GARMAN

Studies on the Fungous Parasites
of the Corn-Field Ant (*Lasius Niger*
L. Var. Americanus Emery)

Entomology

M. S.

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STUDIES ON THE FUNGOUS PARASITES OF THE CORN-FIELD ANT
(*LASius NIGER* L. VAR. *AMERICANUS* EMERY).

BY

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B. S. Kentucky State University, 1913.

THESIS

Submitted in Partial Fulfillment of the Requirements for the
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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

Philip Garman

ENTITLED Studies on the Fungous Parasites of the Corn-field
Ant (Lasius niger L. var. americanus Emery).

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE

DEGREE OF Master of Science in Entomology

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Sporotrichum globuliferum Speg. as a Parasite of the
Corn-field Ant (*Lasius niger* L. var. *americanus* Emery).

Injuries to corn by the corn root-aphis (*Aphis maidiradicis* Forbes) cause the Illinois farmer a very heavy loss every year. This insect infests the roots of the corn, sucks its juice and stunts its growth. The injury being wholly subterranean, it is impossible to apply the usual insecticides without an amount of labor and expenditure such as make^s the procedure unprofitable. The pest, however, is unable to live without the aid of another insect, the cornfield ant. The latter lays bare the roots of the corn for the aphids, keeps their eggs over winter in its burrows, and in spring before corn is planted harbors them upon the roots of near-by weeds.

As the aphids live entirely below the surface during the feeding season, it is impossible to reach them with insecticides. The ants, on the other hand, frequent the surface to some extent; and while it would be impossible to exterminate them they might be reduced in numbers so that the indirect injury caused by them would be comparatively small. The aphid problem then, is two-fold. One part of it is concerned with the destruction of the aphids; the other with destruction of the ant. The first part is attacked with difficulty; the second is more easily approached; and a solution of the second would be a solution of the whole problem.

To destroy the ant, several methods might be used. The common insecticides have been found to be of little use. Fungi might be used to some advantage, but for its successful use a fungus must fulfill several specifications. First, it must be naturally pathogenic for the insect against which it is used.

Second, it must be capable of surviving existing weather conditions and living through periods of drought or cold weather, which seem to be detrimental to the growth of most fungi.

It is an established fact that fungi grow best under moist meteorologic conditions, and insect fungi are no exception. As might naturally be expected, most varied results have been obtained from the artificial application of fungi, in some cases amounting to complete success, in others to complete failure. These varied results may be due to inconstancy of atmospheric conditions or to variation of some unknown factor or they may be due to a lack of virulence on the part of the fungus.

In Florida, noteworthy^y results have been obtained with various ascigerous forms attacking the San Jose scale and the white fly. In Trinidad, the green muscardine (Metarrhizium anisopliae americanum) has been used with remarkable success in destroying the cane frog hopper (Tomaspis postica). Only recently, workers in Europe have discovered a coccobacillus which is particularly effective in despatching various insects. Notwithstanding, workers in the western United States, particularly Kansas, have completely abandoned the use of the white fungus of chinch bugs and those working in the East have done likewise with the gypsy moth disease. The negative results obtained in these regions should not, I think, be taken as conclusive proof that fungi are of no value there, for it may be that the organism tried was not truly parasitic or that the methods of application were at fault.

At any rate, it becomes necessary, first, to determine the true value of the organism as a parasite; and then to find out what are the favorable conditions for its spread. From such data, it would

be easy to determine the best periods for sowing the fungus, and we should avoid much of the unnecessary field work that is usually done to establish the mere effectiveness of a given species.

An attempt is being made by me to obtain such data for fungi which attack the cornfield ant, and so far the work has been confined to a single species. The results obtained can not be taken as conclusive on account of the incompleteness of the study, and no effort will be made to summarize the work in its unfinished state.

Thanks are due to Dr. S. A. Forbes, Dr. R. D. Glasgow, and Mr. P. A. Glenn for their suggestions and general supervision of the work. The experiments have been conducted largely in the insectary of the State Entomologist, and in the entomological laboratory of Dr. A. D. MacGillivray.

Discovery and description of the fungus.- In September 1913 , Mr. P. A. Glenn found upon the Illinois University farm a number of dead ant pupae (species unknown, but not Lasius niger americanus) that were completely covered with a dense, white fungous mycelium. The land where the specimens were found was not particularly low or damp and the soil contained only a moderate amount of moisture. It was thought that the fungus might prove valuable and it was therefore made the object of tests and experiments with a view to determining its real worth.

The conidia were first plated out on beef agar, and a pure culture was obtained from one plate. The fungus was then transferred to potato strips on which medium it grew well and formed a quantity of conidia sufficient for purposes of experimental infection. Stock cultures were kept on the potato and all experimental work was done with the stock cultures as a basis. The original material on the pupae of ants consisted of a dense white mycelium bearing quantities of conidia, a large part of which were cohering in globular masses. The spherical conidia were 1.5 microns in diameter; the globular masses which they formed from 4 to 9 microns. Hanging drop cultures showed that all the conidia were produced on short flask-shaped sterigmata, 3 to 3.5 microns in length. At the end of each are short threads bearing a number of spores. The spore-bearing sterigmata are put out at definite points along the hyphae and when they become dense enough they form, together with the conidia, the spherical masses alluded to above. In cultures, the hyphae are mostly procumbent, the erect ones being comparatively short. They vary to some extent in diameter, depending upon their relation to medium. This is especially true of aerial and non-aerial hyphae.

