THE OIDIA OF COPRINUS LAGOPUS

And THEIR RELATION WITH INSECTS.

By

HAROLD J. BRODIE, B. Sc. (MANITOBA)

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University of Manitoba,

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I. INTRODUCTION.

The researches of Eidam, van Tieghem, Brefeld, Zopf, and others have taught us that, in many Hymenomycetes and Ascomycetes and in certain Pyrenomycetes and Discomycetes, the young mycelium developed from a basidiospore or from an ascospore produces or breaks up into a series of short segments which Brefeld called oidia. Brefeld[1] in his Untersuchungen illustrated the oidia of the following Hymenomycetes:

Coprinus lagopus	Hypholoma fasciculare
Galera tenera	Pleurotus ostreatus
Panæolus campanulatus	Collybia velutipes
Stropharia semiglobata	C. maculata
S. melasperma	C. conigena
Schizophyllum lobatum	Phòliota marginata
Lenzites abietinus	Psathyra spadiceo-grisea
Daedalea unicolor	P. nolitangere
Trametes odorata	Psilocybe spadicea
Polyporus suaveolens	P. semilanceata
P. serialis	Clitocybe metachroa
P. zonatus	Nyctalis asterophora
P. versicolor	N. parasitica
P. quercinus	Naucoria semiorbicularis
Phlebia radiata	Typhula variabilis
Irpex obliquus	Radulum laetum

Brefeld's illustrations represent the oidia as short hyaline rod-shaped cells with a thin cell-wall and dense protoplasmic contents.

In the nineteenth century, when mycologists were searching for sexual organs in the higher fungi, it was believed by

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1. O. Brefeld, Botanische Untersuchungen über Schimmelpilze, Heft III and Heft VIII, Leipzig, 1877.

Eidam[1] and van Tieghem[2] that oidia are male cells or spermatia, even although female organs could not be found. Van Tieghem[3], in 1875, succeeded in germinating the oidia of Coprinus plicatilis and C. stercorarius[4], and he then declared that the oidia serve to reproduce the fungus in an asexual manner, in this respect being comparable to conidia.

Brefeld[5], in 1877, announced that he had been unable to observe germination of the oidia of Coprinus lagopus, although he had made numerous attempts to do so. He came to the conclusion that oidia are vestigial structures which no longer possess the power of germinating and which therefore cannot be regarded as functional spermatia. His observations that the fruit-bodies of certain Coprini owe their origin to the development of a single hypha of the mycelium proved that the co-operation of the oidia in the formation of fruit-bodies is unnecessary.

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1. E. Eidam, Zur Kenntnis der Befruchtung bei den Agaricus-Arten, Bot. Zeit., Bd. 33, pp.649-653, 665-670, 1875.
2. Van Tieghem, Sur la fécondation des Basidiomycètes. C.R. des Sciences, T. 80, p.573, 1875.
3. Van Tieghem, Sur le développement du fruit et sur la prétendue sexualité des Basidiomycètes, C.R. des Sciences, T. 81, p. 877, 1875.
4. Doubtless van Tieghem mis-identified these species, as the true C. stercorarius which develops sclerotia does not produce any oidia.
5. O. Brefeld, loc. cit., Heft III, p. 102, 1877.

Richard Falck[1], in 1902, described and illustrated the oidia of Mucor racemosus, Dacryomyces deliquescens, Ascobolus lignatilis, Phlebia merismoides, Agaricus coprophilus, Chalymotta campanulata, Coprinus ephemerus, Hypholoma fasciculare, Collybia velutipes, C. tuberosa and Oidium lactis.

Falck succeeded in germinating the oidia of the wood-destroying fungi Phlebia merismoides, Hypholoma fasciculare, and Collybia velutipes. Then, using a single oidium to inoculate his culture medium, with each of these species he succeeded in obtaining perfect fruit-bodies. We now know that, in general, oidia are produced only on haploid mycelia and not on diploid. It is therefore possible that Falck's fruit-bodies were all haploid and that the spores produced by each of them were of one and the same sex.

In 1909 Falck[2] showed that the oidia of Lenzites sepiaria would still germinate after they had been kept a year under dry conditions.

So far as coprophilous Hymenomycetes are concerned, Falck did not succeed in germinating the oidia of Coprinus lakopus, Panaeolus campanulatus and Agaricus coprophilus; and he discovered that Coprinus sterquilinus, like Brefeld's C. stercorarius fails to produce any oidia whatsoever. Falck[3] came to the conclusion that

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1. R. Falck, Die Cultur der Oidien und Ihre Rückführung in der höhere Fruchtform bei den Basidiomyceten, Beiträge zur Biologie der Pflanzen, Breslau, 1902.
2. R. Falck, Die Lenzites-Fäule des Coniferholzes, Hausschammforschungen; T. 3, published by Müller, Jena, 1909.
3. R. Falck, loc. cit. in note 1 above, p.312.

the oidia of most of the coprophilous Basidiomycetes have lost the ability to germinate and therefore have nothing to do with the dissemination of these fungi.

In 1918, Mlle Bensaude[1] in her well-known paper on the life-history and sexual phenomena of Coprinus fimetarius[2] incidentally described and illustrated the structure and mode of production of the oidia. She saw some of the oidia germinate and produce short germ-tubes which soon fused with cells of the parent mycelium.

Mlle Bensaude planted two mycelia of opposite sex, A and B, near to one another on nutrient agar, and she observed that occasionally one of the mycelia became diploid before it had come into contact with the other one.

Supposing that the mycelium which became diploid was A, she explained the transformation as follows: oidia from the mycelium B floated across the gap between A and B in the surface film of water covering the agar, and thus reached A; and thus the mycelium A was converted by the oidia from the haploid into the diploid phase.

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1. Mathilde Bensaude, Recherches sur le cycle évolutif et la sexualité chez les Basidiomycètes, Nemours, 1918.

2. Professor Buller has informed me that after personal consultation with Mlle Bensaude he came to the conclusion that her C. fimetarius and the C. lagopus described in his Researches on Fungi (Vol. III) are identical species.

In 1927, J. H. Craigie[1], acting on a suggestion given to him by Professor A. H. R. Buller, discovered that the pycnospores of the Rust Fungi which are produced by haploid mycelia are functional. When (+) pycnospores are carried from a (+) pustule (in the laboratory by hand or under natural conditions by insects) to a (-) pycnium in a (-) pustule, the mycelium in the (-) pustule becomes diploid and produces diploid aecia and aeciospores; and conversely, when (-) pycnospores are carried from a (-) pustule to a (+) pycnium in a (+) pustule, the mycelium in the (+) pustule becomes diploid and produces diploid aecia and aeciospores.

When Craigie's investigations on the Rust Fungi were in progress, Professor Buller conceived the idea that the oidia of the Hymenomycetes might function in a similar way to the pycnospores of the Rust Fungi, i.e. that (+) oidia might be carried, in the laboratory by hand or under natural conditions by insects, from a (+) mycelium to a (-) mycelium where they might germinate and fuse with the (-) mycelium thus converting it into a diploid mycelium; and conversely, that (-) oidia might be carried from a (-) mycelium to a (+) mycelium where they might germinate and fuse with the (+) mycelium and thus convert it into a diploid mycelium.

Since oidia are produced on the haploid mycelia of so many Hymenomycetes, it is obviously of considerable importance to elucidate by experimental means exactly what the function of the oidia is. The problem of the function of the oidia was suggested by Professor Buller to the writer and its solution is offered in the following pages.

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1. J. H. Craigie, Discovery of the Function of the Pycnia of the Rust Fungi, Nature, Nov. 26, 1927.

II. MATERIAL AND METHODS.

The fungus chosen as material for this investigation was Coprinus lagopus. This well-known species is readily obtained on horse--dung cultures and its life-history has been worked out by Brefeld[1], Mlle Bensaude[2], Buller[3], Hanna[4], Miss Mounce[5], Miss Dorothy Newton[6], Oort[7] and others.

