sects and in the suppression of insect populations, one should remember that regardless of man's activities along this line a tremendous toll of insect life is being taken continuously in nature through the pathogenic action of entomogenous micro-organisms. Instances in which nature institutes effective control of an insect species through the agency of disease are common and unceasing. If for no other reason, therefore, it behooves the entomologist and the agriculturist interested in the ecology of insects to be cognizant of those micro-organisms capable of causing disease among these animals and the effect of the diseases on insect populations and activity. Only by including knowledge of this group of enemies of arthropods along with the other more frequently recognized groups can we hope to gain a more complete understanding of insect life.

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The Laboratory of Insect Pathology is a part of the division of biological control in the College of Agriculture at the University of California, and as such it was the first laboratory of its kind to be organized (in 1945) for the purpose of conducting a full teaching and research program in all phases of the subject. The facilities of the laboratory are available to entomologists, farmers, and others interested in having insect specimens diagnosed as to their diseases.

Specimens (subject to quarantine regulations) submitted for diagnoses should be securely packaged, but should not be placed in alcohol or other preservative. They may be sent by fast mail directly to the Laboratory of Insect Pathology, Division of Biological Control, University of California, Berkeley 4, Calif.

Milky Diseases of Beetles

Ira M. Hawley

Grubs of the Japanese beetle live in the ground, where they may be attacked by several milky diseases, of which the type A, caused by *Bacillus popilliae,* is the most widespread and the most important.

That milky disease was first discovered in central New Jersey about 1933 when men who were conducting field surveys found a few abnormally white grubs. On microscopic examination, the late G. F. White, of the Bureau of Entomology and Plant Quarantine, perceived that the blood of the grubs was teeming with bacterial spores. The spores caused the white appearance and led to the designation milky disease for the ailing grubs.

The spores are spindle-shaped bodies about 1/4600 inch long—so small that several billion may exist in one milky grub. When a healthy grub becomes infected with milky disease, the spores give rise to slender vegetative rods, which grow and multiply in the blood by repeated divisions and in a few days develop into the spore form.

As the disease develops, the blood of a sick grub, normally clear, becomes filled with the bacterial forms and milky in appearance. When affected grubs die, the spores, which had filled the body cavity, are left in the soil. They are taken up by other grubs as they feed on the roots of plants, and they in turn become diseased. As the process goes on the number of spores in the soil increases, more and more grubs are killed, and fewer beetles emerge.

In the rod stage the disease organism is comparatively short-lived, but the spores are long-lived. They resist excessive dryness or moisture, cold, and heat.

They may remain alive in the soil for many years.

Many bacterial pathogens—germs that cause disease—of man, animals, and plants can be grown on artificial culture media and thus made to produce in great numbers the organisms that cause diseases. The culture possibilities of *B. popilliae,* which causes the type-A milky disease, have been intensively studied by S. R. Dutky, the bacteriologist of the Moorestown, N. J., laboratory of the Bureau of Entomology and Plant Quarantine, and by workers in State and private laboratories, but they have found no medium in which the rods will develop into spores.

The rods of type-A bacillus, however, will grow on several culture media and great numbers may be obtained. The rods may be transferred from one culture to another, where they will go on producing more. The milky disease may be started in healthy grubs by the injection of the rods into the blood stream with a hypodermic needle. Of the many ingredients for media tested in culture studies, thiamine and tryptophane have been found to be essential for growth and multiplication of the rod form. Other materials help somewhat. However, some element, which is required to bring about the change from rods to spores, has been wanting in all the many culture media we have tested. Work to find a medium in which spores may be obtained has been in progress since 1934.

Early in the study of the type-A milky disease we learned that it occurred largely in a small area where the Japanese beetle had been longest established in this country. In other words, the beetle had spread faster than the disease. We felt certain that the disease would help control the beetle in places where it did not occur if enough spores could be produced.

