OBSERVATIONS ON THE MORPHOLOGY AND CONDITIONS OF GROWTH OF A FUNGUS PARASITIC ON LOCUSTS IN SOUTH AFRICA

R. Sinclair Black M.B., C.M., D.P.H, M.A.

To cite this article: R. Sinclair Black M.B., C.M., D.P.H, M.A. (1895) OBSERVATIONS ON THE MORPHOLOGY AND CONDITIONS OF GROWTH OF A FUNGUS PARASITIC ON LOCUSTS IN SOUTH AFRICA, Transactions of the South African Philosophical Society, 9:2, 68-79, DOI: [10.1080/21560382.1895.9526317](https://doi.org/10.1080/21560382.1895.9526317)

To link to this article: <http://dx.doi.org/10.1080/21560382.1895.9526317>

Published online: 28 Jun 2010.

Submit your article to this journal [↗](#)

Article views: 6

View related articles [↗](#)

OBSERVATIONS ON THE MORPHOLOGY AND CONDITIONS OF GROWTH OF A FUNGUS PARASITIC ON LOCUSTS IN SOUTH AFRICA.

BY R. SINCLAIR BLACK, M.B., C.M., D.P.H., M.A.

Medical Officer Rotten Island.

(Read October, 1897.)

In March, 1896, I had an opportunity of examining some locusts brought from Richmond, in Natal, by Mr. Arnold Cooper to the Bacteriological Institute, Grahamstown, where I was at that time medical officer. These locusts Mr. Cooper found dying and dead in great numbers in a maize-field, many of them attached to the maize stalks in a dying condition. On closely examining them he found that they were all attacked by a fungoid growth, and on a microscopic examination of the tissues of the dead locusts, he found the mycelium of the fungus penetrating the tissues and growing in them. The locust was the large red variety (*Acridium purpuriferum*, Walk.) which had not appeared in these regions for more than thirty years, and which was lamentably destructive both from its numbers and voracity. Recognising the importance of this observation Mr. Cooper, accredited by the Government of Natal, brought a supply of dead locusts to the Bacteriological Institute for further examination. But at this time several observers in various parts of South Africa observed and recorded the presence of the fungus on dead or dying locusts. My part in this investigation was therefore the observation of the modes of growth and the morphology of this fungus.

APPEARANCE OF THE DEAD LOCUSTS.

On the dead locusts the fungus appeared as a grey or buff-coloured fur, in patches about the depression between the thorax and the abdomen, and the joints between the legs and body, and the line of the tracheal stigmata of the abdomen. The growth had a velvety appearance and was in all cases very short. On dissecting and teasing out the connective tissue beneath the integument and examining this microscopically the mycelium of the fungus and round or ovoid conidia were abundantly seen. I found precisely the same appearances in other diseased locusts which were sent to

us for investigation from King William's Town in May, 1896, thus showing that the fungus was spread over a large area of South Africa.

CULTIVATION EXPERIMENTS.

I now made an attempt to get a pure cultivation of the fungus, using as a medium for growth *sloped glycerine-agar* of the ordinary composition. I inoculated the medium with a platinum loop rubbed over with the teased connective tissue in which the mycelium was found. In twenty-four hours, at a temperature of 30° C., small yellowish translucent islets were visible on the surface of the medium; they had a radiate appearance and cog-wheel-like edges; in thirty-six hours they had increased in all directions, forming small circular patches, one-fourth of an inch in diameter, and a slight velvety growth was visible both aerially and into the substance of the medium. Growth in all directions was now vigorous over the surface of the medium, and the aerial growth, and that into the substance of the medium also increased; in forty-eight hours the velvety aerial growth could be seen dotted over abundantly with dark points which on a microscopic examination were found to be spore cases. After this reproductive effort the formation of spore cases apparently ceased but vegetative growth of the aerial filaments continued until the surface was covered with a thick filamentous grey mycelial growth; the strength of this aerial growth evidently depended to a large extent on the nutritive pabulum; for on less nourishing media, such as potato, it was far less abundant and slower in development. I noticed in growing it on potato that the growth continued for some time short and scanty, resembling in appearance that on the integument of the locust, and then the growth suddenly became vigorous and abundant. This increase of vitality I took to be due to self-sown spores growing on the decayed tissues of the earlier fungoid growth, thus obtaining a more suitable pabulum as will afterwards be seen.