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1. O. Brefeld, loc. cit.
2. M. Bensaude, loc. cit.
3. A.H.R.Buller, "Researches on Fungi", Vol. III, 1924; also The Mating Method of Identification of a Coprinus Growing on Germinating Seeds of Mangel and Sugar--beet, Annals of Botany, Vol.XLI, pp. 663-670, 1927.
4. W.F.Hanna, The Problem of Sex in Coprinus lagopus, Annals of Botany, Vol. XXXIX, pp. 431-457, 1925; Sexual Stability in Monosporous Mycelia of Coprinus lagopus, Annals of Botany, Vol. XLII, pp. 379-389, 1928.
5. Irene Mounce, Homothallism and Heterothallism in the Genus Coprinus, Trans. Brit. Myc. Soc., Vol. VIII, Part IV, pp. 256-269, 1922.
6. D.E.Newton, The Distribution of Spores of Diverse Sex on the Hymenium of Coprinus lagopus, Annals of Botany, Vol. XL, pp. 891-917, 1926.
7. A.J.P.Oort, The Sexuality of Coprinus fimetarius, Proceedings of The Koniklijke Academie van Wetenschappen Te Amsterdam, Vol. XXXII, pp. 1-6, 1929.

Spores of Coprinus lagopus were obtained in the following manner: fresh horse dung was procured from a stable in Winnipeg, and was placed in a large crystallizing dish covered with a glass plate. The dish was set upon the laboratory table where it was exposed to light. In about ten days, fruit-bodies began to appear upon the culture and these were identified by Professor Buller as Corinus lagopus. Spores from one of the pilei were allowed to fall on a sterilized glass silde. From this spore-deposit single spores were removed by the dry-needle method described by Hanna[1], and they were sowed in hanging-drops of cleared dung-agar. In this way a series of monosporous mycelia was obtained.

The medium used for cultivating the fungus was dung-agar which was prepared as follows: a litre of water was added to 200 grams of fresh horse dung and this was boiled in an enamel dish for fifteen minutes. The decoction was then filtered once through cheese-cloth and once through cotton-wool, after which 12 grams of agar were added to the filtrate. The mixture was heated in an Arnold sterilizer for one hour to melt the agar, then tubed and finally sterilized in an autoclave for one hour at 15 pounds pressure.

To clarify the medium and thus make it of more use for observation of the mycelium under the microscope, egg-white was employed.

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1. W.F.Hanna, The Dry-Needle Method of Making Monosporous Cultures of Hymenomycetes and other Fungi, Annals of Botany, Vol. XXXVIII, pp. 791-794, 1924.

The whites of four eggs, after being added to 50 cc. of water, were slightly beaten and then poured into the dung-agar decoction. This mixture was heated for one hour in flowing steam, filtered through cotton-wool, and tubed.

Malt-agar was occasionally used as a clear medium. It was prepared by boiling in 25 grams of ground malt in one litre of water and then adding 12 grams of agar, after which it was filtered and tubed as in the preparation of dung-agar.

The mycelium of Coprinus lagopus grows as well upon malt-agar as upon dung-agar.

Each monosporous mycelium after growing for a few days in a hanging-drop of dung-agar was transferred to a Petri dish containing a layer of sterile dung-agar about 2 mm. thick.

To keep mycelia, stock cultures were made by transferring the mycelia to round-bottom glass tubes 3 inches long and 0.9 inch in diameter which had been filled about one-third with fresh horse dung, plugged with cotton-wool, and sterilized in steam at 15 pounds pressure for one hour.

In order to sort out the ten monosporous mycelia into the well-known four sexual groups (AB), (ab), (Ab), (aB), they were mated in all possible combinations and the results recorded. The monosporous mycelia were given numbers from 1 to 10 inclusive.

In the experimental work described in the following pages, the mycelia most employed were those numbered 5 and 10. These were sexually opposite. To mycelium No. 5 was given the symbol (ab) and to mycelium No. 10 the symbol (AB).

III THE STRUCTURE AND DEVELOPMENT OF THE OIDIAL FRUCTIFICATIONS.

From two to three days after the germination of a single spore of Coprinus lagopus in a culture medium, the haploid mycelium which is developed begins to produce oidia on special lateral branches called oidiophores.

On dung-agar plates not covered with a film of water or on horse dung the oidiophores grow more or less perpendicularly outwards from the substratum into the air and there produce their oidia in terminal masses. On the other hand when, as in a hanging-drop of dung-agar in a van-Tieghem cell, the culture medium is covered with a film or thin layer of water, the oidiophores are not able to push out from the medium into the air but instead develop and produce oidia in the film of water. When oidiophores develop in a film of water, the mycelium, the oidiophores and the oidia all lie in one plane, and the stages in the development of the oidiophores can then be readily followed with the microscope.

An oidiophore which develops in a film of water in the manner described above, arises as a hyphal outgrowth of a short cell of the haploid mycelium and attains an average length of 0.05 mm. varying up to 0.1 mm. At its basal end it is 5-6μ thick or as thich as the hypha to which it is attached but it tapers upwards so that its apical end is only 2.5-3μ thick. A little way above its base it is divided by a septum into two cells. Occasionally the oidiophore is divided by two septa into three cells. The protoplasm in the cells of the oidiophore is distinctly vacuolate and exhibits numerous shining particles.

The pointed end of the full-grown oidiophore gives rise to short branches which may be called <u>oidial hyphae</u> (Fig. 2) for these soon break up into a series of 2-5 oidia. The process of oidial formation on an oidiophore which was submerged in a film of water is illustrated in Plate I, Figs. 5. At 10.30 a.m., an oidiophore with its oidial branches (Fig. 5A) was examined and sketched. After twenty minutes (10.50 a.m.) a fine line appeared about one-third of the way from the base of one of the oidial hyphae (Fig. 5B) and after forty-five minutes a clear space or gap in the protoplasm appeared where the line had been (Fig. 5C). Thus an apically-formed oidium became marked off from the basal portion of the oidial hypha. The newly-formed oidium then began to sway from one side to the other about its point of attachment to the hypha. This swaying, apparently due to Brownian movement, continued until the oidium broke loose, which it did forty minutes after the gap was first observed. A gap then appeared on another branch (Fig. 5D) and after thirty minutes, the oidium cut off above it began to sway and was then soon set free. (Fig. 5E).

The oidiophore just described grew in a film of water, i.e. somewhat abnormally: it was shorter than usual and its oidial branches produced only one oidium at a time. Normal oidiophores which project into the air are much longer and their oidial branches break up itno chains of from 2 to 5 oidia.

The formation of a chain of two or three oidia from an oidial branch is due to the division of the protoplasm into two or three separate masses, the shrinking or condensation of these protoplasmic

masses with the formation of short water-filled gaps or spaces between them and at the base of the oidial hypha, the development of a wall at each end of each shrunken mass of protoplasm and the dissolution of the wall of the oidial hypha around the water-filled spaces. The two or three oidia thus set free are so small that if liberated into water they soon separate from one another and move away from their place of origin.

After an oidiophore has ceased to produce oidia and its oidia have been dispersed by immersing the oidiophore in water, the apex of the oidiophore can be seen to be rounded off and not showing any trace of the oidial branches to which it gave rise.

A striking phenomenon in connection with the production of oidia on the end of an oidiophore which is projecting from horse dung or from dung-agar into the air is the excretion of a drop of liquid at the apex of the oidiophore in such a way that the oidia are immersed in it. Neither Brefeld nor Falck who studied the oidia of Coprinus lagopus and other Coprini appear to have noticed the drops in question; but, as we shall see, these drops are of great importance in connection with the dispersal of the oidia and their conveyance to a place where they may function to the advantage of the species to which they belong. The development of the drops on the tips of the oidiophores was observed in hanging-drops of dung-agar and will now be described.

When an oidiophore first pushes upwards from its substratum into the air, it is a simple tapering hypha with one cross-wall near its base. An hour after it has attained its full length there appears on its tip a tiny liquid drop (Fig. 3A) The drop continues to

increase in size until, at the end of ten hours, its diameter may attain 0.08 mm. (Fig.38). From the apex of the oidiophore oidial branches are sent out into the drop where they break up into oidia. In the course of several hours numerous oidia are thus formed and they can be seen with the microscope enveloped in the fluid; apparently the growth of the drop is proportional to the number of oidia that are produced within it. Under normal conditions the drop is always large enough to envelop all the oidia contained within it and at the same time to have a completely spherical surface.

