한국식물보호학회지 : 제22권 제 2 호
Korean J. Plant Prot. 22(2) : 67~73(1983)

Microbiological Control of Insect Pests

Shigemi Kawase

害虫의 微生物學的 防除

川 瀨 茂 實

ABSTRACT

Despite the great importance of chemical insecticides, with the ever-increasing resistance of pest insects to chemical insecticides and the growing concern over environmental pollution, it becomes evident that the problem of pest attack on crops cannot be solved by any one system. Under these circumstances, main pathogens of insects, i.e., viruses, bacteria, fungi and protozoa, have been studied to control many insect pests. Some of these pathogens are now being produced as microbial insecticides at the rate of hundreds of tons per year in the world. Some microbial insecticides are very effective against numerous or target insects under suitable environmental condition, and microbial control has been played an important role in integrated control program. They have many unique properties such as selectivity, multiplication and harmless to higher animals. However, we must be aware also that there are many problems to be solved, such as safety, persistence and difficulty of efficacy, etc. on the microbial insecticides.

INTRODUCTION

From early times man has been aware that insect uffer from diseases, and this awareness has developed nto science of insect pathology. Early investiations were largely concentrated on two domestiated insects, the honey bee and the silkworm, to ure the diseases of these insects. Subsequently, as e main pathogens of insects, viruses, bacteria, ngi and protozoa have been discovered. Gradually ese studies were extended to pest species utilizing seases to control these insect pests,because control insect by chemical insecticides faces on the one nd increase of environmental pollution by poisons, d on the other hand development of resistance of sts to pesticides.

The term "microbiological control" or "microbial ntrol", may be defined as follows: the alterations the balance of nature by the manipulation of crobial agents so that those members of the plant and animal kingdoms that compete wite man are reduced to the lowest acceptable economic level.

With the ever-increasing resistance of pests to chemical pesticides and the growing concern over environmental pollution, it becomes evident that the problem of attack of insect pests on crops cannot be solved by any one system. Despite the great importance of chemical insecticides, none of them are selective, and parasites and predators are frequently killed indiscriminately with the target insect. Chemical insecticides are almost always poisons for higher animals including man. Furthermore, they remain and accumulate in the soil, and may be concentrated in the biological chain of life, and become a hazard in foods that were not originally treated.

SUITABILITY OF PATHOGENS FOR MICROBIAL CONTROL

As stated by several investigators, there are at

Faculty of Agriculture, Nagoya University, Nagoya, Japan

least seven major points to be considered in arriving at an assesment of the suitability of microbial pathogens for insect control.

1) Safety to man, higher animals, fishes and crop plants, both directly at the time of application and indirectly, i.e., there is no accumulation of dangerous quantities of the material nor any formation of toxic degradation products.

2) Specificity for the target insect to be controlled, with minimum effects such as honey bee, silkworm and natural enemies.

3) Persistence of the effect, so that the necessity for repeated application is minimum.

4) No or low increase in resistance of the insect population by selective or otherwise.

5) Ease of application, by conventional methods and apparatus.

6) Cheap cost, including all factors such as elimination of poison residues.

7) High stability under inappropriate environmental condition.

MICROBIAL PATHOGENS AS CONTROL AGENTS

Microorganisms pathogenic for insects belong chiefly to following four groups: viruses, bacteria, fungi and protozoa.

