MORPHOLOGY and CYTOLOGY
of
TYPHULA TRIFOLII ROSTR.

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by
Jessie Donald
MARY J. M. NOBLE, B.Sc.

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- Introduction -

This fungus was described by Rostrup in 1902, as a parasite of Trifolium pratense, T. repens, and of Medicago lupulina. Small dark coloured sclerotia are formed as the resting stage of the fungus and these are so similar to seeds of Brassica that they have very probably been regarded as such in samples of Clover seed. Rostrup (23) found them mixed with Clover seed from the following sources - East Prussia, Moravia, Hungary and Norway, where he considered that the disease was likely to be fairly common but unrecognized. The fungus is further recorded by Jørstad (15) as occurring in samples of seed from Solør in 1921, from Aker in 1924 and as "not infrequently found in samples from Hedmark"

It has also been recorded by seed-testers, Dorph-Peterson (9) reports that in the year 1930-31, 10 samples of clover seed, out of 860 examined, contained the sclerotia; they occurred again in 1930-32, and they have once been found by Doyer (10).

The question of the pathogenicity of the fungus presents an interesting problem. Rostrup in his original description regarded the fungus as a serious parasite most other writers merely quoting from his account. In 1932, however, there is a record from Denmark (8) of Clover being attacked by

Typhula trifolii. One author, Mortensen, (20) on the contrary expresses the opinion that the fungus is not parasitic.

Another species, T. Betae, closely resembles T. Trifolii, the only difference given in the literature being its larger size and its restriction to Beet as a host plant. This species is frequently described as a parasite. The question is further complicated by the occurrence of T. variabilis which is not clearly distinguished from T. Betae and which occurs on several host plants, but most commonly on Asparagus officinalis.

Prillieux (22) describes a disease of Beet in which small ~~se~~erotia are formed like those described by de Bary for T. variabilis. Beet and Carrots when placed in contact with diseased plants of Beet, became infected, but Prillieux also mentions damage caused by the fungus to plants of Asparagus. Brizi (4) then identified this fungus as T. variabilis. More recently Schimdt (27) has carried out experiments in which he infected Beet with T. variabilis and obtained positive results. The Beets were completely rotted in a few weeks and basidia were obtained in culture.

Lind (18) describes T. Betae as very similar to T. variabilis but pathogenic to Beet, while T. Trifolii is somewhat smaller and confined to

Leguminous plants. The only species whose spore size is recorded by Saccardo is *T. variabilis*, thus Sporae 10- 13u X 4- 6 (Britz.), 6- 7u X 2- 5.3u (Schroet.)

The points of distinction between the genera Typhula and Pistillaria are equally ill-defined. Saccardo (24) states that the genera are distinguished by their habit rather than by definite characters, and within Typhula two groups are defined, one possessing sclerotia and the other without sclerotia. Typhula may have either two or four spores on each basidium. According to Gaumann and Dodge (13) Typhula has always four spored basidia and Pistillaria two spored basidia while W.G. Smith (28) defines Typhula as usually springing from a sclerotium.

The only constant difference appears to lie in the form of the fructification, Typhula having a long thin stalk which swells out abruptly in the upper part to form the hymenium. In Pistillaria the stalk, if present at all, is very short so that the whole fructification presents a more or less ovate appearance.

It is interesting to note that Massée (19) includes Typhula in the genus Pistillaria since the separation on account of the presence or absence of sclerotia " has never been suggested in the case of other genera, as Coprinus, Collybia, etc., where

some of the species produce sclerotia. Again it is doubtful as to whether Pistillaria, as here understood, should not be merged into Clavaria; the only distinction is one of relative size only, all the species of Pistillaria varying from minute to very minute."

It appears that both specific and generic distinctions require elucidation.

After some experiments had been carried out, with monospore cultures of Typhula Trifolii it soon became apparent that the fungus was heterothallic.

Although heterothallism had been shown to exist in the Zygomycetes by Blakeslee in 1904 (3), it was not until 1918 that the same phenomenon was demonstrated in the Basidiomycetes. In this year Bensaude (2) published an account of Sexuality in the Basidiomycetes proving by means of monospore cultures that Coprinus fimetarius was heterothallic and showing for the first time the significance of the association between clamp connections and conjugate nuclei.

Knip, (16) in Germany was studying the cytology^{of} certain Basidiomycetes with a view to determining the origin of the first pair of conjugate nuclei. His work was completed very shortly after that of Bensaude whose results it verified.

While discovering that the first conjugate pair of nuclei owed its origin to the fusion of two hyphae neither author observed or described in detail such a fusion. This, however, was successfully accomplished by Lehfeldt (17) who was working under Kniep and who published a detailed account of the origin of the conjugate nuclei in two fungi, Corticium serum and Typhula erythropus. In Typhula, Lehfeldt observed that the behaviour of the nuclei was not such as had been expected by Bensaude or Kniep, although he stated that Coprinus probably behaved similarly to Typhula.

Buller then carried out experimental work with Coprinus (5) and in interpreting his results stated that though he accepted Lehfeldt's results he considered that their confirmation by further work would be desirable. Again, in the course of later investigations (6) Buller states that until Lehfeldt's work is confirmed he prefers to believe that the facts are in accordance with a theory which he, Buller, expounds and that some, at least, of Lehfeldt's observations are incorrect.

Since Lehfeldt was working with a heterothallic species of Typhula, T. Trifolii, being also heterothallic appears to offer a suitable subject for study with a view to confirmation or otherwise of Lehfeldt's work.

Material.

The fungus was obtained in the form of sclerotia which were sent over from Poland where they had been found mixed with Clover seed. The sclerotia were sterilised then cut up and placed on nutrient agar. Growth took place readily and sclerotia were formed in the cultures. By subculturing, many sclerotia were ultimately obtained. These were sown in potting soil and after some time they germinated forming fructifications which bore basidia and basidiospores. Single spore cultures were made of which two, named "F" and "G" have still (i.e. after 2 years) remained in the primary condition and have produced neither sclerotia or fructifications. Later these strains were found to produce sclerotia and fructifications when grown together. The culture obtained by subculturing these sclerotia is termed "FG". Thus the cultures referred to as "F" and "G" are typical of those monospore cultures which remain in the primary state until paired up with a compatible strain when a culture is formed of which "FG" may be taken as a typical example.

Another type of monospore culture occurred. These were obtained in the same way as the "F" and "G" type but in this case, fructifications and sclerotia appear very readily without the strains

being paired. The sclerotia and fructifications are formed on haploid mycelia and will be referred to as "Haploid sclerotia etc". These strains may also be paired as in the first type to form a diploid mycelium.

Of this type are the cultures which will be referred to as "K", "S", and "N".

Microtechnique

Material for microtoming was usually fixed in Flemming's weaker solution. Navashin's fluid was almost as satisfactory as Flemming's while acetic alcohol and chrom-acetic tended to distort the tissues. Sections of the hymenophore were cut at a thickness of 2u and those of the sclerotium at 5 or 7u.

As a nuclear stain for microtomed material Breinl's triple stain was used almost exclusively. Iron alum Haematoxylin was rather confusing as the granules in the protoplasm tended to take up the stain while differentiation was difficult using Flemming's triple stain.

Sass' agar film technique (26) was used in a modified form. It was necessary that the film should be mounted in such a way that it could be examined while growing but not exposed unduly to external contamination. This called for the provision of a chamber such as is used for the germination of spores in hanging drop culture but large enough to carry a microscope slide of 3" by 1".

Experiments were made with strips of glass fixed together at the corners to form a rectangle. This was placed in a Petri dish and sterilised. A slide was then dipped in nutrient agar and placed, film side down, on the glass "box" for which it formed the "lid". ^{PL 13} Fig. 1. When superfine slides are used the film may be observed with a 1/6th inch ob-

jective without exposing the film to contamination.

It soon became apparent, however, that the glass "boxes" were unsatisfactory as no substance could be found to fix the corners in such a way as to remain sufficiently firm after sterilisation. Brass "boxes" were then made; these were slightly wider than the slide and exactly the same length, inside measurements. Thus the slide rested on the end pieces and lay between the side pieces so that only the ends of the film were in contact with the brass. Pl. 13. fig. 2.

Sass used a medium composed of gelatin and agar in his technique but it was found that Typhula Trifolii failed to grow on this medium. Malt extract was added but this upset the colloid system so that the medium became full of little globules which took up the stain very unevenly. Eventually, by trial and error, the following medium was evolved and found to be quite suitable, Malt Extract 2%, Gelatin 2%, Agar .5%, Water 95.5%. This was filtered in the Koch steriliser, tubed in small quantities, and replaced in the steriliser for 30 minutes.

A tube full of medium is ~~now~~ melted and poured into a Petri dish and just before it solidifies again the slide is dipped in it, and after the excess has been drained off, placed in the brass "box". The film is inoculated

immediately. Thus if spores are to be germinated the film may be lightly touched by the fructification without ruffling the surface of the medium.

As this film dries up very quickly the foot of the Petri dish is covered with damp blotting paper from which a rectangle is cut so as to form a damp chamber within the brass rectangle. The blotting paper must not be too damp as this causes the formation of large globules on the surface of the film.

It is necessary to alter the depth of the film according to the inoculum and the nature of the resulting mycelium. Thus if only the early stages of spore germination are required a very thin film is used so that the hyphae spread out more or less in one plane. If, however, two monospore strains are grown together in order that they may produce clamp connections a thicker film is used as otherwise the medium would be exhausted too quickly.

The film is allowed to dry down slightly before fixing. The fixing solution is that recommended by Sass.

The most useful stain for this technique was found to be Heidenhein's Iron-alum Haematoxylin with Light Green as a counter stain. Good results may be obtained by mordanting for 12 to 24 hours, staining in Haematoxylin for a similar period

followed by Light Green in 50% alcohol for about a minute. The advantages of this stain are that the nuclei are very easily stained and that the cell walls also stain fairly readily. Breinl's triple stain may be used but while occasionally excellent results are obtained these are greatly outnumbered by the failures.

The various Types of Mycelia occurring
in *Typhula Trifolii*.

In the following descriptions of the mycelia of Typhula definitions are given merely as a matter of convenience. The various types of mycelia actually merge into one another making accurate definition very difficult.

The terms "Submerged" and "Superficial" will be used as defined below;

Submerged. In any culture the mycelium which first appears is usually of the submerged type. It is closely adpressed to the mycelium, or embedded in it, and appears to be hyaline. This mycelium grows out rapidly in a radial direction around the inoculum.

Superficial. The superficial type usually appears after the submerged. Its most characteristic feature is that it is not closely adpressed to the culture medium. While in the submerged mycelium the majority of the hyphae grow along the surface of the medium and some penetrate below it, in the superficial type a few hyphae grow on the surface of the medium but the majority are raised above it.

The raised part of the mycelium appears in three forms, it may be white and fluffy, white and granular, or brown and crust-like.

These two types of mycelia, submerged and superficial, may be composed of either haploid hyphae (uninucleate with plain septa) or diploid hyphae,

(binucleate with clamp connections). They therefore correspond very roughly to "primary" and "secondary" mycelia as defined by Falck.(11).

(A) Haploid mycelium.

1) Submerged type. The macroscopic appearance of this mycelium is indicated in the definition of the submerged mycelium.

The type of branching is an important microscopic feature of this mycelium. The terminal cell usually puts out a branch but this may be delayed until it is the subterminal or even the third cell from the apex. The branches are apparently of unlimited growth and as they, in turn, subdivide, the mycelium spreads very quickly over, and through, the substrate. Branches which arise from the cells near the apex of the parent hypha form an acute angle with the same, a feature which has been noted by Falck in his definition of "primary" mycelium and by Buller in describing haploid mycelium. It appears, at least in Typhula Trifolii, that the submerged mycelia, both haploid and diploid, spread out over the substrate much more quickly than the superficial mycelia. This may be attributed to two facts, 1) that the branches given off by the apical cells are of unlimited growth and 2) that the acute angle of origin allows them to continue growing along much the same radial line as the parent hyphae.

Branches which arise on the older cells of the mycelium almost always form a right angle with the parent hypha and do not grow nearly so long as the branches given off by the younger cells. These short branches are often involved in anastomoses. They may either fuse with each other or with older hyphae. By doing so they are strengthening the mycelial network and covering those parts of the substrate which were left uncovered by the previously formed branches.

Nuclei. Each cell of the haploid submerged mycelium is typically uninucleate. Cells containing two or even three nuclei are, however, not exceptional particularly near the apices of the hyphae. Bensaude (2), p 45. found similar conditions in Coprinus fimetarius as she states, "Il y a une grande proportion de cellules uninucleées dans ces myceliums primaires. On en trouve cependant beaucoup qui présentent 2, 3 et même 4 noyaux, surtout aux extrémités en voie de croissance active". Bensaude suggests that, in these cases, growth has been so rapid that the formation of the septum dividing the daughter nuclei has been delayed. The same is probably true of multinucleate cells occurring in Typhula Trifolii .

Very few stages of nuclear division have been found in Typhula Trifolii, and of these some were in the basidia. The few cases which have been

found in the haploid mycelia were in a preparation obtained by the agar film method. Two haploid mycelia of opposite sex were anastomosing with one another. This seemed to cause a state of unusual activity in the nuclei even in those not directly concerned in the anastomoses.

Nuclei which are dividing by mitosis are illustrated in Pl.21 figs. 1-3 . In these the persistent nucleolus is visible and in one instance (fig 1.) the small dark coloured points which are presumably the centrosomes.

In Pl.26 fig. 4. a nucleus is shown in division. The nucleolus is very elongated and has a marked constriction in the middle. It is doubtful whether this is a case of direct, or amitotic, division.

