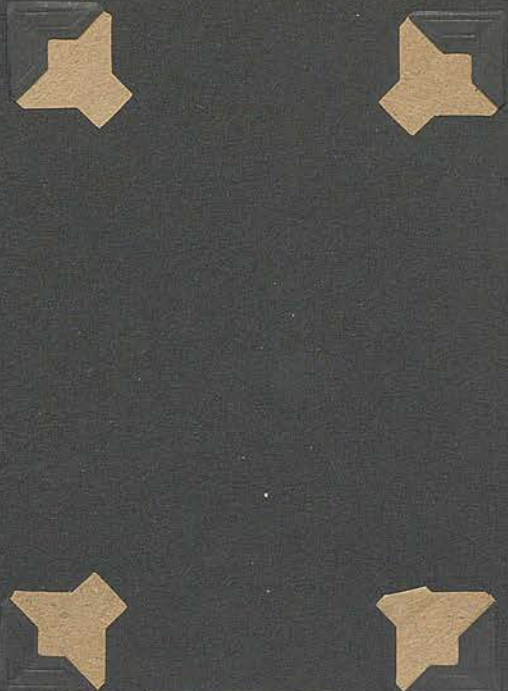


Fig. 42.



Fig. 43.



Fig. 44.



Fig. 45.



Fig. 46.

Fig. 47.

Part of a longitudinal section of the main axis of a diseased panicle, showing a fungal cell complex situated at the base of a multicellular hair. x 275.

Fig. 48.

Part of a section of a prickle borne by the main axis of a diseased panicle. A fungal cell complex is embedded between the epidermal cells, and intercellular hyphae can be seen at (h). x 275.

Fig. 49.

Part of a longitudinal section of the main axis of a diseased panicle, showing part of a prickle cut in oblique section. A pycnidium (p) has been developed directly from fungal pseudoparenchyma situated at (s). x 275.

Fig. 50.

Part of an upper, entire bract of a diseased inflorescence, showing pycnidia of the fungus embedded within the tissues. x 60.

Fig. 51.

Part of a longitudinal section of the base of a scale of an inflorescence bud. An intercellular hypha is shown at (h). x 1000.

Fig. 52.

Part of a longitudinal section of an inflorescence bud, showing germinating pycnospores of the fungus. x 700.



Fig. 47.

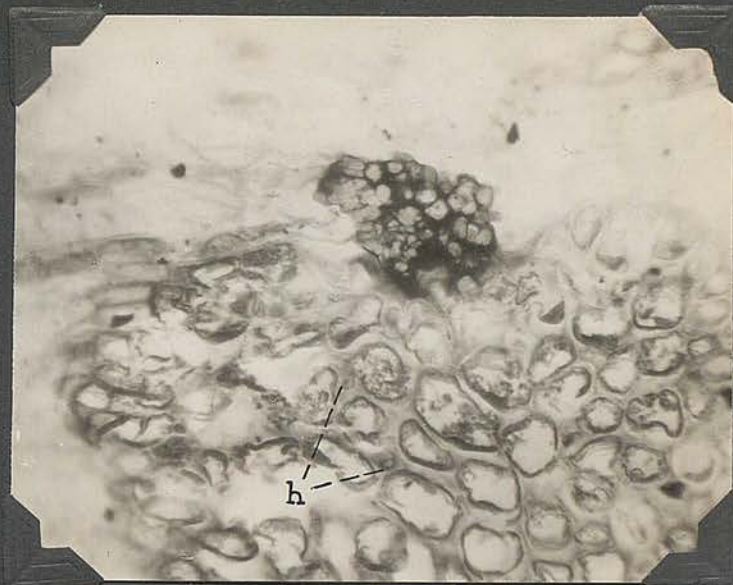


Fig. 48.



Fig. 49.

