THE GREEN MUSCARDINE FUNGUS IN PORTO RICO.

(*Metarrhizium anisopliæ* [Metsch.] Sorokin.)

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The green muscardine fungus is one of the best-known of the fungi attacking injurious insects, and as such has been studied and observed in many parts of the world. It occurs apparently indigenous in some countries, and has been introduced into various others in an endeavor to make use of it in the fight against insect pests. It is probable that with the exception of the work with *Sporotrichum globuliferum*, an enemy of the chinch bug and other insects, more has been done with this fungus than with any other of a similar nature. Although originally described from Europe, most of the studies upon it and attempts at its artificial dissemination have been made in the tropics or subtropics and in connection with sugar-cane insects.

HISTORY OF THE FUNGUS.

The fungus was first noted and described by Metschnikoff (23)[1] in Russia in 1879 as *Entomophthora anisopliae*. Since that time it has been redescribed under several other names or new combinations.[2] Rorer (29) has given a full account of these nomenclatural details. The combination adopted by him in 1910 has been used by other workers since that time, and is also used in this paper.

Since the original discovery in Russia, *Metarrhizium* has been found occurring naturally in France (6), United States (25), Mexico (40), Trinidad (4, 12), Samoa (8), Philippine Islands (2), Queensland (38), Java (26, 41), Hawaii (16, 17), and Porto Rico. In addition the fungus has been introduced for trial under artificial conditions into Mauritius (5), Java (11, 31), Porto Rico (42), Cuba, and Argentina (7). Cultures from this laboratory have been sent

[1] Figures in parenthesis refer to literature cited, p. 28.

[2] *Metarrhizium anisopliae* (Metsch.) Sorokin.
Entomophthora anisopliae (Metsch.) (23).
Metarrhizium anisopliae Sorokin (33).
Isaria destructor Metsch. (24).
Oospora destructor Delacroix (6).
Penicillium anisopliae Vuillemin (43).
Septocylindrium suspectum Massee (22).
Chromostylim anisopliae Sorokin (?).
Penicillium anisopliae Vuillemin (43).

to Java and the two latter countries. Speare (34) reports that no introductions were made into Hawaii from Japan or other sources, as far as the available records show, although statements to the contrary have been made.

HISTORY OF THE INTRODUCTION OF METARRHIZIUM INTO PORTO RICO.

The following paragraph from Bulletin 10 of this Station gives the history of the introduction of *Metarrhizium* into Porto Rico:

"*Metarrhizium* was introduced under the name of Hawaiian beetle fungus by D. L. Van Dine, January 12, 1911, and was identified by the writer, whose identification was confirmed by Prof R. Thaxter. * * * This material consisted of infected beetles, some of which were sent to Mr. C. T. Murphy at Guánica Central. More fungus in the form of infected soil was received by Mr. Van Dine, March 30, 1911. On June 3, a letter accompanying material was received at the Sugar Planter's Station" (now the Insular Experiment Station) "from Mr. D. W. May of the Mayagüez Experiment Station. This material was originally from Hawaii."

It was supposed at that time that the fungus was not indigenous, but since then the finding of infected insects in regions distant from the points where the introduced material has been worked with, makes it appear that it did exist in the Island previous to the Hawaiian importations. The native type has at no time been abundant, having been found on single, isolated insects only.

THE FUNGUS.

Although the fungus has been placed at different times in such widely different genera as *Entomophthora, Isaria, Oospora,* and others, a study of actual material leaves no doubt as to its position near *Penicillium* in the *Moniliaceae.* With one exception, no spore form other than the chains of cylindrical conidia have been reported. Tryon (38) from Queensland makes mention of having found a *Cordyceps* or perfect stage associated with *Metarrhizium.* He assumes the two to be stages of the same fungus, but apparently made no careful cultural studies to prove this assumption, or at least none are given. In as much as no other instances have been recorded, in all of the many references to the subject, of any other spore form such a possibility seems remote. In Porto Rico the fungus has been studied in the laboratory, insectary, and in the field by practically all of the various men who have been connected since 1911 with the

divisions of Pathology and Entomology, and there has never been the slightest evidence to support a theory of another stage in the life history.

