The Oidia of Coprinus lagopus and their Relation with Insects.

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With Plate X and twenty-four Figures in the Text.

I. Introduction.

THE researches of Eidam, van Tieghem, Brefeld, Zopf, and others have taught us that, in many Hymenomycetes and in certain Pyrenomycetes and Discomycetes, the young mycelium developed from a basidiospore or from an ascopore produces or breaks up into a series of short segments which Brefeld called *oidia*. Brefeld (2, 3) in his *Untersuchungen* illustrated the oidia of the following Hymenomycetes:

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

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

In the nineteenth century, when mycologists were searching for sexual organs in the higher fungi, it was believed by Eidam (8) and van Tieghem (18) that oidia are male cells or spermatia, even although female organs

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could not be found. Van Tieghem (19), in 1875, succeeded in germinating the oidia of *Coprinus plicatilis* and *C. stercorarius*,[1] and he then declared that the oidia serve to reproduce the fungus in an asexual manner and that, in this respect, they are comparable with conidia.

Brefeld (2), in 1877, announced that he had been unable to germinate the oidia of *C. lagopus*, although he had made numerous attempts to do so, and that he had come 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.

Falck (9), 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 *P. merismoides*, *H. fasciculare*, and *C. 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 (10) showed that the oidia of *Lenzites sepiaria*, after having been kept dry for a year, are still able to germinate.

So far as coprophilous Hymenomycetes are concerned, Falck did not succeed in germinating the oidia of *C. lagopus*, *Panaeolus campanulatus*, and *A. coprophilus*; and he discovered that *C. sterquilinus*, like Brefeld's *C. stercorarius*, fails to produce any oidia whatsoever. Falck (9) came to the conclusion that 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, Bensaude (1), in her well-known paper on the life-history and sexual phenomena of *C. 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.

[1] Doubtless van Tieghem misidentified this species, as the true *C. stercorarius* which develops sclerotia does not produce any oidia.

[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.

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 then germinated; their germ-tubes fused with the mycelium *A*; and thus the mycelium *A* was converted by the oidia from the haploid to the diploid phase.

In 1927, Craigie (7), acting on a suggestion given to him by Professor A. H. R. Buller, discovered that the pycniospores of the Rust Fungi which are produced by haploid mycelia are functional. When (+) pycniospores 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 (−) pycniospores 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 pycniospores 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.

II. Material and Methods.

The fungus chosen as material for this investigation was *C. lagopus*. This well-known species is readily obtained in horse-dung cultures, and its life-history has been worked out by Brefeld (2), Bensaude (1), Buller (4, 5, 6), Hanna (12, 13), Mounce (14), Newton (15), Oort (16), and others.

Spores of *C. 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 *C. lagopus*.[1] Spores from one of the pilei were allowed to fall on a sterilized glass slide. From this spore-deposit single spores were removed by the dry-needle method described by Hanna (11), and they were sown 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. The whites of four eggs, after being added to 50 c.c. 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 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 *C. 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 test-tubes 3 in. long and 0·9 in. in diameter, which had been filled about one-third with fresh horse-dung, plugged with cotton-wool, and sterilized in steam at 15 lb. pressure for one hour.

Ten mycelia, nos. 1–10 inclusive, were paired on dung-agar in all possible ways; and, a few days later, each pair was examined to find out whether or not clamp-connexions had appeared upon the hyphae. On the basis of this criterion (12), it was then possible to sort out the ten mycelia into the four well-known sexual groups (*AB*), (*ab*), (*Ab*), and (*aB*).

[1] For illustrations of the fruit-body of *C. lagopus*, *vide* A. H. R. Buller, Researches on Fungi, vol. iii, Figs. 130–8, pp. 300–16; also A. H. R. Buller and D. E. Newton, Ann. Bot., vol. xli, 1927, Pl. XXVIII.

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*).

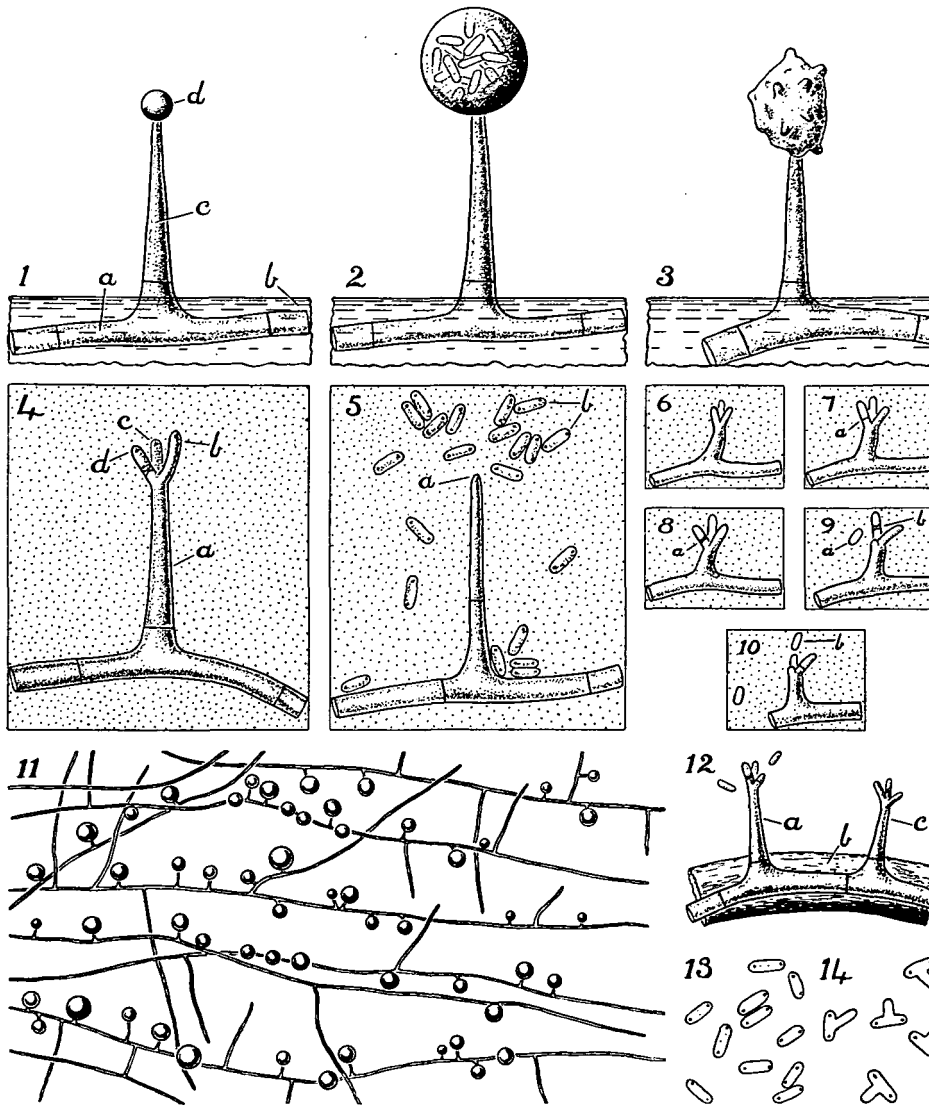
III. The Structure and Development of the Oidial Fructifications.

