NOTES ON SOME SOUTH AFRICAN ENTOMOPHTHORACEAE.

By S. H. Skaife.

(With Plates II—IV.)

The material on which the following notes are based was collected at Cedara, Natal, during the eight months August, 1919, to March, 1920. So far very few records of South African *Entomophthoraceae* have been published, the writings of Pole-Evans, Edington, Black and others on the locust fungus, *Empusa grylli*, being the only publications known to the present writer.

The family *Entomophthoraceae* belongs to the order *Entomophthorales* and includes some fifty or more described species, the great majority of which are parasitic on insects. Only one other family, the *Basidiobolaceae*, all of the members of which are saprophytic or parasitic on the higher fungi, is included in this order.

The two orders, *Entomophthorales* and *Mucorales*, together make up the sub-class *Zygomycetes* of the great class of alga-like fungi, the *Phycomycetes*. These two orders agree with one another in that all the species included in them have isogamous sexual spores, but the *Mucorales* are distinguished by having the conidia borne in sporangia, whereas in the *Entomophthorales* the conidia are borne singly and apically on club-shaped conidiophores.

The family *Entomophthoraceae* has been subdivided into several genera, but some of these are of doubtful rank. *Massospora*, Peck, is an aberrant genus that, so far as present knowledge goes, is restricted to North America; *Tarichium*, Cohn, no longer stands, as it seems to have been based on the resting stage of an *Empusa*; and Nowakowski's genus, *Lamia*, is not valid, according to Thaxter, as the species on which it was founded, *E. culicis*, is a typical *Empusa*.

We are thus left with two genera, *Empusa* and *Entomophthora*. Thaxter, in his classical monograph (1), recognises only the genus *Empusa*, and regards *Entomophthora* as a sub-genus, characterised by the compound, branched conidiophores. More recent writers, however, recognise both genera, the species in which rhizoids are present being classed as *Entomophthora*, and those in which rhizoids are absent being placed in the genus *Empusa* (2). In the present paper the latter classification is followed.

EMPUSA, Cohn.

Cystidia and rhizoids absent.
Conidiophores simple or branched.

Empusa muscae, Cohn. (Plate II, figs. 1–4.)

Conidia bell-shaped, with a broad, subtruncate base, 18–20 × 25–30 μ containing usually a single large oil-globule, and surrounded after discharge by a mass of protoplasm. Conidiophores simple, club-shaped, emerging in white rings between the segments of the abdomen of the host. Secondary conidia spherical, formed by direct budding from the primary conidia. Host attached to substratum by proboscis.

Hosts.—Muscid flies.

Habitat.—Cosmopolitan.

This is perhaps the commonest and most familiar of all the *Entomophthoraceae.* It was first noticed at Cedara on a large species of Anthomyid fly out-of-doors on August 17, 1919. A number of these flies were found dead in the garden on this date, fixed to foliage by their proboscis. The first house-fly killed by this fungus was found in the house on a window-pane on October 18. It was found to be common on house-flies at Umfolozi in Zululand in November. The disease was prevalent at Cedara until the first week in January, when it suddenly disappeared, no specimens dying of the disease being found after the 7th of this month, although house-flies were abundant and the weather damp and warm.

By collecting all the dead house-flies in the house each day and keeping a careful watch it was found that all the victims of the disease died in the evening, somewhere between 6 and 8 p.m. The following notes are typical of several records made:

"Specimen 1, 6.55 p.m.—Adhering to window-pane by its proboscis. When removed could still move its antennae and proboscis.

"7.5 p.m.—Slight twitchings of antennae only signs of life.

"7.10 p.m.—Quite dead.

"7.30 p.m.—Intersegmental membranes of the abdomen distended and gleaming white.

"9.30 p.m.—Protrusion of conidiophores well marked.

"10.15 p.m.—Large numbers of conidia have been thrown off.

"Specimen 2, 6.55 p.m.—This individual could buzz its wings and walk quite freely, but could not fly when found.

"7.10 p.m.—Can walk feebly.

"7.20 p.m.—Proboscis and antennae still retain power of movement.

"7.30 p.m.—Dead.

"10.15 p.m.—Conidiophores prominent.

"11 p.m.—A few conidia have been thrown off.

"Specimen 3, 6.55 p.m.—This individual was sluggish and easily captured, but could still fly.