Mr. Johnston (14) records two conidial forms, forma *major* and forma *minor* occurring on different hosts. Sufficiently complete accounts of the appearance and behavior of the fungus on artificial media as well as technical descriptions will be found in several of the articles cited in the bibliography, in particular those by Rorer (29), Speare (35), Johnston (14), and the earlier papers by Metschinkoff (23, 24).

HOSTS.

The number of species of insects attacked is very large, and includes many that are of considerable economic importance. A list of the more important host species, together with the country from which the report was made, follows:

Adoretus compressus, Java (41).
Adoretus tenuimaculatus, Hawaii (16–18).
Adoretus umbrosus, Hawaii (35).
Agriotis manci, New York (25).
Anisoplia austriaca, Russia (22).
Anomala, sp., Hawaii (35).
Cleonus punctiventris, Russia (32).
Cyrtacanthracris nigricornis, Java (31).
Holotrichia helleri, Java (11).
Lachnosterna sp., Illinois, reported by Wolcott.
Lepidiota albohirta, Queensland (38).
Leucophilus rorida, Java (31).
Oryctes rhinoceros, Samoa (8).
Phytalus smithi Mauritius, (?) (5).
Rhabdocnemis obscura, Hawaii (29).
Tomaspis postica, Mexico (40).
Tomaspis varia, Trinidad (9, 28).

It will be noted that this series includes the froghopper of Trinidad, as well as a number of other serious cane pests; the rhinoceros beetle, a pest of the coconut; the cockchafer of Europe, and other well-known depredators. The silk-worm has also been reported as a host by Delacroix in France (6).

A considerable range of hosts has also been found in Porto Rico but for the most part only insects in confinement in the breeding cages. These have been collected by the several entomologists of

the Station, but more particularly by Mr. E. G. Smyth (32) who carried on work with the white grubs over a number of years.

The insects found diseased by *Metarrhizium* in Porto Rico are as follows:

Aphodius sp.
Canthon sp.
Dyscinetus barbatus.
Lygyrus tumulosus.
Metamasius hemipterus.
Phyllophaga citri.
Phyllophaga guanicensis.
Phyllophaga portoricensis.
Phyllophaga vandinei.
Phytalus insularis.
Strategus titanus.
Tiphia inornata. (Received from Illinois.)

The fungus has been found in addition on a number of undetermined Scarabaeids, an earwig, a roach, and some other unnamed hosts. The localities of the many collections made have been Río Piedras (Experiment Station), Santa Rita (Guánica), and Añasco, localities into which the fungus was introduced. Specimens have also been taken at Fajardo (*Phyllophaga* sp.), Hacienda Santa Isabel of Aguirre (*Phyllophaga* sp.), and Sierra de Naguabo (earwig), localities into which the fungus was not introduced, apparently tending to prove that the fungus is indigenous to the Island.

Further information on the stages of the various hosts attacked, prevalence, progress of the disease on the individual insects, and related points will be found in the report by Smyth (32).

EXPERIMENTS IN ARTIFICIAL DISSEMINATION.

Metschnikoff (23) conducted the first experiment with *Metarrhizium*, using it to fight the cockchafer of wheat. He obtained his spore material from infected insects. Krassilstchik (19, 20) used the fungus in a similar fashion in this work on the sugar beet curculio and reported from fifty to eighty per cent of the insects infected. These earlier experiments were necessarily on a limited scale because of the difficulty of obtaining spore material in quantity.

During recent years extensive tests have been carried out in Java, Hawaii, Trinidad, Porto Rico, and other regions.