When hyphal fusions occur between branches of the haploid mycelia of Typhula they are often followed by nuclear migration, even when there is no question of heterothallism or "diploidisation". This is interesting in view of Buller's remark, "To what extent, if any, in ordinary vegetative hyphal fusions nuclei pass through the junction-places from one hypha to another still remains to be determined by exact observation and experiment. As will be shown.. ..great quantities of cytoplasm often pass through hyphal bridges from one mycelium to another; but to what extent, if any, the streams of cytoplasm carry

nuclei with them is at present unknown".

From observations made on Typhula Trifolii it may be concluded that when two cells of ordinary vegetative hyphae anastomose, the nucleus of one cell usually passes over to the other cell.^{Pl. 20.} It will be shown later that the same movement of nuclei occurs in the diploid hyphae on anastomosis, again even when there is no question of heterothallism. Anastomosis between different heterothallic strains will be discussed later. In the case of vegetative anastomosis between diploid hyphae, the nuclei of one of the two cells involved disintegrate and are replaced by the nuclei from the other cell.

It is very difficult to determine whether similar degeneration of nuclei takes place in the haploid cells. Normal cells with two nuclei are not exceptional so that in the case of a binucleate cell involved in anastomosis the origin of the nuclei cannot easily be determined.

Oidia. Oidia are formed on this type of mycelium. They are very small, measuring $4\frac{1}{2}u$ by $1\frac{1}{2}u$, and they are colourless. Each oidium contains one nucleus, situated centrally, whose diameter appears to be very nearly equal to that of the oidium.

The cells of hyphae bearing oidia are very much shorter than those of ordinary hyphae. The oidia are either borne directly on such cells or on short branches which arise from them. In any case the oidia are formed in little groups which, however, fall

apart easily so that the oidia may move away from the place of origin. Pl. 5 fig. 3 .

2) Superficial type. The most important difference between the submerged and the superficial mycelia lies in the type of branches produced. In the superficial mycelium these are very short and abundant. They are, however, rather longer in the fluffy form than in the granular or crust-like form of the mycelium.

The fluffy form; this is usually the first of the superficial mycelia to appear in culture. It is formed at first around the inoculum and spreads out slowly over the submerged mycelium. Pl. 5 figs 1-3. When subcultured this fluffy superficial mycelium gives rise to the submerged mycelium. While the hyphae of the fluffy form together compose a tangled network owing to the frequent and irregular branching, the intercellular spaces are greater than in the other two forms.

Granular form. This is similar to the fluffy form but owing to the cells being shorter, the hyphae are much more closely interwoven. The granular form may arise within the fluffy form (Pl. fig. —.) or it may arise independantly. (Pl. 16 fig. 3) The little granules which make up this mycelium are actually the fundaments of sclerotia.

The hyphae in those little granules are very strongly branched and lobed. The cells in this form of mycelium are often swollen and distorted in shape, especially in those hyphae which later form the inner part of the sclerotium.

A few of the granules, or fundaments, become much larger and, at the same time, become darker in colour, eventually forming into sclerotia. The outer hyphae become thick-walled and, later, become the rind or outer protective layer of the sclerotium.

Brown crust-like form; Under dry conditions in culture a brown crust-like growth appears. Pl. 15 fig. 2. It is formed of hyphae exactly similar to those which make up the outer rind of the sclerotium. In agar slope cultures it may be formed around the upper end of the slope where the agar is thin and dry while sclerotia appear on the thick part of the slope. It may also be formed, especially on oat agar, as a crust over the surface of the medium in a Petri dish culture.

It is suggested that this is a diffuse sclerotium, formed where the conditions are unfavourable for the building up of a true sclerotium. This is further borne out by the occurrence of small abnormal fructifications growing up from the crust.

The cytology of the superficial mycelium presents an interesting point; that many of the short branches do not contain a nucleus. It has been observed that in the submerged as well as the superficial mycelium, a branch is not provided with a nucleus until it has attained a certain length, i.e. until its length is approximately four to six times greater than its breadth.

It therefore seems reasonable to suppose that in this superficial mycelium many of the branches never grow long enough to be provided with a nucleus.

The granular type of mycelium contains a great number of these short branches without nuclei. This mycelium, since it forms the centre of the sclerotium, functions as a reservoir of food material for the fructification which will be formed later. This function is fulfilled by virtue of the numerous short branches which contain a great amount of protoplasm within a small space.

B. Diploid mycelium.

1) Submerged type. The two important diagnostic features of this mycelium are, 1) the presence of two nuclei in each cell, 2) the presence of clamp connections.

Clamp connections will be described later.

under a separate heading.

The branches are of the same type as in the haploid submerged mycelium, they are long and regular in form and they leave the parent hypha at an acute angle. These long branches arise on the terminal cells of the hyphae. As in the haploid mycelia the branches do not contain nuclei until they are relatively elongated. Then the conjugate pair of nuclei from the parent cell enter the base of the branch and divide forming a clamp connection. The parent cell is temporarily uninucleate until the completion of the clamp connection. A branch may thus arise from a clamp connection itself so that two clamps are formed on top of one another.

Although the cells of the diploid mycelium are typically bi-nucleate they have been observed to contain a ^{greater} number, three or even four nuclei occurring in one cell. Occasionally a uninucleate cell with plain septa is found.

While cells with four nuclei only occur very rarely, (Pl. 22 fig / .) except where anastomosis has taken place, it is not difficult to find cases where three nuclei are present. The most reasonable explanation of these abnormalities seems to be that where four nuclei are present, both members of the conjugate pair of nuclei have divided independantly after the formation of the clamp

connection and, where three nuclei are present, that one member of the pair has divided independently. This explanation is borne out by the occurrence of cells such as are figured in pl. 26 fig. 4. in which one of the two nuclei appear to be dividing.

Buller states that, in Coprinus, each member of a conjugate ^{pair} ~~pair~~ of nuclei may divide independently of the other member whenever such a division is able to promote the diploidisation of a haploid mycelium. It appears that in Typhula independent division can take place quite apart from any question of the promotion of diploidisation.

Branches which arise a few cells back from the apex do not form an acute angle with the parent hypha but usually come off at approximately right angles. These branches are much shorter than usual and frequently anastomose with one another or with cells of the main hyphae. After the anastomosis is complete the nuclei of one of the cells thus connected pass over to the other cell. The cell which is thus invaded at first contains four nuclei but this number is soon diminished. One of the conjugate pairs gradually disintegrates leaving one pair in the cell. So far as can be ascertained it appears that the remaining pair is that which migrated so that the indigenous pair disintegrate. Pls 23-24 figs. 1-2. The disintegration of the nuclei may commence even before the other nuclei have entered

the cell. This migration of nuclei in diploid mycelia has been described by Bensaude as occurring in Coprinus.

The nuclei are usually to be found near the middle of a cell and while the members of a conjugate pair are sometimes closely associated, they are more frequently fairly far apart. Thus when a branch arises one nucleus may pass into it while the other member of the ^{pair} ~~pair~~ is some distance away in the parent hypha. Pl.²⁵ fig. 4. This point will be referred to again in the description of the superficial mycelium.

2) Superficial mycelium. Three forms of the diploid superficial mycelium are found. These are, macroscopically, exactly similar to the forms occurring in the haploid mycelium, namely, fluffy, granular and crust-like. As in the case of the submerged mycelium the only points of distinction between the haploid and diploid are the number of nuclei in each cell and the presence of clamp connections. It is therefore unnecessary to repeat the description of each of the three forms.

The cells of the superficial diploid mycelia are usually shorter than those of the submerged and they are wider. Branches are of limited growth and very numerous, they are also very irregular in shape.

Nuclei. The cells normally contain two nuclei but quite frequently only one is present. The nuclei assume abnormal shapes in these cells and many appear to degenerate. Sometimes both members of a conjugate pair undergo this process or, on the other hand, one nucleus survives; in the latter case the nuclei are usually found to have been widely separated.

This separation of conjugate nuclei may be caused by the formation of several branches on one cell. There must be some tractive force which causes a nucleus to enter a young branch and it has been noted that in the diploid mycelium of Typhula one member of a conjugate pair may enter a branch while the other member of the pair is some distance away in the parent hypha. If, in such a case, a second branch should arise near the second nucleus, as is shown in Pl. 25, fig. 4, it is reasonable to assume that the nuclei will separate and each enter a branch.

However, a second factor is involved, the force which holds together the two conjugate nuclei. It appears that, in Typhula the nuclei sometimes separate and enter the branches, at others, they remain conjugate.

A branch which thus contains one nucleus is capable of giving rise to a haploid mycelium. This is borne out by the appearance of oidia in an agar film which had been inoculated with a diploid mycelium. The inoculum in this case was taken from a polyspore culture which had been several times subcultured. It is therefore very improbable that the inoculum happened to consist of hyphae which had never been "diploidised", i.e. containing single nuclei which had never encountered other compatible nuclei which which they might become conjugate.

The oidia were quite similar to those which typically occur on haploid mycelia and they were actually borne on haploid cells. This points to the conclusion that diploid mycelia may revert to the haploid condition.

Although a small haploid mycelium may thus be formed within a diploid it never becomes extensive as it is reconverted to the diploid condition by means of anastomoses and nuclear migrations.

It has been shown that anastomosis with nuclear migration exists to a limited extent in the typical haploid mycelium and in the typical diploid in circumstances where there is no question of

"diploidisation". The anastomoses which lead to the "diploidisation" of a whole mycelium very probably originate in the same way but the dissimilarity of the nuclei thus fortuitously brought together promotes more extensive migration.

Clamp Connections.

The majority of the clamp connections in Typhula Trifolii are formed according to Bensaude's first type the stages of which are as follows; The conjugate nuclei approach one another and a small protuberance appears on the cell wall at this point. Fig 1 . Pl. 25. If the cell is the terminal cell of a hypha the clamp is formed in the middle of the cell but in the case of a branch the clamp appears close to the base.

The small protuberance now grows backward to form a hook, one nucleus passes into the base of the hook while the other remains in the parent hypha. Bensaude observed that the nuclei appeared to be drawn into these positions by the centrioles, one case which occurred in Typhula and might be so interpreted is shown in Figure 1 . Pl. 25.

Conjugate division now occurs, the spindles lying obliquely one in the base of the hook and one in the parent hypha. Septa are formed along the equator of each spindle and the clamp connection is completed by the fusion of the hook cell with the parent hypha. Both pairs of daughter nuclei are now reconstituted.

These stages are shown in Figures 1 to 3 Pl. 25. Although many slides have been examined, in only one case has the stage of conjugate division been found.

In this case, unfortunately, the clamp was seen in surface view so that it presented a very confused appearance. However it was possible to distinguish the spindles ^{and} ~~as~~ as the stage of late anaphase had been reached the septa were also visible.

In watching the formation of clamp connections in agar films of living hyphae it seemed that the septa were not always formed before the hook fused with the parent cell. McDonald (21.) also observed this delay in the formation of septa in the case of Typhula gyrans. In stained preparations of Typhula Trifolii cells have been found bearing a hook which had apparently grown out then curved back and fused again with the parent hypha. No septa were present. Two nuclei were lying close together in the parent hypha, division had not yet begun thus the absence of septa is explained. Pl. 26. figs 1-2.

These observations confirm, at least in part, Bensaude's second type of formation of clamps, as she states, ^{p65} "Dans le second cas, souvent dès la prophase, le pont d'anastomose est déjà formé; les deux mitoses simultanées se font, dans ce cas, tout à fait parallèlement, l'une dans l'anse et l'autre juste à côté."

Buller, on the contrary, states that he never observed in Coprinus the fusion of the hook before the formation of the septa and so considers that Bensaude's second type as described above has

been due to incorrect observation or to misinterpretation of her microscopic preparations.

Buller also notes that, in *Coprinus*, the hook does not fuse directly with the parent cell but with a small peg-like outgrowth produced by the latter apparently in response to some stimulus caused by the hook. Bensaudé had previously observed this type of fusion. She does not give a description but figures it in plate 9 fig. 14.

An exaggerated form of this type of fusion has been observed in *Typhula Trifolii*. Here the hook instead of forming a semi-circle has grown straight backward so that it has the appearance of a small branch. Fig ^{Pl 27}~~14~~. A very small bridging hypha sent out by the parent hypha fused with the "hook" just behind the apex.

This seems to indicate what may have been the origin of the clamp connection. The little hook may be actually a branch. The conjugate division takes place and the septa form between the daughter nuclei. The terminal cell is now binucleate, but both the sub-terminal and the hook-cell are uni-nucleate. The nuclei in these last two cells are compatible being daughter nuclei of a conjugate pair so that when they are brought into association with one another by means of a cell fusion, they again form a conjugate pair. In the case just described in *Typhula* there

would be considerable delay in bringing together the nuclei as a Bridging hypha had to be formed. There would be less delay in Bensaude's first type where the bridging hypha is reduced to a peg.

In Bensaude's second type which she considers to be derived from the first, this delay in reconstituting the conjugate nuclei in the subterminal cell is reduced to the minimum; in fact the nuclei in the subterminal are reassociated as quickly as those in the terminal. Bensaude does not attach much importance to this point in her description but merely states, ^(p66) "Ce deuxième cas, étant moins explicite, est évidemment dérivé du premier cas par condensation et empiètement des différentes phases les unes sur les autres."

Although the second type has been observed in Coprinus and Typhula, the first type is, in both fungi, the more common. Thus there is usually a phase in which the subterminal cell is uni-nucleate. If the hook, instead of growing backward from the apex of the parent cell, grew towards it, the terminal cell would suffer the delay with regard to the nuclei instead of the subterminal. As the terminal is more intimately concerned in the growth of the hypha than the subterminal it is important that it should be provided with its conjugate pair of nuclei as soon as practicable. This is accomplished by the

hook growing backward from the apex.

