QUEENSLAND.

BUREAU OF SUGAR EXPERIMENT STATIONS.

DIVISION OF ENTOMOLOGY.
BULLETIN No. 12.

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Bureau of Sugar Experiment Stations,
Brisbane, 1st May, 1921.

The Under Secretary,
Department of Agriculture and Stock, Brisbane.

Sir,—I have the honour to submit for publication as Bulletin No. 12 of the Division of Entomology of the Bureau of Sugar Experiment Stations "A Study of Natural Methods of Control for White Grubs," by Dr. J. F. Illingworth.

I have, &c.,
H. T. EASTERBY, General Superintendent.

Approved: E. G. E. Scriven, Under Secretary.

A Study of Natural Methods of Control for White Grubs.

By J. F. Illingworth.

Disease organisms in the soil have long been recognised as useful allies in the control of white grubs. Hence, I was gratified, soon after my arrival in Queensland, to find that approximately one-third of the grubs on the Greenhills Estate had recently succumbed to Muscardine fungus. Naturally this discovery led to further investigations, especially as to the cond.tions under which these friendly agents became effective. Later, artificial propagation of the disease was undertaken with some success; but this discontinued in 1920, when I found that the peculiar climatic conditions had brought about an epidemic wherever spores of the disease occurred naturally in grubby soils.

The year 1920 was abnormal in many ways. First of all the rainy season was very late in starting, so that it was the middle of January before the first beetles emerged. The delay did not curtail their numbers, however, for they came out in swarms, and, furthermore, when it became time to oviposit, they were exceedingly erratic in their flight, especially on the Greenhills Estate, where they extended far into fields which had been regularly immune. As a result of this peculiar distribution of the grubs, I have been able to work out some important facts as to the normal location of disease organisms in the soil there.

The Greenhills Estate

The Greenhills Estate has about 1,000 acres under cultivation. Nearly half of this area—the portion with timber to the north and east—has suffered for a number of years from excessive attacks of grubs. The soil is of a uniform chocolate colour, of volcanic origin and great depth. It is excellent ground to work, mellow as a garden, and so well drained that it can be cultivated even during the rainy season. Splendid crops can be grown without manures; yet the soil is very deficient in humus, a fact which probably explains the tremendous destruction to the crops from white grubs.

The shaded area on the plan of the estate (Fig. 1) shows the distribution of these pests in normal years, and it will be noted that there appears to be a definite relation to the prevailing winds, as indicated by the arrows. This sketch, too, is especially interesting since it appears to demonstrate that the beetles do not usually fly much over half a mile from the feeding trees to oviposit. Thus it will be observed that the inner limits of the shadow conform fairly well to the contour of the cultivated area on the infested side of the estate, and, furthermore, that there is no destruction in those fields that lie along the south-western portion, even where feeding trees are adjoining. Another interesting feature is the unshaded area about the quarters, adjoining the horse paddock. I have called attention to this area in my reports, because it was most remarkable to find such a spot without grubs right in the midst of the worst-infested fields. I finally came to the conclusion that this immunity was due to the beetles being destroyed by the hundreds of fowls, especially during the morning flight, when the gravid female beetles rest upon the cane leaves for a considerable period before descending to lay their eggs, at the roots of the plants. This hypothesis would appear to be correct for just before the 1920 flight of the beetles all the fowls had been disposed of, hence the beetles escaped and, naturally, the grubs covered this former immune area. Furthermore, as indicated by the small circles, we were unable to find any disease organisms there, as was the case in other unshaded areas shown by the sketch.

In the early days this land was used for growing bananas; and I have learned that when planted to sugar-cane, in 1904, there was no noticeable injury from grubs. The 1905 crop was certainly a tremendous one, for it ran well over 30,000 tons. The third year, however, grubs began to give trouble, and there has been no material let-up ever since, the last year being the worst of the lot, with a harvest of only 2,400 tons. The shortness of this latter crop was due to the abnormal distribution of these pests, for many of the beetles flew far past their old limits in the shaded area, to deposit their eggs in the normally immune portion of the estate, indicated on the sketch by stippling.

It is possible that this erratic flight of the beetles was largely due to the terrific cyclone, which occurred on the night of 2nd February, just about a fortnight after the main emergence of the beetles, hence just at the time when they were ready to oviposit. Naturally, this gale would carry them far past their normal locations: at any rate, after studying the problem from every angle, it is the only explanation that suggests itself to me, and it appears to fit the case perfectly.

A Study of Conditions under which the Muscardine Fungus is Effective.

On 10th August, 1917, we started experiments in the laboratory to determine the relation of temperature and moisture to mortality from the