The average diameter of the former is somewhat more than 1.5 microns and increase^s to double that diameter in hyphae growing below the surface. Within the host, short rod-like bodies are formed that have been called conidia by some; but it is doubtful whether these are anything more than unusually thick and septate hyphae. In the stalks of the coremia described below, the hyphal threads also become swollen, though not so much so as in material from the interior of the host. Septation is not, as a rule, noticable in young hyphae but becomes evident with age and may be easily demonstrated by stains. *

On plain beef-peptone agar, the fungus produces colonies which appear above the surface (if sown below it) in three or four days and begins to produce new conidia. The colony then becomes convex, if sufficient moisture is present, and may grow to the diameter of several centimeters. Eventually the colonies show a tendency to sink in the middle. On potato the growth is much more dense than on agar and the mass is decidedly cream-colored. Conidia, too, are produced much more freely than on agar; and in from twenty days to a month cultures begin to send up sterile bunches of hyphae, tightly bound together by a glutinous substance. These coremia sometimes become three or more centimeters in height, and where they touch the glass they take on a color at first greenish yellow and finally becoming a shade between a light orange yellow and a deep chrome. In the bases of coremia not touching the glass a color is produced that is similar to that

* A simple method of staining consists in placing a bit of mycelium on a cover-glass in 80% alcohol, teasing it apart, allowing it to dry and fixing it by heating gently. The cover is now flooded

first described. The coremia become clavate, and are at first pure white, contrasting strongly with the cream colored masses of spores about them. Conidia are produced at length upon the surface of the coremia.

Conidia sown in agar germinated in from eighteen to twenty hours when kept at a room temperature of from seventy to eighty degrees Fahrenheit, the germination taking place with the production of one or two, occasionally three, germ tubes. In three days, the fungus appears above the surface and sends out aerial hyphae which form new conidia. In no case were conidia produced before the end of the three days.

Several *Cecropia* pupae were artificially inoculated with the fungus and in a short time died and became shrunken and hard. Some of them were then placed in a moist chamber, and covered with damp sphagnum moss. Pieces of one specimen were also placed in a petri dish on moist blotting paper. In both instances mycelia developed and bore conidia freely. When examined, the growth of mycelium seemed to be largely from the layers directly beneath the cuticle. The specimens placed in the damp moss sent out bunches of hyphae which came to the surface and produced conidia in abundance, but failed to grow anything like characteristic *Isaria* "sporophores", even after several months time. On ants the growth is essentially

with dilute carbol-fuchsin and allowed to stand for a few seconds; two or three is sufficient. Next the stained mass is washed in water and is allowed to dry, and the whole ^{is} mounted in balsam. This method, while open to the possible objection of plasmolizing the cell contents, has been very useful in demonstrating the presence and nature of sterigmata.

the same as on the *Cecropia* pupae but differs in the character and location of the internal mycelium. Sections in the region of the gaster showed that mycelium completely replaces the internal organs and forms short elliptical (sometimes dumb-bell-shaped) bodies. It appears to gain exit through the membranes connecting the segments or between the joints of the legs, etc., and frequently develops extensively on the outside of the insect where the usual spore masses are produced. The exact point of entrance has not been determined but it seems likely that it is by way of the mouth, although no definite data have been secured.

Name of the fungus.- Material was sent to Dr. Roland Thaxter of Harvard University for identification and was named by him *Sporotrichum globuliferum* Speg. The systematic position of the fungus seems to me to be somewhat uncertain owing to the manner of conidia-production described above, and the appearance of coremia on certain media. Saccardo does not mention the presence of sterigmata in describing the species. Burrill* in his description of the *Sporotrichum* from the chinch bug mentioned the spore bearing sterigmata, "basidial cells". In stained preparations they are exceedingly distinct and there can be no doubt as to their presence. Again, there seem to be differences in the morphology and growth of fungi almost identical with this *Sporotrichum* that indicate separate species or at least varieties. The production of coremia, itself seems not to have occurred in the earlier cultures of *Sporotrichum* from the chinch bug. (Forbes** and Snow***), though cultures of the same species.

* 17th Rep Sta. Ent. Ill. p. 81 (note). 1889-1890.

** Univ. Kans. Exp. Sta. 4th Ann. Rep of Director, 1894.

*** 19th Rep. Sta. Ent. Ill. p. 95, Figs. 1-4, 1893-1894.

from May-beetles did show fasciation to some extent*. Cultures now in the possession of Dr. Glasgow show two distinct types; one rarely producing coremia in potato cultures, the other frequently fasciated. Both of these fungi have been authoritatively determined as Sporotrichum globuliferum Speg. It has been suggested that such differences are due to variations in the media; but according to my own observations neither form can be made to produce the other, and the differences still exist when the two fungi are grown on the same media and under similar conditions of moisture.

Several forms^s of *Isaria* possess similar sterigmata to those of *Sporotrichum*, and when grown on such media as beef agar and potato strips can hardly be distinguished from the latter. Pettit** established a new species of *Isaria* on the basis of "sporophore" production and described cultures of *Sporotrichum* from the chinch bug and a carabid as being entirely without "sporophores". He says further that a microscopic examination of the fungus (*Isaria vexans*) showed the fructification to be exactly as in the case of typical Sporotrichum globuliferum. Other cultural features, too, seem to be identical, the only difference being in the production of "sporophores". Evidently here are two separate species, or at least varieties, the question remaining as to whether the two forms should be referred to separate genera. The fungus from ant pupae identified by Dr. Thaxter produces "sporophores" abundantly on potato strips and to some extent on artificially inoculated *Cecropia* pupae. It agrees in every particular with Pettit's description of Isaria vexans. It is possible that herein lies one of the unknown factors in the chinch-bug-*Sporotrichum* mystery, namely, that two varieties or species exist, one of which is

* 18th Rep. Sta. Ent. Ill. p. 95, Figs. 1-4, 1893-1894.

** Cornell Agr. Exp. Sta. Bull. 97 p. 365, 1895.

actively parasitic, the other saprophytic or only occasionally parasitic. The whole group needs further investigation, and our knowledge of it would be helped materially by a thorough and complete study of the Hyphomycetes or Moniliales, and by a monograph of the particular families that include the Isarias and Sporotrichums.

History of Sporotrichum globuliferum Speg. in America.-

The first record of a fungus anything like *Sporotrichum globuliferum* was made by H. Shimer* in 1867. From his description the form is not identifiable but it was probably this species. Between 1867 and 1882 the fungus received little attention. In Jan. 1884 Joseph Leidy** exhibited before the Academy of Natural Sciences of Philadelphia an ant, *Camponotus*, covered with a fungus. It, too, can not be identified from the brief mention given it, but may have been a species of *Sporotrichum*. In 1888 Forbes*** distinguished three diseases of the chinch bug, one of which was characterized by a profuse growth of *Sporotrichum globuliferum*. He further described the disease and figured the fungus in 1889, 1895 and 1898#. In this period came also the reports of Snow,## (1891 to 1897) setting forth the results of experiments with the disease. About this time Pettit### of Cornell described various *Isarias* and *Sporotrichums* from artificial cultures. Among others, he obtained a fungus on wasps which he doubtfully called *Isaria* sp. and which seems to me to be nearly the same as the fungus now under investigation. After this period of first discovery and

* Proc. Acad. Nat. Sci. Phil. V. 19, pp. 75, 234. 1867.