Buller considers that, in Coprinus, the hook must grow backward in order to maintain the nucleo-plasmic ratio in the terminal cell. Growth would be adversely affected if the terminal cell alternated between the uninucleate and the binucleate conditions. This appears to be rather an extreme view of the case, since the terminal cell of any haploid hypha must to a certain extent suffer this variation in nuclear content. Thus, when the nucleus divides, if there is any delay in the formation of septa between the daughter nuclei the cell will be temporarily binucleate. Binucleate terminal cells are recorded by Bensaude as occurring quite frequently in Coprinus; ^(p45) " Une des cellules terminales a trois noyaux..... On en trouve cependant beaucoup qui présentent 2,3 et même 4 noyaux, surtout aux extrémités en voie de croissance active". As binucleate terminal cells are also quite frequent in Typhula in normal cells which are growing rapidly it seems unlikely that the upsetting of the nucleo-plasmic ratio is very deleterious. There may be more significance in the fact that the ^{nuclei of the} terminal cell are capable of conjugate division some time before those of the subterminal. As is shown in Fig.5, Pl. 25 the terminal cell has forked and conjugate division of the nuclei is imminent while the subterminal cell has not yet received its second nucleus,

The function of the clamp connection has been a question of debate for many years. Kniep (*) considers that the clamp assures the proper pairing of the daughter nuclei after conjugate division. In fungi which do not form clamps the two spindles lie parallel to one another so that after division a single straight septum is sufficient to separate off the appropriate pairs of daughter nuclei. If the spindles are not thus situated within the cell then the two septa of the clamp connection are necessary. Kniep does not suggest any reason why in the latter case the spindles do not lie parallel as before.

In examining stained preparations of the hyphae of Typhula it is at once apparent that the diameter of the normal nucleus is almost equal to that of the hypha. If the size of the living nucleus bears the same relationship to the size of the living cell then it would be impossible for the cell to accomodate, without any distortion, two such nuclei side by side. It is therefore improbable that two spindles could be formed under the same circumstances. However, if a protuberance is formed such as that which marks the beginning of the clamp connections the diameter of the hypha is greatly increased. In the first type of clamp as described above, the spindles lie obliquely but in the second case they again lie parallel and each spindle occupies a hypha.

The Uredineae may be quoted as a group in which conjugate division occurs without the formation

of clamp connections. In these fungi the diameter of the nucleus is usually very much smaller than the diameter of the hypha, thus the two spindles can be accomodated side by side so that the need for the clamp connection never arises.

Again, in some fungi which only occasionally form clamp connections it is probable that the ratio between the diameters of the nucleus and the hypha do not remain constant.

Many authors, including Kniep and Bensaude have drawn a parallel between the clamp connection of the Basidiomycetes and the hook of the Ascomycetes and find evolutionary significance in the similarity. In drawings of the Ascus hook, such as those given by Clausen,⁽⁷⁾ the nuclei appear to be too large to allow of two spindles being formed parallel to one another. This is, however, accomplished in the terminal cell after the hook has formed and thus doubled the available space. The suggestion is now offered that the similarity between the ascus hook and the clamp connection is due to their similarity of function.

Buller (6)^{P41} ascribes quite a different function to the clamp connection, that of facilitating the transport of protoplasm, and therefore food material, through the hypha. This theory is prefaced by the statement that each septum, either of the parent hypha or the hook, possesses a central pore. Thus a plain septum only presents one such

pore to the protoplasmic stream while the clamp connection presents two.

The question of the presence of the pore is intimately connected with the method of formation of the septum. Baum (/.) quoted by Bensaude, described two such methods. The first occurred in young, actively growing cells which were therefore full of protoplasm. The septum was laid down simultaneously over its whole length. Such a septum did not have a central pore. The second type occurred in old cells whose protoplasmic content had diminished. In this case the septum formed first in the peripheral protoplasm then gradually grew in towards the centre like an iris diaphragm.

In Typhula Trifolii any septa which have been observed in the course of formation were in young hyphae and they seemed to conform to Baum's first type. The septa of the clamp connection also appeared to be formed in this way. If so, then they would not possess a central pore.

The function of the transport of protoplasm has been previously ascribed to the clamp connection by Falck (12). But the means by which this was accomplished was different from that described by Buller. Falck discovered clamp connections in which the septum of the hook had degenerated and disappeared so that the hook was transformed into a by-pass around the septum of the parent hypha. Bensaude

failed to find any such clamp connections in Coprinus while Buller does not mention them. It is believed, however, that such a clamp connection has been found in Typhula Trifolii, Fig. ^{3 Pl. 26}~~24 Pl. 25~~. This clamp connection, it is interesting to note, occurred in a secondary diploid hypha such as is present in the sclerotium and therefore concerned in the transport of food material.

There is also the possibility that the septum had never formed at all. As the nuclei of an ordinary hypha can divide without the formation of a septum between the daughter nuclei it seems quite likely that the same may be true of nuclear division in the clamp connection.

Multispore Cultures.

The mycelium which is first formed in multispore culture is the submerged type. Pl. 2 fig. 1. If the inoculum consists of many spores of different sexes the mycelium is at first haploid but rapidly changes to the diploid condition. This change is only apparent microscopically, as is shown in Plate 6 fig. 2. where one of the mycelia is haploid and the other diploid.

The superficial mycelium soon appears when it may assume any of the three forms already defined, fluffy, pl. 4 fig. 1. at F, granular Pl. 2 fig. 3 at G or the crust-like form Pl. 2 fig. 4. at A.

The granular form quickly develops into sclerotia such as are illustrated in Pl. 2 fig. 5. The formation of sclerotia is considerably affected by temperature. In Plate 3 fig. 1-2. four subcultures are shown; these were grown under various temperature conditions other conditions being constant. From this experiment it is seen that sclerotia are formed most perfectly at low temperatures, in this case 13 °C, while their formation is stopped altogether at 25 °C.

Sclerotia are formed freely in such cultures as that shown in Pl. 2 fig. 5 where the agar is deep. In Pl. 4 fig. 2 the agar is not so deep and only a few small sclerotia are formed but many abnormal sterile fructifications have appeared. If the

agar is very thin the brown crust-like mycelium is formed. Pl. 2 fig. 4. This brown crust seems to be formed more readily on oat agar than on malt agar. Masses of fused sclerotia are also found frequently on oat agar.

The fructifications appear almost immediately after the sclerotia are mature, but if the latter are removed from culture and planted in soil, sand or damp blotting paper a resting period is usually necessary before the fructifications are formed. Fertile fructifications are produced in soil and in sand, such specimens are normal in shape. Those produced on damp blotting paper are, on the contrary, sterile and their shape is correspondingly altered. Pl. 1 fig. 3.

In only one case has a fertile diploid fructification been formed in culture. It is illustrated in Plate. 2 figs 1 & 2. In the second figure the fructification is seen to have branched at the apex. Such branching occurs very often in culture, Plate 8 fig. 3 showing two specimens from oat agar which are very strongly branched. Although it must be admitted that such specimens are due to the artificial culture conditions it is interesting to note their similarity to some members of the genus Clavaria, in which it is suggested, Typhula should be included.

As has been stated, the fungus grows well on malt or oat agar. Satisfactory growth is also obtained on a medium composed of an extract of clover seed stiffened with agar. A similar medium made with Bean seed instead of clover was also used.

On soil extract agar the fungus grows very slowly and long very slender fructifications are formed. Pl. 3 fig. 3 . As ^{is} ~~was~~ noted in dealing with haploid fructifications in monospore cultures p 43, the fungus is only able to grow for a short time on a medium of agar alone.

Monospore Cultures.

As has been stated under the heading of Material (p 6), monospore cultures generally be divided into two classes, those which produce sclerotia and fructifications and those which do not. "F" and "G" belong to the first class and "K", "S" and "N" to the other.

All these strains were isolated by the usual method of spreading spores on a thin layer of filtered malt agar, punching out small pieces of agar containing single spores and transferring to fresh culture medium. Owing to the small size of the spores, particularly those of the haploid fructifications it was necessary to allow the spores to germinate before removing them from the filtered malt agar, it was then much easier to ascertain that only one spore was transferred each time.

"F" and "G" were thus isolated early in 1933 and they still retain certain characters which distinguish them from one another. "F" is a typical example of the first type of monospore culture. When subcultured the mycelium grows out fairly rapidly and quickly covers the surface of the culture medium; in tube cultures it also grows up the sides of the tube. It never forms superficial mycelia of any kind so that the culture always has a flat

unbroken surface. Pl. 18 fig. 2 .

The microscopic appearance of "F" is also characteristic. The cells are very elongated and regular in size; its branches are typical of the submerged type of mycelium, very long and regularly formed. The edge of a colony of "F" is very even, only here and there does a hypha apparently outgrow the others so that it projects beyond the line formed by the hyphal tips.

The strain termed "G" is not quite so typical of the first class of monospore cultures, since it may produce the brown superficial hyphae which is often formed in cultures of the second class. In cultures of "G" the colony is formed very slowly and has quite a characteristic appearance. Instead of covering the the surface of the medium like "F" it forms a little raised growth often with radial indentations. Pl. 18 fig. 1 . The young outer part of the colony is usually light yellow in colour while the inner portion which is oldest and more raised up, is light to dark brown. The whole colony forms a compact, tough, mass.

Microscopically "G" is again quite different from "F". The hyphae are much more slow growing and more irregular in branching. The edge of the colony does not present such an even line as in "F" owing to the more irregular branching.

Oidia are not formed so freely in "G" as in "F". It might be thought that these characteristic features of "G" are due to age as the same strain has been in culture for so long but as "F" has been in culture for an equally long time and has been treated in the same way it appears that in some measure at least they must be regarded as individual features.

Bensaude in her description of her two monospore cultures of Coprinus also observed that they were not absolutely alike but differed in certain small points just as do "F" and "G". As the other two monospore strains which she was able to isolate only survived for 15 days she was unable to determine whether the variation was due to individual differences or whether they would also be found in other strains of similar sex.

Blakelee (3) working with Mucor found that monospore strains which were sexually alike and to which he applied to sign (+) were more vigorous than those of the other sex which he termed (-). Bensaude thus compares Coprinus with Mucor^{P¹⁰⁶}:- "Il est probable que les différences morphologiques que nous avons constatées entre le mycélium "A" et le mycelium "B" sont caractéristiques des formes (+) et des formes (-), c'est-à-dire constituent de véritables caractères sexuels secondaires... Mais

il est possible cependant que les caractères morphologiques différentiels des mycéliums "A" et "B" soient individuels et non sexuels et que les thalles (+) et (-) de notre Coprin comme ceux de quelques Mucors, ne soient pas morphologiquement reconnaissables ". "Seules des études ultérieures pourront éclairer ce point".

Buller has since made many experiments with monospore strains of the same species of Coprinus and does not remark upon any individual differences between the strains. He has shown that there are four classes, or sexes, of spores instead of the two as in Mucor so that Bensaude appears to be correct in her supposition that the monospore strains of the same sex cannot be distinguished morphologically in Coprinus.

It has not yet been possible to determine whether there are two sexes or four in Typhula Trifolii as the results of a sufficient number of cross inoculations are not yet available. Up to the present, however, it appears that as in Coprinus the strains of similar sex cannot be distinguished morphologically.

Monospore cultures of Typhula Trifolii of which "K", "S" and "N" are examples:- In these cultures the mycelium which is first formed is submerged, i.e. the type which is constantly present

in "F". This grows out quickly and soon covers the medium, it is followed shortly after by the fluffy or granular type of superficial mycelium. The young sclerotia appear as very small knots of hyphae which rapidly darken in colour, a small quantity of liquid is exuded and after three to four weeks the sclerotia reach maturity. Fructifications which may have been forming on the substrate at the same time as the sclerotia are also fully formed after three weeks. Fructifications which arise from sclerotia do not appear until some time after the sclerotium has reached maturity. The fructifications thus formed do not appear to be any more vigorous than those which arise on the substrate.

When monospore strains of this type are grown on agar films haploid fructifications sporing vigorously may appear seven days after the inoculation was made. Small sclerotia are often formed on such films but they do not appear so consistently as the fructifications.

The following experiment was carried out in order to investigate the reaction of these strains to reduced food supply. Basidiospores from a monospore culture were sown in two Petri dish cultures, one of malt agar and one of plain agar ($2\frac{1}{2}\%$ agar in water). Two days later single germinated spores were removed from the malt agar dish and some were sown again in

malt agar and some in plain agar; similarly those from plain agar were sown, some in plain agar and some inbmalt agar.

After three weeks the strains which had been transferred from malt agar to plain agar, or vice versa, not only produced many fructifications but these spored so freely that a second crop of hymenophores had appeared as the progeny of the first generation of spores.

The strains which had been transferred from malt to malt were not so prolofic but the fructifications which were produced were larger than in the first case.

Of the strains which were on plain agar and transferred to plain agar only one survived more than three weeks. In this culture a very small abnormal fructification appeared before growth stopped.

These results indicate that, in Typhula Trifolii, restriction of food supply to a certain point, causes a decrease in the size of fructifications produced but an increase in their numbers. Restriction below this point results in complete cessation of growth. A very fine degree of adjustment between the food supply and the growth of the fungus is indicated.

Diploid Fructifications and Sclerotia.

A normal diploid fructification is illustrated in Pl. 1 fig. 1. Such specimens are obtained by causing the sclerotia to germinate in soil or damp sand. The average size of such fructifications is 1.5 cms, the largest being 1.7cms and the smallest .7 cms.

Then stalk is usually clearly defined but in some cases, as is shown in Pl.9 fig. 1, the hymenium may only be distinguished by the presence of spores. Hairs occur at the base of the stalk and hymenium. In one case, Pl. 7 fig. 3, a band of hairs is found in the middle of the stalk. Earlier stages in the formation of this fructification are illustrated in figs. 1 and 2 of the same plate. It is seen that the hairs replace the basidia which failed to form owing to staling of the substrate. When the fructification was transferred to fresh sand, growth recommenced so that finally a fertile fructification was superposed upon the sterile fructification.

