On Stigeosporium Marattiacearum and the Mycorrhiza of the Marattiaceae.

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With Plate III and nine Figures in the Text.

NOTWITHSTANDING the attention which has for many years been bestowed upon the Marattiaceae and the frequent references to the presence of a mycorrhiza, our present knowledge of the endophytic fungi which are commonly associated with the roots of this important group of Ferns is very incomplete, and, apart from the observations of Kühn and of Gallaud, practically nothing is known of their morphological and biological characters or of their systematic position.

In his thesis on the Marattiaceae, Kühn (28) reserved a section for a short account of the fungi which he observed in roots of *Angiopteris evecta*, Hoffm., *Kaulfussia aesculifolia*, Bl., and *Marattia alata*, Sm., and, in the case of the last-named genus, claimed to have succeeded in isolating an endophyte which produced spores when grown on artificial nutrient media. But, for reasons mentioned below (p. 90), it seems certain that this botanist was dealing not with the endophytic but with the epiphytic fungal flora of the marattiacean roots. Gallaud (22, p. 40) very briefly described the endophyte which he found in the roots of *Angiopteris Durvilleana*.

The present writer, who was fortunate in having abundant material at his disposal, has therefore attempted to give a more detailed account of these fungi.

MATERIAL AND METHODS.

Material of *Angiopteris evecta*, Hoffm., *Danaea alata*, Sm., *Danaea nodosa*, Sm., *Kaulfussia aesculifolia*, Bl., and *Marattia Cooperi*, Mre., was examined, together with a few pieces of root from a herbarium specimen of *Archangiopteris Henryi*, Chr. et Gies., which was soaked out in 80 per cent. NH_3OH ¹ with most successful results.

The greater part of the material was fixed in 75 per cent. alcohol + glycerine: for ordinary histological work this mixture gave very good results, but rendered a careful cytological investigation impossible.

¹ According to the method described by M. Raciborski in Flora, Bd. lxxxi, 1895, p. 153.

Both hand- and microtomed sections were cut ; the former were found useful for demonstrating the grosser features, whilst the latter were necessary for determining the finer details.

A considerable number of stains and reagents were used, as it was found necessary to employ a variety of staining methods in order to successfully differentiate the younger parts (especially haustoria) of the fungal mycelium from the tissues of the host-plant.

Fairly good results were obtained by the use of Durand's¹ haematoxylin-eosin combination for differential staining, but the cotton blue + lactic acid method recommended by Jones, Giddings, and Lutman (27, p. 30, foot-note) did not give satisfactory results.

The following stains were also employed either singly or in combination : Kleinenberg's haematoxylin, safranin, azo-blue, picro-nigrosin, methylene blue, and Congo red.

For determining the chemical nature of the cell-walls, &c., various reagents, including chlor-zinc-iodide, H_2SO_4 , iodine, phloroglucin + HCl, osmic acid, alkannin, Sudan III, Congo red, and ruthenium red were found useful.

The sections were mounted in Canada balsam or in glycerine jelly.

A. ON THE ENDOPHYTE OF THE ROOTS OF ANGIOPTERIS EVECTA, HOFFM., ARCHANGIOPTERIS HENRYI, CHR. ET GIES., KAULFUSSIA AESCULIFOLIA, BL., AND MARATTIA COOPERI, MRE.

The roots of the first order of all genera and species of Marattiaceae are relatively robust and frequently branch in a monopodial manner at some distance from the caudex. The roots of the second and higher orders are usually long and fibrous, and are provided with multicellular root-hairs (*r.h.*, Text-fig. 9 ; Pl. III, Fig. 3), which are rather abundant on the fine threadlike terminal rootlets.

In all the above-named genera the roots are characterized by the complete absence of a cortical zone of stereome (cf. *Danaea*), although isolated 'stone-cells' are not infrequently met with near the periphery of the larger roots. The ground-tissue of the cortex consists of typical large parenchymatous cells, many of which are packed with starch.

The endophyte, a brief description of which the present writer (44) has already published, is of general occurrence in the primary and earlier adventitious roots, but its presence appears to be less regular in the later roots of each genus.

It seldom occurs in the aerial portion of the main roots, and is invariably absent from the terminal (i. e. meristematic) region of all roots.

¹ Durand, E. J. : The Differential Staining of Intercellular Mycelium. *Phytopathology*, i, 1911, p. 129.

A few plants were examined in the roots of which no trace of the fungus could be detected; this condition also obtains in a small proportion of the roots of all plants which have passed their juvenile stage. This may explain Kühn's (28, p. 494; 29, p. 149) failure to find infected roots in his specimens of *Marattia fraxinea*, Sm., and of *Danaea alata*, Sm., and the statements of Campbell (14, p. 215) and of Charles (15, p. 84) concerning the distribution of the endophyte in older roots.

The presence of the mycorrhizal fungus, which can be detected only by an examination of sections of the host-root, does not influence in the slightest degree the external appearance of the host, infected and uninfected plants appearing to be equally healthy and vigorous. Neither does the nature of the soil bear any relation to the presence or absence of the endophyte, which is found in the roots of plants growing in soils ranging from light rich humus to red clay from disintegrated gneissic rocks.¹

Thus it would appear that in the above-named genera of Marattiaceae symbiosis is not obligate, but facultative.