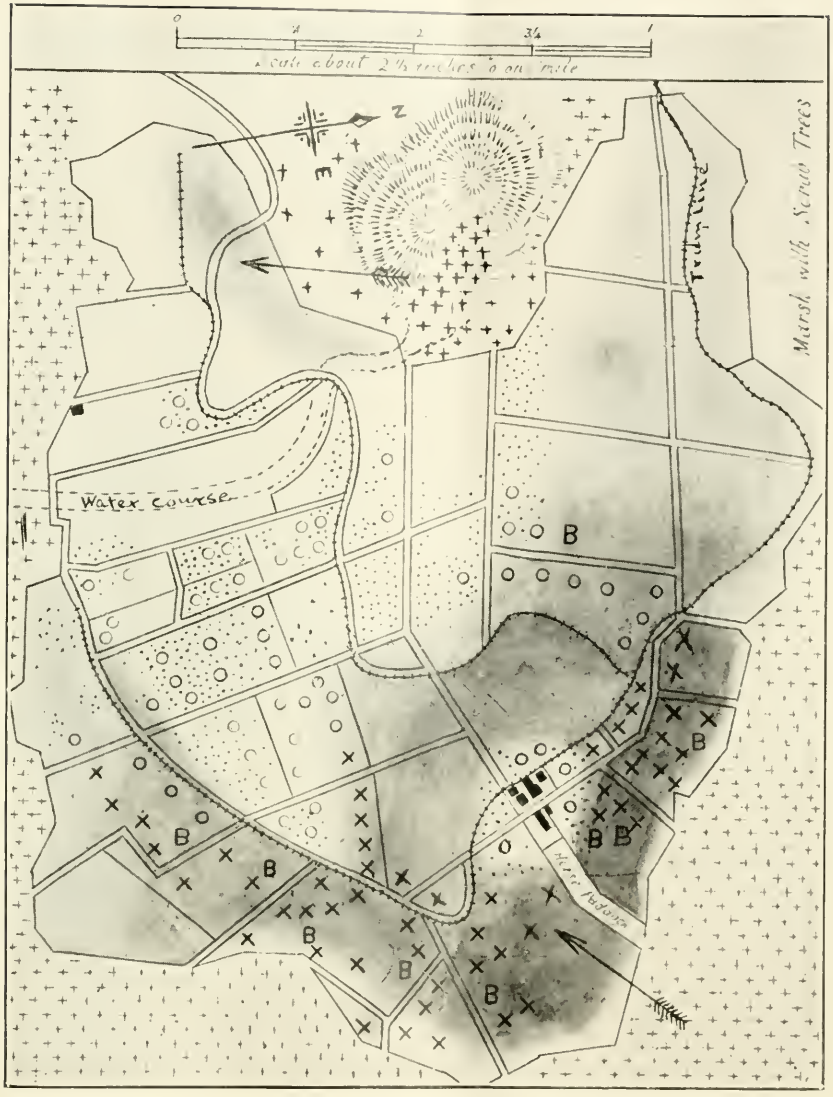

FIG. 1.—SKETCH OF THE GREENHILLS ESTATE.

Arrows indicate direction of prevailing winds. Shaded areas regularly injured by grubs. White immune. Stippling indicates additional areas injured, following abnormal flight of the beetles in 1920.

+ + Feeding trees of the beetles.
= = Roads and headlands.
× Muscardine fungus destroying grubs.
B Bacterial disease destroying grubs.
O None of the grubs diseased.

Muscardine fungus. In these tests we used second-stage grubs of *Lepidiota frenchi*. Twelve pots were prepared with sifted red volcanic soil, and six larvæ were placed in each.

Nos. 1 and 2, infected by mixing spores uniformly with the soil.

No. 3, check for Nos. 1 and 2.

Nos. 4 and 5, same as Nos. 1 and 2, but considerably damper.

No. 6, check for Nos. 4 and 5.

These six pots were kept as cool as possible on a shady verandah which was open to the trade wind.

Nos. 7 and 8, had the same infection as Nos. 1 and 2.

No. 9, check for Nos. 7 and 8.

Nos. 10 and 11, same treatment as Nos. 7 and 8, but considerably damper.

No. 12, check for Nos. 10 and 11.

Pots Nos. 7 to 12 were put in a warm, sunny room, which averaged 5° Fahr. higher temperature than that recorded on the verandah.

The pots were examined and the results noted as follows :—

17th August—Pot No. 5 had two grubs rotting, apparently due to mechanical injury. There was no sign of fungus in any of the pots.

24th August—Pot No. 1, two grubs dead, apparently killed by mates, though there was fungus showing on one of them. Pot No. 2 had a grub covered with ripe fungus spores. Pot No. 10 had one grub dead and rotten, but no signs of fungus. Water was added to pots Nos. 4 and 5, as the soil had dried out more quickly on the verandah than inside.

29th August—Pot No. 3 (check), one grub had been killed by its mates ; no sign of fungus. Pot No. 6 (check), three grubs rotten ; no sign of fungus. Pot No. 9 (check), two grubs were dead ; both showed fungus, though one had evidently been mangled by its mates. Pot No. 12 (check), one grub had been killed by its mates.

13th September—Pot No. 1, two grubs on top of soil covered with fungus. Pot No. 2 had three more grubs dead and covered with fungus spores, though one appeared to have been mangled by its mates. Pot No. 6 (check), two more grubs killed by biting, but no sign of fungus. Pot No. 9 (check), two grubs dead, one killed by fungus and the other by biting.

22nd September—Pot No. 2, the last two grubs dead from fungus ; the soil nearly dry. Pot No. 3 (check), soil very dry ; one grub dead ; and dry on top ; supplied with water. Pot No. 4, soil very dry ; five grubs dead from fungus ; supplied with water. Pot No. 6, one rotten

grub present; no sign of fungus. Pot No. 10, soil waterlogged; four grubs dead and rotten as though killed by excessive moisture. Pot No. 12 (check), one dead from biting.

1st October—Pot No. 4, the last grub dead; no fungus showing. Pot No. 7, two grubs have been killed by biting, but there is no sign of fungus. Pot No. 11, one grub has been killed by biting, but no sign of fungus.

At this stage the experiment was discontinued. It will be noted that the disease was not very virulent, probably because the temperature in each case was too high. This conclusion is borne out by the fact that practically all of the grubs that succumbed to the disease were in the cooler temperature of the verandah. The three deaths from fungus in the checks was evidently due to a lack of care when examining the pots, for it would be a simple matter to transfer the spores on one's hands.

Artificial Propagation of the Fungus.