The stalk, in normal specimens, swells out rather sharply to form the hymenium which entirely covers the upper part of the fructification. Pl.1 fig. 1. The slight constriction in the middle of the part bearing the hymenium is not a constant feature. The basidium

is of the usual club shape and bears at the top four spores which are supported by slender sterigmata.

The approximate size of the basidium is 7u to 8u by 20u, the sterigmata appear about twice the length of the spore, average spore measurement 4.9μ by 10.94μ . The breadth of the spore varies from $3.8\frac{2}{3}\mu$ to 6.36μ and the length from 8.58μ to 12.4μ .

The basidiospores are transparent and oval; one side is often slightly flatter than the other, this side faces inward on the basidium.

The normal fructification of Typhula Trifolia as described above is a typical example of the genus Typhula since it has a slender stalk which swells out to form the hymenium. In some, however, the stalk is only distinguished from the hymenium by the absence of spores. Pl. 9 fig. /. This specimen was grown in damp sand so that its shape cannot be entirely due to abnormal conditions.

A fungus which occurred in pots containing plants of *Rhododendron* spp. is illustrated in Pl. 10 figs. 1-3. In some of these fungi the stalk is more or less defined but in others it appears to merge into the hymenium. Another fungus which was found in pots of *Boronia* sp. is shown in Pl. 11 figs. 1-2. Again it is very difficult to decide whether the stalk is sufficiently well defined to warrant the fungus being placed in the genus Typhula.

In Plate 12 fig. 3 . specimens of Pistillaria quisquiliaris are illustrated. Comparing these specimens with those in figs. 1 & 2, the difficulty of determining the genera is very apparent.

Fructifications of both Typhula and Pistillaria may be branched. This makes the identification of the specimen illustrated in Pl. 12 fig. 1 . all the more difficult. This fructification might be considered to approach the genus Clavaria, while the specimens shown in Pl. 12 fig. 2 are almost certainly members of this genus.

If, as Masee suggests, Typhula and Pistillaria were included in Clavaria the number of species would be greatly reduced as many fungi, which are undoubtedly identical, are described under both Typhula and Pistillaria. Again many species of Clavaria are synonymous with corresponding species of Typhula and Pistillaria. It is therefore desirable that the three genera should be included in the one genus Clavaria.

Sclerotia; These are small dark brown or black bodies whose diameter varies from 1 to 2 mm. The outer wall, or rind, is quite hard in mature sclerotia, and has a rough outer surface. The formation of this rind has been described by McDonald in his work with Typhula gyrans which in this point appears to be very similar to T. Trifolii.

The mature sclerotia are quite easily

detached from the substrate owing to abstriction by the formation of the wall. The point of attachment to the substrate is marked by a small white patch very closely resembling the hilum of a Brassica seed. This feature adds considerable to the difficulty of ^{identifying} ~~detecting~~ sclerotia in samples of seed. The sclerotia are fairly easily seen in samples of Wild White Clover seed as the latter are rather smaller and of a uniform colour whereas Red Clover seed is of varying colour and about the same size as the sclerotia. Pl. / fig. 2 .

When the sclerotium is cut open the rind is seen to be relatively thin and that the bulk of the sclerotium consists of a whitish mass of compacted hyphae. The rind is continuous but is slightly indented at the "hilum" . Pl. / fig. 4 at H. The small white patch at the "hilum" consists of thin-walled hyphae which previously attached the sclerotium to the substrate.

Large sclerotia are often hollow as is ^{shown} ~~shown~~ in Pl. / fig. 4.

The cytology of these structures, fructifications and sclerotia, does not present any unusual feature.

The vegetative cells of the fructification are binucleate. In the young basidium, however, the two conjugate nuclei fuse to form one large nucleus. Pl. fig. . Meiosis doubtless occurs at this point as two divisions rapidly succeed one another, (pl. 21 fig. 5-6.) resulting in the formation of four nuclei of which one passes out to each spore.

The sclerotium is composed of the "superficial" type of diploid mycelium. The white central part is made up of the granular superficial hyphae and the rind of the crust-like superficial hyphae. The nuclear condition of these types of hyphae is discussed under the corresponding headings pp 23-25.

Haploid Fructifications and Sclerotia.

Monospore cultures do not usually produce either fructifications or sclerotia. In February 1934 monospore cultures were made from a fructification which was produced in soil. At first the cultures, of which three were termed "K", "S" and "N" appeared to be of the usual type, the hyphae had plain septa. However, after about three weeks sclerotia began to appear in all the cultures followed immediately by fructifications. Except that these appeared to be smaller than usual the cultures were now very similar to multispore cultures. There was one very important difference, the septa appeared to be plain, no clamp connections could be found on any of the hyphae. This indicated that the fungus had not become homothallic or given rise to a homothallic strain as was at first suspected. It was still possible that this might be a homothallic strain which did not produce clamp connections but by using the agar film technique it has been shown that the cells are uninucleate. The only possible explanation which remains is that the sclerotia and fructifications are haploid. A monospore, (S). culture is shown in Pl. 16 fig. 1.

Fructifications.

The most striking feature of a typical haploid fructification of Typhula Trifolii is its very small size. Specimens have been obtained in culture measuring 1.5 mm. in length. As these fructifications were sporing actively their small size cannot entirely, if at all, be ascribed to immaturity. These specimens were, however, exceptional as the average size of the fructifications in culture is about 1 to 1.5 cms. i.e. about the size of a small diploid fructification.

While diploid fructifications never attain their normal shape or size in ^{artificial} culture the reverse is true of the haploids. Thus the two types of fructification may not be very different in culture as regards size. However, when the sclerotia of each type are germinated in soil or sand the difference between the resulting fructifications is marked, the diploids being at least two or three times as large as the haploids. Pl. / fig / + Pl. 127. fig. 1

In culture the haploid fructifications appear as miniatures of normal diploids. The stalk is slender and swells out at the top to form the hymenium. The shape of the basidium appears to resemble closely that of a diploid fructification while the four basidiospores are borne on very slender sterigmata.

One feature of the typical diploid is not repeated very faithfully in the haploid fructification. The stalk, instead of bearing hairs at the top and bottom, is either downy all over or more or less glabrous. When hairs occur, however, they are rather longer at the top and bottom of the stalk than in the middle. Pl. 14 fig. 4.

When grown in diffuse light the stalk elongates considerably, attaining a length of several centimetres. In this case the hymenium remains quite as small as in the normal fructifications; thus while the total length of this fructification is equal to that of a normal diploid its proportions are quite abnormal.

As in the diploid, a haploid fructification may give rise to another which is borne on the stalk of the original fructification. Other exceptional specimens are those in which several seem to have fused together, as is shown in Pl. 14. fig. ~~4~~². This specimen was 8 mms. in length and at the time of photographing was sporing actively. The little colonies seen at C in the illustration had already been derived from spores thus produced.

The hymenium of a haploid fructification is quite as fertile as that of a diploid. As the spores germinate rapidly secondary fructifications sometimes appear on the mycelium below those from

whose spores they have been derived.

In fructifications which arise directly from the culture medium (without having a sclerotium at the base) the lower half of the stalk is often dark brown in colour. The colour is darkest below and gradually shades off into the normal yellowish white of the upper part. When examined microscopically the brown part of the stalk is found to consist of thick-walled hyphae such as make up the outer layer of the sclerotium. It appears as if in some way or other these thick-walled hyphae were substituted for the sclerotium.

It is interesting to note that the nature of haploid fructifications apparently varies from one fungus to another. Hanna (14) referring to Coprinus lagopus writes "After transference to sterilised horse-dung, diploid mycelia fruit(produce fruit-bodies which expand) with great regularity within about 14 days whereas, while certain haploid mycelia fruit just as quickly, many others fruit more slowly, so that three weeks or a month not infrequently elapses before a single fruit-body opens its pileus. The rudimentary fruit-bodies on some haploid mycelia never expand at all. On the whole, therefore, it appears that the diploid mycelia fruit not only more certainly but also more

quickly than haploid."

Hanna also found that as a general rule, diploid fruitbodies are more vigourous than haploid, as many of the haploid never form a normal hymenium and "even the most highly developed haploid fruit-bodies produce but relatively few spores".

This lack of vigour in the haploids, Hanna explains as follows; "The greater vigour of diploid fruit-bodies as compared with haploid is correlated with the fact that the cells of diploid fruit-bodies contain conjugate nuclei of opposite sex, while the cells of haploid fruit-bodies contain non-conjugate nuclei only. If we suppose that each nucleus possesses a factor for vigour which shows itself in fruit-body development, then diploid fruit-bodies, in general, should be more vigourous than haploid."

In discussing haploid and diploid fructifications as they occur in nature, Buller also gives an explanation of the fact that haploids in Coprinus rarely reach maturity. In a dung-ball infected with many spores the resulting mycelium may only become partially diploidisedso that only a limited number of diploid fructifications can be formed. These then monopolise the food materialand so reach maturity. If , however, the spores gave rise to haploid mycelia capable of forming haploid fructifications a great number of rudimentary fruit-

bodies would appear simultaneously and there would be an insufficient food supply to enable them all to mature.

Nothing is known of the occurrence of haploid fructifications of Typhula Trifolii in nature but in culture they show no lack of vigour such as has been described in Coprinus. This may be partially explained by the fact that they are capable of producing viable spores while still very small. It has already been mentioned that the haploids vary in size from ^{1.5}mm to 1.5 cms. The large specimens are found in petri dish cultures of malt agar where abundant food material is available and the very small ones on agar films where the medium not only contains less food material but is less abundant. Pl. 13 figs 3 & Pl. 14 f2.

This suggests that in Coprinus lagopus the available food material is used up in constructing a large fructification which cannot then obtain the further nourishment necessary for spore formation; while in Typhula Trifolii if the available food supply is limited a very small fructification is formed which is capable of forming many spores.

Spores of the haploid fructification:- As in the diploid fructification the basidium carries four spores each of which is borne on a slender sterigma, all being proportionally smaller than in the diploid. The spores are hyaline and ovate, with one side not so rounded as the other, the less rounded sides face inward on the basidium.

The spores are smaller than those formed on diploid fructifications, measuring approximately 3.03u by 1.8u. These figures were obtained by measuring spores which had been smeared on to a slide prepared with albumin, fixing at once in Flemming's weaker solution and staining with Heidenhein's Iron Alum Haematoxylin. Spores from diploid fructifications treated in exactly the same way measured 10.9u by 3.5u. ; it is therefore improbable that the small size of the spores is due to the treatment.

This method was adopted as it was impracticable to measure such spores accurately except by staining and using an oil immersion objective.

It is interesting to find that in this point also the haploids of Typhula differ from those of Coprinus. Hanna records that "The most fully developed fruit-bodies, therefore, differ from the normal fruit-bodies in the smaller number of spores which they bear, but not in the size of the spores".

The spores are viable and have now been grown through several generations, although the later generations grow more slowly than the first they still produce many fructifications which spore vigorously. Pl. 16 fig. 2 .

Like the spores from diploid fructifications they are uninucleate. They germinate quite readily on culture media and produce a very slender germ tube which later attains the diameter of the typical haploid mycelium. Plate 14 fig. 3 . shows a haploid fructification which , while sporing, was laid on an agar film. The basidia and the sterile hairs are seen growing out into the agar in the immediate proximity of the fructification. From the lower end of the stalk the hyphae of a normal haploid mycelium are growing. All these hyphae are much larger than the small hyphae seen at G. These are the germ tubes produced from spores at the time that the film was inoculated.

No oidia have yet been found on the mycelia producing "haploid spores", but as only relatively few haploid strains have been studied this may not be a constant character of such mycelia. Actually the basidiospores have become the equivalent of oidia since they are all of one sex.

This similarity of sex is easily proved by sowing many spores from one hymenium in an agar

film. They germinate readily and mingle with one another as is shown in Plate 14 fig. 3 . at G. In such a film as this, where the spores are all from one hymenium, clamp connections never develop and the cells remain uninucleate.

Clamp connections have been formed, however, on bringing together the mycelia of two different strains exactly as was done in the case of the normal haploid mycelia. Two strains were grown together in an agar film, after eleven days the mycelia were observed to be in close contact with one another and three days later the film was fixed and stained. Clamp connections were found on many of the hyphae. An anastomosis from this film is shown in plate 31 fig. 1 . In another case the hyphae first fused after nine days, thirty hours later clamp connections were observed, the film was fixed and stained and the fact verified that anastomoses with the formation of clamp connections had occurred.

Haploid sclerotia :- These are, in shape, structure and colour, very similar to diploid sclerotia. As in the case of the fructifications there are two important differences between the haploid and the diploid. The haploid sclerotium is composed of haploid hyphae and it is smaller

than the diploid sclerotium. This is shown in Pl. 16 fig. 4. where the haploid and diploid sclerotia are photographed together. The specimens illustrated are each typical of their class.

The haploid fructifications, spores and sclerotia are all very similar to the corresponding diploid structures but they differ in size and in the nature of the hyphae with which they are constructed. In dealing with Coprinus, Hanna considers that the haploid condition of the hyphae is reflected in the diminished vigour of the haploid fructifications. It appears that, in Typhula, vigour remains as great in the haploid as in the diploid, but size is considerably reduced.

Heterothallism.

The following points indicate that Typhula Trifolii is heterothallic. Single spore cultures remain indefinitely in the haploid condition. No diploid sclerotia or fructifications are formed in such cultures, the cells have isolated nuclei, usually one in each cell and the septa are plain.

The two monospore cultures "F" and "G" already described, p 38 were found to be compatible. When these two strains were allowed to come in contact with one another in culture the hyphae anastomosed, nuclear migrations took place and a diploid mycelium arose. Pl. 18 figs. 1, 2, & 3. This was marked microscopically by the appearance of clamp connections on the hyphae and the presence of conjugate nuclei in the cells. Normal sclerotia were then formed on the diploid mycelium, Pl 18, fig.4. Subcultures of "F" and "G" alone which had been made at the same time as the double inoculations remained in the primary condition.