From two to three days after the germination of a single spore of *C. 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 (Text-figs. 1–3 and 11). 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, they develop and produce oidia in the film of water (Text-figs. 6–10). 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 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. (*cf.* Text-figs. 4–5 and 6–10). At its basal end, a well-developed oidiophore (Text-figs. 1 and 2) is 5–6 μ thick, or as thick as the hypha to which it is attached, but it tapers upwards so that its apical end is only 2·5–3·0 μ 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 a full-grown oidiophore gives rise to short branches which may be called *oidial hyphae* (Text-fig. 4, *b*, *c*, *d*) and these soon break up into chains of from two to five oidia. The process of oidial formation on an oidiophore which was submerged in a film of water is illustrated in Text-figs. 6–10. At 10.30 a.m. an oidiophore with its oidial branches (Text-fig. 6) 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 (Text-fig. 7, *a*) and, after forty-five minutes, a clear space or gap in the protoplasm appeared where the line had been (Text-fig. 8, *a*). Thus an apically-formed oidium became marked off from the basal portion of the oidial hypha. The newly-formed oidium then



TEXT-FIGS. 1–14. *Coprinus lagopus*. 1. A young oidial fructification produced aerially on a four-days-old haploid basidiosporous mycelium growing on dung-agar: *a*, a hypha of the basidiosporous mycelium; *b*, the dung-agar medium in which it is growing; *c*, the oidiophore; *d*, a drop of liquid just beginning to be excreted. × 933. 2. The same oidial fructification ten hours later. The drop has increased greatly in size, and oidia which have been formed within the drop are seen floating about in it. × 933. 3. An oidial fructification which has been exposed to dry air so that the liquid surrounding the oidia has dried up leaving their outlines projecting from the mass. × 933. 4. A young oidiophore on a ten-days-old haploid mycelium. The oidiophore has been immersed in the film of water covering the surface of a dung-agar plate: *a*, the oidiophore; *b*, *c*, and *d*, oidial branches which break up into from two to five oidia. × 933. 5. An old oidiophore on a ten-days-old haploid mycelium. The oidiophore has been immersed in the film of water covering the surface of the agar, and the oidia which were formerly in a mass on the apex of the oidiophore have been scattered: *a*, the rounded tip of the mature oidiophore; *b*, the oidia. × 933. 6–10. Successive stages in the process of oidia formation on an oidiophore developed in the film of water covering the

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 (Text-fig. 9, *b*) and, after thirty minutes, the oidium cut off above the gap began to sway and was then soon set free (Text-fig. 10, *b*).

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 (Text-figs. 1–3 and 11) are much longer and their oidial branches break up into chains of from two to five oidia. In the oidial fructifications shown in Text-figs. 1–3 and 11, the oidial branches are obscured by the drop of liquid in which they are immersed.

The formation of a chain of two or three oidia from an oidial branch is due to: (1) the division of the protoplasm into two or three separate masses; (2) 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; (3) the development of a wall at each end of each shrunken mass of protoplasm; and (4) the dissolution of the wall of the oidial hypha around the water-filled spaces. The two or three oidia set free from an oidial hypha are so small that, if liberated into water, they soon separate from one another and move away (Brownian movement) 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 (Text-fig. 5).

A striking phenomenon in connexion 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, both of whom studied the oidia of *C. lagopus* and other Coprini, appear to have noticed the drops in question; but, as we shall see, these drops are of great importance for the dispersal of the oidia and their conveyance to a place where they may function to the advantage of the

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surface of a hanging-drop of dung-agar. In Fig. 7 a line has appeared at *a*; in Fig. 8 the line has widened into a gap, *a*; in Fig. 9 the oidium *a* has floated freely away from the oidial branch on which it was formed, and another gap is appearing at *b*; in Fig. 10 the oidium *b* has also been freed. × 400. 11. A forty-eight-hours-old haploid mycelium growing on the surface of a poured-plate of dung-agar showing the hyphae with their wide angle of branching and the oidial fructifications. × 87. 12. Oidial fructifications found under natural conditions on horse-dung, sketched from material in water on a glass slide: *a* and *c* the oidiophores; *b*, a bit of straw from the horse-dung. × 466. 13 and 14. Oidia of *Coprinus lagopus* sketched in water on a slide: Fig. 13, some normal oidia showing a refractive granule at each end of each oidium: Fig. 14, a few branched oidia which are occasionally produced. × 600.

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 (Text-fig. 1). The drop increases in size until, at the end of ten hours, it has become about 0·08 mm. in diameter (Text-fig. 2). From the apex of the oidiophore oidial branches are sent out into the drop, where they break up into oidia (cf. Text-fig. 4). 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 spherical, and it completely envelops all the oidia contained within it.

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 drops of liquid on the apices of oidiophores developed on a dung-agar plate (Text-fig. 11), 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 was found: (1) that whirling can be started by breathing upon the drops, and that the whirling ceases as soon as the drops are no longer breathed upon; and (2) that one can cause a drop to whirl in a clockwise or a counter-clockwise direction by breathing more on the left or on the right of the drop respectively. Thus whirling can be caused by friction with air-currents.

When one looks down on the top of an oidial drop with the high power of the microscope, one sees an enlarged image of some of the oidia immersed in the drop. This is due to the drop acting like a magnifying glass.

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

observes 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 liquid (Text-fig. 20). It was found that, if the cover-glass was dipped in water before the oidiophore liquid had dried, the liquid dissolved in the water and the oidia were dispersed. Two days after some drops were collected and allowed to dry on a cover-glass, it was found that they could not be dissolved either in water or in 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.

Very large oidial drops may be formed by the fusion of several smaller drops and by further excretion of liquid. A continuation of this process may result in almost the entire surface of a haploid mycelium growing on a plate of dung-agar becoming covered with closely-packed oidia.

A photograph showing the appearance of oidial fructifications when seen with a magnification of 100 is reproduced in Pl. X, Fig. 2. When the drop on an oidiophore is allowed to dry it shrivels and assumes a rough appearance owing to the fact that the ends of the oidia project (Text-fig. 3).

The individual oidia of *C. lagopus* are 1 μ in width and may be from 2 μ to 10 μ long. The average length is about 5 μ. Occasionally an oidium is produced which has a side branch, as shown in Text-fig. 14. Two refractive granules are usually to be seen in the protoplasm within each oidium (Text-figs. 5, 13, 14).

Bensaude (1) stained the oidia of *C. fimetarius*, and she illustrated the stained oidia in her paper on Pl. II, Figs. 3 and 4, and on Pl. IV–V, 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 *C. lagopus* are produced by haploid mycelia 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 advent of the diploid phase, the mycelium ceases to produce oidia. The same is true for *C. niveus* and *C. curtus*—two other heterothallic species of *Coprinus* which have been examined by the writer.

Brefeld (2) showed that *C. stercorarius*, now known to be a homothallic species, never produces oidia; and Falck (9) demonstrated that *C. sterquilinus*, also now known to be homothallic, is entirely without oidia.

The writer kept monosporous mycelia of *C. stercorarius* under observa-

tion from the time of germination of the spores onwards for several days. Three days after the germination of each spore the mycelium which developed from it became diploid. No oidia whatever were produced.

It is a rather remarkable fact that a *very young* haploid mycelium of *C. 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 the four sexual types (*AB*), (*ab*), (*Ab*), and (*aB*), were sown together in a hanging-drop of cleared dung-agar. Twelve hours later about one-fourth of the spores had germinated. When two to five 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 to produce oidia. It may be concluded that a young spore-mycelium passes through a period of oidia-production of about forty-eight hours' duration, and that it will not unite with a mycelium of opposite sex until this period has been completed.

The number of oidia produced by a haploid mycelium developed from a spore on one square millimetre of dung-agar varies for different mycelia and for different parts of the same mycelium. Some mycelia produce far more oidia 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 millimetre of dung-agar surface. At this rate, on a mycelium covering a square with each side 5 cm. (2 inches) long, the number of oidial fructifications would be 2,500. Reckoning twenty oidia to each oidial 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. Some idea of the great number of oidia which a haploid mycelium of *C. lagopus* may produce may be gained by a glance at the photomicrograph reproduced in Pl. X, Fig. 2.

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

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

The cover was removed from an agar plate in which a haploid mycelium was growing, and the plate was allowed to rest in an inverted position on two corks on the laboratory table. The dish and corks were covered with

a bell-jar under the edge of which a small slice of cork was placed to allow free access of air. Thus the surface of the agar was exposed to air much drier than that in a closed Petri dish, and yet not dry enough to dry the agar surface completely. At the end of three days the oidiophores were being produced by the leading hyphae in as great numbers as under the conditions of excess of moisture existing in a closed Petri dish. Under the dry conditions, however, there were on the oidiophores not as many large compound drops due to fusion of individual drops as may be observed under more moist conditions.

Light is not essential for the production of oidia, for it was found that oidia were produced in cultures kept for ten days 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 to determine whether or not the ring-formation is due to the alternating influence of light and darkness. Mycelia on agar plates were exposed to sunlight for one hour each day for ten days, and were kept in the dark for 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 on dung-agar, &c., the laboratory conditions are somewhat artificial, and it seemed desirable to find out whether or not the oidial fructifications are produced under natural conditions.

