Microbial Control in Hawaii[1,2]

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Insect pathology refers to that field of entomology that studies the abnormalities of insects. As defined by Steinhaus (1963), who is largely responsible for the interest and activity in the field today, insect pathology encompasses matters relating to etiology, pathogenesis, symptomatology, gross pathology, histopathology, physiopathology, and epizootiology.

The principal applications of the field of insect pathology are in the fields of agriculture, medicine and general biology. We are here, of course, primarily concerned with its application in agriculture. However, this concern is not only for the use of microorganisms for the control of noxious insects (properly called microbial control) but also for the protection of beneficial insects such as bees, parasites, predators and other insects introduced for the control of weeds.

It is altogether fitting that Hawaii move with dispatch into microbial control, which is of course related to biological control. Hawaii has been, and still is without doubt, one of the world's foremost proponents and exponents of biological control. This includes not only the mere introduction of parasites and predators to control pests but also the intelligent manipulation of these parasites and predators to attain the significant successes exemplified in Hawaii. The entomologists of the HSPA, the Department of Agriculture, and indeed all of the other entomologists of Hawaii who have in one capacity or another been connected with biological control in Hawaii, can justly be proud of the record of Hawaii in biological control.

Although microbial control had its true beginning about the same time as the rest of biological control, until recently there were very few real concerted efforts to utilize microbes for the control of pests. One of the first documented attempts to utilize a pathogen to control a pest was a project conducted by Metchnikoff in Russia when he used *Metarrhizium anisopliae* against the cockchafer, *Anisoplia austriaca.* Subsequently, Krassilschik in 1884 established a special laboratory for producing spores of the fungus on a large scale for use in field experiments (Steinhaus, 1949).

Even in microbial control, Hawaii was one of the early pioneering areas. In 1897, Albert Koebele, of the vedalia beetle fame, reported that a greyish green fungus, now thought to be *Metarrhizium anisopliae*, was disseminated

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in Hawaii for the control of the Chinese rose beetle, *Adoretus sinicus*, by distributing diseased beetles in the field. This fungus, however, apparently was already present in Hawaii. From 1901 to 1915, the Board of Agriculture and Forestry carried out a program in which Chinese rose beetles, inoculated with the green muscardine, were distributed to all islands whenever populations of the rose beetle became high and destructive. This fungus today can be found, especially during the rainy months, infecting both adults and larvae of the rose beetle.

In addition, Koebele, in 1897, introduced two species of fungi for the control of coccids. This was the first purposeful introduction of a microorganism for the control of a pest in Hawaii. This introduction was followed by series of others, primarily fungi, but including bacteria and nematodes, involving many of the distinguished members of this society such as Dr. Cyril E. Pemberton and Mr. R. H. Van Zwaluwenberg of the HSPA, Dr. Walter Carter of the PRI and the late Dr. David Fullaway of the State Department of Agriculture.

Some years ago, Dr. Yoshinori Tanada started a project in Insect Pathology at the Department of Entomology at the University of Hawaii. I was the happy recipient of this dynamic program when "Joe," as he is known to most here, left to take a position with Steinhaus' group at the University of California.

Dr. Tanada introduced *Bacillus thuringiensis* into Hawaii for the control of the cruciferous pests, the imported cabbage worm, *Pieris rapae*, and the cabbage looper, *Trichoplusia ni* (Tanada, 1956). He also compiled a list (Tanada, 1957) of known insect diseases in Hawaii and did research on a number of other insect diseases including those on several species of armyworms (Tanada, 1955a, 1955b, 1956). He gave the field in Hawaii a tremendous impetus and got it going again (a Kennedy phrase) in the right direction.

This brings us up to the field as it stands in Hawaii today. For today's address, I would like to discuss some of the pathogens and the diseases they cause and speculate on the potentials of groups of pathogens that can be introduced into Hawaii for the control of some of our pests. It is necessary, however, for ease of presentation and to facilitate discussion, to subdivide microbial control into two types.

