The Fungi of the Bee-Hive.

BY

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With 28 Figures.

The fungus-flora of the bee-hive is doubly of interest. From the point of view of the mycologist, it might be expected that a habitat providing such unusual conditions, as regards temperature and substratum, would contain some interesting forms. From that of the bee-keeper it is very desirable that our knowledge of the organisms found in the hive should be as thorough as possible, in order to facilitate the study and suppression of diseases of the honey-bee. In spite of these considerations, it appears that the bee-hive fungi have never been thoroughly worked out, though various species have from time to time been recorded as present in hives, mostly in connection (or supposed connection) with bee-diseases.

The following is believed to be a full account of all the hive-fungi hitherto described. Since many of the records occur in non-mycological periodicals, the descriptions are in many cases inadequate or even wanting; these cases have nevertheless been included, for the sake of completeness.

Berkeley and Broome (3) in 1854 described a new species, *Oidium favorum,* as follows:—"Floccis erectis septatis, sporis flavis brevibus subcylindricis. On honey-comb, near Woolwich, Mrs. Col. Jones. Flocci erect, white, septate and slightly torulose below, above bearing a few short cylindrical yellow spores. These spores when fallen seem to acquire a septum, and then to be gradually attenuated at either end. A new septum is then formed in each division, constituting an irregularly fusiform body." Cooke (5) follows Berkeley and Broome in his account of this species. Saccardo (25, iv, 22) transfers it to the genus *Oospora.* His diagnosis is: "Hyphis erectis septatis; conidiis luteis, brevibus subcylindricis ultimis sphæroideis. Hab. in favis Apum in Britannia." Massee (22) describes *Oospora favorum* as follows: "Tufts minute, white,

unconspicuous, hyphae branched, intertwined, septate, fertile branches erect bearing short chains of yellow, subglobose conidia, 4-5 μ. . . . On honey-comb. Rare (Type in Herb. Berk. Kew). An examination of type specimen shows the conidia subglobose and concatenate in short chains. When quite young the conidia are filiform."

Dönhoff and Leuckart in 1856 discovered a parasite in the chyle-stomach of the honey-bee. This organism was described by Hoffmann (15), and named *Mucor melittophthorus*; a supposed "oidium-form" being designated *Oidium leuckarti*. It has recently been pointed out[1] that the supposed *Mucor* spores were in all probability a stage of the protozoan parasite *Nosema apis*; and it is fairly obvious from Hoffmann's figures and description that he mistook detached cells of the stomach-epithelium full of *Nosema* spores for sporangia. *Mucor melittophthorus* must therefore be struck off the list of the Fungi. *Oidium leuckarti* apparently consisted of fragments of mycelium, which it is impossible to identify. These may have been merely ingested by the bees, but it is possible that the fungus was growing in the stomach (*cf.* Graham-Smith and Bullamore's experiment (12, p. 85) where *Aspergillus niger* and *Penicillium* were in some cases apparently induced to grow in the stomachs of *Nosema*-infected bees).

Higgins (14) in 1858 published an account of the death of some bees, caused, it was presumed, by a fungus. His description is, unfortunately, insufficient to determine the species which made its appearance on the dead bees, and which he did not name.

Preuss (24) in 1869 found *Penicillium crustaceum* to be common in hives in the spring.

Zorn and Hallier (30) in 1870 found *Thamnidium elegans* and *Myxotrichum chartarum* growing on diseased brood-combs. They erroneously supposed that these fungi were the cause of "foul-brood."

Cowan (6) in 1881 described a bee-disease, which occurred in Denmark in 1880, and appears to have been due to a fungus which was either a species of *Claviceps* or of *Cordyceps*. The drone-brood was the first to be attacked; the disease spread to the worker-brood; and finally the adult bees became diseased. The pupae dried up in their cells, and were permeated by mycelium. Stromata were subsequently developed. It is noted that ergot of rye was very

[1] W. Hein, in a paper read at the Conference of German, Austrian and Hungarian bee-keepers, at Constance, August 8th, 1911. *Nosema apis* appears to be the causal organism of the "Isle of Wight" bee-disease (*11*).

plentiful in Denmark that year, and it is suggested that this was the source of the disease. Lack of material prevented a continuation of the work, so that the exact species concerned was not determined.

Bennemann and Hübner (2) in 1881 described *Mucor mucedo* as the cause of a bee-disease. According to their figures, this species had branched sporangiophores, a smooth sporangium-wall and globose spores. No dimensions are given. The *Mucor* was itself attacked by a parasite, a species of *Chaetocladium* (?); and the authors mention *Penicillium glaucum* as being also present on the bodies of the dead bees.

Howard (16, 17) in 1896 and 1900 recorded several fungi as present on diseased combs examined by him. Among them were *Penicillium glaucum,* an *Aspergillus* ("*Aspergillus pollinis*"), a species of *Mucor, Dactylium roseum,* species of *Hendersonia* and *Massaria,* and others. I have unfortunately been unable to see the 1896 paper, so cannot give any particulars as to the dimensions, etc., of *Aspergillus pollinis,* which, I believe, are there given.

Maassen (20) in 1906 described *Aspergillus flavus* as the cause of the bee-disease known in Germany as "Steinbrut." The course of this malady is somewhat similar to that of the disease described by Cowan (6). The brood becomes mummified and permeated by mycelium; the adult bees succumb later, at about the same time that the fungus on the dead brood develops its conidial stage. Hein (13) describes the disease; and adds (l.c., foot-note, p. 7) that, in a case of "Steinbrut" investigated by the Royal Institute for Bee-keeping at Erlangen, Bavaria, in 1910, the causal fungus was *Aspergillus fumigatus.* A case, evidently of the same disease, is described in the *Bienen-Zeitung* of 1860, p. 232. The species of fungus concerned was not, however, ascertained.

Material and Methods.

The present research was begun in 1909, and some knowledge of the species of fungi present in bee-hives was gained by examination of specimens of mouldy comb from healthy stocks. Reliable methods of sterilization were not adopted till July, 1910, however; and the results given in this paper are based on work done in 1911 and 1912, the material being nearly all derived from the combs of stocks which died during the winters of 1910-11 and 1911-12, of the prevalent "Isle of Wight" bee-disease.

Cultures were started by transferring samples of fungus from the combs, with sterilized implements, to test-tubes (more rarely to

Petri dishes or flasks) containing various nutritive media. In some cases, pure cultures were at once obtained; more usually, several sub-cultures had to be made before the species were separated. Drop cultures were made use of occasionally, chiefly in order to watch the germination of spores. Since December, 1911, the poured plate method has been almost exclusively employed in making cultures direct from the combs.

All the 1911 test-tube cultures were reliable, the tubes being sterilized as described below. The Petri dishes and flasks were unfortunately not sterilized with adequate thoroughness.[1] In the few cases, however, where my cultures of a species were originally derived from a culture made in a flask, Petri dish, or drop-culture cell (and consequently not absolutely reliable), subsequent work with poured plates (as well as an examination of combs) has verified the presence of the fungus on the combs, thus confirming the 1911 results.

Throughout the course of the work on which this paper is based, the methods of sterilization were as follows. All glass-ware (except drop-culture cells) was sterilized in an oven, test-tubes and flasks being previously plugged with cotton wool. After being filled with a suitable quantity of the nutritive medium, all tubes, whether intended for sloped tubes or for poured plate work, were sterilized by boiling in water for at least twenty minutes on three successive days (time being reckoned from beginning of ebullition of the water).[2] Forceps, wires, etc., were sterilized in a spirit-lamp flame.

The usual precautions were observed when removing and replacing the cotton wool plugs of culture-vessels.

The following media were used:—

Pollen-decoction. This was made by boiling in water pieces of comb in which the bees had stored pollen, and straining repeatedly till the wax, cocoons, and bulk of the pollen-grains were removed. It was made up with 1 to 2 grammes of bar agar-agar per 100 ccm. of decoction, or with gelatine,[3] and was usually left acid. This medium was used a good deal; but has latterly been for the most part abandoned in favour of the honey media.

Honey, diluted with 3 or 4 times its volume of water, was found to be an excellent medium for most of the fungi studied. It was generally made up with gelatine, but agar was also used. An

[1] The medium was boiled, and poured into the dishes and flasks, which were then used for cultures without further sterilization. They were nearly always kept for some days before use.

[2] The flasks used during 1912 were also treated in this manner.

[3] In all cases where gelatine was used, the proportions were: 10 grammes gelatine (Gold Label) to 100 ccm. of liquid.

attempt was made to cultivate the majority of the fungi on nearly pure honey, but it was not successful. This result was to be expected; as honey in the hive is very rarely, if ever, attacked by fungi (other than yeasts).

