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FUNGI PARASITIC UPON ALEYRODES CITRI

By
HOWARD S. FAWCETT

A THESIS FOR THE DEGREE OF MASTER OF SCIENCE
SUBMITTED TO THE FACULTY OF

THE UNIVERSITY OF THE STATE OF FLORIDA

JUNE, 1908.

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FUNGI PARASITIC UPON ALEYRODES CITRI.

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I. INTRODUCTION.

Since taking up the investigation of Citrus diseases in the fall of 1905, in connection with work in the Florida Experiment Station, the author has given considerable attention to the study of the fungi parasitic on *Aleyrodes citri* R. & H., and on scale insects. Since the beginning of the work three species of fungi have been discovered to be parasitic upon the larvae and pupae of *Aleyrodes citri*; two of these, *Aschersonia flavo-citrina*, and an undetermined species of Microcera, were first noticed by Prof. P. H. Rolfs, and the third, *Verticillium heterocladum*, by the author. With the addition of these three, the number of known fungus parasites of this insect is increased to six, namely:

1. *Aschersonia aleyrodis* Webber,
2. *Aschersonia flavo-citrina* P. Henn.,
3. *Verticillium heterocladum* Penz.,
4. *Sphaerostilbe coccophila* Tul.,
5. *Microcera* sp.,
6. The sterile Brown fungus of Webber.

Cultures of all these fungi except the Brown fungus have been grown in the laboratory by the author. One of these, *Sphaerostilbe coccophila*, had been previously studied in pure cultures by P. H. Rolfs, who published his results in Bulletin 41 of the Florida Experiment Station under the title of "A Fungus Disease of the San Jose Scale". For this reason a further study of this fungus in cultures was not undertaken, and it is, moreover, only rarely parasitic upon *Aleyrodes citri*.

It is the purpose of this thesis, after a brief review of previous investigations of the fungus parasites of insects, to describe the results of recent study of the fungi that are parasitic upon *Aleyrodes citri* in Florida. With the description of each fungus there is given its distribution and the names of its insect hosts. A bibliography of the six fungi is added. The illustrations, except Figs. 1, 2, and 42, are original. Technical descriptions and a general review of previous literature, when any such has been published, are included with the account of each fungus.

ENTOMOGENOUS FUNGI.

The fact that certain low forms of plant growth, such as fungi and bacteria, are at times the cause of the destruction of great numbers of insects, has created much popular as well as scientific interest for many

years. As early as 1754, according to M. C. Cooke,¹ a popular description was written by Father Torrubia of a fungus growing out from the bodies of wasps. Father Torrubia had collected specimens of this at Havana, Cuba, as early as 1749. Cooke thinks that this fungus was *Cordyceps specocephala*. He quotes Torrubia's account from Edwards' "Gleanings in Natural History," published in 1758, and says that this species represents the rather celebrated vegetable wasp which had a romantic history a century before. Cooke also gives an account of a species of *Isaria*, which had been known as early as 1782 on the Sphinx moth.¹ He mentions as many as 197 species, representing 39 genera of fungi, that have been found growing upon various insects. The majority of these species are included by Cooke under 5 genera: *Cordyceps*, with 48 species; *Laboulbenia*, with 34 species; *Isaria*, with 24 species; *Entomophthora*, with 22 species; and *Empusa*, with 13 species. Since then many new genera have been added to the entomogenous fungi. Species of *Cordyceps* found on wasps, bees, ants, caterpillars, and scale insects, have been mentioned by many scientists and popular writers from the last-mentioned date down to the present time. Cooke refers to the "History of Insects" by Murray, published in 1838, as containing interesting accounts of fungi growing upon insects.

The Tulasne brothers were probably among the first to study the parasitism of these fungi. They published descriptions of some of them in 1857,² and of others in 1865.² Among those described in 1865 was *Sphaerostilbe coccophila* Tul., which is one of the species treated of in this thesis as sometimes parasitic on *Aleyrodes citri*. The work of Pasteur on the flacherie of the silkworm, marked a great advance in our knowledge of insect diseases. This disease was due to a species of bacterium. Pasteur's work was taken up with the view of protecting the insect from parasitic growth, while most subsequent work has been carried on with the view of destroying insect pests by means of their parasites. A work of much scientific importance was that of Roland Thaxter,³ "The Entomophthoraceae of the United States", published in 1888. The genera which he studied attack flies, beetles, moths, caterpillars, grasshoppers, and plant lice.

Since the institution of Experiment Stations in the United States, experiments have been made with several species of fungi, with a view of using them in combating insect pests. Up to this time the greatest degree of success has been attained in Florida, where the conditions of temperature and moisture are conducive to the spread of fungi. The use of fungi to combat insect pests has met with only limited success in the Northern States. A number of years ago the employment of a species of *Sporotrichum* for spreading disease among chinch-bugs, received much attention. In 1895,

¹ Vegetable Wasps and Plant Worms, pp. 41-43, London, 1892.

² Cooke, M. C. Vegetable Wasps and Plant Worms, pp. 5, 33, 41, 73, 96, 112, 183, and 281.

³ Memoirs of the Boston Society of Natural History, Vol. IV, No. 6.

S. A. Forbes⁴ of the Illinois Experiment Station gave a detailed account of cultures and infection experiments carried on with this fungus. A few sentences from his discussion of the results are here quoted:

The white muscadine will not spread among vigorous chinch-bugs in the field in very dry weather to an extent to give this disease any practical value as a means of promptly arresting serious chinch-bug injury under such conditions. * * *

It is most likely to catch in low spots, where the soil is kept somewhat moist by dense vegetation, a mat of fallen herbage, or the like. * * *

If decidedly wet weather follows upon its introduction, even after an interval of several weeks, it is likely to start up and take visible effect; but continuous rains, depressing the vital energies of the insect, seem commonly requisite to its efficient action.

Some investigation has been carried on with a number of other entomogenous fungi grown in pure cultures. G. F. Atkinson⁵ and R. H. Pettit⁶ studied cultures of *Cordyceps*, *Isaria* and *Sporotrichum*. R. H. Pettit in his bulletin issued in 1895 included a long bibliography of the literature on entomogenous fungi, to which any one interested in the history of this subject is referred.

In 1897, P. H. Rolfs,⁷ in his bulletin "A Disease of the San Jose Scale," demonstrated that the fungus *Sphaerostilbe coccophila* could be used in a practical way in combating the San Jose scale in Florida. In 1906, a valuable paper by J. Parkin,⁸ "Fungi Parasitic on Scale Insects," gave a general review and the distribution of the fungus parasites of Coccidae and Aleyrodidae which had been described in all countries up to that time. He referred to a recent publication by Guéguen⁹ in France, which is said to be an exhaustive work on the fungus parasites of man and animals. A recent contribution by Dop¹⁰ on a new fungus parasite in Martinique that has saved the coconut industry of that island is also briefly mentioned.

In Florida, insects belonging to the orders Coccidae and Aleyrodidae are very subject to attacks of fungi. In addition to the six fungus parasites of *Aleyrodes citri* here treated of, there are two other fungi which are not found on this insect, although they are quite common on scale insects of Citrus. These are *Ophionectria coccicola* E. & E., and *Myriangium duriaci* Mont., both of which are illustrated in Bulletin 94 of the Florida Experiment Station. Webber also found on the wax scale *Ceroplastes floridensis*, the fungus *Aschersonia turbinata*; and he mentions finding on various in-

⁴ Experiments with the Muscadine Disease of the Chinch-bug, etc., Ill. Agr. Exp. Sta., Bul. 38, 1895.

⁵ Artificial cultures of an Entomogenous Fungus. Bot. Gaz., Vol. XIX, pp. 129-145, 1894.

⁶ Studies in Artificial Cultures of Entomogenous Fungi. Cornell Univ. Agr. Exp. Sta. Bul. 97, 1895.

⁷ A Fungus Disease of the San Jose Scale. Fla. Agr. Exp. Sta. Bul. 41, 1897.

⁸ Annals of Roy. Bot. Gard. Peradeniya, Vol. 3, Part 1.

⁹ Guéguen, F. Les Champignons parasites de l'homme et des animaux, pp. 252, Paris, 1904. Bot. Centralbl., XCVI, p. 644.

¹⁰ Dop, P. Bull. Sci. France et Belgique, XXXIX, p. 135, 1905. Bot. Centralbl., XCIX, p. 505.

sects other *Aschersonias*, which, as far as we know, he has not identified. Prof. H. H. Hume sent to the author in 1906 a species of *Aschersonia* found on what appeared to be an *Aleyrodes* on the leaves of *Ilex Dahoon*. It is quite probable that further observation will reveal a number of other species of fungi parasitic upon insects of these two orders in Florida and the adjoining States.

ALEYRODES CITRI.

This insect, the larval and pupal stages of which are parasitized by the fungi to be discussed later, has been a serious pest of orange groves in Florida and the adjoining States for a number of years. It had been observed by C. V. Riley in 1878 in the greenhouses at the United States Department of Agriculture at Washington. In 1885, Mr. Ashmead wrote an account of it for the "Florida Despatch", in which he gave it the name of *Aleyrodes citri*. In 1895, Riley and Howard¹¹ first described this species in "Insect Life". In this publication it is reported as having been received from Mississippi, Louisiana, North Carolina and many points in Florida. Gainesville and Crescent City are places mentioned at which it had been studied by Jas. Voyle and H. G. Hubbard, before 1893. It is also stated that during the years 1892 and 1893 it had so multiplied in parts of Louisiana and Florida as to deserve immediate attention. It is of interest here to note that it was about this time, 1893, that Webber discovered the first fungus parasite of this insect.

This insect is not a true fly, as the name might imply, but belongs to the Order Hemiptera, which also includes the plant lice and scale insects. The following summary of the life-history of *Aleyrodes citri* is from Bulletin 88 of the Florida Agricultural Experiment Station, by E. W. Berger:

There are three well-defined broods of the whitefly, with an interval of several days to several weeks between each brood, when few or none are seen on the wing. The first brood generally appears some time during March, April or May; the second during June, July or August; and the third during September and October.

Larvae and pupae of the whitefly are to be found on the under surfaces of the leaves, and seldom elsewhere. The larvae are scale-like and closely appressed against the leaf. They vary in size from the very young, just visible to the unaided eye, to the fully matured larvae which measure about one-sixteenth of an inch in length.

The larvae are white and translucent with a tinge of yellow, and almost invisible upon the leaf. The pupa (Fig. 2, No. 8) is the transformation stage from the larva to the adult winged fly. The pupae are readily visible as yellowish-white, plump, oval bodies with a dark reddish spot on the back. From the pupa emerges the adult winged fly. The little white cases, with a T-shaped split on the back, found on the under surface of a leaf, are the empty pupa cases from which the adults have emerged (Fig. 2, No. 10). The eggs (Fig. 1, Nos. 3 and 4) are just visible to the unaided eye as a fine dust upon the under surface of the leaves. An ordinary hand lens will show them as little egg-shaped bodies much resembling grains of wheat. * * *

¹¹ Riley, C. V., and Howard, L. O. Insect Life, U. S. D. A., Div. of Entomology, Vol. V, No. 4, p. 220, Washington, D. C., 1893.

Fig. 1.—*ALEYRODES CITRI*.*

No. 1 and 2, Adult female; No. 3, Egg; No. 4, Egg-shell; No. 5, Claspers at tip of abdomen of male; No. 6, Antenna; No. 7, Fore margin of front wing.

INSECTS SOMETIMES MISTAKEN FOR *ALEYRODES CITRI*.

No. 8, Larva of *Aleyrodes floridensis*; Nos. 9 and 10, Margin of larva; No. 11, Larva of *Lecanium hesperidum*.

* From Fla. Agr. Exp. Sta. Bul. 67, by H. A. Gossard.

Fig. 2.—*ALEYRODES CITRI*.*

Nos. 1 and 2, Larva, first stage, at different magnifications; No. 3, Larva, second stage; No. 4, Larva, third stage; No. 5, Larva, fourth stage; No. 6, Margin of advanced larva; No. 7, Vasoform orifice of fourth stage; No. 8, Pupa; No. 9, Adult emerging from pupa-case; No. 10, Empty pupa-case.