A large number of fruit-bodies of *C. lagopus* were noticed coming up on unsterilized horse-dung which had been brought into the laboratory and placed in a covered glass vessel sixteen days previously. This culture was examined 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 in appearance those which had been studied in laboratory cultures of haploid mycelia of *C. lagopus*. Some oidial fructifications were found on a bit of straw, and the straw was then removed from the rest of the dung with fine forceps and placed on a glass slide in a drop of water. Hyphae with simple cross-walls, from which oidiophores bearing oidia projected, were then seen (Text-fig. 12).

On three other occasions oidial fructifications have been found in unsterilized stable-dung cultures. The dung was obtained fresh from a stable and oidial fructifications exactly resembling those of *C. lagopus* were found upon it two to three days after it had been deposited. About a week later, on the same dung, appeared numerous fruit-bodies of *C. lagopus* as well as some fruit-bodies of *C. curtus*, &c. From these observations it

is clear that oidial fructifications are produced under natural conditions in the open even in competition with other fungi, bacteria, &c. Since, in the cultures under discussion, *C. lagopus* fruit-bodies were more numerous than those of any other fungus, and since the structure of the oidiophores and oidia exactly resembled that of the oidiophores and oidia produced in pure cultures of *C. lagopus*, there can be little doubt that the oidiophores and oidia found in the wild cultures were produced by *C. lagopus*. So far as the writer is aware, this is the first time that oidiophores and oidia of any species of Hymenomycetes have been observed under natural conditions.

VI. The Germination of the Oidia.

Some spores of *C. lagopus* were sown separately in hanging-drops of cleared dung-agar and malt-agar. The spores germinated, and each of the monosporous mycelia soon began to produce numerous oidiophores and oidia. Owing to the fusion of oidial drops, and to the drops coming into contact with the dung-agar, great numbers of the oidia became scattered over the surface of the medium. When these cultures were ten days or more old oidia were observed germinating in them *in situ*.

An oidium when about to germinate becomes much swollen (Text-fig. 17, *b*, *c*), rather more at the ends than in the middle, and the refractive granules at the ends are then more prominent than in ungerminated oidia (Text-fig. 17, *b*). The swollen oidium usually develops a single germ-tube at one end (Text-fig. 17, *d*), but sometimes it sends out two germ-tubes, one from each end (Text-fig. 17, *f*). The width of the germ-tube is about two-thirds that of the swollen oidium ; and, as the germ-tube is produced, large vacuoles appear in the oidium, thus indicating that the germ-tube is being produced at the expense of the protoplasm of the oidium (Text-fig. 17, *d*). Germ-tubes which (as in ten-days-old cultures) are produced on an exhausted medium develop slowly, often soon cease to elongate, and not infrequently anastomose with one another (Text-fig. 17, *e*).

An attempt was made to germinate the oidia in a freshly prepared nutrient medium. Oidia of various ages were sown in hanging-drops of one or other of the following: water, sugar solution, cleared and uncleared dung-agar, and 2·5 per cent. malt-agar. The results in these first experiments were all negative, this notwithstanding that similar oidia to those employed had been seen germinating *in situ*.

The effect of heat on the germination of oidia was next investigated. Oidia were obtained on each of a number of cover-glasses by bringing the cover-glasses lightly into contact with the surface of a thirty-days-old culture of a haploid mycelium. The cover-glasses with the oidia attached to their lower surfaces were then placed on van Tieghem cells which had been partly filled with water. The preparations were then heated from above

by means of a desk-lamp to 50° C., were kept at this temperature for one minute, and were then allowed to cool. Some control preparations were not heated.

In the course of twenty-four hours the oidia which had been heated had swollen and germinated, while the oidia in the unheated control preparations had not germinated. This experiment was repeated several times, always with the same result. It thus appears that heating in some way assists oidia to germinate.

Next, some oidia taken from a three-weeks-old culture of the haploid mycelium No. 5 were set in hanging-drops of cleared dung-agar and malt-agar. They germinated rapidly and in large numbers. After this, oidia of various ages (quite young to several weeks old) were taken from the haploid mycelia Nos. 5 (*ab*), 10 (*AB*), 2 (*Ab*), and 7 (*aB*) and were sown in hanging-drops and poured-plates containing the same media; again germination took place readily. Not a single one of upwards of twenty of these cultures yielded a negative result, for germinating oidia were observed in every one of them.

Five months after the experiments recorded above were performed, oidia were taken from the haploid mycelia Nos. 3 (*AB*), 6 (*ab*), 4 (*Ab*), and 1 (*aB*) and were sown in hanging-drops of freshly prepared dung-agar. Again, in every drop, the oidia germinated freely.

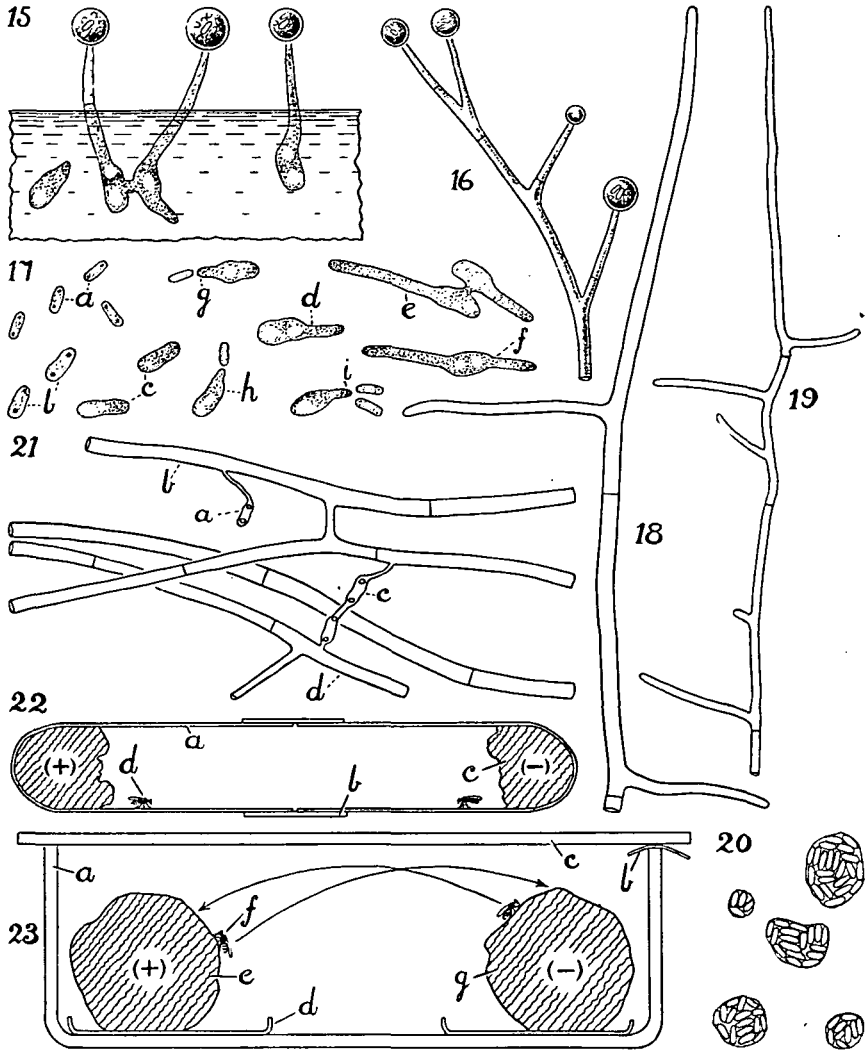
The two sets of experiments just described showed that the oidia of eight of the ten monosporous mycelia isolated for use in the present investigation germinated readily when subjected to favourable conditions.

One may ask: why is it that in the first set of experiments in which the culture medium consisted of hanging-drops of dung-agar and malt-agar the oidia failed to germinate, whereas in similar experiments made subsequently the oidia germinated well? Possibly the answer is to be sought in the variability of the horse-dung used in making the media. It is unlikely that the media made at different times with horse-dung are ever quite alike in composition. Even after it is made, dung-agar undergoes change, for its colour gradually becomes darker.

There can be little doubt that, when subjected to suitable conditions, the oidia of *C. lagopus* germinate readily. This conclusion is supported not only by the germination experiments already described, but by other experiments made with the help of flies, to be described in a later section.

When oidia were sown in hanging-drops of dung-agar or malt-agar the minimum time for germination after sowing was found to be eight hours, and it was estimated that in each of the cultures about 80 per cent. of the oidia germinated.