In a modification of this experiment two identical subcultures of "F" were used. After a certain time "G" was introduced into one of them and the point of inoculation marked. Clamp connections were then formed on the hyphae at this point and

four months later fructifications appeared. Pl. 18 fig. 5. The subculture of "F" which had not received the "G" strain was still in the primary condition.

Hyphae from the two strains were now placed together in a hanging drop of nutrient solution. In the young hyphae which grew out from each strain the septa were observed to be plain. After some time the hyphae of the two strains mingled to form a tangled network and later clamp connections were observed.

This has been repeated using the agar film technique when clamp connections were found thirty hours after the leading hyphae of the two strains had come into contact. In this case it was possible to confirm the presence of clamp connections and conjugate nuclei by fixing and staining the mycelia.

In reviewing the literature concerning heterothallism (p.4) it has been shown that Lehfeldt was the only investigator who described the mechanism by which the nucleus of one mycelium passes into another mycelium and causes the formation of clamp connections. It is now proposed to summarise Lehfeldt's work, particularly that dealing with Typhula erythropus. This will be followed by an

account of the results obtained in the present investigation of Typhula Trifolii. A discussion of the significance of these results will then be given. 1).

Lehfeldt's work was principally concerned with the two fungi, Corticium serum, already studied by Kniep, and Typhula erythropus. In Corticium, when a quite young germ tube comes in contact with another they anastomose, and it is usually the apical cells which are involved. The nuclei in these cells then approach one another and divide conjugately to form the first clamp connection. Thus, in Corticium, there is a close connection between the passing of a nucleus from one hypha to another and the formation of the first clamp connection. In Typhula erythropus germ tubes are, for some unknown reason, unable to anastomose thus the hyphal fusions with passing of nuclei are delayed until the mycelia are older. In this fungus there is, almost without exception, no such correlation, either in time or space, between

1) Considerable difficulty has been experienced in elucidating Lehfeldt's conclusions and it appears that this difficulty has not been confined to the author alone - In consequence extensive quotations have been given so that there shall be no doubt as to the source of the interpretation given here.

the point of anastomosis and the first clamp connection. The few exceptions were found in anastomosis between very young cells :- "Es ware denkbar, dass hier nur alles so glatt vonstetten ging, weil es junge Zellen, nahe der Fadenspitze, waren, die von der Anastomose betroffen wurden" p.51.

In the investigation of the processes involved in the fusion between heterothallic forms a more detailed consideration of the results obtained by Lehfeldt is necessary. Two hyphae come into contact with one another and anastomosis occurs. In all the cases illustrated and described fusion is accomplished by a short bridging hypha :- p. 40 "Es fanden sich einige Stadien, wo nur eben erst eine Brücke zwischen + and- Myzel hergestellt war.. " He does not say whether the bridge is always present.

A nucleus from one hypha now passes through the bridge to the other hypha, leaving behind an empty cell;- p. 40. "Weiter fanden sich Phasen, in denen ein Kern bereits durch die Brücke geschlüpft war, also die eine der zwei Zellen an der Brücke kernlos war."

The invading nucleus divides and one of the daughter nuclei may move towards the apex of the hypha while the other moves towards the base. After such movement, during which the migrating nuclei frequently divide, the septa of the invaded

hypha are found to be very irregular in that some are partially broken down and other new septa are formed, sometimes close beside the broken ones. It is considered that the invading nuclei cause the partial or complete dissolution of the septa in order that they may pass through the hyphae and the plain septa are then formed after their passage: p. 42.

"Erkennt man nun die eine Wand von beiden als halbe, im Abbau begriffene, so fragt man sich sofort: Was soll die volle Wand daneben? Ich halte sie für später gebildet, als die halbe Wand schon durchbrochen war und einem Kerndurchtritt Raum gegeben hatte." Sometimes the side wall opposite such a half wall is bulged out by the passing nucleus:- p.43
"Häufig ist die Längswand der Zelle, gegenüber der unvollkommenen Querwand, ausgebaucht, als ob sich der Kern dadurch den Engpass erweitert hätte."

Rawitscher, to whom Lehfeldt refers, has described a similar migration of nuclei through "half walls" in Ustilago Maydis. According to Lehfeldt, if, in an optical section, the position previously occupied by the missing part is in front or behind the remaining part then it is only by measuring the depth of the wall by means of a micrometer that it may be distinguished from a complete wall. Again, some walls may be completely broken down leaving no trace so that afterwards there is no sign of any disturbance at all. If a single nucleus

should be found in a cell with plain septa it might be one of the indigenous nuclei between the original septa or one of the invading nuclei between septa of later origin:- p. 44. "Da der Grad der Auflösung sich ganz verschieden stark findet, ist es leicht möglich, dass einzelne Wände auch spurlos verschwunden sind. Vollen Wänden ist ferner nicht anzusehen, ob sie primär oder sekundär entstanden sind, ebenso wie wir leider keinen Anhaltspunkt haben, um Kerne in einem Fadenstück als myzelfremd oder myzeleigen anzusprechen, wenigstens ihrem Aussehen nach nicht." The position of nuclei relative to other nuclei and to clamp connections sometimes indicates their possible origin. At this stage the nuclei occur in very irregular positions since the equilibrium of the haploid state has been upset.

The migrating nuclei will now have reached the younger parts of the hyphae, the apices of the main hypha and its branches. The invading nuclei then form conjugate pairs with some of the indigenous nuclei, and when they divide the first clamp connections appear. Lehfelddt states that in Typhula erythropus there is no great difference in length between haploid and diploid cells, and that when a haploid mycelium is converted into a diploid, some of the indigenous nuclei degenerate :- p.47.

"Schnallenzellen und Einkernzellen zeigen bei Typhula erythropus keine in die Augen fallenden Längenunterschiede. Wenn also aus einen primären Myzelstück später ein sekundäres mit etwa gleicher Zellenzahl entsteht, müssen zahlreiche primäre Kerne zugrunde gehen, denn das sekundäre Myzel erhält seine Kerne aus konjugierten Teilungen neu. "

If a hypha which possesses both kinds of nuclei, invading and indigenous, anastomoses with another hypha of the same mycelium which has only indigenous nuclei then invading nuclei pass from the former hypha to the latter. This occurs even if the former hypha does not yet bear clamp connections :-

"
p. 49. "Aste eines Myzels, die auch nur einen myzel-fremden Kern enthalten, müssen natürlich auf noch rein primäre - auch wenn diese dem gleichen Myzel entstammen - wirken wie ein andersgeschlechtiges Myzel und bei Anastomose Schnallenbildung im primären Myzel hervorrufen können, selbst wenn sie selber noch gar keine Schnallen tragen."

In a rather general statement Lehfeldt says that when two hyphae are involved in anastomosis such as has been described above, the transference of nuclei is eventually mutual, both hyphae being converted from the haploid to the diploid state. He does not say however, whether nuclei pass in both directions through one hyphal bridge:- p. 46. "Im ganzen lässt sich also nach Bild 2, und ähnlichen vermuten, dass nache einem Brückenschlag zwischen

sexuell verschiedenen Myzelien lebhaft Kernwanderungen und -umlagerungen einsetzen, und zwar erst im einen Myzel, später im anderen auch." His drawings rather suggest that nuclei only pass one way in a hyphal bridge but that many such bridges are formed through which exchange of nuclei is possible. Further, it has been shown in the preceeding paragraph that branches containing both kinds of nuclei can act in this respect as primary branches and thus form another channel for the passage of nuclei.

He has also shown that there is a close connection in Corticium serum between the anastomosis and the first clamp connection resulting from it; in Typhula, on the other hand, several clamps may appear simultaneously at different places on the hyphae at some distance from each other:- p. 50. "Sicherlich verhalten sich die einzelnen Pilze in dieser Hinsicht durchaus nicht gleich. Dass zwischen Corticium serum und Typhula erythropus in der Anlage der ersten Schnelle in Wechselbeziehung zur ersten Anastomose ein grosser Unterschied besteht, glaube ich erwiesen zu haben. Coprinus fimetarius steht in seinem Verhalten bei diesen Vorgängen vermutlich der Typhula viel näher, und der Umstand, dass auch Kniep bei Corticium varians und Collybia conigena gleichfalls keine einfachen Verhältnisse vorfand, lässt schliessen, dass auch diese beiden Pilze, bei denen nach meinen Ergebnissen Heterothallie nicht ausgeschlossen ist, obwohl der zytologische Befund

zunächst auf Homothallie deutet, sich mehr dem Typhula- Typus nähern. Identisch ist ihr Verhalten mit Typhula allerdings auch nicht, denn die halben Querwände und Ausgebauchungen scheinen zu fehlen, die leeren Zellen scheinen zum mindesten erheblich seltener zu sein." This quotation is important since Coprinus fimetarius is synonymous with Coprinus lagopus with which Buller later carried out his researches on heterothallism etc.

An account of results obtained with Typhula Trifolii is incorporated in the following discussion of some examples of anastomoses between hyphae of different sex. In these attempted explanations of the origin and nature of nuclei and septa involved in anastomosis it is almost impossible to be dogmatic. There are so many possible explanations that only suggestions can be offered with occasional indications of that explanations which is the most likely to be correct.

(Note- Although the fungus illustrated in the first two examples is probably T. Trifolii its origin is not quite certain. It appeared in pots of soil in the same greenhouse as that in which T. Trifolii had been growing and it ~~was~~ indistinguishable in morphology and cytology from T. Trifolii. As spores of the T. Trifolii ^{of known origin} were not available at the time, the spores of unknown origin were used in the interim.)

In the first example, illustrated in Pl. ~~28~~²⁹ fig. , conditions are relatively simple. Two haploid hyphae have fused at the point marked F. The terminal cell of one hypha has fused with either the terminal or the subterminal of the other. A nucleus has passed over to the latter hypha and moved up to its apex. Since there are now three nuclei in the apical cell it is probable that this cell was originally binucleate. The invading nucleus became conjugate with one of these indigenous nuclei and divided to form the clamp connection. The septum at S may have been formed either before or after the passage of the nucleus. It appears to be entire but may actually be a half wall of which the missing part was either behind or before the remaining part in which case it is very difficult to detect. The nucleus at F is probably a daughter nucleus of the invading nucleus.

Young cells are also involved in the second example, illustrated in Pl. ~~28~~²⁹ fig. . Fusion between two haploid hyphae appears to have taken place at F but this is not quite clear in the preparation. The branch AB is without a nucleus so it is quite probable that the missing nucleus is responsible for the abnormal conditions in the hypha CD. It is not possible to trace the hypha CD any further back from the apex as it is obscured by other hyphae. The cells towards the apex of CD will now be discussed.

The nucleus at N1 is in the course of degeneration, only the nucleolus persisting, it is therefore probable that this is an indigenous nucleus. Of the two nuclei in the next cell, N3 is in the same condition as N1 but N2 is clearly defined and quite normal in shape, therefore N3 is likely to be indigenous and N2 an invading nucleus. An empty cell then follows. Either the nucleus of this cell has entirely disintegrated or one of the septa is secondary in origin, i.e. it has been formed after the passage of the invading nucleus.

At H there is a fairly well defined half-wall close to an entire wall; the latter is probably secondary in origin. It is not clear whether the space in the protoplasm on the other side of the whole septum from the half wall is merely a vacuole or another half wall. Of the three nuclei in the next cell, N4 and N6 are normal but N5 is degenerating. N4 and N6 may be invading nuclei and N5, indigenous.

The terminal cell contains five nuclei, of which N7, N8 and N10 are normal while N9 and N11 are degenerating. The position of N8 and N7 suggests that they are conjugate. This cell provides a good illustration of the difficulty which may be experienced in accounting for each nucleus in such cases where the normal haploid condition is upset.

In the third example, illustrated in Pl. 30 fig. the hyphae involved are of the two strains "F" and "G". The subterminal cell of F has fused with a comparatively old cell of G. Anastomosis occurred at the point marked A, probably by means of a short bridging hypha which is now hidden by the hyphae at A.

A "G" nucleus evidently passed over into the "F" hypha. It divided at once and one of the nuclei in the binucleate cell at S is probably one of the daughter nuclei of this division. The terminal cell FT is as yet undisturbed and contains its original F nucleus. The G nucleus now in the subterminal (S) cell will probably divide and a daughter nucleus will pass into the terminal cell (T), form a conjugate pair with the F nucleus and on division the first clamp connection will appear. Another possibility is that the pair of nuclei in S may divide conjugately and form a clamp connection.

Another daughter nucleus of the invading G nucleus has moved away from the apex in the F hypha. The nucleus named N1 seems to be disintegrating thus it is probably an F nucleus. A half wall appears at H accompanied by a bulge on the opposite wall therefore the G nucleus has probably passed this point. N2 may be either a G or an F nucleus but since it shows no signs of degeneration it is probably a G nucleus the product of a division which occurred at this point.

The other daughter nucleus of this division is either N3 or N4. This is the furthest point reached by the G nuclei as the cells containing N5 and N6 are undisturbed.

In the fourth example, Pl. 31 fig. the hyphae involved are of the strains S and K which are two of the haploid strains capable of producing haploid fructifications. Two points of fusion are visible, two different hyphae of S have fused with one K hypha, at the points F1 and F2. F1 is actually a double fusion as the apex of S has forked and each branch of the fork has fused with K. There are very few nuclei in the vicinity of F1 which suggests that a nucleus from K may have moved into S or that migration has not yet taken place and that N1 and N2 are both indigenous.

Nuclei have almost certainly passed from S to K at the second fusion, F2, as clamp connection formation has begun in the apical cells of K. The structure at L is rather difficult to explain. No septa can be seen such as are present on a typical clamp connection. Further, if it were a true clamp connection one would expect to find two nuclei instead of the one at N6. Even N6 is rather faint as if it had begun to degenerate. The structure, L, may be one of the pseudo-clamp connections which Lehfelddt mentions as being very easily confused with true clamp connections in living material.