** Ibid. p.7. Jan. 1884.

*** Psyche, V. 5 p. 110.

16th Rep. Sta. Ent. Ill., pp. 1,45,46; 19th Rep. pp.16-176;
20th Rep pp. 25-74.

Ann. Rep. Kans. Univ. Exp. Sta. nos.1-6. 1891-1897.

primary investigation, little was done with the fungus until about 1910 except to make a few casual observations upon it. In 1911 Billings and Glenn* concluded, from experiments, that it was useless to sow *Sporotrichum globuliferum* by artificial means, because it was naturally present in the soil in quantities greater than could be obtained by such dissemination. Still later Headlee and McCulloch** of the same station studied the problem and their results were published in Bull. 191 of that station. They include in this work a list of insects attacked by the fungus, which contains representatives of Coleoptera, Hemiptera, Lepidoptera, Hymenoptera, Orthoptera and Diptera.

Experiments with infection only.— The ant material for the following experiments consisted largely of ^{specimens of} the species *Lasius niger americanus* collected on the farm of the University of Illinois.

They were obtained by simply digging up hills of corn and locating their nests. In the laboratory the most convenient way of keeping them prior to starting an experiment was found to be in small two- or three-quart tin buckets in which were placed several inches of soil. The formicaries used were modified Janet nests consisting of a double chamber connected by a small opening. The brood cell was darkened by placing over it a piece of black paper or a piece of dark glass while the runway cell was left well lighted and covered only by a single piece of clear glass. Water was supplied to the ants by placing it in a small chamber at one end near the brood nest and allowing it to soak through. In this way it was easy to keep the cells reasonably moist. The exterior of the formicaries was coated with shellac or paraffin which served to keep the water from evap-

* U.S. Dept. Agr. Bull. 167 1911.

**Kansas Agr. Exp. Sta. Bull. 191, 1913.

orating from the outside and made frequent watering unnecessary. It was sufficient to water the cages only once every three or four days. A rim on each side of the formicary allowed a number to be stacked together, thus economizing space.

Temperature in the insectary where the first experiments were made , ranged from 50 degrees Fahrenheit to near 100, the average being a trifle below 70. The ants were fed granulated sugar-which they seemed to eat to some extent- and small larvae and pupae. They seemed very fond of the latter and carried them to their young in great haste, or else set busily to work gnawing off pieces and eating them themselves. Larger pupae, such as those of *Samia cecropia* were also tried, but the ants did not eat them readily and piled dirt and other refuse about them. Freshly killed flies were also eaten by the ants.

1. October 10, 1913, a colony of thirty workers and thirteen pupae (*Lasius niger americanus*) were placed in one of the formicaries described above, and spores of the fungus were dusted in the outer cell. After two weeks time, twenty dead adults were found, many of which showed the typical white powdery mycelium of the original fungus. Eighteen days from the date of infection all of the colony were dead. The fungus growing on the ants was isolated and obtained in pure culture. It is without doubt the same as the one used to infect.

2. On the same date , October 10, 1913, 33 workers, 62 winged queens and 3 larvae, (*Lasius sp.*) obtained on the University campus , were dusted with spores and placed in a formicary. After a period of one week the ants were found dying in numbers and thirty dead queens were removed from the cage. One queen had tufts of mycelium

growing on various parts of the body. In two weeks 37 queens and 29 workers had died, and by the end of the third week 32 workers and 61 queens, or nearly the whole colony succumbed. The fungus was isolated from the dead queens and obtained in pure culture.

3. October 10, 1913, a colony of 35 workers, 45 pupae, and 10 larvae (Lasius niger americanus) was placed in a cage and kept beside the first two. These ants received no treatment. Eighteen days from the date of starting the experiment the cage was accidentally left open and the colony escaped. No dead were left in the cage.

4. October 31, 1913, a colony of about 45 workers (Lasius niger americanus) were treated by placing a small piece of fungus covered medium in the outer cell. No effect was noticed until the third week, when twenty dead workers were seen, two or three of which showed a growth of white fungus resembling that used. After one month eighteen live ants still remained and the experiment was discontinued.

5. November 17, 1913 a colony of Lasius niger americanus with 75 to 100 larvae (estimated) and a number of workers were treated with the fungus as in the preceding experiment. In one week a pile of dead, pinkish larvae were found in the inner cell. (Note that the fungus was placed in the outer cell). In two weeks, only 2 adults and 10 larvae remained alive, and ⁱⁿ one month the colony had perished completely.

6. November 17, 1913, a colony of Lasius niger americanus with 30 to 40 workers and about 75 larvae were infected by placing pieces of media covered with fungus in the outer cell. After one week 13 dead workers and 11 dead larvae were noticed, the larvae being pinkish in color. One month from the date of infection there remained 19 live workers and a few larvae. A fungus was isolated from the dead

ants which proved to be identical with the original.

7. November 17, 1913 a colony of sixty workers (Lasius niger americanus) , over one-hundred larvae and one queen, was established as a check, receiving no treatment. In one week, 2 dead workers were seen, but at the end of one month no more had died and no fungus appeared upon the dead ants. One month and a half from the day when the experiment was started, 20 dead workers were found, but the colony seemed to be otherwise healthy. Two months and a half from that date a large portion of the colony was still alive and the experiment was discontinued.

8. November 17, 1913, a colony (Lasius niger americanus) with over 100 workers and more than 100 larvae was treated by placing a piece of fungus-covered medium in the outer cell. After one week 12 dead workers were noted and 30 dead larvae that were pink in color. Three weeks from the initial date, there was no increase in the number of dead, though several of those already dead showed signs of fungus. After one month and a half the colony had died completely but the fungus was scanty on the dead material and nothing was isolated that resembled the original fungus.