"7.10 p.m.—Can no longer fly, but can walk freely.

"7.20 p.m.—Can cling tenaciously, but when turned on its back cannot right itself.

"7.55 p.m.—Dead.

"10.30 p.m.—Conidiophores prominent.

"No conidia given off yet."

Individuals which were still quite active, but which had distended, white abdomens, were found on dissection to have the abdomen filled with spherical, hyphal bodies of regular shape and size, measuring about 40 μ in diameter. Just as the host is on the point of death these hyphal bodies germinate and grow with extraordinary rapidity, giving rise to the conidiophores and in three to four hours producing conidia.

Although the house-fly fungus has been much studied in different parts of the world, the occurrence of resting spores has only been recorded once. Winter (3) states that they are spherical, colourless, and 30–50 μ in diameter. No resting spores were found by the present writer, although some dozens of specimens were examined and several attempts were made to induce the formation of these spores by placing infected flies in conditions unfavourable to the fungus. It was found quite easy to inhibit the formation of conidia by placing specimens, immediately after death, in tightly-corked glass tubes containing a little calcium chloride. Exposure of only an hour to this dry atmosphere served to arrest the development of the fungus inside the body of the host. No conidia were formed when several infected flies were placed together in a small tightly-corked tube, and submersion in water also served to prevent conidia formation. It was found that when once the development of the fungus had been checked in any of the above ways it failed to develop further, even when placed under the most favourable conditions. In the moist chamber the hyphal bodies broke down in the course of a few days, and in no cases were any signs of conjugation or the formation of resting spores seen.

Both sexes of the house-fly seem to be attacked impartially, and vigorous individuals seem just as liable to infection as those that are spent. Several dead females were found whose abdomens contained large numbers of eggs.

In 1912 Hesse (4) claimed to have succeeded in artificially cultivating *E. muscae*, and Bernstein (5) confirmed his results in 1914. No further work on this subject seems to have been done since the latter date. Hesse used as his culture medium the yolk of egg spread on glass slides and kept in a moist chamber. He states that he invariably obtained a profuse growth of the common mould *Mucor racemosus* from *Empusa* conidia sown on this medium. By feeding these *Mucor* spores back to adult house-flies he was able to produce epidemics of the disease at will.

The present writer repeated Hesse's experiments many times, but never once was a growth of *M. racemosus* obtained. From Bernstein's account of the experiments conducted by himself and Hesse it would seem that the initial cultures were obtained by placing the infected flies on the slides bearing the culture medium. He definitely states (p. 28) that "it was impossible to sterilize the flies from which the cultures were obtained." Obviously a grave source of error was introduced in this way. In order to overcome this the writer sterilised the flies used in the experiments by soaking them for fifteen minutes in a 1 per cent. solution of corrosive sublimate, the specimens being afterwards well washed in sterile water. The soaking was not long enough to prevent conidia formation provided the specimens were so treated immediately after death, yet it served to kill any foreign spores or bacteria adhering to the exterior of the flies.

Besides egg-yolk colostrum was also used as a culture medium, this being also a highly concentrated nutritive substance. The tubes containing the colostrum were sterilised in a slanting position, and in this manner excellent slopes of the coagulated milk were obtained. The infected flies, after sterilisation, were dropped into the tubes containing the egg-yolk and colostrum slopes, and next morning, after large numbers of conidia had been thrown off in each tube, the flies were removed. In no case was a growth of *M. racemosus* obtained. The conidia germinated freely and grew for two or three days, but no increase in bulk took place, and finally the germ tubes died and disintegrated.

A large number of flies that had not been sterilised were dropped into the tubes, and in many cases profuse fungous growths were obtained. These were found to consist of *Mucor*, *Rhizopus* and *Penicillium*, spp., besides other saprophytic fungi that were not identified, but in no instance was a growth of *M. racemosus* found in the cultures.

The *Mucor* spores obtained in the above cultures were mixed with a solution of sugar in water (as recommended by Hesse), and fed to fifty or more house-flies bred in the insectary and kept in a roomy cage. None of these flies died of *Empusa*, although in the house numbers of flies were dying of the disease at the time.

Empusa conglomerata, Sorokin. (Plate II, figs. 5 and 6.)