Pollen, strained out of the pollen-decoction, freed as far as possible from wax and cocoons, and with a little agar (less than 1 per cent.) to stiffen it, was used on one occasion for some of the fungi.

Prune-decoction was prepared according to Duggar's recommendation (9), 12 grammes of dried prunes to 100 ccm. of water. It was made up with either 1 or 3 grammes of agar per 100 ccm.; gelatine was tried, but presented no advantages. This medium was generally neutralized, and was frequently used with the addition of 2 ccm. of litmus solution to every 100 ccm. as a test for the production of acid.

Sugar-solution (10 grammes sugar to 100 ccm. water) with 2 grammes agar and 2 ccm. litmus solution per 100 ccm., was also used.

In addition to these, the following were experimented with in the case of several of the fungi :—

Bread, potato, apple, milk gelatine, bouillon agar, rice, sterilized horse-dung, decoction of horse-dung made up with agar, and portions of brood-comb (chiefly wax and cocoons) boiled down and sterilized.

Owing no doubt to the use, as a rule, of acid media, bacteria did not give any trouble; but many cultures were rendered useless by being overgrown with *Penicillium* or *Citromyces*, which were very plentiful on the material.

The fungi were tested for cuticularization as follows. Specimens were placed in drops of concentrated sulphuric acid, hydrochloric acid, and saturated caustic potash solution, on slips, and covered with cover-glasses. The effects of these liquids were noted, both when cold, and after heating over a flame till bubbles were expelled.

An incubator not being at my disposal, accurate determination of the germination-optima was not possible. Some experiments were however made with a view to getting some idea of the behaviour of the various fungi at different temperatures. Three exactly similar cultures of each species were started; one being exposed to temperatures ranging between 26°—42° C., the second to room-temperature (15°—19° C.), and the third being placed out of doors.[1] This experiment was repeated, in some cases more than once; the results agreed together very fairly.

[1] Mild winter weather; or during cool weather in May.

All measurements were made from pure-culture material (except in the case of *Gymnoascus setosus* and *G. ruber,* when the material was taken direct from the combs, attempts to cultivate these species not having been very successful).

Stains were used but little. Some specimens were stained with Haidenhain's Haemotoxylin in order to make more evident the structure of the young fruiting stages. Hoffmann's Blue was also employed occasionally.

The Relation of the Fungi to their Habitat.

It is difficult to determine which of the species here described are true bee-hive fungi; and which are merely casual saprophytes, only able to gain a footing in the hive after the death of the bees. Light may perhaps be thrown on the question by the consideration of the conditions prevailing in the hive, and of the behaviour of the fungi when growing at different temperatures and on various media; some account of the former matter will therefore not be out of place here.

The temperature in a bee-hive while the colony is active is maintained at 32°—34° C.[1]; in the winter months, when the bees hybernate in a cluster and breeding is at a standstill, the temperature appears to be about 12° C. in the cluster,[2] and is of course lower in the outer parts of the hive. There are no very exact determinations of the hygrometric state of the hive-atmosphere extant (so far as I can find); but there seems to be little doubt that it is decidedly dry. During the summer months the air is being continually changed, partly by convection, but chiefly by the bees' own efforts in "fanning" at the entrance, and so drawing the stale air out of the hive. In the winter the air is doubtless changed more slowly; but can evidently never be quite stagnant so long as the colony is alive. In a hive where the bees have died, the conditions are considerably different. The air is stagnant, and probably damper than in a healthy stock; and the temperature is that of the outside atmosphere. These conditions were reproduced with fair accuracy in the cultures referred to on p. 4, which were placed out of doors. The tubes were provided with rubber caps; in spite of this, the plugs became saturated with moisture in most (if not all) cases. The cul-

[1] M. Parhon. Ann. Sci. Nat. (Zool.), Series 9. Vol. IX, p. 39.

[2] Parhon gives 32°C. as the winter temperature (in the cluster), but the balance of opinion seems to be in favour of 12°C. Should the bees be disturbed, the temperature will rise temporarily to 32° (Tseselsky, Revue Int. d'Apiculture, 1894); whence perhaps Parhon's result, the insertion of the thermometer having disturbed the cluster.

tures at room temperature and at 26°—42° C. also had rubber caps; it is probable that they did not at all accurately reproduce the conditions prevailing in the healthy stock, perhaps because the air in them was too moist. At any rate, fungi which are known to be capable of growth (or at the least of survival in the form of spores) in the hive in summer, were killed in the high temperature series, even in cases where the maximum temperature attained probably did not exceed 38° C.

Fungi are found growing on various substrata in the hive. The stored pollen is perhaps the chief; it is the chosen pabulum of *Pericystis alvei* (4), and possibly of other species also. The most luxuriant and conspicuous fungous growth in the hive is usually to be found upon it. The rubbish, consisting largely of fragments of waste wax, which accumulates on the hive-floor, is full of spores, and is often over-grown by a film of mycelium. These are the only situations in which fungi can establish themselves in healthy stocks; dead bees, which form an excellent substratum, are usually ejected from the hive before any fungi can develop on them. In hives where the bees have died, however, almost any of the contents may become mouldy. (The exception is the honey, which appears to be immune from the attacks of fungi other than some yeast-forms which cause it to ferment[1]). In particular, when the stock has succumbed to the "Isle of Wight" disease, and the cluster has died *in situ* during the winter, many fungi flourish on the dead bees adhering to the combs; several of these have not been met with in other situations in the hive.

But little is known as to the sources from which the various fungi are brought into the hive. Some are probably not found elsewhere, and must be carried from hive to hive principally by the bees themselves. That swarms do carry fungus spores with them, as was suggested in the case of *Pericystis alvei* (4), has been confirmed by an experiment made in May, 1912. Bees, taken direct from a swarm, were shaken up in two flasks and a large test-tube containing some honey gelatine; the bees were then liberated, and the vessels put aside at room temperature for a few days. No growth resulted in the tube, perhaps because the layer of gelatine was thin and became too dry for germination to take place. In the flasks, however, *Citromyces subtilis*,[2] *Aspergillus glaucus*, and

[1] No attempt has been made in this paper to deal with the hive yeasts; but several species are known to be present, either constantly or occasionally.

[2] This *Citromyces* had the morphological characters of the species believed to be identical with *C. subtilis;* its acid-producing capacities were not, however, investigated.

(in one of them) *Penicillium crustaceum* made their appearance. No mould had been noticed in the combs when the hive from which the swarm issued was inspected in April; this perhaps accounts for the absence of *Pericystis alvei* from the cultures.

Some of the fungi which are brought into the hive seem unable to establish themselves in it. This is naturally the case with specialized parasites such as *Ustilago* (the spores of which are sometimes mistaken for pollen by the bees and collected as such); but it occurs also in instances where a different result might be expected. The most striking is that of a species of *Cladosporium,* the conidia of which are frequently present on the bodies of bees and in the pellets of pollen carried into the hive during the summer and autumn (as has been proved by cultures).

None of the species here discussed appear to be pathogenic. The presence of much mould in a stock is, however, if not a cause, at any rate a sign of unhealthy conditions. It indicates either that the hive is not weather-proof, or that the colony is weak; and is moreover a source of much labour to the bees in the spring, when they have to remove the hard plugs of mouldy pollen from the combs, a process which often necessitates the breaking down of the cell-walls.

The Fungi.

The twelve species here described probably include all those which are frequent in bee-hives, besides several less common fungi. The list does not, however, pretend to be an exhaustive one; some further species are known to occur in the hive, but have not been sufficiently worked out for inclusion in the present paper.

Eremascus fertilis, Stoppel.

This species is not common in bee-hives, but has been met with once or twice.

The appearance and dimensions agree well with those given by Stoppel (27). The fungus consists of delicate septate hyphae, bearing numerous asci, which (in younger cultures) may be found of all ages on a single hypha. The ascus arises as follows. Processes grow out from two adjacent cells, close to the septum between them (Fig. 1). These fuse, and the tip of the flattened loop so formed swells, is cut off by septa from the supporting hyphae, and finally develops into the ascus (Figs. 2, 3). Each ascus contains eight spores. Possibly the number of spores, as also the method of

formation of the ascus, may vary occasionally (Stoppel, pp. 337-8, 335). The spores are of an asymmetrical pointed oval form, and usually measure 6 × 3 μ. (Stoppel gives 5.2 × 3 μ as the average). The ascus is 10 μ in diameter.