Since the Muscardine fungus was again in evidence at Greenhills, and I had had reports of its activities in other districts, I decided, toward the end of 1918, to try propagation of the spores artificially. Upon presenting the matter to the Cairns Canegrowers' Association, the plan was heartily endorsed and funds supplied for the construction of a large cabinet, built on the lines of those used so successfully in Trinidad.* Ours was built of hardwood, hence very strong to withstand the pressure of steam for sterilizing. This metal-lined box was 6 feet high, with ten shelves made of narrowly-corrugated iron 2 feet 9 inches square. (Fig. 2.) The food for the growth of the spores was deposited on these shelves and subjected to sterilization by steam on three successive days before inoculation with spores from a pure culture, which had been previously prepared from grubs that had succumbed to the disease.

The first trial, using cooked rice as a substratum, placed about half an inch thick on the shelves, did not prove satisfactory for some reason. At any rate, impurities got in to such an extent that the Muscardine growth was practically eclipsed by other moulds.

I experimented with various other starchy substances, since the price of rice was almost prohibitive. Corn-meal had been used in this sort of work in Illinois,† and I found that it did even better than rice, when finely ground, for the mat of spores on this substance was perfect. Furthermore, I had almost equal success with sliced sweet potatoes, which were placed in the cabinet raw—the sterilising steam being sufficient to cook them.

Under laboratory conditions here, during the summer season, the fungus begins to show as compact white spots one day after planting, and signs of the green spores are noticeable in the centres on the second day; within a week the whole substance is a compact mass of the green fruiting.

* Board of Agric., Trinidad, Circ. No. 8.

† Ill. Agric. Exp. Sta., Bul. No. 38.

In our most comprehensive experiment, 10th February, 1920, the spore-laden material from the cabinet was applied in a field at Greenhills by placing it in drills made with a pony-plough close alongside the stools of cane. This was just about the time the young grubs were hatching.

On 27th February, 1920, a further trial was made by using a quantity of this pure culture material under individual stools, in a grubby field at Meringa.

A. After digging the soil away from the stool to a depth of about 6 inches, we replaced fourteen first-stage grubs of *Lepidoderma albohirtum* in the bottom: after covering slightly, a small quantity of the spore-dust was placed on the soil and covered.

B. This second stool was prepared in the same way, except that the spores were dusted directly on to seven first-stage grubs of *L. albohirtum* before covering the soil.

C. Six third-stage grubs of *Lepidiota frenchi* were dusted with the spores before covering in with soil.

D. Twelve third-stage grubs of the same species were thoroughly dusted with the spores in this fourth stool, before covering.

16th March, 1920—All the above stools were dug out and the soil carefully searched for evidence of the action of the fungus on the grubs. In each case we dug a considerable area about the stool, trying to locate the grubs, which had largely disappeared. The results follow:—

A. We found six *L. albohirtum* grubs, four in the first stage and two in the second. There were no indications to show what had become of the others, and no sign of the fungus.

B. None of the grubs could be located. The drier portions of the old stool were somewhat mouldy, as if affected by the fungus.

C. None of the grubs could be located.

D. There was an ants' nest (*Pheidole megacephala*) in the soil near the stool. One grub was found dead and eaten out by these ants, a fact which may be suggestive as to the disappearance of those in the other stools. Two other living healthy third-stage grubs were all that remained of the twelve that we had placed there.

These results were most discouraging, for I did not find a single grub showing signs that it had been killed by the fungus. It was rather strange, however, that the grubs disappeared as they did. Of course, they may have travelled away to other stools, though this does not appear to be their habit, especially when the soil is in such a dry condition. It is just possible, however, that the ants got them as fast as they became diseased, and removed their bodies. At any rate, these results were far from satisfactory.