* From Fla. Agr. Exp. Sta. Bul. 67, by H. A. Gossard.

Twenty thousand eggs have been estimated on a large orange leaf. From observations made in the laboratory, egg-laying begins when the female is from eighteen to thirty hours old; and from seventeen to twenty-five eggs are deposited. These eggs are generally all laid within twenty-four hours after the first egg has been laid. Her length of life has been estimated at from three days in warm weather to three weeks in cool weather, and the complete length of life cycle from egg to adult is from forty or fifty days in summer to six months in winter.

The origin of the whitefly pest in Florida is only a matter of conjecture. It is not definitely known whether it is a native species or was introduced from the East. A recent report that *Aleyrodes citri* has been discovered in Asia gives some weight to the latter view. H. A. Gossard, in Bulletin 67 of the Florida Experiment Station, "The Whitefly", 1903, said with regard to its advent in Florida:

The fly seems to have been first known in Florida throughout the region comprised in Volusia, Marion, Lake, Alachua, and Orange counties; from which, I have little doubt, it was transferred to Manatee county and to local centers along the northern borders of the State.

It had therefore become widely distributed before it attracted any considerable attention. At the present time it is widely distributed in many parts of Florida, and is spreading slowly to parts not before infested, in spite of the work that is done by the growers to keep it out of the groves.

Several investigations are being carried on at the present time by the Florida Experiment Station, and by the United States Department of Agriculture in order to work out practical methods of controlling the pest. Spraying with insecticides, fumigation with hydrocyanic acid gas under tents, and infection with fungus parasites have all been used. Fumigation and fungus infection are the most promising remedies known at the present time. The use of the fungus parasites in destroying this insect will be briefly discussed later, when the various fungi are described.

The most serious injury caused by the whitefly is the sooty mold that always follows the insect. This is a species of *Meliola*, a soot-colored fungus that lives in the honeydew secreted by the whitefly larvae. Since the larvae are found upon the under surfaces of the leaves, the honeydew collects upon the upper surfaces of the leaves below and furnishes a suitable medium for the growth of *Meliola*. The sooty mold spreads in a black layer over the surface. The fruit is also blackened, so that it must frequently be washed before it is shipped. The vitality of the tree is lowered, not only by the loss of the nourishment sucked out by the insects, but also by the shutting off of the sunlight from the surfaces of the leaves. An account of this fungus is given by Webber in Bulletin 13 of the Division of Vegetable Physiology and Pathology, Washington, D. C.

II. ASCHERSONIA ALEYRODIS WEBBER.

This species was first discovered by H. J. Webber in August, 1893, at Crescent City, in the grove of J. H. Harp. Mr. Webber¹² published a preliminary notice of the entomogenous nature of this fungus in 1894, referring it to *Aschersonia tahitensis* Mont. In 1896, under the same name, he mentions it in the bulletin, "The Principal Diseases of Citrus Fruits in Florida".¹³ Finding it after further study to be a distinct species, he described it in 1897 in his bulletin, "Sooty Mold of the Orange and its Treatment," as *Aschersonia aleyrodis*,¹⁴ as follows:

Aschersonia aleyrodis Webber. Stroma hypophyllous, depressed hemispherical, pinkish buff or cream colored, coriaceous, 1—2½ mm. in diameter; mycelial hypothallus grayish white, forming a thin membrane closely adhering to the leaf and extending about 1 mm. beyond the stroma; perithecia membranaceous, at first superficial, later becoming immersed, irregular, reniform or orbicular in mature specimens, and opening by small, round, or elliptical pores or slits; basidia crowded, filiform, slender, continuous, 28—40 microns long, 0.94—1.5 in diameter; paraphyses abundant, slender, projecting beyond the basidia, 65—100 microns long, ¾—1 micron in diameter; sporules fusiform, continuous, mucilaginous, hyaline, sometimes obscurely 3—4 guttulate, 9.4—14.1 microns long by 0.94—1.88 microns wide, very abundant and erumpent, forming conspicuous coral red or rufus masses. (Parasitic on *Aleyrodes citri* R. & H. infesting citrous leaves in Florida).

HISTORY.

Some species of *Aschersonia* have been reported to be conidial stages of the genus *Hypocrella*, an Ascomycete. Massee¹⁵ speaks of having shown that the ascigerous forms of species of *Aschersonia* were produced on fallen leaves on which the conidial stages had grown. Parkin¹⁶ also mentions this genus as the probable perfect stages of species of *Aschersonia* and refers to a species of *Hypocrella* (*H. Raciborskii*) described by Zimmerman,¹⁷ with a conidial stage referable to *Aschersonia*. No ascigerous forms have as yet been discovered in connection with the *Aschersonias* in Florida.

In 1905, F. S. Earle¹⁸ reported *Aschersonia aleyrodis* on *Aleyrodes citri* in Cuba. In 1906, J. Parkin¹⁹ reported finding an *Aschersonia* on several undetermined species of *Aleyrodes* in Ceylon, which closely resembled *Aschersonia aleyrodis*. He said, "Numerous forms of *Aschersonia* have been found in Ceylon on species of *Aleyrodes* and *Lecanium*". In February, 1908, Cook and Horne²⁰ reported *Aschersonia aleyrodis* on *Aleyrodes citri* and on *Aleyrodes howardii*, in Cuba.

¹² Webber, H. J. Journal of Mycology, Vol. VI, No. 4, p. 363, 1894.

¹³ Webber, H. J. Div. of Veg. Phys. & Path., Bul. 8, p. 27, Washington, D. C., 1896.

¹⁴ Webber, H. J. Div. of Veg. Phys. & Path., Bul. 13, p. 21, Washington, D. C., 1897.

¹⁵ Massee, G. Jour. Bot. Vol. 34, p. 151.

¹⁶ Parkin, J. Ann. Roy. Bot. Gard. Peradeniya, Vol. III, Part I, pp. 29 and 35, 1906.

¹⁷ Zimmerman. Centralb. für Bakt., Abth. II, Vol. VII, p. 872.

¹⁸ Primer Informe Anual de la Estacion Central Agronomica de Cuba, 1904 and 1905, p. 169, 1906.

¹⁹ Annals Roy. Bot. Gard. Peradeniya, Vol. III, Part 1, p. 36, 1906.

²⁰ Cook, M. T., and Horne, W. T. Cuban Exp. Sta. Bul. 9, p. 31, 1908.

Previous to Webber's publication,²¹ the entomogenous nature of the genus *Aschersonia* was not known, although up to that time there were 19 species of this genus described, as recorded in Saccardo's "Sylloge Fungorum". This species was therefore the first known parasite of *Aleyrodes citri*, and was probably the first fungus that had been reported on any species of *Aleyrodes*. In the course of his investigations on the sooty mold, Webber reported that he had found three other species of *Aschersonia*, parasitic on other insects in Florida; one of which was *Aschersonia turbinata* on the Wax Scale (*Ceroplastes floridensis* Comst.). The others were not determined by him. In 1897, when he wrote his bulletin on sooty mold, Webber reported that *Aschersonia aleyrodis* was found in Florida at Crescent City, Bartow, Panasoffkee and Gainesville. He also stated that no sign of the fungus was apparent in groves infested with *Aleyrodes citri* at Ocala, Orlando, Evinston and Ormond. In the same bulletin, the development of the fungus, the probable method of spore dissemination, and the methods of introducing the fungus on the orange trees, are discussed at some length. The description of the development of the fungus in the next paragraph is taken with slight changes from this bulletin.²²

The first indication of the effect of the fungus on the larva of the whitefly is the appearance of slightly opaque, yellowish spots unusually near the edge of the larva. In the early stages of infection the larva becomes noticeably swollen, and appears to secrete a greater abundance of honeydew than normally. As the fungus develops, the interior organs of the larva appear to contract away from the margin, leaving a narrow circle, which becomes filled with hyphae. Shortly after this the hyphae burst out around the edge, forming a dense marginal fringe. This may form all around the larva at about the same time, or develop at one portion of the margin sooner than the others. Death usually ensues, it is believed, before the hyphae burst out. The fungus does not spread over the leaf to any extent, but grows upward in a mass, gradually spreading over the larval scale. It is not uncommon to find the pycnidia, with their bright coral-red masses of sporules, formed in a circle around the edge of the larva while it is yet visible. As the *Aschersonia* develops, the hyphae spread over the larva forming a dense compact stroma, which ultimately entirely envelops the larva. The stroma in this stage is thin and disk-like, the fructification being usually borne in a circle near the edge. The hyphae, which make up the main mass of the stroma are from 3.5 to 7.5 micro-millimeters in diameter. Within the body of the insect and near the pycnidia they are somewhat smaller.

METHODS OF INTRODUCTION.

Two methods of introducing the fungus into groves infested with *Aleyrodes citri* were used by Webber with fair success. (1) Pinning fungus-bearing leaves into trees infested with *Aleyrodes citri*, in such a way as to cause the fungus spores to come in contact with larvae not yet infected. (2) Planting small trees with fungus infected larvae in a grove, so that the fungus-bearing leaves came in contact with the leaves on which

²¹ Webber, H. J. Div. of Veg. Phys. & Path., Bul. 13, p. 20, Washington, D. C., 1897.

²² Webber, H. J. Div. of Veg. Phys. & Path., Bul. 13, pp. 23-24, Washington, D. C., 1897.

it was desired to start the fungus. Further methods of introducing this *Aschersonia* by spraying the trees with water containing fungus spores, obtained either from previously infected larvae or from artificial cultures, have been recently carried on by E. W. Berger²³ of the Florida Experiment Station. Webber²⁴ had tried infecting larvae by spraying a mixture of conidia in water, but had failed to reproduce the fungus in this way. E. W. Berger has found that to succeed with this method it is best to have a spray pump that contains no copper parts, and that has also not been previously used for spraying fungicides or insecticides. Fairly good infection of this fungus has been obtained by Berger, by the spore-spraying method, at St. Petersburg, Leesburg, New Smyrna, Gainesville and Lake City. The fact that infections may be made from cultures that have grown under artificial conditions in the laboratory for long periods of time, suggests the possibility of using these cultures in a practical way at the very beginning of the rainy season, when fresh fungus on leaves is hard to obtain. Considerable quantities of this fungus may be grown artificially on various media, as will be shown in the following pages.

GERMINATION OF CONIDIA.

Conidia of this fungus were germinated in hanging drop cultures of distilled water, tap water, and various solutions of glucose. In all of these cultures the germination was very slow, scarcely ever beginning in less time than 20 hours. Germination in distilled water and tap water was very feeble, while that in solutions of glucose was much stronger, as is explained more fully under the germination tests for *Aschersonia flavo-citrina*.

Trials were made at various times to germinate spores in hanging drop cultures from pustules that had dried in the atmosphere of the laboratory. The following is a record of these tests:

Fig. 3.
Conidia of *Aschersonia aleyrodis*,
X 1000.

1. On November 10, 1906, cultures were made from leaves collected on October 12, 1906, which had remained in the laboratory 28 days. The tests were made in glucose, in distilled water, and in tap water. On December 11 (31 days) sporids formed in 5 per cent. glucose. The growths in distilled water and in tap water had not proceeded far.

2. On January 5, 1907, cultures were made from leaves collected October 12, 1906, which had remained in the laboratory for 85 days. The test was made in distilled water. No germination took place.

²³ Fla. Exp. Sta. Bul. 88, pp. 57-63, 1906.

²⁴ Webber, H. J. Div. of Veg. Phys. & Path., Bul. 13, p. 26, Washington, D. C.

3. On April 18, 1907, cultures were made in tap water from leaves collected October 12, 1906, which had remained in the laboratory 189 days. No germination took place.

4. On April 18, 1907, cultures were made in tap water from leaves collected on December 12, 1906, which had remained in the laboratory 128 days. No germination took place.

5. On April 18, 1907, germination trials were made from a culture isolated December 7, 1906, which was transferred to potato on January 14, 1907, and had not entirely dried out. A few spores germinated.

Fig. 4. Conidium of *Aschersonia aleyrodis* after 6 days in 5 per cent glucose solution at 25°C., X 1000.

CULTURES.