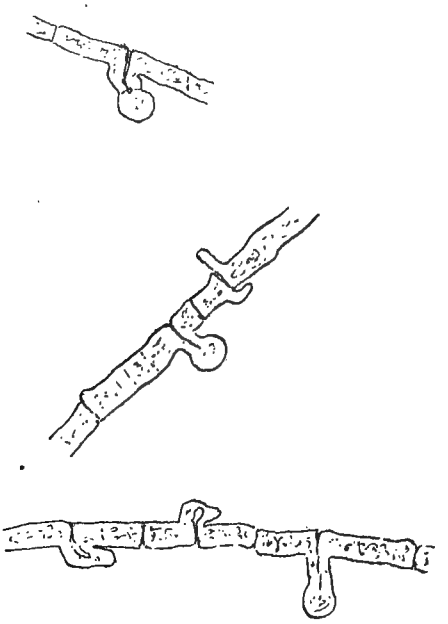

Fig. 1.—*Eremascus fertilis.* Formation of ascus. × 1,400.

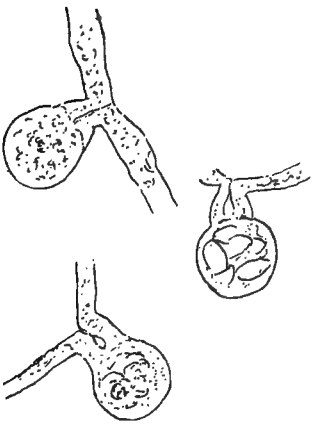

Fig. 2.—*Eremascus fertilis.* Formation of ascus; older stage. × 1,400.

Fig. 3.—*Eremascus fertilis.* Ripe ascus and ascospores. × 1,400.

An old culture has the appearance of a mass of asci; the fungus, as stated by its discoverer, being exceedingly prolific. The naked-eye colour is whitish. The spores, before germinating, swell up considerably and become globose (27, p. 334).

The asci and spores are not immediately affected by immersion in hydrochloric acid or caustic potash solution, even when heat is applied, except that they are rendered more transparent. In sulphuric acid they are rendered very transparent, and on the application of heat are disorganized.

The fungus was only once cultivated on gelatine; but appeared to grow more luxuriantly on this than on agar (as is noted by Stoppel). Gelatine is not liquefied. No acid is produced, so far as could be ascertained. Germination took place sooner at room-temperature than out of doors. The fungus appears to dislike high temperatures.

This species has not been found often enough for any very definite statements to be made as to its favourite pabulum in the hive; but it appears to grow on the pollen, and was once found associated with *Pericystis alvei*. It has not so far been recorded from healthy stocks; but there is no reason whatever to suspect it of being pathogenic.

Gymnoascus setosus, Eidam.

A fungus believed to be identical with the above is fairly common in bee-hives. My specimens differ in some respects from *G. setosus*; but the differences were not considered sufficient to warrant the making of a new species.

The fungus does not grow in tufts or balls, but forms a smooth layer, whitish at first, becoming pale sulphur-yellow when the asci are formed; when oidia are plentiful, the colour is greenish-grey. The hyphae are of two kinds; thick-walled, much branched, spiny, olive-brown hyphae, 4 μ in diameter (Fig. 4), which are embedded in a tangle of thin-walled, somewhat granulated hyphae of a yellow colour, 1-4 μ in diameter; on some of the thicker of these the asci are borne, aggregated in small groups. The asci are globose, 9-10 μ in diameter; each contains eight ascospores. The ascospores are oval, 5 × 3 μ, tinged with yellow (Fig. 5). (The dimensions given by Massee and Salmon (23) for *G. setosus* are:—asci 7-8 μ, spores 5-7 × 2 μ). The oidia occur frequently, and are of the type figured by Dale for *G. candidus* (8, Pl. xxviii, fig. 56). The oidium-hyphae are branched, and break up easily when mature. The oidia are cylindrical or globose, 2.5-3 μ (Fig. 6). There is probably a

conidial form of this fungus; but this cannot be stated positively, as pure cultures of the other forms have not yet been obtained.

The formation of the asci seems to follow the course usual in *Gymnoascus* (Fig. 7).

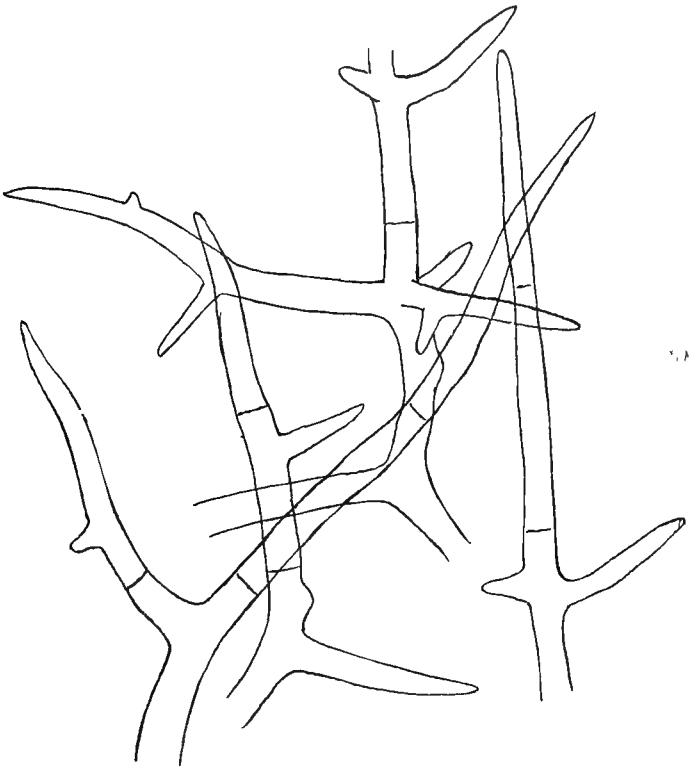

Fig. 4.—*Gymnoascus setosus*. Thick-walled brown hyphae. × 1,400.

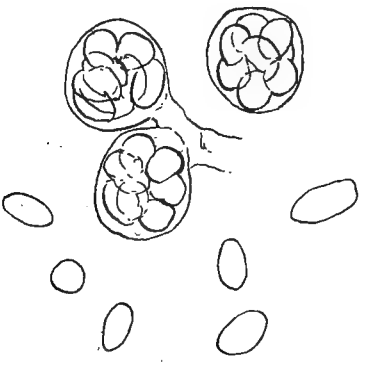

Fig. 5.—*Gymnoascus setosus*. Asci and ascospores. × 1,400.

The thick-walled brown hyphae are not affected by heating in sulphuric acid. Cold sulphuric acid dissolves the thin-walled yellow hyphae, and the liquid in their vicinity is coloured a rich magenta.

The fungus appears to be specialised for growth in the hive, as attempts to obtain pure cultures on artificial media have hitherto failed. It is interesting to note that the oidium-form grew well, and the ascus-form occasionally, in cultures heavily infected with *Penicillium* (which was present on the combs with the *Gymnoascus*).

The fungus appears to prefer empty brood-cells to any other substratum in the hive, but has been found on the cappings of dead pupae; also in pollen-cells, mingled with *Pericystis alvei*. It has been met with in a healthy stock, but evidently flourishes best after the death of the bees.

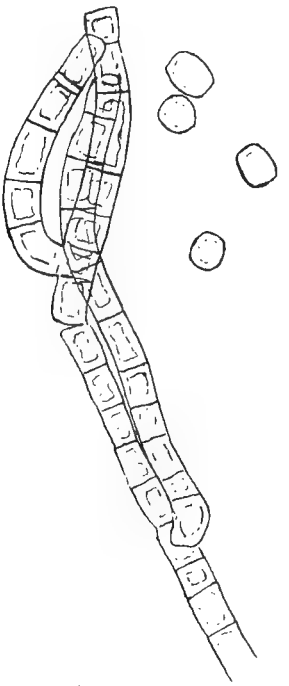

Fig. 6.—*Gymnoascus setosus*. Oidium-hypha and oidia. × 1 400.

Fig. 7.—*Gymnoascus setosus*. Conjugation of hyphae. From specimem stained with haematoxylin. × 1,400.

Gymnoascus ruber, Van Tieghem.

This species has only once been met with; it was growing on the dead cluster in a stock which died out during the winter of 1911-12. The fungus formed dull brick-red tufts on the dead bees or on the edges of the cells where they are clustered.

