BOARD OF AGRICULTURE,
TRINIDAD AND TOBAGO.

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CIRCULAR No. 8.

# THE GREEN MUSCARDINE FUNGUS AND ITS USE IN CANE FIELDS,

BY

JAMES BIRCH RORER,

MYCOLOGIST.

*Issued March 31st, 1913.*

TRINIDAD:
PRINTED AT THE GOVERNMENT PRINTING OFFICE, PORT-OF-SPAIN.

1913.

### Inquiries about Fungous and Insect Pests.

In forwarding specimens planters and others can greatly facilitate the work connected with the inquiry by observing the following directions:—

1. A letter should always accompany the specimens giving a short account of the nature and extent of the damage done, the part of the plant attacked, and when first noticed.

2. All packages should be plainly marked with the name and address of the sender.

3. Specimens of parts of plants attacked by fungi may be sent green if packed in a well ventilated box and if they can be delivered within 24 hours.

4. Specimens of the insect, its work, and of plants injured should be sent.

5. Caterpillars, grubs, maggots, and any other larvæ should be sent alive with a supply of food.

6. For packing caterpillars use wooden boxes for long distances, and tin boxes where the journey is not very long.

7. When in doubt about the manner of forwarding or packing specimens of any kind, write to the Secretary, Board of Agriculture, for detailed instructions.

8. All packages will be forwarded, post free, if addressed to the "Secretary, Board of Agriculture, St. Clair Experiment Station, Port-of-Spain." Printed labels for this purpose will be supplied on application to the Secretary.

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# LETTER OF TRANSMITTAL.

BOARD OF AGRICULTURE,
Port-of-Spain, Trinidad.

GENTLEMEN:

I am herewith submitting a paper entitled the "Green Muscardine Fungus and its Use in Cane Fields," which is published as Circular No. 8.

This fungus has been known as an insect parasite since 1878 but was first noted on the froghoppers in 1890. However no serious attempt to use it as a means of control of this pest was made until 1910 when pure cultures were obtained and the method of growing the fungus in larger quantities in culture cabinets was devised. The fungus is also now being experimented with in Hawaii, Porto Rico, and Barbados as a possible means of control of the small moth borers, beetles and other cane pests.

About 25 cabinets for growing the froghopper fungus have been built on various estates in Trinidad within the past six months and it is hoped that this paper will be of value to those who will look after them during the coming season.

I have not given details for growing the fungus in pure culture in test tubes as I hope to be able to supply the spores for inoculating the cabinets from the Laboratory at Saint Clair.

Every effort should be made to start an epidemic of the Green Muscardine among the first brood of froghoppers. If this can be done successfully the fungus should prove a valuable ally in combatting this pest.

Respectfully submitted,

JAMES BIRCH RORER,
Mycologist.

February 1, 1913.

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# THE GREEN MUSCARDINE FUNGUS AND ITS USE IN CANE FIELDS.

## Introduction.

THE disease of insects caused by the fungus *Metarrhizium anisopliæ* and commonly called the green muscardine was first discovered in Russia in 1878 among the larvæ of certain beetle pests of wheat. Since that time the same fungus has been found in nearly all parts of the world attacking a great variety of insects belonging to widely different families. In Trinidad it was first noted by Hart* in 1890, who reported it as one of the natural enemies of the froghopper. In 1906 it was again common here and Urich† stated that it seemed to have checked the blight on Orange Grove Estate during the latter part of that year.

The parasitism of the fungus on froghoppers was first tested in Trinidad by Collens‡ in 1908, who placed nymphs in cages with adults which were covered with the fungus. The nymphs contracted the disease and died.

The writer § first found the fungus in the latter part of 1909 on dead froghoppers at Caroni Estate and since that time has constantly had it growing in cultures. At the beginning of the rainy season in 1910 small quantities of spores were obtained from cultures in tubes and flasks and used for inoculation purposes. Large numbers of insects were confined in breeding cages and dusted with spores. Later on a small area of growing canes, about 100 stools, badly infected with froghoppers was also dusted with spores. The results of this work has already been published and showed that under favourable weather conditions froghoppers were very susceptible to the disease and that an epidemic could be started by spreading the fungus in a cane field. Attention was then turned to devising a suitable method of growing the fungus in large quantities and a trial culture cabinet was built and proved satisfactory.

Gough‖ towards the latter part of 1910 found the fungus at Woodford Lodge and Tarouba Estates. He made cultures, repeated many of the writer's inoculation experiments, and suggested the pudding pan method for growing the fungus in large quantities.