Pure cultures of *Aschersonia aleyrodis* were first obtained in January, 1907. On December 7, 1906, petri dishes of neutral 5 per cent. glucose agar were poured. These were made by introducing into the melted agar a platinum loop, that had been thrust several times into a test-tube containing spores from several stromata shaken up in sterile water. In the first dilution, on January 5 (29 days), at a temperature of 15° to 25° C., minute fungus mycelia appeared, yellow in the center, with a fringe of delicate white hyphae projecting outward. On January 8 (32 days), the largest of these had turned red in color. They were raised, hemispherical, and had the upper surface dotted with little white lumps. Larger stromata were 2 to 4 mm. in diameter. On January 15 (39 days) the stromata were 5 to 7 mm. broad, with a wide fringe of straight hyphae projecting outward over the agar. The stroma by this time contained pycnidial cavities with spores (Plate I, Fig. 20).

On April 10, 1907, this fungus was again isolated. Leaves were picked at Orlando, on April 6. Pustules were broken up in water in a watch-glass, and a dilution set of three petri dishes A, B and C, was poured with agar (1 per cent. normal acid to phenolphthalein), to which 5 per cent. glucose sugar had been added. Petri dish A was overrun with bacteria and quick-growing fungi. Petri dish B contained, on April 26 (16 days), about 50 centers of growth just beginning, and a few bacteria. Petri dish C, on April 26 (16 days), contained no visible growth. On May 11 (31 days), C contained a fine growth of 11 mycelia, which probably first showed a few days before. No further record was kept.

On September 23, 1907, this fungus was isolated for the third time in petri dish cultures A, B and C. Four or five small pustules were shaken up in 7 cc. of water until it became milky in appearance. Five loopfuls were washed into A, etc. A was contaminated with other fungi, C developed one mycelium of *A. aleyrodis*, and one of another fungus. Petri dish B developed a pure culture as follows:—On October 8 (15 days), one point of growth was just appearing. On October 18, there were four mycelia, five to six mm. in diameter, with rings of reddish pycnidia; and seven others just starting. On October 28 (35 days), twenty very red pustules with abundant spores and light gray fringes of outgrowing hyphae had developed.

From these isolation tests, it appears that on 5-10 per cent. glucose agar in the laboratory, it requires from 30-40 days for the fungus to mature a pustule and produce pycnidia. This time corresponds somewhat closely to the time for the fungus to develop upon larvae of *Aleyrodes citri*, as

shown by the infection experiments of E. W. Berger. (See Bulletin 88, Florida Experiment Station, pp. 57, 58.) The fungus is extremely slow in developing in the petri dishes, thus increasing the liability to contamination with other fungi and bacteria. Its slow-growing habit demands therefore a strictly pure culture, since the rapidly growing fungi and bacteria will otherwise crowd it out completely.

The many previous failures of the author and of others before him to grow this fungus in cultures, were probably due to the fact that the petri dishes were rejected too soon, or were allowed to dry out before the spores had time to form mycelia and stromata. This fungus was transferred from petri dish cultures to test-tubes of sweet potato (Plate I, Fig. 22), Irish potato, rice, white cornmeal (Plate I, Figs. 21, 23), and bread. On all of these media, the growth was similar in general appearance to the growth of *Aschersonia flavo-citrina*, except that the color of the stroma and spore-masses was red instead of yellow. The most luxuriant growth was on sweet potato plugs. The characteristic red color of the fungus stroma rarely appeared on Irish potatoes. This would seem to indicate that sugar was necessary for the proper development of both the red pigment of *Aschersonia aleyrodis*, and the yellow pigment of *A. flavo-citrina*.

The growth on sweet potato plugs is given here for comparison with the growth of *Aschersonia flavo-citrina* on this same medium.

1. On April 8, 1907, spores were transferred from cultures made on January 14, 1907. The spores were streaked onto the surface of the sweet potato plug with a platinum needle. On April 17, abundant growth all along the streak had begun. On April 26, a large raised red mass, 40 mm. long, had formed.

2. On April 8, spores were transferred as in No. 1. On April 17, very abundant growth had started, and on April 26, a very large red mass had formed.

3. On April 8, spores were transferred as in No. 1. On April 17, good growth with thickened points had started. On April 26, a very large mass of red growth had formed.

4. On April 8, spores were transferred as in No. 1. On April 17, a cream-colored to orange, mealy appearance was evident, spreading to each side of the streak. On April 26, an abundant growth, red in color, had formed.

EXPERIMENTS IN GROWING ASCHERSONIA ALEYRODIS IN LARGE QUANTITIES.

In April, 1907, experiments were begun in order to grow large quantities of fungus for infection of *Aleyrodes citri*. Ten large moist chambers, nine wide-mouthed bottles of 500 cc. capacity, and four petri dishes five inches in diameter, were prepared with sweet potato medium. The potatoes were washed, peeled, washed again and put through a meat chopper. This ground-up mass was then washed in running water to get out fine particles, and the moist medium was sterilized in the autoclave at 110° C. for about 20 minutes. After sterilization, the medium appeared well cooked.

On April 5, these were inoculated, in the transfer closet, by spraying the surface of the medium with a hand atomizer with a mixture of conidia in

sterile water. The conidia were obtained from an old culture of the fungus grown on sweet potato. All of the cultures, except two large petri dishes, finally became contaminated with a growth of bacteria. This was probably due to insufficient sterilization of the interiors of the large masses of medium in the moist chambers and in the 500 cc. bottles. The fungus in the two petri dishes which were successful, appeared in 15 days as a creamy white, felt-like growth over the surface of the medium, with no red color. Very little red color appeared later, and no large spore-masses developed. It seemed that the spores had been sown too thickly over the surface to develop the characteristic pustules with pycnidial cavities. This experiment was practically a failure.

On June 12, 1907, five bottles of ground-up sweet potato, and one bottle of bread, which had been sterilized in an autoclave for about half an hour at 120° C., were inoculated by streaking the surface with a large platinum loop from cultures previously made on sweet potatoes. Three of these were successful, two on sweet potato, and one on bread. The notes on these are as follows:

1. On June 12, 1907, a culture on bread was made from a culture on sweet potato plug, which had been transferred three times from an isolation culture in petri dish poured December 7, 1906, and described above.

Transfers had been made on January 14, 1907, April 10, 1907, and June 3, 1907. On June 19, the fungus was growing well at one point.

On July 1, a large raised mass $\frac{1}{2}$ inch in diameter was formed. On July 20, a large area 1 inch in diameter, and red in the interior, with abundant spores, had grown.

2. On June 12, 1907, a culture on sweet potato was made from another culture with same history as above. On June 19, the fungus had started at seven points. On July 1, masses 1-3 inch in diameter had formed on one side of the bottle.

3. On June 12, 1907, a culture on sweet potato was made from a sweet potato plug, which had been transferred once from a petri dish culture of April 10, 1907, being transferred on June 3, 1907. On June 19, the fungus was just starting at three points. On July 1, raised areas one inch in diameter had formed on one side. On July 20, a growth $1\frac{1}{2}$ inch in diameter had formed.

On June 19, six bottles of sweet potato and one of bread were inoculated from sweet potato test-tube cultures which had been transferred twice from an isolation culture made not later than April 10, 1907. Four bottles developed pure cultures.

1. On June 19, 1907, the surface of a bottle of sweet potato medium was streaked with a small needle from a culture of June 3, 1907. On July 1, three or four areas of growth had appeared. On July 20, a good white growth had formed all around the base of the potato plug.

2. On June 19, 1907, sweet potato medium was streaked as in No. 1, from a culture made June 3, 1907. On July 1, a good growth had formed on the surface, white and lumpy in appearance. On July 20, the growth had formed all over the surface of potato, and spores had grown in the pycnidia. On August 12, spores were very abundant.

3. On June 19, 1907, sweet potato medium was inoculated by shaking up conidia in a test-tube of water and pouring it over the plug. On July 1, very small areas on side of glass appeared. On August 12, areas of large size had grown, but no conidia could be found.

4. On June 19, 1907, bread medium was inoculated as the sweet potato in No. 3, with conidia from a culture made on June 3, 1907. The old potato plug from the same culture was also dropped in. On July 1, a good growth out from old plug had started. On July 20, growth over nearly entire surface, reddish in patches, had formed. On August 12, no conidia could be found.

INFECTION OF ALEYRODES CITRI FROM CULTURES.

In two localities in the State, this fungus was started on larvae of *Aleyrodes citri*, from cultures which had grown for some time on culture media in the laboratory. This infection work was done by E. W. Berger at Gainesville and St. Petersburg, from cultures grown by the author.

The following records are taken from E. W. Berger's field notes:

1. August 10, 1907. Gainesville. From culture on sweet potato made June 23, having been isolated not later than April 10, 1907. Culture mixed up in water and sprayed with hand sprayer on under surface of leaves on lower branches of an orange tree. October 6, no fungus found. On November 16, three pustules of fungus found. On December 31, sprinkling of pustules evident. April 3, 1908, quite a sprinkling of fungus pustules evident.

2. August 10, 1907. Gainesville, Florida. From old cultures in three test-tubes. Age of cultures unknown. No start by April 3, 1908.

3. August 15, 1907. St. Petersburg, Florida. From culture made June 19, on bread in large bottles and transferred twice before from cultures isolated not later than April 10. The culture was washed to fine pulp in water and strained. One and a half to two quarts of the liquid was used. Sprayed on the under side of leaves with a compressed-air sprayer. On October 23, quite a sprinkling of fungus was found. Some twigs had several leaves well covered with pustules, mainly on the newest growth. On December 10, about the same condition. On February 8, 1908, an abundance of fungus was found on northwest side of tree, and fresh pustules were appearing.

4. August 15, 1907. St. Petersburg, Florida. From culture on sweet potato, made June 19, 1907. Culture washed to fine pulp and strained. Two quarts of solution were sprayed on with compressed-air hand sprayer. On October 23, a good sprinkling of fungus was found with some leaves well covered. Best catch was on west side, mainly on newer growth. On December 10, 1907, pustules more mature, but apparently not spreading. On February 8, 1908, an abundant catch of fungus was evident.

5. August 15, 1907. St. Petersburg, Florida. From a mixture of two cultures, one of *Aschersonia aleyrodidis* made June 19 on potato plug and transferred twice before; the other of *Aschersonia flavo-citrina* made May 23, on sweet potatoes in a bottle, and transferred twice before. Cultures mixed, washed and strained, making one gallon of the solution. On October 23, fair sprinkling of the fungus, probably only the red, mainly on newer growth. On December 10, same, but pustules more matured. On February 8, 1908, all red pustules, no yellow present.

6. August 14, 1907. St. Petersburg, Florida. From culture made June 19, 1907, and transferred twice before. No start by February 8, 1908.

7. August 14, 1907. St. Petersburg, Florida. From culture made April 8, 1906, which had been dried for two months. The fungus had been transferred twice before. Isolated from petri dish culture made December 7, 1906. No start of fungus by February 8, 1908.

DISTRIBUTION OF ASCHERSONIA ALEYRODIS IN 1908.

In Florida it has been reported from or seen at the following places, occurring only on *Aleyrodes citri*:

Alva, Apopka, Bartow, Bradentown, Buckingham, Citra, Fort Myers, Gainesville, Glen St. Mary, Jacksonville, Lake City, Leesburg, Manatee, McIntosh, New Smyrna, Oneco, Orlando, Oviedo, Palmetto, Sarasota, St. Petersburg, St. Augustine.

Outside of Florida it was reported by Cook and Horne from Cuba on *Aleyrodes citri* R. & H., and *Aleyrodes howardii* Quaintance; from Java by Kirkaldy & Kotinsky, doubtfully upon *Aleyrodes longicornis* Zehntner; from Brazil by the same authority, doubtfully upon *Aleyrodes horridus* Hempel; from Ceylon by Parkin on various undetermined species of *Aleyrodes*; and from Jamaica by Cockerell under the name of *Aschersonia tahitensis* Mont., which is probably *A. aleyrodis* Webber.