Some oidial fructifications which pointed vertically upwards were examined laterally through a horizontal microscope with a magnification of about 400. It was then seen that the oidia practically filled the interior of each drop.

When oidia have been produced in the drops of oidiophores on a dung-agar plate and one removes the cover and examines the drops with the high power of the microscope, one can often observe that the oidia in the drops exhibit a more or less violent movement. In some drops the movement is typically Brownian; but in others the whole mass of oidia, in the course of a few seconds may whirl round and round and thus display an activity which to the writer was at first very unexpected. It is possible that evaporation from the surface of the drop is the cause of the whirling movement; for it was found that whirling could be started by breathing upon the drops, and that the whirling ceased as soon as the drops were no longer breathed upon.

When one looks down on the top of the drop with the high power of the microscope, an enlarged image of some of the oidia appears above the upper surface of the drop owing to the sphere acting as a converging lens for the light coming from the oidia.

Since the drops on the oidiophores are only about 0.05 mm. in diameter, it has not been possible to analyse them chemically, but some simple observations seem to show that the fluid of which they are composed is not pure water but contains colloid matter. When a cover-glass is lowered gently so that it just touches the liquid drops of numerous oidiophores projecting above the surface of dung-agar on which the parent mycelium is growing and is then lifted up and examined with the microscope, one finds that the drops with the oidia have come away intact, have dried up, and are attached to the cover-glass as flat circular masses of oidia and dried-up oidiophore fluid. Two days after the drops were collected and dried on a cover-glass it was found that they could not be dissolved either in water or alcohol.

The oidia in individual drops obtained on a cover-glass were counted. Large drops contain eighty or more oidia and very small drops five or ten oidia. Drops of average size with a diameter of 0.05 mm. contain about twenty oidia.

The very large oidiophore drops may be formed by the fusion of several smaller drops. A continuation of this process of fusion may result in almost the entire surface of a haploid mycelium growing on an agar plate becoming covered with closely-packed oidia.

A photograph showing the appearance of oidial fructifications when seen with a magnification of 100 is reproduced in Fig. 14.

When the drop on the oidiophore is allowed to dry, it shrivels and assumes a rough appearance owing to the fact that the ends of the oidia project as shown in Fig. 3C.

The individual oidia of Coprinus lagopus are 1μ in diameter and may be from 2μ to 10μ long. The average length is about 5μ. Occasionally an oidium is produced which has a ~~sied~~ side branch as shown in Fig. 7A. Two refractive granules are usually to be seen in the protoplasm within each oidium.

Mlle Bensaude[1] stained the oidia of Coprinus fimetarius and illustrated the stained oidia in her paper on Plate 2, Figs. 3 and 4, and on Plate 4-5, Fig. 4. In these illustrations one nucleus can be seen in each oidium and some of the nuclei can be seen undergoing division.

IV THE CONDITIONS UNDER WHICH OIDIAL FRUCTIFICATIONS ARE PRODUCED.

The oidia of Coprinus lagopus are produced by haploid mycelium only, never by diploid. It is true that a haploid mycelium which has been converted into a diploid mycelium may still have attached to it oidia which were produced when the mycelium was haploid, but with the diploid phase the mycelium ceases to produce oidia. The same is true

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1. M. Bensaude, loc. cit.

for Coprinus niveus and C. curtus, two other heterothallic species of Coprinus which have been examined by the writer.

Brefeld[1] showed that Coprinus stercorarius, now known to be a homothallic species, never produces oidia; and Falck[2] demonstrated that C. sterquilinus was also entirely without oidia.

The writer kept monosporous mycelia of Coprinus stercorarius under observation from the time of germination of the spore for several days. Three days after the germination of the spore, the mycelium which developed from it became diploid. No oidia whatsoever were produced.

It is a rather remarkable fact that the very young haploid mycelium of Coprinus lagopus produces oidia for a time even when it is growing in contact with mycelia of opposite sex. A large number of spores (two hundred or more) and therefore spores of all four sexual types (AB), (ab), (Ab), (aB), were sown together in a hanging-drop of cleared dung-agar. Twelve hours later, about one-fourth of these had germinated. When 2-5 days old, the young mycelia were vigorously producing oidia, each mycelium behaving as though it were isolated from the rest of the mycelia. Fusion between the mycelia of opposite sex then began to take place and in the course of twenty-four hours all of the leading hyphae in the hanging-drop developed clamp-connexions and ceased producing oidia. It may be concluded that the young spore--mycelium passes through a period of oidia production of about 48 hours duration, and that it will not unite with mycelium of opposite sex

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1. O. Brefeld, loc. cit., Bd. , p. .

2. R. Falck, Die Cultur der Oidien..etc.., loc. cit., p. 313.

until this period has been completed.

The number of oidia produced by a haploid mycelium developed from a spore on one square millimeter of dung-agar varies for different mycelia and for different parts of the same mycelium. Some mycelia produce oidia in far greater numbers than others but all haploid mycelia produce them in great numbers. In what seemed to be an average mycelium, a count showed that there were fifteen to twenty oidial fructifications per square millimeter of dung-agar surface. At this rate, on a mycelium covering a square with each side 5 cms. (2 inches) the number of oidial fructifications would be 2500. Reckoning twenty oidia to each oidiophore drop, such a square of mycelium would give rise to 50,000 oidia. It is possible that after the drops become fused together in the older part of the mycelium in the almost continuous fluid layer so produced still more oidia are developed thus greatly increasing the calculated number.

The oidial fructifications on haploid mycelia derived from spores always develop in the air and never under the surface of the medium. It therefore appears that air is necessary for their development.

If sterilized dung-balls be inoculated with haploid mycelium, the mycelium grows very vigorously, becoming fluffy owing to the production of aerial hyphae. The oidial fructifications are produced in the same way as on dung-agar and the surface of the dung-ball becomes covered with them, the oidiophores projecting away from the substratum.

An agar plate on which a mycelium was growing was exposed to the dry atmosphere of the laboratory for three days as shown in Fig. 12. At the end of that time the oidiophores were being produced by the leading hyphae in as great numbers as under the conditions of excess moisture existing in a closed Petri dish. Under the dry conditions, however, there were not as many large liquid drops on the oidiophores as were produced under more moist conditions.

Light is not essential for the production of oidia since they are produced both in the light and in the dark. Occasionally the mycelium on agar plates shows concentric rings, the oidial fructifications being produced to a greater and lesser extent alternately. Some experiments were undertaken in order to investigate the possibility of these rings being due to the alternating influence of light and dark. Mycelia on agar plates were exposed to sunlight for one hour each day for ten days and were kept in the dark the rest of the time. This treatment failed to produce the concentric rings. Further investigation is desirable to explain the phenomenon of the concentric ring growth habit.

V THE OCCURRENCE OF OIDIA IN NATURE

Although oidial fructifications are produced in the laboratory, the laboratory conditions are somewhat artificial, and it seemed desirable to show whether or not the oidial fructifications are produced under natural conditions.

A large number of fruit-bodies of Coprinus lagopus were noticed coming up on a dish of unsterilized horse dung which had been brought into the laboratory sixteen days previously. This culture was

in the hope of finding oidia. Under the low power of the microscope, many oidial fructifications were seen projecting above the dung surface, and there were many more on strands of aerial mycelium. These oidial fructifications resembled very closely those which had been studied in laboratory cultures of haploid mycelium of Coprinus lagopus. Some oidial fructifications were seen on a bit of straw which was then removed from the rest of the dung with fine forceps. The straw was then placed on a glass slide in a drop of water. Hyphae showing simple cross-walls, oidiophores and oidia were identified under the microscope and were sketched (Fig. 6).

On three other occasions oidia have been found on wild dung cultures, hance it can be stated definitely that oidial fructifications are produced in nature even in competition with other fungi, bacteria, etc. Since in the wild cultures in which the oidia were found, Coprinus lagopus fruit-bodies were more numerous than those of any other fungus; and since the structure of the oidiophores and the oidia was so like that of the oidiophores and oidia produced in pure laboratory cultures of Coprinus lagopus, it is highly probable that the oidia found in the wild cultures belonged to that fungus.