*Report on sugar cane blight. Agricultural Record of Trinidad. 2:127. Reprinted in Bulletin of Miscellaneous Information, Botanical Department, Trinidad. 7:153. October, 1906.

†Froghoppers. *The Mirror, Port-of-Spain*, Trinidad, September 19, 1906.

‡Sugar cane blight and the froghopper. Proceedings of the Agricultural Society of Trinidad and Tobago. 8:152. 1908.

§The froghopper fungus. Report to Board of Agriculture, Trinidad. Issued September 25, 1910.

§The green muscardine of froghoppers. Proceedings of the Agricultural Society of Trinidad and Tobago. 10:467–482. 1 Pl. 1910.

‖Results of experiments with the froghopper fungus. Proceedings of the Agricultural Society of Trinidad and Tobago. 10:463. 1910.

‖The froghopper fungus and its practical application. Circular No. 6, Department of Agriculture, Trinidad, January 1911.

‖Results obtained in the study of the froghopper during the wet season of 1910. Circular No. 8. *Ibid.* January 1911.

During the past two years the writer has tried both the cabinet and pan method and finds the former much easier and more economical for getting large quantities of spores, for a single cabinet will yield as much fungus as 180 pans. About 25 cabinets have been installed on various estates and those which the writer has seen have given good cultures when looked after properly.

Although the fungus was not tried during the early part of last year for the reason that no cabinets were built in time, during the latter part of the year, after the severe outbreak of blight in September, many fields at Esperanza estate were treated with the fungus with the result that no more blight appeared on this estate while on other estates where the fungus was not used, new outbreaks of blight continued to occur well into November. The same is true of Orange Grove Estate. In one field of twenty acres on the latter estate which was dusted with spores during the first week in August actual counts made six weeks later showed an average of 92 dead insects per cane stool, and infection was still taking place.* This is a far higher percentage than is ever found in cases of natural infection.

Observations made this year show that infected female froghoppers frequently die before oviposition, a point of great practical value. A number of larvæ of the small moth borer have also been found killed by the fungus.

If by spreading large numbers of spores about at the beginning of the rainy season an epidemic of the muscardine can be started in the first brood of froghoppers the fungus should prove of great value in the control of the pest.

## Construction of Culture Cabinets.

The unit cabinet is a wooden box 2 feet 9 inches square and 6 feet high † lined with galvanized iron, with a steam pipe running down through the centre and containing a number of galvanized shelves. (Plate I.) The shelves should not be more than 6 inches, nor less than 4 inches apart. They are supported on wooden laths screwed to the sides and back of the cabinet. From 2 to 3 inches above each shelf on either one of the sides or the back, it is immaterial which, two holes, ½ inch in diameter are made through to the outside and fitted with galvanized tubes 1½ inches long which are soldered to the galvanized lining and plugged with corks on the outside. The cabinets are inoculated through these tubes.

The central steam pipe is soldered to the lining at top and bottom and has 6 or 8 small perforations not more than $\frac{1}{16}$ to $\frac{1}{32}$ inch in diameter through which steam enters the cabinet. A valve must be placed on the lower end of the pipe outside of the cabinet to let condensation water escape, while a small tube, similar to those used for inoculation, should be placed in the bottom of the cabinet to draw off the water which collects there.

The door should fit with a groove and tongue. The groove is partly filled with cotton or oakum which has previously been soaked in a solution

* Urich. Minutes of the Board of Agriculture, September 20, 1912.

† This is found to be the most convenient size on account of the measurements of the sheets of galvanized iron used in the lining.



of corrosive sublimate, one in one thousand, and dried, so as to make a tight antiseptic joint. It is necessary to have several glass panels in the door as the fungus produces spores more quickly in light than in darkness. The so-called fire proof glass is best for this purpose as it stands the heat without cracking. The panels should be bedded with cotton to allow for expansion and should be protected on the outside with sheets of asbestos during sterilization. The door is fastened in place by bolts or battens.

The cabinet, or series of cabinets should be placed in as clean a place as possible; a specially built room is preferable, but is not necessary. It is essential that they should not be exposed to the wind, rain or sun.

Live steam for sterilization, thirty or forty pounds pressure, is necessary.

## Method of using Cabinets.

Before a cabinet is used it should be washed out thoroughly with clean water. So far, rice has proved the best medium on which to grow the fungus. About 40 pounds is used for each cabinet. The rice is well washed and then boiled from 10 to 15 minutes, either in a kettle over an open fire, or better, by allowing live steam to escape from perforated cross pipes in the bottom of a pot, or half barrel, in which the required amount of water has been placed.