1. *Distribution, Organization, and probable Life-history of the Fungus in the Tissues of the Host.*

i. *Mycelium.*

Microscopical examination of transverse or longitudinal sections of infected roots shows that fungal hyphae from the surrounding soil may enter the root through any of the epidermal cells in spite of the cuticularization of

TEXT-FIG. 1. Part of a longitudinal section of a root of *Archangiopteris Henryi*, Chr. et Gies., showing infecting hypha of *Stigeosporium Marattiacearum*. $\times 270$.

the outer wall of these cells (Text-fig. 1; cf. Noël Bernard, 10, p. 243). The mycelium is both inter- and intracellular within the tissues of the host, exhibits very few septa, and is copiously branched (Text-fig. 2, *a* and *b*).

¹ The author is indebted to Professor J. B. Farmer, F.R.S., for information regarding the habitat of a number of plants of *Angiopteris evecta*, Hoffm., gathered by him in Ceylon.

When young, the hyphae are hyaline and possess extremely thin delicate walls with a very irregular contour (Text-fig. 2, *a*). At this stage, their contents consist of a finely granular cytoplasm in which many relatively large nuclei are irregularly distributed; in other words, the mycelium of this fungus exhibits the multinucleate condition typical of the Oomycetes (Text-fig. 2, *a*, *b*).

The walls of the hyphae thicken up with age, assume a light-brown coloration, and become highly refractive. Meanwhile the cytoplasm becomes

TEXT-FIG. 2. Mycelium of *Stigeosporium Marattiacearum*. *a*, *b*, and *c* are drawn to the same magnification in order to show remarkable variation in size and form of the hyphae. From a root of *Angiopteris evecta*, Hoffm. *n.* = nucleus.

The contents of the host-cell are not shown. $\times 750$.

vacuolate and tends to disappear, whilst the oldest hyphae are invariably empty (Text-fig. 2, *c*). In diameter the hyphae are extraordinarily variable, ranging from $1\ \mu$ in the younger to $12\ \mu$ in the older regions (Text-fig. 2, *a*, *b*, *c*).

As stated above, the mycelium within the tissues of the host is both inter- and intracellular. In the outer layers of the cortex the hyphae are usually intracellular, apparently experiencing no difficulty in penetrating the cell-walls of the host (Text-fig. 1). No obvious constriction of the hyphae is noticeable at the point where they penetrate the cell-wall, nor does the latter develop a tubular sheath around the invading hypha. It is probable that the secretion of enzymes, which results in the solution of the cell-wall, is confined to the apical region of the hyphae, for, even in very old roots, the presence of the fungus never leads to isolation and disorganization of the

host-cells. But a curious effect is produced upon many of the large parenchymatous cells of the inner cortex, which are stimulated to further division, as is shown in Text-fig. 3, *a*, *b*.

However, on reaching the inner layers of the cortex, the hyphae for the most part become intercellular (Pl. III, Fig. 5 A). Several hyphae (eight or more) may frequently be found traversing a single intercellular space (Text-fig. 4, *a*).

With few exceptions the principal intercellular hyphae pursue a course more or less parallel with the long axis of the host-root (Text-fig. 5 ; Pl. III, Fig. 5 A), but cross-connexions between two or more of these main

TEXT-FIG. 3. Cortical cells undergoing further division as a result of the presence of the endophyte. *a*. From a transverse section of *Marattia Cooperi*, Mre. *b*. From a longitudinal section of *Archangiopteris Henryi*, Chr. et Gies. The cell-contents are not shown. $\times 200$.

hyphae are not infrequent. From the intercellular hyphae numerous branches of limited growth penetrate the walls of the surrounding cortical cells. These short hyphal branches ramify repeatedly within the invaded cell, where they form peculiar tufts of exceedingly fine mycelial threads. Structures of this nature, for which Gallaud (l.c., p. 223 et seq.) has proposed the term 'arbuscules', appear to be characteristic of most endotrophic fungi, and are undoubtedly homologous with the haustoria of the Peronosporaceae. The ultimate ramifications of these intracellular branches are so fine, and possess such an exceedingly delicate membrane, that even under a very high magnification their true form can be distinguished only after careful treatment. Judging from the infrequency with which it is found, this stage is quickly passed over ; the fine threads of the ultimate branches soon lose their individuality, and finally become transformed into structureless, granular conglomerations ('sporangioles' of Gallaud, l.c.,

p. 231 et seq.), which stain a dirty yellow after treatment with iodine and completely resist the action of concentrated sulphuric acid or potassium hydrate. The 'stalk' portion of the 'arbuscules' is more resistant to the

TEXT-FIG. 4. Disorganizing 'arbuscules' of *Stigeosporium Marattiacearum*, as seen in (a) a transverse section of a root and in (b) a longitudinal section of a root of *Marattia Cooperi*, Mre. n. = nucleus of host-cell. $\times 700$.

TEXT-FIG. 5. Diagram showing position of the fungus within the tissues of the host-root. Note: Relative proportions are greatly exaggerated.

enzymes, which are presumably secreted by the cytoplasm of the mycorrhizal cell, and persist for a considerable time after the complete disorganization of the ramifications (Text-fig. 4, a, b).¹

¹ The cytological features connected with the digestion of fungal endophytes by their hosts have been carefully studied by Magnus (32), Shibata (40), and Kusano (30).