MICROSCOPIC EXAMINATION ON GROWTH OF MEDIUM.

On examining the edges of the growing islet of fungus it was seen with a low power to be composed of the interlacing filaments of a fungus; the mycelium was composed of filaments branching alternately; the filaments being formed of elongated cells with no other differentiation of structure; the protoplasm in some of the filaments was clear and homogeneous, in others very granular, with large vacuoles scattered through the protoplasm of the cell; experience showed that this granularity and vacuolation was an accompaniment of active growth. Wherever active growth, either vegetative or sexual,

went on there the growing cell showed granularity of protoplasm ; a faint yellowish colouring of the protoplasm was often seen in these conditions. Such was the appearance of the filaments twenty-four hours after inoculation ; but after forty-eight hours very considerable differentiation of structure was apparent. Then a form of asexual reproduction made its appearance, rapid segmentation of the filaments taking place, followed by the throwing off of buds. This gemmation takes place on the filaments growing along the surface of the medium, on the perforating and on the aerial filaments. The formation of conidia is preceded by the rapid segmentation of the terminal mycelial filaments which at the same time show granularity, pigmentation, and vacuolation of the protoplasm. Once the process of segmentation begins, it goes on with the most extraordinary energy ; buds are thrown off in all directions and on separating reproduce the process. The conidia thrown off are ovoid or circular in contour, and of varying sizes, some being small, not measuring much more in diameter than a white blood corpuscle, while others are from three to six times larger. The margins show a double contour, this appearance being no doubt due to a thin cell wall ; the enclosed protoplasm is often vacuolated and irregularly granular ; what appears to be a nucleus is often seen, the protoplasm of which is darker and more homogeneous. These separated conidia repeat the vegetative cycle with great rapidity either by budding or by elongating ; a process being pushed out by the cell wall which takes part in the prolongation. It appeared to me that the initial division nearly always began with the nucleus when that was visible, the cell then elongating, and a septum forming which separated the new from the germinating cell. Actual gemmation, however, often seemed to take place, portions of protruded protoplasm being cut off by constriction of the cell wall. The new cells and buds could be separated with great ease from the germinating cell and then they immediately reproduce the same process ; in this way from a single cell long filaments were formed which branched alternatively, the branches often forming conidia and throwing them off. Such is the vital process of the formation of mycelium as is found on the surface of the nutritive medium and the filaments which, penetrating root-like into the medium, show the same characters and formation. These penetrating filaments, however, always ceased to invade the medium deeper than $\frac{1}{4}$ to $\frac{1}{3}$ of an inch. This stoppage of growth probably points to the necessity of considerable oxygenation as requisite to the vital process.

The aerial growth, however, which begins in the same way and goes through the processes above described, soon takes on a true

sexual process of reproduction ; that is to say, the protoplasm of two separated individual cells unites and rapid segmentation of the protoplasm of the conjugating cell takes place, an oospore being formed. The manner in which I have observed this process to take place is as follows :—

This reproductive function is established about forty-eight hours after the mycelial growth has begun on the surface of the nutritive medium. An aerial filament is observed branching, one of the short branches being very granular and terminating in a large granular conidium ; from the division of the stem another branch, the protoplasm of which is usually clear, and which may or may not be terminated with an enlargement or globular swelling, runs up and approaches the conidium, which it touches, and the protoplasm of the two cells in contact intermingles. The process, however, may very likely be more promiscuous, and I have seen appearances which make me suspect that terminal conidia were fertilised from cells or conidia of neighbouring filaments with which they came in contact.