Because spores could not then (and still cannot) be obtained in artificial culture media, Dr. Dutky developed a new technique for obtaining spores. In the process, the grub itself is used as the culture medium. Thousands of healthy grubs are dug in the field each fall and stored in cold cellars at the laboratory. They are removed as needed, and each grub is inoculated hypodermically with about 1 million type-A spores from milky grubs. The injected grubs are held individually at a temperature of 86° F. in cross-section boxes with soil and with sprouted grass seed as food for 10 to 12 days. In that time, each grub usually contains 2 billion or more spores. The United States letters patent covering the main features of the process were granted to Dr. Dutky, who assigned them to the Secretary of Agriculture.

Occasionally enough milky grubs are found in a field to justify collecting them for processing, but we seldom find a place with enough diseased grubs in the proper condition to pay to dig.

When the disease has developed to just the proper stage, the grubs are screened from the soil, washed to remove dirt particles, and placed in jars of ice water, which are stored in a refrigerator at about 35° F. The cooling quiets the grubs and prevents deterioration. When enough injected grubs have accumulated, the excess water in the jars is poured off and the grubs are ground up in a meat chopper. Samples are taken of the resulting mixture of spores and grub parts and are checked to see how many spores exist in each unit of the mixture. Enough chalk is added to standardize the mixture at 1 billion spores per gram. Then it is passed through a blower to break up the masses of particles. The mixture is dried by a blast of warm air and a filler, usually talc, is added to standardize the powder at 100 million spores per gram, roughly 2.8 billion spores per ounce. The powder is known as spore dust and it is ready for packaging.

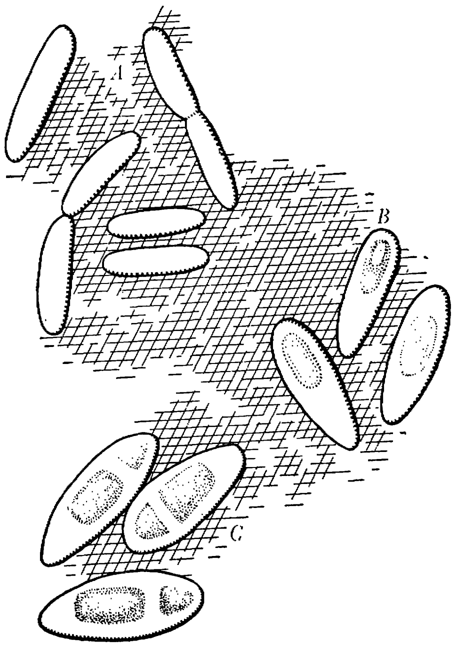
If there were no loss in the making, 23 grubs containing 2 billion spores each would produce 1 pound of spore dust. Spore dust has been held in a dry condition for as long as 10 years without noticeable deterioration and it is always ready to use.

A program to colonize the disease in areas where it did not occur was started in 1939 by workers at the Moorestown laboratory in cooperation with State and Federal agencies. By the close of the 1950 season, more than 166,000 pounds of spore dust had been applied at nearly 122,000 colony sites. Nearly 93,000 acres had been treated in 199 counties in 14 Eastern States and the District of Columbia. At least 15,500 pounds of spore dust were used in treating properties owned or maintained by the Federal Government.

State entomological agencies have sometimes assisted in the production of spore dust by digging and inoculating grubs, which were then shipped under refrigeration to Moorestown, where all the processing into spore dust has been done. The spore dust from the grubs was then returned to the States for distribution. Records of the number of colonized sites in each county were supplied to the Moorestown laboratory. Workers at the University of Maryland have carried on a large-scale program of grub inoculation and distribution of spore dust since 1940.

Because spore dust is difficult to make and costly, it is seldom applied to the soil as a complete coverage. In experimental applications it has been spread evenly over the ground with commercial fertilizer or some other filler and good disease infection has been obtained. There was no evidence that the fertilizer reduced the action of the disease spores. However, spore dust is usually distributed in spots with a modified rotary hand corn planter, which drops about 2 grams of the dust each time it is tripped. In treating a small yard, the powder may be applied in spots with a teaspoon. It is merely placed on top of the ground; the next rain will wash it in.