9. November 17, 1913, a colony of 30 to 40 workers and about 100 larvae (Lasius niger americanus) was treated as in the preceding experiment. After two weeks, 5 dead workers were noted and 6 dead larvae that were pink in color. A mold soon appeared which proved not to be the fungus used to infect. After one month there were 9 dead workers and a number of dead pinkish larvae. At this time there remained 26 workers and 6 larvae. After a month and a half all the ants were dead. The fungus could not be isolated from the dead ants.

10. November 17, 1913, a colony of Lasius niger americanus with 10 to 20 workers and 30 larvae, were treated as in the preceding experiment. After one week seven dead workers were noted and 10 pink larvae. After one month all were dead and the fungus was subsequently isolated from the dead ants.

11. November 17, 1913, a colony of Lasius niger americanus containing 75 to 100 workers and 50 to 75 larvae, were treated as in the preceding experiment, by placing a piece of fungus covered medium in the outer cell. After a week 36 dead workers and 9 dead larvae were removed from the cage. Two weeks from the beginning of the test 18 more dead adults were noted, After three^{weeks,} 30 workers and 5 larvae remained alive, and after one month there was no increase in the number of dead. In one month and a half, however, all were dead. The fungus was isolated from the dead ants, and proved to be the same as that used to infect.

12. November 17, 1913, 30 workers and 50 larvae (Lasius niger americanus) were treated as in the preceding experiment. After one week 18 dead adults and 9 dead pinkish larvae were noted, Two weeks from the initial date there were 30 dead workers and the dead larvae were covered with a dense felt of white mycelium. In three weeks all were dead. The fungus was isolated from dead larvae and appeared identical with the original.

13. November 17, 1913, a colony of Lasius niger americanus with 40 workers and 20 larvae and a queen was taken as a control, receiving no treatment. One week from the above date seven dead workers were seen in the cage. Subsequent observations showed no increase in the number of dead until a month and a half from the day of starting the experiment, when the colony suddenly died. No fungus

was isolated from this experiment that resembled the original.

14. December 2, 1913, a colony of Lasius niger americanus with 30 workers and over 100 larvae was started as a check. Three weeks from date no change had taken place in the number of individuals and no dead were seen. One month from the initial date there remained twelve workers and a large pile of larvae and nearly two months from that date the colony was still alive and of approximately the same size as before.

15. December 2, 1913, 40 to 50 workers and over 100 larvae of Lasius niger americanus were established without treatment. At the end of three weeks there were no dead, and after one month there remained 33 workers and a large pile of larvae. After two months the colony was still alive and in good condition.

16. December 2, 1913, 10 workers and 20 larvae of Lasius niger americanus were exposed to fungus spores placed in the outer cell. After two weeks all the ants were dead and the experiment was discontinued. The fungus found growing upon the dead ants was isolated and a pure culture was obtained which was identical with the original.

17. December 2, 1913, a colony of 20 workers and 20 larvae of Lasius niger americanus was established with no treatment. After two weeks no dead were found. At the end of the third week the colony escaped, but left no dead.

18. December 2, 1913, 50 to 75 workers and from 50 to 75 larvae were treated by placing a small piece of fungus covered medium in the outer cell. In two weeks several dead workers were noticed, and in one month only three workers remained alive. A fungus was isolated from the dead ants which proved to be the same as that used for

infection.

19. December 4, 1913, a colony of Lasius niger americanus was established as a check. After three weeks no dead ants were found. After one month there remained 5 or 6 workers and 40 larvae. No fungus was found on the dead ants, however, and after two months, the colony being still alive, the experiment was discontinued.

20. December 4, 1913, a large colony of corn-field ants was placed in a two-quart tin bucket half full of soil. In this bucket the colony had lived for some time and seemed to be in good condition. Moisture conditions were ideal on account of the tight cover allowing little evaporation from the soil within. Spores were mixed with the soil in the bucket. In two weeks all the ants and their larvae were dead and piles of them could be seen scattered about over the surface that were covered with a growth of white mycelium. Isolations were made from the dead ants and the fungus obtained in pure culture. It is undoubtedly the same as the one used for infection.

22. December 4, 1913, a colony similar to the one of the preceding experiment was established in a similar bucket as a check. This colony lived for over two months without the appearance of a single dead ant.

In drawing conclusions from the above experiments the fact must be kept in mind that during the experimentation very uneven and perhaps unfavorable temperatures prevailed in the insectary where the work was done. Nevertheless, while having a greater range of variability than occurs out of doors, the upper and lower limits of this range were certainly not above or below the maximum and minimum temperatures often experienced by the corn-field ant.

The number of experiments showing positive results is nine and

includes experiments 1, 2, 5, 10, 12, 20, and 21. The number showing negative or partially negative results is five and includes experiments 4, 6, 8, 9, and 11. In all of these unsuccessful experiments the fungus had some effect upon the ants but since no growth appeared upon the dead and since no fungus was isolated from them the fact can not be definitely stated. Some of the ants in these experiments lived for a considerable length of time in the presence of the fungus and seemed to have become immune to the disease. Such facts are paralleled by cases which have been observed in the field and there is reason to believe that natural immunity to disease often occurs. The percentage of immunity seems rather high in this case though not so high when we consider the total number of individuals treated. Experiments 2 and 20 and 21 were perhaps the most successful of any and show clearly that *Sporotrichum globuliferum* can parasitize the corn-field ant effectively under certain conditions. I have tried to determine these conditions somewhat more accurately than was possible in the above tests, and to this end have devised the apparatus and planned the experiments which are described in the following pages.