The cooked rice should be drained dry and immediately spread in a layer about ¼ to ½ inch thick on all the shelves in the cabinet. The door is then put in place and steam turned on. A thermometer in a cork should be inserted in one of the inoculating holes and the temperature of boiling water, 212° F. or 100° C, maintained for at least an hour. This sterilization is repeated on four successive days, *care being taken that the steam valve is tightly closed* after each heating.

The rice should be inoculated as soon as it has cooled down after the fourth sterilization.

## Inoculating the Cabinets.

The cabinets are inoculated with pure spores of the fungus contained in a flask fitted with a cork and glass tubes which has been previously sterilized in a hot oven. (Figure 1). An atomizer bulb with a piece of sterile cotton tied over the intake valve is attached to one tube while the other is inserted in turn into all the holes in the cabinet and a charge of spores blown in quickly by a slight pressure of the bulb. The corks must be taken out and replaced as quickly as possible in order to avoid contamination of the rice from the outside air.

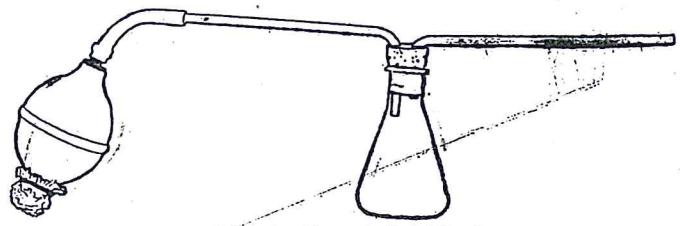

FIG. 1. Inoculating Flask.

If the inoculating is done thoroughly as soon as the cabinet has cooled after the last heating, within four days the whole surface of the rice should be covered with a white felt like growth which will assume an olive green colour, due to the formation of spores, after 8 to 12 days. Spore

production will be at its maximum (in tropical temperature) within 20 days after inoculation. The cabinet is then ready for opening. (Plate I, fig. 2.)

## Taking off the Spores.

The spores may be taken off from the rice as soon as the cabinet is open, but it is better to let them dry for a day or two *in situ* leaving the door open. As the shelves are taken out from the cabinets they should be dusted over with cassava starch or flour, the former preferably, and then the whole mixture is gradually brushed off the tray. A white wash brush with the bristles cut to about half length is very suitable for this purpose. The rice is then sifted out from the starch and spore mixture. Mr. C. Connell has devised a convenient apparatus for taking off the spores. (Plate II, fig. 1.)

A cabinet with ten shelves about three feet square well covered with the fungus, should give at least 70 pounds of the starch and spore mixture, while there should be about 1/3 of a barrel of rice.

## Applying the Fungus in the Field.

Many different ways of using the fungus in the field have been tried and two proved successful during the latter part of the past year in starting epidemics of the disease. The first may be called hand infection and works well where the infested area is more or less limited in extent. A number of boys with tubes containing spores walk through the cane field and wherever they see a froghopper resting on a leaf they catch him in the tube and let him jump out again. Ten or twelve boys can cover a fairly large area in a day. The infected insects soon die and within ten days the fungus is fruiting on their bodies, so that they in turn, become sources of infection for others. By this method the fungus was well established in fields at Forres Park Estate.

Spreading the spores with dusting machines is the method which has given most success in the treatment of larger areas. A machine of the "Cyclone" type made by the Kansas City Dust Sprayer Company, may be mounted on a cart and driven along the traces and the spore mixture blown out over the canes, or the "Furet" duster (Figure 2) may be used, the men walking between the cane rows. (Plate II, fig. 2.) The spore and starch mixture should be applied at the rate of about three pounds per acre.

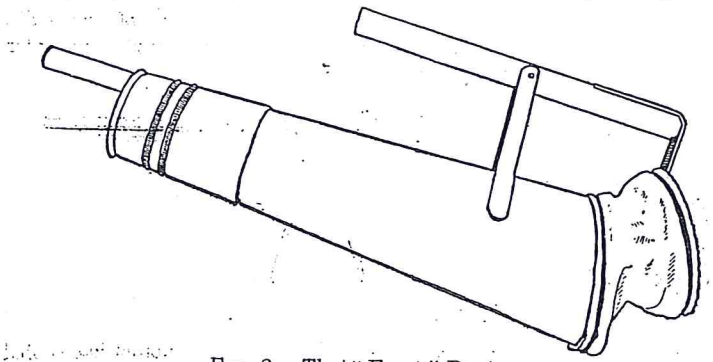

FIG. 2. The "Furet" Duster.