Conidia ovoid, usually with a single large oil-globule, 20–25 μ × 25–40 μ, average length about 35 μ. Conidiophores simple. Secondary conidia like the primary, produced by direct budding. Resting spores not observed at Cedara, but according to Thaxter they are "azygospores, produced from spherical hyphal bodies, and borne on a neck-like process of variable length."

Host.—Imago of *Nephrotoma umbripennis*, Alex. (Tipulid).

Habitat.—South Africa, U.S.A., and Europe.

Only one specimen was found, the host clinging to a pine-needle by

means of its legs, on August 8. The above determination is based entirely on the shape and size of the conidia, which agree very closely with Thaxter's description and figure. Until the resting spores are observed, however, the determination must be regarded as somewhat doubtful, especially as Thaxter states that his specimens (Tipulid larvae and imagines) were found floating on water.

Empusa grylli, Fresenius. (Plate III, figs. 7–12.)

Conidia ovoid to pear-shaped, 25–45 μ × 20–35 μ, average about 35 × 28 μ, containing one or more large oil-globules. Conidiophores simple, club-shaped. Secondary conidia like the primary and produced by direct budding. Resting spores spherical, colourless, very regular in shape and size, 30 μ in diameter, with thick, double, hyaline walls. Host attached to tips of grass, etc., by the contraction of its legs.

Hosts.—Orthoptera (according to Thaxter, also *Lepidoptera* and *Diptera*).

Habitat.—South Africa, U.S.A., Europe.

This disease was first noticed on grasshoppers on January 10 at Cedara. It was exceedingly common from this date until the end of March, attacking impartially several different species of Acridiids.

According to Sacharov (6) and Perez (7) this fungus only attacks spent adults, but at Cedara all stages from the second instar to the adult stage were found dead of the disease, and several dead females were found whose ovaries contained eggs.

As was noticed in the case of *E. muscae*, all the infected individuals that were kept under observation died in the afternoon, the great majority dying between 5 and 7 p.m. Sluggish individuals could be found in the field clinging to grass-stems at 1 p.m. Earlier in the day no dying individuals could be found. This remarkable characteristic of the fungus is probably explained by the climatic conditions at Cedara. During the summer the mornings are usually warm and dry, but in the afternoon mists and thunderstorms came up, making the atmosphere very moist.

During the period that this fungus was kept under observation about 1 per cent. of the individuals that were found infected failed to throw off conidia, even when kept under favourable conditions. On dissection these individuals were found to contain numerous resting spores (fig. 12). The mode of formation of these spores was not observed. Attempts to germinate them in water all failed, although hyphal bodies and conidia germinate quite freely in drops of water.

In view of Hesse's claims regarding *E. muscae*, it is interesting to recall the work of Edington and Black on the locust fungus, carried out at Grahamstown over twenty years ago. These two authors cultivated a fungus on a large scale, and this was distributed to farmers as the locust fungus. The reports of the efficiency of this fungus as a means of control

were conflicting, but several farmers announced excellent results from its use. In 1899 D. McAlpine, the plant pathologist of New South Wales, secured some of the cultures and pronounced the fungus to be *M. racemosus*, Fres. (9)—that is to say, the same fungus that Hesse claims to have secured from artificial cultures of the house-fly fungus.

Lounsbury sent some of the cultures to Kew in 1900, and Massee (10) states that they consisted of pure cultures of a new species of *Mucor*, which he named *M. etiosus*. In his paper describing this species he gives some interesting details of some experiments he carried out with the fungus on *Periplaneta australasiae*. Cockroaches which were sprinkled with the spores or which were made to ingest them died within twenty-four hours. Unfortunately Massee does not state whether he obtained a typical growth of *E. grylli* on the dead insects or not.

Cultures of the South African locust fungus (so called) were tried on a large scale in the United States in 1900 (11), and once again conflicting reports of its utility were obtained. On the other hand, Stockman (12) definitely states that the *Mucor* proved useless against locusts in India.

The writer repeatedly tried to cultivate *E. grylli* artificially, using the methods and media described under *E. musca*. In no case was a growth of *M. racemosus* or *M. etiosus* obtained. Fresh conidia, hyphal bodies and resting spores were used in these attempts, but only the first two germinated, and even these failed to grow in the real sense of the word, as no increase in bulk took place.