1) **Viruses:** Insect viruses are classified into several groups for their properties, namely, baculoviruses including nuclear polyhedrosis virus (NPV), granulosis virus(GV) and *Oryctes* virus; cytoplasmic polyhedrosis virus (CPV); entomopox virus (EPV); iridescent virus, parvovirus and picorna virus(Table 1). The NPV,GV, CPV and EPV form inclusion body, polyhedron or capsule. In NPV, CPV and EPV numerous virus particles are occluded in the polyhedron, but in GV only one virus particle is occluded in the capsule. As the viruses in the inclusion body are very stable under unsuitable environmental condition, these viruses have been mainly used for microbial control. There are numerous

Table 1. Classification and basic properties of insect viruses

Virus group	Particle symmetry	Nucleic acid	Inclusion body
Family: BACULOVIRIDAE			
Genus: *Baculovirus*	Bacilliform	ds DNA	
A. Nuclear polyhedrosis virus (NPV)			+
B. Granulosis virus (GV)			+
C. Oryctes virus			−
Family: POXVIRIDAE			
Genus: *Entomopoxvirus* (EPV)	Ovoid or Brick-shaped	ds DNA	+
Subgenera, A, B and C			
Family: IRIDOVIRIDAE			
Genus: *Iridovirus*	Isometric	ds DNA	−
Family: PARVOVIRIDAE			
Genus: *Densovirus*	Isometric	ss DNA	−
Family: REOVIRIDAE			
Genus: *Cypovirus* (CPV)	Isometric	ds RNA	+
Family: PICORNAVIRIDAE			
Genus *Enterovirus*	Isometric	ss RNA	−
Family: RHABDOVIRIDAE			
Genus *Sigmavirus*	Bullet-shaped	not known	−
UNCLASSIFIED VIRUSES	Isometric or Ovoid	ss RNA	−
		ds RNA	−

ds: double-stranded; ss: single-stranded successful records on control of insect outbreaks by viruses until now. Of course, it can not be said that all insect viruses control their host insects, nor that all insects can be controlled by viruses. A well known case of control of insect outbreak is that of two sawflies, *Diprion hercyniae* and *Neodiprion sertifer* in Canada and the United States. About four decades ago, the major pest insects of the forest in North America were these sawflies, but there was no natural enemy of these insects in these area in those days. Subsequently, NPV of these sawflies were introduced from Europe to Canada and the United States. It spread rapidly in the forest to cause a spectacular disease epizootics that reduce the sawfly populatoin. A large number of successful results controlled by artificial dissemination of NPVS have been shown on *Heliothis zea, Trichoplusia ni, Mamestra brassicae, Porthetria dispar,Spodoptera exigua* and *Orgyia pseudotsugata,* and GV of *Pieris rapae.* Most of their preparations are produced largely and commercially available in the United States, the U.S.S.R. and other countries. Recently, palm rhynoceros beetle,*Oryctes rhinoceros* which is major pest of coconut palms occurs throughout Southeast Asia has been accidentally introduced into a number of South Pacific countries. The damages of palms were so large that efforts of discovery of the natural enemies of this insect pest were made. A baculovirus of this insect was first discovered in Malaysia. Microbial control using this virus during several years was successful for the decrease of *Oryctes* populations.

The persistence of virus infection in a population is a major advantage, probably of greatest importance in control of forest insects, where the need for repeated applications of pesticides can make the cost prohibitive. Generally it seems to be a result of continuous transmission both within and between generations. This is quite different from pollution by chemical insecticides, which are merely added to by successive applications. Generally speaking, viruses are very easy to apply, because they initiate infection and then multiply in the host cells. As the host range is rather restrictive, we can kill only target insect without consideration to beneficial insects.

One of the demerit of virus preparations is following point: they can not produce in the artificial media, so that the cost is rather expensive. However,it has been reported that viruses obtained from 5~10 dead larvae are usually considered sufficient to make a satisfactory preparation to spray one acre of crop. Therefore, even if the insect must be reared and infected artificially, this should not be very costly. Recently, production of insect viruses in cultured cells has been carried out in the United States. Probably, the most serious deficiency of the virus preparation is the length of time required to cause death following infection, i.e., a period that vary from 3~12 days. During this time, the insects continue to feed and considerable damage may be done. Another problem, especially in food crops, in the tendency of virus-killed larvae to adhere to the plant after death. In a heavy population this can be rather serious, because presence of larval cadavers on such crops as cabbage and cauliflower would reduce their market price.