The relation of moisture to the growth of fungi upon insects.-

The importance of atmospheric conditions in causing epidemics among insects was noted by the first workers in this field of study. As early as 1867 Shimer* expressed the belief that there was a contagious disease working among chinch bugs and gave as a cause of it the abundance of damp weather at that time. In 1882 Forbes** stated that the epidemic found by him among chinch bugs was directly influenced by wet weather. His observations were repeated in his later work and were verified by others in different parts of the country. Snow (l.c.), in Kansas made thousands of tests with the "white fungus" and seemed to have such success with ^{it} that the state legislature made appropriations to enable him to supply it to farmers throughout the state. He too observed that wet weather influenced the spread of the disease. Webster later duplicated Snow's work in Ohio*#. However, no attempts were made to determine what temperatures and what amounts of moisture were necessary for the appearance of epidemics, and it was not until recently that actual records of meteorological conditions in connection with the fungous diseases of insects were made. Probably the first of these in America were made in Florida, on fungi parasitic upon the San Jose scale and the white fly**#. Here the relative humidity ranged from fifty to one-hundred per cent, and at the time when successful infections were made, remained above ninety per cent much of the time. The tempera-

* Proc Acad. Nat Sci. Phila. V. 19 pp. 75, 234.

** 12th Rep. Sta. Ent. Ill. p. 47. 1882.

*# Ohio Agr. Exp. Sta. Bull. 77, pp. 33-52, 1897.

**# U. S, D, A, Bureau Entomology Bull, 102, 1912.

ture ranged from seventy to ninety degrees Fahrenheit. Success, as far as could be expected with any fungus, was the result there. Notwithstanding that fact, however, climatic conditions are not greatly different there from those of the North and West where complete failures have been recorded. The following table will serve to show the temperatures, relative humidity, and amounts of rainfall in the two parts of the country. The months chosen are representative summer months and are typical of summer weather in the two regions* .

	Month.	Range of precipitation.	Av. relative humidity.	Temperature.
Florida. (1913)	May .	.91 in.-9.34 in.	70%	75.7F.
	June.	1.80 " -10.40 in.	76%	78.9 F.
	July.	1.09 " -11.59 in.	76%	81.7 F.
Illinois. (1913)	May.	.41 in.-8.32 in.	67%	61.4 F.
	June.	.81 in.-6.18 in.	62%	73.4 F.
	July.	.10 in.-5.85 in.	65%	76.7 F.

It will be seen from the above, that the precipitation in Florida is frequently more than double that of Illinois; that the average relative humidity is not more than ten per cent higher than that of Illinois; and that there is a somewhat constant difference in temperature of about ten degrees. The variations in humidity and temperature alone, it seems to me, are not enough to explain a complete success in the use of fungi in the one state and a complete failure in the other. The amount of precipitation seems to be a more likely cause of the difference, but even here there is a doubt. It is probably true that other factors influence the spread of disease; and if we are to regard the weather as the sole cause of the appearance or absence of insect diseases, we must determine the exact point at which such disease will appear or fail to appear. A tentative plan

* Monthly Weather Review. 1913.

then, for field investigations of insect diseases would be somewhat as follows:-

I. Regarding metereological conditions.

1. Temperature; thermograph or maximum and minimum thermometer readings.
2. Humidity; psychrometer readings.
3. Evaporation; evaporimeter observations.
4. Barometer readings.
5. General condition of the sky, cloudiness.
6. Amount of precipitation, (government records.)
7. Dew; presence or absence of.

II. Regarding the insect and the fungus.

1. Extent of the epidemic and the per cent of insects attacked.
2. Examination and identification of the fungus.
3. A careful examination of surrounding vegetation and soil for fungi similar to the supposed parasite.
4. Examination of the affected insect noting age, etc.

The relation of moisture and temperature to the growth of

insects.- The relation of temperature to the development of insects has been investigated by numerous workers. Ants are able to withstand temperatures below freezing and as high as 96 degrees F. but on the whole are more susceptible to high temperatures than to low*. The optimum temperature for them lies between 70 and 80 degrees F. It is an interesting fact that temperatures below the optimum, while not injuring the insect to any extent usually cause them to develop much more slowly. In numerous cases this fact is so marked that it

* Wheeler, Ants, p. 83. 1906.

has led to the advancement of the hypothesis of accumulated temperature or "thermal constant". This is essentially as follows;- The amount of heat expressed in degrees above a certain point known as the critical point multiplied by the number of days required to complete the life cycle, or period of development, is constant for the particular species. The hypothesis has been disproved by Hennings* and others, but we can obtain from it some ideas as to the effect of the factor on insect metabolism. Hennings attempted to secure data upon the effect of moisture on the growth of insects. The form studied was the scolytid, Tomicus typographus. He also experimented with temperature, and some of his more important conclusions regarding the subject follow.

I. The egg-laying period can be lengthened one to eight days by decreasing the temperature from 24 to 14 degrees C., while a rise of humidity (55% to 96%) causes a lengthening of this state five days.

II. The embryonic period is retarded $5\frac{1}{2}$ days by high temperature, while humidity has little effect.

III. The larval period is lengthened $32\frac{1}{2}$ days by lowering the temperature three or four degrees below 24 degrees C. A moist atmosphere (96% rel. hum.) lengthens it $9\frac{1}{2}$ days compared with a dry atmosphere of 55% relative humidity.

IV. The pupal state is very little influenced by either moisture or temperature.

V. In the length of life cycle from egg to adult a difference of four degrees C. causes an increase of from one to two weeks, a change from dry to moist atmosphere lengthens it one to two weeks

*Biologisches Centralblatt Bd.27 pp. 324-337, 1907.

and the effect of moisture becomes more and more apparent the lower the temperature.

These facts indicate that a low temperature accompanied by a high humidity retards the metabolism of coleoptera in a remarkable degree. Sheldon* and Headlee** have studied the reactions of insects to various amounts of moisture. Headlee determined the optimum relative humidity for Toxoptera graminum to be about 60%. Little work, however, has been done on such problems, and such results as have been obtained up to the present time will need verification by others before they can be accepted.

It will be seen that the advantage of knowing the optimums of temperature and humidity for the insect lies in the fact that parasitism by fungi of insects becomes effective or ineffective according as the vitality of the insect is increased or decreased by one factor or another. Temperature and humidity are the most important meteorological causes of a lessened vitality of the insect and it is, therefore important to know what temperatures and humidities cause a slowing down of the vital processes. This knowledge, together with that acquired of the optimums for fungous growth and a knowledge of atmospheric conditions in the field, would enable one to form an accurate idea of the value of any parasitic fungus which he might encounter and to plan practical experiments which would settle the question immediately.