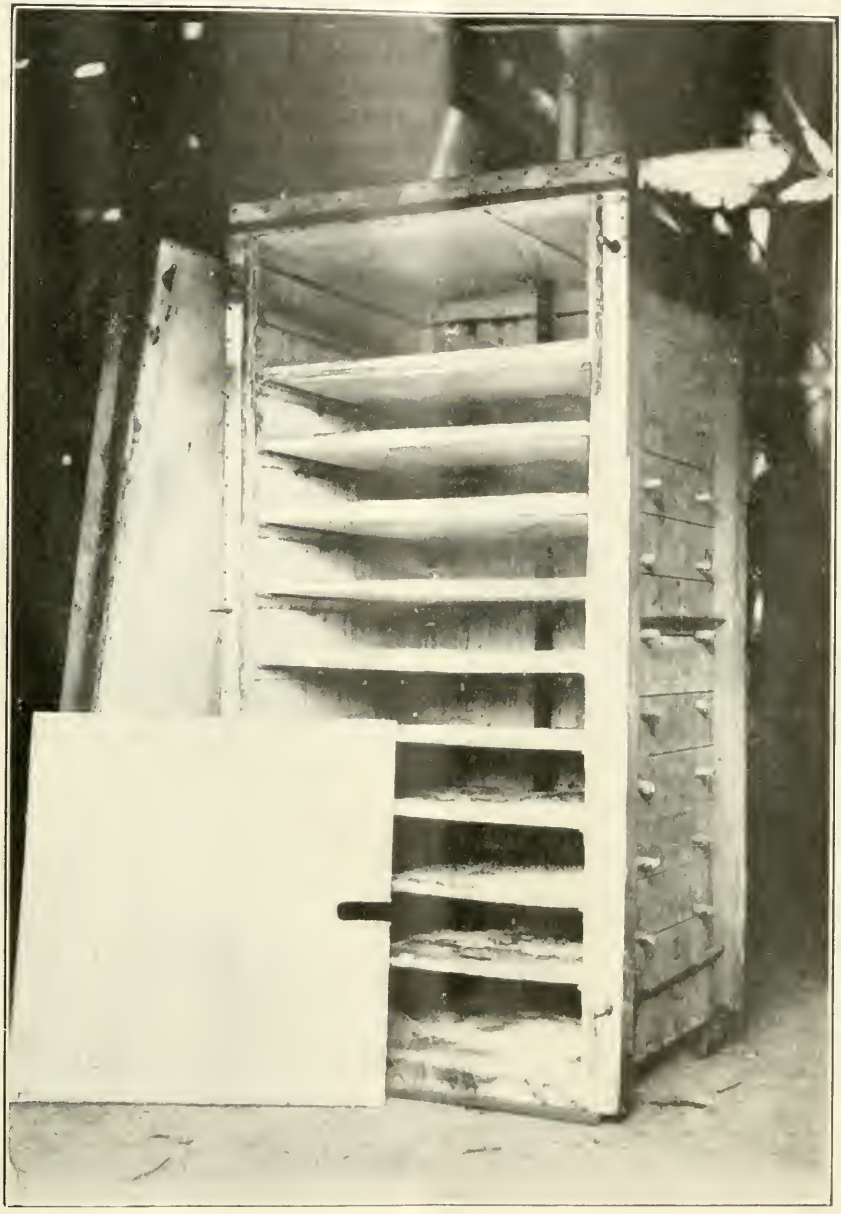

FIG. 2.—Cabinet used for breeding the Muscardine fungus in the Mulgrave Central Mill, Gordonvale. The heavy door stands at the left, and one of the corrugated iron shelves in the foreground. The twenty small tubes through which the cabinet is inoculated are shown on the right side.

Unfortunately, results from the Greenhills fungus plot were also inconclusive. This was largely due to the fact that during the 1920 season this disease became a real epidemic extending practically throughout all of the fields covered by the shaded area in the sketch. The cane treated, in a grubby field just north of the quarters (the cluster of black rectangles near the horse paddock), gave no greater percentage of mortality than any of the adjoining untreated blocks; hence I could draw no conclusions in this particular experiment.

Epidemic at Greenhills during 1920.

Since the grubs did not hatch until fully two months past their regular season, they were compelled to continue their activities among the cane roots well into the winter, before descending into the lower levels of the soil to hibernate. This unusual procedure was their undoing; for, wherever there were fungus or bacterial spores in the soil, fully 98 per cent. of the grubs succumbed to these diseases before they were ready to pupate. This fortunate devastation in their ranks resulted because they were subjected to a continuation of a peculiar season. Unusual rains occurring during the cooler months of May, June, and July developed ideal conditions both for the activities of the fungus and other death-dealing organisms, such as bacteria, &c.

Even as early as March I began to find grubs that had been killed by the fungus, and by the middle of June there was a real epidemic, for I then found 37½ per cent. of them succumbing to it. This was the greatest mortality that I had ever observed, up to that time.

Nevertheless, by 1st July I found this average greatly increased. The first stool that I examined in the field just north of the quarters had twenty-six grubs, all third stage *L. albohirtum*; eighteen of them were already dead and mouldy with the Muscardine fungus, *i.e.* 70 per cent. mortality among the number located; and undoubtedly many had already disintegrated, for these stools formerly had an average of 100 grubs or more per stool a short time before—in fact, in one case, I found 134.

About 9th July weather conditions became rather dry, and there was a consequent decrease in the death rate. Then, too, those that had been dead for a short time were rapidly breaking up into humus, so that they were very hard to locate when digging. Nevertheless, there was a marked decrease in the number of grubs in the soil on every side. At first I supposed that they might have gone down to hibernate, but digging failed to disclose any of them deeper than 12 inches. Where, a short time before, the grubs had been exceedingly numerous, going well over 100 per stool, it suddenly became difficult to find more than about four. For example, a typical stool gave—2 to 6 inches deep, two alive and one dead by fungus; 6 to 12 inches deep, one alive, one dead by fungus, one sick with black spots on skin indicating a bacterial disease, and one dead, spotted and very soft—undoubtedly due to bacterial disease. No grubs were found deeper, though we excavated to a depth of over

3 feet. The soil was rather dry and crumbly, so that the dead grubs were easily broken up and difficult to discover—only the chitinous head-shield remaining for a time after the decomposition of the soft body.

Furthermore, experiment demonstrated that the diseased grubs often came to the surface before dying, and, hence, were easily removed by predators—ants, ground beetles, mammals, and birds. On one occasion I watched a flock of fully 500 ibises assiduously probing about the grubby stools ; and in almost every case the soil was dug up at the roots by the omnivorous bandicoot in his search for the grubs. With all these grub-destroyers at work it is not hard to understand the rapid disappearance of the pest.

Though I had often found grubs destroyed by the Muscardine fungus at Greenhills and elsewhere, as noted above, I had never observed such remarkable mortality. Under normal conditions they go down to hibernate in March or April, before the cool weather sets in ; and they appear to be fairly immune to diseases when they are thus located deep in the moist subsoil. In our experiments it was noted that mortality increased with the lowering of the temperature, and was also stimulated by the addition of moisture ; hence, the heavy death-rate in the field, with this combination of factors, is just what we might expect.

During the time that the Muscardine fungus was most virulent, following the chilly rains of early July, it was very conspicuous in the soil. The greenish-white mycelium extended out on every side of the grub for 2 inches or more. In some cases this mycelium had attached itself to the underground portions of the cane, wherever the diseased grub had been in contact ; in fact, we often discovered the disintegrated grub by seeing the grey-green spores or mycelium on some portion of the root system.

By the middle of July the percentage of dead grubs had greatly increased. The ibises were in the infested fields in hundreds, gathering up the sick larvae within reach of their long beaks. In digging we found many stools in which every grub had succumbed (100 per cent.).