The amount needed for any area is regulated by the distance between the spots, which are usually 3 to 10 feet apart. When applied with a corn planter at a 3- by 3-foot rate, 20.6 pounds of spore dust will be required to cover an acre. At a 5- by 5-foot interval, 7.0 pounds will be needed. When the spots are 10 by 10 feet, 1.75 pounds are required. The size of the treated tracts has varied greatly. Treatment of at least 2 half-acre plots per square mile is desirable in open agricultural country. In city areas, the treatment of the properties on at least 1 block in 10 is considered good. These rates of treatment are heavier than it has been possible to use in many places. Often the spore dust has been applied in only a few locations where grub counts were highest. In a few places where beetles have been present in destructive numbers, tracts of 100 acres or more have received a complete coverage with the spots close together. That was done to get a quick establishment of the disease.



Milky disease spores. A, vegetative rods; B, sporulating rods; C, mature spores.

In colonizing new areas, places are selected for treatment which have a permanent turf and a high count of grubs. Milky disease is not usually applied to fields that might be plowed or cultivated soon after treatment because the spores are scattered and buried before the disease can become established. Furthermore, the grub count is usually low in such places. The rapidity of disease build-up will depend on the number of grubs per square foot, the closeness of the spots in the treated area, and the number and size of treated tracts. Since grubs become diseased largely by feeding in infected soil, some time must elapse before the disease will spread over an entire field or larger area from the first grubs infected. Milky disease will be spread from those initial points by any natural or artificial movement of topsoil containing spores, by the movement of diseased grubs through the soil, and by birds and animals that feed on diseased grubs. It has been shown experimentally that live spores are present in the droppings of birds that have fed on diseased grubs. Spores of the type-A milky disease can withstand wide ranges of soil temperature and moisture conditions, but may lose some of their infective power after direct exposure to the rays of the sun for several days.

THE UNITED STATES DEPARTMENT OF AGRICULTURE does not have spore dust for distribution to residents of beetle-infested areas, but two companies have been authorized to make it under license from the Secretary of Agriculture.

Their products may be bought at many of the seed and hardware stores throughout the beetle-infested area. Samples of the spore dust made by the companies are tested by the Moorestown laboratory several times each year to see that a proper standard is maintained. Such samples have always been found to be satisfactory. Spore dust made by the companies has been used to treat small yards, estates, golf courses, parks, and similar areas. In some places garden clubs or other civic organizations have stimulated the purchase and distribution of spore dust. At times State and municipal agencies have purchased spore dust and used it to supplement that supplied by the Moorestown laboratory in cooperative programs.

In some counties the county agents and boards of supervisors have bought quantities of spore dust for sale to farmers at a fraction of the usual retail price. Sometimes the county agents have arranged to have the dust distributed on fields.

INEXPERIENCED OBSERVERS would probably see little difference in the appearance of healthy grubs and those which have milky disease, especially in the early stages. The hollow body cavity of a beetle grub is filled with blood, which is kept in circulation by the pulsations of the dorsal blood vessel, a tubelike organ that serves as a heart. It runs lengthwise just beneath the semitransparent upper body wall and may readily be seen in a healthy grub. Because of the spores in the blood, it becomes increasingly difficult to see the dorsal blood vessel as the milky disease develops. In the final stage of the disease, the entire grub (even the legs, through which the blood circulates as it does through other parts of the body) has a milky white appearance. If one of the legs is snipped off by pressing it with a fingernail, a drop of blood will be formed on the remaining stump. The drop will be clear and watery in a healthy grub and opaque and white in a milky one. The final test for milkiness is to examine a drop of blood with a compound microscope for the presence of disease organisms.