When fertilised the oospore and its sporophore grow with great rapidity, the sporophore shooting up and outstripping the fertilising branch. The oospore develops a thick cell wall, which assumes a dark or brownish colour, and the protoplasm, which can be seen rapidly segmenting, also takes on a golden yellowish colour. The size of the oospore increases rapidly, and its cell wall becomes thickened ; the sporophore also increases in thickness out of proportion to the mycelium branch from which it springs ; with its growth it forces itself up into the oospore, forming a secondary globular enlargement or capitellum.

FURTHER DEVELOPMENT OF THE OOSPORE.

After fertilisation the oospore rapidly increases in size, the wall thickens, and the enclosed protoplasm becomes densely and finely granular and of a yellowish colour ; then segmentation makes its appearance, the enclosed protoplasm apparently resolving itself by condensation at numerous spots into numerous globular masses. These became more defined in shape, and, losing their circular contour, appeared ovoid or ellipsoidal. These ovoid light brown bodies are the spores, and these individually now increase in size, causing the rupture of the spore cyst. The spore cyst ruptures with violence, as the cyst wall has by this time become dry and elastic ; this is shown by the fact that the ruptured wall curls back after splitting. The spores are thus thrown broadcast over the surrounding area ; from the appearance of the ruptured cyst wall it is evident that it is at the time of rupture composed of two layers, the outer fine and transparent, the

inner thick and pigmented ; the capitellum stands out conspicuously from the floor of the ruptured spore cyst.

THE SPORES.

These, as I have said, are ovoid or ellipsoidal bodies of light brown colour, and semi-transparent, the pigmentation is evidently chiefly in the cell wall ; many of them appear more pointed at one end than at the other, and there is also a want of bilateral symmetry, in the majority, one side being the more flattened ; their sizes equal the diameter of a red blood corpuscle in length and one-half or one-third as broad. I have calculated that there are often as many as 200 spores in a single spore cyst. When in the space covered by a square surface of one-quarter of an inch of the fungus growing on nutrient medium I have counted as many as 100 spore cysts the enormous fecundity of the fungus may be imagined ; 20,000 spores being produced in this area, or 320,000 to the square inch.

DEVELOPMENT OF THE SPORES.

The spores when they reach maturity, and are ejected from the spore cyst, are ovoid or boat-shaped in contour. For examining the development of the spores I used a fine film of gelatine pressed between two cover glasses separated to the extent of the thickness of a cover glass ; a rim of Canada Balsam round the edges of the cover glasses made a closed cell which could be fixed to a glass slide. The development of the spores shown in the gelatine could then be easily watched from day to day under the microscope. Observation showed that at ordinary temperature in twelve hours the ovoid spores had become much larger and had lost the ovoid form and become circular. In twenty-four hours further a still greater increase in size had occurred and the spores had begun to sprout ; in twenty-four hours further the sprouts had greatly increased in length and showed the characteristic alternate branching and the segmentation of the mycelium. Examined with a high power the spores at the end of the first twenty-four hours were not distinguishable from the conidia of the fungus ; the cell walls had become thin and the protoplasm from being homogeneous had become granular and vacuolated. The vegetative process was from this stage exactly as I have described it in the case of the vegetative conidia ; sprouting, elongation of the sprout, and branching of the mycelium thus formed taking place ; thus the growth was as given above : first the formation of mycelium, then the throwing off of conidia, finally the formation of spores by sexual reproduction. The cycle from spore to spore occupied, at room temperature, about five to six days. There may possibly have

been some phases of the reproductive process which I may have overlooked, either from inaccuracy of observation or inexperience in working microscopically with fungi. But I may here state that I made repeated experiments with the fungi from many separate cultivations, so that I over and over again clearly made out and verified the phenomena above described by numerous separate observations on many different occasions.

SUITABILITY OF MEDIA FOR GROWTH OF THE FUNGUS.