Relation of moisture and temperature to the growth of fungi.-

The optimum temperature and the thermal death points of fungi have been determined for many species. The humidity and moisture problem has however been attempted by only a few, and the results obtained

*Biological Bull. 25 pp. 79-114, 1913.

**Ann. Ent. Soc. America. V. 3 pp. 147-153, 1910.

in this field have not been checked sufficiently to inspire confidence in them. The main difficulty with the problem lies in the complicated and expensive apparatus that is required for work along this line. The optimum temperature for Sporotrichum globuliferum has been found to be between 70 and 80 degrees F. and corresponds in general with the optimums for other fungi of the same group. The thermal death points of the spores of the same fungus, as determined by Headlee, Duggar* and others, are somewhere near 60 degrees and -10 degrees C. ** The optimum relative humidity seems to have been worked out by Headlee in Kansas and, as stated by him, lies between 90% and 100% saturation.

Types of apparatus used to maintain constant moisture conditions.- Hennings in his experiments made use of an apparatus somewhat as follows:- Two incubators separated by a plate of glass were fitted with thermostats and heating connections. Humidity was partially controlled and only two degrees of saturation were maintained. This was done by placing calcium chlorid within one of the incubators and circulating the air over it by means of a fan. The other incubator was kept at nearly complete saturation (96%) by placing vessels of pure water within. Headlee has attempted to construct a similar apparatus which consists of an iced incubator fitted with a thermostat. Within this incubator he maintained a rising humidity by introducing plants and kept the air at a certain point of saturation by drawing it over calcium chloride with an electric fan. Contact closing the circuit to the dynamo operating the fan was made by the arm of an hygrometer. Sheldon(l.c.) in working upon the reactions of various animals to different gradients of evaporating power of

* Botanical Gazette No. 2. pp. 131-135, 1899.

** Kansas Sta. Agr. Coll. Exp. Sta. Bull. 191 p. 322, 1913.

air , contrived an apparatus in which he maintained three degrees of relative humidity. These he obtained by driving air through pumice-stone saturated with sulphuric acid, which gave dry air. For his second degree of moisture he used unaltered air and for his third he drove air through pumice-stone saturated with water. Mr. Lehenbauer of the Botany Department of this University maintained for experimental work a constant temperature and humidity by drawing air through bottles filled with water. The whole train was immersed in water kept at a constant temperature by means of electric heaters and thermostats. The ideal apparatus would be one that would maintain all degrees of moisture and humidity. Headlee's comes nearest to this ideal theoretically but does not seem to have worked satisfactorily. Shelford's apparatus maintained only three degrees of moisture and only one of temperature, while Lehenbauer's was constructed mainly for the control of temperature. In the experiments conducted by the writer, moisture control has been sought primarily and no attempt has been made to control temperatures other than to place the apparatus in a room in which heat was regulated by a thermostat.

In collecting ant material for the experiments it was thought advisable to note how the insect lived, what were its immediate surroundings in the cornfield where injury is done, and what are the facts concerning the life history of the species. The possibilities of testing humidities within the soil have been canvassed, but there seems to be no accurate method of determining it, now in use. The following notes show in general the conditions under which the cornfield ant is able to live and thrive.

The habits of *Lasius niger americanus*.— A colony of corn-field ants contains anywhere from a few to six thousand or more individuals

including larvae, pupae, and queens. The ants inhabit cultivated, waste, and grass lands. They are sensitive to light, avoid it for the most part during the day, and remain within their cells and burrows. The depth to which they burrow in the soil varies to some extent according to conditions. In damp summer weather, pupae and larvae have been obtained from tunnels only a fraction of an inch below the surface. In dry weather they may penetrate to a depth of nearly two feet. In winter they retreat into the ground and are rarely seen on the surface; in freezing weather the ground in which they live may be frozen without doing them especial injury. According to Miss. Fielde*, ants may be frozen without killing them. Collections made by me as late as November and as early as Jan 29 show that they rarely go more than a foot below the surface. The nest of the ant is comparatively simple and is made up of one or a number of cells with tunnels radiating in all directions.

A single family group occupies as much as three hundred ** square feet, within which area are probably several queens and a number of colonies. The different colonies are connected to some extent by tunnels.

The life cycle of the corn-field ant is also dependant upon physical and atmospheric conditions. Colonies kept in modified Janet nests at a temperature averaging below 70 degrees Fahrenheit produced no pupae although they were fed ^{an} abundance of food in the form of granulated sugar and other material, and it was not until these colonies were placed in a warmer average temperature that any pupae were noticed. This indicates that temperature plays a large part in the rapid-

* Wheeler, Ants, p. 80. 1906.

** 25th Rep Sta. Ent. Ill. 1909.

ity with which the development proceeds. In summer the life cycle is completed in about one month, while in winter it may require six, or even longer, to complete the life cycle. We have records of ants passing as much as a whole year in the larval state.*

The food of the ant consists of both proteids and carbohydrates, the carbohydrates being supplied by the secretion of captive aphids, and the proteids by dead insects obtained from various sources.

Activity of the ants ceases with the onset of cold weather and they retreat within their earthen cells and become semi-torpid. They cling closely together and hover over their larvae, seldom venturing more than a few feet from the cells in which the latter are kept. In spring, when warm weather begins, their activities are evidenced by the appearance of little craters of pellets thrown out as the insects construct newer burrows.

From collections in the field it would seem that considerable moisture is necessary for the welfare of *Lasius*. In several cases brood cells with larvae and pupae were uncovered in exceedingly wet soil, but in no case were ants found in places showing less than the average moisture content. This observation is borne out by the moisture conditions which must be maintained in order to keep the ants alive in captivity.

* Tanquary. Bull. Ill. Sta. Lab. Nat. Hist. V.IX. p.437,
May 1913.

Description of the apparatus used in maintaining different degrees of relative humidity.- Air was drawn through solutions of sulphuric acid of various strengths or through pure water and through the ant "cages", by means of a filter pump attached to the laboratory faucet. The sulphuric acid or water was found to add enough moisture to the air passing through it to raise the humidity of the air to near the desired point. This point for the acid was first calculated from tables giving vapour pressures over different per cents of sulphuric acid, and tested by means of a large hair hygrometer. The two checked fairly well. The strength of the sulphuric acid solutions was determined by means of a hydrometer.