The germ-tubes of oidia show a lesser tendency to anastomose in a fresh culture medium than in an exhausted one. In a fresh medium some of the germ-tubes do not grow much longer than the oidia which



TEXT-FIGS. 15–23. *Coprinus lagopus.* 15. Oidia of one sex which have germinated in dung-agar and have given rise to short germ-tubes. The germ-tubes have projected into the air to become oidiophores, and each of them bears at its apex the usual mass of oidia and liquid excretion. × 933. 16. A branched oidiophore produced on a mycelium derived from oidia of one sex, growing on dung-agar or horse-dung. × 400. 17. Oidia sown in a hanging-drop of cleared dung-agar; *a*, ungerminated oidia; *b* and *c*, much swollen oidia about to germinate, eight hours after having been sown; *d*, a germinating oidium showing two vacuoles within and producing a germ-tube from one end only; *e*, two germinating oidia which have anastomosed; *f*, a germinating oidium which has produced a germ-tube from each of its ends; *g* and *h*, oidia each with a single germ-tube from the end of which has been constricted off a second-generation oidium; *i*, a germinating oidium with a germ-tube which has formed two new oidia. × 933. 18. A leading hypha of a haploid mycelium derived from a basidiospore, developed on dung-agar. The hypha is 4 μ to 5 μ in diameter. × 400. 19. A leading hypha of a mycelium derived from an oidium, developed on dung-agar. The hypha is 2 μ to 3 μ in diameter. × 400. 20. Masses of oidia which have become attached to a cover-glass by allowing the cover-glass to come lightly into contact with the surface of a haploid mycelium growing on a dung-agar plate. Each mass of oidia is held together by dried-up oidiophore fluid. × 400. 21. To show the fusion of the oidial germ-tubes of the

produced them before they begin to form new oidia, as shown in Text-fig. 17 *g*, *h*, *i*. Often these germ-tubes grow out of the medium into the air, and there soon develop into oidiophores bearing the usual drop of fluid containing oidia (Text-fig. 15). The oidiophores thus produced are usually smaller in size than those produced on ordinary haploid mycelia developed directly from basidiospores. While some oidia develop in the manner just described many others in the same cultures give rise to an extensive mycelium which grows vigorously, spreads indefinitely through the medium, and produces great numbers of aerial oidiophores bearing the usual oidial drops.

The oidia which are developed on a mycelium derived from a basidiospore may be considered as *first-generation* oidia. If one sows these oidia, the mycelium which they produce soon develops a fresh crop of oidia—*second-generation* oidia. If one sows second-generation oidia one can obtain *third-generation* oidia ; and so forth. Twelve successive generations of oidia were thus obtained, and the twelfth-generation oidia did not differ appreciably in size and power of germination from those of the first generation.

The technique for sowing the twelve successive generations of oidia was as follows. A drop of cleared dung-agar was placed on the under-side of a sterilized cover-glass and then the drop was brought lightly into contact with the surface of a haploid mycelium growing on dung-agar in a Petri dish. The cover-glass was then set on the glass ring of a van Tieghem cell which contained a little water. The germination of the oidia required about eight hours, and in the next four hours the germ-tubes and mycelia produced new oidia in abundance. From one to a few days elapsed between the sowings of any two successive generations of oidia.

The mycelium derived from a basidiospore, No. 5 (*ab*), which produced the first of the twelve successive generations of oidia, developed clamp-

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oidia *a* and *c* of one sex (+) with the hyphae *b* and *d* of a mycelium derived from a basidiospore of opposite sex (−). 22. Diagram of an apparatus used for investigating the relation of flies with the oidia of *Coprinus lagopus*. Two wide test-tubes *a*, each containing a mass of sterilized horse-dung *c* have been fitted mouth to mouth, and have been bound together by means of the cardboard collar *b*. On one dung-mass is a haploid mycelium derived from a basidiospore of one sex (+), and on the other dung-mass is another haploid mycelium derived from a basidiospore of opposite sex (−). The (+) mycelium has developed (+) oidia at its surface, and the (−) mycelium (−) oidia. Some *Drosophila* flies *d* have been introduced into the chamber. They carry (+) oidia from the (+) mycelium to the (−) mycelium and (−) oidia from the (−) mycelium to the (+) mycelium, with the result that both the (+) mycelium and the (−) mycelium become diploidized. One-third the actual size. 23. Diagram of another apparatus used for investigating the relation of flies with the oidia of *Coprinus lagopus*; *a*, a large glass crystallizing dish with a glass cover *c* under which a slip of paper *b* has been inserted to make a slight space between cover and dish with a view to ventilation; *d*, one section of a small Petri dish; *e*, a horse-dung ball on which is growing a mycelium derived from a basidiospore of one sex (+), and *g*, another horse-dung ball on which is growing a mycelium derived from a basidiospore of opposite sex (−). The (+) mycelium bears (+) oidia and the (−) mycelium (−) oidia. Some *Drosophila* flies *f* have been introduced into the chamber. They carry (+) oidia from the (+) mycelium to the (−) mycelium and (−) oidia from the (−) mycelium to the (+) mycelium, with the result that both the (+) mycelium and the (−) mycelium become diploidized. One-third the actual size.

connexions when paired with another mycelium derived from a basidiospore, No. 10 (*AB*). The mycelium derived from the first-generation oidia and the mycelium derived from the second-generation oidia also developed clamp-connexions when paired with mycelium No. 10 (*AB*). Hence the two mycelia derived from the oidia had the same sexual constitution (*ab*) as the mycelium No. 5 from which they were primarily derived. From these observations we may conclude that successive generations of oidia are all of one and the same sex.

VII. The Mycelium produced by Oidia.

For each of the haplonts Nos. 5 (*ab*) and 10 (*AB*), the mycelium derived from a *basidiospore* and the mycelium derived from *oidia* have been carefully compared with a view to finding out what differences, if any, there are between them.

As a result of the comparison it has been found: (1) that, whereas the individual hyphae of a basidiosporous mycelium are about 5 μ thick, those of an oidial mycelium are only about 2 μ thick, and are therefore relatively *very fine* (*cf.* Text-figs. 18 and 19); (2) that, under the same culture conditions, an oidial mycelium *grows more slowly* over the culture medium than a basidiosporous mycelium; (3) that, whereas a basidiosporous mycelium produces many aerial hyphae—on this account becoming fluffy—an oidial mycelium, except for its oidiophores, *produces no aerial hyphae* whatever; (4) that, whereas a basidiosporous mycelium produces fruit-bodies (haploid and usually imperfectly developed), an oidial mycelium *remains sterile indefinitely*; (5) that an oidial mycelium produces *far more oidiophores* per unit area than does a basidiosporous mycelium; (6) that, whereas a basidiosporous mycelium produces its oidiophores always aerially or in a film of moisture on the surface of the medium, an oidial mycelium produces oidiophores not only aerially or in a film of water on the surface of the medium, but sometimes also *beneath the surface of the medium*; and (7) that, whereas the oidiophores of a basidiosporous mycelium (at least when first formed) are never freely branched, those of an oidial mycelium are *frequently branched* (*cf.* Text-figs. 1–5 and 16).

Evidence of (4) the sterility of an oidial mycelium so far as fruit-bodies are concerned was obtained as follows. Oidia taken from the haploid mycelium No. 5 were sown on sterile dung balls in crystallizing dishes and in wide test-tubes (3 × 1 inch). At the end of twenty-four hours the oidia had grown into a fine white oidial mycelium, and this eventually covered the whole surface of the substratum and produced many thousands of oidiophores. These cultures were kept under observation for three months, yet, during this long period, they showed not the least sign of producing fruit-bodies. On the other hand, a mycelium derived from a basidiospore,

after being set on a sterile dung ball, usually fruits within ten to fourteen days.

In comparative cultures, a glance is sufficient to convince one of the fact that oidial mycelium produces far more oidiophores per unit area of the culture medium than does a basidiosporous mycelium. It was observed that, whereas a basidiosporous mycelium rarely produces more than fifteen oidial fructifications per square millimetre, an oidial mycelium may produce upwards of sixty-five oidial fructifications per square millimetre.