THE FEMALE Japanese beetle lays eggs in the ground in summer. The grubs that hatch from the eggs are one-sixteenth inch long at first, but they feed and grow until they are about an inch long. As they grow, they pass through three stages, or instars. The change from one instar to the next is brought about by shedding the outer skin. The grub stage starts in midsummer and continues through the fall and winter and until the change to pupa late the following spring or early sum-

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mer. During the pupal stage, the insect is inactive and goes through the changes that produce the beetle. The cycle from egg to adult beetle requires nearly a year and is spoken of as one generation or brood of the insect. For example, the eggs laid in the summer of 1951 gave rise to the beetles of 1952, and this constituted the 1951–52 brood.

Grubs of the Japanese beetle may become infected by milky disease at any time during the long span of grub life. In experimental feeding tests, more than one-half of all milky grubs found became diseased in the first and second instars. If a grub becomes diseased in one instar, it seldom lives to change to the following instar, but continues on for some time in a fully milky state before it finally dies. Grubs that become infected in late summer or fall frequently live through the winter with the disease in a dormant state. As the temperature rises in spring, the disease again becomes active. Milky disease has been produced experimentally in prepupae, pupae, and adults of the beetle, but natural infection in these stages is probably not common.

Since grubs contract milky disease and die over such a long period, an inspection of the grubs in the soil will show only those that are milky at that particular time. You will find no trace of the ones that have become infected and died, because their bodies will have decomposed in the soil. The time that must elapse after the grub first takes in the spores until it is fully milky varies with the temperature. At 86° F., grubs will show the first symptoms of milky disease in 6 to 9 days. At lower temperatures it will take longer. Milky disease organisms will not develop at temperatures above 97° or so; therefore they cannot infect man, domestic animals, or wild game, whose body temperatures are higher than that. It also will not develop when temperatures fall below about 62°. The milky disease will not show up in spring-infected grubs until the soil temperature has been above this point for about 2 weeks. You would have to make soil examinations nearly every week during the time grubs are present to get a complete picture of the action of milky disease. You can estimate the mortality due to milky disease by making soil examinations in August, when the soil population is the highest for a brood, and then making later examinations to determine the drop in numbers.

A grub count made in the fall will show the number present just before hibernation. A count in May or June of the following year will show the number present as the time nears for the change to beetles. The number of grubs that are milky is usually highest at that time. The change in the number of grubs per square foot found in examinations in August of one year and June of the next year will give some idea of the number killed by milky disease in any brood. Of course grubs die from causes other than milky disease, and due credit should be given to any other known agent causing death.

Ralph T. White of the Moorestown laboratory has been in charge of investigations of the milky disease under field conditions for many years. I give a few examples of the action of the disease as he found them.

Beetles were so abundant at the Glen Brook Country Club in Stroudsburg, Pa., in 1941 that the grubs caused serious damage to the turf. White uncovered no evidence of milky disease at that time. Using the spot method, plots were treated at 5- by 5-, 5- by 10-, and 10- by 10-foot intervals with the milky disease in October 1941. Soil surveys made all over the course on May 26, 1942, showed an average grub population of 66 per square foot. On the more heavily treated plots the count was 81 per square foot. If there had not been an abundance of rain the turf on the course would have been severely damaged by grub feeding.

When the new brood was in the ground in August 1942 the grub population on the course averaged 88 per square foot. By early June 1943, there had been a drop from 130 grubs per square foot to 31 in the 10- by 10-foot plot, from 53 to 11 in the 5- by 10-foot plot, and from 82 to 11 per square foot in the 5- by 5-foot plot. In two untreated areas, grubs averaged respectively 62 and 66 per square foot in June and there was turf damage there but not in the treated plots. The disease incidence ranged from 30 to 62 percent in treated areas in late June and a few milky grubs were found in untreated areas, showing that the disease had begun to spread. By the fall of 1943, 2 years after the treatment with spore dust, milky disease had spread all over the course and there was no evidence of turf injury. The entire course was examined in June 1949 and the grub population averaged only 1.7 per square foot; 69 percent of all grubs found were milky. In 1952 so many spores were in the soil that turf damage by beetles is unlikely in the future.