The 1st is a long term type, i.e., the usual concept of biological control where the pathogen is introduced into a new area, becomes established and exerts a controlling influence on the pest without any further help from man. The pathogen exerts long term control and operates as a typical density-dependent mortality factor.

The 2nd type of microbial control is one in which repeated applications of a pathogen must be made using the same methods and principles as a chemical insecticide. This is a method of application that is more suited to some bacteria and viruses on short term crops. There are gradations, of course, between the 2 types of control. A pathogen may be applied at the beginning of a growing season and remain there and exert its effect for the rest of the season.

The problems encountered by this 2nd approach are not unrelated or unlike those confronting ordinary chemical control.

The major groups of pathogens involved in microbial control programs are: (1) bacteria, (2) fungi, (3) viruses, (4) protozoa, and (5) nematodes. For ease of presentation, each of these groups will be taken up in the order given and discussed.

THE BACTERIA

There have been very few bacterial pathogens reported from Hawaii. *Bacillus larvae*, the causative organism for American foul brood of honey bees was reported in 1931 by Fullaway. The milky disease organism, probably *B. popilliae*, was tested against *Anomala orientalis* and *Adoretus sinicus* by Carter in 1941 and Tanada tested *Bacillus thuringiensis* var. *thuringiensis* against cruciferous pests in 1953. Both *B. thuringiensis* and *B. popilliae* are produced commercially and available for use in the field. *B. thuringiensis* is known to be pathogenic to at least 100 species of *Lepidoptera* and some Diptera and Coleoptera.

B. thuringiensis var. *thuringiensis* is a spore-forming aerobic rod that forms a crystalline para-sporal body when it sporulates. This para-sporal body or crystal is in large measure responsible for the pathogenicity of this bacillus. When the insect pest eats the spores and crystals, and this is the normal route of entry for all bacterial, viral, and protozoan pathogens, the crystal dissolves in the alkaline gut juices of the host. In the great majority of cases, the crystal causes a paralysis of the gut so that the insects no longer are able to feed. This can occur within a few minutes to a couple of hours after the insect ingests the bacteria. The speed of action depends on the species of host. The crystal also causes a breakdown of the midgut cells allowing the bacteria which germinates in the gut to penetrate into the hemocoel. The death of the host occurs within 24 to 48 hours. Bacterial diseases in general are rapid fulminating types and an infected host usually does not last very long once infected.

B. thuringiensis is a pathogen that will have to be periodically applied like a chemical insecticide. In none of the situations where it has been introduced has it become "established" and continued to exert control against the pest on which it was applied. Ecologically, although the spore of the bacillus is able to survive for quite a while in the field, subsequent generations of the pest do not contact a sufficient dosage of the pathogen to initiate more infections and an epizootic. Moreover, in some insects such as *Cactoblastis cactorum*, the bacillus is unable to sporulate and dies after killing the host (Huang and Tamashiro, 1966).

This pathogen has been approved for use on a number of crops and should be utilized especially against the imported cabbageworm and the cabbage looper. Not only is this bacillus extremely pathogenic to these 2 species but also does not leave any toxic residue so the material can be applied up to the day of harvest. Moreover, the bacillus has been found to be effective even in the cooler crucifer growing areas of the State such as at the Volcano on Hawaii.

There are other bacterial pathogens that should be tested and used against Hawaiian pests. Throughout the world, there have been approximately 90 species of bacteria that have been reported to be pathogenic to insects. Some of these are too specific or are not pathogenic enough to be used here but there are others, especially among the crystal bearing spore formers that could be effective against some of our chewing pests. The spore formers are not adversely affected by weather conditions. Moreover, there is a possibility that a bacillus, once introduced, may become established and permanently control a pest just as *B. popilliae* has done against the Japanese beetle. The pathogen, in order to exert this type of control will in all probability have to be one that is quite specific for that pest.