The hyphae vary in size and appearance; they are branched in an irregular manner, are apparently not rigid, and do not end in hooks or spines. They are usually 1-4 μ in diameter; some of the

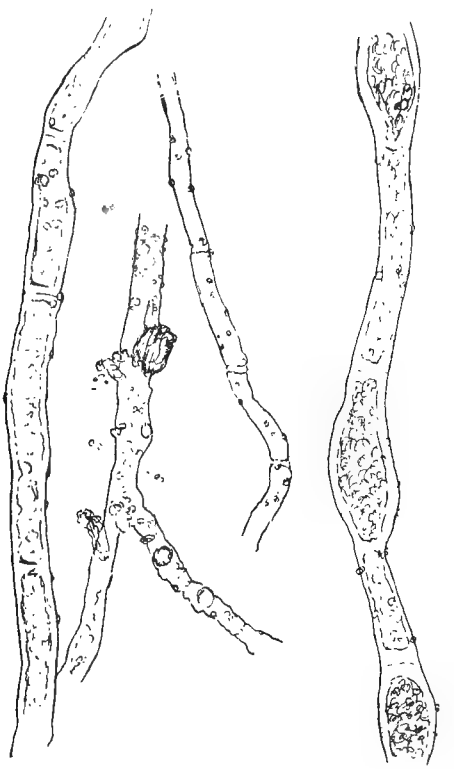

Fig. 8.—*Gymnoascus ruber.* Granulated hyphae. × 1,400.

thicker ones have swollen cells at intervals (Fig. 8). The surface bears scattered orange granulations. The asci are globose, 9-10 μ in diameter, and are borne on the hyphae in groups of about six; each ascus contains eight ascospores. The ascopores are globose or oval, tinged with yellow, 3.5-5 μ in diameter (Fig. 9). (These dimensions agree well with those given for *G. ruber* by Saccardo (25): asci 10-12 μ in diameter, spores 4.5-5.5 μ). Asci have so far not been

produced in cultures; but in one culture (on honey agar) oidia were developed. The vegetation was sparse, and of a pale salmon colour; the oidia are globose or subglobose, 5 to 6.5 μ. The oidium-hyphae fall to pieces very easily when immersed in weak spirit (Fig. 10). It is probable that there is a conidial form, but this remains to be verified.

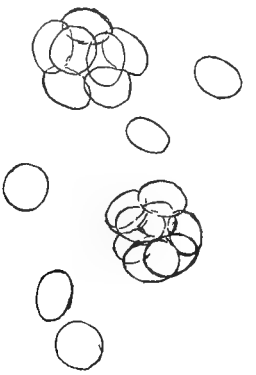

Fig. 9.—*Gymnoascus ruber*. Asci and ascospores. × 1,400.

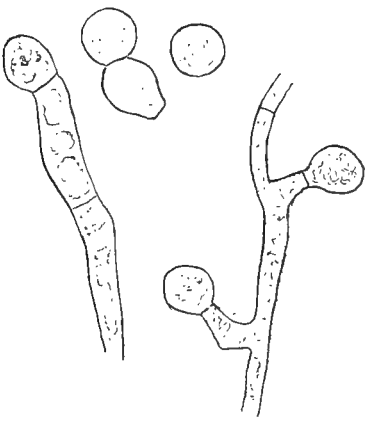

Fig. 10.—*Gymnoascus ruber*. Oidium-hyphae and oidia. × 1,400.

The red colour of the hyphae is destroyed by immersion in sulphuric and hydrochloric acids; this effect is also produced by caustic potash solution on the application of heat. Heating in sulphuric acid destroys the hyphae, and renders the asci and spores very transparent.

Aspergillus glaucus, Link.

This species is frequent in hives, especially after the death of the colony.

The dimensions of my specimens agree well with those given for *Aspergillus glaucus*. The conidiophores are from 0.5 mm. to over 1 mm. in height, and 5-10 μ (average 8 μ; an exceptional one as much as 15 μ) in thickness. The inflated apex is spherical, 25-40 μ in diameter. The sterigmata are oval or bottle-shaped,

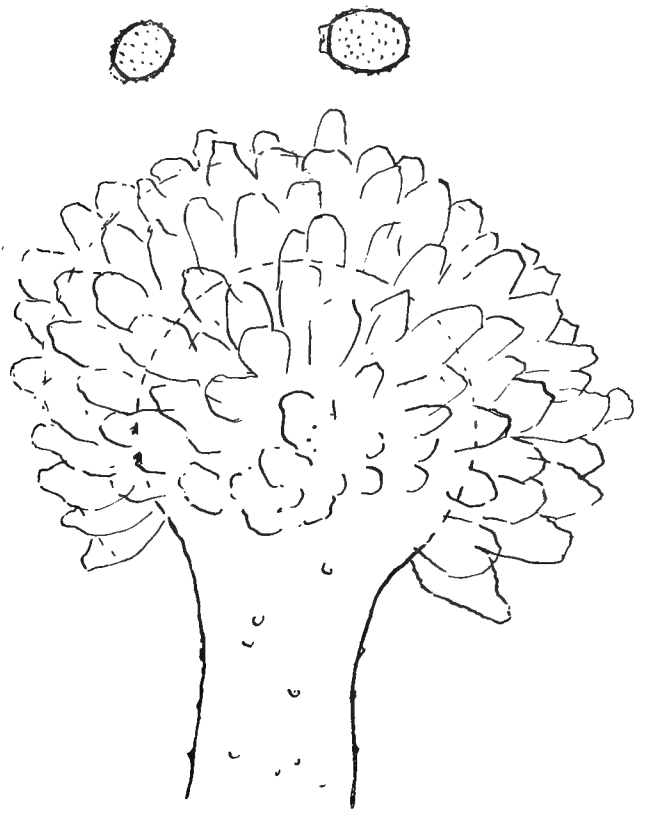

Fig. 11.—*Aspergillus glaucus*. Conidiophore and conidia. × 1,400.

10 × 4 μ; they cover most of the surface of the sphere, and are directed radially outwards (Fig. 11). The conidia are elliptical or subglobose, 10-6 × 8-4 μ (in old cultures some are irregular in shape, *cf.* Mangin, 21, p. 337). They have a projection at one end where the adjoining conidium was attached, are echinulate, and greenish in colour (Fig. 11). The perithecia are of the usual *Eurotium* type, and are produced plentifully in most cultures. They are globose or

subglobose, 240 × 200 μ to 70 μ (average about 140-180 μ) in diameter. The asci are numerous, spherical or subglobose, 15-20 μ (average 17-18 μ) in diameter; each ascus contains eight ascopores of the usual form (Fig. 12), 8 × 5 μ.

The naked-eye colour of the vegetation is at first white, then bluish-green, later of a dull (brownish) green. The perithecia are bright sulphur-yellow in colour.

In some cultures a violet colouring matter made its appearance; in others, the culture when viewed from behind appeared reddish-orange. These were evidently the phenomena described by Mangin (21, pp. 349-351) as occurring with some varieties of *Aspergillus glaucus*. He states that the violet coloration appears in neutral or slightly alkaline, the red in slightly acid cultures; and that the pigment concerned is very sensitive to variations in the acidity or

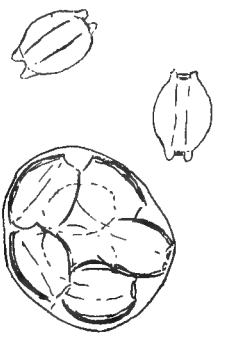

Fig. 12.—*Aspergillus glaucus*. Ascus and ascospores. × 1,400.

alkalinity of the medium, so much so that alterations taking place during the growth of the fungus affect it. As my estimations of the acidity and alkalinity of media were only made roughly, with litmus paper, no conclusions can be drawn from the results of the cultures as to this last point.

The conidiophore-stalk, sterigmata, and conidia survive heating in sulphuric and hydrochloric acids, but the former dissolves the basal part of the conidiophore-stalk. The perithecium wall, and especially the ascospores, partially resist hydrochloric acid, but disappear entirely on heating in sulphuric acid. In hot concentrated solution of caustic potash, all parts except the asci and ascospores are more or less disorganized; the perithecium-wall is ultimately coloured red-brown. The conidia become resistant earlier than the conidiophore-stalk and sterigmata; for, if young specimens be heated in sulphuric acid, only the conidia remain.

This species appears to dislike high temperatures, but is able to germinate, on being transferred to room temperature. At room temperature the fungus does well. Growth takes place under outdoor conditions, but germination is sometimes delayed.

This species does not liquefy gelatine (in one culture some liquefaction occurred). No acid is produced, as far as could be seen from cultures on litmus media.

Aspergillus nidulans, Eidam.

A fungus which is believed to be this species has been met with occasionally in dead stocks; it has not so far been demonstrated with certainty to be present in healthy stocks.