C1 and C2 are true clamp connections; the diploid mycelium is now established.

It is clearly seen in this case that ~~none~~ one hypha (the K hypha) may be involved in more than one fusion so that nuclei from a strange mycelium may enter and form conjugate nuclei at various points. Or, alternatively, the hypha may receive a nucleus at one point and at another transfer one of its own nuclei to the other ~~hypha~~. In the same preparation from which this last example was taken a hyphal branch was found which had fused four times with hyphae of the opposite strain.

The fifth example is again provided by the strains F and G, but the stages which will be described were all observed in the ~~living~~ living hyphae. This was brought about in the following manner.

An agar film was inoculated on January 9th with hyphae from the strains F and G. As usual, F grew much more quickly than G so that on the 19th January when the two mycelia came into contact with one another, F had formed a fairly large mycelium and G a very small one. On the 22nd, the hyphae of F had mingled with those of G at various points and were growing tangentially around the sides of the G mycelium. A hypha from the G mycelium was observed to be growing towards one of the tangential F hyphae. At 11 a.m. on the 22nd the apex of the

G hypha reached the F hypha at a point several cells back from the apex and anastomosis appeared to take place. Pl.32 ~~figs.~~

The anastomosis was, however, not completed until later since at 4.50 p.m. the protoplasmic contents of the apical cells of G were very suddenly and forcibly expelled into F. The septum at S1 broke down and some of the protoplasm passed through. The movement then subsided. S1 remained in the position shown, and it was observed that the septum S2 was also bulged out towards the apex of the F hypha. The septum still nearer the apex from S2 was however quite straight. As the F hypha was rather old the conditions on the ^{other} side of the fusion, (i.e. the basal cells of F) were difficult to observe but no protoplasm was seen to pass in that direction.

At 7.30 p.m. the protoplasm became denser in a straight line at SF, and it is believed that a septum was formed. In the photographs shown in Pl.33 fig. 1 + fig. 2, a very fine line is visible in this position. At 7.40 two highly refractive objects, which appeared as small points of light, were observed in the cell at P. Pl.33 fig. 1 + 2. At 7.59 one of them moved along the side of the cell to S1 passing without any difficulty the point where the septum SF had been previously observed. A third bright spot now became apparent at Q. Pl.34 fig. .

Between 8.5 and 8.9 p.m. the first two bright spots became very active, they moved backward and forward several times in the cell, all the while revolving about each other. At 8.9 they became slower in their movements and together approached the bright spot at Q. This spot now became very active and slipped round into the fusion cell of G. It travelled up this cell until it reached the septum SG where it seemed to rebound and then return to the point of fusion. The movement, from the time of entering the cell occupied 30 seconds and as the distance traversed was .15 mm, the speed is equal to 18 mm per hour.

The branch B was meanwhile growing very slowly. At 8.19 the protoplasm appeared to be massing up around the paired bright spots as if a branch was forming but at 8.50 the spots moved out of this part and came to rest in the middle of the cell. The third spot now began to travel slowly and steadily up the (G) fusion cell again.

The continuation of the F hypha towards the apex became very faint and by midnight it could not be traced at all. At this time the spots in the F cell either coalesced or became superposed as only one was distinguishable and the side wall was bulged out around the position which they occupied. The third spot had now reached the

septum in G again. The branch at B was degenerating and becoming difficult to trace. Pl. 34 fig. .

At 1.20 p.m. the spot at the septum in G had moved up the hypha and the septum was no longer visible but by 2.30 another septum was observed at approximately the same position which had been occupied by SG. This septum, however, as well as another which had been observed at S3, later disappeared. The spots now became less bright and at 3.15 those in F had almost disappeared and were lying at the original point of fusion while the third spot was also fading and moving very slowly back in the G hypha.

Another F hypha now approached the point of fusion and by 9 a.m. had grown across it. This hypha was kept under observation until 6.50 p.m. but, although it encountered 6 hyphae of the G strain no anastomosis occurred. As the film was now becoming rather dry it was fixed.

The slide was re-examined after staining in order to determine the nature of the "bright spots".

Unfortunately the cell in which the fusion had taken place was hardly visible, no doubt owing to degeneration of the protoplasm. At the position previously occupied by the bright spots two rather indeterminate darkly stained objects were found.

On observing another growing film, bright

spots were found as before. A photograph was taken recording the position of the spots. Pl. 33 fig. 3. and the film immediately fixed and stained. In place of the spots were found nuclei. Pl. 33 fig. 4. This was confirmed by repeating the process with yet another film when again the bright spots were replaced by nuclei in the stained preparation. The conclusion is therefore reached that the spots were actually nuclei. Since the only nuclei thus observed in the living condition have been those involved in anastomosis and nuclear migration it indicates that such nuclei are probably in an abnormal condition. This is another result of the disturbed equilibrium of cells involved in such anastomoses.

The movement of the spots, or nuclei, now becomes of significance. It is possible that two of them entered the cell from the G hypha along with the extruded protoplasm and that the third was indigenous in the F cell. Two of the nuclei were apparently compatible and became conjugate while the third returned to the G hypha which presumably was at the moment without a nucleus.

On considering the nature of the cells involved in this anastomosis, (one an apical and the other comparatively old), and the movements of the nuclei, the most reasonable interpretation appears to be that this was a more or less abortive

attempt at diploidisation. If the cell into which the protoplasm rushed had not been so old there is little doubt that a branch would have been formed or that the rudimentary branch already present at B would have grown on to form a diploid branch. This hypha however being too old to initiate the diploid mycelium, gradually disintegrated. The third nucleus, as is suggested by its movements, was probably an F nucleus which passed over to the G mycelium in which it might eventually conjugate with a G nucleus.

The formation of what might be called temporary septa is an important point. Lehfeldt deduced from the study of stained preparations that a migrating nucleus caused either the partial or complete disintegration of septa but these might be replaced by other "secondary" septa. In the case described above the cell in the F hypha was probably too old to build up a complete septum at SF to replace that which had been broken at Sl. This new and probably incomplete septum at SF disintegrated at once on the approach of the nuclei. Again the septum at SG at first resisted the passage of the nucleus which was observed to rebound as if a substantial obstacle had been encountered. However when the nucleus approached more slowly the second

time it passed this point quite easily and again an unsuccessful attempt was made to replace this septum.

The last example of fusion between haploid hyphae is illustrated in Pl. 36 . Again the strains F and G are involved but the hyphae are rather difficult to follow as they occur deep down in the agar film. The main hypha which is running horizontally cannot be seen on either side of the part shown as it is obscured by other hyphae.

The hyphae are very confused at the point Fl and it is possible that fusion took place here between the apices of an F hypha and a G hypha. It is unlikely that the main hypha consists entirely of one strain since it is old and vacuolated on each side of the part shown. This indicates that the fusion took place somewhere near Fl. For ~~the~~ the present discussion it will be assumed that the fusion occurred at this point.

The hypha AB which arisen at this point is almost regularly binucleate. As has been stated, the hyphae at the point of origin of this branch are very confused and it is possible that nuclei are present in the cell at A. The next cell, towards the apex from A, has two conjugate nuclei, then a branch occurs. There may be a clamp connection on the under side of this hypha but as it soon be-

comes merged in a tangled mass of haploid hyphae it is possible that the "branch" is actually a haploid hypha which has anastomosed at this point with the diploid. The nucleus at N1 is very small and apparently becomes disintegrated. It may be one of the nuclei which were present in the hyphae while it was haploid. The two apical cells of this hypha AB are typically diploid and will give rise to a diploid mycelium.

The hypha CD1, CD2 is only partially diploid and this indicates that the invading nucleus, or one of its daughter nuclei, reached this branch later than the branch AB. The basal cell has no nucleus but there is a very well defined nucleus at C. This nucleus may be one of the conjugate pair which should be present in this cell, while the other member of the pair, an invading nucleus, has passed along the main hypha.

Septa are not shown in the clamp connections in the branch CD1, CD2, but this is probably due to unsatisfactory staining as the presence of paired nuclei in the cells vouches for their being true clamp connections. The terminal cells of CD1 are still in the haploid condition.

If the fusion actually took place at F1 then this is a case of exchange of nuclei between the two mycelia since the branch EF has also become diploid. The bulge on the main hypha at H? may be a half wall with bulge such as occurs at H but as it

has formed obliquely on the hypha it cannot be viewed in one focal plane. There is the possibility that it is a clamp connection but the absence of conjugate nuclei in its vicinity is evidence in favour of its being a half wall.¹ At H,H two very clearly defined half walls with bulges are shown. These were observed in the living hyphae as little bulges, the half walls were not visible. After fixing and staining however, it was apparent that these structures corresponded exactly those in Lehfeldt's description to which reference is made on p 63 of this paper.

The nuclear matter at N2 has several explanations, part of it is likely to be indigenous and part invading, but it is impossible to identify these parts.

Since the branch at K is haploid the invading nucleus has probably still to reach this point, and this further indicates that the transference of nuclei occurred at F1 and that the migration of invading nuclei is proceeding away from that point.

Two examples of anastomosis between haploid and diploid hyphae will now be briefly described.

The first is illustrated in Pl. 37 fig. ~~Pl. 38~~.

Fusion occurred at the point marked F. Both nuclei from the diploid cell passed over to the haploid hypha leaving behind an empty cell. The condition of the haploid hypha indicates that it is only a

short time since this fusion occurred. The two nuclei at N1 and N2 are probably the conjugate pair which migrated from the diploid hypha and which now replace the nucleus originally present in this cell. One of the nuclei in the branch AB may be a daughter nucleus either of N1 or N2.

In the other case of anastomosis between a haploid hypha and a diploid, Pl. 38 ~~fig.~~, a clamp connection has been formed at the point of fusion F, and again two conjugate nuclei entered the haploid cell. The nuclei N1 and N2 appear to be conjugate; either they are both invading nuclei or one of them is indigenous. In the latter case, the invading pair must have separated and it is possible that N3 is the other member of the pair. The invading nuclei will now divide and migrate further through the haploid hypha.

Further examples were found in which the originally haploid hyphae had been more completely converted to the diploid condition but, owing to the presence of many other hyphae not concerned in the anastomoses, the original points of fusion could not be traced.

General consideration of the Results
obtained with Typhula Trifolii.

The various stages of the process of anastomosis and nuclear migration are, in Typhula Trifolii, as follows:- Fusion may take place directly between two hyphae as in the anastomosis which was observed in the living condition, Pls 32-35 fig. or a small bridging hypha may be formed.

Once the hyphae are in open communication with one another the transference of nuclei takes place. In most cases in Typhula Trifolii the nucleus of one of the cells passes over to the cell of the other hypha leaving behind an empty cell. It has been observed that this transference of nuclei may not always be a matter of independant movement of the nucleus through the protoplasm but a violent expulsion of protoplasm and, incidentally, nuclei, from the one hypha to the other.

The invading nucleus now divides, perhaps once or several times. This is followed by the progression of the daughter nuclei of such divisions through the hyphae. To allow of the passage of these nuclei the transverse septa are either partially or

wholly dissolved, thus "half walls" are formed. New "secondary" septa are sometimes formed to replace the "primary" septa.

If the anastomosis occurs on a young hypha the nuclei may become conjugate without extensive migration and the first clamp connection is formed quite quickly. When older cells are involved in anastomosis there is a period between the first transference of nuclei and the formation of the first clamp connection during which the nuclei of the invaded hyphae are disturbed from the equilibrium of the haploid state. As soon as the invading nuclei reach the younger cells they become associated with the indigenous nuclei in conjugate pairs and the hyphae again reach a state of equilibrium, this time in the diploid condition.

These results confirm Lehfelddt's results with Typhula erythropus in the following points:- Anastomosis is not usually followed immediately by the formation of clamp connections, but only after a period of unstability and irregularity in the nuclei of the hyphae involved. Nuclei can move through the hyphae of a mycelium of opposite sex. Nuclei do not require to migrate extensively as anastomoses occur at several points on the hyphae and the transference of nuclei may take place at any or all of these points.

It has not been found possible to detect half walls in T. Trifolii by measuring with a micrometer as Lehfelddt describes in T. erythropus; the only half walls found were those in which the remaining part could be seen in the same focal plane as the space left by the missing part.

Buller, in Vol. 1V of his Researches (5) devotes a chapter to the subject of "The effect of diploid on haploid mycelia in Coprinus lagopus and the biological significance of conjugate nuclei in the Hymenomycetes and other higher fungi". In his introduction Buller gives a short account of the occurrence of conjugate nuclei in the Basidiomycetes, particularly the Rusts, and says that it occurred to him that the non-fusion of nuclei of opposite sex in the diploid mycelium of Mushrooms and Toadstools is not only an interesting cytological fact, but:-
(pp. 188-189) " may also be something of great physiological importance in that it may permit of a diploid mycelium diploidising an appropriate haploid mycelium in dung-balls.... And it further occurred to me that it would be possible to test the value of the theory just propounded in a very simple manner i.e. by pairing a diploid with a haploid mycelium of the same species and observing whether or not the diploid mycelium is able to diploidise the haploid mycelium".

This theory might, however, have been suggested by Lehfeldt's work since he showed that a diploid hypha does actually "diploidise" another hypha of the same mycelium which is yet in the haploid state. (See p.65 paragraph / of this paper)

Buller's introduction finishes with the statement that the "organisation of the nuclei in the diploid mycelial hyphae of the Hymenomycetes in conjugate pairs is essential for the diploidisation of any haploid mycelium whatever, regardless of the kind of mycelium, haploid or diploid, which may initiate the diploidisation process".