2) **Bacteria :** Of numerous insect pathogenic bacteria, spore and toxin forming species are useful for microbial control, since the spore can persist under unsuitable environment, and the toxin kills pest insects directly as well as well as chemical pesticides. The first successful case may be the control of Japanese beetle, *Popillia japonica* using *Bacillus popilae.* In the United States, large quantities of this bacterium have been produced and distributed for control of this insect since 1940. The spore can persist and remain viable in the soil for several years, germinating only when are ingested by a suitable host.

The most famous bacterium for microbial control is *Bacillus thuringiensis* (*B. t,*), which have now been developed to the first truly industrial production of a microbe for general use in insect control. This microbial insecticide is now being produced very largely in several countries for use on a number of crops. However,there are several problems to be solved on this preparation.In the case of *B.t.* a biological rather than a chemical process is involved. with the possibility that gentle or developmental changes may occur. *B.t.* is a complex species divisible into over twenty subspecies by serological

Table 2. Varieties of *Bacillus thuringiensis* and productivity of toxins

Variety	Serotype	α-exotoxin	β-exotoxin	Titer of Endotoxin
thuringiensis	H_1	+	+	1.00
amuscatoxicus		+	–	
finitimus	H_2	+	–	
alesti	H_3 3a	+	–	0.01
kurstaki	H_3 3a, 3b	+	–	
sotto	H_4 4a, 4b	+	–	0.003
dendrolimus	H_4	+	–	
kenyae	H_4 4a, 4c	+	–	
galleriae	H_5 5a, 5b	–	+	0.74
canadensis	H_5 5a, 5c	–	+	
entomocidus	H_6	–	–	0.94
subtoxicus	H_6	–	–	
aizawai	H_7	+	+	0.05
pacificus	H_7	+	–	
morrisoni	H_8	–	+	1.00
tolworthi	H_9	+	+	0.33
darmstadiensis	H_{10}	–	+	
toumanoff	H_{11}	+	+	
thompsoni	H_{12}	+	–	
pakistani	H_{13}	+	–	
israensis	H_{14}	+	–	

and biological tests (Table 2), but they are not all equally effective as insecticide.

The *B.t.* preparation have been recommended for use against a number of insects, and unlike some other microorganisms, it has a broad spectrum. Namely, almost all susceptible insects belong to the order, Lepidoptera, including silkworm, However, new subspecies, *israelensis*, which was isolated in Israel recently, kills some mosquitoes such as *Anopheles*, *Culex* and *Aedes*, but was nontoxic to Lepidoptera. The mass production of this *B.t.* preparation is now being carried out in Israel. *B.t.* produces several insecticidal toxins, two of which are used in agriculture, i.e., crystal endotoxin and β-exotoxin (thuringiensin). The feature is further complicated because different subspecies can produce more of the same crystal toxin than others, and these difference between subspecies are very difficult to determine.

The principal insecticidal component of present commercial preparations of *B.t.* is the crystalline protein body formed during sporulation. When *B.t.* is grown in artificial media, a period of rapid vegetative growth is followed by formation of an environmentally resistant endospore. The crystal toxin of *B.t.* has an activity spectra limited to certain Lepidoptera, mosquitoes, chironomids and blackflies. The crystal is not toxic until dissolved, and when is solubilized by alkali or gut juice of host insects,it becomes toxic for susceptible insects. Quantitative analyses showed that the crystal was composed largely of protein, which had molecular weight of 230,000 daltons. No nucleic acid and lipid were detected in the crystal. Amino acid compositions from several subspecies of *B.t.* were slightly differnt from each other, cut methionine was the amino acid present in smallest proportion. Gut juice protease may contribute to the host toxicity spectrum exhibited by each taxonomic *B.t.* group. The toxicity of crystal toxin from several subspecies is different from each other.