THE FUNGI

According to Dr. F. X. Williams, entomogenous fungi probably destroy more mealybugs in the tropics than all of the parasites and predators combined. The statement could probably be enlarged to include many other soft bodied insects. This is obviously a truism to anyone who has worked in Hawaii long enough, especially if he has had the chance to observe the effect of the fungus *Aspergillus parasiticus* on sugarcane mealybugs. It is not unusual to find entire populations of mealybugs completely devastated by the fungus. In other crops, such as coffee and guava, the green scale, *Coccus viridus*, has been eliminated by the fungus *Verticillium lecanai.* House flies and aphids have been reduced by *Entomophthora muscae* and *aphidis* respectively.

It is not surprising, therefore, that fungal pathogens were noticed early and most of the early work in microbial control was concentrated on the fungi. There have been approximately 20 to 25 species of fungi, more than in any other group, reported from insects in Hawaii. Over the entire world, however, there have been approximately 500 species of fungi reported pathogenic to insects. There are, therefore, a large number of fungi that could potentially be of use in Hawaii.

Interest in the fungi has been reawakened in recent years after a relatively long spell of inactivity. The fungi after enjoying great initial popularity and giving all of insect pathology a great initial push was also in part responsible for the temporary decline of the field. A study conducted in Kansas to determine why some fungi were so effective one year and ineffective the next revealed that fungal epizootics were in large measure dependent upon certain weather conditions. Also they found that artificial dissemination of spores of a fungus that was already present in the area was of minimal benefit. From these studies, other workers made generalizations stating that not only all fungi but all other insect pathogens were completely subject to the vagaries of the weather so that they were not worth consideration.

Certainly, this is not true of the spore-forming bacteria, the inclusion body viruses and the spore-forming protozoa. Even with the fungi, the microclimatic situation, the condition at the leaf surface or at the surface of the integument of the insect is of more importance than the general climate. Moreover, the conditions need to remain ideal only long enough for the fungal spore to germinate and permit the germ tube to penetrate directly through the integument. In most of our crop areas, the weather conditions are favorable for fungi throughout most of the year. Irrigation can be manipulated to raise the humidity to the required level in other areas if this is necessary.

Since the fungi penetrate the insect integument directly, these pathogens can attack any type of host regardless of the type of mouthparts possessed by the insect. General pathogens such as *Beauveria* and *Metarrhizium* attack a wide range of hosts, including Lepidoptera, Diptera, Coleoptera and many other orders of insects. Others such as the *Coelomomyces* are almost exclusively pathogens of groups like the mosquitoes. We have tested a species of *Coelomomyces* against our local mosquitoes but have not been able to obtain an infection. Other species will be imported and tested. There are other soft bodied colonial type insects that can potentially be controlled by fungi. All of the species of termites in Hawaii were found to be susceptible to *Metarrhizium* and *Beauveria*. Moreover, these fungi were able to infect and kill the termites in complete darkness, a situation that would be normal for a termite gallery. Sporulation under these conditions, however, was sparse with the strains of the fungus used. Other strains may be able to sporulate more profusely and initiate an epizootic in the warm moist termite galleries.

The fungi, therefore, merit much more research and study. Conditions in Hawaii appear to be highly favorable for entomogenous fungi. The fungi would be used primarily in the biological control sense although *Beauveria* has been sprayed on crops like a chemical insecticide.

THE VIRUSES

The viruses have been the glamour organisms in insect pathology for the past decade and a great deal of time and effort has been expended to determine how they can be mass produced and used in microbial control. Insect pathologists generally agree that, as a group, the viruses hold the greatest promise for microbial control.

The important insect viruses are divided into 5 different groups depending on viral morphology. These are: (1) the nuclear polyhedrosis virus, (2) the cytoplasmic polyhedrosis virus, (3) the granulosis virus, (4) the polymorphic inclusion virus and (5) the non-inclusion virus.

The nuclear polyhedrosis viruses are the most commonly known and cause the spectacular wilt diseases. The virus attacks the nuclei of certain cells and eventually causes a complete dissolution of all of the internal tissues of the host. The insect becomes negatively geotropic and climbs to a high place to die. The combination of the wilt (the insect in essence is like a paper bag filled with liquid) and the negative geotropism form an admirable method of spread for the virus.