The conidiophore-stalk is 6-10 μ in diameter; it seldom, in my specimens, exceeded 1 mm. in height. The apex thickens somewhat gradually; the inflated tip is 14-21 μ in diameter (18-20 μ are about the average dimensions). The upper part of the stalk is thick-walled and is often brown in colour. The sterigmata are compound, and are borne on the upper surface of the conidiophore-apex (Fig. 13); the primary sterigmata are 10-8 × 2 μ; the secondary, 9-7 × 2 μ. The conidia are oval or subglobose, very slightly echinulate, 3-7 μ in diameter (4.5 × 3 μ being perhaps the average) (Fig. 13).

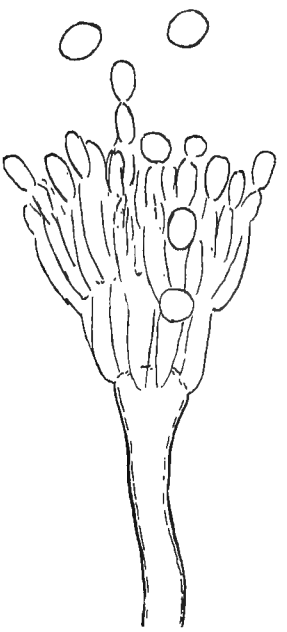

Fig. 13.—*Aspergillus nidulans.* Conidiophore and conidia. × 1,400.

On honey agar on two occasions sclerotia were produced. They are irregularly globose sulphur-yellow bodies, 1-2 mm. in diameter; and were crowded together at the foot of the agar slope. Each consisted of a tangle of numerous thick-walled cells, 12-30 μ in diameter (average 20-25 μ; some large oval ones to 40 × 35 μ; see Fig. 14). Their appearance is exactly that figured by Saito (26; Pl. iii, Fig. 11g) for the sclerotium of *A. nidulans*. The asci were not developed, even in one culture which was kept for about five months after the sclerotia first began to form.

The naked-eye colour of the vegetation is at first greenish-yellow, later a deep bright green, which does not become brownish and dull as in the case with *Aspergillus glaucus*.

The upper (brown) portion of the conidiophore-stalk, and the conidia, survive heating in sulphuric acid; the sterigmata seem able to resist this acid when cold, but disappear on heating. The upper

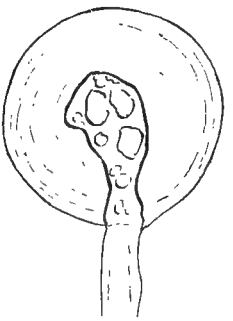

Fig. 14.—*Aspergillus nidulans*. Thick-walled cell from sclerotium. × 1,400.

part of the stalk-apex is more fragile than the rest of the conidiophore, and is of a lighter brown colour; it is very apt to break away when the stalk is heated in sulphuric acid. Hydrochloric acid does not seem to affect any parts. Heating in caustic potash solution extracts a yellow colouring-matter from the mature green conidia.

The conidia are apparently killed by exposure to temperatures of 26°—42° C., or even 38° C.; for cultures under these conditions not only did not germinate, but seemed incapable of doing so when transferred to room temperature. Under outdoor conditions germination took place, but was delayed. The fungus did well at room temperature. (In the matter of their behaviour at higher temperatures, my specimens differ from *A. nidulans* as described by Lindau 19, p. 139); he gives 38—42° C. as the optimum. He also gives 8 μ and 7 μ as the lengths of the primary and secondary sterigmata

respectively; and describes the conidia as spherical, 3 μ in diameter; otherwise the agreement is close).

This fungus grows well on honey agar, prune decoction agar, and apple; on the other media tried it either grew poorly or produced much sterile mycelium. Gelatine was invariably liquefied; consequently the fungus did not do well on gelatine media. No acid was produced.

Crookshank (7, p. 588) remarks that "bread and potatoes acquire a reddish-brown colour" when *A. nidulans* is cultivated on them. This was verified in both cases; but my fungus did not do well on either substratum. The effect was also observed in the case of apple, which was turned a dark-red brown; and a tendency to a darkening of the medium was also noticed in a culture on sugar solution with litmus.

Citromyces glaber, Wehmer.

This species was originally obtained from a living stock, but has since been cultivated from material from dead stocks, and is probably common in hives.

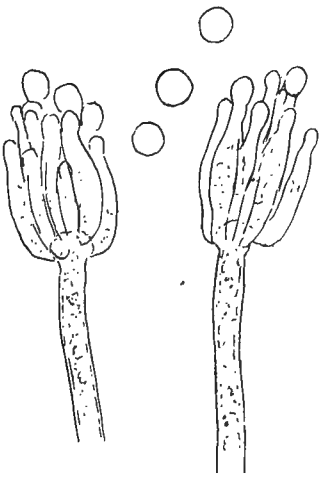

Fig. 15.—*Citromyces glaber.* Conidiophore and conidia. × 1,400.

The conidiophore is 2.5—3 μ in diameter, septate, rarely if ever branched. The apex is slightly inflated, 5—8 μ in diameter. The sterigmata are elongated oval, pointed at their distal ends, directed parallel to the long axis of the conidiophore, 10 × 3 μ (Fig. 15). The conidia are spherical, smooth, 2.3 μ to barely 3 μ in diameter, often forming long chains. Bodies probably of a sclerotial nature were met with in one culture on prune decoction agar. They were oval,

tawny-yellow in colour, 680-500 × 560-400 μ, and were partially embedded in the mycelium. They were evidently immature when examined; asci were not found.

The naked-eye colour of the vegetation is similar to that of *Penicillium*, but lighter and greyer. The fungus produces a yellow or brown coloration on some media (prune decoction agar and honey gelatine, for example); when grown on rice, it colours the rice a bright yellow (see Wehmer, 28).

The conidia and hyphae survive immersion in cold sulphuric acid; on the application of heat, the conidia only remain. A similar result ensues on heating in hydrochloric acid. Cold hydrochloric acid and caustic potash solution do not produce any immediate effect on the specimens. Hot caustic potash solution extracts a yellow colouring matter from the conidia.

This species is unable to endure high temperatures; the cultures at 26°-42° C. were all killed. Germination took place out of doors in May as quickly as at room temperature (in three days), but was inhibited during the winter. At room temperature the fungus did well. These observations are in accordance with Wehmer's results (28); he gives the limits of germination as 8°-32° C., optimum 20°-25° C. The swollen apex of the conidiophore, in his specimens, measured 4-15 μ, the sterigmata 9-12 × 3-4 μ. In other respects the fungus here described agrees well with Wehmer's.

This species liquefies gelatine; a considerable quantity of acid is produced, as is shown by the reddening of media containing litmus. The vegetation was grey, instead of green, on bouillon agar. The fungus grew well on a 5 per cent. solution of citric acid to which 2 grm. of agar per 100 ccm. had been added. (The medium remained liquid).

Citromyces subtilis, Bainier and Sartory.

This species is very common in hives, whether more so than the last it is not possible to say, as they resemble each other closely, and have doubtless often been confused.

The conidiophore is 3 μ in diameter, generally branched, having a septum just above the branch. The apex is slightly inflated, 3-5 μ in diameter. The sterigmata are of the usual form, 8-10 × 2 μ, directed parallel to the conidiophore-stalk. (Fig. 16). The conidia are spherical or subglobose, 2.5-3 μ (a few 4 μ) in diameter. No sclerotia have so far been observed.

The naked-eye colour of the vegetation is approximately that of *Penicillium*, that is to say slightly darker and less grey-green than

that of *C. glaber*. This species does not appear to produce any colouring matter, nor to discolour the medium on which it grows.

In sulphuric acid all parts of the fungus are rendered very transparent; on heat being applied, the conidia only remain. Hydrochloric acid produces no immediate effect; on heating, the conidia remain, also some hyphae; but the latter are considerably disorganised. Caustic potash extracts a yellow colouring matter from the conidia even when cold.

This species is more tolerant of high temperatures than *C. glaber*. Cultures at 26°-42° C. germinated, and in some cases produced a few conidiophores. At room temperature the fungus did well, also under outdoor conditions (May).