The most important of the efforts in this connection has been Rorer's (27–29) work in Trinidad directed against the froghopper

(*Tomaspis varia*). Entomologists and others (1, 3, 4, 9, 10, 13, 39) connected with the agricultural work of that Island have also assisted in the tests. In the preliminary experiments adult froghoppers in wire cages were inoculated by spraying, and a high percentage of mortality resulted. Results were also obtained in infecting the nymphs. A field experiment, using a mixture of flour and spores, in which over one hundred cane plants were dusted, resulted very favorably in the death by the fungus of a large number of the insects.

Work was then commenced on the production of spore material in great quantities so as to permit of the dusting or spraying with spores of entire fields. For this purpose large cabinets capable of being sterilized by steam were devised. The manner of construction of these cabinets and the course of procedure in producing the spore material have been fully described by Rorer. The results obtained were sufficient to cause these spore-producing plants to be erected on a number of the sugar estates. These are operated at such times as conditions seem favorable for rapid increase of the froghoppers.

The experiments in Java have been carried on in several sections of the Island by different workers but have all been confined to tests on a small scale, mostly in breeding cages apparently. Groenewege (11) infected soil with the spores and then added larvæ of various insects, varied proportions of which were killed by the fungus. He concludes that since most of them were killed near the close of the larval period, and after the full damage to the host would have been caused, that the method is not efficacious. He furthermore stated that the cost would be prohibitive for field operations.

Rutgers (31) carried out experiments at practically the same time, using cultures obtained from Hawaii. For infection he employed spores mixed with a double quantity of flour. The insects used were *Leucophilus rorida* and locusts (*Cyrtacanthacris nigricornis*). In one experiment a mortality of eighty per cent was obtained, but succeeding tests gave only slight results. It was found that infection, even when the insects were enclosed in a small space and were in intimate contact with quantities of spores, was dependent upon external conditions, particularly the moisture content of the air. For this reason and since *Metarrhizium* is found under natural conditions attacking a wide range of insects he concluded that it is a dangerous parasite only under favorable conditions, and that attempts to spread the fungus artificially would be useless.

Speare (35) in Hawaii carried out an extensive series of laboratory inoculations with the fungus, working with *Rhabdocnemis obscura* as the host. The mortality varied considerably in the different trials, not exceeding sixty per cent, however, in any case. No field tests are reported.

Tryon (38) has also conducted tests upon the parasitism of *Metarrhizium*, using the spores mixed with a fine soil rich in organic matter. Final results and conclusions are not given beyond the statement that the fungus appears to have possibilities.

In Samoa (8) excellent results were reported in controlling on an extensive scale the rhinoceros beetle, an enemy of the coconut. Infected beetles were placed in trap piles of rotten coconut husks and other debris, scattered about through the coconut groves. The beetles gathered in these piles for egg laying, and it was claimed that practically all the larvæ were ultimately attacked and killed by the fungus.

Porto Rico.

Work with this fungus was begun immediately upon the receipt of material from Hawaii. Infected beetles and soil containing spores were sent to Mr. C. T. Murphy in charge of experimental work at Central Guánica. In June, 1911, he reported as follows: "The Hawaiian beetle fungus seems to be working well and the beetles kept under control in the cages seem to take it up rapidly At present several thousand are under control and in a few days time, I shall start letting them loose in the fields * * *. I am also propagating the spores artificially so as to more thoroughly infect the beetles." About a year later (April, 1912) he reports that "Inoculating beetles with the Hawaiian fungus started earlier in the month. The fungus took readily and appears to be increasing in virulence; about a fortnight after inoculating the cage, eighty-seven beetles were found killed by the disease. During the next month liberations of dead and sickly beetles will be made about every ten days in a field where the work can be watched."

Mr. Murphy stated that in May he found evidence of beetles having been killed by the disease, and in July a final mention of Metarrhizium occurs in his reports to the effect that "Beetles infected with the Hawaiian fungus have been liberated during the month in cane fields, and at the roots of trees around the nursery

beetles killed by the fungus have been buried so as to infect the soil." It is not apparent that any practical results came from this work.