All stages in the degeneration of the 'arbuscules' are irregularly distributed throughout the inner cortical cells of the host-root. In sections of infected roots, the cells which contain these masses of partially digested fungal mycelium can easily be distinguished, even with the naked eye, as pale yellowish strongly refractive spots, which stand out in sharp relief against the dull brown background of the other cells of the cortex. These clumps or masses of disorganized mycelium persist throughout the life of the host-root, yet the invaded cells show no obvious signs of injury, unless the complete disappearance of starch-grains from these cells can be interpreted as such. On the other hand, the host-cells not only keep the fungus in check, but presumably utilize as food part at least of the products of disorganization of the fungal hyphae. Moreover, the mycorrhizal cells exhibit no signs of approaching death until the root as a whole decays, their nuclei retaining a healthy appearance to the last (Text-fig. 4, *a*, *b*).

It is interesting to find that the mycelium is invariably absent from the tannin-cells of the host even when hyphae are abundant in adjacent cortical cells, and again, the fungal zone of the larger roots of *Marattia Cooperi*, Mre., is always situated *outside* the ring of mucilage-canals (Pl. III, Fig. 7).

Cook (18) has shown that tannin exerts a deleterious effect upon certain fungi, whilst Gallaud (l. c., p. 317) remarks of endotrophic fungi in general that they are repelled by secretory cells or by chlorenchyma. On the other hand, Peklo (35) found that the fungal symbiont of the mycorrhiza of *Carpinus* and of *Betula* appears to show a preference for this substance.

ii. Vesicles.

The terminal portion of many of the hyphae swell up and give rise to structures which are generally known as vesicles (= 'vésicules' of Janse (25), N. Bernard (10), and others). These swellings, which are locally abundant and assume various shapes and dimensions, occupy either an inter- or an intracellular position amongst the cortical cells of the host-root (Text-fig. 6, *a*, *b*, *c*). They are never delimited by a septum from the supporting hyphae, from which they differ only in shape and size. When young they possess a thin hyaline membrane and contain a granular vacuolate cytoplasm in which numerous minute deeply staining bodies of irregular form can be distinguished (Text-fig. 6, *a*, *c*).

As the vesicles increase in size, their wall irregularly thickens up and assumes a yellowish coloration exactly similar to that of the older hyphae, and, as in the latter, the contents of old vesicles tend to disappear. Numerous empty vesicles were observed in some of the roots (Text-fig. 6, *d*). Now the fact that the vesicles rapidly lose their contents militates against the view that they are reproductive bodies of a conidial nature (cf. Campbell (13); also Noël Bernard (10), Fig. 9, p. 249). Neither is there any reason for supposing that they are secretory products of the fungus, and they

obviously are not homologous with the structures designated vesicles by Kusano (l. c., p. 32 et seq.). Gallaud (l. c.) claimed for these structures, which are almost universally distributed amongst mycorrhizal fungi, a function as temporary reserve organs, and, in the opinion of the present writer, this interpretation is the most satisfactory which has yet been given.¹

TEXT-FIG. 6. Vesicles of *Stigeosporium Marattiacearum* in cortical cells of (a, b) roots of *Angiopteris evecta*, Hoffm., and of (c, d) roots of *Archangiopteris Henryi*, Chr. et Gies. Contents of host-cells not shown. $\times 300$.

iii. *Resting spores.*

Special interest attaches to this mycorrhizal fungus, inasmuch as it produces *under natural conditions distinct reproductive bodies* (other than 'vesicles') *within the tissues of the host-root*.

Distributed among the inner cortical cells of infected roots of every age and size, characteristic thick-walled spores were frequently noticed (Text-figs. 7 and 8; Pl. III, Figs. 1, 2, 5, and 6).² These spores, which are usually spherical ($32-45\ \mu$), but occasionally ovoid, pyriform, &c., occur

¹ For a full discussion of this question the reader is referred to the works of Gallaud (22, p. 130 et seq.) and of Kusano (30, pp. 36, 37).

² The isolated spores observed by Kühn (28, p. 493, Taf. XX, Fig. 39) in *Kaulfussia aesculi-folia*, Bl., were probably identical with these.

either in a terminal (Text-fig. 7), or more often in an intercalary (Text-fig. 8) position on the hyphae, and were at first mistaken for ripe oospores, to which they bear a remarkably close resemblance, especially during the earlier stages of their development. However, the complete absence both of an oogonial wall and of antheridia is sufficient proof of their non-sexual nature.

a. Development of the resting spores.

A terminal resting spore originates as a more or less spherical enlargement of the terminal portion of an ordinary young hypha, from which it is usually delimited by a septum (Text-fig. 8, i, ii, iii). The dense granular contents of this enlargement are multinucleate. At this stage the immature resting spore closely resembles a young spherical vesicle, from which, however, it can readily be distinguished by its denser contents and by the uniform thickness of its wall (cf. Text-fig. 8, i, ii, with Text-fig. 6, *a*). The intercalary resting spores (= chlamydospores) are formed in the following manner: Swelling takes place in the course of a young hypha, either equally all round, in which case globular or ovoid enlargements are produced, or in one direction only, in which case the swollen portion has a sub-globose form. In either case the enlargements may or may not be delimited by septa from the supporting hyphae (Text-fig. 7, i). The wall of these swellings, whether terminal or intercalary, is at first similar in thickness to that of the hyphae from which they arise, but differs slightly in its staining capacity. For example, the wall of the young spore stains readily with Kleinenberg's haematoxylin, whereas the hyphal walls take up this stain with difficulty. Very early in the development of the resting spore the middle wall originates as a thin highly refractive layer, in which irregular deep yellow areas can easily be distinguished (Text-figs. 7, ii, and 8, iv, v). At a slightly later stage the first indications of canals or pores can be identified in this wall (Text-figs. 7, ii, and 8, iv, v). The thickening of the middle wall proceeds apace: meanwhile the contents of the spore become more densely granular, and oil globules,¹ which are so conspicuous in the contents of the ripe spore, appear in the cytoplasm and completely mask the subsequent behaviour of the nuclei (Text-figs. 7, iii, iv, and 8, vi, vii).