On the extensive grounds of the Perry Point Veterans' Administration and Facility at Perryville, Md., the grubs in the soil averaged 37 per square foot in August 1939, and there was marked browning of the grass due to their feeding. A low incidence of milky disease occurred at that time. Soil surveys were made at Perryville several times each year from 1939 through 1949, except during some war years.

In surveys covering six broods, White found reductions of 86.1 to 94.4 percent in the grub populations, due largely to milky disease. At the time of the June examinations, the number of milky grubs varied from 27.7 to 67.0 percent. In computing the figures, all milky grubs were counted as dead, since milky grubs almost always die. The question has often been raised how, with grub mortality approaching 90 percent in each brood as it did at Perryville, it is possible to have enough beetles emerge from the disease-laden soil to produce such high grub counts by August each year. One reason for this is that a female beetle deposits from 40 to 60 eggs in the ground, and if conditions are favorable a few beetles can concentrate many eggs in a small area. Another reason is that so many favored food plants grow at Perryville that beetles move into the area to feed from other places and, as they lay their eggs in the well-cared-for turf, grub populations are high in August each year.

In parts of the District of Columbia, the beetle had become so abundant by 1940 that feeding grubs had injured turf. The disease was well established in one small area and 55 percent of the grubs were diseased. Some disease occurred in a few other places but there was no evidence of it in most of the District. The application of spore dust, started in 1940, was continued. By 1949 a total of 3,784 pounds had been applied to 2,929 acres by Federal agencies. Spore dust also was applied in places near the District by entomological agencies of Maryland and Virginia, and commercially made spore dust was purchased and applied by civic groups.

In August 1941, the grub count in soil surveys made at seven treated places averaged 31.5 per square foot. By June 1942 the count at the same places was 7.0 per square foot; 12 to 70 percent of the larvae were milky. Information is also available from surveys made at 10 widely scattered places twice each year from 1946 through 1949. The grub population at those places averaged 5.0 in June 1947, 5.4 in June 1948, and 1.6 in June 1949. The disease incidence at the time of the surveys averaged 46.0 percent in 1947, 45.5 percent in 1948, and 57.0 percent in 1949. There has been a noticeable reduction in beetles in the District in recent years, as would be expected from the low grub populations, despite weather conditions that favored an increase in numbers. Surveys made in 1948 at several points where no spore dust had been applied showed the disease to be present at all of them. Milky disease probably occurs to some extent wherever there are grubs in this area. The Japanese beetle was present in great numbers in 1951 in places a short distance from the District of Columbia; it was believed that the low populations in most parts of the District could be attributed largely to the high incidence of milky disease there.

THE MORE GRUBS there are to the square foot the faster the disease becomes established and the more rapid the spread. There had been high grub populations in the three locations I have described and the number of disease spores in the soil had become large. Grubs will usually be plentiful in a place where there are many favored food plants on which the beetles can feed and plenty of good turf for egg laying. This was the case at all the places where disease activity has been described. If the conditions are less favorable, there will be fewer beetles, grub populations will be lower, and it will take longer to build up effective numbers of spores in the soil. Therefore, the situation may not always be so favorable as in the instances given. In order to get the disease established as soon as possible, spore dust has sometimes been introduced where the grub population was as low as one or two per square foot. The build-up of disease in such places is likely to be slow. Under highly favorable conditions, good establishment of the disease has been obtained by the second season after introduction. A longer time has been required in less favorable situations.

SINCE THE SPORES can survive under adverse weather conditions, the dust will not have to be reintroduced once the disease has become established in the grub population. Even though unfavorable weather conditions have sometimes reduced grub populations to as low as one grub per square foot, the disease still persisted and became active again as the grub count rose. In some newly colonized places, milky disease is not yet so effective as it eventually will be because of the time needed for it to become generally established. Because of lower temperatures in the northern part of the beetle-infested area, some observers feel that a longer time will be required for the disease to become established there than in warmer places to the south. However, we have every indication that the disease can become established wherever the beetle exists.