Oidia of opposite sex were sown together in the following manner. A drop of cleared dung-agar was suspended on the under-side of a cover-glass and the drop was allowed to come lightly into contact first with the surface of an agar plate on which the haploid mycelium No. 5 (*ab*) was growing and then with the surface of an agar plate on which the haploid mycelium No. 10 (*AB*) was growing. The cover-glass with the agar on the under-side was then placed on the glass ring of a van Tieghem cell containing a little water. The oidia germinated in the usual manner, and a network of mycelium was produced in which there were many fusions between the individual hyphae. The mycelium which developed after the fusion of germ-tubes of oidia of opposite sex was relatively fine like mycelium derived from oidia of one sex, and it did not develop any clamp-connexions. Many of the hyphae of the network bore the usual oidiophores and oidia.

Oidia taken from the haploid mycelia Nos. 6 and 2 (sexually opposite) were also sown together, with the same result as before: no clamp-connexions appeared on the mycelium developed from the oidia.

It has not been possible up to the time of writing to repeat the above experiments or to investigate the matter further. However, the results of the experiments so far made seem to indicate that, for *C. lagopus*, haploid mycelia of opposite sex derived from oidia of opposite sex do not unite with one another to form a diploid mycelium, and that a diploid mycelium arises through the agency of oidia only when oidia of one sex come into contact with a mycelium of opposite sex derived from a basidiospore.

VIII. The Effect of Sowing Oidia on a Haploid Mycelium of Opposite Sex.

When (+) oidia (oidia of one sex) are deposited on a (−) mycelium (a mycelium of opposite sex) they germinate, and the germ-tubes fuse with the (−) mycelium (Text-fig. 21), and the (−) mycelium is converted into a diploid mycelium. Conversely, when (−) oidia are deposited on a (+) mycelium they germinate, and the germ-tubes fuse with the (+) mycelium, and the (+) mycelium is thereby converted into a diploid mycelium. The evidence which led to these conclusions will now be adduced.

A fragment of the haploid mycelium No. 10 (*AB*) was transferred to

malt-agar in a Petri dish where it was allowed to grow until it was about 4 cm. in diameter. A platinum loop was then touched lightly to the surface of a culture of the haploid mycelium No. 5 (*ab*), and oidia from this mycelium adhered to it. The loop with the oidia on it was then touched to the surface of the agar in the plate containing the mycelium No. 10 (*AB*) at a point just in front of the leading hyphae.

Three days after the (*ab*) oidia were added to the plate at the periphery of the (*AB*) mycelium all the leading hyphae of the (*AB*) mycelium had become diploid, as was indicated by the presence of clamp-connexions on them.

The experiment just recorded was successfully repeated four times, i. e. in each experiment mycelium No. 10 was diploidized [1] through the agency of oidia derived from mycelium No. 5.

In another experiment oidia from mycelium No. 10 (*AB*) were sown at the periphery of mycelium No. 5 (*ab*); and the haploid mycelium (*ab*) was soon diploidized thereby.

So many oidia were used as inoculum in the two previous sets of experiments that it was found very difficult to observe clearly just where the germ-tubes from oidia fused with the 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 experiments. The oidia were watched continuously, and in several places the germ-tubes were seen to *fuse with the spore-mycelium*, as illustrated in Text-fig 21.

In another set of experiments, oidia from an (*AB*) mycelium, which was growing on a dung ball in a crystallizing dish 12 cm. wide, were gathered on a sterile platinum loop by touching the loop lightly to the mycelium. The oidia were then deposited on an (*ab*) mycelium growing on a dung ball in another crystallizing dish; and, conversely, the oidia from an (*ab*) mycelium were deposited on an (*AB*) mycelium. Both the (*AB*) mycelium and the (*ab*) mycelium became diploid within a week. Control haploid mycelia, which were grown on dung balls in crystallizing dishes but were not inoculated with oidia, remained haploid. This experiment was repeated several times, always with the same result.

The foregoing experiments definitely prove that oidia of one sex are capable of causing a haploid mycelium of opposite sex to become diploid.

IX. Wind and Rain and the Dispersal of Oidia.

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

[1] For the first use of the terms *diploidize* and *diploidization process*, *vide* A. H. R. Buller (5).

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 an oidial drop is in the moist condition it can be detached from its oidiophore by *violent* shaking. This was demonstrated as follows. The bottom section of a dung-agar Petri dish in which a large haploid mycelium bearing numerous oidiophores was growing was inverted over the bottom section of another Petri dish containing freshly-poured dung-agar. Then the inverted upper Petri-dish section was violently struck a number of times with the hand. A few of the oidial masses were thereby detached and fell on to the nutrient agar in the lower dish, where they germinated and produced new oidial mycelia. 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.

The experiments just described indicate clearly that the oidia of *C. lagopus* are not normally dispersed by the wind.

Since the drops in which the oidia are developed are soluble in water when they have just been excreted and have not dried up, it is obvious that rain may scatter the oidia locally. However, rain is a phenomenon of uncertain occurrence and must tend to wash the oidia to the ground rather than to transport them from place to place.

X. The Transportation and Deposition of Oidia by Flies and the Action of the Deposited Oidia on Haploid Mycelia.

The oidial drop on an oidiophore is very mucilaginous and adheres readily to any object brought into contact with it. When a cover-glass is lowered so as to touch the oidial drops on a mycelium they come away from their oidiophores intact. The end of a glass rod, after having been rubbed lightly over the surface of a haploid mycelium, is covered with oidial fluid and oidia. Oidial drops can also be readily removed from their oidiophores by means of a platinum loop.

It seemed probable that, if an insect were to come into contact with some oidial fructifications, the oidia would adhere to its legs and body. The experiments described in the following pages show that insects actually do transport oidia from one place to another.

The species of insect used in these experiments was *Drosophila melanogaster*, the celebrated fly employed by Morgan and his pupils for the study

of genetics. The flies were reared in pint sealers (glass jars) plugged with cotton-wool. Over-ripe banana was provided as food.

The bottom section of a Petri dish in which a haploid mycelium (*ab*) was growing on a layer of dung-agar was fitted against the bottom section of another Petri dish containing a layer of sterile dung-agar, and a fly was introduced into the chamber thus formed. The two Petri dish sections were then fastened together by means of a wide strip of adhesive paper pasted around their rims. The fly was allowed to walk over the mycelium contained in one of the plates for half a minute. Then the chamber was suddenly held up vertically and turned by the hand, so that light from a window streamed through the base of the plate containing the sterile agar. Immediately the fly, in response to the heliotropic stimulus, left the plate containing the mycelium for the plate containing the sterile agar, and here it walked about over the surface of the medium. The plates were then disjoined, the fly was removed, and a cover was placed over the plate which, until the advent of the fly, had contained sterile agar.

One day after the fly had walked on the sterile plate the bacteria which had been deposited from the fly's feet on the agar had formed bacterial colonies. These colonies clearly marked out the track of the insect. On the agar surface where the fly had walked large numbers of oidia were observed with the microscope. These germinated and developed mycelia which soon pushed their way far beyond the bacterial colonies and rapidly developed new oidiophores and oidia. Five days after the fly had walked over the plate the plate was photographed, and the photograph, reproduced in Plate X, Fig. 1, clearly shows the fly-tracks with bacterial colonies down the centre and oidial mycelium growing outwards on all sides. Some of this mycelium was removed and paired with some (*AB*) mycelium derived from a basidiospore, with the result that clamp-connexions were formed. It was thus proved that the oidial mycelium had the constitution (*ab*), i. e. the same constitution as the mycelium over which the fly had walked. The fly which had walked over the (*ab*) mycelium was examined under the microscope, and masses of oidia were found clinging to its feet, especially to the tarsal claws and the hairs on the tarsi.

The experiment just described shows conclusively that flies can transport oidia and that the oidia, after transportation, can germinate and produce mycelia.