The toxin acts as the surface of gut epithelial cells to cause a rapid loss of ATP from the cells stimulating respiration and glucose uptake. Soon thereafter, the microvilli swell and the cell apices begin to swell in the gut lumen.

Almost two decades ago, a low molecular weight insecticidal component, β-exotoxin(thuringiensin), was reported in cultured media of a few subspecies of *B.t.* Recently the chemical structure of the toxin has been determined to be a ATP analogue. Later occurrence of two kinds of the toxin, thringiensin A and B was reported. The mode of the action of thringiensin is due to the specific inhibitor of DNA polymerase. It inhibits by competition with ATP for the binding site of the enzyme. Thuringiensin is toxic to a much broader range of insects than that of crystal toxin. It kills species of the order Lepidoptera, Diptera, Coleoptera, Hymenoptera Isoptera, and Orthoptera. The effect varies greatl with dose, mode and time of application, and sub lethal dose often produce anomalies, deformities an teratologic changes. The toxicity is most marke during molting, pupation and metamorphosis. It i not damaged or absorbed by the gut of domest animals, therefore, their food including the toxi would pass through the gut and control flies in feces.

On account of the relative virulence of *B.t.* preparations produced by different companies is different from each other, the toxicity of international standard (E61; subspec. *thringiensis*) by Institute Pasteur is determined to be 1000 IU (international unit). Thus, the potency of the sample is calculated by the following formula:

$$\text{Potency of sample} = \frac{LD_{50}\text{ of E61}}{LD_{50}(\mu g/ml)\text{ sample}} \times 1000\ Iu/mg$$

n the United States, new US standard (HD-1-S-980) is used. The subspecies used is *kurstaki* HD-1 ain and the potency is determined to be 16000 mg.

evelopment of resistance would have an ortant effect on the use of *B.t.* in pest insects. ough slight resistance to β-exotoxin had been n in *Musca domstica*, no acquired resistance observed in some larvae treating by *B.t.* for and 30 generations, respectively. Therefore, substantiated resistance to the spore, crystal complex f *B.t.* in Lepidoptera is not to be expected in the eld within a reasonable time.

Although large scale investigations using *B.t.* reparation have been carried out during past two ecades, there is no evidence of developing resistance susceptible populations. However, the danger is at contaminants or mutants might become dominant during large scale production. Continual testg will be needed to guard against such an occrrence, but so far as we know, it is harmless to gher animals and man. The primary effect of the eparation is that of the crystal toxin as stated ove. When this toxin is solubilized by alkaline ndition in gut juice of host insect, it acts a toxic ect. This causes, in most susceptible insects, an nost immediate cessation of feeding, even though ath may not occur for hours or even days. This aracteristic reaction is of great practical value, cause crop damage is kept to a minimum.

Generally speaking, the bacterial insecticides are plied in much the same as any other pesticides the form of either a dust or aqueous suspension. wever, this preparation is toxic for silkworm, therefore, careful application is necessary in those countries where the silkworm is economical importance.

3) **Fungi**: Although there are many reports on natural epizootics of fungi that reduced or eliminated insect outbreaks, there are both successful and unsuccessful records by fungi for microbial control.

Very large number of insect pathogenic fungi have been isolated until now. *Entomophthora*, *Beauveria*, *Metarrhizium* and *Aspergillus* are the genera most commonly encountered in nature, and all four major taxonomic groups of fungi contain insect pathogenic species. Of these fungal species, *Beauvaria bassiana* is most widely used for microbial control, because it attacks about five hundred insect species. This fungal preparation, "Boverin", is used practically in the U.S.S.R. The second is *Metarrhizium anisopliae*, the host range of which exceeds two hundreds insect species. *Nomuraea rileyi*, which kills mainly many lepidopterous insects, is a likely candidate for a microbial insecticide, because it is not virulent against beneficial insects, and is not toxic or pathogenic for mammals.