Definitions of the terms "Diploidisation" and to "Diploidise" are then given as follows :- "The word diploidise is used to designate in the Hymenomycetes the process by which a haploid cell is converted into a diploid cell, or a haploid mycelium into a diploid mycelium, by the formation of conjugate nuclei within the cell or within the mycelium." The verb "to diploidise" is thus used "A haploid mycelium of one sex may be said to diploidise a haploid mycelium of opposite sex, or two haploid mycelia of opposite sex may be said to diploidise one another" (p 190)

Under the heading of Methods (p. 194), Buller defines the haploid and diploid mycelia of Coprinus lagopus. He states that in the present investigation

no cytological work has been done", Only morphological features were used to determine whether the hyphae were in the diploid condition . "When paired mycelia growing on dung agar in a Petri dish were examined.. the Petri dish was usually turned upside down on the stage of the microscope and the mycelium was viewed with the low-power objective. .. Only occasionally, for special or very critical observations or when the mycelia were not further required, the Petri dish was opened and the mycelia were examined from above with the high-power objective". In working with T. erythropus, found, on the contrary, that in such investigations it is necessary to study the cytology, (p.40) :- "Bei der schwachen Vergrößerungen, die man zur Kontrolle der Objektträgerkulturen in geschlossenen Petrischalen nur anwenden kann, täuscht man sich anfangs sehr leicht darüber, ob schon Schnallen gebildet werden oder nicht. ... Selbst da, wo eine Anastomose zwischen verschiedenen Myzelien noch gar nicht denkbar ist, oder in unzweifelhaft reinen Einsporkulturen treten kleine Häkchen auf, die man bestimmt für Schnallen halten möchte, bis der zytologische Befund des gefärbten , dann aber auch leider schon toten Objekts zeigt, dass man es mit Pseudoschnallen zu tun hat." In the present investigation on T. Trifolii this opinion was found to be fully justified. Even in exceptional

cases where the movement of living nuclei could be observed the hyphae were watched continually with the high-power objective and subsequently fixed and stained. The few examples of diploidisation in Typhula Trifolii which are described are the result of the examination, both in the living condition and after staining, of eighty agar films.

Buller now describes the experiments in which he paired haploid and diploid mycelia with the result that the haploid mycelia were diploidised by the diploid mycelia. In these experiments he varied the relative sizes of the haploid and diploid mycelia and their position relative to one another. Thus, in one experiment, he used a large haploid mycelium, in whose centre he set a small diploid mycelium, in order to determine "whether or not a diploid mycelium can be effective through the older portions of a large haploid mycelium" (p 207). "From the result of this experiment it is clear that a diploid mycelium is able to diploidise a haploid mycelium when it has come in contact with only the oldest part of the haploid mycelium ...". It may be remarked, however, that even in the central part of a mycelium nine days old, such as that used in the experiment, there would be present younger hyphae which were probably responsible for the diploidisation process.

The next point which Buller investigates is the rate of movement of nuclei through a haploid mycelium which is being diploidised by 1) another haploid mycelium or by 2) a diploid mycelium. Eight comparative experiments were carried out using the haploid and diploid mycelia, the method of pairing these and the results obtained are given in Table 11 (p 217 Vol 1V). The most significant figure is that of the "Average rate of movement of nuclei through the two sets of haploid mycelia which in individual experiments varied from .79 mm. per hour to 1.18 mm per hour. At this point, after summarising Lehfeldt's work, Buller makes the following statement p. 216; - "It is on the assumption that, during its diploidisation, a haploid mycelium of Coprinus lagopus behaves essentially in the same manner that I have collected the data embodied in Table 11 and have made the calculations of the speed of movement of the nuclei shown there in the last two columns". The word "essentially" is rather important as Lehfeldt did not claim that Coprinus fimetarius (C. lagopus) behaved in exactly the same manner as Typhula. His statement regarding Coprinus is quoted on page of this paper and it will be recalled that this was further qualified by the remark that the behaviour of individual fungi doubtless varied in this respect.

In comparing the rate of movement of nuclei through 1) a haploid mycelium being diploidised by another haploid mycelium with the rate through 2) a haploid mycelium being diploidised by a diploid mycelium, Buller shows that the difference comes within the limits of experimental error. Hence it appears that the diploidisation of a haploid mycelium can be affected as rapidly by a diploid mycelium as by a haploid mycelium. The actual rate of movement of the nucleus Buller calculates to be 2.5 mm per hour. In Typhula Trifolii a nucleus has been observed to move at the rate of 18 mm per hour.

The next point which is considered is the septation of the mycelium through which the nuclei pass. p.227. :-"In a hypha of one piece of haploid mycelium there were about 14 septa to one mm of length. Assuming that, on the average, each mm of each haploid hypha contains 14 septa, a nucleus passing for 6 cms. in a straight course through a haploid mycelium would be obliged to pass through a hyphal passage-way which initially was provided with 840 septa ". In plates III and IV at the end of Vol IV, Buller illustrates the passing of a nucleus through a haploid mycelium. There is a nuclear division at each septum which is passed. If the passage of 840 septa is to be accomplished in this way in 42 hours, the speed of nuclear division must be very great. Buller concludes that the

nuclei pass the septa in the same way as Lehfeldt describes for T. erythropus, by the formation of half walls, but "this supposition remains to be verified or refuted by further observations. "

In further experiments on the direction taken by the diploidising nuclei in passing through a haploid mycelium Buller shows that the nuclei pass much more quickly through the peripheral hyphae than through the older central part of the haploid mycelium.

In Vol. IV, pp 237-241, Buller gives an account of his observations on the conversion of haploid into diploid hyphae, illustrated by three drawings. The younger branches of a leading hypha were observed to be haploid, no clamps were present, while the older branches were diploid as was shown by the presence of clamp connections. The pregressive diploidisation of the younger branches was observed. No septa are shown on these younger branches until the clamp connections are formed but Buller does not state whether the branches were actually non-septate before diploidisation. This is rather an important point since in Vol. V pl67. Buller states :- "When watching haploid hyphae of Coprinus lagopus being diploidised I never once observed a bulging of hyphae at a septum which had to be passed by a nucleus, nor did I see any septa

disappear"(1).

In this case the absence of plain septa in the drawings is difficult to understand. If they were actually present and their part in the diploidisation was observed then there may be justification for Buller's further remark (Vol V p. 167) :- "In my Volume IV in discussing the diploidisation process, I accepted Lehfelddt's conclusions; but now, as a result of my own observations upon, and the study of the literature upon, the structure of septa in the higher fungi I ^{feel} ~~fell~~ that Lehfelddt's work needs verification before it can be accepted." On the other hand, if the drawing is correct then there is obviously no evidence either for or against Lehfelddt's conclusions.

It has been shown that, in Typhula Trifolii, the septa may be partially broken down to form half walls such as Lehfelddt described and in some cases such as that which was observed in the living condition, septa were completely broken down by the passage of the nucleus. Bulging of the side walls

(1) At this point the reference is given to Lehfelddt in Hedwigia pp. 237-241. I am informed by Prof. Buller that this is an error and that the Reference should be to Vol. IV of his own researches pp. 237-241.

was also observed. Therefore while Buller did not observe such phenomena in Coprinus, this does not detract in any way from the value of Lehfeldt's work with Typhula since he did not claim that the two fungi behaved in exactly the same manner. In figure 135 of Vol. 1V Buller gives a camera-lucida drawing of diploid hyphae mingling with haploid hyphae, and in several places, anastomosis has occurred. Although this drawing was apparently made under the same conditions as Figure 133, septa are shown on all the hyphae, plain septa occurring not only on the haploid hyphae but on the diploid. Although several anastomoses have occurred and the strains are compatible, or to use Buller's term "legitimate", the haploid hyphae are still without clamp connections. It is possible, however, that the anastomoses are too recent to allow of the formation of clamps.

In the plates at the end of Vol. 1V. Buller represents diagrammatically the mode of union, in Coprinus, of mycelia which are involved in diploidisation. In the first two figures anastomosis is shown between the apical cells of two haploid hyphae; the nucleus of one cell has moved over to the other cell with whose nucleus it becomes conjugate and divides to form the first clamp connection.

Lehfeldt found that this method of fusion

with immediate formation of the clamp connection was typical of Corticium but not of Typhula.

In plates 111 and 1V a haploid hypha is shown in process of diploidisation by a diploid hypha. In this case the apical cell of a diploid branch fuses with the third cell from the apex of the main haploid hypha. One of the conjugate pair of nuclei in the diploid cell divides and a daughter nucleus passes over to the haploid cell with whose nucleus it is compatible. The invading nucleus again divides and a daughter nucleus migrates into the next cell. Two septa are thus passed in turn after which the apical branch of a haploid branch is reached on which the first clamp is formed. This is the origin of the diploid mycelium. Invading daughter nuclei move both towards the base and apex of the haploid hyphae. The movement is very well regulated, there never being more nuclei in a cell than can be accounted for, and, on the other hand, no empty cells occur. In Typhula erythropus and T. Trifolii conditions are not ~~so~~ at all similar to those illustrated as occurring in Coprinus. In them it has been shown that there is a period of disturbed equilibrium during which some cells may contain as many as five nuclei while others are empty. Further, the first clamp connection is only rarely formed as close to the point of

anastomosis as is figured in Coprinus.

In giving the results of a mating experiment in Vol IV pp 221, Buller makes the following statement "It doubtless took several hours for the two haploid mycelia to meet and fuse, but by the end of 42 hours after the^(ab) inoculum had been added to the plate, clamp connections were observed at the periphery of the (AB) mycelium proceeding away from the (ab) inoculum to a distance of 6 cm. on one side and 5.3 cm on the other side. " It is difficult to understand from this, when and where clamp connections were first observed. If the diploidisation proceeded according to the diagrams discussed above(pl. 1 to IV in Vol IV) then the first clamp connection would certainly ~~not~~ have been formed before 42 hours had elapsed and it would not have been separated from the inoculum by a distance of several centimetres.

Buller appears to consider, however, that the invading nucleus does migrate for a relatively great distance before the formation of the clamp connections, as has been already discussed under the question of the septation of the mycelium p 89 . Again in Vol V. pl66 the statement is made "If Lehfeldt's observations were correctly made,... the diploidisation of an (AB) mycelium of Coprinus lagopus would be as follows. An (ab) nucleus, on approaching a septum in an (AB) hypha, in some way causes the septum to break down partially or causes the (AB) hypha to bulge and so leave a space between the septum and

the cylindrical wall; a passage-way having thus been made, the (ab) nucleus passes from one cell to the other; and this mode of making and using a passage-way is employed in respect to each of a long series of septa which must be passed through or passed by as the (ab) nucleus moves along (AB) hypha several centimetres in length". Thus Buller conveys an impression of a nucleus which migrates from one hypha to another through which it moves rapidly for a distance of several centimetres when it suddenly begins to produce clamp connections on the leading hyphae, while in the plates I to IV the nucleus divides before passing each septum of which only two are actually passed before the first clamp connection is formed.

Lehfeldt, in considering that Coprinus probably behaved similarly to Typhula did not infer either that the clamps were formed so close to the point of anastomosis as Buller figures in Plates I to IV or, on the other hand, at a distance of "several centimetres" as is stated in the above quotation. Lehfeldt's contention was that the invading nucleus migrated from the point of anastomosis to parts of the mycelium suitable for the formation of conjugate nuclei and clamp connections. In most cases such parts were found to be the apical cells of hyphal branches, (p.48) :- "so dass die Annahme,

die jüngeren Zellen seien besser zur Aufnahme des Schnallenzustandes geeignet als die alten Myzelteile, begründet erscheint", Thus the first clamp connections appear after the nuclei have moved from the point of anastomosis to the young cells of the same hypha; this distance may only be equal to the length of one or two cells but it is highly improbable that it ever equals several centimetres.

After stating that Lehfeldt's work needs verification before it can be accepted Buller offers an alternative suggestion as regards the mode of passage of nuclei through mycelia in the process of diploidisation (p. 167 Vol V) :- "I now wish to offer the suggestion that, during the diploidisation process in the Hymenomycetes, the open pores are actually used for the passage of nuclei from one cell to another". If such open pores are present in the hyphae of these fungi it seems very reasonable to suppose that the nuclei could pass through them but it has been shown in Typhula erythropus and in Typhula Trifolii that the partial or complete dissolution of the septa also occurs during the diploidisation process.

Until further more detailed information is available regarding Coprinus there seems no reason to suppose that Lehfeldt was wrong, either in his conclusions from his work on Typhula, or in

suggesting that Coprinus behaved similarly. Further analysis of Buller's work might be made but the above indicates sufficiently clearly the complexity of the subject. The writer concurs with the opinion expressed by Lehfeldt that the observation of hyphae in Petri dish cultures is quite unsatisfactory in the investigation of these fungi. Stained preparations are absolutely essential for the exact determination of the position of the nuclei. Even when nuclei are visible in living hyphae only one part of the mycelium can be watched continuously, so that other parts which may affect that under observation must be ignored. Only after fixing and staining can the hyphae be thoroughly examined.

In conclusion, this work on Typhula Trifolii has confirmed, in at least the most important points, Lehfeldt's work with Typhula erythropus.

Summary.

Typhula Trifolii was obtained from Poland in the form of sclerotia which had been found in samples of Clover seed. Both generic and specific criteria have been found insufficient in identifying this, and allied fungi. pp. 45-46.

Using the sclerotia as inocula, the fungus has been grown in pure culture. Haploid and diploid mycelia are only distinguishable by cytological characters, macroscopically the mycelia are alike. The haploid mycelium has plain septa and usually one nucleus in each cell, the diploid mycelium has clamp connections at most septa and usually one pair of conjugate nuclei in each cell. Anastomosis and movement of nuclei between the two cells thus connected occurs in both haploid and diploid mycelia even where there is no question of heterothallism. pp. 12-26.

Monospore cultures usually produce neither sclerotia or fructifications but in some these structures are formed which are therefore made up of haploid hyphae. Haploid sclerotia are smaller than diploids but otherwise very similar. Haploid fructifications are also smaller than diploids but

while haploids are always fertile in artificial culture, diploids are sterile. Spores borne on haploid fructifications are smaller than those on diploid fructifications. pp 38-49.