Following on this important evidence, I started a careful survey of all the infested fields at Greenhills, to earn if this valuable disease was distributed throughout the plantation. This was done by digging out numerous stools in each of the infested fields, the result being indicated on the plan of the estate by an X for fungus and 0 for none. By this method I developed an interesting discovery—the fungus appeared to be well distributed in all of the areas regularly attacked by grubs (*i.e.*, those deeply shaded on the sketch), but we were not able to find it outside of this well-defined region, especially where the pest in its erratic flight had caused the devastation of fields that had usually been immune (*i.e.*, the portions stippled on the sketch).

Apparently, the disease spores persist in the soi where they have once been introduced, and continue from year to year, ready to bring about an epidemic when conditions are favourable. Therefore, since we

have learned that the mortality is increased by lowered temperature and abundant moisture, it would appear that we might easily add to the destruction in the field by irrigation, especially after the cool weather sets in, which is normally the dry season.

During the survey we also found many grubs that had died of some bacterial disease, which occurred in the same areas with the fungus. The locations of this second disease were indicated with a B on the sketch of the estate. Many of these dead grubs were also found on the surface. By digging under such stools specimens were found presenting all stages of the disease. This infection usually appears at one of the spiracles, or in the membrane between the segments of one or more of the legs; in the latter case, the affected appendage soon drops off and the disease rapidly progresses upward into the body. The affected parts have a peculiar shiny black appearance, which coincides with that described by Zae Northrup* who gave the name *Micrococcus nigrofaciens* to the organism causing the disease; this friendly organism was found to be well distributed in the United States. Finally, at death the body becomes very soft and black all over (Fig. 3), totally different in appearance from specimens that have died from the fungus (Fig. 4), the latter being hard and cheesy, with a soft grey-green covering of spores.

Naturally, such macerated specimens quickly decompose in the soil, and it is almost impossible to find them after a few days. This fact helps to account for the way that the grubs so suddenly disappear, as if by magic in some instances, when climatic conditions are just right. The remarkable disappearance of the grubs at Fairymead, in 1909, is a case in point. I have discussed this matter with Mr. Howe, Manager of the Mulgrave Central Mill, who was located at the Lynwood Estate at that time, and who made a careful study of the mortality of the grubs.† From all that I can learn, the mites which appeared in such numbers on the sick grubs were only an after effect, the real cause of death being apparently due to a bacterial disease similar to the above. Mr. Howe also informed me that that district had since been free from grubs for years, but that they had just begun to give trouble again.

Due to favourable rains, these diseases continued active in the field right through July, and even on 3rd August we found considerable evidence of them at Greenhills. Digging near the quarters, in one of the worst-infested locations, we found the following :—

12 inches deep—One *L. frenchi*, stage III.; two *L. frenchi*, stage II.; one *L. albohirtum*, stage III., killed by fungus.

18 inches deep—One *Anomala*, stage III., in cell; one *L. albohirtum* pupa in cell; one *L. frenchi*, stage III., in cell.

24 inches deep—Three *Anomala*, stage III., in cells; one *L. frenchi*, stage III., in cell.

* Mich. Tech. Bul. No. 18.

† Aust. Sugar Journ., I., 65.

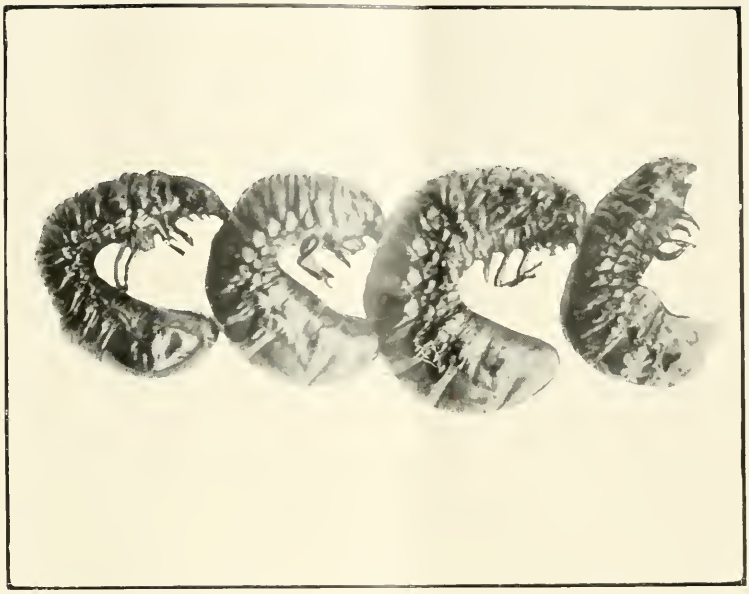

FIG. 3.—Full-grown grubs of *Lepidoderma albohirtum* killed by the bacterial disease. Note the shiny black skin and the stumps of the appendages that have dropped off.

FIG. 4.—Grubs of all three stages of *Lepidoderma albohirtum* that have succumbed to the Muscardine fungus. Nat. size.

We dug over 3 feet deep without finding any more.

A trench with five stools gave the following :—

6 inches deep—Ten *L. albohirtum*, stage III., eight of them killed by fungus ; one *Anoplognathus*, stage III. ; one *L. frenchi*, stage III.

12 inches deep—Eight *L. albohirtum*, stage III., three of them killed by fungus, and two killed by bacteria ; one *L. albohirtum* pupa ; four *Anomala*, stage III., in cells ; one *L. frenchi*, stage III. ; one *Campsomeris* wasp cocoon.

18 inches deep—Two *L. albohirtum*, stage III., one of them killed by fungus ; six *Anomala*, stage III., in cells ; one *L. frenchi*, stage III.

24 inches deep—One *L. albohirtum*, stage III., pupating ; two *Campsomeris* wasp cocoons.