So far the only indication of the innermost wall is the closer aggregation of the granular contents in the peripheral zone of the cytoplasm (Text-figs. 7, iii, iv, vi, and 8, vi, vii). A number of spores which were artificially crushed at this stage showed no signs of an inner wall; hence it must be concluded that the innermost wall is laid down very late in the development of the spore. Thickening of the middle wall continues until an average thickness of 6μ is attained, but shortly before this result is arrived at, the inner wall begins to develop.

¹ It may be noted in passing that the presence of oil as a reserve material is characteristic of 'resting' bodies.

TEXT-FIG. 7. i-vii. Intercalary resting spores of *Stigeosporium Marattiacearum* in various stages of development. From roots of *Angiopteris evecta*, Hoffm. $\times 750$. viii. Part of the wall of a ripe resting spore in surface view showing canals. $\times 1700$. c. = canal; cyt. = cytoplasm; i.w. = inner wall; m.w. = middle wall; o.w. = outer wall; n. = nucleus; o.gl. = oil globules.

TEXT-FIG. 8. i-ix. Terminal resting spores of *Stigeosporium Marattiacearum* in various stages of development. From roots of *Angiopteris evecta*, Hoffm., and *Archangiopteris Henryi*, Chr. et Gies. c. = canal; cyl. = cytoplasm; i.w. = inner wall; m.w. = middle wall; o.w. = outer wall; n. = nucleus; ogl. = oil globules. $\times 750$.

Whilst the above-described changes have been taking place in the spore wall, the cytoplasm, which at first consisted of dense granular protoplasm completely filling the interior of the spore, gradually decreases in bulk until in the ripe spore a condition is arrived at where the cytoplasm (proteid) occupies only a fraction of the interior of the spore, the remaining space being filled with oil (cf. Text-figs. 7, ii, and 8, ii, with Text-figs. 7, vii, and 8, ix).

Thus, it would appear that the innermost wall is deposited at the expense of part of the original spore contents, whilst the greater part of the remainder of these cell contents is converted into oil. The innermost wall rapidly increases in thickness until in the mature spore an average thickness of $2.5\ \mu$ is attained (Text-figs. 7, vii, and 8, ix).

β. Structure of the mature resting spores.

The ripe resting spores possess three distinct walls, namely—

1. An extremely thin smooth outer wall. This wall is colourless and is closely applied to the middle wall, from which it is indistinguishable in unstained preparations, but can readily be identified in stained preparations of crushed spores.

2. A thick ($6\ \mu$) middle wall. This wall consists of a transparent straw-coloured matrix which is traversed by a large number of canals or pores (average diameter $0.8\ \mu$), which radiate from the interior of the spore (Text-figs. 7, vii, and 8, ix; Pl. III, Fig. 1, *c, d, e, f, g*). Each canal is not of uniform diameter throughout its length, but tapers towards the periphery of the spore (Text-figs. 7, ii–viii, and 8, iv–ix).

The chemical nature of this substance could not be determined with certainty. It is very resistant to every reagent to the action of which the ripe spores were subjected. These included KOH, chlor-zinc iodide, eau-de-Javelle, concentrated HCl, &c., &c., but Kleinenberg's haematoxylin stained it with difficulty (cf. Dastur (19), pp. 207–8).

Irregular bands and markings of a deeper yellow colour ramify through this matrix and are clearly visible both in surface view and in sections of the spores (Text-figs. 7, ii–vii, and 8, iv–ix; Pl. III, Figs. 1, *a–g*, 2, and 5B). The substance of which these bands are composed is equally resistant to the above-mentioned reagents, and all attempts to stain it failed.

3. A moderately thick ($2.5\ \mu$) inner wall. This wall develops very late, is generally not closely applied to the middle wall, and presents many of the reactions of cellulose. It is exceedingly tough, and even when the thick middle wall of the spore is crushed artificially this inner wall remains unbroken.

On reaching maturity the spores become detached from their supporting hyphae, and are then found lying free within or between the cells of the host-root, which are sometimes forced apart by the growth of the spores, as is shown in Text-fig. 8, *i*.

On the other hand, it is probable that the pressure of the cell-walls of the host upon the developing spores is responsible for the unusual shape of some of the mature spores (Text-fig. 7, vii).

iv. *The probable life-history of the endophyte.*

In no case, even in very old decaying roots, has a resting spore been observed to germinate *in situ*; it therefore seems probable that germination never takes place until, on the complete decay of the host-root, the spore is eventually deposited in the soil.

Dastur (l. c., p. 199) states, with reference to *Phytophthora parasitica*, that the 'resting' conidia, if kept moist, retain their vitality for over nine months, when they germinate by one or more germ-tubes, but do not necessarily require a period of rest.

It is not unlikely that a similar statement holds good for the resting spores of our fungus; these spores when finally set free in the soil, where they would be preserved under moist conditions, probably germinate direct after a varying period of rest, giving rise to one or more germ-hyphae, which, either at once or after a while, meet with a suitable host-root, the epidermis of which they penetrate in the manner described above.