III. ASCHERSONIA FLAVO-CITRINA P. HENN.

The discovery that *Aschersonia flavo-citrina* P. Henn. was parasitic upon *Aleyrodes citri* R. & H., was made by P. H. Rolfs²⁵ by means of specimens sent to him from J. F. Adams, Winter Park, Fla., in September, 1906 (Plate II, Fig. 24). Later in the same year E. W. Berger found this fungus effectively parasitizing *Aleyrodes citri* in several other localities in the eastern part of Florida, and since that time he has succeeded in introducing it into still other localities. This fungus was first described by P. Hennings in 1902, as occurring on leaves of *Psidium* from the botanical gardens of Sao Paulo, Brazil. No mention is made by him of any insect associated with its presence on the guava leaf. This species is described by Hennings²⁶ as follows:

Aschersonia flavo-citrina P. Henn. Stromatibus carnosus, hypophyllis, subdiscoideo-pulvinatis vel hemisphaerico-depressis, citrinis, 2—2.5 mm. diameter, pruinosis, superne punctulato-pertusis, intus subaurantiis, subiculo membranaceo, flavo; pycnidiiis immersis oblongis, paraphysibus filiformibus, flexuosis, hyalinis 140-180×1—1.5 micr., continuis; conidiis fusoidis, utrinque acutis, continuis, hyalinis, 12-18×2 micr.; conidiophoris brevibus, hyalinis, fasciculatis.

It was found in the botanical garden of Sao Paulo on a leaf of *Psidium* sp., October, 1901.

That *Aschersonia flavo-citrina* may be able to attack other Aleyrodidae besides *A. citri* is shown by the fact that in June, 1907, Stene of the Rhode Island College of Agriculture, was able to infect larvae of *Aleyrodes vaporariorum*, on cucumber leaves in the greenhouse, from material sent from Florida by E. W. Berger.²⁷ These are the only two species of *Aleyrodes* on which it has yet been observed. It is probable, however, that further ob-

²⁵ Rolfs, P. H. Fla. Exp. Sta. Rept., p. xvi, 1907.

²⁶ Hennings, P. Hedwigia, Vol. 41, p. 307, 1902.

²⁷ Berger, E. W. Fla. Agr. Exp. Sta. Rept., p. xxxiv, 1907.

servations will reveal its presence on other Aleyrodidae native to the woods of Florida.

COMPARISON OF ASCHERSONIA FLAVO-CITRINA WITH *A. ALEYRODIS*.

The two species are very much alike in their general appearance, and also approach each other closely in the measurements of their structural parts. The most evident distinction is in the color. *A. aleyrodis* is usually red or pink, while *A. flavo-citrina* is yellow and never contains any reddish pigment. The stromata of *A. aleyrodis*, under similar conditions, average less in diameter, and the pycnidial cavities are usually more sunken than in *A. flavo-citrina*. The spores of *A. aleyrodis* also average a little smaller than those of *A. flavo-citrina*. Measurements of Florida specimens show that the spores of *A. aleyrodis* are about $9-14 \times 2-3$ microns, while those of *A. flavo-citrina* measure about $12-15 \times 2-3$ microns. Cultures of these two Aschersonias on similar culture media, under similar conditions, showed them to be distinct forms.

CULTURES.

Soon after the discovery of *A. flavo-citrina* on *Aleyrodes citri*, attempts were made by the writer to produce cultures of this fungus on ordinary culture media. Attempts to isolate *Aschersonia aleyrodis* on neutral peptonized agar had been made without success. The beneficial effect of the addition of sugar on the germination of the spores in hanging drops had been noticed. On the addition of 10 per cent. glucose to the agar, petri dish cultures of *A. flavo-citrina* were successfully grown. The growth on 10 per cent. glucose agar, poured on September 14, 1906, at a temperature of about 28° to 30° C., was as follows:

On October 2 (18 days) small, whitish delicate-fringed colonies appeared, just visible to the unaided eye. On October 11 (25 days), a distinct elevated stroma had formed, waxy and pale, with a yellow center containing pycnidia with spores. Surrounding this was a white fringe of outgrowing mycelium. These stromata became 2 to 6 millimeters in diameter, and closely resembled the stromata growing naturally on leaves bearing larvae (Plate III, Fig. 26). Later cultures grown on the same medium bore stromata 25 millimeters in diameter, indicating that the size of any individual stroma depends on the amount of the medium taken, and the length of time before it is dried out (Plate III, Fig. 27). The fungus was then transferred to test-tubes containing other media. Cultures were obtained on sterilized sweet potato, Irish potato, bread and rice. The most luxuriant growth was obtained on sweet potato plugs, this being probably due to the presence of sugar in that medium (Plate IV, Figs. 28, 29 and 30).

The progress of the fungus on the various media was as follows:

On sweet potato plugs, transferred from petri dish culture by inserting a platinum needle into a spore mass, there was no evident growth in three days. In six days, numerous small white points appeared along the scratch made by the needle. In fourteen days, stromata 2 mm. in diameter, and turning yellow, were formed. In twenty days these had become typical

stromata with spores. In forty-seven days, these separate points had grown together into one waxy, yellow mass, fringed and tipped with a white velvety growth of vegetative hyphae, as shown in the photograph (Plate IV, Figs. 28, 29, 30). Numerous pycnidial masses were also present. On Irish potato plugs, the growth of the fungus, for the first fourteen days, was about the same as on sweet potato plugs. From that time on, the growth was more feeble, and the yellow color rarely appeared. Typical spore-masses were not so abundant. On sterilized rice there appeared in ten days a slightly yellow growth, not much raised above the surface. In eighteen days, the growth had spread out considerably over the surface of the rice, and the color had become a decided yellow, without presenting any raised mass as described for sweet and Irish potatoes. In thirty-four days, there appeared small whitish patches over the surface of the yellow growth. On white cornmeal, the growth of the fungus was the same as on rice. On bread, the fungus grew well and formed yellowish masses in a few weeks.

Fig. 5.
Hyphae of stroma,
X 700.

GERMINATION OF CONIDIA.

The germination of the conidia of this fungus was very slow as compared with that of many fungi. In September, 1906, germination tests were made of conidia placed in hanging drops of distilled water, tap water, agar, and various solutions of glucose in water. These were prepared by placing a number of minute drops of the solution on each sterilized cover glass, and putting in them by means of a sterile needle a few conidia from fresh pustules of the fungus. These were then placed in a moist chamber to keep them from drying out. In all of these solutions some germination took place. In distilled water and tap water, the germination was slow and feeble, the hyphal tube not advancing far. The addition of sugar appeared to increase germination up to 10 per cent. of sugar. Above 10 per cent. germination was retarded, and at 30 per cent. only a few spores were seen to germinate. In October, 1907, part of the same test was repeated with the same general results; except that in this case the conidia in 5 per cent. glucose solution appeared to germinate a little more readily than in 10 per cent. and the conidia in 30 per cent. solution refused to germinate at all. Parallel tests in part were made with conidia of *Aschersonia aleyrodis* with similar results.

Fig. 6. Conidia from
dried pustules of
Aschersonia flavo-
citrina, X 1000.

The table below gives the result of the tests for *Aschersonia flavo-citrina*. In germinating, the first hyphal tube usually pushed out just behind one of the acute ends of the boat-shaped spore (Figs. 7, 8 and 9). The acute end,

which seemed to lack protoplasm, was bent back in the opposite direction.

The hyphal tube then grew very slowly, and in four days, in 10 per cent. glucose solution, began to form sporids at the distal ends (Figs. 8 and 9).

Fig. 7. Conidium of *Aschersonia flavo-citrina* germinating in 30 per cent glucose solution after 27 days. (a) Original conidium, (b) spore-like bodies, X 1000.

Fig. 8.

Figs. 8-9. Conidia germinating. (a) conidium, (b) hyphal tube, (c) sporid, X 1000.

Fig. 9.

ASCHERSONIA FLAVO-CITRINA—GERMINATION OF CONIDIA.

MEDIUM.	24 TO 28 HOURS.	44 TO 48 HOURS.	
Distilled water..	No germination.	Germination just beginning.	
Tap water	No germination.	Germination just beginning.	In 6 days good germination, some hyphae 2 or 3 times longer than the conidia.
Glucose 1 per cent.	Germination just beginning.	Fairly good germination.	
Glucose 2 per cent.	Germination just beginning.	(Dried out.)	
Glucose 3 per cent.	Germination advanced farther than in 2 per cent. glucose.	Very good germination of many conidia.	
Glucose 5 per cent.	Good germination in 28 hours, hyphal tubes as long as conidia.	Good germination, hyphae growing slowly.	
Glucose 10 per cent.	Very good germination, hyphal tubes 2 to 3 times length of conidia.	Very good germination, hyphae growing slowly.	In 4 days sporids forming on ends of hyphal tubes.
Glucose 20 per cent.	Only a few just beginning to germinate.	Hyphal tubes as long as conidia.	
Glucose 30 per cent.	No germination.	No germination.	A very few finally germinated. Sporids formed in 27 days.

Germination tests of conidia from dried pustules made at various times showed the following results:

1. On November 10, 1906, hanging drop cultures were made from specimens sent in from Winter Park on September 28, 1906, which had remained in the laboratory exposed to the atmosphere for 46 days. Tests

were made in distilled water, in tap water and in 5 per cent. glucose at a temperature of 15° to 20° C. No germination occurred up to December 11, 1906, when the slides were discarded.

2. On January 5, 1907, hanging drop cultures of conidia in distilled water were made from leaves picked September 28, 1906, which had remained in the laboratory exposed to the atmosphere for 68 days. No germination was noticed up to January 9, 1907, when the slides were discarded.

3. On January 5, 1907, hanging drop cultures in tap water were made from leaves collected by E. W. Berger on December 18, 1906, and kept in the laboratory 18 days. On January 7, many conidia had germinated, and on January 9, some hyphal tubes were twice as long as the conidia.

4. On April 18, hanging drop cultures were made from leaves collected on September 28, 1906, at Orlando, and kept in the laboratory 203 days. No germination had occurred by April 29, 1907, when the slides were discarded.

A SUPER-PARASITE OF *ASCHERSONIA FLAVO-CITRINA*.

A super-parasite belonging to the genus *Cladosporium* has been noticed as of common occurrence on *Aschersonia flavo-citrina*. Attention was first called to it by A. W. Morrill, who afterwards observed it at Orlando overrunning this *Aschersonia*, in the summer of 1906. Later on a brief report was given by E. W. Berger. Plate VII, Fig. 43, shows a leaf from Orlando with pustules of *Aschersonia flavo-citrina* covered over with the dark brown *Cladosporium*. This same fungus has also been observed by the writer associated with sooty mold (*Meliola*) in secretions of honeydew from *Aleyrodes citri*, and it appears to aid at times in smothering the white-fly larvae, as does also the *Meliola*. Cultures of this fungus were readily obtained in 5 per cent. glucose agar, and the fungus was transferred to potato plugs. The growth was rapid and the color was the same as on the leaves.

DISTRIBUTION OF *ASCHERSONIA FLAVO-CITRINA* IN FLORIDA IN 1908:

Altamonte Springs,
Gainesville (introduced),
Largo (introduced),
Maitland,
New Smyrna (introduced),
Orlando,
Oviedo,
St. Petersburg (introduced),
Winter Park.