30 inches deep—One *L. albohirtum*, stage III., killed by fungus ; one *Campsomeris* wasp cocoon.

36 inches deep—One *L. albohirtum* pupa ; one *L. albohirtum* beetle (1919) dead in cell.

This gives for the remaining grubs of *L. albohirtum* located in this excavation : 20 per cent. killed by *Campsomeris* wasps, 40 per cent. killed by fungus, and 5 per cent. killed by bacteria, hence, a total mortality of 65 per cent. ; of the remainder, 15 per cent. were pupating and 20 per cent. were apparently healthy grubs. As indicated above, these figures do not show the total mortality due to the diseases, because most of the deceased grubs had disintegrated before the excavation was made. The true percentage of mortality can only be determined by comparing the remaining living grubs and pupæ with the average number that were there before the epidemic commenced. Since the original infestation was well over 100 per stool, and at the time of this excavation it was reduced to less than two per stool, we must conclude that fully 98 per cent. had succumbed to these various death-factors.

Further Experiments with these Diseases in the Laboratory.

In order to note more closely the conditions under which these diseases were effective, I started a number of experiments in pots of soil in the laboratory. By this means our field observations were thoroughly confirmed.

First Experiment.—On 10th July, 1920, three pots were prepa ed with Greenhills soil, and three healthy grubs were placed in each. Pots Nos. 1 and 2 were each inoculated with a grub that had succumbed to the bacterial disease, the bodies being soft and black. Pot No. 3, no inoculation, except what might occur naturally in the soil.

13th July an examination was made, finding—

Pot No. 1—Two grubs dead and black, one alive.

Pot No. 2—One grub dead and black, two alive.

Pot No. 3—One grub dead and black, two alive.

Note—In two cases the dead grubs were on top of the soil, just as I had observed in the field.

17th July—
Pot No. 1—No change.
Pot No. 2—One grub dead and black, one alive.
Pot No. 3—One grub dead from fungus, the other alive but infected with black spot on thorax.

21st July—
Pot No. 1—No change.
Pot No. 2—No change.
Pot No. 3—No change.

31st July—
Pot No. 1—Grub dead and black.
Pot No. 2—No change.
Pot No. 3—No change.

2nd August—
Pot No. 2—Grub dead from fungus.
Pot No. 3—Grub dead from fungus.

Second Experiment.—On 10th July, 1920, thirty-five apparently healthy stage III. grubs of *L. albohirtum* were collected with a quantity of soil at Greenhills and placed in a large pot.

14th July—Examined these, and found eleven grubs dead and black from the bacterial disease several being on the surface; three more were badly infected on the legs and around the thoracic spiracles; also two grubs had succumbed to the fungus.

Note—This is 38 per cent. dead from the bacterial disease in three days, under moist, cool conditions.

17th July—Of the twenty-two grubs alive on the 14th, four had succumbed to the bacteria and four to the fungus, leaving fourteen alive, but two of these had bacterial infection on the front legs, one being somewhat blackened on the body near the infected leg.

21st July—Four more had died of the bacterial disease and six of the fungus; of the four remaining alive, two had bacterial infection on the legs.

31st July—The last grubs were dead, one from the bacterial disease and three from the fungus.

Third Experiment.—14th July, 1920, twenty-four pots were prepared with soil from a Meringa field, where no disease organisms were known to occur. Each pot was supplied with a healthy stage III. grub of *L. albohirtum*. Pots Nos. 1 to 18 were then infected by placing a grub which had succumbed to the bacterial disease in each. Pots Nos. 19 to 24 were checks.

17th July—All the pots were examined, but there was no change.

22nd July—In No. 10, the grub had the front and middle legs black at the tip. No. 12, the grub had the left hind leg dead, the black starting on the body. No. 13, the grub was dead, blackened around the spiracles.

25th July—In No. 12 the grub was almost dead, the infection greatly extended, the spiracles blackened.

27th July—No. 12 was dead, blackened along the line of spiracles.

9th August—No. 18 was dead ; Nos. 3, 7, and 17 were infected.

21st August—No. 4 was infected on the back left leg. Check No. 20 was infected on the right front leg, which had gone black : also Check No. 23, which had the left hind leg black. Nos. 1 and 6 had succumbed to fungus.

Note—This infection in the checks was undoubtedly due to a lack of care in examining the grubs, for it would be an easy matter to infect them from the hands. The pots were in a warm place on a table in the sun, which probably accounts for the low mortality.

25th August—No. 3 was dead of bacteria. No other changes.

30th August—Checks Nos. 20 and 23 were dead of fungus, following the bacterial infection.

4th September—No. 4 was black on the under side of the thorax. Nos. 2, 9, and 10, dead of fungus.

10th September—The weather has been very warm. No. 4 dead of fungus, following the bacterial infection. No. 11 dead of fungus.

17th September—Weather warm. No. 16 dead of bacteria. Check No. 22 pupated. Nos. 7 and 17, no change, except that the infected legs had dropped off.

The experiment was discontinued at this point, since the weather was too warm to get results with the disease. There were three checks and five of the grubs in the infected pots still alive.

Fourth Experiment.—2nd August, 1920, nineteen healthy grubs from Meringa fields were placed in nineteen pots of soil from the infected area at Greenhills. The soil was then made very moist, and covered to prevent evaporation.

5th August—No change ; this lack of mortality is evidently due to the prevailing warm weather, the pots being in the sun.

9th August—Two of the grubs dead of bacteria. The pots were removed to a place under the laboratory, where the temperature was about 10 degrees cooler.

21st August—Five grubs were dead of the fungus, and one of bacteria.

25th August—Four grubs dead of fungus, and one of bacteria.