VI THE GERMINATION OF THE OIDIA

Germination of the oidia of Coprinus lagopus was first observed by the writer in hanging-drop cultures of cleared dung-agar and malt-agar. In several such cultures which were ten days or more old, oidia were observed to germinate in situ. The germinating oidia are rather different in appearance from the ungerminated ones. Oidia

about to germinate are swollen, rather moreso at the ends than in the middle, and the refrective granule at either end (see this paper, page 14) is more prominent than in ungerminated oidia. The germ-tube is about two-thirds as wide as the oidium which produces it. A germ-tube may develop from one end or from both ends of an oidium. Large vacu~~oes~~oles appear in the oidium as its protoplasmic contents are sent out into the germ-tube. Since oidia germinating ~~g~~ in situ do so on medium which is exhausted of nutriment, the germ-tubes produced by the oidia do not develop rapidly and often soon cease to grow. Germ-tubes from several oidia may anastomose when growing in the exhausted medium.

Effort was then turned to causing the oidia to germinate. Oidia from agar plate cultures of various ages were planted in a variety of media; water, sugar solution, cleared and uncleared dung-agar and 2.5 per cent. malt-agar. At first none of these efforts met with success. The oidia did not appear viable, although they had been seen to germinate in situ as described above. The effect of heat was then tried. Oidia were obtained from a thirty day old culture of a haploid mycelium by touching cover-glasses lightly to the surface of the mycelium. The cover-glasses were then placed on Van-Tieghem cells with water in the bottom of each cell. No nutrient medium was added to the oidia. The preparations were then heated under a desk lamp to 50° C., kept at that temperature for one minute and then allowed to cool. Other preparations kept as controls were not heated.

In 24 hours, the oidia which had been heated had swollen and germinated. The oidia in the control preparations which had not been

heated did not germinate. The experiment was repeated several times and the oidia always germinated when heated.

Some oidia from a three weeks old culture of haploid mycelium No. 5 were then found to germinate spontaneously. They germinated readily in hanging-drops of cleared dung-agar and malt-agar.

At present it is not possible to offer an explanation of the real cause of the germination of these oidia and the failure of oidia to germinate in previous experiments. It may well be due to the variability of the dung-agar medium. This medium is variable in that the dung from which it is made is probably never exactly of the same composition twice it succession. Even after it is made up, the dung--agar undergoes change, being light in colour when freshly made up and gradually becoming darker. This colour change shows that some chemical changes, probably including oxidation, are slowly altering the nature of the medium. The writer is confident that if the correct conditions for germination are supplied, the oidia will germinate readily enough. Numerous experiments will be discussed in the following pages in which oidia have germinated readily under natural conditions on horse dung.

The minimum time for germination of oidia from this particular culture was found to be eight hours. It was estimated that about eighty per cent. of the oidia in each hanging-drop germinated.

The appearance of the germinating oidia has been described above. When oidia germinate **in situ**, many of the germ-tubes anastomose due to the exhausted condition of the medium. In fresh medium however,

only a small proportion of the germ-tubes anastomose. Some of the germ-tubes do not grow much longer than the oidia which produced them before they begin to form new oidia as shown in Fig. 10. Many of the germ-tubes grow out of the medium into the air to become new oidiophores on which the usual oidia and mucilage drops appear (Fig. 11.). The oidiophore produced in this manner is not usually as large as the oidiophore produced on haploid mycelium derived from a spore. However, after the oidial mycelium has become well established and is growing vigorously, it produces oidiophores which are quite as large as those developed on spore mycelium.

The oidia of the second generation are identical in sex and appearance to the oidia from which they were produced. Twelve generations of oidia were grown and studied. The oidia for the first cultures were obtained by affixing a drop of cleared dung-agar to a cover-glass and then lightly touching the agar drop to the surface of a haploid mycelium growing on a dung-agar. The cover-glass with its drop was then placed on a van-Tieghem cell in the bottom of which there was a drop of water. The oidia germinated and produced a mycelium which in its turn produced oidial fructifications. The oidia from these fructifications, that is the oidia of the second generation were isolated by allowing them to touch, very lightly, the surface of another drop of sterile agar.

A period of about twelve hours elapsed between the planting of oidia of one generation and the production of oidia of the next generation.

The oidia of each new generation germinated as readily as those of the generation before, and there was no change in the time required for germination. The mean length of time required for germination for the twelve generations was eight hours: the first generation oidia required eight hours, the twelfth generation seven hours.

No difference was observed between the size or shape of the oidia of the twelfth generation, and those of the first generation.

VII THE MYCELIUM PRODUCED BY OIDIA

In many respects the mycelium which develops from an oidium of Coprinus lagopus is different from the mycelium which develops from a spore.

Many cultures of oidial mycelium produced from the sexually opposite haplonts 5 and 10 have been examined critically with a view to making comparisons between the oidial mycelium and the spore-mycelium developed from a spore.

The individual hyphae of the oidial mycelium are finer than those of the spore-mycelium, being about one-half as wide. The haploid spore-mycelium averages about 5μ in width while the oidial mycelium is but 2μ wide.

The oidial mycelium branches more frequently than the spore-mycelium but the angle at which the branches come off is the same for the two kinds of mycelium.

Whereas the haploid spore-mycelium under suitable conditions grows fairly rapidly (about 3 mm. in 24 hours), the oidial mycelium grows much more slowly.

The spore-mycelium generally produces many aerial hyphae and on this account presents a fluffy appearance. The oidial mycelium grows either on the surface of the agar or under the surface and except for the oidiophores produces no fluffy aerial hyphae.

Oidia were sown on sterile dung-balls in crystallizing dishes and in round-bottom glass tubes. At the end of twenty-four hours, a fine white oidial mycelium had been produced. The mycelium eventually covered all the substratum and produced numerous oidia, but although kept under observation for two months it showed no signs of producing sporophores. Haploid fruit-bodies are produced by haploid mycelium derived from a spore within fourteen days from the date of transfer to sterile dung. It would appear therefore, that the oidial mycelium of Coprinus lagopus does not produce sporophores.

The oidial mycelium produces far more oidia per unit area than does the spore-mycelium. The spore-mycelium probably never produces many more than fifteen oidial fructifications per square millimeter of mycelium, whereas the oidial mycelium produces far greater numbers. Some counts were made which gave sixty-five oidial fructifications per square millimeter and it is probable that this number may be exceeded. In some cultures of oidial mycelium, the oidial fructifications appear to stand almost as close to one another as is possible without the oidial drops touching.

So exuberant is the production of oidia by the oidial mycelium that many oidia are produced beneath the surface of the agar.

Spore-mycelium has never been observed to produce oidia beneath the surface of the agar.

Whereas the oidiophores of the haploid spore-mycelium are single hyphae, the oidiophores of oidial mycelium are very frequently branched or compound as shown in Fig. 9. From two to five oidiophores have been found to be formed on a common stalk. The individual oidiophores of such a compound structure are not different from oidiophores coming up individually on haploid spore-mycelium.

VIII THE EFFECT OF SOWING OIDIA ON A HAPLOID MYCELIUM OF OPPOSITE SEX.

When (+) oidia are deposited on a (-) mycelium they germinate, the germ-tubes fuse with the (-) mycelium and it is converted into diploid mycelium. Conversely, when (-) oidia are deposited on (+) mycelium, the (+) mycelium is converted into diploid mycelium.

This was proved in the following manner. A bit of the haploid mycelium No. 10 was transferred to a plate of Malt-agar in a Petri dish. The mycelium was allowed to grow until it was about 4 cms. in diameter. A platinum wire loop was then touched lightly to the surface of a culture of haploid mycelium No. 5 and oidia from this mycelium adhered to the wire loop. These oidia were opposite in sex to the mycelium No. 10 growing on the agar plate. The loop with the oidia on it was then touched to the surface of the agar plate at a point just in front of the leading hyphae of mycelium No. 10.