At the Experiment Station at Río Piedras in connection with cultural studies of the fungus Mr. Johnston, then pathologist, carried on in 1912 a series of inoculation tests in screened cages. Beetles and larvæ (*Phyllophaga vandinei* for the most part) were obtained from the vicinity of Añasco, and Santa Rita, Guánica. These were stored in three cages until transferred to the inoculating cages. It may be noted that ten beetles were found infected in these supply cages as the transfer was being made, pointing again to the natural occurrence of the fungus.

In addition to the *Metarrhizium* trials, other entomogenous fungi secured from France were used in a similar fashion. These were *Sterigmatocystis ferrugineus, Sporotrichum globuliferum, Isaria densa*, and *Botrytis Bassiana*. No positive results were obtained with these, but on the other hand the beetles in each of the boxes in which these fungi were employed showed infection with *Metarrhizium*, as will be noted hereafter.

The *Metarrhizium* material used was a transfer from an isolation made from infected insects received from Hawaii. In each case the fungus material was scraped off the surface of the medium (yam cylinders) and placed with distilled water in an atomizer. The surface of each box was thoroughly sprayed with the spore suspension, and the culture medium itself placed on the surface of the soil. The beetles were then added from the supply boxes and observations taken from time to time. All beetles found dead were held for full development of any fungus that might be present, so as to permit of exact determinations.

As the results obtained were much the same for all, details of the examination of but one box are given, as per the following table:

Infection of May-Beetles by *Metarrhizium* (Box No. 1).

Date	May 24	June 1	6	10	15	21	26	July 1	8
Dead on surface.	1			6		5	2		
Infected.....						1			
Dead in soil.....	9	18	17	16	7	9	3	5	2
Infected.....	5	8	8	6	3	3	1	2	1

Total dead, 100; infected by *Metarrhizium*, 38.

A Summary of results from all the boxes follows:

Infection of May-Beetles by *Metarrhizium*.

Box No.	Inoculated with	No. of beetles dead	No. infected with *Metarrhizium*	Per cent infection
1	Metarrhizium.....	100	38	38
2	"	113	22	19
3	Sterigmatocystis.....	98	35	35
4	Sporotrichum.....	102	18	17
5	Check.....	97	29	29
6	Supply	628	93	14
7	Isaria	92	31	33
8	Botrytis.....	104	29	27
9	Supply	1132	190	16

It can very clearly be seen from these results that the fungus was, first of all, not especially virulent toward the May-beetles and that above all it occurred independent of inoculatons.

In order that thorough field trials might be carried out, there was constructed at the Station an apparatus (see Fig. 1) consisting of two cabinets and a five-horse-power upright boiler, following the plans of Rorer (27, 30). The medium used was rice, spread out in thin layers on the shelves and cooked *in situ*. No particular difficulties other than mechanical ones were encountered, and the first batch of spore material was taken off by Mr. Johnston in August, 1913. A low-grade flour was used to dilute the spore mass and to make removal from the rice media more easy.

The resulting material consisting of about fifty pounds of the flour-spore mixture and a similar amount of the rice residue was taken to Yauco on the south coast, and applied to one of the fields of the Guánica Central. About an acre of young plant cane was dusted, using two types of hand dusters, the Furet and the Cyclone. The former was the more convenient and serviceable. The rice residue material was applied by hand around stools of cane adjoining the dusted area.

In October a considerable number of adults were collected from the dusted area by Mr. Smyth and confined to Santa Rita. Only one of the entire number showed at any time signs of *Metarrhizium*.

The following year another lot of spore material was prepared by the writer and again applied to the field previously dusted at Yauco and in the same manner. It has never been apparent that any infection resulted among the beetles in this field. A third lot

of material was some months later applied at Río Piedras not only to cane, but around the bases of a number of coconut trees, which had been severely attacked by *Phyllophaga* sp. Infected insects have never been recovered.