The thickness of the wall and the absence of a definite papilla indicate that the resting spores are not sporangia, but specialized conidial growths. It is possible that the suppression of vegetative vigour and of sporangia formation on the part of the fungus, due to lack of oxygen or to some other cause (or causes) at present unknown, are determining factors in their production.

According to Magnus (32, p. 216) certain portions of thick-walled hyphae of the endophyte of *Neottia Nidus-avis* persist throughout the winter after the decay of the host-root and germinate ('zu neuem Leben zu erwachen') in the following spring and thus infect fresh host-plants. He refers to these structures as 'sclerotia' or 'cysts'. This fact is interesting, inasmuch as it points to the possible evolution of highly specialized resting spores by the local modification of ordinary vegetative hyphae for a reproductive function. On the other hand, the complex nature of the wall and the striking resemblance which the spores under discussion bear to oospores, especially during the later stages in their development, suggests their origin from parthenogenetic oogonia (= parthenospores), with complete suppression of the male organs.

2. *Cultural Experiments.*

Many attempts to grow this fungus on various artificial media, both in Petri-dishes and in hanging-drops, met with no success. No growth of the fungus resulted when pieces of root of *Marattia* or of *Angiopteris*, the surface of which had *previously been sterilized*, were placed in or upon various artificial media (cf. Rayner, l. c., p. 119). The surface of the root

was sterilized by immersion for varying short periods in very dilute solutions of formalin, mercuric chloride, or hydrogen peroxide, followed by thorough washing in running water. Failing this preliminary superficial sterilization of the host-root, a number of forms appeared in the cultures. The numerous genera represented in such cultures obviously constitute the epiphytic mycoflora of the roots in question and have no necessary connexion with the mycorrhizal fungus.

Bearing these results in mind, it is only reasonable to suppose that the fungal growths which Kühn (28, p. 493, Taf. XX, Fig. 40) succeeded in isolating from roots of *Marattia alata*, Sm., were of a similar nature, for they were obtained from roots which apparently had not been previously sterilized.

In this connexion it is interesting to find that Gallaud (l. c.) invariably failed to obtain cultures of the numerous mycorrhizal fungi which were investigated by him. This observer suggests that the mycelium of the endophyte undergoes some vital change during its sojourn within the tissues of the host-plant. The present writer is of the opinion that Gallaud's explanation is correct.

Noël Bernard (1, 3, 4, 5, 6, 7, 8, 9), however, in the course of a series of cultural experiments extending over a number of years, succeeded in isolating a number of mycorrhizal fungi from the roots of various genera of Orchidaceae and brought to light the remarkable degree of specialization which obtains in these plants. This botanist proved that the germination of the seeds of certain Orchids and the subsequent development of the root-system of the seedlings depended upon their association with their particular fungal symbiont, and in this way solved the problem which in the past had puzzled Orchid growers, namely, the apparent impossibility of raising certain genera of Orchidaceae from seed.

An ingenious theory that tuberization in a number of higher plants depended upon their infection with a fungal endophyte, which brought about an increase in the concentration of the cell-sap, was advanced by this observer (2, 9, 10). Many of these experiments were afterwards repeated and confirmed by Burgeff (11).

Rayner (l. c., p. 120) succeeded in isolating an endotrophic fungus from seeds and from pieces of ovary tissue of *Calluna vulgaris* and in effecting the synthesis of sterile seedlings of the higher plant with this fungus, thereby inducing development of the root-system and vigorous growth under aseptical conditions in closed tubes.

There is good reason to believe that the only parts of our fungus capable of an independent existence outside the tissues of the host are the resting spores and germ-hyphae; hence it was unfortunate that in the only living material available for investigation the vegetative structures of the endophyte were alone represented. On this account no experimental work

on the germination of the resting spores and the subsequent infection of the host-plant could be attempted.

3. *Biological Relations between Host and Endophyte.*

A. *Historical.*

Leaving out of consideration the research upon various ectotrophic mycorrhizas, an abundant literature dealing with the many views put forward to explain the biological significance of the association of endotrophic fungi with the root, or other absorbing organ, of a number of Phanerogams and Vascular Cryptogams, has accumulated during recent years.

But full summaries and discussions of the more important results obtained in this field of investigation, including those of Frank (21), Noël Bernard (2, 7, 8, 9, 10), and Burgeff (11), are to be found in the recent works of Kusano (30) and of Rayner (38). Detailed reference to the literature is therefore unnecessary.

The theories put forward to explain the biological significance of endotrophic mycorrhizas is very briefly summarized below:

1. The mycorrhiza is a real symbiosis with reciprocal advantages; in other words, there is a mutual interchange of material (or other advantages) between host and endophyte (Groom (23), Macdougall (31), Shibata (40), &c., &c.).

A. The higher plant provides the fungal symbiont with one or more of the following:

- i. Habitation.
- ii. Carbohydrate (especially starch).¹
- iii. Shelter from excess of oxygen.²

B. The fungus benefits the higher plant in one or more of the following ways:

- i. The fungus takes up humus products from the soil by means of its external mycelium. These humus products are conducted to the internal hyphae, where they are converted into proteids which are in part utilized by the fungus in its own metabolism and in part yielded to the tissues of the host-plant.³
- ii. The fungus is capable of fixing nitrogen directly from the air, with which it builds up organic nitrogenous substances (i. e. proteids) which it yields to the host-plant in exchange for carbohydrate material.⁴
- iii. The fungus absorbs mineral salts from the surrounding soil.⁵

¹ *Corallorrhiza arizonica*, Macdougall (l. c.); *Thismia Aseroë*, Groom (l. c.).