27th August—Four grubs dead of fungus ; only two left alive.

30th August—The last two grubs were dead of fungus.

Conclusions.—From the above experiments, it was quite evident that low temperature and abundant moisture are most important factors in the virulence of these diseases. The bacterial contagion was far less destructive than Muscardine fungus, under the same conditions. Nevertheless, as noted above, these two diseases often go hand in hand, the grubs dying by a complication of ailments. Often the legs of a grub became blackened, one or more falling off—unmistakable symptoms of the bacterial complaint—then the grub would quickly succumb to the fungus, his body becoming hard and cheesy, and, finally, turning green as the spores developed. The fungus spores are exceedingly small ; even when viewed under a microscope with a magnification of 400 diameters, they appear as exceedingly tiny oval bodies (Fig. 5). Ordinarily,

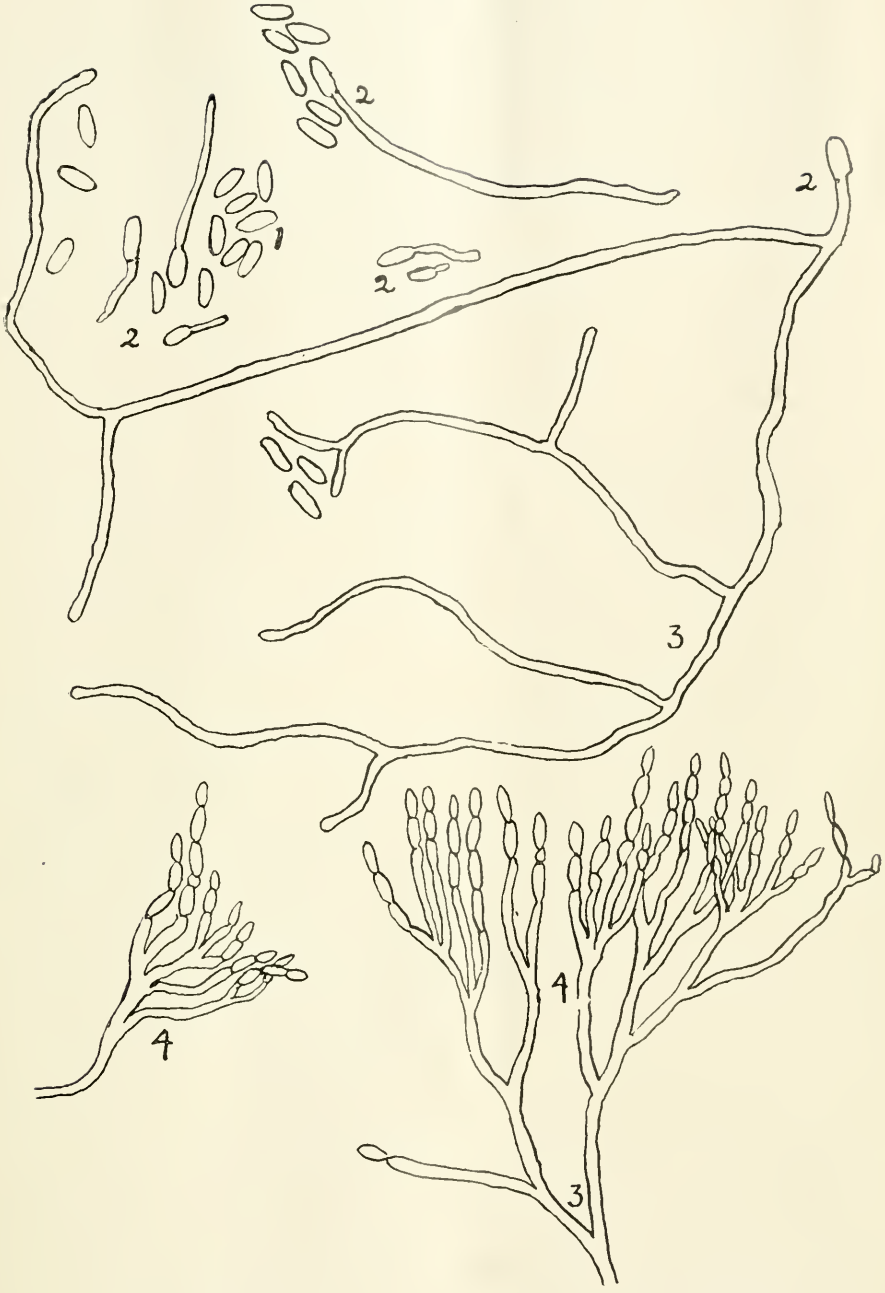

FIG. 5.—Microscopic characters of the Muscardine fungus; magnified about 800 diameters.

1. Mature spores. 2. Spores germinating. 3. Mycelium, the vegetative structure, which finally forms a compact white mat; the ultimate tips on the surface are the sporophores (spore-bearers). 4. Sporophores, showing how the mycelium becomes constricted at the tips like strings of sausages, forming the spores. These drop off as they ripen; the green powder on the dead grub is composed of myriads of them.

unless broken apart, they are attached end to end, like a string of sausages, for these fruiting bodies are formed from the branching tubes of the mycelium (the vegetative part of the mould). Wherever the mycelium comes to the surface of the dead grub, it branches profusely, and each of the tips then becomes constricted into a line of numerous spores. Hence, the numbers produced by a single grub are unthinkable—a tiny speck of this green powder taken on the point of a pin and placed under the microscope is a revelation, for the mass of spores is beyond counting. Therefore, it is easy to understand how the soil becomes thoroughly inoculated with this contagion as it is ploughed and cultivated. Furthermore, probably a single spore is sufficient to bring about the disease, when ingested with the soil by the grub, if the climatic conditions are right.