Three days later, all the leading hyphae of the mycelium in the

plate had become diploid as was indicated by the presence of clamp--connections on them. This experiment was successfully repeated four times diploidizing mycelium No. 10 with oidia from mycelium No. 5. The experiment was also performed putting oidia from mycelium No. 10 on mycelium No. 5., and again diploid mycelium was produced. So many oidia were used as inoculum in these experiments that it was found very difficult to observe clearly just where the germ-tubes from oidia fused with haploid spore-mycelium. To overcome this difficulty, a few oidia were germinated beforehand in hanging-drop cultures and transferred to the mycelium of opposite sex in an agar plate by touching the hanging-drop to the agar in the plate at a point just in front of the leading hyphae, as in the previous experiment. The oidia were watched continuously, and in several places the germ-tubes were seen to fuse with the spore-mycelium as illustrated in Fig. 8.

In another set of experiments, oidia from a (+) mycelium which was growing on a dung-ball in a crystallizing dish 12 cms. wide, were gathered on a sterile platinum wire loop by touching it lightly to the mycelium. The oidia were then deposited on a (-) mycelium in another crystallizing dish. Conversely, the oidia from a (-) mycelium were deposited on a (+) mycelium. Both the (+) and the (-) mycelium became diploid within a week. Control haploid mycelia which were undisturbed remained haploid.

This experiment was repeated several times, always with the same result.

It has therefore definitely been proved that oidia of one sex are capable of causing a haploid mycelium of opposite sex to become diploid.

IX THE DISPERSION OF OIDIA

It has been shown in the preceding pages that when oidia of one sex germinate in the presence of mycelium of the opposite sex, a diploid mycelium may be produced. In order to prove that oidia are carriers of sex from one mycelium to another under natural conditions, it is necessary to show whether or not oidia may be dispersed and if so, by what agency they are dispersed.

Wind plays such an important part in the dissemination of seeds and spores that the possibility of oidia being dispersed by wind has been investigated.

Oidial fructifications were watched under the microscope while a sharp puff of air was applied to them by blowing through a rubber tube. The oidiophores shook violently so that some of them were flattened out on the substratum, but neither when the oidial drops were moist nor when they had been allowed to dry, were oidia detached by blowing on them.

When the oidial drop is in the moist condition, the oidia can be detached by violent shaking. This was demonstrated by inverting the bottom section of a Petri dish over the bottom section of another Petri dish containing sterile nutrient agar, and violently striking the upper dish with the hand. A few of the oidial masses were thus detached and fell on the nutrient agar of the lower plate, there to germinate and produce new oidial mycelium. No oidia could be shaken off in this manner when the oidial drops were first allowed to dry. The adhesive nature of the liquid in which the oidia are produced causes the oidia to be bound securely to the oidiophore when the liquid dries so that the oidia cannot be detached. Wind, then does

not disperse the oidia of Coprinus lagopus. Since the liquid in which the oidia are produced is soluble in water when it is fresh, rain might disperse the oidia. Rain would probably not serve to carry them very far, however.

The oidial drop on the oidiophore is very mucilaginous and adheres readily to any object brought into contact with it. When a cover-glass is lowered to touch the oidial masses, they come away from the oidiophore intact. The end of a glass rod if rubbed lightly over the surface of a haploid mycelium picks up oidia, and they adhere similarly to a platinum wire loop.

It is evident that if an insedt were to come into contact with the oidial fructifications, the oidia would adhere to the legs and body of the insect. The experiments described in the following pages show that insects do transport the oidia.

Flies were used in these experiments, the species being Drosophila melanogaster, the fly employed by Morgan and his pupils for the study of genetics. The flies were reared in pint sealers plugged with cotton-wool. Over-ripe banana was provided as food.

The bottom section of a Petri dish in which a haploid mycelium was growing on a layer of dung-agar was fitted against the bottom section of another dish containing a layer of sterile dung-agar and a fly was placed in the vessel thus formed. The two bottom sections were held together by a wide strip of adhesive paper pasted around their rims. The fly was allowed to walk over the mycelium in one plate for one-half minute, and then by turning the plate containing the sterile agar toward the light, the fly was attracted in this direction and it then

walked over the sterile agar surface.

The plates were disjoined and the fly removed. A cover was placed over the sterile agar plate and one day later, the bacteria which had been on the fly's feet had formed large colonies which made the track of the insect visible. On the agar surface where the fly had walked large numbers of oidia were found. These germinated and produced mycelium on which were borne new oidia. The oidial mycelium was proved to be of the same sex as the original haploid mycelium over which the fly had been allowed to walk. This was done by mating the oidial mycelium against mycelium of the opposite sex when clamp-connections were formed. The agar plate was photographed to show the oidial mycelium growing where the fly had walked, and is shown in Fig. 15. This experiment conclusively shows that flies can transport oidia and that these oidia can germinate and produce new mycelium.

When the fly used in the above experiment was examined under the microscope, masses of oidia were found clinging to the feet, the tarsal claws and the hairs on the tarsi.

In another experiment, six flies were allowed to go without food for twelve hours. They were then placed in a Petri dish in which a haploid mycelium was growing on dung-agar, and the behaviour of the flies was observed with the low power of a binocular microscope. Immediately upon being set free in the dish, the hungry flies began to suck up the oidial drops with their probosces. The lower end of the proboscis of the Drosophila fly is about 0.3 mm. wide: the oidial fructifications are but 0.1 mm. in length and the oidial drops but 0.05 mm. in diameter. The end of the fly

proboscis therefore touched perhaps a dozen oidial drops at the same time.

The flies walked along, vigorously sucking up the drops, leaving the oidiophores pressed down on the agar surface and cutting in this manner a swath, as it were, through the projecting oidial fructifications.

The stickiness of the drops seemed to bother the flies, for one often stopped feeding to rub its proboscis with its feet to clean off the liquid.

A clean glass plate was substituted for the cover of the Petri dish and the culture plate was inverted to allow the flies to walk on the clean plate. The flies were watched, and when several drops of excrement had been deposited which had not been touched by the flies' bodies or feet, the plate was removed.

The fly-drops were examined under the microscope and were seen to contain many oidia and some yeast cells, normally carried in the alimentary canal and on the bodies of fruit-flies. To some of the fly-drops malt-agar was added as a nutrient medium. Other of the drops were enclosed in van-Tieghem cells without additional nutriment. Under both of these conditions the oidia in the fly-drops germinated within twenty-four hours and the mycelium produced gave rise to new oidial fructifications.

The flies may transport the oidia, therefore, in two ways: first, on their legs and bodies; second, by using the oidial liquid as food, sucking up the oidial drops, passing the oidia through their alimentary canals and depositing the oidia in viable condition in fly-drops.

Other dung-inhabiting flies, beetles and other insects must also be capable of transporting oidia on their legs and bodies.

XI EXPERIMENTS DEMONSTRATING THE TRANSPORTATION OF OIDIA BY FLIES.

It has been shown above that flies are capable of transporting oidia. Experiments will now be described in which it will further be shown that flies can carry oidia from a (+) mycelium to a (-) mycelium or from a (-) mycelium to a (+) mycelium and that the oidia may function in such a way as to convert haploid mycelia into diploid mycelia; in short, that oidia are sex carriers.

In the first set of experiments, two balls of fresh horse dung were placed as far apart as possible (a distance of 12 cms.) in each of four large crystallizing dishes, 23 cms. in diameter and 8 cms. deep, the bottoms of which were lined with moistened filter-paper. Each ball was placed in one half of a shallow Petri dish 7cms. in diameter to prevent mycelium from growing from the dung-ball over the surface of the moist filter-paper in the bottom of the crystallizing dish and in this manner reaching the mycelium from the other dung-ball and mating with it. The filter-paper was placed in the dishes to give the flies which were to be put in later a rough surface to walk on and so prevent them from being drowned in the water which accumulated on the bottom of the crystallizing dishes.

Each crystallizing dish was covered with a glass plate and the dish along with its contents was sterilized in steam at 15 pounds pressure for one hour. Fig. 13 shows diagramatically the appearance

of the crystallizing dish and the dung-balls.

One ball in each dish was inoculated with a bit of the haploid mycelium No. 5 and the other ball in each dish inoculated with haploid mycelium No. 10 of Coprinus lagopus. Two of these dishes were used for experiment with flies, and labeled A and B, and two were kept for controls.

Three days after inoculating the dung-balls, the mycelium which had developed on each covered an area of about two square centimeters. Eight flies were then placed in each of the experiment dishes A and B. The flies were first removed from the glass jar in which they had been raised to another jar where they were etherised to make it easy to transfer them to the crystallizing dishes.