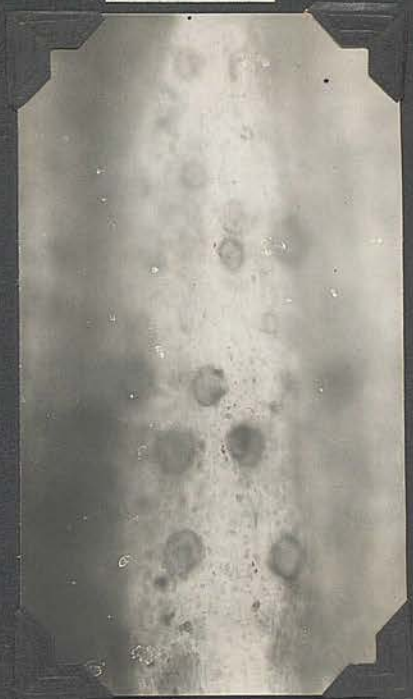


Fig. 50.



Fig. 51.



Fig. 52.

Fig. 53.

Part of a longitudinal section of an inflorescence bud, showing a leaf base attacked by the hyphae of the fungus. An appressorium is shown at (a).
x 1400.

Fig. 54.

A longitudinal section of part of an inflorescence bud, showing a fungal cell complex embedded within the epidermis of the bud primordium. x 400.

Fig. 55.

Part of a longitudinal section of a young inflorescence bud, showing the development of fungal pseudoparenchyma(s), between the bud scales. x 250.

Fig. 56.

Germinating pycnospores of the fungus. Anastomoses occur between the germ tubes. x 1000.

Fig. 57.

A section through part of a culture of the fungus, showing pycnidia arising from the basal layer of pseudoparenchyma. x 95.

Figs. 58 & 60.

Pycnidia of the fungus obtained from culture. x170, x100.

Fig. 59.

The mycelium of the fungus situated between the flower parts, showing the production of conidia. x 600.

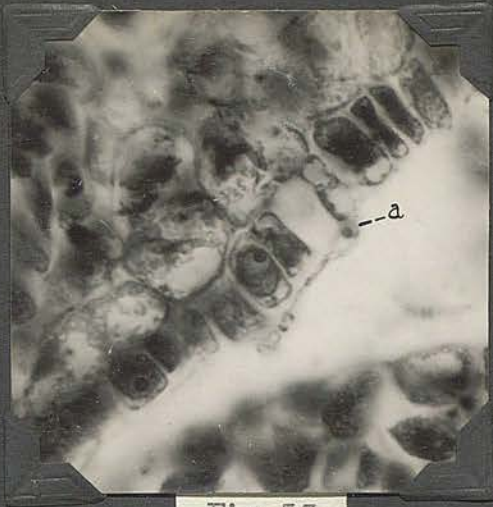


Fig. 53.



Fig. 54.

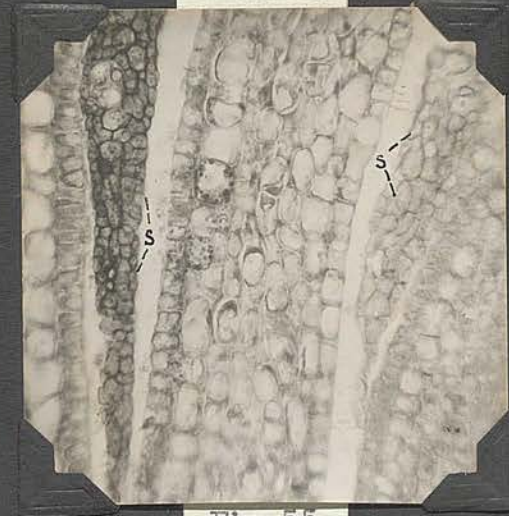


Fig. 55.



Fig. 57.



Fig. 58.



Fig. 59.

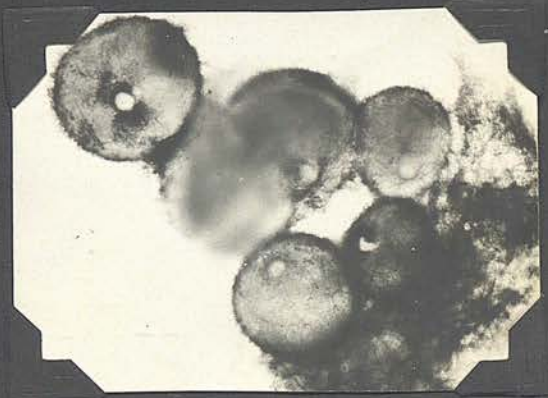


Fig. 60.

ACKNOWLEDGEMENTS

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