As I have said, the primary cultivation experiments were made on glycerine-agar peptone, and that medium was always found very suitable; but the fungus grew well on many other media, such as gelatine peptone, sugar agar, a solid medium made with crushed locusts: in fact, on all nitrogenous solid media it grew well. On bread paste and on potato it grew slowly at first, but after the lapse of a fortnight the growth suddenly became fairly luxuriant. I have stated above the cause to which I attribute this phenomenon. In nitrogenous fluid media, such as beef bouillon, a bouillon made from dead locusts, &c., it grew most vigorously; gradually filling the upper part of the tube with a fine flocculent growth, which close to the surface became a dense felted network. Only the asexual forms of growth took place in the fluid, but these were produced with the greatest exuberance, conidia being thrown off and mycelium being formed in all directions. When the growth came to the surface aerial filaments were formed with the production of spores in the way above described. The more vigorous growth close to the surface supports what I stated above with regard to the growth in solid media that the appearance of air is necessary for active growth.

In media made from non-nitrogenous substance, such as starch and sugar, the growth was not nearly so abundant, and soon ceased. In comparing tubes of bouillon and tubes of strong starch and sugar solution sown with fungus spores on the same day, after a week's growth this difference was very remarkable: in the nitrogenous tube the growth being vigorous and abundant, in the non-nitrogenous it being feeble and scanty. Besides, on microscopic examination, the mycelium in the carbohydrate solution was very pale, extremely vacuolated, and the granularity of the protoplasm was absent, and also in the older mycelium there was marked fatty degeneration of the protoplasm, large yellow globules of oil being abundant in the cells, the protoplasm of which was collapsed. It would thus appear that the fungus is one which needs nitrogenous matter for its healthy growth. From the foregoing considerations, the ease with which the fungus grows on various media, at ordinary room temperature,

and also from the following experiment it would seem that there is reason to believe that the fungus may be saprophytic over a wide area in South Africa. The ease with which it can be cultivated makes it difficult for me to believe otherwise.

RESISTANCE OF THE FUNGUS TO DRYING AND TO HEAT.

I took the felted pellicle of growing fungus from a tube of beef bouillon and dried it thoroughly *in vacuo* over sulphuric acid. After the interval of a month I broke up the flinty mass thus produced, and on sowing particles of it on glycerine-agar, the characteristic fungus growth was produced. The exposure to moisture or dry heat over 150° F., however, quickly killed the fungus whether dried or not.

INOCULATION OF HEALTHY LOCUSTS, &c., WITH THE FUNGUS.

I obtained some healthy locusts from the surrounding district, and inoculated them by rubbing the connective tissue of locusts dead from fungus disease over them, and others I inoculated with the cultivated fungus. Within a week a considerable proportion of the locusts, both those inoculated with the cultivated fungus and those inoculated from the locusts dead of fungus disease, showed symptoms of illness, and a large proportion, about 40 per cent. died. These dead inoculated locusts showed the characteristic mycelium and conidia in their tissues, thus proving the identity of the cultivated fungus with that found in the original dead locusts. The greyish growth which I above described as appearing on the locusts brought from Natal, was also apparent in several of the inoculated locusts. This surface growth I found to possess the true sexual mode of reproduction, and showed the characteristic spores.

I fed some healthy locusts on the cultivated fungus, and some of these also died showing the characteristic appearances, but whether in the process of feeding some spores got on the surface of the locusts and grew from there, or whether the infective process spread from the alimentary canal I do not know. The process of infection from locust to locust is probably by the ovoid spores which get into the tracheal stigmata and then begin to sprout, penetrating into the connective tissues surrounding the tracheal tubes, and there forming the vegetative mycelium and conidia. I did not, however, microscopically demonstrate the actual presence of the spores in the trachea. There can however be, in my opinion, no hesitation in affirming the identity of the growth inside the locust with the cultivated fungus.