The apparatus through which the air was drawn consisted of the following pieces:- First beyond the filter pump was attached an empty bottle holding about 300 c.c. This was used merely as a suction bottle. It was fitted with a four-hole rubber stopper which provided for connection to the pump and also to three trains of apparatus in each of which a different relative humidity could be maintained. Only two of these connections have been used, so far, in the experimental work. Directly beyond the suction bottle, were placed, in each train, two small bottles of about 250 c.c. capacity, the first one of them containing sulphuric acid or pure water, the per cent of the sulphuric acid depending upon the humidity kept in the train. The second bottle was empty and was used merely to catch anything which might be sucked back from the first. Next were joined two Novy jars containing ant or fungus material. Glass wool was placed in the tubing between the Novy jars. Beyond the Novy jars were connected two small bottles, both filled with saturated pumice stone for the higher

humidities, and one of them filled with glass wool for the lower humidities, where sulphuric acid was used. Finally one or more bottles of two and three liter capacity, two thirds full of sulphuric acid or water, were connected to the trains. The air passing through the trains was tested after passing the glass wool filters and Novy jars by allowing it to bubble through methyl orange indicator. Slightly alkaline phenolphthalein was also used but with neither test did any change of color take place after four hours time. To test the humidity of the air passing through the apparatus a large hair hygrometer was placed in a tubulated bell jar with a ground glass base. The jar was provided with a 2-hole rubber stopper and was placed on a glass plate and sealed around the bottom with vaseline or paraffin. Smaller hygrometers were put into each Novy jar to keep track of the humidity there in a general way. Air passed into the large bottles at the end of the trains at the rate of three or four bubbles per second. The bubbles contained on an average of about $\frac{1}{2}$ c.c. In the higher humidity experiments it was found necessary to double the rate of flow of the other trains. The rate of flow in the different series was controlled by regulating the flow of water at the faucet and by means of screw clamps placed on the tubing connecting the jars.

A difficulty arising in the operation of the low humidity apparatus was experienced in supplying drinking water to the ants without changing the moisture of the air within the cage. To do this a small bottle, of the kind used for the preservation of alcoholic specimens, was filled with water, and a small capillary tube threaded with a wick was inserted through the cork. The bottle was then placed

within the Novy jar alongside of the ant colony and the necessary amount of drinking water~~was~~ was thus provided.

Experiments with the moisture factor in the growth of *S. globuliferum* upon the corn-field ant.- The object of the following experiments has been to discover, first under what conditions of moisture *Sporotrichum globuliferum* is able to live parasitically upon the corn-field ant; second, what conditions of moisture the ants prefer; and, third, what are the moisture requirements of the fungus itself. The material used was the same as that used in the experiments with infection only, the species of ant being *Lasius niger americanus*, and the fungus, except in one case, that obtained from stock cultures. The temperature during the experiments stood between 70 degrees and 80 degrees F., a range considered to be favorable for both ant and fungus.

1. On Feb. 19, 1914, a large colony of ants with a quantity of larvae were placed in one of the Novy jars of the humidity apparatus. About an inch of soil was put into the cage along with the ants. The soil was then thoroughly saturated with water so that when squeezed in the hand water would flow readily from it. On Feb. 25 eight or nine ants that were covered with a white mycelium were seen in the jar. Examination showed the fungus to be the same as that obtained from ant pupae. Some of the material was sent to Thaxter, along with specimens of the original fungus from ant pupae, and was identified by him as *Sporotrichum globuliferum* Speg. As the fungus was present already in the jar (introduced perhaps along with the soil) no more material from the stock cultures was added. On March 13, the jar was opened and examined. The colony which had previously consisted of a

large number of workers and larvae had dwindled to a few wandering individuals. No larvae could be found and a number of piles of dead ants with growths of the white fungus were noticed scattered about over the soil. The fungus was isolated from the dead ants and kept in pure culture.

2. Feb. 18, 1914, a colony similar to that used in experiment 1 was placed under similar conditions of moisture. The colony, in fact, was a part of the one used in the preceding, the original having been divided into two parts. The soil was not kept as moist as in the preceding experiment, but the saturated air passed through this jar before entering the jar of the first experiment. March 13, the cage was opened and a considerable number of ants were found to be still alive. March 20, some of the ants and a few of the larvae were^e found still alive. No sign of any sort of fungus was seen in the cage. The soil was moderately moist, but not as moist as in the preceding experiment. April 8, the colony was again examined and ten or more ants were found to be alive. April 13, all of the colony were dead; but there was no evidence of fungus growth within the cage and no fungus was obtained from the dead ants. On this date another colony was added to the jar. It contained approximately fifty larvae and fifty workers. The soil was then saturated as in the preceding experiment, and the humidity of the air kept at the same point as before. April 28, the ants in the jar were still alive and in possession of both larvae and pupae. No fungus was apparent, although the earth contained enough water to become pasty when stirred with a glass rod. May 10 some of the colony were still alive and in good condition, at which time the experiment was discontinued.

3. Feb. 18, 1914, a moderately large colony of ants were placed in a Novy jar with an inch of soil and subjected to an atmosphere of eighty per cent relative humidity. The cage was treated with fungus spores on March 5, at which time the ants seemed to be in a thriving condition. March 13, all live ants were gone and a number of dead ones were observed which bore tufts of white fungus mycelium. The fungus proved to be the same as the one used to infect.

4. Feb. 18, 1914, a check experiment to 3. The colony was placed in 80% air. March 13, the colony was still in good condition and consisted of a considerable number of workers and some brood. March 20, the colony was still alive and the experiment was discontinued.

5. March 27, 1914, a single colony from the field was divided and one-half was placed under this experiment and the other under experiment 6. Treatment was given the ants by dusting spores of the *Sporotrichum* from ant pupae inside the jar. White sterile sand was used in place of the soil used in the previous experiments. The humidity of the air was held at 80 per cent of saturation and the temperature between 70 and 80 degrees F. April 2 all ants were dead, though no fungus was apparent on any of them. Examination failed to reveal the presence of any fungus parasite whatsoever. Twenty to thirty adults and a number of larvae were now added. April 8, this colony was also dead but as in the preceding case no fungus could be found anywhere about the dead ants. On this date another colony of equal size was added. The sand was removed and a watering bottle to provide drinking water was placed within. April 24 nearly all of the ants were alive and no fungus was evident. The colony continued to live until May 10 when the experiment was discontinued.