No flies were placed in the control dishes.

In the course of the next few days, the flies in each dish flew back and forth, from the mycelium on one dung-ball to the mycelium on the other. They could also be seen sucking up the drops of liquid containing the oidia.

One week after the flies had been put in the dishes, the mycelia on all the dung-balls were examined by taking a bit of each mycelium, mounting it in water on a glass slide, and examining it under the microscope for the presence of clamp-connections.

It was found that the control mycelia were still all haploid, whereas all the mycelia in the experiment dishes were diploid.

This means that the flies carried oidia from the mycelium No. 5 and deposited them on mycelium No. 10 and also carried oidia from mycelium No. 10 and deposited them on mycelium No. 5. The oidia must have germinated and the germ-tubes fused with the mycelium on which they were deposited.

The experiment was successfully repeated in the manner described above. However it was found that it was not necessary to use large crystallizing dishes. Further, when a small piece of paper was placed under the cover at the edge of the dish to allow sufficient ventilation for the mycelium growing in the dish it was difficult to prevent the culture from becoming contaminated with mould. The experiment was therefore modified in the following manner. The haploid mycelia were grown in round-bottom glass tubes as has already been described on page 8 of this paper. Four days from the time when the tubes of dung were inoculated with mycelium, six flies were placed in the chamber formed by fitting together tubes containing mycelium No. 5 or mycelium No. 10, these being of opposite sex. The tubes were held together by means of a cardboard collar 5 cms. long which fitted tightly over the tubes. The flies were free to wander from the mycelium in one end of the chamber to the mycelium at the other end. Four such experiments were made up. The flies were allowed to remain for different lengths of time in each chamber; in one 24 hours, in one 48 hours and in the other two 72 hours. One tube containing mycelium No. 5 and one containing mycelium No. 10 were kept as controls, no flies being placed in these tubes.

The mycelia were all examined five days after the flies were first put in the tubes.

The mycelia in all four experiments were all diploid when examined with the microscope, whereas the control mycelia were still haploid.

The experiment was repeated, this time allowing the flies to remain in the tubes only fifteen minutes. The results were equally satisfactory, the mycelia visited by the flies becoming diploid, the control mycelia remaining haploid.

To further extend the experiment, flies were caused to go from one haploid mycelium on dung in a glass tube through a cardboard tube of slightly greater diameter and 3.5 feet long to a haploid mycelium of opposite sex in a glass tube at the other end of the cardboard tube.

The flies were allowed to crawl over the (+) mycelium in the glass tube in one end of the cardboard tube and the other end was then held in the sunlight. The light attracted the flies to the (-) mycelium and they then walked over it. The flies were allowed to remain on each haploid mycelium only fifteen minutes. The mycelia visited by the flies had become diploid four days later as was shown by examination, while control mycelia still remained haploid.

Furthere experiments were carried out with dung-balls in large crystallizing dishes, one dung-ball of each pair in a dish being inoculated with haploid mycelium, the other dung-ball being left sterile. In each of three of such dishes flies were placed, but no flies were placed in one dish kept as a control. At the end of one week, oidial mycelium was growing on the sterile dung-ball in each of the three experiment dishes. Oidia had been carried by the flies from haploid mycelia to sterile dung-balls. No mycelium appeared on the sterile dung-balls in the control dishes.

To make the above experiments still more convincing, it was necessary to show whether or not the flies might be transporting bits of mycelium on their legs and bodies, and whether or not this

mycelium rather than the oidia, when carried to mycelium of opposite sex caused diploid mycelium to be produced.

In order to investigate this point, experiments were designed using the same apparatus as was used in the preliminary fly experiments, namely dung-balls in large crystallizing dishes.

This time, one of the balls in each dish was inoculated with diploid mycelium and the other ball was left sterile. Diploid mycelium was used because it bears no oidia, and therefore if the flies transported anything, it must be bits of mycelium. As in the previous experiments, flies were placed in two of the dishes and one dish was kept as control. The flies were allowed to remain in the dishes for ten days. No mycelium appeared on the sterile dung-balls although observation was continued for fifteen days. The same result was obtained in a duplicate experiment performed later. It can therefore be said that flies do not transport bits of mycelium and the conclusion that oidia only are transferred is strengthened.

XII GENERAL REMARKS ON THE LIFE-HISTORY OF COPRINUS LAGOPUS.

From the facts presented in the preceding pages, it may be concluded that the oidia of Coprinus lagopus play an important part in the life-history of that fungus.

The oidiaaare produced on special hyphae which project perpendicularly outwards from the substratum into the air; they are enveloped in a drop of liquid which causes them to adhere readily to any object which comes in contact with them; and the liquid drpp is attractive to flies which may suck it up as has been described. The oidia may be carried from their place of origin by rain but are probably not dispersed to any extent by rain, and they are not carried away by wind. In these respects the oidia may be considered to be dependent upon insects for their dispersal.

Flies of various species may usually be seen flying about or crawling over horse dung on the street or in the fields. The writer has notiwed several times that when fresh horse dung is exposed on the laboratory table even during the winter months, whatever fruit-flies are at large in the laboratory are soon attracted to the dung. Many beetles also frequent horse dung. Among these may be mentioned many members of the family Staphylinidae and several species of the genus Aphodius of the family Scarabeidae. It has been shown that flies are capable of transporting oidia from place to place and doubtless the beetles may do the same thing.

The oidia function not in the actual dissemination of Coprinus lagopus from place to place but in the dissemination of sex: they are sex carriers, being capable of converting a haploid mycelium into a diploid mycelium.

It is to the advantage of the fungus for every mycelium arising from its spores to become diploid since a haploid mycelium produces feeble fruit-bodies whose spores are all of one sex, whereas a diploid mycelium produces vigorous fruit-bodies on which are borne spores of all four sexes.

To consider just how the oidia of Coprinus lagopus may function in converting a haploid into a diploid mycelium, suppose first, that in nature a (+) spore and a (-) spore have been deposited near to one another on horse dung. They may germinate and produce mycelia, the (+) mycelium producing (+) oidia and the (-) mycelium producing (-) oidia before the (+) and (-) spore-mycelia unite.

It has been shown in the preceding pages that the (+) oidia may be carried by insects to a (-) mycelium on another dung-ball where they may germinate and convert the (-) mycelium into a diploid mycelium. If however, the (+) oidia are carried to another (+) mycelium or to a dung-ball on which there is no mycelium of Coprinus lagopus, they may germinate and produce a (+) oidial mycelium on which in turn may be produced (+) oidia capable of being further disseminated.

Conversely, the (-) oidia may be carried to a (+) mycelium on another dung-ball and convert the (+) mycelium into a diploid mycelium, or if they are carried to another (-) mycelium or to a dung-ball on which there is no mycelium of Coprinus lagopus, they may germinate and produce more (-) oidia capable of being further disseminated.

The (+) and (-) mycelia derived from the spores mentioned above may unite to form a diploid mycelium which will then cease to produce oidia.

The diploid mycelia produced in any of the above ways may develop diploid fruit-bodies which are vigorous and which shed spores of all four sexes.

Again, consider what happens to a single (+) spore which germinates on horse dung in the absence of any other spores of the same species. About the third day after it germinates the (+) mycelium which develops from it begins to produce oidia. These (+) oidia may be carried by insects to a (-) mycelium on another dung-ball and cause the (-) mycelium to become diploid; or, they may be carried to another (+) mycelium or to a dung-ball on which no mycelium of Coprinus lagopus is growing and there produce oidial mycelium and more (+) oidia capable of being further disseminated. Also, the (+) spore-mycelium may produce haploid fruit-bodies bearing only (+) spores.

In a like manner, a single (-) spore may give rise to a (-) mycelium which may produce oidia. These (-) oidia may be carried to a (+) mycelium and cause it to become diploid, or they may be carried to another (-) mycelium or to a dung-ball on which there is no mycelium of Coprinus lagopus and there produce oidial mycelium and more (-) oidia. The (-) spore-mycelium may produce haploid fruit-bodies bearing only (-) spores.

may be carried to a (+) mycelium and cause it to become diploid, or they may be carried to another (-) mycelium or to a dung-ball on which there is no mycelium of Coprinus lagopus is growing and there produce oidial mycelium and more (-) oidia. The (-) spore mycelium may produce haploid fruit-bodies bearing only (-) spores.