Abundant growths of two or three undetermined species of *Mucor* and of a species of *Rhizopus* were obtained by freely exposing bread-paste and potato-slices to the air. The spores of these saprophytic fungi were spread over slices of carrot and fed to cockroaches of an undetermined species that were common under stones at Cedara. These insects flourished in captivity, and none died during the three weeks they were kept under observation, although they must all have swallowed myriads of spores in this time. Similarly individuals that were liberally sprinkled with spores failed to become infected, although kept in a damp atmosphere. On the other hand, out of five nymphs that were inoculated with the spores suspended in sterile water, four died within three days, and all were found after death to contain ovoid and spherical bodies. These bodies were very numerous, some occurring singly, but the majority being in chains, and they were apparently the cause of death in each case. As a very similar growth was obtained in several instances from *Mucor* spores grown in hanging drops of nutrient solutions, it seems legitimate to conclude that the cockroaches in this experiment were killed by the *Mucor* spores injected into them. None of the five individuals in the control experiment that were inoculated with sterile water died.

The experiments carried out by the present writer failed to confirm the

results obtained by Edington, Black, Hesse, and Bernstein, yet it seems safest to reserve judgment in the matter, for it is a remarkable fact that these workers, experimenting with two different species of fungi in widely separated localities, should have obtained such similar results. Mycologists may find it very difficult to accept the theory that an *Empusa* becomes a *Mucor* when grown saprophytically, but the two orders to which these species belong are nearly allied, and similar cases of alternation of generations are familiar to all biologists. Furthermore, it is of interest to note in this connection that the torula form of *Mucor* bears a marked resemblance to the hyphal bodies that are characteristic of the *Entomophthoraceae.*

ENTOMOPHTHORA, Fresenius.

Cystidia and rhizoids present.
Conidiophores simple or branched.

Entomophthora aphidis, Hoffman. (Plate IV, figs. 13, 14.)

Conidia long ovoid, commonly asymmetrical, very variable, containing one to several oil-globules, 20–35 μ × 10–15 μ. Conidiophores simple or branched. Cystidia long and generally tapering at their extremities. Secondary conidia spherical, containing usually a single large oil-globule, produced by direct budding. Resting spores not seen at Cedara, but according to Fresenius and Sorokin they are "spherical, 33–45 μ, and borne terminally or laterally on hyphae." Host attached to substratum by rhizoids, few in number, and terminating in a disc-like expansion.

Hosts.—Several species of aphides.

Habitat.—South Africa, U.S.A., and Europe.

This species was first noticed at Cedara on November 11 on a species of large green aphis common on peas. It was common on certain species of aphides found on sweet peas, roses, maize, and *Datura stramonium* throughout the summer, serving as a very effective check on these pests. On the other hand, no specimens of the common cabbage aphis nor of a black aphis common on chrysanthemums were found infected with the disease.

The cystidia are not numerous, but are readily recognised by the fact that they are much longer than the conidiophores and contain very little protoplasm. Apparently they are hyphae, which would have developed into rhizoids if they had come into contact with the substratum. The rhizoids are long and comparatively stout, and lose their protoplasm soon after forming the disc-like expansion at the end.

Entomophthora apiculata, Thaxter. (Plate IV, figs. 15, 16.)

Conidia spherical, with a prominent papillate base, from 30–45 μ in diameter. Conidiophores simple. Secondary conidia like the primary, produced by direct budding. Resting spores were not seen at Cedara, but

according to Thaxter they "are formed laterally or terminally from hyphae, spherical, hyaline, 30–45 μ." Host attached to substratum by long and conspicuous rhizoids, few in number, and terminating in a disc-like expansion.

Hosts.—*Lepidoptera*, imagines of *Lycophotia muscosa*, Geyer (Noctuid), of an undetermined Geometrid, and a Lycaenid; larvæ of *Pachypasa capensis*. *Diptera*, imagines of a large Anthomyid fly, and of *Nephrotoma unicingulata*, Alex.; *Coleoptera*, imagines of *Trocalus fulgidus*, Fabr., and of *Adoretus ictericus*, Burm.; *Hemiptera*, adults of *Locris arithmetica*.

Habitat.—South Africa and U.S.A.

This species was exceedingly common at Cedara during the latter half of the summer, the first example of it being found on a larva of *Pachypasa capensis* on December 22. During the months of February and March it caused the death of large numbers of the beetles named above, the victims being found mostly on the trunks of wattle trees, fixed by means of rhizoids, with their wings partially spread.