The cytoplasmic polyhedrosis viruses attack only the cytoplasm of the midgut. It is usually carried in an attenuated state in the insect population and can be activated by certain stress conditions. The granulosis viruses also attack the nucleus of certain cells and may or may not cause any wilt symptoms. As the name implies they have granular inclusion bodies. The polymorphic inclusion virus was only seen by Paillot, a Frenchman, and very little is known about this virus. The non-inclusion viruses generally develop in the cytoplasm of most cells of the host. They can be quite pathogenic but do not last very long out of the insect since they are not enclosed in an inclusion body.

Of all the types of viruses, the nuclear polyhedrosis and the granulosis are considered to hold the most promise for use in microbial control. Not only are they generally the most virulent of the viruses but also they are quite host specific. Moreover, the virus rods are enclosed in the polyhedral or granular protein allowing them to persist in the field for prolonged periods. The cytoplasmic polyhedrosis viruses are less virulent and less host specific.

There have been several virus diseases reported from insects in Hawaii including those on the armyworms, *Spodoptera mauritia acronyctoides*, *S. exempta*, *S. exigua* and *Pseudaletia unipuncta*. Most of the diseases have been nuclear polyhedrosis although both granuloses and cytoplasmic polyhedroses have been found. The viruses have been found in field populations causing, at times, great epizootics in the field.

Artificial dissemination of virus by spraying has been made for the control of the corn earworm, *Heliothis zea*, by Dr. Wallace Mitchell, for the imported cabbageworm by Tanada, and for the cabbage looper by myself. In the latter 2 instances, excellent control was obtained with very low dosages. In the first case, the commercial material that was obtained was completely inactive so the tests failed. They will be repeated.

There are at present approximately 260 different insect viruses known. Among these are many that will become very important. The viruses are so pathogenic that only a small dosage is required to initiate an infection. Moreover, because of the virulence, they can easily be transmitted from a diseased to a healthy host by parasites during oviposition (Laigo and Tamashiro, 1966). The small amount of inoculum carried on the parasite's ovipositor is sufficient to infect several hosts. Also, because of viral specificity, they will not infect the parasite. There are numerous records of parasites emerging from virus diseased hosts.

There are, however, some drawbacks. The viruses are rather slow acting so that unless application is timed correctly, there can be considerable damage to a crop before the host is killed. Also, the host in death may smear the plant so that they would have to be cleaned up after harvest. This residue, however, is non-toxic and is not in the same class as a chemical residue.

THE PROTOZOA

There are protozoa, especially some of those in the order Microsporidia, that are highly pathogenic to insects. There are others that reduce the fecundity of the females, sterilize the males, or reduce the ability of the insect to withstand temperature extremes. This is a group that apparently has potential for use in microbial control but due to certain difficulties have not had the attention they deserve. The protozoans, like the viruses, are obligate pathogens so cannot be reared on artificial media.

In Hawaii, all of the protozoans have either been accidentally introduced with the pest or transferred on to the pest from another host. There have been no purposeful introductions for biological control except for those brought in by our laboratory for experimental use. That the protozoans can be extremely effective is shown by the effect of a *Nosema* on the lawn armyworm. This pathogen, which was discovered shortly after the armyworm was found in Hawaii, is quite effective and easily transmitted through the egg. The eggs laid by an infected female give rise to infected larvae, which all die before they reach the 4th instar. The incidence of this pathogen was not very high at the beginning, but by 1964 up to 80% of the egg masses collected on the University campus contained spores of the protozoan. It has become, without doubt, a major factor in the regulation of the populations of armyworm. The *Nosema* apparently can become "established" in a given area and increase in effectiveness with time.

Other species of *Nosema* have been found on the cabbage looper, imported cabbageworm, cosmopolitan armyworm and melon fly. The role played by these protozoans in the regulation of these populations is not known but laboratory studies indicate that they are sufficiently pathogenic to have a major effect.