IV. *VERTICILLIUM HETEROCLADUM* PENZ.

The attention of the writer was first called to this fungus in November, 1905, by E. H. Sellards, Entomologist of the Florida Experiment Station, who brought in specimens of Brown fungus from Palmetto, Florida, with which was associated a cinnamon-colored fungus. At the time of its dis-

covery on *Aleyrodes citri*, it was thought that this fungus might possibly be the spore-bearing stage of the sterile "Brown fungus" described by Webber, because of its close association with the stroma of the Brown fungus, and since it also resembled the Brown fungus somewhat in color. Cultures of the *Verticillium heterocladium*, however, together with inoculations of larvae of *Aleyrodes citri* in the greenhouse, showed that this fungus was distinct from the Brown fungus found by Webber in 1896. Further evidence of its distinct character was subsequently obtained when it was found in other localities on *Aleyrodes citri* and on other insects, in no way associated with the Brown fungus. At Palmetto, where it was first discovered, it was seen to be attacking the long scale, *Mytilaspis citricola*, that occurred on the same leaves with larvae of *Aleyrodes citri*. In 1907 it was found on *Mytilaspis gloverii* at Gainesville, quite independent of the Brown fungus. During the same year, it was found on a species of *Diaspis* on the leaves of *Euonymus Americanus* in the woods near Gainesville. Later on it was observed unaccompanied by Brown fungus on *Aleyrodes citri* at St. Petersburg, Fla. It was introduced by the writer on larvae of *Aleyrodes citri* on a privet hedge at Gainesville in the fall of 1907. It was also introduced by Mr. Gaitskill along with the Brown fungus on larvae of *Aleyrodes citri* at McIntosh, during the summer of 1907. Specimens of the same fungus on *Aleyrodes citri* were sent in from Apopka and Citra, Florida, early in 1908. In a few cases only has it been found in large quantities, and as yet its efficiency as a parasite of *Aleyrodes citri* is not fully determined. In its parasitism it differs from the three fungi previously described, by attacking other insects not belonging to the genus *Aleyrodes*. Several other species of the genus *Verticillium* have been reported as occurring on insects. *Verticillium aphidis* Bauml.²⁸ on plant lice, and *Verticillium minutissimum* Corda²⁹ on larvae of a small insect, were described from Europe. An undetermined species of *Verticillium* is reported by J. Parkin³⁰ on a scale insect *Asterolecanium miliaris* which was infesting the leaves of a bamboo bush in Ceylon. Parkin also refers to Guéguen as authority for the statement that *Verticillium heterocladium* had been found in Africa and the Antilles. It would therefore appear to be a widely distributed species.

DESCRIPTION.

This fungus was first described and figured by O. Penzig,³¹ in 1882, occurring on *Lecanium hesperidum* on lemon leaves in Italy. His description is as follows:

Verticillium heterocladium Penzig (Fung. Agrumic. N. 108, Fig. 1193). Hyphis

²⁸ Saccardo, P. A. Sylloge Fungorum, X, p. 546, 1892.

²⁹ *Ibid.* IV, p. 152, 1886.

³⁰ Annals Roy. Bot. Gard., Peradeniya, Vol. III, Part I, p. 45, 1906.

³¹ Studi Botanici sulle Agrammi e sulle Planti Affini, p. 398, Tavola XLI, Fig. 3, Roma, 1887.

repentibus, elongatis, paullum ramosis, continuis; ramis fertilibus adscendentibus, ramulosis; ramulis ternis vel quaternis, oppositis vel alternis, patentibus, rectis, apice attenuatis; conidiis in ramulorum apice solitariis, geminatis vel ternis, saepius pedicellis brevissimis suffultis, oblongis, hyalinis, 5.5-6 micr. long., 2-3 micr. crassis.

Fig. 10. *Verticillium heterocladium*. (a) Conidiophore with conidia forming, X 450. (b) Conidiophore with mature conidia, X 450. (c) Conidia, X 1000.

3 to 4 microns, several times septate. The conidia are oblong, hyaline, 4 to 6 microns long by 1.5 to 2.5 thick. The main body of the cinnamon-colored stroma when mature becomes powdery in appearance, and under the microscope it is found that the hyphae have broken up into short pieces irregular in shape and length with rounded ends, some of them quite closely imitating spores (Fig. 11). These have thicker walls than the conidia, and probably act as reproductive bodies in carrying the fungus through a period of dry weather.

Fig. 11. Short hyphal bodies into which the central portion of a mature pustule breaks up, X 450.

CULTURES.

Pure cultures of this fungus were made in November, 1905, soon after the fungus was discovered on *Aleyrodes citri*. The next year other cultures were made and spores of the fungus transferred from cultures to larvae of *Aleyrodes citri* in the greenhouse. The fungus was readily grown in 5 per cent. glucose agar by drawing a moistened platinum needle over the top of the upright conidiophores and then washing it off into the melted agar.

On November 12, 1906, two petri dishes (A and B) of 5 per cent. glu-

cose agar were poured. A moistened needle was drawn over the upright brush of conidiophores and washed into a little bouillon in a test-tube. Inoculations were made from this with a platinum loop, nine loops being transferred to A, and nine from A to B.

On November 20 (8 days), 120 pure white mycelia appeared in A and 4 in B, 3 to 4 mm. in diameter, with reddish brown center and a brush of upward growing white conidiophores. On December 5 (23 days), the mycelia in B were 25 mm. in diameter and reddish brown. On December 12 (32 days), the mycelia in B were 30 to 35 mm., and cinnamon-colored almost to the edges. By this time a pustule was formed composed of closely interwoven hyphae and closely resembling the pustules upon larvae of *Aleyrodes citri* (Plate V, Fig. 34).

The fungus was transferred to test-tubes of sterilized Irish potato, sweet potato, rice, white cornmeal, stems of canna and of caladium, and bread; on all of which the fungus grew to some extent. It grew best, however, on sweet potato and bread, over the entire surface of which it formed a felted cinnamon-colored stroma (Plate V, Figs. 32, 33). On rice the color was that of ocher, and on caladium stems it was brick-red. On the other media the color was nearly that of the growth on 5 per cent. glucose agar.

The growth on sweet potato plugs was recorded as follows: On November 22, 1906, conidia from cultures made November 12, were rubbed on the surface of the potato. On December 5 (13 days), a brown colored growth had formed over the surface of potato. By December 12 (20 days), the entire surface was yellowish brown. On January 15, 1907 (39 days), the entire surface was covered over with a thick cinnamon-colored mat of fungus.

GERMINATION OF CONIDIA.

Conidia were placed in distilled water in hanging drops. It was found that they germinated much more rapidly than did the conidia of *Aschersonia*. In distilled water, in 24 hours, conidia had just begun to elongate; in 48 hours a few spores were found with short hyphal tubes. In bouillon, in 24 hours a slight germination took place, the hyphal tubes becoming as long as the spore; in 3 days, the hyphae were 120 to 200 microns long and 1 to 1½ microns wide, and branched once or twice.

In 5 per cent. glucose, in 24 hours, the tube had extended one to two times the length of the spore, and in three days, the growth had proceeded farther than those in distilled water.

When germinating, the spores first swelled, elongated, and then sent out a hyphal tube from one or both ends.

INFECTION OF LARVAE OF ALEYRODES CITRI.

On December 6, 1906, small badly infested orange trees which had previously been in the greenhouse, were covered with large belljars. Conidia from a petri dish culture poured November 12, 1906 (24 days old), were shaken up in sterile water, and this was sprayed on to the plants with a small atomizer. In 35 days several leaves were found with stromata in a spore-bearing condition, identical with those from which the culture had originally been obtained. These had evidently developed sooner than 35 days, and had been overlooked. They became powdery in appearance, and in all particulars were like the natural pustules.

On September 19, 1907, leaves containing *Verticillium heterocladium* were tied to a twig of privet (*Ligustrum ovalifolium*) bearing abundant larvae of *Aleyrodes citri*. The weather was moist for two weeks after.

On October 5 (16 days), pustules were very evident on many larvae on leaves adjacent to those tied in. Under the microscope a mycelium was to be seen inside the larvae. On October 25 (36 days), the fungus with conidia was well established on a few neighboring leaves, but was not spreading rapidly (Plate V, Fig. 35).

On November 16, leaves of *Euonymus Americanus* bearing a species of Diaspis attacked by *Verticillium heterocladium* were pinned on to an orange tree at Gainesville by E. W. Berger. On December 31, 1907, three pustules of freshly grown *Verticillium* were found (one on a scale insect, probably *Lecanium*, and the others apparently on larvae of *Aleyrodes citri*), on a tree next to the one in which the fungus had been pinned.

INSECTS PARASITIZED, WITH LOCALITIES.

Aleyrodes citri: Palmetto, Manatee, St. Petersburg, Gainesville, Apopka, Citra, McIntosh.

Mytilaspis gloverii: Gainesville.

Diaspis sp. on leaves of *Euonymus Americanus*: Gainesville.

Lecanium sp. on orange leaves: Gainesville.

Lecanium hesperidum on lemon leaves: Italy.

Mytilaspis citricola: Palmetto, Citra.

In Africa and the Antilles the host insect is not known.

V. SPHAEROSTILBE COCCOPHILA TUL.

This fungus, which has a world-wide distribution, and has been reported as a parasite on no less than fifteen different species of scale insects, has been found in a few instances attacking larvae of *Aleyrodes citri*.

Fig. 12. Perithecium of *Sphaerostilbe coccophila* showing asci within, X 75.

The conidial stage of this fungus (Figs. 14 and 15) was discovered by Desmazières³² on scales of a coccid on young willow-stems in France, as early as 1848. It was described under the name *Microcera coccophila*. The perithecia (Figs. 12 and 13) were discovered by Tulasne³³ and described by him in 1865 as occurring on scale insects on species of *Laurus*, *Salix* and *Fraxinus* in France, Italy and America. Desmazières' description is as follows:

Microcera, Desmaz. Velum externum persistens, membranaceo-floccosum, dein supra in lacinias plures rumpens; receptaculum clavatum carnosum e fibris subsimplicibus sporidiiferis formatum; sporidia fusiformia, acuata.

Microcera coccophila Desmaz. M. minutissima, subcaespitosa cornuto-conica, simplex, lateritio-rosea, basi membrana tenuissima albida vaginato-connata. Sporidiis paucis, hyalinis, elongatis, utrinque acutis. Hab. in coccis. Hieme.

Fig. 13. Ascus from perithecium, X 200.

³² Desmazières. Ann. des Sc. Nat., Tome X, p. 359, 1848.

³³ Tulasne. Carpologia, Vol. III, 105, 1865.

The ascus stage is described in Saccardo's *Sylloge Fungorum*³⁴ thus:

Sphaerostilbe coccophila Tul. Carp. III., 105.—Peritheciis permultis supra et prope stromata conidiophora nascentibus, minimis globosis, obtusis, brevissime papillatis, glaberrimis, nitide rubris, saepe 4-5 sociatis, senio collabentibus; ascis linearibus, 60-80×6½; sporidiis oblique monostichis, ovatis, 10×5, 1-septatis, subhyalinis, leniter constrictis.—Stat. conid. *Microcera coccophila* Desm. Stromate e crusta coccorum solitarie oriundo, crasse teretiusculo, obtuso, rubro 2 mi'l. alt.; conidiis linearilanceolatis 4-6 locularibus, 65×6 subhyalinis.

In 1892, Ellis and Everhart³⁵ in "North American Pyrenomycetes" gave nearly the same description in English, and reported this fungus on a specimen of *Alnus serrulata* collected in Pennsylvania, adding:

The conidial stage (*Microcera coccophila* Desm.) which has been sent from Florida by Dr. Martin and collected in Carolina by Ravenel (F. Am. 286), has stroma arising from various species of dead bark-lice. It is red, obtuse and about 2 mm. high. The conidia are linear lanceolate, 5-7 septate and 56-65×5-6 microns, nearly hyaline.

Fig. 14. Sporodochium, or conidial fructification of *Sphaerostilbe coccophila*, X 75.

D. McAlpine³⁶ in describing the conidial stage of this species in Australia gives the measurements of the conidia as 75—103×5½—8½ microns. Measurements made of a number of Florida specimens from different localities gave 70—112×3.5—6 microns. Measurements of the perfect stage from Florida specimens were as follows:—Perithecia 350—390 long by 300 microns thick. Divisions of perithecial wall 6—10 microns. Asci, 70—98×8—12 microns. Spores 12—18×7—9 microns.

Fig. 15. Conidia, X 200.

HISTORY.

This fungus has been found in nearly every country in the world. In 1892, M. C. Cooke³⁷ spoke of it as common in Europe and as a well-known parasite on dead Coccus. In the same year it was reported from Jamaica by T. D. A. Cockerell.³⁸ In 1894, Henry Tryon³⁹ reported it on the long scale, the red scale and the circular black scale in Queensland, Australia. In 1897, it was reported by P. H. Rolfs⁴⁰ as a parasite of the San Jose Scale, *Aspidiotus perniciosus* Comst. In 1899, it was reported by D. McAlpine⁴¹ on *Aspidiotus aurantii*

³⁴ Saccardo. *Sylloge Fungorum*, II, p. 513, 1883.

³⁵ Ellis, J. B. and Everhart, M. B. *North American Pyrenomycetes*, p. 111, 1892.