The Cercopid, *Locris arithmetica*, is exceedingly common on grasses at Cedara during the summer months, yet only two individuals were found killed by this fungus. Both specimens were fixed to grass stems by means of rhizoids, and both had their wings outspread.

The fact that various species of hemipterous insects are liable to attack by *Entomophthoraceae* indicates that the host is infected by contact with the conidia, and not by their ingestion, as maintained by Hesse and others. It is difficult to understand how insects which are provided with mouth-parts such as those found in the *Hemiptera* and which feed on the sap of plants could swallow the comparatively large conidia of these fungi.

Entomophthora megasperma, Cohn. (Plate IV, figs. 17, 18.)

Conidia long ovoid, of irregular shape, with bluntly rounded apex and base, containing numerous small oil-globules, 10–20 μ × 15–35 μ. Conidiophores simple or branched. Cystidia not observed. Secondary conidia like the primary and produced by direct budding. Resting spores spherical, 35–40 μ in diameter, with thick, opaque, dark-brown epispore, borne laterally or terminally on the hyphae. Host fixed to substratum by rhizoids.

Hosts.—Larvae of *Euxoa segetis*, Schiff.

Habitat.—South Africa, U.S.A., and Europe.

In 1875 Cohn described a new parasitic fungus found in the larvae of *Agrotis segetum*, which he named *Tarichium megaspermum*. Only the resting spores were found, and Cohn's description of these agrees with that given above. Thaxter, in his monograph, describes a new species which he found on the larvae of *Agrotis fennica* and names it *E. virescens*. In this case only the conidial form was observed.

At Cedara an *Entomophthora* was found to be common on cutworms from October to January. It occurred in two forms; in the one conidia were thrown off in the usual manner, whilst in the other no conidia were formed, but the body of the host became filled with blackish brown resting spores. In both cases the hosts were fixed to the upper sides of leaves, etc., by means of rhizoids, but in the latter case the victim's body was blackened and flattened on to the substratum. There could be no doubt but that the two forms belonged to the same species of fungus. They occurred simultaneously on similar hosts, in each case the rhizoids were exactly similar, and in the bodies of cutworms containing the resting spores the remains of typical hyphal bodies could be found.

Thaxter expresses the belief that *E. virescens* and *T. megaspermum* are identical, but could not be sure, as he never found the resting spores. Both forms were found side by side at Cedara, although no specimens were found which bore resting spores and conidia simultaneously. Apparently the fungus exhausts itself in producing either one or the other form of reproductive body, but not both.

If these two forms do both belong to the same species—and every indication points that way—then Thaxter's name *E. virescens* falls before Cohn's *megaspermum* and the species becomes *E. megasperma*. According to Burger and Swain (12) *E. chromaphidis* also produces resting spores very similar to the above, and no individuals were found bearing both conidia and resting spores at the same time.

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EXPLANATION OF PLATES II—IV.

PLATE II.

Fig.

1. *Empusa muscae*, Cohn. Hyphal bodies from a still active fly.
2. „ „ „ Hyphal bodies immediately after death of host.
3. „ „ „ Hyphae from abdomen during conidia formation.
4. „ „ „ Conidia, showing mass of protoplasm surrounding each. Two conidia forming secondary conidia.
5. *E. conglomerata*, Sorokin. Conidia, one forming secondary conidium.
6. „ „ Resting spores (copied from Thax).

PLATE III.

7. *E. grylli*, Fresenius. Hyphal bodies from living grasshopper.
8. „ „ Hyphal bodies immediately after death of host.
9. „ „ Internal hyphae during conidia formation.
10. „ „ Conidia, some forming secondary conidia.
11. „ „ Conidiophores.
12. „ „ Resting spores.

PLATE IV.

13. *Entomophthora aphidis*, Hoffman. Conidia and secondary conidia.
14. „ „ „ Cystidia and rhizoids.
15. *E. apiculata*, Thaxter. Conidia.
16. „ „ Rhizoid.
17. *E. megasperma*, Cohn. Conidia.
18. „ „ Resting spores and one of the peculiar empty hyphae that often accompany them.

All figures, with the exception of No. 6, redrawn from camera lucida drawings. × 500.