Because of the time needed for effective build-up, the use of the disease as an immediate control measure is not advised in situations where beetle grubs are so numerous that turf is being injured. A rapidly acting poison should be used to kill the grubs and protect the grass. The poison will not kill any spores that are present. Spore dust, applied at a heavy dosage to such places, has reduced the number of grubs and checked the injury, but not always in time to prevent additional damage to the grass.

Milky disease organisms probably occurred in our native white grubs before the Japanese beetle was introduced into this country. When the type-A disease was first found in grubs of the Japanese beetle, grubs of many of our native species of May beetles, or June beetles, were examined closely and disease was found in several of them. Apparently several kinds of bacteria caused milky diseases in different kinds of white grubs. The type-A organism may have existed in some of the native grubs and then, with the arrival of the Japanese beetle, found its grubs favorable to its development. As far as we know, only certain species of white grubs develop the milky disease. We have no records of a condition just like it in other insects.

MY DISCUSSION has dealt largely with the important type-A organism because it is the one usually found in field-colonizing work. Another organism sometimes found in grubs of the Japanese beetle is *Bacillus lentimorbus*, which causes the type-B milky disease. Attempts to produce the spore form of this organism in artificial culture, as with type A, have been unsuccessful. Spore dust made of the type-B organism has been tested in field plots, but the results obtained were not so good as those with type A.

BESIDES THE MILKY DISEASE, many biological agents destroy beetle grubs—other bacteria, parasitic fungi, nematodes, parasitic and predaceous insects, birds, and other animals. The amount of summer rainfall is an important factor, for beetles are always reduced in number following summers with low rainfall. Among all of these factors, the milky disease is perhaps the most effective in checking the build-up of the Japanese beetle, as it spreads into new areas, mostly free from its natural enemies. Any agent that slows down or checks this initial build-up makes the pest less destructive. The milky disease is such an agent and this is the reason for colonizing it in newly infested places as soon as the grub population reaches the point where it will support the disease. Diseased spores will then increase in numbers in the soil as more and more beetle grubs become diseased. The number of beetles will decrease again and the Japanese beetle will cease to be the serious pest it has been.

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For further reading on milky disease Dr. Hawley suggests Two New Spore-forming Bacteria Causing Milky Diseases of Japanese Beetle Larvae, *by S. R. Dutky, in the Journal of Agricultural Research, volume 61, pages 57–68, 1940, and the Bureau of Entomology and Plant Quarantine publication E–801,* The Effect of Milky Disease on Japanese Beetle Populations Over a 10-Year Period, *by R. T. White and P. J. McCabe, issued in 1950.*

The Vapor-Heat Process

A. C. Baker

The Mediterranean fruit fly invaded Florida in 1929 and spread rapidly across the big citrus region of the State. A campaign to wipe out the Medfly began immediately. It was the first campaign in history that was successful in eradicating a widespread insect pest.

But it was 19 months before the quarantine was lifted. Meanwhile a crop was maturing, which the country needed and on which the economy of Florida depended. A way had to be found to market the crop without risk to the rest of the country. Only embargo had been considered safe against this fly, and fruit from countries where it occurred was excluded.

What should be done? All possible information about the fly was gathered. The cold winters of the Northeastern States made those States seem safe for the shipment of fruit from protective zones—the 9-mile zones surrounding the known infested zones. But the Southern States, from North Carolina and Tennessee westward, and the Pacific States, from Utah and Idaho westward, were looked upon differently. The occurrence of the fly there might mean disaster. Idaho was included as a barrier. It was decided to let no citrus from Florida enter any of those States. In order to open the markets there, a treatment had to be developed that would guarantee fruit free from any living stages of the fly.

Time was short. The new crop was hanging on the trees. To develop a process before the fruit would be ready to move seemed impossible. We had one hope. Larvae of the Mexican fruit fly, which also attacks citrus, had been killed when fruit infested with them had been heated to 110° F. or above.