³⁶ *Fungus diseases of Citrus Trees in Australia*, Dept. of Agr., Victoria, p. 113, 1899.

³⁷ *Vegetable Wasps and Plant Worms*, p. 322, London, 1892.

³⁸ *Bul. Botanical Dept. of Jamaica*, No. 36, p. 6, 1892.

³⁹ *Queensland Dept. of Agr.*, Bul. 4, p. 15.

⁴⁰ *A Disease of San Jose Scale*, Fla. Exp. Sta. Bul. 41.

⁴¹ *Fungus Diseases of Citrus Trees in Australia*, Dept. of Agr., Queensland, pp. 27 and 28, 1899.

Mask. in Australia. In 1900, P. H. Rolfs⁴² reported that *Mytilaspis citricola* Pack., *Mytilaspis gloverii* Pack., and *Parlatoria pergandii* Comst. were all attacked by this fungus in Florida. The same year F. S. Earle⁴³ found it common on *Aspidiotus obscurus* Comst. on water oaks in Alabama. It also occurs naturally in Georgia, and has been found as far north as Philadelphia, Pa. In 1901, Fuller⁴⁴ reported finding the same fungus in Natal, South Africa. In 1903, H. A. Gossard⁴⁵ first reported its occurrence on *Aleyrodes citri*. In 1903, F. S. Earle⁴⁶ found it in Porto Rico, and in 1904⁴⁷ in Cuba; in both places on *Mytilaspis citricola*. In 1904, S. I. Kuwana⁴⁸ reported the fungus as present upon *Aspidiotus perniciosus* Comst. and on *Diaspis pentagona* Targ. in Japan. He spoke of finding it on the last-named insect in the mountain districts. In 1906, it was reported by J. Parkin⁴⁹ as occurring upon *Mytilaspis citricola* Pack. and *Aspidiotus aurantii* Mask. in Ceylon. In 1908, it was reported by C. W. Howard⁵⁰ from the Transvaal. It has also been found by E. W. Berger on *Aspidiotus hederae* (Vall) and *Aspidiotus ancylus* Putnam, in Florida.

Both the conidial and ascus stage of this fungus (Plate VI, Figs. 36, 37, 38) are found commonly in Florida, although the latter is not at all common in other parts of the world. In Ceylon, the perithecia have not been found by Parkin; and one would infer, from the name *Microcera*, which is used by D. McAlpine, that the perfect stage is not common in Australia.

RELATION OF S. COCCOPHILA TO THE SAN JOSE AND OTHER SCALE INSECTS.

The use of this fungus as a practical remedy in combating the San Jose scale was first brought out by P. H. Rolfs in 1897, in Bulletin 41 of the Florida Experiment Station. This fungus was discovered by him upon the San Jose scale in northern Florida during the previous year. By means of pure cultures and infection experiments, Rolfs demonstrated that the San Jose scale could be readily infected with spores of this fungus grown upon sterilized bread or other media. The results of these experiments are given in the bulletin referred to.

This fungus was also discovered to be a common parasite of the obscure scale, *Aspidiotus obscurus* Comst., on the twigs of water oaks (*Quercus aquatica*). A simple method of infection has been developed in Florida by one of the peach-growers, Mr. F. P. Henderson, by tying into the top of

⁴² Proc. Fla. Sta. Hort. Soc., p. 65, 1900.

⁴³ Ala. Exp. Sta. Bul. 106, p. 198, 1900.

⁴⁴ First Rept. of Govt. Entomologist, Dept. of Agr., Natal, p. 99, 1901.

⁴⁵ Fla. Exp. Sta. Bul. 67, p. 623, 1903.

⁴⁶ Annual Rept. Office of Exp. Sta., Washington, D. C., p. 457, 1903.

⁴⁷ Primer Informe Annual de la Estacion Central Agronomica de Cuba (1904-05), p. 162, 1906.

⁴⁸ Japan Imperial Agr. Exp. Sta. Rept., p. 26, 1904.

⁴⁹ Annals Roy. Bot. Gard., Peradeniya, Vol. III, Part I, p. 50.

⁵⁰ Dept. of Agr., Transvaal, Agr. Journal, p. 6, 1908.

an infested tree a short piece of wood whose bark bears a good supply of the fungus. This has proved to be a very effective way of distributing this fungus, and has already saved thousands of dollars to the peach and orange growers of the State. In addition to attacking *Aspidiotus perniciosus* and *Aspidiotus obscurus*, this fungus is effective in checking a number of other scale insects in Florida. It is, under favorable moisture conditions, an effectual parasite of *Mytilaspis citricola*, *Mytilaspis gloverii*, *Aspidiotus ficus*, *Aspidiotus hederæ* and *Parlatoria pergandii*. There are times, in dry weather, when these scale insects get ahead of the fungus; but a moist period of a few days will quite often enable the fungus to kill them off almost completely. (Plate VI, Figs. 36, 37 and 38.)

The effective work of this fungus, and two others, *Ophionectria coccicola* E. & E., and *Myriangium duriaei* Mont. upon the orange scales, is readily shown by spraying an orange tree very thoroughly with Bordeaux mixture. During the summer and fall of 1907, the author sprayed a number of orange trees with Bordeaux mixture for another purpose. The trees were sprayed very thoroughly, once in May, once in July, and once in September. Before the first spraying, the trees were practically unhurt by *Mytilaspis citricola*, only a few individuals of this scale being found on any part of the trees. After the first spraying this scale insect began to spread, and increased slowly in numbers until November, when the trees were badly attacked by the scale. Other trees near by, that had received no spray, were as free from scale as at the first. The fungicide had evidently destroyed, on the sprayed trees, the fungi that had been all along working upon the unsprayed trees.

Soon after the discovery of this fungus in Florida by P. H. Rolfs, experiments were made by S. A. Forbes⁵¹ of the Illinois Experiment Station, B. Smith⁵² of the New Jersey Experiment Station, J. Craig⁵³ of the Canadian Experiment Station, and F. M. Webster⁵⁴ of the Ohio Experiment Station, to introduce *Sphaerostilbe coccophila* on San Jose scale in the North, but the climate of these States did not prove to be conducive to its spread, and the work was abandoned.

S. COCCOPHILA AS A PARASITE OF ALEYRODES CITRI.

As was stated previously, *Sphaerostilbe coccophila* seems to have been first reported as parasitic on *Aleyrodes citri* in 1903 by H. A. Gossard. It was found on larvae of *Aleyrodes citri* on orange leaves received from Orlando. In 1905, E. H. Sellards⁵⁵ also reported having received it from

⁵¹ Ill. Exp. Sta. Bul. 56, pp. 270-280.

⁵² N. J. Exp. Sta. Rept. 18, pp. 470-479, 1897. Rept. 19, pp. 445-446, 1898. Rept. 24, p. 567, 1903.

⁵³ Canada Exp. Farm Rept., p. 119, 1897.

⁵⁴ Ohio Exp. Sta. Bul. 103, p. 198, 1898.

⁵⁵ Fla. Exp. Sta. Rept., p. 27, 1905.

Orlando. In 1906, E. W. Berger found it on a few whitefly larvae at Leesburg, Florida.

The effect of this parasite upon *Aleyrodes citri* seems to be of little practical value. It has only rarely been observed attacking this insect, and then does not occur in quantity, as do the other fungi before spoken of. It is not uncommon to find a large amount of this fungus upon *Mytilaspis citricola* in trees that are at the same time infested with larvae of *Aleyrodes citri* on which no fungus can be found.

LIST OF INSECTS PARASITIZED.

A list of insects reported to be attacked by this fungus is here given, together with the authority for the report and the localities in which they were found parasitized:

NAME OF INSECT.	LOCALITY.	AUTHORITY.
<i>Aleyrodes citri</i> R. & H.....	Florida	H. A. Gossard.
<i>Aspidiotus ancyclus</i> Putnam	Florida	E. W. Berger.
<i>Aspidiotus articulatus</i> Morgan	Jamaica	T. D. A. Cockerell.
<i>Aspidiotus articulatus</i> Morgan.....	West Africa	J. Parkin.
<i>Aspidiotus articulatus</i> Morgan	West Indies	J. Parkin.
<i>Aspidiotus aurantii</i> Mask	Australia	D. McAlpine.
<i>Aspidiotus aurantii</i> Mask	Natal	Fuller.
<i>Aspidiotus aurantii</i> Mask	Ceylon	J. Parkin.
(<i>Chrysomphalus</i>) <i>aurantii</i> Mask.....	Transvaal	C. W. Howard.
<i>Aspidiotus ficus</i> Comst	Florida	
(<i>Chrysomphalus</i> <i>aonidum</i>) Linn.....	Transvaal	C. W. Howard.
<i>Aspidiotus hederæ</i> (Vall).....	Florida	E. W. Berger.
<i>Aspidiotus obscurus</i> Comst.	Florida	P. H. Rolfs.
<i>Aspidiotus perniciosus</i> Comst.	Florida	P. H. Rolfs.
<i>Aspidiotus perniciosus</i> Comst.	Japan	S. I. Kuwana.
<i>Chionaspis citri</i> Comst.	Cuba	Cook and Horne.
<i>Diaspis pentagona</i> Targ.	Japan	S. I. Kuwana.
<i>Florinia floriniæ</i> Targ.	Mauritius	J. Parkin.
<i>Ischnaspis filiformis</i>	West Indies	J. Parkin.
<i>Mytilaspis citricola</i> Pack.	Ceylon	J. Parkin.
<i>Mytilaspis citricola</i> Pack.	Cuba	F. S. Earle.
<i>Mytilaspis citricola</i> Pack.	Porto Rico	F. S. Earle.
<i>Mytilaspis citricola</i> Pack.	Florida	P. H. Rolfs.
<i>Mytilaspis gloverii</i> Pack.	Florida	
(<i>Lepidosophes</i>) <i>gloverii</i> Pack.	Transvaal	C. W. Howard.
<i>Parlatoria pergandii</i> Comstock.....	Florida	P. H. Rolfs.

VI. MICROCERA SP.

Microcera sp.: Mycelium pure white; hyphae delicate, septate, loosely branching, hyaline, 4—5 microns thick; conidia at first borne on the ends of the branches, one and two-celled, oval to oblong, 7—12×3 microns; conidial tubercles various in size, cushion-shaped, pink, made up of a compact mass of conidia; conidia lunate, acute at both ends, 3 to 5 septate, mostly 28—40×3.5—5 microns. A few conidia reach 52 microns. On *Aleyrodes citri* R. & H., on citrus trees in Florida, U. S. A.

DISCOVERY.

In September, 1907, Prof. P. H. Rolfs, when visiting the orange grove of Mr. F. Wills at Sutherland, Florida, noticed that a great many larvae of *Aleyrodes citri* were dead or dying. None of the known parasitic fungi were to be seen, but by the use of the hand lens a whitish fringe could be noticed growing from the edges of the larvae. Specimen leaves were brought back to the laboratory on September 13, and on microscopic examination, the larvae upon these leaves were found to be diseased by a species of *Microcera*. Leaves sent in by Mr. Wills on September 26, were found to have even more abundant fungus, and nearly all the larvae were dead. Orange leaves sent in from Manatee a few days later were also found to bear larvae infected with the same fungus. This *Microcera* has since been found at Largo, Bayview, and Safety Harbor by the author, and has been brought in from Orlando, DeLand and Leesburg by E. W. Berger.

Fig. 16. Hypha of *Microcera* from edge of larva showing branching and formation of one- and two-celled conidia, X 1000.

Afterwards there are formed on the edge of the larvae pinkish spore-masses, which are made up of a compact mass of lunate spores (Fig. 17). These are 3- to 5-septate, and 28 to 40 microns long, by 3.5 to 5 microns thick, in fresh specimens. When the larvae are placed in distilled water on a microscope slide, the fungus spores are seen to float apart and spread out in the water.

A preliminary notice of this fungus was published by the author in Press Bulletin 68 of the Florida Experiment Station, "A New Whitefly Fungus". This fungus presents a fringe of delicate white hyphae growing outward from the edges of the larvae (Plate VI, Fig. 41). These hyphae at first bear one-, two-, or three-celled conidia, which are oval to fusiform in shape (Fig. 16). Afterwards there are

Fig. 17. Conidia of *Microcera* from larvae dried on leaves of Citrus, X 450.