Invasion of fungi into host insects is primarily through the integument. Conidia once lodged on the cuticle swell and then produce a slender invasion hypha. Penetration of hypha through the cuticle involves both mechanical and enzymatic factors. In many cases small black spots on the integument are the first overt sign of infection of a larva. Growth of fungus after reachs the hemocoel is by budding, which produce discrete yeast like hyphal bodies. They are eventually transported throuout the hemocoel and give rise to localized concentrations of mycelia. Some fungi produce several toxins by hyphal bodies or early mycelial growth, by which death is hasty and metamorphosis is delayed. After death, conidiophores are produced which erupt through the cuticle, by which conidia are formed on the outside of the insect. The larval body is completely mumified and coverved by a white mycelial mat, thereafter, shrouded a pile of conidia, which are easily dislodged and distributed by wind. The conidia are considerably unstable under unsuitable environmental condition. Owing to these circumstances, there are many problems on the application for microbial control.

Chemical structures of toxins, beauvericin, destruxins and others produced from some insect pathogenic fungi, have been determined. The beauvericin is somewhat toxic to mosquito larvae and some other insects.

One of the demerit of fungal insecticides is the unstability under unsuitable environmental conditions. It has been generally believed that fungal conidium germinates only under suitable temperature and high humidity.In relatively suitable temperature and humidity, fungi cause over 99% mortality, but when tests are done under inappropriate condition, such as low temperature and low humidity, the results are generally unsuccessful. Furthermore, as insect pathogenic fungi are much less host specific than viruses and bacteria, careful application needs for sericultural area. On the other hand, one of the merit of fungal insecticides may be cheap cost of the preparation, because it can be easily produced largely in artificial media as well as bacteria. Generally, insect pathogenic fungi do not cause and ill effects in man, higher animals and crop plants.

For these reasons, several fungal insecticides have been produced largely during recent years. For example, in the U.S.S.R. fungal pesticide "Boverin",which is mass-produced from *B. bassiana* is recommended for use with reduced dosage of chemical insecticides. Mean mortality during four consecutive years (30×10^9 conidia/g; 1.5 kg/ha) reaches 92% in the case of control of the Colorado beetle. Mass production of conidia of *M. anisopliae* (name of preparation: "Metaquino") has been done in Brazil for control of spittlebugs, such as *Mahanarva posticata* on sugar cane. The area treated with the product "Metaquino" (50 l/ha; containing 10^{12} conidia) was increased from 500 to 50,000 ha in a State of Brazil alone from 1972 to 1978.

4) **Protozoa:** The characteristics of protozoan pathogen suggest that they can not be used as short-term, quick activity microbial insect icides. They can be produced only in vivo, and large quantities of the pathogen are relatively difficult to produce. The primary route of infection by a protozoan is the alimentary tract by ingestion of the spore, but in some cases transovarial transmission from diseased insects is also important for microbial control. Th[illegible] symptoms are mild, and in some cases diarrhe[illegible] or loss of activity occurs.

Several trials on pest control by protozoa suc[illegible] as *Nosema locustae* for a pathogen of grasshoppers and crickets, *Vairimorpha necatrix* for many lepidopterous larvae, and *Nosema fumiferanae* fo[illegible] spruce budworm, *Choristoneura fumiferana* hav[illegible] been carried out in some countries.

Protozoa are most likely to be useful for insec[illegible] control as introducers of low vilurence, mostly un[illegible] spectacular, some exerting major control and other[illegible] reducing populations to level that permit econom[illegible] control by other means. The most importa[illegible] factors in successful control were transmission [illegible] disease, namely, directly from parent to offspri[illegible] some cases, and persistence of spores, result[illegible] infection of a sufficient proportion of the pop[illegible] for decimation by disease to counteract the [illegible] ability to increase.