In another experiment six flies were allowed to go without food for twelve hours. They were then put into 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 each hungry fly began to suck up the oidial drops with its proboscis. The lower end of the proboscis of the Drosophila

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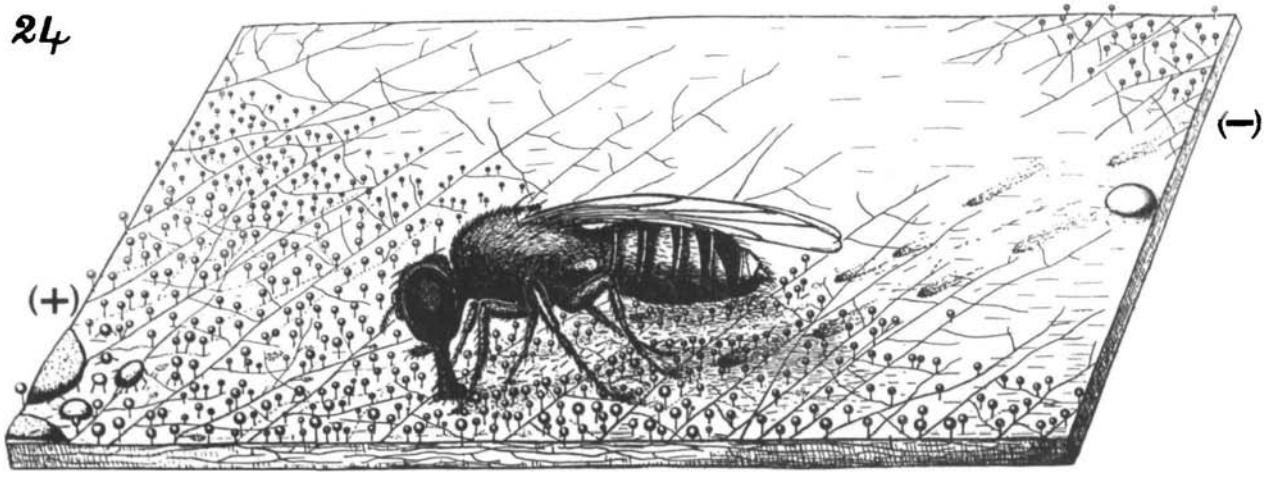

TEXT-FIG. 24. A diagram to illustrate the relation of flies with the oidia of *Coprinus lagopus*. A (+) mycelium (mycelium of one sex), derived from a basidiospore, and a (−) mycelium (mycelium of opposite sex), also derived from a basidiospore, are growing near one another on dung-agar in a Petri dish. Only a small portion of each mycelium is shown. Both of the mycelia have produced numerous aerial oidiophores, each crowned with a drop containing many free-floating oidia. At the extreme left in the foreground are some large masses of oidial fluid. These have been formed by the fusion of oidial drops which have grown and touched one another and the surface of the agar. A fly (*Drosophila melanogaster*) has walked over the (−) mycelium (right background) and has carried (−) oidia on its feet and proboscis to the (+) mycelium. The (−) oidia thus deposited will germinate, their germ-tubes will fuse with the hyphae of the (+) mycelium and, as a consequence, the (+) mycelium will be converted into a diploid mycelium. The fly has deposited a drop of excrement on the surface of the agar between the two mycelia. This drop contains large numbers of oidia which will germinate and produce a mycelium. The fly is now walking over the (+) mycelium and is engaged in sucking up the oidial drops on the tops of the oidiophores. As it feeds, it presses down the oidiophores and so leaves a trail which marks its passage. Oidial drops are clinging to the last segment of the hind legs and will be deposited elsewhere when the fly moves on. The oidia which the fly is now taking into its alimentary canal will be deposited in a viable condition in its excrement. × about 20.

fly is about 0·3 mm. wide, while the oidial fructifications are about 0·1 mm. in length, and the oidial drops about 0·05 mm. in diameter. The end of the fly's proboscis, therefore, touched perhaps a dozen oidial drops at the same time. The flies walked along, vigorously sucking up the drops from the oidiophores and pressing down the oidiophores on to the agar surface, thus leaving a cleared trail through the projecting oidial fructifications (Text-fig. 24). Often a fly stopped feeding to rub its proboscis with its feet and so clean off the adherent liquid.

A clean glass plate was now substituted for the cover of the Petri dish and the Petri dish was inverted to allow the flies to walk on the glass plate. The flies were watched, and as soon as several drops of excrement had been deposited which had not been touched by the flies' bodies or feet the plate was removed (a fly-drop is shown on the right-hand side of Text-fig. 24).

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

The foregoing observations justify the conclusion that flies transport oidia in two ways: (1) on their legs and bodies; and (2) 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.

Experiments will now be described in which it will 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 the first set of experiments (Text-fig. 23) two balls of fresh horse-dung were placed as far apart as possible (a distance of 12 cm.) in each of four large crystallizing dishes, 23 cm. in diameter and 8 cm. deep, the bottoms of which were lined with moistened filter-paper. 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 crystallized dishes. Each ball was placed in an uncovered shallow Petri dish 7 cm. in diameter, an arrangement which prevented the mycelium of one dung ball from growing over the surface of the moist filter-paper in the bottom of the crystallizing

dish and so meeting and mating with the mycelium of the other dung ball. 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.

In each dish one dung ball was inoculated with the haploid mycelium No. 5 (*ab*) and the other with the haploid mycelium No. 10 (*AB*). Two of these dishes were used for experiments with flies and were labelled A and B, and two were kept for controls.

Three days after inoculating the dung balls the mycelium which had developed on each ball covered an area of about two square centimetres. Some Drosophila flies were then removed from the glass jar in which they had been reared to another jar. Here they were etherized, and then eight of the etherized flies were placed in each of the experimental dishes A and B (cf. Text-fig. 23). 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, a piece of the mycelium on each dung ball was mounted in water on a glass slide and examined under the microscope for the presence of clamp-connexions. It was found that the control mycelia were still all haploid, whereas *all the mycelia in the experimental dishes were diploid.* This indicates: (1) that the flies had carried oidia from the mycelium No. 5 (*ab*) and had deposited them on mycelium No. 10 (*AB*) and had carried oidia from mycelium No. 10 and had deposited them on mycelium No. 5; (2) that the oidia must have germinated; and (3) that the germ-tubes or hyphae of the mycelium derived from the oidia must have fused with the mycelium on which the oidia had been deposited.

The experiment just described was successfully repeated four times. However, the apparatus employed was found to be defective in several respects. The large crystallizing dishes were cumbersome to handle, and when a small piece of paper was placed under the glass cover at the edge of each dish to allow sufficient ventilation for the mycelium growing in the dish it was found to be 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 wide test-tubes containing sterilized horse-dung. The dung in each tube was inoculated with a fragment of one of the following stock mycelia: Nos. 1, 2, 5, 6, 9, and 10. Four days after the six tubes of dung had been inoculated their plugs were removed and they were fitted together mouth to mouth in the manner shown in Text-fig. 22, so as to form three chambers. Each pair of tubes contained two mycelia of

opposite sex, and the pairings were as follows: No. 5 (*ab*) with No. 10 (*AB*), No. 6 (*ab*) with No. 2 (*AB*), and No. 1 (*Ab*) with No. 9 (*aB*). Into each of the three chambers six Drosophila flies were introduced, and then the two tubes forming each chamber were bound together by means of a tight cardboard collar 5 cm. wide. The flies were free to wander from the mycelium in one end of each of the chambers to the mycelium at the other end (cf. Text-fig. 22); and they were kept in the chambers for forty-eight hours and then removed. No flies were placed in tubes of each mycelium kept as controls.

The mycelia in the three sets of tubes were examined with the microscope five days after the flies were introduced into the chambers, and all six of them were found to have developed clamp-connexions and therefore to have become diploid. On the other hand, the six mycelia in the control tubes had not developed clamp-connexions and were therefore still haploid.

The experiment was repeated, but this time the flies were allowed to remain in the tubes only fifteen minutes. The results were equally satisfactory: the mycelia visited by the flies became diploid, while the control mycelia remained haploid.

To find out whether or not oidia adhered to the legs and bodies of the flies when the flies were allowed to crawl a greater distance than in the above experiments, some flies were caused to go from a (−) mycelium on dung in a test-tube through a cardboard tube of slightly greater diameter and 3·5 feet long to a (+) mycelium in a test-tube at the other end of the cardboard tube. The flies were allowed to crawl over the (−) mycelium, and the other end of the cardboard tube 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. When the mycelia visited by the flies were examined four days later, they were found to have become diploid, while control mycelia remained haploid.

Further experiments were carried out with pairs of separate dung balls contained in each of the four large crystallizing dishes. One dung ball of each pair in a dish was inoculated with a *haploid mycelium*, while the other dung ball was left *uninoculated* and sterile. Into three of the dishes flies were introduced, but no flies were introduced into the fourth dish, which was kept as a control. At the end of one week it was found that in the control dish the uninoculated dung ball was still quite sterile, whereas in all of the three experimental dishes the uninoculated dung-ball had an oidial mycelium growing upon it. These results are easily explained, on the supposition that the flies in each dish carried oidia from the haploid mycelium of the one dung-ball to the surface of the other uninoculated dung ball and that the oidia there germinated and produced a mycelium.