² Janse (l. c.).

³ Macdougall (l. c.), and others.

⁴ Janse (l. c.); *Podocarpus*, Nobbe and Hiltner (34), Ternetz (42).

⁵ Stahl (41).

2. The host-plant (Phanerogam) derives the entire, or by far the greater, advantage from the association.¹
3. The advantages of the association are entirely, or almost entirely, on the side of the fungal partner.²

B. *The present investigation.*

The biological relations between the marattiacean roots and the endophyte which inhabits them will now be briefly considered in the light of previous work on mycorrhizal fungi.

Hyphae are never found leaving the host-root. This is shown by the fact that few hyphae are found in the outer cortical layers, and these are obviously only the infecting hyphae, since they are relatively older than the mycelium found in the inner cortical layers of the host-root. This fact may be urged against the hypothesis that the fungus absorbs humus products or mineral salts from the surrounding soil and liberates them, with or without previous modification, in the tissues of the host. This also militates against the view that the mycelium of the endophyte takes the place of root-hairs in the higher plant. Moreover, the distribution of the latter bears no relation whatever to the presence or absence of the fungus. Again, root-hairs are sometimes relatively abundant on young infected roots.

To the question whether the fungus is a facultative anaerobe capable of fixing free nitrogen from the air, no definite answer can be given in the absence of experimental evidence.

But whilst admitting that the fungus is a true parasite, it cannot be said to cause any obvious injury to the plant attacked (cf. the fungus found in the seed and vegetative organs of *Lolium temulentum*, Hanausek (24), Nestler (33)). Kusano (l. c.) has proved conclusively that the facultative parasite *Armillaria mellea* may behave towards the Orchid *Gastrodia elata* either as a true parasite or as an endotrophic symbiont. There is no reason for doubting that the host-plant utilizes part at least of the products of disorganization of the 'arbuscules', but there is no intimate relation between the endophyte and the nucleus of the mycorrhizal cell, such as Groom (l. c.) claims for the mycorrhiza of *Thismia Aseroë*.

Now, apart from its rôle as habitat (and possible shelter from excess of oxygen) for the endophyte, the host-plant provides the latter with all, or at least the greater part, of the food material it requires, because the mycelium in the outer cortical layers of the root, which provides the only connexion between the living mycelium in the inner cortical layers of the root and the free hyphae in the soil, is usually dead and without contents, and therefore cannot function as a channel for the passage of food-material. Moreover, starch invariably disappears from the invaded cells; all stages in the solution of the starch granules, which do not reappear even after the complete collapse of the 'arbuscules' (cf. Groom, l. c., p. 348, and Kusano, l. c.,

¹ *Gastrodia elata*, Kusano (l. c.).

² Gallaud (l. c.).

p. 36), can be seen in these cells. But this circumstance, whilst indicating that the presence of the endophyte is in some way responsible for the absence of starch from the host-cell, does not necessarily prove that the carbohydrate in question is utilized by the fungus as a food-material.

Since no visible injury is done to the cells of infected roots, it would appear that this fungus shares with the great majority of mycorrhiza-forming fungi investigated by Gallaud (l. c.) the capacity for obtaining all the food it requires from the non-living cell-contents (e. g. starch, sugars, elaborated sap, &c.) of its host.

And the resting spores, with their oily contents, are formed at the expense of the higher plant; hence the advantage of the association is almost entirely on the side of the fungus, the host-plant thriving *in spite of* the presence of the endophyte.

4. *Systematic Position of the Fungus.*

The systematic position of the fungus is of special interest, inasmuch as our knowledge of the phylogeny and relationships of the various mycorrhiza-forming fungi has hitherto been exceedingly meagre.

From the mycorrhiza of the Orchidaceae Noël Bernard (8) obtained cultures of a number of fungal endophytes which he placed provisionally in the genus *Rhizoctonia*. In the same year (1909), Burgeff (11), working on the mycorrhizal fungi of Orchid roots, came to the conclusion that they formed a distinct morphological group, for which he proposed the descriptive name *Orcheomyces*.

Gallaud (l. c., p. 433) observes that, except in the Orchids, the reproductive bodies of the fungal partners of all mycorrhizas remain to be discovered. Dangeard (18 A, 18 B), however, described and figured (l. c., Pl. XV, Fig. 15) not only the mycelium, but also the spores, of a fungus which occurs in the rhizome of *Tmesipteris*, and provisionally referred the endophyte to the genus *Cladochytrium*. Peklo (l. c.), however, claims to have isolated a species of *Penicillium* from the mycorrhiza of *Carpinus* and of *Betula*, whilst Rayner (l. c.) has recently isolated from *Calluna vulgaris* an endophyte for which the new sub-genus *Phyllophoma* has been established.

Judging from its mycelial characters alone, our fungus undoubtedly belongs to the Oomycetes, and exhibits many of the essential features of the sub-family Peronosporae of the Peronosporaceae, where for the present it may conveniently be placed.