6. March 27, 1914. One half of the colony used in 5 was placed in a Novy jar on sterile sand. No treatment with fungus was given this part. Humidity was kept at 80 per cent. April 2, the entire colony was dead and there was no evidence of any fungus. The dead ants were removed and another colony added which consisted of twelve workers and a large number of larvae. April 8, all of these were dead. On this date still another colony was added to the jar but the sand was removed and the ants were placed on the bare glass. A watering bottle was introduced as in 5. The relative humidity as recorded by the hygrometer was about 87%. Observations on the ants in this experiment were made until May 10, at which time a number of live workers (about a dozen) were noted gathered round a pile of brown, shrunken and apparently dead larvae.

Statement of the results of the experiments.- Some interesting facts are shown by the above experiments. Briefly these facts are;- First, The fungus (Sporotrichum globuliferum Speg.) flourishes upon ants under complete saturation of the soil and atmosphere (experiments 1 and 2). Second, The ants (Lasius niger americanus) live well in completely saturated soil and atmosphere (experiments 1 and 2) but can not live under 80 to 90 per cent relative humidity (3 and 4) and succumb quickly where no drinking water is provided (5 and 6). Third, Sporotrichum globuliferum is apparently present in some soils but not present in others (1 and 2).

Experiments with the effects of moisture on Sporotrichum globuliferum.- The following series of experiments have been intended to clear up doubtful points regarding the optimum of moisture for the growth of the fungus. Plate cultures of the fungus were made on plain beef agar and placed within the jars of the humidity apparatus.

The various growths on the different plates were observed, and especially the amounts of spore production.

The rapid production of conidia may be looked upon as indicating that the fungus has about reached the extent of its growth and is providing for a continuation of the species. When this stage appears it usually means one of several things. Either nourishment is exhausted or moisture, light or heat are unfavorable. If, now, light is excluded, the temperature held at what has previously been determined to be the optimum for fungous growth and with abundant food material present the hastened production of spores must necessarily mean that moisture conditions are unfavorable. This is what was done in the following experiments.

April 23, 1913 three plate cultures (beef-peptone-agar) of the *Sporotrichum* were made. April 25, just before the colonies had appeared above the surface, one culture was placed at 80 to 90 % relative humidity, another in a saturated atmosphere and the third was left at room conditions. The humidity in the room as tested with the hygrometer showed a much smaller moisture content than either of the two jars where the cultures were placed. The temperature ranged during this time from 70 to 80 degrees F. April 28, all three were examined. The plate kept at 100 % showed a rather extensive growth of mycelium above the surface of the agar, but there were very few spores or spore masses anywhere to be found. The culture placed at 80 to 90 % showed nearly the same growth of mycelium, with a few distinct bunches of spores and spore masses. The third plate, kept under room conditions, showed much less mycelial growth than either of the other plates, but a large quantity of spores and spore masses.

April 29 three more cultures were made on the same medium. One of these was subjected to an atmosphere of 60 to 70 %, relative humidity, another to 80 to 90 %, and the third ^{was} left to grow at room conditions. As in the preceding experiment, the culture left open to room conditions of moisture showed by far the greatest number of spores and spore masses; the one kept under 60 to 70 % was next in point of numbers, while the third, kept at 70 to 80 %, showed the fewest spores and the largest amount of aerial mycelium.

From these experiments it would seem that *Sporotrichum* flourishes best under extremely moist atmospheres, and that 90 to 100% saturation is more favorable to its growth than a saturation of 80 to 90%, and that this per cent of saturation is in turn more favorable than one of from 60 to 70 per cent.

General conclusion.— Following is a brief statement of the facts brought out in this paper.

1. A species of *Sporotrichum* (*S. globuliferum* Speg.?) is able to live parasitically upon the corn-field ant under certain conditions of moisture, and temperature.

2. The parasitism takes place best where there is complete saturation of the soil and of the surrounding air.

3. Infection may take place at a temperature of from 70 to 80 degrees F.

4. The ants (*Lasius niger americanus*) are able to live in an atmosphere containing a high per cent of moisture, and do not live so well under conditions where the humidity of the air is lower.

5. The fungus grows best under complete saturation of the air.

Explanation of Figures.

Figure 1.

1. Sterigmata and spores of *Sporotrichum* from ant pupae, hanging drop culture.
2. Sterigmata and spores of the same from potato culture.
3. Germinating conidia at the end of 24 hours, room temperature, 70 to 80 degrees F.
4. The same at the end of 30 hours.

Figure 2.

1. Growth from a single spore at the end of 48 hours 70 to 80 degrees F.
2. Hyphae from the base of a coremium.

Figure 3. Diagram of formicary .

- a. Cell for water supply.
- b. Brood cell, (kept dark)
- c. Passageway between cells
- d. Outer runway cell (left lighted).

Figure 4.

1. Potato culture of *Sporotrichum* from ant pupae showing coremia.
2. Dead queens attacked by the fungus.

Figure 5. Apparatus used in maintaining different degrees of relative humidity.

- A. View from above.
 - a. High humidity train.
 - b. and c. Low humidity trains.

Figure 5. (con). Numbers refer to trains b and c.

1. Bottle with solution of sulphuric acid.

2. Empty catch-bottle.

3 and 4. Novy jars.

5. Bottle with glass wool.

6. Bottle with pumice stone saturated with solution of sulphuric acid.

7. 3-liter bottle with solution of sulphuric acid.

B. Tubulated bell-jar with hygrometer ready for connection into any of the trains.

C. Side view of the high humidity train.

1. Suction bottle.

2. Bottle with water.

3. Empty bottle.

4 and 5. Novy jars.

6 and 7. Bottles with pumice stone saturated with water,

8. 3-liter bottle with water.

FIG. I.

1.

2

3

4

FIG. 2.

1

2

FIG. 3.

1.

FIG. 4.

2.

FIG. 5.

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