CULTURES.

Culture of this *Microcera* were grown much more easily and quickly than those of any of the previously described fungi. This fungus grew rapidly on nearly all kinds of media which were tried.

Fig. 18. Conidia from test-tube culture of *Microcera* on bread, X 450. (Culture had dried out.)

On September 14, 1907, the day after the fungus had been examined and recognized as a new parasite on *Aleyrodes citri*, three sets of petri dish cultures of three each, A, B, and C, were prepared according to the usual method for isolation. The first set was made by touching an infected larva with a moist platinum needle, and washing the needle off in test-tube A. B received three loops from A, and C three loops from B. In three days on petri dish A, two mycelia and a number of bacterial colonies were evident. In nine days, the fungus had overrun almost the entire dish, in spite of the bacteria present, and was producing an abundance of conidia. B and C developed no fungus.

The second set was inoculated with spores from a test-tube in which an infected larva had been shaken up in water. In both A and B, a good growth of fungus appeared. The fungus just showed after 24 hours. In A, in three days, there were about 50 mycelia. In B, in three days, the mycelia, three in number, had become 10 to 12 mm. in diameter, loosely tufted, with numerous conidia on the upwardly projecting, irregularly branching hyphae. In nine days, the growth had covered the entire surface of the medium.

The third set was inoculated from a test-tube containing a little sterile water, in which two infected larvae had been placed. Only the first petri dish A developed a growth of *Microcera*, which grew as described for the second set.

The fungus in all cases was of a pure white color. It grew in loose tufts, with upwardly growing, very delicate hyphae, forming a loose, fluffy mass, which soon collapsed when the cover of the petri dish was removed. A microscopic examination of this growth showed that it was made up of irregularly branching hyphae bearing conidia.

All intermediate shapes of spores from the oval one-celled conidium, to the septate lunate conidium, could be found in the same culture. (Figs. 16, 17, 18). It may be remarked in this connection, that in the cultures of *Sphaerostilbe coccophila* made by P. H. Rolfs in 1897, the conidial stage of which has been referred to *Microcera*, these one- and two-celled conidia appeared, and are figured by him in Bulletin 41 of the Florida Experiment Station, Plate II. Judging from the growth of *Microcera* sp. in cultures, it would probably fit into the genus *Fusarium*, but since the distinction between *Microcera* and *Fusarium* is rather vague, we prefer to hold to the name *Microcera* until the perfect stage is worked out. Perithecia of this fungus appear to be developing at the present time on culture media.

On September 18, two test-tubes of standard agar, one of Irish potato, and one of rice, were inoculated by drawing a moist needle over the top of culture B of the second set poured on September 14. On agar tubes there seemed to be some evidence of growth in three or four hours. On September 21 (3 days), tufts of white mycelium were formed over the entire surface. On November 20, the agar had begun to shrink away from the sides of the tube, and the fungus had grown down over the sides of the medium. On Irish potato, September 28, almost the entire surface was covered with a snowy white growth of fungus. On rice in a 50 cc. flask, by November 11 (54 days) the entire surface was covered with growth, and the spaces between the rice grains were packed with a fungus mycelium. The mycelium was very thick, matted, and pink on the sides near the glass (Plate VI, Figs. 39 and 40).

On September 25, a test-tube and a flask of rice, and two tubes of bread, were inoculated from the same culture as before. On rice, September 28 (3 days), a delicate growth 10 mm. high was formed in the test-tube. The rice had turned pinkish from the top to $\frac{3}{4}$ inch down on the sides. In the flask almost the entire surface was covered, and was pinkish at the base on the sides of the rice. By October 18 (23 days) the fungus had grown through all the available spaces in the medium, forming a pinkish matted

growth. On November 11, numerous conidia were present in pink masses on the sides of the rice. On the bread, on September 28 (5 days), a delicate white growth was formed over the upper surface. On October 10 (15 days), the growth had taken up every available space in the pores of the bread, giving it a pinkish matted appearance.

On October 11 the following cultures were made by transferring spores from the bread cultures of September 25, which had been transferred once before. Three tubes of agar, one of sweet potato, one of Irish potato, one of rice in a 50 cc. flask, and one of bread were used. Notes were taken on October 19 only. In the three agar tubes, which reacted 1.0, 1.5 and 2.0 respectively to phenolphthalein, the growth was about one inch high with abundance of conidia. There was no noticeable difference in the growth. On sweet potatoes, a pure white, delicate growth appeared; on Irish potatoes, a very abundant growth; on rice, an abundant growth with a pinkish color on the surface, and thick mycelium between the rice grains.

INFECTION OF ALEYRODES CITRI.

On September 19, 1907, infection experiments were made on healthy whitefly larvae from cultures of this fungus. The larvae were on the leaves of a privet hedge (*Ligustrum ovalifolium*) in Gainesville. The following is taken from notes made at the time of the experiments:

Healthy larvae were very abundant on the privet leaves. The weather was damp after a rain. Inoculations were made from 3 to 5 P. M.

No. 1. Inoculation was made from culture of September 14; conidia were penciled on the under side of the leaves on one branch with a moist camel's-hair brush; a piece of cheese cloth was tied around the inoculated branch. On September 21 no conidia were found; the cheese cloth was removed. On September 28, no conidia were found. On October 5, abundant conidia of *Microcera* were present. On October 25, about 50 per cent. of the larvae were dead.

No. 2. Inoculation was made from same culture, penciled on as in No. 1, but not covered with cheese cloth. On September 28, no spores were found. On October 1, spores of *Microcera* were present. On October 5, pink spore-masses were developed on the edges of larvae. By October 25, about 50 per cent. of larvae were dead.

No. 3. Conidia were penciled on as above, and not covered with cheese cloth. On September 21, no spores were found. On September 28, no spores were found. On October 1, *Microcera* spores were present. On October 5, pink spore masses were present on the edges of the larvae. On October 25, about 50 per cent. of the larvae were dead.

No. 4. Inoculation was made from larvae on citrus leaves from Manatee. No cheese cloth was tied around. On September 21, larvae seemed to be attacked by a small fungus, which looked like the *Microcera* of the cultures. The conidia were one- and two-celled. On September 28, abundant spores of *Microcera* on dead larvae were found, and live larvae with filaments of fungus within. About 60 per cent. estimated dead. On October 5, pink conidial masses were evident on edges of larvae.

No. 5. A branch as a check was not inoculated, but tied up with cheese cloth. On September 21, no conidia of *Microcera* were to be found. On September 28, no conidia of *Microcera* to be found.

No. 6. A branch near No. 5 was neither inoculated, nor tied with cheese cloth. On September 21, no spores of *Microcera* were to be found. On September 28, no spores of *Microcera* were found.

No. 7. A branch above No. 4 was neither inoculated, nor tied with cheese cloth. When examined on September 28, no *Microcera* could be found.

During the first two weeks after these infection experiments were made there were frequent rains, and the weather was quite moist. This was followed by two weeks of drier weather during which the fungus apparently ceased to grow. These inoculation experiments show that under favorable climatic conditions like those under which they were carried on, the larvae of *Aleyrodes citri* may readily be infected either directly from previously infected larvae, or from pure cultures.

GERMINATION OF CONIDIA.

The conidia of *Microcera* germinated quite readily in water. On October 3, a hanging drop culture was made with conidia from a potato culture. In 24 hours, one of the cells of the conidia, usually the end cell, sent out hyphal tubes to a distance of one to four times the length of the conidia. On the end of a number of these were seen small oval sporids (Fig. 19). In 48 hours the hyphae were 6 to 7 times the length of the conidia, some of them branched as in Fig. 19, and many sporids had formed. In six days the branching hyphae were prominent, with many sporids. The segments of the conidia had become swollen, thus causing constrictions at the septa.

Fig. 19. Conidium of *Microcera* germinating and forming sporids. (a) Conidium, (b) hyphal tube, (c) sporid, X 450.

VARIATION IN SIZE OF CONIDIA.

The measurements of conidia at various times and under various conditions indicated a considerable variation in size. The greatest variation was in the length. As has been said, the conidia varied from oval one-celled spores to long lunate spores. Measurements of lunate spores on larvae were as follows:

On September 23, the first specimen from Sutherland after drying bore conidia measuring $28-40$ by $3.5-5$ microns. On September 26, the second lot of specimens from Sutherland, while still fresh, contained conidia measuring $36-45 \times 3.5-4.5$ microns. On October 5, privet leaves from inoculation experiment No. 4, at Gainesville, while still fresh, bore conidia measuring $31-52 \times 3.5-4.5$.

Measurements of lunate spores in cultures were as follows:

On September 17, conidia from cultures made September 14 measured $21-32 \times 3.5-4.5$ microns. On November 21, conidia from culture on potato, measured 12 to 30 microns long; two-celled conidia were 12 microns, the others longer. On February 2, 1908, from cultures 101 days old, made October 26, 1907, on white cornmeal cultures not dried out; conidia in pink

cushion-shaped masses, 5-septate, 40—60 microns long; most of them 40 microns, few 60 microns. On June 2, 1908, from culture on bread made September 25, 1906, dried out; conidia $15-30 \times 3-4.5$ microns.

DISTRIBUTION OF MICROCERA SP. IN FLORIDA.

Sutherland.....	September 13, 1907.
Manatee.....	September 18, 1907.
Gainesville.....	September 21, 1907. (Introduced.)
Leesburg.....	October 11, 1907.
Orlando.....	November 25, 1907.
Largo.....	November 1907.
Titusville.....	December 3, 1907.
Safety Harbor.....	March, 1908.

VII. THE BROWN FUNGUS OF ALEYRODES CITRI.

In March, 1896, H. J. Webber⁵⁶ discovered this fungus on whitefly larvae in the grove of J. H. Viser, Manatee, Florida. During the summer of that year the fungus spread rapidly through the Viser grove, and was observed to be a very effective parasite of *Aleyrodes citri*. As no fructification of any kind has been found in connection with this fungus, it has not been classified. It has been known since its discovery as the "Brown Fungus" of the whitefly (Plate VII, Fig. 42).

DESCRIPTION.

Webber⁵⁷ gave a general description of this fungus in Bulletin 13 of the Division of Vegetable Physiology and Pathology, as follows:

The mature stroma is compressed hemispherical, frequently having a slight depression in the apex over the center of the insect, where the hyphae come together as they spread from the edges of the scale in their development. The size varies greatly, according to the stage of development of the insect attacked. In very young larvae it is from $\frac{1}{4}$ to $\frac{1}{2}$ a millimeter in diameter. In mature larvae and pupae it frequently reaches 2 millimeters in diameter. The thickness, or height, also varies in like manner, specimens on mature larvae or pupae being usually from 175 to 260 microns, while those on young larvae are much thinner. * * * The stroma is commonly seal brown, with a shade of chestnut, but becomes slightly darker with age. * * * The hyphae, which make up the body of the stroma, are light brown, very tortuous, and but slightly branched. Those in the body of the insect are of similar character, but a much darker brown. From the base of the stroma a ground mycelium, or hypothallus, spreads out in all directions on the surface of the leaf, forming a compact membrane near the stroma, but becoming gradually dispersed into separate filaments. * * * The hyphae of the hypothallus are colorless, sparingly branched, mostly continuous, having only an occasional septum, and are from 5 to 7 microns in diameter. In some places in the hypothallus, where the hyphae are apparently somewhat massed and knotted, they become light brown, similar in color to the isolated hyphae of the stroma.

⁵⁶ Div. of Veg. Phys. & Path., Washington, D. C., Bul. 13, p. 27, 1897.

⁵⁷ *Ibid.* pp. 28-30.

BROWN FUNGUS OF PARKIN.