Clamp connections are usually formed according to Bensaude's first method but it is believed that some are formed according to her second method. pp 26-35.

Typhula Trifolii is heterothallic; certain compatible haploid strains have been grown together to form diploid mycelia. p.59. The cytology of anastomoses between compatible mycelia has been studied by means of a modification of the agar film technique as used by Sass. p.8.

When anastomosis occurs between two compatible haploid mycelia migration of nuclei occurs, followed by the upsetting of the equilibrium of the cells of invaded hyphae. Migrating nuclei appear to be in an abnormal physical state since they may be seen unstained in living hyphae. p. 72-78. Some indigenous nuclei in invaded cells disintegrate.

Nuclei migrate by means of the partial or complete dissolution of septa. In the former case "half walls" are formed, sometimes the side wall at such a partially dissolved septum is bulged out apparently to increase the aperture through which the nucleus passes. When the invading nuclei have

reached the younger parts of the hyphae, which may be quite close to the point of anastomosis or several cells distant, they conjugate with indigenous nuclei and the first clamp connections appear. Anastomosis between diploid and haploid hyphae has much the same result in causing migration of nuclei from the former to the latter so that it also becomes diploid.

Thus the work of Lehfeldt on Typhula erythropus has been confirmed. A discussion is given on the significance of this confirmation with regard to recent work on the method of diploidisation in Coprinus.

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PLATE 1.

PLATE 1.

- Fig. 1. Typical fructification of Typhula Trifolii $\times 3$
- Fig. 2. Sclerotia compared with seeds of Red Clover, and
seeds of Brassica sp.
Sclerotia at S.
Clover seed at C.
Brassica seed at B.
- Fig. 3. Fructifications obtained by germinating
sclerotia on damp blotting paper.
- Fig. 4. Dissection of sclerotia. "Hilum" at H.
-

Plate 1.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

PLATE 2.

Plate. 2.

- Fig. 1. Malt agar culture with submerged diploid mycelium , diploid sclerotia and fructification. Mycelium at Sm. Sclerotia at Sc. Fructification at F. This fructification produced a few spores.
- Fig. 2. The same culture as in Fig. 1, at a later stage. Fructification has forked.
- Fig. 3. Typical culture on malt agar. The fluffy and granular forms of diploid mycelium are shown, also sclerotia in various stages of formation. Fluffy form of superficial diploid hyphae at F. Granular form " " " " at G.
- Fig. 4. Culture containing the Brown Crust form of diploid superficial mycelium at B.
- Fig. 5. Culture showing the occurrence of sclerotia.
-

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

PLATE 3.

Plate. 3.

Fig. 1. Upper tube, culture grown at 25°C.

Lower tube, culture grown at approx. 13°C

Fig. 2. Upper tube, culture grown at room temperature,
approx. 18°C.

Lower tube, culture grown at 20°C.

Fig. 3. Culture on soil extract agar with very long,
thin fructifications.

Fig. 1.

Fig. 2.

Fig. 3.

PLATE 4.

Plate. 4.

Fig. 1. Fluffy form of superficial diploid mycelium containing little knots of hyphae which may be classed as "granular".

Fig. 2. The same culture at a later stage, the knots of granular hyphae are more prominent.

Fig. 1.

Fig. 2.

PLATE 5.

Plate 5.

- Fig. 1. Photomicrograph showing the type of branching in submerged mycelium, either haploid or diploid.
- Fig. 2. Photomicrograph showing the type of branching in superficial mycelia, either haploid or diploid.
- Fig. 3. Photomicrograph showing oidia borne on haploid submerged mycelium.

Plate 5.

Fig. 2.

Fig. 1.

Fig. 3.

PLATE 6.

Plate 6.

Fig. 1 Colonies of haploid mycelia, strains S and K,
S has produced fructifications whose spore
deposit is seen on the left.

Fig. 2. Colonies of the haploid mycelium strain F,
and the diploid mycelium FG.
F is on the left, and FG on the right.

Plate 6.

Fig. 1.

Fig. 2.

PLATE 7.

Plate 7.

- Fig. 1. Sterile fructifications formed in sand in which the fungus has been growing for some time.
- Fig. 2. The three fructifications on the right in Fig. 1, growth has been renewed and the middle fructification is becoming fertile.
- Fig. 3. The two fructifications on the right in Fig. 2 after a few days. Both have now developed fertile hymenia.
-

Fig. 1.

Fig. 2.

Fig. 3.

PLATE 8.

Plate 8.

- Figs 1 & 2. Young diploid fructifications growing in soil.
- Fig. 3. Branched fructifications from oat agar culture. These fructifications are sterile.
- Fig. 4. This fructification was growing in sand which was so dry as to cause it to wither up. The sand was then watered and another fructification is appearing on the stalk of the withered specimen.
-

Plate 8.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

PLATE 9.

Plate 9.

- Fig. 1. Fertile fructification growing in sand.
The hairs which are typically borne at the base of the stalk are here a little way above that point. The hymenium is not club shaped as in the typical fructification.

Fig. 1.

PLATE 10.

Plate 10.

Figs. 1& 2. Pots of Rhododendron sp. in which an unidentified fungus is growing.

Fig. 3. A few specimens of the fructifications magnified approx. 3 times.

Fig. 1.

Fig. 2.

Fig. 3.

PLATE 11.

Plate 11.

Figs. 1 & 2. Specimens of a fungus, as yet unidentified,
which occurred in pots of Boronia sp.

Fig. 1.

Fig. 2.

PLATE 12.

Plate 12.

Figs. 1 & 2. Specimens of fungi occurring in pots
of Rhododendron sp. Magnified approx.
3 times.

Fig. 3. Pistillaria quisquilaris on Braken.
Magnified $1\frac{1}{2}$ times.

Plate 12.

Fig. 1.

Fig. 3.

Fig. 2.

PLATE 13.

Plate 13.

Agar film technique, in each photograph the lid of the Petri dish has been removed.

- Fig. 1. Rectangle or "Box" which carries the slide is made of strips of glass.
- Fig. 2. "Box" is made of brass and the sides are recessed so that the slide rests on the end pieces only.
- Fig. 3. Side view of "box" showing an agar film with haploid fructifications. The agar film has been inverted for photographic purposes.
-

Fig. 1.

Fig. 2.

Fig. 3.

PLATE 14.

Plate 14.

Haploid fructifications.

- Fig. 1. Haploid fructification growing in sand. The small swelling at the top is the hymenium. Magnified 3 times.
- Fig. 2. Abnormally large fructification which is probably composed of several individuals which have coalesced. The hymenium is bilobed. This specimen is growing in culture medium and the small white patches are colonies derived from the spores deposited by the fructification shown.
- Fig. 3. Photomicrograph showing a fructification which has been laid down on an agar film. The cut end of the stalk has given rise to normal haploid hyphae while on the left side of the hymenium are shown the very thin germ tubes. *C.*
- Fig. 4. Photomicrograph of the apex of a haploid fructification. The top of the stalk, with hairs, is shown, also the hymenium.
-

Plate 14.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

PLATE 15.

PLATE 15.

Cultures of haploid mycelia.

Figs 1 & 3. The fluffy form of superficial mycelium.

Fig. 2. The brown crust-like form of superficial mycelium.

Fig. 1.

Fig. 2.

Fig. 3.

PLATE 16.

Plate 16.

- Fig. 1. Monospore culture of the strain "S", haploid sclerotia are shown.
- Fig. 2. Culture obtained from spores borne on haploid fructification, this culture therefore represents the "second generation". Haploid fructifications are seen at the top of the agar slope, and their spore deposits are seen as white streaks on the lower part of the slope.
- Fig. 3.. Cultures inoculated at the same time, that on the left with cells from a haploid sclerotium and that on the right with a similar inoculum from a diploid sclerotium.
- Fig. 4. Sclerotia from culture, on the left haploid sclerotia, and on the right, diploid sclerotia.
-

Plate 16.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

PLATE 17.

Plate 17.

- Fig. 1. Part of a haploid culture producing abundant fructifications.
- Fig. 2. Haploid culture showing submerged mycelium at S, and the fluffy form of superficial mycelium in the centre of the colony. The fructifications are arising directly from the substrate but they have a small brown patch of hyphae at the base of the stalk.
- Fig. 3. Typical haploid fructification as it is formed in culture.

Fig. 1.

Fig. 2.

Fig. 3.

PLATE 18.

Plate 18.

Heterothallism.

- Fig. 1. Culture of strain F.)
Fig. 2. Culture of strain G.) Haploid.
- Fig. 3. These two strains growing together and forming diploid fluffy superficial mycelium at C, clamp connections are found on these hyphae.
- Fig. 4. Later stage of a culture similar to that in Fig. 3. Sclerotia are forming on the diploid mycelium at the point of contact. Sclerotia at S.
- Fig. 5. Culture of F, which was inoculated with hyphae of G at the point Fr, where fructifications are now appearing.
- Fig. 6. Typical diploid culture of FG hyphae, obtained by mating F and G as shown in Figs. 1 to 5.
-

Fig. 2.

Fig. 3.

Fig. 1.

Fig. 4.

Fig. 5.

Fig. 6.

PLATE 19.

Plate 19.

Fig. 1. Small colony of G strain and the edge of a large colony of F strain, the colonies are of equal age.

Fig. 2. Small part of the colonies shown in Fig. 1 at a later stage. Clamp connections were formed on the hyphae at C.

Plate 19.

Fig. 1.

Fig. 2.

Plates 20 to 38.

The descriptive letters and numbers in these drawings are fully explained in the text. It has been necessary to photograph some of the drawings as they were too large to be mounted in the original size.

Plate 20.

Plate 20.

Figs. 1 & 2. Anastomoses between hyphal branches
 of the same haploid mycelium,
 followed by nuclear migration.
 p. 16.

Plate 20.

Fig. 1.

Fig. 2.

Plate 21.

Plate 21.

Figs. 1 - 3. Mitosis in haploid cells.

Fig. 4. Fusion of conjugate nuclei in the
young basidium.

Figs. 5 - 6. Meiosis in the young basidium.

Fig. 1.

Plate 21.

Fig. 2.

Fig. 3.

Fig. 5.

Fig. 4.

Fig. 6.

Plate 22.

Plate 22.

Hyphae from the diploid mycelium ; a cell with
four nuclei is present. Referred to on p. 20
of the text.

Plate 23.

Plate 23.

- Fig. 1. Anastomosis between two diploid cells.
 Four nuclei are present in the fusion cell.
- Fig. 2. As Fig. 1.
 p. 21 of text.
-

Fig. 1.

Fig. 2.

Plate 24.

Plate 24.

Fig. 1. Anastomosis between diploid cells,
indigenous nuclei disintegrating.

Fig. 2. Similar anastomosis, indigenous nuclei
, almost completely disappeared.

p. 21 of text.

Plate 24.

Fig. 1.

Fig. 2.

Plate 25.

Plate 25.

Figs. 1 - 3. Stages in the formation of the first type of clamp connection.

Fig. 4. Young branches which tend to separate the conjugate pair of nuclei.
p. 23 of text.

Fig. 5. Apical forking before completion of clamp connection.
p. 30 of text.

Fig. 3.

Fig. 1.

Fig. 2.

Fig. 4.

Fig. 5.

Plate 26.

Plate 26.

Figs. 1 & 2. Clamp connections of the second
type. p. 27 of the text.

Fig. 3. Clamp connection in which the
septum of the hook is missing.
p. 34 of the text.

Fig. 4. Diploid cell in which one of the
nuclei appears to be dividing.
p. 15 of the text.

Fig. 1.

Fig. 3.

Fig. 4.

Plate 27.

Plate 27.

Abnormal form of clamp connection, p 28
of the text.

Plate 28.

Plate 28.

Anastomosis between two young haploid hyphae,
fusion at F, between the apical cell of
one hypha and the subterminal of the other.

See p. 68 of the text.

Plate 29.

Plate 29.

Anastomosis between young haploid hyphae showing
an empty cell and a half wall. Nuclei very
irregular. p. 68 of the text.

Plate 30.

Plate 30.

Anastomosis between hyphae of the strains F and G.

Nuclēi irregular in F as result of anastomosis,
and a half wall has been formed. p 70 of the
text.

3.

Plate 31.

Plate 31.

Haploid hypha of strain K (vertical) has fused at
F1 and F2 with hyphae of the strain S. Apical
cells of K have become diploid.

p. 71 of the text.

Plate 31.

Plate 32.

Plate 32.

Stages of the anastomosis between hyphae of
the strains F and G which was watched
in the living state.

p. 73 of the text.

Plate 33.

Plate 33.

Figs 1 & 2. Photomicrographs of the hyphae showing the "bright spots", broken septum at ^PSl and the rudimentary branch at B. Fig 1 is a mirror image of Fig 2 owing to the agar film having been inverted. Fig. 2 was taken approximately 10 minutes after Fig 1 and it will be noticed that the spots have changed their position during that time.

p. 73 of the text.

Figs. 3 & 4. Photomicrographs of hyphae showing "bright spots" before and after staining. Spots at B. p. 76 of the text.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

.Plates 34 and 35.

Plates 34 and 35.

Further stages in the anastomosis between
hyphae of F and G. as in plates 33 and 32.
pp. 74 - 76 of the text.

Plate 36.

Plate 36.

Anastomosis, at Fl, between apical cells of F and G
hyphae, resulting in the formation of half
walls and clampconnections.

p.p 78 - 80 of the text.

Plate 36.

Plate 37.

Plate 37.

Anastomosis between a diploid and a haploid
hypha, nuclear migration has resulted.
p.79 of the text.

Plate 38.

Plate 38.

Anastomosis between a haploid and a diploid hypha.

Nuclear migration has followed.

p. 80 of the text.

1.VIII.35