According to Clements (16, p. 17), six genera are included in this sub-family; of these, the genus *Phytophthora* most closely resembles the fungus under consideration, sharing with it several important characters, the most noticeable of which are mentioned below.¹

¹ Treub (43) considered the fungal endophyte of *Lycopodium Phlegmaria* prothallia to belong to the Peronosporaceae, while Noël Bernard (10, p. 252) suggests that the fungal endophyte which occurs in the roots of *Solanum Dulcamara* has affinities with this family; Nobbe and Hiltner (34) claim a similar position for the endotrophic fungus of *Podocarpus* nodules.

Perhaps the most striking character which these genera share in common is the production of thick-walled 'resting' spores. About one-half of the species of *Phytophthora* hitherto described form thick-walled 'resting' spores. Spores of this nature have been described for the following species of *Phytophthora*, viz.:

Species :	Mentioned by :
<i>P. Faberi</i> , Maublanc	Dastur (19), Petch (36)
<i>P. infestans</i> , de Bary	Jones, Giddings, and Lutman (27), Dastur (19 A)
<i>P. parasitica</i> , Dastur	Dastur (19)
<i>P. Colocasiae</i> , Raciborski	Butler and Kulkarni (12)
<i>P. Nicotianae</i> , de Haan	Pethybridge (37)
<i>P. Fatrophae</i> , Jensen	Pethybridge (37)
<i>Cacao Phytophthora</i> (<i>P. omnivora</i> ?)	Rorer (39).

And it is not improbable that the parthenogenetic oospores, described by Coleman (17) for *Phytophthora Theobromae*, Coleman, are of a similar nature.

The description and figures of the structure and development of the resting spores of *Phytophthora infestans* published by Jones, Giddings, and Lutman (l. c.), except for a few minor differences, could be applied equally well to the resting spores of the endophyte of these marattiacean roots. And the discovery of ramified haustoria in *Phytophthora infestans* by Delacroix (20, p. 361, Fig. 2) and by Dastur (19 A, p. 5) is also of interest.

The chief points of difference between the two genera may be attributed to the peculiar habitat of our fungus, which no doubt has become associated with special adaptations, amongst which we find an almost complete suppression of certain reproductive bodies generally characteristic of the Peronosporae, namely, the sporangia, oogonia, and antheridia, the modification of the conidia, and a remarkable specialization of the vegetative parts.

Since, however, we unfortunately have no available evidence regarding sexual reproductive bodies in this fungus, nothing precise can be stated as to its affinities, but the marked difference in habit between this fungus and *Phytophthora* is sufficient to make its inclusion in the latter genus unjustifiable.

I therefore propose to establish a new genus, *Stigeosporium*,¹ for its reception, and, since it is undoubtedly normally associated with roots of the Marattiaceae and, so far as is known, is confined to that habitat, I suggest for it the specific name *Stigeosporium Marattiacearum*.

5. Geographical Distribution of the Fungus.

This fungus has a remarkably extensive geographical distribution, the same species being found in the marattiacean Ferns occurring in such widely

¹ Or *Stygeosporium*.

separated localities as Eastern Asia (Java, South-West China), Australasia, and Ceylon.

DIAGNOSIS: ¹

Stigeosporium, gen. nov.

Mycelium ramosum ex hyphis inter- et intracellularibus continuis rarissime septatis constans; haustoriis numerosis, extremitatibus in ramulis radiatis valde dissectis; sporis perdurantibus solitariis plerumque globosis raro subglobosis et cet., membrana crassissima irregulariter intenseque colorata.

Differt a *Phytophthora*, cui arcte affine, habitu symbiotico, qua de causa nulla conidia normalia producuntur.

Sp. unica.

Stigeosporium Marattiacearum, West, sp. nov.

Hyphae primum hyalinae demum flavo-brunneae vacuatae 1-12 μ crassae; sporis vel intercalaribus vel terminalibus plerumque globosis 32-45 μ diametro, raro subglobosis vel ovoideis vel pyriformibus; exosporio tenuissimo hyalino levi, mesosporio crassissimo 6 μ crassitudine minute punctulato flavo irregulariter intenseque colorato, endosporio tenue 2.5 μ crassitudine.

Hab. In radicibus subterraneis quae ad quasdam marattiacearum generum orientalium omnium adhuc cognitorum species pertinent.

Distrib. Asia orientalis, Australasia, Zeylania.

B. ON THE ENDOPHYTE OF THE ROOTS OF DANAEA ALATA, SM.,
AND OF DANAEA NODOSA, SM.

The principal roots of both species of *Danaea* examined by the present writer are characterized by the presence of a zone of fibrous cells which is separated from the epidermis by a few layers (2-6) of large parenchymatous cells which constitute the outer cortex (Text-fig. 9; Pl. III, Figs. 3 and 4): in the smaller roots, however, no fibrous zone is found.

In other respects the roots of *Danaea* very closely resemble those of the other genera of Marattiaceae.

An endotrophic fungus was found in nearly every root examined, irrespective of age and size; it is invariably present in the primary and first few adventitious roots of the young sporophyte, but a few of the older roots were found to be free from fungal mycelium.²

The mycelium is irregularly distributed throughout the cortex of the smaller roots which do not possess a cortical zone of stereome, but in the larger roots the mycelium is always confined to the few layers of parenchyma *outside* the fibrous zone of the cortex (Text-fig. 9; Pl. III, Figs. 3 and 4).

¹ The above diagnosis is an extended form of that which the author published as a note in *Annals of Botany*, vol. xxx, 1916.

² An endophyte exhibiting similar characters was observed in the cells of the prothallia.

In *Danaea* also, the presence of the endophyte does not in any way alter the external appearance of the roots.