When two spores of the same sex germinate side by side, the myceliawwhich develop may anastomose. The fruit-bodies produced will all be haploid and bear spores of only one sex. Oidia may also be produced and these too will all be of one sex.

XIII SUMMARY

1. The oidia of Coprinus lagopus are produced on oidiophores which project away from the substratum into the air and they are developed in a drop of adhesive liquid which is of impertance in the dissemination of the oidia. Some observations concerning the nature of the liquid drop have been recorded.

2. Oidia ere produced only on haploid mycelium, never on diploid. On mycelium derived from a spore oidia are produced only aerially or on the surface of the nutrient medium, never undert the surface of the medium.

3. When many spores of Coprinus lagopus of diverse sex are sown together, the mycelia developing from them do not fuse when they are very young but produce oidia before uniting to produce diploid mycelium.

4. Some observations have been made of the process whereby oidia are formed from the hyphal branches at the apex of the oidiophore.

5. The oidia of Coprinus lagopus are capable of germinating and of producing oidial mycelium on which may be developed more oidia of the same sex as the parent mycelium.

6. The mycelium developed from oidia is different from the mycelium developed from a spore of Coprinus lagopus. Although kept under observation for two months the oidial mycelium produced no sporophores whereas mycelium derived from a spore produces haploid fruit-bodies in from ten to fourteen days. Oidial mycelium has thinner hyphae than the mycelium derived from a spore and produces more oidial fructifications on one square millimeter of its surface than does spore-mycelium.

7. Oidial fructifications have been found coming up naturally on horse dung on four occasions.

8. Oidia are not transported by wind. They may be transported by rain but are mainly disseminated by insects such as dung-inhabiting flies and possibly some beetles.

9. Experiments have been described which prove that flies can carry oidia on their feet and bodies and may use the liquid drops on the oidiophores as food, the oidia in the drops passing through the alimetrary canals of the flies and being deposited in viable form in the excreta of the flies.

10. Experiments have been described which prove that when flies are enclosed in a large crystallizing dish and are allowed to walk on a (+) mycelium and then on to a (-) mycelium and vice versa, both the (+) mycelium and the (-) mycelium are converted into diploid mycelia. Flies have been caused to transfer oidia in this way between mycelia of opposite sex 3.5 feet apart.

11. Oidia do not function in the dissemination of Coprinus lagopus but they are sex carriers, capable of converting a haploid mycelium into a diploid mycelium.

12. It has been proved that flies transport only oidia and not bits of mycelium.

The investigations recorded in the preceding pages were carried out in the Botanical Laboratory of the University of Manitoba. Professor A. H. R. Buller suggested the problem and gave generously of his time throughout the course of the work, and his stimulating advice and helpful criticisms are gratefully acknowledged.

EXPLANATION OF PLATE

All figures are those of *Zoophagus insanus*.

Fig. 1. An oidiophore from a fourteen day old haploid mycelium which was immersed in water to free the oidia and to show the apex of the oidiophore; Magnification 350.

Fig. 2. A young oidiophore from a four day old haploid mycelium grown on horse dung and mounted in water on a slide for sketching; Magnification 350.

Fig. 3. Studies of the drop on the oidiophore produced on a three day old haploid mycelium growing in a hanging-drop of dung-agar; the dotted line on each figure indicates the surface of the agar above which the oidiophore projects; Magnification 400;

A. the drop just beginning to form.
B. a fully formed drop showing the oidia within it.
C. a drop which has dried up.

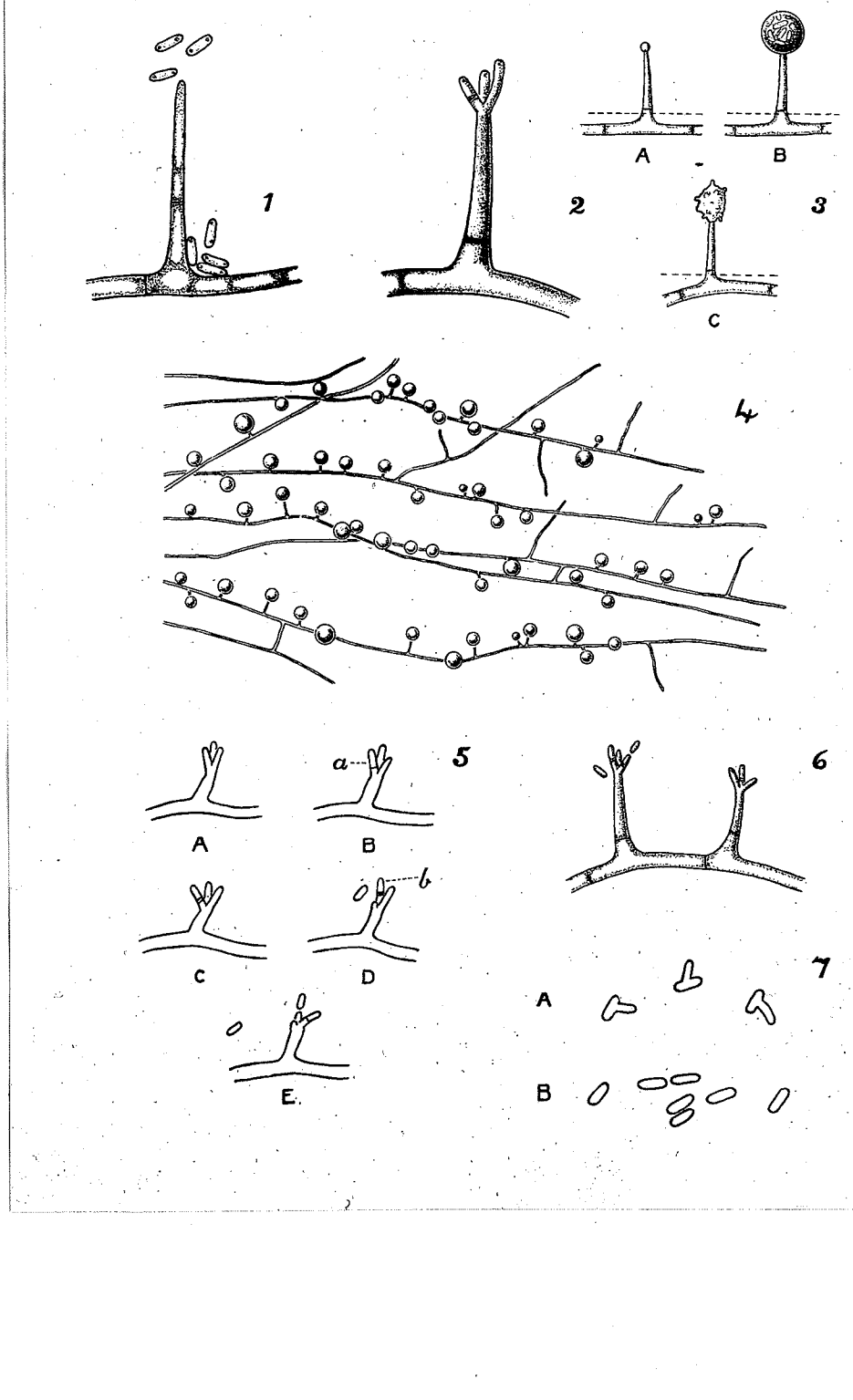
Fig. 4. The disposition of oidiophores on a 48 hour old diploid mycelium growing on the surface of a hanging-drop of dung-agar; Magnification 67.

Fig. 5. Development of oidia on an abnormally short oidiophore produced in the film of water on the surface of a hanging-drop of dung-agar; Magnification 400;

A. initial condition.
B. appearance of a fine line near the base of the oidial branch *a*.
C. showing the gap in the protoplasm developing on the oidial branch *a*.
D. an oidium set free from the branch *a*, another gap developing in branch *b*.
E. an oidium set free from branch *b*, another gap beginning to form on the third oidial branch

Fig. 6. Oidial fructifications which developed of their own accord on horse dung under natural conditions; sketched from material in water on a glass slide. Magnification 400.