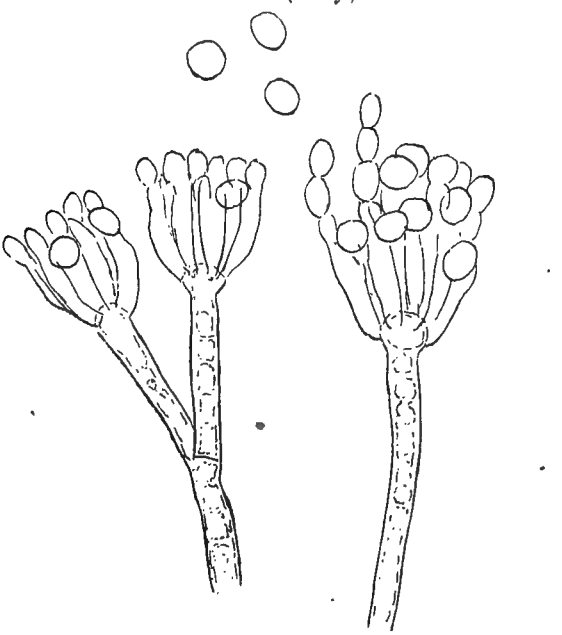

Fig. 16.—*Citromyces subtilis*. Conidiophore and conidia. × 1,400.

Only one gelatine culture was made; after 15 days some liquefaction had taken place. Several cultures on media with litmus were made; in no case was any reddening of the medium seen, so that acid is evidently not produced. The fungus is therefore probably *C. subtilis*; Bainier and Sartory (1, p. 46) describe this species as peculiar in that it produces no citric acid. Their specimens had conidia 2-2.5 μ, inflated apex 8-10 μ; in the manner of branching they resembled the fungus here described. Bainier and Sartory's species liquefied gelatine slowly; liquefaction began 16 or 17 days after germination.

Penicillium crustaceum, Linn.

This ubiquitous species is, as might be expected, common in bee-hives. It will grow on nearly any part of the contents of the hive, but is not usually found in great quantity until after the death of the stock.

An interesting point is the occurrence of two varieties differing chiefly in the size of their conidia. The one, which is probably more

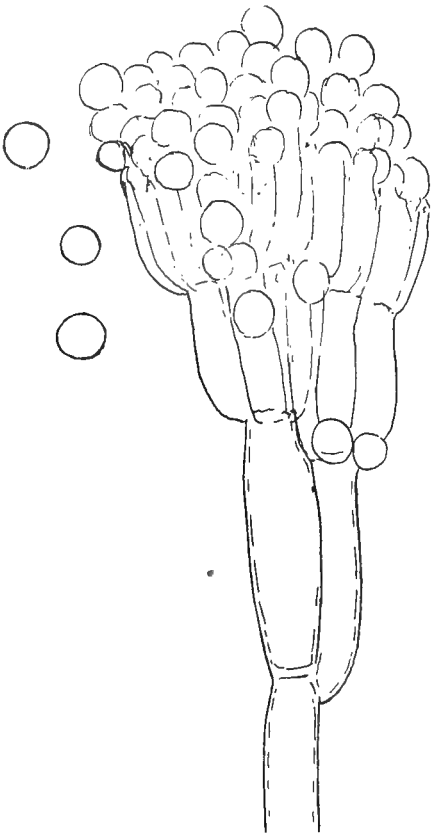

Fig. 17.—*Penicillium crustaceum*. Conidiophore and conidia (3-5 μ). × 1,400.

prevalent, has conidia 3-5 μ in diameter. The conidia of the other are 2.5-3 μ in diameter; this is perhaps the species studied by Brefeld, and referred to by him as *Penicillium glaucum* (Lafar, 18, p. 333).

The conidiophores are much alike in the two cases, and are of the usual *Penicillium* form (Figs. 17, 18). The sterigmata are 10-15 μ in length in both varieties (perhaps 10-12 μ in the form with small conidia). The branches bearing them are 15-17 μ in

length (15-16 μ in the form with small conidia). In both the conidiophore-stalk is about 5 μ in diameter.

The colour of the vegetation is very similar in both forms, but there seems to be a slight tendency for the form with smaller conidia to produce a paler, greyer, or more bluish-green vegetation than that of the other.

Only the conidia appear able to resist the action of sulphuric acid when heat is applied. No particular effect is produced by hydrochloric acid or caustic potash solution.

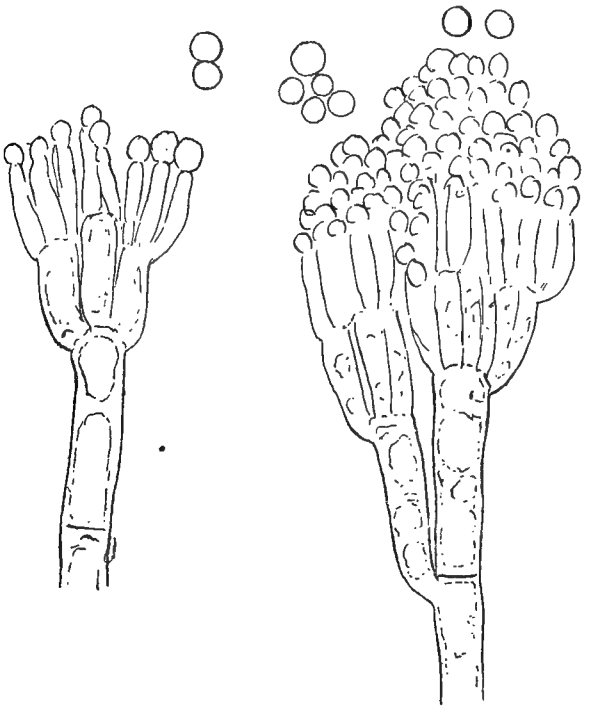

Fig. 18.—*Penicillium crustaceum*. Conidiophore and conidia (2·5-3μ). × 1,400.

This species seems to dislike high temperatures; in one case a culture of the form with larger conidia germinated and flourished at 26°-38° C., while the form with small conidia in a similar tube did not germinate and appeared to have been killed; but as a rule, cultures exposed to high temperatures failed to germinate even when transferred to room temperature. At room temperature and under outdoor conditions the fungus did well.

Gelatine is not liquefied. In some cases litmus media were reddened, but the production of acid is evidently not so great as in the case of *Citromyces glaber*. The vegetation on bouillon agar was grey instead of green.

Sordaria fimicola, Rob.

This species has been cultivated once or twice from mouldy combs, but is evidently only very occasionally present in the hive. Its presence in healthy stocks has not been demonstrated with certainty. It is probably carried into the hive by bees seeking water in places to which horses have access.

The dimensions here given are for the most part taken from material cultivated on horse-dung. The perithecia are pear-shaped, or globose, with a curved neck, 280 μ in diameter; on some media they only attain 75 μ. The asci are cylindrical, narrowing to a stalk (Fig. 19); the sporiferous portion is 145 × 14 μ; the tip is slightly thickened. The asci in each perithecium are, in a fairly young

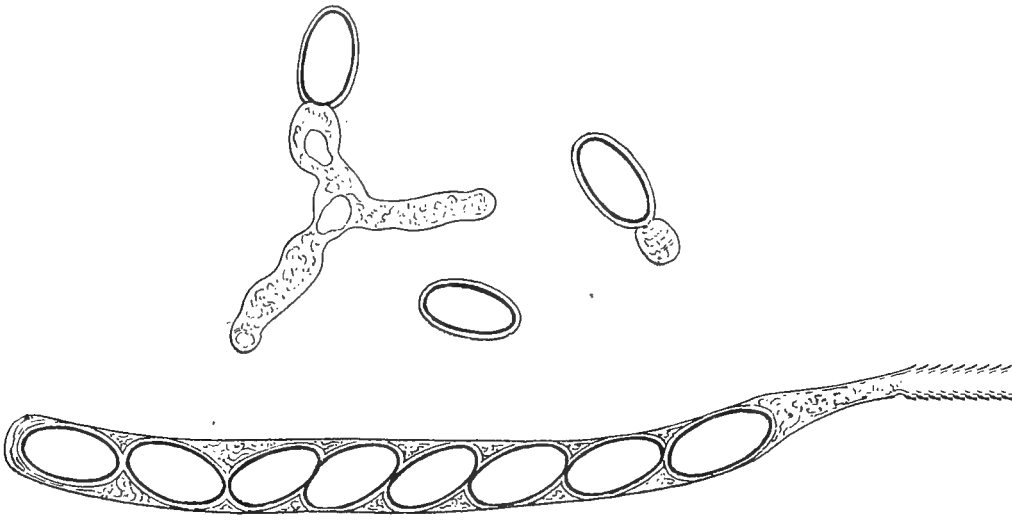

Fig. 19.—*Sordaria fimicola.* Ascus, ascospores, germination. × 640.

culture, of various ages. The paraphyses are irregularly swollen, septate, transparent hyphae. The ascospores are eight in each ascus, in a single row; each spore has its germination-pore directed towards the stalk-end of the ascus. The spores are dark brown in colour, 20 × 12 μ; they have a gelatinous integument, and (usually) one vacuole. They germinate by the emission from the pore of a spherical bladder, from which a hypha or hyphae then proceed (Fig. 19).