5) **Rickettias and Nematodes:** Both rickett[illegible] nematodes pathogenic for insects may be use[illegible] microbial control. However, the former is d[illegible] for safety to man and higher animals,and the [illegible] have several problems concerning the practica[illegible] for the difficulty in obtaining large number of [illegible]ocula. Recently, some trials on a nematode, *Ro[illegible] anomermis culicivorax*, which is pathogenic f[illegible] mosquitoes, have been carried out to contr[illegible] mosquitoes in North America and Taiwan.

MICROBIAL CONTROL IN JAPAN

In Japan, recognition of the importance for t[illegible] application of insect pathogenic microbes w[illegible] rather delayed, because sericulture played an i[illegible]portant role in the agriculture.

1) **Viruses:** CPV of pine caterpillar, *Dendrolim[illegible] specutabilis*, was used for the forest insect in 19[illegible] ~1969. Dissemination of polyhedra by helicop[illegible] at the rate of 2×10^{11}/ha seemed to be m[illegible] effective for the control of the caterpillar. Aque[illegible] preparation was sprayed at the rate of 200~300 (polyhedron conc., 1.7×10^6/ml)/ha in the case [illegible] aerial spray. The dust was also used at the rate [illegible] 30~60 kg/ha both in the case of ground and ae[illegible]

spray. The product named "Matsukemin" containing :ytoplasmic polyhedra is commerciallized already. Several control experiments by NPVs of *Hyphantria cunea*, *Spodoptera littoralis*, *Pieris rapae* and *Prodenia litura*, and GVs of *Pieris rapae* and *Adoxophes orana fasciata* are now being carried out by several investigators.

2) **Bacteria:** Preparation of a spore forming bacterium, *Bacillus moritai*, which is effective for the control of the house fly, *Musca domestica*, has been manufactured. The *B. moritai* is not pathogenic for man, higher animals, the silkworm and the honey .ee. Its product, "Rabirus" was registered as an .dditive in domestic animal's food. The bacterium ' ·uld pass through the gut of animal and kill the tr. from their feces. *Bacillus thuringiensis* has U/ een easily used in Japan owing to its toxicity D· e silkworm. The selection and breeding of mp ith low toxicity for the silkworm have been \ltl. and a product of such a strain has been ap- how ·xperimentally in the field since 1971. Owing ras se efforts, *B.t.* has been registered in Japan ly, and is hoped to be marketed soon.

*ʼ***ungi:** Up to 1960, experiments on the obial control of insect pests in Japan were all ncerned with the utilization of insect pathogenic ngi, such as *Isaria kogane* for some chafers. *picaria fumoso rosea*, which is scarcely pathogenic ›r the silkworm, was used to control the silkworm chnid fly, *Crossocormia zebina*. This fungus kills ıe fly, a parasite of the silkworm, in the soil.

FUTURE PROSPECTS OF MICROBIAL CONTROL

Microbial insecticides have been supplied a new thal factor for the control of some insect pests the agricultural and forest environments. In comrison with chemicals, microbial insecticides are w in number. However, for a limited number of es, they are as effective as the best chemicals, ough they are often more expensive. Products ntaining *B.t.* are registered and used com:rcially against some lepidopterous pests in many countries. However, owing to the range of Lepidoptera known to be susceptible is wide, careful application is necessary. Generally, introduction of pathogens and their use as microbial insecticides is a valuable method of control, and those pathogens are important factor in the natural limitation of insect numbers.

We are acquiring a more thorough knowledge of ecology, because the effect of microbial control is often slower and more subtle than that of chemicals. For example, the greatest effect of an introduced pathogens may be delayed for a year following treatment, as after introducing some fungi and the polyhedrosis viruses.

The future of insect control lies in the integration of all available control methods. The use of chemicals alone is unlikely to solve pests problems permanently. A balance between the pest and all controlling forces, physical and biological, is essential. Pahogens applied as microbial insecticides can restore this balance when pests begin to increase, while pathogens applied as introductions and as supplements to enzootic organisms form part of the natural controlling complex. We anticipate that microbial control will play a useful part in integrated control, while not replacing chemical methods against the majority of pests.

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