I also inoculated the large green grasshopper (*Phymateus leprosus*, Fab.) so commonly found on garden sub-tropical plants, with the

cultivated fungus, and this insect also contracted the mycosis and died. I found mycelium in its tissues and the conidia; also it was very interesting to find that its excreta after being discharged in a few days became covered with a luxuriant mouldy growth, from which I was able on glycerine-agar to cultivate the characteristic fungus afresh. I was unable at the time to pursue my experiments further in this direction, but the one case is sufficient evidence to show that the mycosis is not confined to the locust species. The fact also that I found the characteristic mycelium and the spores in locusts sent from King William's Town in May, 1896, and that the fungus grows so freely on albuminoid and decaying animal matter would lead us to suppose that a careful search would demonstrate that this fungus in certain seasons is fairly widespread in South Africa, and that it attacks from year to year, during the warm season, locusts and other allied insects. But whether the fungus is carried to these parts by the swarms of locusts, or whether it is saprophytic on decaying albuminoid matter throughout these regions and then attacks the locusts which arrive in these parts, is a matter for further investigation.

CLASSIFICATION.

I was at first inclined to believe that the fungus belonged to the class *Entomophthoræ* with which it has, as will be seen, many strong resemblances, but it has also several characters in common with the *Mucorini*, so that my limited experience will not allow me to say that it is not closely connected with the latter class.

PATHOGENICITY.

Sufficient evidence has been given to show that the fungus is pathogenic. But what we know of the pathogenicity of this class of parasites does not encourage me to believe that through this agency destruction of locusts will take place on an extensive scale. When I brought the fact of the ease with which the fungus could be cultivated to Dr. Edington's notice, he suggested that it should be sent out in small tubes of glycerine-agar to districts where locusts were abundant and destructive. Large numbers of such tubes were distributed throughout Cape Colony during the latter half of 1896, and, as will be seen from Dr. Edington's report of the Bacteriological Institute for 1896, very favourable accounts were returned of the pathogenic action of the fungus on swarms of locusts in certain districts. But in spite of this positive evidence I do not think that sufficient data for a positive induction as to the value of the fungus in destroying locusts in large numbers have been collected; it is

doubtful, in my opinion, whether this method of spreading a pathogenic mycosis can compete with the usual methods of destroying locusts *en masse*.

The general bearing of the evidence as to the life-history of the fungus which I have laid out above, would be to show that we have here to do with a fungus saprophitic on decaying animal or vegetable matter, which occasionally becomes parasitic on and pathogenic to locusts and other allied insects in South Africa, and which has probably acted in this way for an indefinite time in the past, though it has for the first time in 1896 been brought under actual observation.

APPENDIX.

REPORT ON THE DISEASE AMONGST LOCUSTS IN NATAL CAUSED BY A FUNGUS *EMPUSA ACRIDII*.

BY ARNOLD W. COOPER, F.R.M.S.

In the month of February last (1896) specimens of locusts, which had died from disease, were sent to me for examination by Mr. Willie Nicholson, of Thedden, near Richmond. The locusts were covered with a fungoid growth which resembled Muscardine. The fungus is now determined as belonging to the Order *Entomophorææ*, Genus *Empusa*; the species is probably a new one, and it is proposed to name it *Acridii*.

Microscopical examination showed that the locusts, now identified as *Acridium purpuriferum*, had died from this fungus disease, which nearly resembles Muscardine, which broke out amongst the silkworms in Southern Europe many years ago, and threatened the destruction of the silkworm industry in the countries affected by it. It was found that a careful removal of the dead silkworms was the most ready method of checking the ravages of the disease, thus showing that the infection or contagion spread.

Experiments made here with the locusts showed that the local fungus disease was easily communicated from the dead locusts to healthy ones, and death ensued in five or six days; the experiments were on a small scale only, but repeated satisfactory results showed me that there was little doubt of the value of the discovery.

Specimens of the diseased locusts were forwarded, by his Excellency the Governor, to Cape Town, and a report upon them was made by M. Péringuey, of the Museum, in which he recommended that I should set to work in earnest to endeavour to spread

the disease, and also stated that it was the best satisfactory announcement he had been able to make for the last seven years regarding the destruction of locusts by natural enemies.