The residual rice is best used by scattering it about the ground around the cane stools.



Another method of using the fungus which was suggested by the writer in 1910 and later recommended by Gough should be given a thorough trial. This consists of catching the insects with lights, inoculating them with the fungus and letting them go. This method was tried in 1911, but had to be abandoned on account of the lack of spores in sufficiently large quantities.

## Impurities in Cabinets.

If cabinets are properly built, thoroughly sterilized and carefully inoculated they should always yield practically pure cultures of the green muscardine fungus. Whether or not the cabinet is going to give a good culture can generally be ascertained on the third or fourth day after inoculation by an examination of the trays through the inoculation tubes, or through the panels in the door if plate glass has been used. If the culture is pure there will be no spores produced by this time but the whole surface of the rice will be covered with a compact white growth. There should be no patches of colour either pink, black, yellow or green, and no fluffy white cotton like masses.

The fungus which is the commonest impurity in the cabinets is a species of Aspergillus. At first sight this fungus is sometimes mistaken for the froghopper fungus but can be readily distinguished from it by the fact that it produces its spores within a week, the spore masses are light yellowish at first and then become brownish green and are borne in round heads at the ends of white stalks. Microscopically they are spherical in shape, while the muscardine spores are cylindrical.

Other common impurities are species of mucors. These can always easily be distinguished for within two or three days they will completely cover the rice with a fluffy white growth two or three inches thick.

Cephalosporium is another rapidly growing fungus which is sometimes met with. It is fluffy somewhat like the mucor but is pink in colour.

Sterigmatocystis forms round black patches on the rice.

Penicillium, the common bluish green mould is sometimes met with in cabinets but not so often as one might suppose. It can be distinguished by its colour and the rapidity with which it produces spores.

The froghopper fungus itself at times may be mistaken for an impurity. For some unknown reason, occasionally it will not produce spores within the given time. The white felt like growth will gradually become greenish yellow and give rise to a great number of small white globular knobs all over the surface. Eventually a small amount of spores will be produced.

## Cabinet Hints.

Be sure that the outer ends of the galvanized tubes through which the inoculations are made are perfectly round so that the corks fit tight.

Always draw off the condensation water from steam pipes and cabinets after each sterilization but do not let the cocks remain open as ants may get into the cabinet in this way.

Be sure that steam valves can be closed absolutely tight, otherwise it is better to disconnect.

Tighten the bolts of the door evenly so that there will be no strain on the glass panels.

Use clean water for washing and cooking the rice.

Wait until water is boiling before putting in the rice to cook.

Use a kettle of large diameter rather than a kerosine tin.

In sterilizing be sure that a boiling water temperature is maintained for an hour.

Inoculate cabinets as soon as they have cooled down after the last sterilization.

Use enough spores for each shelf but don't overdo it.

*Don't examine the cabinets too often, for each time a cork is taken out, especially during the first week there is danger of impurities getting a foothold. Examine two or three shelves on the fourth or fifth day and if they are all right don't remove any corks until the fourteenth or fifteenth day when it is almost time to open the cabinet.*

## Description of Plate I.

Fig. 1.—Culture cabinet with door removed. This is one of a series of 6 cabinets installed at the Usine Ste. Madeleine. The small tubes through which the cabinet is inoculated are well shown.

Fig. 2.—Open cabinet showing growth of fungus. This is the first cabinet which was built on a sugar estate in Trinidad and is now one of 6 in use on Esperanza estate. The steam pipes are on the sides toward the back of the cabinet. The rice is well covered with a pure growth of the fungus.