CONCLUSIONS.

As a result of the field observations and the varied experiments carried out by the members of the Station staff and others who have been connected with the project, the conclusion seems justified that the green muscardine will not serve as a practical means of controlling the white grubs or May-beetles in Porto Rico.

It is true that in confinement various stages of *Phyllophaga* spp. are subject to attack, as are also other cane pests, but even in these instances the disease has not been virulently parasitic. No positive results have been obtained in the field tests and it appears that the fungus is indigenous, but so dependent upon humidity and other natural conditions that it is a negligible factor in controlling insect pests and will remain so.

This conclusion is borne out by the reports of workers in Java, both Rutgers (31) and Groenewege (11) stating that while considerable numbers of insects were attacked in confinement, results in the field were so absolutely dependent upon the weather that no artificial attempts at dissemination of the disease would avail. The favorable results obtained in Samoa in the control of the coconut beetle may be easily accounted for by the fact that conditions approximated confinement, the piles of debris retaining moisture, so that for all practical purposes they were no different than so many insect cages. None of the Porto Rican insects lend themselves to this method of trapping.

In Trinidad most favorable results have been reported, it is true, but in this case the insect pest involved has an entirely different mode of life from the May-beetles, which it is thought will account for the difference in the efficiency of the fungus in the two regions. It is also quite possible that the weather conditions prevailing at times of severe froghopper infection may favor the fungus.

It does not seem advisable to carry out any further work with the green muscardine in Porto Rico, at least in connection with the white-grubs or May-beetles.

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PLATE I.

THE GREEN MUSCARDINE FUNGUS IN PORTO RICO.

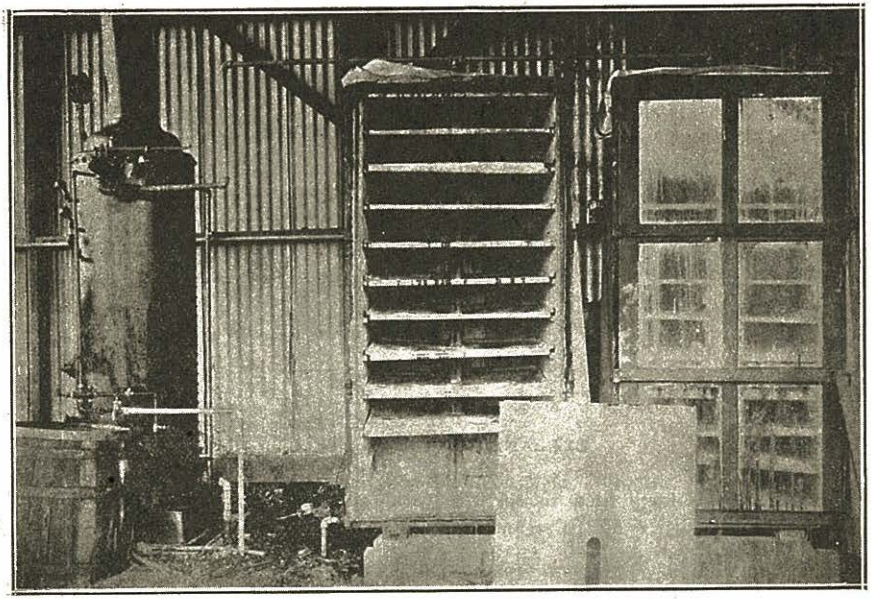

FIG. 1.

FIG. 2.

FIG. 3.

EXPLANATION OF PLATE I.

Fig. 1.—Cabinets used in the production of *Metarrhizium* spore material, and boiler, the source of the steam supply.

Fig. 2.—Pupa of *Ligyrus tumulosus* infected with *Metarrhizium*, showing also molted larval skin.

Fig. 3.—Pupa of *Strategus titanus* infected with *Metarrhizium*, showing characteristic conidial masses.

Figs. 2 and 3 from photographs by Smyth.