Early in April I proceeded to Cape Town, to confer with M. Péringuey on this subject; on my arrival there I found that the Agricultural Department of the Cape Colony had arranged for the resources of the Bacteriological Institute at Graham's Town to be placed at the service of the Natal Government, for the purpose of research into the disease. At the request of the Cape Government, and authorised by Sir John Robinson, I left Cape Town after making the necessary investigations there, and arrived at Graham's Town on the 23rd of April. Before leaving Cape Town I had telegraphed to Richmond for suitable material for the investigation, which arrived at Graham's Town on the 24th of April.

Dr. Edington, the Director of the Institute, being absent, Dr. Sinclair Black took in hand the cultivation of the fungus; several media were tried with more or less success, and pure cultivations were obtained of the fungus. Locusts having been obtained in the neighbourhood of Graham's Town, experiments were made to ascertain whether the cultivations would be successful against them. The results of the experiments are quite satisfactory, the affected locusts died in five or six days, and on microscopical examination I was gratified to observe the same results and conditions as I had seen in the dead specimens sent to me in February by Mr. Nicholson, and which had appeared in the successful experiments I had made at Richmond, and as appeared in locusts destroyed at Graham's Town in an experiment in which locusts obtained there had been affected directly by some of the material sent to me from Richmond.

The results of the investigations so far must be considered very satisfactory, and justify further research and experiments with the view to spreading the disease in other localities, and over South Africa.

Measures of destruction have been taken from time to time against injurious insects in other countries, by means of diseases somewhat similar to the one now under consideration with more or less success, but I am not aware of any great measure of success having been attained. From information which I have gathered, and from reports which I have seen, I learn that in most instances these measures were tried with fungi which were not natural either to the injurious insects it was sought to destroy or to the localities or countries.

In the case under consideration the fungus appeared naturally as

an epidemic amongst the locusts, and is probably indigenous. The only case which appears to be at all parallel to the present one is that of the Muscardine disease amongst the silkworms, before mentioned; in that case the object was to stay the disease, which was done with difficulty, in this case the object is to disseminate it, and there appears to be good grounds to hope that this may be done.

The fungus, although not the same as that causing "Muscardine," grows very rapidly in artificial cultivations in suitable media, and developes an immense quantity of spores, which, on coming into contact with the locust, immediately germinate, the growth at once penetrating the outer skin and the mycelium rapidly spreads through the fatty matter in the body of the locust, and through the internal organs, thus preventing their proper and natural action, and causing death. It is probable that climatic influences may either lengthen or diminish the term of five or six days observed in the experiments, although much variation is not likely.

The life-history of the fungus, with illustrations, and a further account of its action will form the material for a more detailed report when investigations are more complete.

As the result of the observation and investigations up to the present time the following facts may be learned:—

1. That the fungus disease broke out amongst the locusts in an epidemic form, and appears to be natural to the country.
2. That it is fatal to the locusts in all stages of growth (except the egg).
3. That the fungus can be easily cultivated artificially in quantity in suitable media, and artificially communicated to locusts.
4. That when the locust is attacked by the disease it is, from the nature of the fungus, fatal.
5. That no evil results to stock or pasture are likely to ensue from the dissemination of the fungus.

RECOMMENDATIONS.

That investigations be continued in Natal, with suitable experiments, in order to ascertain the extent and localities of the fungus. The manner in which, and the extent to which, locusts may be best destroyed.

That provision be made for the cultivation of the fungus, and that field experiments be undertaken.

REMARKS.

Most, if not all, the material required to carry out these suggestions and recommendations may be had in the Colony or Cape

Town. The expenses of the investigations will not be heavy, and there are good grounds upon which satisfactory results may be looked forward to.

Dr. Edington considers that the spores of the fungus, which are very numerous when grown in proper media, may be easily obtained from cultivations and spread on a large scale by means of spray pumps, the locusts when sprayed would carry, and further spread the disease; there would be no practical difficulty in the way of carrying out this experiment, as a good pump would throw the medium, with which the spores should be mixed, to a considerable distance.

RICHMOND, *May* 11, 1897.