Infecting hyphae enter the root either through ordinary epidermal cells or through the root-hairs (Text-fig. 9).

The hyaline non-septate copiously branched mycelium consists for the most part of fine intracellular hyphae which are fairly uniform in diameter ($2-4\mu$). These hyphae have a delicate membrane and finely granular contents in which no nuclei can be identified with certainty (Text-fig. 9; Pl. III, Fig. 4, *myc.*; and cf. *Stigeosporium*).

TEXT-FIG. 9. Part of a transverse section of a moderately large root of *Danaea alata*, Sm., showing endophyte (*end.*) in outer cortical cells. *fibr. l.* = cortical zone of stereome; *n.* = nucleus of host-cell; *r.h.* = root-hair; *s.gr.* = starch grains; *sp.* = foreign spore-like bodies; *v.* = vesicle; *x.* = disorganized 'arbuscules'. $\times 300$.

From the relatively few intercellular hyphae 'arbuscules' essentially similar to those already described for *Stigeosporium Marattiacearum* are produced. These rapidly collapse, their disorganized remains forming irregular structureless granular masses *within* the host-cell (Text-fig. 9, *x*; Pl. III, Figs. 3 and 4). Vesicles are occasionally found: they are generally globular in form and are never delimited by a septum from the supporting hypha, upon which they occupy a terminal position (Text-fig. 9, *v*; Pl. III, Fig. 4, *v*). Intercalary vesicles were never observed in this endophyte. The hyphae often form peculiar coiled bunches within the mycorrhizal cells (Text-fig. 9): the real significance of this phenomenon, which is not uncommon among endotrophic mycorrhizal fungi, is not yet understood.

So far as it is possible to judge from an examination of sections of infected roots, the hyphae of this endophyte exert no deleterious effect upon

the invaded cells, the only obvious result of their presence being the complete disappearance of starch from such cells.

Unfortunately, no reproductive bodies were observed, consequently the systematic position and relationships of this endophyte could not, even approximately, be determined. No living material of either species of *Danaea* was available for investigation; it was therefore impossible to carry out cultural experiments with this fungus.

That the endophyte which is associated with the roots of *Danaea* spp. is quite distinct from *Stigeosporium* is proved by the following characters, which belong only to the former :

1. *Mycelial characters.*

- A. Uniform diameter of the hyphae ;
- B. Comparative rarity of intercellular hyphae ;
- C. The hyphal wall does not materially thicken with age ;
- D. Absence of *distinct* nuclei from contents of the mycelium.

2. *Apparent absence of definite reproductive bodies.*

3. *Absence of vesicles of irregular form.*

SUMMARY.

1. A new fungus, *Stigeosporium Marattiacearum*, which forms an endotrophic mycorrhiza with roots of certain genera (*Angiopteris*, *Archangiopteris*, *Kaulfussia*, *Marattia*) of Marattiaceae, is described, and its biology, probable life-history, and systematic position discussed.

2. An unnamed mycorrhizal fungus, which enters into association with roots of the genus *Danaea*, is briefly described.

I take this opportunity of expressing my sincere thanks to Professor J. B. Farmer, F.R.S., for many helpful suggestions, and for allowing me to carry out the present investigation in his laboratory.

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EXPLANATION OF FIGURES ON PLATE III.

Illustrating Mr. C. West's paper on *Stigeosporium Marattiacearum* and the Mycorrhiza of the Marattiaceae.

Fig. 1. *a-g*. Resting spores of *Stigeosporium Marattiacearum*, showing different stages in development. *a, b, c, d* in optical section ; *e, f, g* in surface view. From roots of *Angiopteris evecta*, Hoffm. × 500.

Fig. 2. Mature resting spores of *Stigeosporium Marattiacearum* in a root of *Archangiopteris Henryi*, Chr. et Gies. × 350.

Fig. 3. Transverse section of a small root of *Danaea alata*, Sm., showing endophyte (*end.*) in the cortex. *e.* = endodermis ; *fibr. l.* = fibrous layer of the cortex ; *rt. h.* = root-hair. × 60.

Fig. 4. Part of a transverse section of a small root of *Danaea nodosa*, Sm., showing endophyte (*end.*) in the outer layers of the cortex. *fibr. l.* = fibrous layer ; *myc.* = intracellular mycelium ; *t.c.* = tannin cell ; *v.* = vesicle. × 120.

Fig. 5 A. Resting spores (*sp.*) of *Stigeosporium Marattiacearum* as they appear in a longitudinal section of a root of *Angiopteris evecta*, Hoffm. At *x* the intercellular mycelium of the fungus is clearly shown. × 30.

Fig. 5 B. Part of the same more highly magnified to show wall-markings. × 120.

Fig. 6. Part of a transverse section of a moderately large root of *Angiopteris evecta*, Hoffm., showing endophyte (*end.*) in the cortex. *e.* = endodermis ; *sp.* = resting spore ; *t.c.* = tannin cell. × 35.

Fig. 7. Transverse section of a root of *Marattia Cooperi*, Mre., showing endophyte (*end.*) in the cortex. *e.* = endodermis ; *m.c.* = mucilage canal ; *t.c.* = tannin cell ; *v.* = vesicle. × 30.

C. West photo.

C. West photo.

WEST-MYCORRHIZA OF MARATTIACEÆ.

Huth coll.

West, Cyril. 1917. "On Stigeosporium marattiacearum and the mycorrhiza of the Marattiaceae." *Annals of botany* 31, 77–99.

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