These dimensions agree well with those given by Winter (29, p. 166) for *Sordaria fimicola.*

On heating in sulphuric acid, a reddish colouring matter is extracted from the ascospores, and the ascus-wall is dissolved; the other parts of the fungus resist the action of the acid. On heating in caustic potash solution, oil-drops appear to exude from the ascospores.

This species has a preference for warmth, cultures at 26°-38° C. germinating more quickly than those at room temperature, at which, however, the fungus did well. Under out-door (winter) conditions, germination was delayed and the fungus did not flourish.

This species grew well on horse-dung, potato, and pollen. It seems to dislike acid media, but could grow on them. Gelatine was liquefied. No acid was produced.

Mucor erectus, Bainier.

This species is very common in and about hives, occurring principally as a saprophyte on dead bees. It is improbable that it is able to flourish in the living stock; but its spores are frequently present on the alighting-boards and about the entrances of hives. The fungus has also been cultivated from pellets of pollen taken from home-coming bees, and from the bodies of the bees themselves.

The vegetative hyphae are of the type usual in the genus, branched, non-septate, and containing oil-drops. At intervals swellings occur. The sporangiophores are branched, having always a septum above the branch; in thickness they vary from 9 μ to 20 μ (in exceptional cases portions of the sporangiophore may attain 38 μ). They are not rigid, but lean up against each other in a tangled

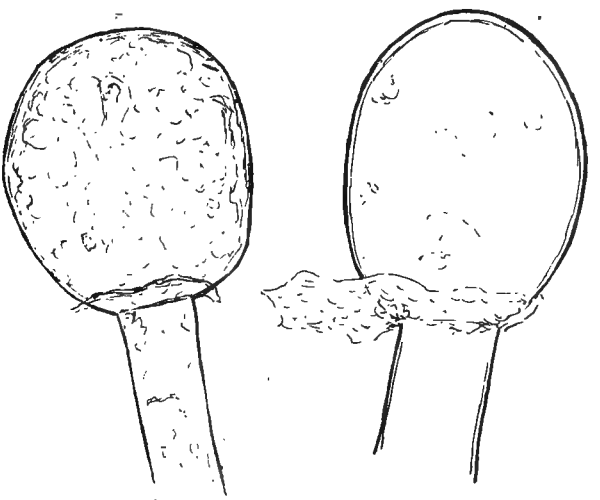

Fig. 20.—*Mucor erectus*. Columellae. × 1,400.

manner; they exhibit strong positive heliotropism. The sporangium is spherical, greenish-grey when mature, 70-190 μ in diameter. The sporangium-wall is semi-transparent, smooth, and very fugitive when mature; it usually deliquesces if it so much as touches a neighbouring hypha. The columella is spherical to oblong, or sometimes slightly pear-shaped, 55-22 × 50-20 μ, the longer diameter being the vertical one (Fig. 20). There is a basal collar. The spores vary in

Fig. 21.—*Mucor erectus*. Spores. × 1,400.

Fig. 22.—*Mucor erectus*. Zygospore. × 640.

size and shape (Fig. 21), 11-7 × 6-3 μ (average 7 × 5 μ; some are almost spherical, 2-3 μ in diameter). In the mass they are grey in colour.

The zygospores and azygospores were produced in cultures on potato and on bread, but on no other media. They are ornamented with star-like thickenings of a darker red-brown than the rest of the surface (Fig. 22). Good specimens of zygospores are 90 μ in

diameter; the smaller ones average about 60-65 μ. The azygospores are generally unequally developed, one of the pair being often aborted; they are 60-70 μ in diameter when well developed.

The zygospores have not been germinated, neither has it been as yet determined whether this species is homo- or heterothallic.

On heating in sulphuric acid all parts of the fungus are dissolved, excepting the exospore of the zygo- and azygospores. Similar treatment with hydrochloric acid is survived also by the spores and hyphae, sometimes by the columellae (but these last are rendered very brittle). Caustic potash does not produce any marked immediate effect on any parts.

This species dislikes high temperatures; the spores appear to be killed by exposure to 26°-38° C. At room temperature and out-of-doors the fungus does well.

Growth was luxuriant on most media; on potato and bread, as stated, zygospores are produced The fungus would not grow on pollen, and in most cases grew poorly on honey media; it did not flourish on pollen decoction gelatine or on apple. Gelatine is liquefied, and the resulting liquid is often coloured a tawny yellow (about the colour of Flemming's fluid). A culture on bouillon agar was also coloured yellow. Acid is probably not produced.

The description here given agrees in most points with that given by Fischer for *Mucor erectus* (10, p. 197).[1] The principal difference is that Fischer describes the spores as uniform in shape ("gleichgestaltet"), whereas in my specimens there is a decided tendency to variableness in the form of the spores. He also gives the zygospore diameter as 40-65 μ. Otherwise the agreement is close.

Fischer mentions echinulate gemmae, which did not occur in my cultures; also a spherical yeast-form. This latter, it is believed, was met with occasionally in the earlier course of the research; but has not been observed lately.

Pericystis alvei, Betts.

This species is apparently a true bee-hive fungus, occurring only on the pollen stored in the combs, and adapted to life in the hive. The appearance, both of the mycelium (Fig. 23), and of the dark-green cysts (Fig. 24) containing numerous spherical spores (Fig. 25), is very characteristic and peculiar, and makes the identification of the fungus an easy matter. The process of development of the

[1] The dimensions given by Fischer for *M. erectus* are greater than those in Bainier's original description (see *10*, p. 197).

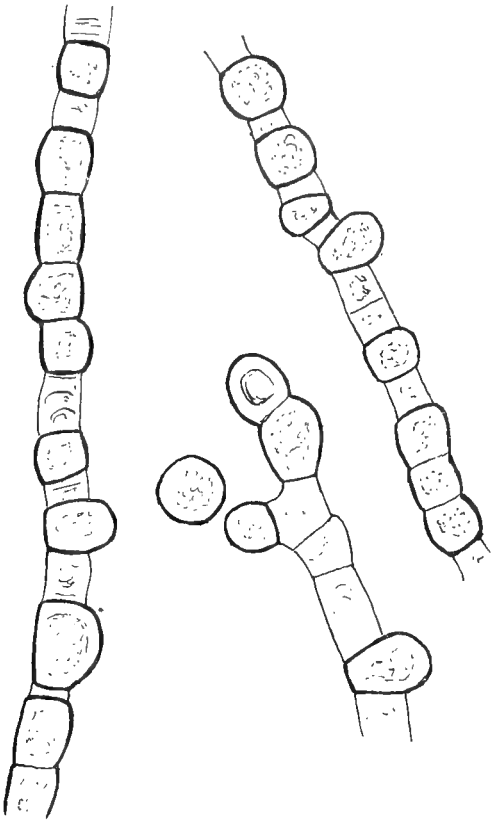

Fig. 23.—*Pericystis alvei.* Hyphae and chlamydospores. × 1,400.

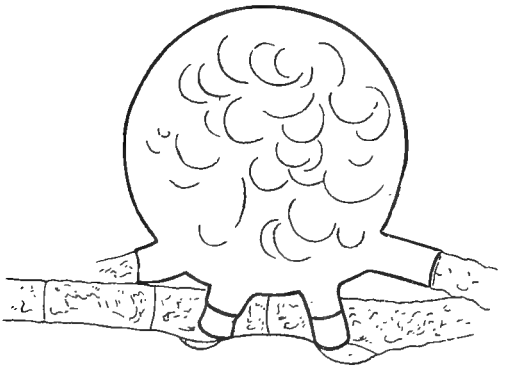

Fig. 24.—*Pericystis alvei.* Cyst. × 1,400.

cysts, and the probable life-history, have been dealt with elsewhere (4).

Pericystis alvei is one of the most frequent of the bee-hive fungi, and is probably the species chiefly responsible for the white "pollen-mould" so well known to bee-keepers.

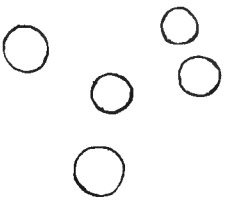

Fig. 25.—*Pericystis alvei*. Cyst spores. × 1,400.

It would be of interest to ascertain the distribution of this fungus; whether it is confined to the British Isles or is of world-wide occurrence. The type specimens were collected in North-western Surrey.