One may ask: Do flies carry away from a haploid mycelium oidia and oidial fluid only or, in addition, are they able to carry away pieces of the mycelium? To solve this problem two separated dung balls were placed in each of the three large crystallizing dishes and were sterilized as in the first set of experiments in which flies were used (cf. Text-fig. 23). Then one of the dung balls was inoculated with a *diploid* mycelium and the other dung ball was left *uninoculated* and sterile. A diploid mycelium was employed in these experiments because it *does not produce any oidiophores or oidia.* After the inoculation of the dung-balls had been accomplished and the mycelium on the inoculated balls had grown over the dung sufficiently, some flies were introduced into two of the dishes. The other dish, into which flies were not introduced, served as a control. The flies were allowed to remain in the two experimental dishes for ten days.

At the end of fifteen days after the introduction of the flies into the experimental dishes neither in the experimental dishes nor in the control dish was there any mycelium growing on the uninoculated dung ball, and a similar result was obtained in another similar set of experiments made subsequently to the first set.

Had the flies broken off hyphal fragments from the diploid mycelium and deposited them on the uninoculated dung-ball doubtless these fragments would have grown and would have developed into vigorous mycelia; and the fact that, in the experimental dishes, the uninoculated dung balls remained free from mycelium is strong evidence that flies do not remove hyphal fragments from a mycelium. The experiments just described justify the conclusion that, when a fly visits a haploid mycelium, it carries away only oidia and oidial fluid and nothing else.

XI. The Oidia of *Coprinus lagopus* compared with the Pycniospores of the Rust Fungi.

The basidiospores of *C. lagopus*, under natural conditions, germinate in freshly-deposited horse-dung balls. The germ-tubes rapidly develop into haploid mycelia, and these haploid mycelia, some 2–3 days after the germination of the spores, develop oidiophores and oidia. The oidiophores project upwards from the substratum into the air and the oidia are set free into a drop of oidial fluid at the tip of each oidiophore. Even when haploid mycelia of opposite sex intermingle, they continue in the haploid state and produce oidia for 2–5 days after the germination of the spores (*vide* Section IV). The oidia are the first kind of spore to be produced on the young mycelia of *C. lagopus* and, sexually, they are haploid. In these respects they resemble the pycniospores of the Rust Fungi.

The oidia of *C. lagopus* are embedded in a fluid greedily sucked up by flies. This fluid therefore resembles the nectar which encloses the pycniospores of the Rust Fungi.

There can be no doubt that the function of the oidia of *C. lagopus* resembles the function of the pycniospores of such heterothallic Rust Fungi as *Puccinia graminis* and *P. helianthi*, i.e. the oidia, like the pycniospores, after being carried by flies from a mycelium of one sex to a mycelium of opposite sex, germinate and diploidize the mycelium of opposite sex on or near which they have been deposited.

In *C. lagopus*, just as in a heterothallic Rust Fungus, two mycelia of opposite sex, derived from two basidiospores of opposite sex, may (1) develop in contact with one another, or (2) develop far apart from one another. Let us consider how the sexual process is initiated in both these cases.

(1) Let us suppose that two haploid *C. lagopus* mycelia of opposite sex, say (*AB*) and (*ab*), derived from two basidiospores of opposite sex, have begun their development *close together* at or near the surface of a dung ball dropped in a pasture. As the mycelia grow in size they will meet and fuse and mutually diploidize one another; and the oidia will play no part in the diploidization[1] process. The same kind of thing happens in the heterothallic Rust Fungi; for, as Craigie has shown, in *P. graminis* or *P. helianthi*, when two mycelia of opposite sex, derived from two sporidia of opposite sex, develop close together in a leaf so that they come into contact with one another, even when the pycnial nectar containing the pycniospores is left undisturbed diploid aecia are produced under each mycelium.

(2) Let us now suppose that two haploid *C. lagopus* mycelia of opposite sex, say (*AB*) and (*ab*), derived from two basidiospores of opposite sex, have begun their development *far apart* at or near the surface of a single dung ball dropped in a field or one mycelium on one dung ball and the other mycelium on another dung ball. As the mycelia grow, owing to their distance apart it will be impossible for them to meet and fuse and mutually diploidize one another, and they will remain in the haploid condition unless and until flies or other insects carry (*AB*) oidia from the oidiophores of the (*AB*) mycelium to the (*ab*) mycelium and, vice versa, carry (*ab*) oidia from the oidiophores of the (*ab*) mycelium to the (*AB*) mycelium. When flies have thus transferred the oidia, the oidia will germinate and the oidial germ-tubes or mycelia will fuse with the mycelium of opposite sex to which the oidia have been brought and will diploidize it. In the heterothallic Rust Fungi, again, the same kind of thing happens; for, as Craigie has shown, in *P. graminis* or *P. helianthi*, when two mycelia of opposite sex, say (+) and (−), derived from two sporidia of opposite sex, are separated from one another on a single leaf or by one being on one leaf and the other on another leaf, so that they cannot intermingle, flies may transport (+) pycniospores from the pycnia of the (+) mycelium to

[1] For the origin of this term, *vide* A. H. R. Buller (5).

the (−) mycelium, and, vice versa, (−) pycniospores from the pycnia of the (−) mycelium to the (+) mycelium, with the result that diploid aecia are developed beneath each of the two formerly haploid mycelia.

The Hymenomycetes and the Uredineae both belong to the Basidiomycetes and are allied groups. The functional similarity of the oidia of *C. lagopus* and the pycniospores of the Uredineae is so great as to suggest the possibility that the oidia of the Hymenomycetes and the pycniospores of the Uredineae are related phylogenetically.

In one respect there is a marked difference between the oidia of *C. lagopus* and the pycniospores of the Rust Fungi. We know but little about the germination of the pycniospores, but it is certain that the germ-tubes or mycelia produced by pycniospores do not give rise to any further pycniospores. On the other hand, when oidia of *C. lagopus* germinate on sterilized dung-agar or horse-dung the mycelium derived from them soon produces a new crop of oidiophores and oidia; and, if these second-generation oidia are sown, the mycelium derived from them soon gives rise to third-generation oidia; and so forth. It may well be that, under natural conditions, when oidia are transported by flies to freshly-dropped dung balls, such oidial multiplication actually takes place.

In the Rust Fungi it seems certain that pycniospores, through the germ-tubes or mycelia to which they give rise, can cause diploid aecia to be developed only on mycelia of opposite sex derived from sporidia. As already recorded, in two experiments with *C. lagopus* where oidia of opposite sex were sown together in the same culture medium, the resultant mycelia of opposite sex failed to diploidize one another. Further experiments are needed to decide whether or not the results of these two experiments are normal. If they are normal, the mycelium derived from the oidia of *C. lagopus* is able to diploidize only those mycelia which have been derived from basidiospores, in which case the oidia behave like the pycniospores of the Rust Fungi.

Ráthay (17), in 1883, in Austria, identified 135 species of insects which visited the pycnia of various Rust Fungi; but, doubtless, a still greater number of species of insects visit recently-dropped horse-dung. The oidiophores and oidia of *C. lagopus* begin to appear on horse-dung two or three days after its deposition, and at this time insects can often be seen crawling over the dung in large numbers. It was noticed that, when fresh horse-dung is exposed on the laboratory table and Drosophila flies are about, the flies are soon attracted to the dung, doubtless by its odour. Under natural conditions in the open not only flies but many species of beetles, especially Staphylinidae and Scarabeidae, frequent horse-dung; and in all probability these beetles, like flies, transport oidia from place to place. We may conclude that the insect agents required to carry the oidia of *C. lagopus* to mycelia of opposite sex are usually abundantly present in

pastures at the time when the haploid mycelia may be benefited by their services.

XII. Summary.

1. The oidia of *C. lagopus* are produced on oidiophores which project away from the substratum into the air, and they are developed in, and are set free into, a drop of adhesive liquid which is of importance in the dissemination of the oidia.

2. The oidia of *C. lagopus* are produced only on haploid mycelia, never on diploid mycelia. On a mycelium derived from a spore oidia are produced only aerially or on the surface of the nutrient medium, never under the surface of the medium.

3. When many spores of *C. lagopus* of diverse sex are sown together in a culture medium, the haploid mycelia, in the first few days of their growth, produce oidiophores and oidia in abundance but do not fuse with one another. Then mycelia of opposite sex fuse with one another and mutually diploidize one another with the result that the production of oidiophores and oidia ceases.