J. Parkin⁵⁸ in writing of the Ceylon forms of fungi parasitic on *Aleyrodes*, mentions having found on three different kinds of leaves a brown sterile fungus, which he thinks is similar to the one described by Webber on *Aleyrodes citri*. He also states that these brown pustules were in many cases closely associated with *Aschersonia aleyrodidis* of Webber, and suggests the possibility of one being a form of the other. In regard to this point he writes:

Intermingled with the brightly colored *Aschersonia* stromata on the leaf of *Flemingia strobilifera* were other brown ones. Many of these latter were evidently old or arrested *Aschersonia* stromata, as sections of them revealed closed pycnidia. Others again were flatter, more nearly resembling Webber's brown fungus, thus suggesting the possibility of all these sterile pustules being really connected with *Aschersonia*. The two fungi often appear in association on the same scale and even on the same leaf. Webber mentions that *A. aleyrodidis* was present on those orange bushes containing also the "brown mealy wing fungus". In the Ceylon specimen on *Meme-cylon* the two were intimately associated. Atmospheric conditions such as dryness may also influence the development of the *Aschersonia* as to induce it to assume a sterile resting form. This, when conditions are again favorable, might send out infecting hyphae over the leaf surface. Webber's account of how this brown fungus develops and spreads hardly favors such a view. However, its close association with *Aschersonia* is a point to be kept in mind. By cultures perhaps this sterile form might be induced to form some fructifications, and so a clue to its nature and relationship might be obtained.

The cultures of *Aschersonia aleyrodidis* and *Aschersonia flavo-citrina* made by the author on various media and at different times of year with varying amounts of water, never showed any tendency to develop the brown sterile form of the Brown fungus. In Florida there seems to be no evidence to indicate any connection between this sterile Brown fungus and the *Aschersonias* parasitic upon *Aleyrodes citri*.

METHODS OF INTRODUCTION.

Webber, in the bulletin previously referred to, describes in some detail the method of introducing this fungus into trees infested with *Aleyrodes citri* by pinning in leaves, or by planting young fungus-bearing trees in such a way that their leaves would come in contact with the larvae-bearing leaves to be infected with fungus. E. W. Berger⁵⁹ has recently produced some infection by grinding up the brown stromata, stirring with water, and spraying this water upon infested leaves.

A number of attempts have been made by the writer to grow cultures of this fungus in the laboratory, but so far he has been unsuccessful. In one case where stromata of the brown fungus were placed close to a drop of agar in a hanging drop culture, short tortuous hyphae were seen to grow

⁵⁸ Annals Roy. Bot. Gard. Peradeniya, Vol. III, Part I, p. 52, 1906.

⁵⁹ Fla. Exp. Sta. Bul. 88, p. 64, 1906.

out from the edge. This is the only growth that has so far been observed under artificial conditions.

DISTRIBUTION OF THE BROWN FUNGUS.

Florida.—Alva, Bartow, Buckingham, Bradentown, Fort Myers, Largo, Leesburg, Manatee, Oneco, Orlando, Palmetto, St. Augustine and St. Petersburg, on *Aleyrodes citri*.

Ceylon, as reported by J. Parkin, on various species of *Aleyrodes*.

SUPPLEMENTARY NOTES.

1. Since the above has been written, what appear to be the spores of the *Brown* fungus of *Aleyrodes citri* have been discovered. These spores are germinating in hanging drop cultures of sugar solutions, and are producing hyphae that seem to be identical with those of the *Brown* fungus. Further study is needed to prove the relationship of these spores to the *Brown* fungus.

2. A species of *Sporotrichum* has been discovered upon the adult and larva of *Aleyrodes citri*. This fungus seems to be responsible, during damp weather, for the premature death of great numbers of adults. Cultures and inoculation experiments are being carried on, the results of which will be published later.

3. Since the writing of this thesis it has been shown by Dr. E. W. Berger, Entomologist of the Florida Experiment Station, that the insects hitherto designated as *Aleyrodes citri* comprise two distinct species of *Aleyrodes*; one with smooth eggs and clear wings, and the other with rough eggs covered with a delicate net of five- and six-sided meshes, and wings with a smoky-colored area at the end of each fore wing. Specific differences have also been observed in the first and fourth stage larvae. (See Press Bulletin 97, Fla. Agri. Exp. Sta., and Proc. Fla. Sta. Hort. Soc., p. 86, 1908.) Dr. Berger's observations and also those of the writer seem to show that these two species of *Aleyrodes* are differently attacked by the *Aschersonias* (*A. aleyrodis* and *A. flavo-citrina*), and also by the *Brown* fungus and *Microcera* sp. *Aschersonia flavo-citrina* attacks readily only the smoky-winged *Aleyrodes*, but will attack the clear-winged species. *Aschersonia aleyrodis* and the *Brown* fungus attack both species of *Aleyrodes* in an equal degree, but the greater number of the specimens so far observed have been found upon the clear-winged species. The *Microcera* has been found on both species of *Aleyrodes*, although it is more effective upon the smoky-winged species. The infection experiments described in the foregoing pages with cultures of *Microcera* sp. (page 32) and *Verticillium heterocladium* (page 24) were made upon the clear-winged species. The infection experiments also made by E. W. Berger at St. Petersburg and Gainesville (page 16) were also upon the clear-winged *Aleyrodes*.

October 1, 1908.

SUMMARY.

1. Entomogenous species of fungi representing many different genera have been described in the past hundred or more years.

2. These have not, until recent times, been studied from an economic standpoint. The greatest success in the use of fungi to combat insect pests seems to have been attained in Florida, where proper conditions of temperature and moisture are present.

3. *Aleyrodes citri* R. & H., known since 1885 as a pest to citrus trees in Florida, has spread to many citrus districts since that time, doing much damage chiefly by creating conditions favorable to the growth of *Meliola*.

4. A study of the fungi parasitic upon *Aleyrodes citri* shows that there are at least six species, five of which have been grown upon culture media in the laboratory.

5. *Aschersonia aleyrodis* Webber is the most widely distributed fungus parasite of *Aleyrodes citri*. It is easily isolated and grown in pure cultures in 5 to 10 per cent. glucose agar medium, maturing a stroma in 30 to 40 days.

6. Healthy larvae of *Aleyrodes citri* may be infected from cultures of this fungus by spraying a mixture of conidia in water on trees infested with *Aleyrodes citri*.

7. *Aschersonia flavo-citrina* P. Henn., which was recently found in eastern Florida, is also an important parasite of *Aleyrodes citri*. Its growth on culture media is the same as that of *A. aleyrodis*.

8. Conidia of both of the *Aschersonias* germinated best in a 5 to 10 per cent. solution of glucose in water. Percentages of sugar above or below this retarded germination. Conidia from stromata dried in the laboratory for more than 28 days failed to germinate.

9. *Verticillium heterocladum* Penz. has been recently shown to occur parasitically upon *Aleyrodes citri*. It has also been observed on a number of other insects. Cultures and inoculation experiments show that this fungus is distinct from the "Brown fungus", which it somewhat resembles in general appearance, and with which it is frequently found associated.

10. The growth of this fungus in cultures is much more rapid than that of the two species of *Aschersonia* described.

11. *Sphaerostilbe coccophila*, known since 1848, is world-wide in distribution, and has been reported on no less than fifteen species of scale insects, in addition to being found on *Aleyrodes citri*.

12. *Sphaerostilbe coccophila* has been used in Florida as an effective parasite in controlling the San Jose scale and other scale insects. In more northern States it has not proved to be effective. It is possibly a weak parasite of *Aleyrodes citri*.

13. *Microcera* sp., recently discovered, has been found in a number of places in Florida attacking larvae of *Aleyrodes citri*. Abundant cultures of

this fungus may be grown in a few days, and larvae of *Aleyrodes citri* may be infected from these cultures. Conidia of this fungus vary greatly in size under different conditions of growth.

14. The Brown fungus, known in Florida since 1896, has never been observed to produce spores and is therefore unclassified. Its growth and development on *Aleyrodes citri* were described by Webber. All attempts to grow this fungus in pure cultures have failed. (See, however, Supplementary Note 1.)

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EXPLANATION OF PLATES.

PLATE I.

- Fig. 20.—*Aschersonia aleyrodinis*. Culture in petri dish of 5 per cent. glucose agar poured December 7, 1906; showing mature stroma 36 days old. The central raised portion was red, and contained the pycnidial cavities with spores.
 Fig. 21.—*Aschersonia aleyrodinis*. Culture on rice after 5 weeks, grown at room temperature.
 Fig. 22.—*Aschersonia aleyrodinis*. Culture on sweet potato plug, after 5 weeks. Grown in refrigerator after first few days.
 Fig. 23.—*Aschersonia aleyrodinis*. Culture on white corn meal after 5 weeks. Grown at room temperature.

PLATE II.

- Fig. 24.—*Aschersonia flavo-citrina*. Citrus leaf showing stromata of the fungus in the position originally assumed by larvae of *Aleyrodes citri*. The hemispherical raised center was yellow with a fringe of outgrowing hyphae.
 Fig. 25.—*Aschersonia aleyrodinis*. Citrus leaf showing stromata. The raised center was red instead of yellow, as in the previous figure.

PLATE III.

- Fig. 26.—*Aschersonia flavo-citrina*. Culture on petri dish of 5 per cent. glucose agar made +0.5 to phenolphthalein, after 33 days. Culture poured September 14, 1906; showing the mature stromata one and a half times natural size.
 Fig. 27.—*Aschersonia flavo-citrina*. Culture on petri dish of 5 to 10 per cent. glucose agar, after 40 days, showing how stromata will spread when given room to grow.

PLATE IV.

- Figs. 28-30.—*Aschersonia flavo-citrina*. Cultures on sweet potato plugs, inoculated November 1, 1906, after 60 days.
Fig. 31.—*Verticillium heterocladium*. Citrus leaf showing fungus pustules on larvae of *Aleyrodes citri*.

PLATE V.

- Fig 32.—*Verticillium heterocladium*. Culture in test-tube on bread.
Fig. 33.—*Verticillium heterocladium*. Culture in test-tube on Irish potato plug.
Fig. 34.—*Verticillium heterocladium*. Culture on glucose agar, showing brown pustules.
Fig. 35.—*Verticillium heterocladium*. Leaf of privet bearing larvae of *Aleyrodes citri* infected with the fungus by means of a citrus leaf containing pustules.

PLATE VI.

- Fig. 36.—*Sphaerostilbe coccophila*. Sporodochia of the fungus on bodies of *Aspidiotus hederæ*, on Chinaberry twig.
Fig. 37.—*Sphaerostilbe coccophila*. Sporodochia of fungus on bodies of *Mytilaspis citricola* on citrus twig.
Fig. 38.—*Sphaerostilbe coccophila*. Perithecia of fungus on *Mytilaspis citricola* on bark of citrus. Twice natural size.
Fig. 39 and 40.—*Microcera* sp. Cultures on rice showing loose fluffy growth on top of medium. Natural size.
Fig. 41.—*Microcera* sp. Citrus leaf with larvae of *Aleyrodes citri* infected with the fungus. The fungus is shown by white fringes on the edges of a number of the larvae.

PLATE VII.

- Fig. 42.—The Brown Fungus of Webber. Citrus leaf with brown pustules that have formed on larvae of *Aleyrodes citri*. (Photographed by H. H. Hume.)
Fig. 43.—*Cladosporium* sp. A super-parasite found growing over *Aschersonia flavo-citrina* on leaf of citrus.

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Figs. 20-23. Cultures of *Aschersonia aleyrodidis*.

Fig. 24. *Aschersonia flavo-citrina* on citrus leaf.

Fig. 25. *Aschersonia aleyrodidis* on citrus leaf.

Fig. 26. Cultures of *Aschersonia flavo-citrina*.

Fig. 27. Cultures of *Aschersonia flavo-citrina*.

Figs. 28-30. Cultures of *Aschersonia flavo-citrina*.

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Figs. 32-34. Cultures of *Verticillium heterocladium*.
Fig. 35. *Verticillium heterocladium* on *Aleyrodes citri* on privet leaf.

Figs. 36-37. Sporodochia of *Sphaerostilbe coccophila*.
 Fig. 38. Perithecia of *Sphaerostilbe coccophila*.
 Figs. 39-40. Cultures of *Microcera* sp.
 Fig. 41. *Microcera* sp. on *Aleyrodes citri* on citrus leaf.

Fig. 42. Brown fungus on *Aleyrodes citri*.

Fig. 43. A super-parasite (*Cladosporium* sp.) upon *Aschersonia flavo-citrina*.

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