As far as could be ascertained by means of cultures on litmus media, no acid is produced by this species.

Oospora favorum, Berkeley and Broome.

The type specimen having disappeared from the Berkeley Herbarium, I was unable to make a direct comparison with it of my

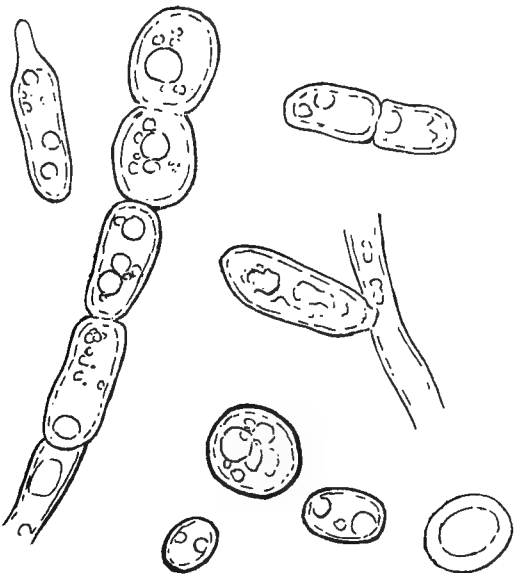

Fig. 26.—*Oospora favorum*. Conidia and torulose cells. × 1,400.

specimens; but there seems little doubt that the fungus here described is identical with Berkeley and Broome's species.[1]

Oospora favorum is found chiefly on old brood-combs, on the wax of the cocoons; it sometimes also occurs on the stored pollen. On wax or cocoons it has the appearance described by Berkeley and Broome—small yellow tufts. On pollen it forms a yellow wrinkled growth, having a velvety surface owing to the presence of numerous conidiophores, which consist of a short hyphae bearing chains of conidia. The hyphae are septate, and have a strong tendency to torulose growth, particularly in damp cultures. In such cultures the resemblance to a yeast is striking; no true hyphae are developed, but only strings of torulose cells, which are often indistinguishable from

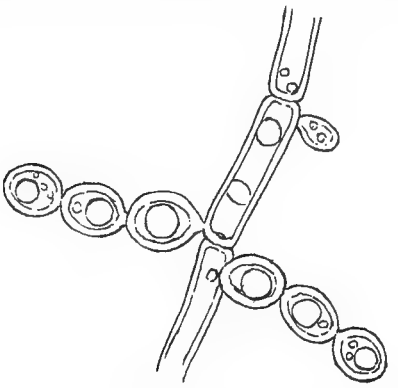

Fig. 27.—*Oospora favorum*. Conidia. × 1,400.

the conidia, save by their more irregular and elongated shape (Fig. 26). The fusiform bodies noticed by Berkeley and Broome were probably cells of this kind.

The conidia are borne in short, simple or branched chains (Figs. 27, 28). They are spherical when young, becoming larger and subglobose or oval as they grow older. The filiform conidia seen by Massee (22) were perhaps of that form in consequence of having been long dried. The size of the conidia varies. In specimens from a culture on pollen they measured 6 × 4 μ; but on various artificial media they range from 8 × 6 μ (or even 10 × 7 μ) to 7 × 5 μ. Owing to the difficulty of distinguishing between torulose hyphal cells and conidia, it is impossible to be certain of the maximum dimensions.

The fungus is at first white, later yellow. In some cultures (*e.g.*, some of those on pollen and on honey media) this is the final colour.

[1] My thanks are due to Miss Wakefield for her kindness in searching for the type specimen in the Berkeley Herbarium.

In others, however, it deepens to a dull mustard colour, olive-yellow, or (in many cases) a deep brown (almost black). The torulose hyphae undergo this change as well as the conidia.

The alteration in colour is accompanied by a progressive alteration in the chemical constitution of the cell-walls. Young conidia (that is, from white or light yellow cultures) are dissolved in sulphuric acid, often without the application of heat. After a culture has reached the dull mustard-yellow or light olive stage, however, the conidia are not destroyed by heating in sulphuric acid. Similar results are obtained with hydrochloric acid, except that the young conidia do not entirely disappear even on heating, but traces of them remain. Caustic potash solution does not appear to produce any marked effect on the conidia at any stage.

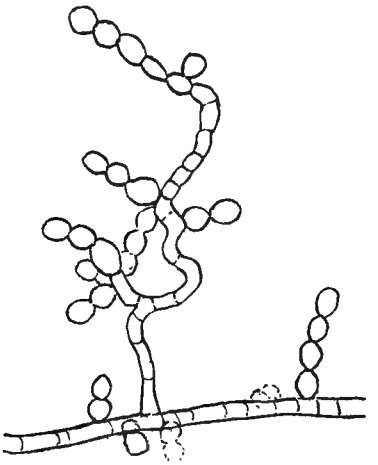

Fig. 28.—*Oospora favorum*. Conidiophore. × 640.

This species appears to be variable. I cultivated two varieties, derived originally from the same culture. One of these tended to colour and cuticularize earlier than the other, and to attain ultimately a darker colour. They were transferred to various media, and in nearly every case the difference referred to was apparent.

The results of the temperature-experiments were somewhat contradictory, and no certain conclusions can be drawn from them. The fungus did well at room temperature in all cases.

This species appears to do best on pollen; cultures on honey media were, however, fairly successful. Normal conidia were produced on fairly dry potato, in small quantity. On prune decoction media the growth is torulose. Gelatine is liquefied. From the results of cultures on litmus media it appears that acid is occasionally produced.

General Conclusions.

Of the foregoing twelve species, *Pericystis alvei* is probably a bee-hive fungus in the strict sense. This conclusion seems warranted by the frequency of its occurrence in hives, taken in conjunction with the fact that it has not been observed elsewhere; and is confirmed by what is known as to its requirements in the way of temperature and pabulum (4).

Of the others, *Oospora favorum* is very probably also confined to bee-hives; it is not a common species. *Gymnoascus setosus* seems to be adapted to life in the hive, judging by its luxuriant growth in dead stocks, and by its unwillingness to grow on artificial media. It has been previously recorded from the nests of other Hymenoptera (8, p. 571). *Aspergillus nidulans* was originally found by Eidam on a humble-bee's nest; it is one of the less common of the bee-hive fungi, however, and has only once been observed to grow luxuriantly on a mouldy comb, being generally found in but small quantity, when present at all. It is probably absent from healthy stocks.

Mucor erectus is chiefly, if not exclusively, found on dead bees. It is probably not able to flourish on the combs.

Sordaria fimicola and *Gymnoascus ruber* are coprophilous; the former, as has been stated, may very likely be carried into the hive by bees visiting stagnant water. *G. ruber* has only once been observed; its spores were probably adhering to some of the bees of the cluster, and developed on their bodies after death.

Nothing is known as to the probable original source of *Eremascus fertilis*. Stoppel found it on paper which had been soaked in rum and used to cover some apple and currant jelly pots, and it seems capable of normal growth on various media; hence can hardly be considered specially a bee-hive fungus.

Aspergillus glaucus and *Penicillium crustaceum* are ubiquitous, and their presence in the hive needs no explanation. It is possible that the same considerations may to some extent apply to the two species of *Citromyces*.

In conclusion, my thanks are due to Dr. Rendle for permission to use the library at the Cryptogamic Herbarium, British Museum; to Miss A. Lorrain Smith for her unfailing kindness in advising me on systematic and other points; and to Mr. J. Ramsbottom for much kind assistance in naming the fungi. I also desire to thank Mr. T. W. Cowan for valuable information as to previous work on the bee-hive fungi, and for the loan of several periodicals cited in this paper.

The figures illustrating this paper were drawn from fresh material with the aid of a camera lucida, and the magnification is approximately × 1,400 except where otherwise stated.

SUMMARY.

1. An account is given of the previous work that has been done on the fungi present in bee-hives; and some questions arising from these records are discussed.

2. A general description of the conditions prevailing in the hive, and of the distribution of fungous growth in it, are given.

3. The following twelve fungi are described :—

Probably confined to the hive :
Pericystis alvei, *Oospora favorum*.

Adapted to hive-life, but not confined to this habitat :
Gymnoascus setosus ; Eremascus fertilis (perhaps).

Common, but not specially adapted to life in the hive :
Penicillium crustaceum, *Aspergillus glaucus*, *Citromyces subtilis*, *C. glaber*, *Mucor erectus*.

Occasionally present :
Aspergillus nidulans, *Sordaria fimicola*, *Gymnoascus ruber*.

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The fungi of the bee-hive.

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