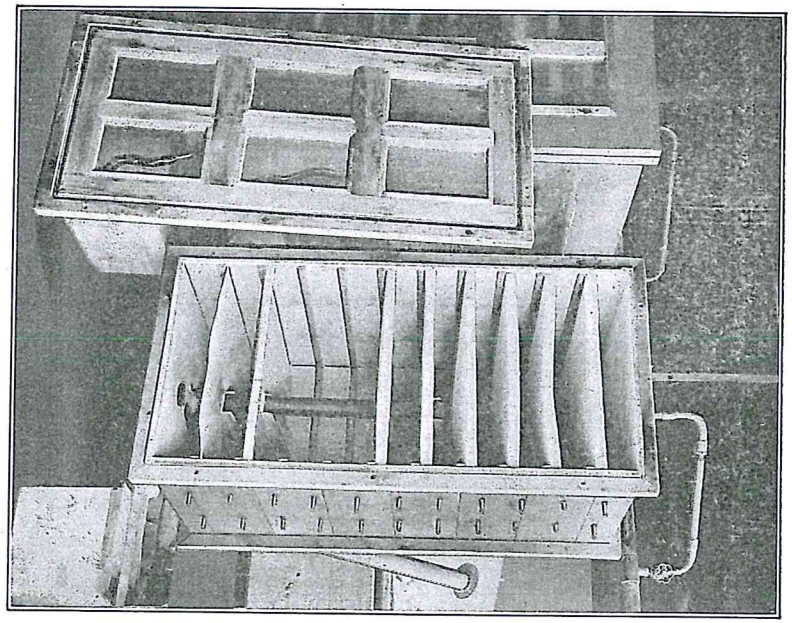

FIG. 1. Culture cabinet with door removed

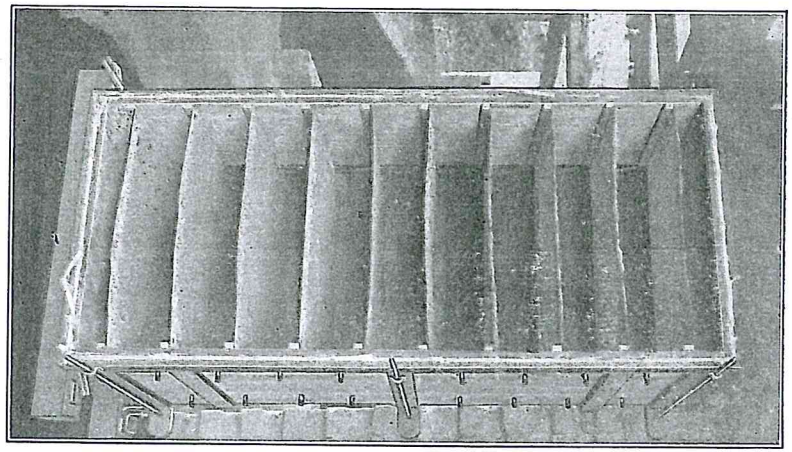

FIG. 2. Open cabinet showing growth of fungus.

## Description of Plate II.

Fig. 1.—Brushing spores from the rice. This method of separating the spores from the rice was devised by Mr. C. Connell of Esperanza estate. Cassava starch is dusted over the culture and the whole is brushed gradually from the tray, which is held in a slanting position, into the rectangular screen, which fills the top of a box.

Fig. 2.—Applying spores to sugar cane with the "Furét" duster. The "Furet" duster is of French manufacture and is well adapted for use in cane fields. The receptacle should be filled about ⅓ full with the spore mixture which may be then blown out in a cloud by a quick movement of the wrist. The apparatus should be held at the angle shown in the picture.



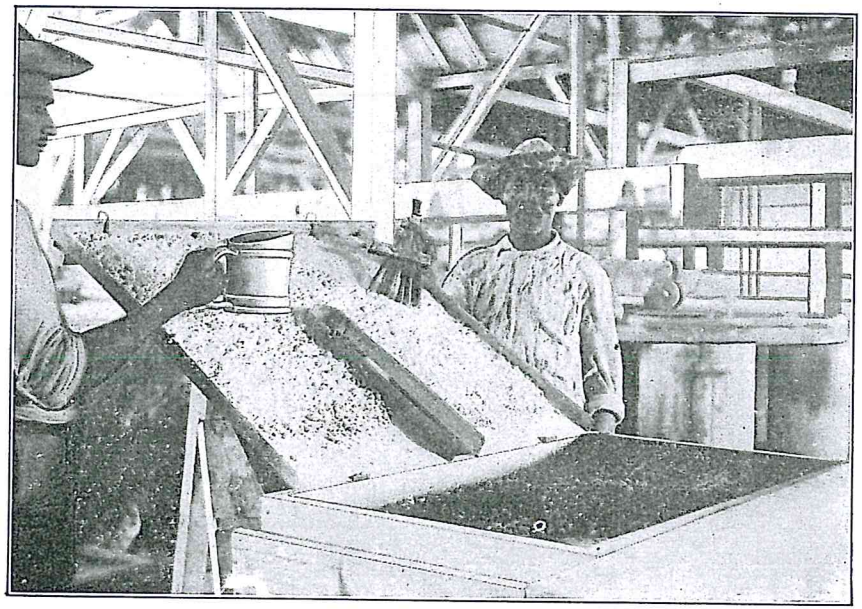

FIG. 1. Brushing spores from the rice.