There are about 210 species of protozoans known to be pathogenic to insects in the world including several on mosquitoes. We have tested a species on our local mosquitoes without any success. The major difficulty is the lack of information on how these pathogens normally infect their hosts in the field.

THE NEMATODES

Insect parasitic nematodes have been reported by many members of our Society including Pemberton, Van Zwaluwenburg, Sakimura and Sherman. The nematodes, however, for the most part have remained laboratory curiosities and no work has been done on them in Hawaii. Recently, however, Dr. Samson Dutky working on a nematode called DD-136 has found that this parasite will attack a large number of insects. The pathogenicity of this nematode is associated with a bacterium, *Achromobacter nematophilus*, which is carried in the gut of the infective 2nd stage nematode. When the nematode penetrates into the hemocoel, the bacterium is released and kills the host.

This nematode has been field tested against the codling moth and many other species with considerable success. Dutky found that the nematode can enter stems, seek out the host and kill them in the plant tissues. It is also effective against house fly larvae in chicken manure. We are now in the process of importing this nematode for use against *Rhabdoscelus obscurus* the New Guinea sugarcane weevil, house flies and other pests.

SUMMARY

Summarizing, microbial control can be effectively utilized in Hawaii. The pathogens, even aside from long term biological control, offer advantages that will warrant their introduction and use. Some of these advantages are: (1) The pathogens are highly specific so they will not affect other beneficial insects or upset an existing biological control, (2) They are nonpathogenic to man, his animals and his plants so there is no danger to the man handling the pathogen, and also there is no possibility of leaving a toxic residue. *B. thuringiensis*, for example, is exempt from tolerance. (3) The pathogen may become established and exert permanent control of the pest. (4) No insect has in all the years developed a resistance to a pathogen to which it is susceptible. (5) The pathogen can be applied with conventional equipment and even can be put on together with chemical insecticides. (6) The cost of the pathogen can be made competitive with chemical insecticides.

The pathogens, however, obviously will not completely replace chemical insecticides. There are many situations where a chemical insecticide will be superior. Moreover, many insects will not be amenable to microbial control, either because of behavior or because a suitable pathogen is not available.

It will be a while before microbial insecticides become readily available for many insects. There are still many problems involving production, formulation, etc., that have to be worked out. There is also need for a great deal of information on the biology and pathogenicity of the microbes. The ecological relationships of pathogen and host need to be investigated. Without this type of information, field trials would be doomed to failure.

At this point, also, the USDA is requiring a series of very stiff tests, especially for the viruses, to be sure that the pathogens will not be harmful to man or animals. Tests such as those performed on *Heliothis* virus and *Trichoplusia* virus (this included intracerebral, intravenous, intraperitoneal, and subdermal injections of the virus into 5 species of vertebrates) must be made for all viruses. All of the results obtained so far indicate that insect viruses are not pathogenic to man or his animals. There has been no record of a specific insect pathogen causing a disease in man or his animals, in spite of the great number of contacts occurring when an epizootic occurs on a crop.

Although there is need for reasonable precautions before clearing a pathogen for field use, it does seem paradoxical that a pathogen could cause a natural epizootic in the field and the crop would be accepted; but the same pathogen could not be sprayed on the same crop in the same field.

From a biological control standpoint, there are other phases of microbial control that should be investigated aside from the introduction of additional pathogens. Ways must be sought to improve the effectiveness of those pathogens that are already here, by increasing virulence, improving distribution, increasing transmission, extending field life, etc. As an example, since it is known that parasites and predators can act as vectors of the pathogens, the parasites and predators may be reared, contaminated with the pathogen, and released in the field to initiate epizootics. The idea would not be unlike an insect transmitting any other pathogen to a plant or animal. By this method, epizootics may be initiated much earlier than when the pathogen is allowed to go naturally.

In closing, I would like to state that although it is a very rosy picture I paint for microbial control in Hawaii, I would hope that it is from recognizing the tremendous potential of this method of control rather than from a personal bias causing me to look at microbial control with rose-colored glasses.

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