Therefore, in spite of our apparent lack of success in multiplying the Muscardine fungus artificially, in the cabinet, it would at least appear worth while to attempt to assist nature in the distribution of these friendly allies, especially at the time that dead grubs are so abundant in the field. While the epidemic was on, it was an easy matter to gather up any quantity of the spore-laden soil ; and we distributed this to several other infested localities where the diseases were not known to occur.

Recent observations (January, 1921) at Greenhills demonstrate that the fungus is already becoming virulent again. Many of the newly-hatched grubs have succumbed to it ; and in spite of the rather high temperature, we have found it attacking and destroying the third-stage grubs of *Lepidiota frenchi*. Then, too, the young grubs that we have brought to the insectary, in soil from these infected fields, have died off in great numbers—in some instances the whole lot succumbing after a few days. The extreme virulence is not so hard to understand, for the insectary is shaded and open, hence considerably cooler than the field ; then, too, the pots have a more constant supply of water—the ideal conditions for the activities both of the fungus and other diseases.

Suggestions for making use of these Friendly Allies.

Though there is no hope of producing an annual epidemic, even in the most favoured local ties, I nevertheless regard these parasitic organisms as our most valuable allies in the control of white grubs. I feel certain that they are accountable for the sudden total disappearance of these pests in certain districts ; and they undoubtedly are a most important factor in holding grubs, as well as other insects, in check. It is a fact well recognised that if almost any insect pest were permitted to multiply absolutely without restriction, there would soon be no room for man or other living creatures upon the earth. Since these diseases have such possibilities of assisting us, it certainly behoves everyone with grub-infested land to have his soil inoculated as soon as possible, if, perchance, nature has not already done it for him.

Fortunately, as I have indicated above, these same organisms are widely spread, occuring in most parts of the world ; hence, undoubtedly, they are somewhere in each cane district. Therefore it is simply a matter of help one another. Everyone should be on the lookout for dead grubs, and as soon as they are noticed in a field, the fact should be advertised among the growers of the district. In this way each and every sufferer could use some of the soil to inoculate his fields. Of course, I would not advocate the doing of this work blindly, but undoubtedly there is someone in each district who is familiar with the characteristic appearance of grubs that have succumbed to the Muscardine fungus. Furthermore, the main function of this bulletin is to make this work more sure and easy.

I have not had time to make a general survey for these diseases in the various sugar-growing districts, but the few definite records that I have secured may be of use, so I shall enter them here.

28th May, 1918, I found the Muscardine fungus doing effective work at Fishery Creek, on the farm belonging to the late Mrs. Hobson—fully 30 per cent. of the third-stage *Lepidoderma albohirtum* grubs had been killed.

29th September, 1918, this fungus was found doing effective w rk at Mossman, out at Saltwater, on Donnelly's farm.

12th August, 1920, I found the bacterial disease also at Mossman, on the Mango Park Estate, and also at Saltwater, on the farm belonging to Kadow.

3rd September, 1920, Mr. G. M. Tooth, now farming on the Atherton Tableland, informed me, when I showed him the dead grubs, that both these diseases were very effective on the Herbert River, near Ingham, in 1912. At his place on the Long Pocket line, near the Stone River, he said he ploughed up quantities of both sorts of dead grubs. The land had been planted to beans, and overflowed, shortly before planting. This farm now belongs to an Italian named Menshaca.

7th October, 1920, I learnt that the Muscardine fungus was abundant at Innisfail. Mr. Davis, across the river at Daradgee, reported that he had ploughed up the dead hardened grubs in great numbers on his farm.

Numerous other reports have come to me during the past three years, but in most instances they did not appear to be definite enough to record.

Method of Inoculating the Soil.—I have considered various ways of doing this, the main object being to cover the spores in the soil of grub-infested fields so that they will have an immediate opportunity to multiply and become effective. Since the fungus probably depends upon grubs and other insects for food in the field, I would not advocate the inoculation of land where grubs do not already occur in destructive numbers Observat'ons at Greenhills would indicate that these diseases do not multiply and continue under such conditions. I mention this because some have taken the advice as a cure-all, and wanted spore-infected soil to inoculate their ground, perchance it might some day be troubled with grubs.

Given, however, a grubby field, the method of treating may be with a shovel, taking out a shovelful of earth, so that a pinch of the spore-laden soil may be dropped in and covered. This inoculation should be no more than a chain or so apart in the field, the closer the better, for quick results. An easier method of applying would be when ploughing. By having a tin of the inoculating soil on the plough, a pinch could easily be dropped here and there in the furrow—say, about every tenth row or closer, if the spore-laden soil was easily procurable.

Let me finally advise, however, that immediate results must not be looked for. A few dead grubs may be found from time to time ; but the hope that I am working on is the unusual season, such as that of 1920, when the presence of the spores over most of the infested cane areas would probably do much toward a marked reduction in this terrible pest. Furthermore, since I am compelled shortly to give up the investigation of this tremendously important problem—control of the pests of sugar-cane, due to which the industry loses hundreds of thousands of pounds annually—I wish to recommend for the benefit of those most interested, the further introduction of other similar friendly organisms. I have had considerable correspondence on the subject, and would urge immediate investigation in Java. They have two species of grub-destroying bacteria, which might prove very beneficial here, since our rains come at the time that the grubs are active. At the same time, it would be possible to collect and breed a number of other natural enemies of the grubs, for introduction here. The Philippine Islands are also a rich source of similar organisms, as demonstrated by the Hawaiian Sugar Planters' entomologists. If we consider the economical production of sugar, the introduction of friendly organisms will be pushed as speedily as possible.

By Authority: ANTHONY JAMES CUMMING, Government Printer, Brisbane.