Fig. 7. A. Branched oidia which are occasionally formed.
B. Normal oidia. Magnification 600.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EXPLANATION OF PLATE

All figures are those of *Pythium hypogynum*.

Fig. 8. The fusion of germ tubes produced by (-) oidia with (-) mycelium developed from a spore. Magnification 400.

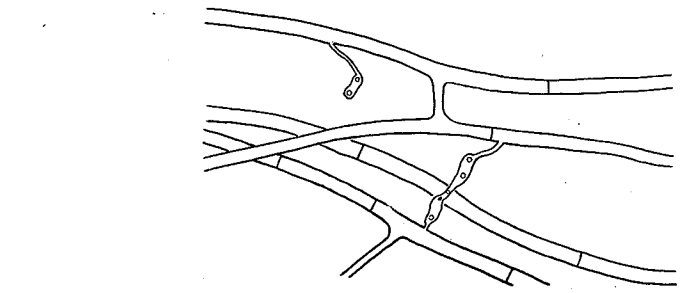
Fig. 9. A branched oidiophore produced on a seven day old haploid mycelium growing on dung-agar; the mycelium produced from oidia. Magnification 400.

Fig. 10. Oidia germinating twenty-four hours after having been planted on cleared dung-agar, the germ tubes of some already producing new oidia. Magnification 935.

Fig. 11. Oidia germinating on cleared dung-agar and producing new oidia on aerial oidiophores; dotted lines indicate the surface of the agar out of which the oidiophores project. Magnification 935.

Fig. 12. Apparatus to show the effect of dry atmosphere on the production of oidiophores; b, an inverted Petri dish standing on cones c; a, the layer of nutrient agar in the Petri dish; d, the table; one-third natural size.

Fig. 13. Diagram of apparatus for illustrating the transportation of oidia from a (-) mycelium to a (-) mycelium and *vice versa*; b, a large crystallising dish; c, its glass cover with a slip of paper, e, between the dish and the cover to allow ventilation; c, a ball of horse dung; d, a shallow Petri dish cover; one third natural size.



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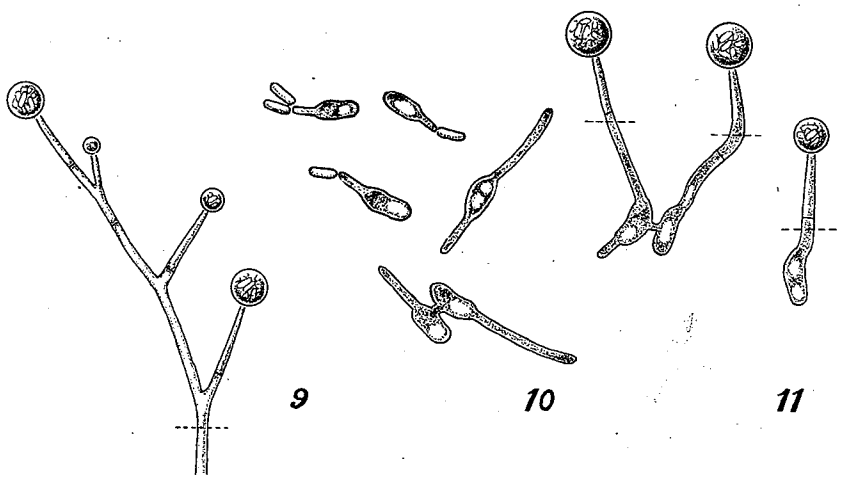

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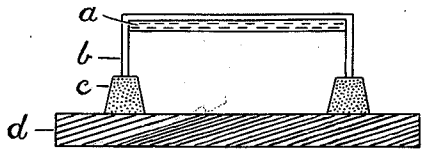

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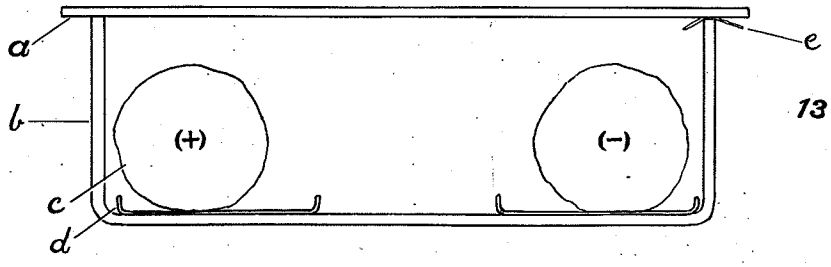

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EXPLANATION OF PLATE

Both figures are those of *Coprinus lagopus*.

Fig. 14. A haploid mycelium four days old in a hanging-drop of malt-agar showing the oidia in drops on oidiophores and some lying about on the surface of the agar. Magnification 100.

Fig. 15. A fly, *Drosophila melanogaster* was allowed to crawl on a haploid mycelium for about one-half minute and then on to a dung-agar plate where it made tracks with its body and feet. The plate was photographed five days later. The oidia everywhere produced mycelium bearing new oidia. Natural Size.

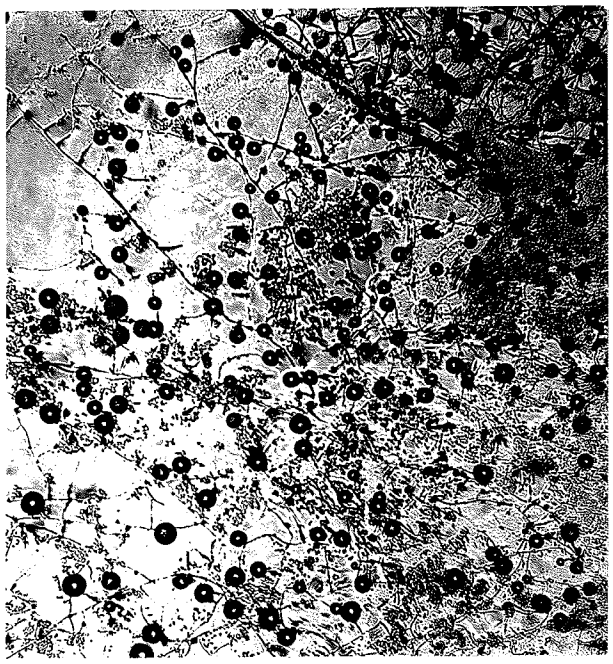

Fig. 14.

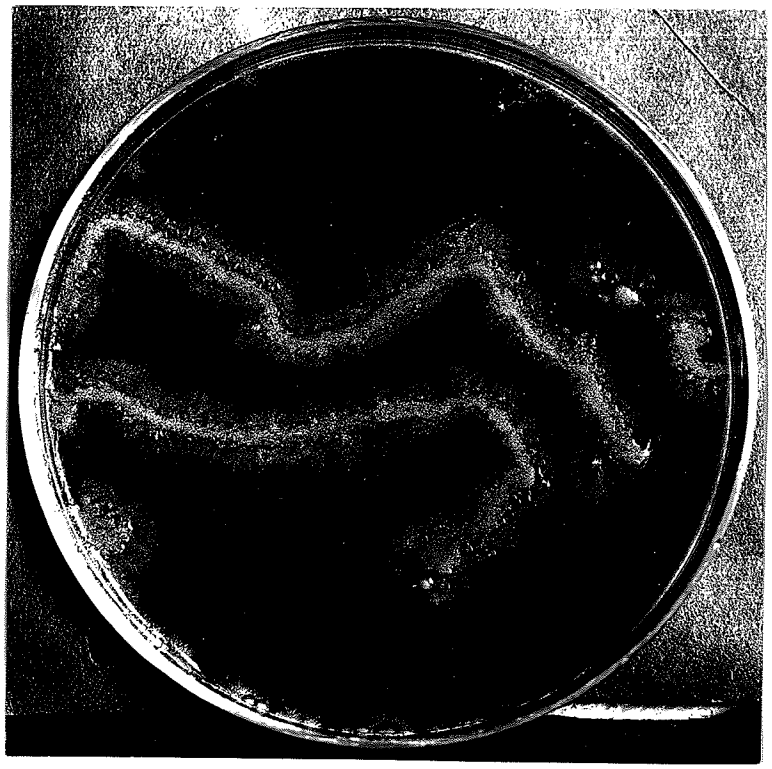

Fig. 15.