4. Oidia are formed from the oidial branches at the apex of the oidiophore by: (1) the division of the protoplasm of the branch into two or three separate masses; (2) the shrinking or condensation of these masses with the formation of short water-filled gaps or spaces between them and at the base of the oidial branch; (5) the development of a wall at each end of each shrunken mass of protoplasm; and (4) the dissolution of the wall of the oidial hypha around the water-filled spaces.

5. The oidia of *C. lagopus*, under suitable conditions, germinate readily. Their germ-tubes, in culture media, develop into a mycelium which soon produces numerous oidiophores and oidia. By sowing oidia, twelve successive generations of oidia were obtained.

6. In *C. lagopus*, the mycelium developed from an oidium, as compared with a mycelium derived from a basidiospore, has thinner hyphae, produces more oidial fructifications per unit of surface, develops no aerial hyphae except oidiophores, grows more slowly, and remains sterile so far as ordinary fruit-bodies and fruit-body rudiments are concerned. Haploid mycelia derived from basidiospores, when grown separately on sterilized horse-dung, usually produce haploid fruit-bodies in ten to fourteen days, whereas a mycelium derived from oidia grew on sterilized horse dung for three months without any sign of fruit-bodies appearing.

7. Oidial fructifications of *C. lagopus*, associated with ordinary fruit-bodies of *C. lagopus*, were found on unsterilized horse-dung on four occasions. Thus, possibly for the first time, the oidia of a hymenomycetous fungus have been observed and recognized as such under natural conditions.

8. The oidia of *C. lagopus* are not transported by the wind; they may be scattered to a limited extent by rain, but they are mainly disseminated by coprophilous insects and, in particular, by flies.

9. Flies use the oidial drops on the oidiophores of *C. lagopus* as food. They carry away oidia on their feet and bodies, and they deposit oidia in a viable condition in their excreta.

10. From the mycelia of *C. lagopus* flies carry away oidial fluid and oidia only, and not any hyphae.

11. It has been proved experimentally for *C. lagopus* that, when flies walk on a (+) mycelium (mycelium of one sex) and then on a (−) mycelium (mycelium of opposite sex), they transfer (+) oidia to the (−) mycelium and (−) oidia to the (+) mycelium, with the result that both the (+) mycelium and the (−) mycelium become converted into diploid mycelia. The diploidization of haploid mycelia through the agency of flies took place when two mycelia were separated by a distance of 3·5 feet.

12. The oidia of *C. lagopus* very closely resemble the pycniospores of the Uredineae in their early appearance on haploid mycelia, in their being immersed in a fluid which is attractive to flies, in their transportation by flies from one mycelium to another, and in their ability to bring about the diploidization of mycelia of basidiosporous origin and of opposite sex.

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 entire course of the work, and his stimulating criticism and helpful advice are gratefully acknowledged.

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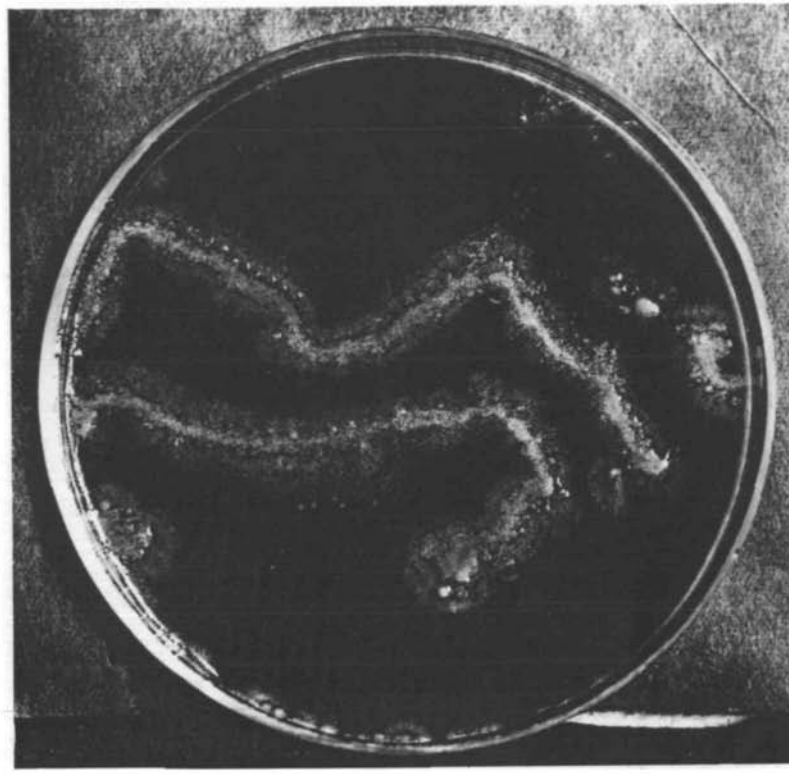
EXPLANATION OF PLATE X.

Illustrating Mr. H. J. Brodie's paper on the Oidia of *Coprinus lagopus* and their Relations with Insects.

Fig. 1. The dissemination of oidia of *Coprinus lagopus* by a fly (*Drosophila melanogaster*). A fly was allowed to walk for half a minute over a haploid mycelium (*ab*) contained in a Petri dish and then over a freshly-poured dung-agar plate where it made two main tracks with its body and feet. This second plate was photographed five days later, and the photograph is here reproduced. The fly deposited on the agar from its feet and body great numbers of oidia taken up from the mycelium (*ab.*) and also bacteria with which it was contaminated before walking over the Petri dishes. The main tracks of the fly over the agar are now clearly marked out by bacterial colonies down the centre and mycelia growing away from the bacterial colonies on all sides. The mycelia originated from the oidia deposited by the fly, and they are already developing a new crop of oidiophores bearing the characteristic oidial drops. By mating experiments it was found that the mycelia had the same sexual constitution, namely (*ab*), as the mycelium in the first of the two Petri dishes visited by the fly. Natural size.

Fig. 2. Photomicrograph of a haploid mycelium, No. 10 (*ab*), of *Coprinus lagopus*, to illustrate the production of oidia. The mycelium had grown for seven days in a hanging drop of malt-agar. The photomicrograph was then taken through the cover-glass and the culture medium. The drops, which appear as black balls, are all filled with oidia, and they are borne on the ends of odiophores projecting into the air. The very numerous oidia in the film of moisture at the surface of the culture medium arrived at their present position: in part by oidial drops coming into contact with the film of moisture, in part by being developed from the first on oidiophores lying in the film of moisture, and in part by floating about in the film of moisture. Magnification, 100.



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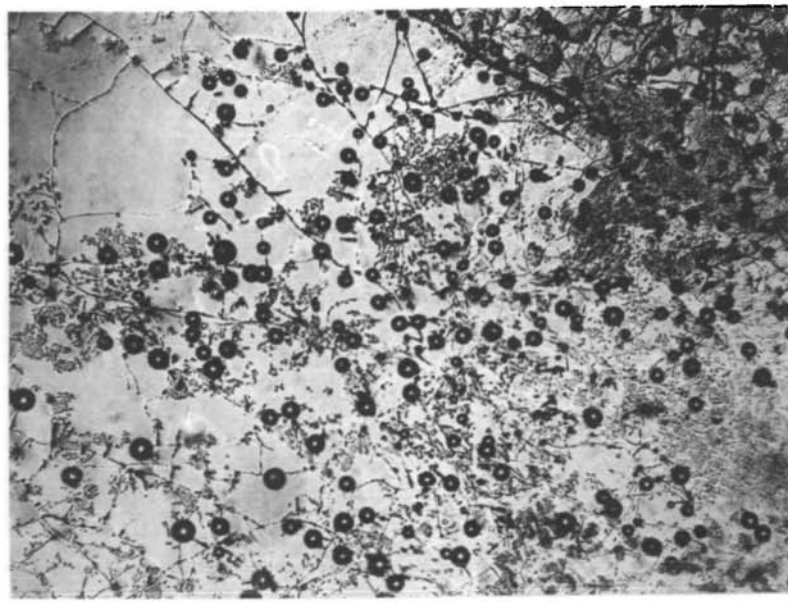

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Huth, coll.

BRODIE — OIDIA OF COPRINUS LAGOPUS.