INSECT PATHOGENS: THEIR SUITABILITY AS BIOPESTICIDES

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ABSTRACT

This chapter focuses on groups of insect pathogenic bacteria, viruses, fungi and nematodes that have shown potential as inundative biocontrol agents. Each group will be roughly characterised and examples of successful use as biocontrol agents are given. *Bacillus thuringiensis* is the most widely used microbial control agent because of its activity against important lepidopteran and dipteran pests and its relatively easy industrial production in fermentors. Viruses are highly selective control agents but as mass production depends on living hosts few products are available on the market. Fungi can be useful pathogens but their dependency on specific environmental conditions severely limits their applicability. Nematodes are successfully used against insects living in soil but the high production costs so far limit application to high value crops such as ornamentals and strawberries.

INTRODUCTION

Every single insect has its diseases caused by pathogenic micro-organisms, viruses or nematodes and all of these pathogens are potentially useful as biological control agents for that particular insect. In practice representatives of only a few groups of pathogens are actually studied and used as biocontrol agents. Factors such as pathogenicity, speed of kill, host range, possible side effects on non-target hosts (in particular plants and vertebrates) by the pathogen itself or other members of the same group, potential for in vitro propagation, potential for mass production all together determine the potential of the pathogen as a subject for study and commercialisation. A large heterogeneous group of pathogens that cause more chronic diseases, leading to some reduction of fertility and life-span such as many protozoans and some more obscure pathogens such as mycoplasmas and rickettsias are not well studied, often overlooked or even not recognised by experienced insect pathologists. Because these organisms do not show spectacular short-term effects they get little chance to compete with and serve as alternatives for chemical pesticides. In nature and integrated farming systems of the near future these more chronic diseases may well prove their use in providing stable and long-term control of pest insects. This chapter, however focuses on the four main groups of insect pathogens often used as microbial pesticides: bacteria, viruses, fungi and nematodes.

INSECT PATHOGENIC BACTERIA

Though bacteria are the most successful group of insect pathogens from a practical and commercial point of view the number of species so far proven to be useful for biological control is surprisingly small. Three species of spore forming *Bacillus*, *B. thuringiensis*, *B.*

sphearicus and *B. popilliae* are of practical importance, with the *B. thuringiensis* (Bt) being of prime importance (Entwistle *et al.*, 1993, Cannon, 1995). Many other species of insect pathogenic bacteria, often belonging to the genera *Serratia, Clostridium* or *Pseudomonas*, have been found and described from insects but most of these are either opportunists with low virulence or they are closely related to vertebrate or plant pathogenic bacteria and therefore pose safety risks. In most cases bacteria affect their hosts after ingestion with food, and often produce toxic metabolites that damage the gut wall. The following paragraphs will mainly concentrate on *B. thuringiensis* because of its prime importance. *B. sphearicus* is important because it can be used against mosquito larvae. Its usefulness and host range are comparable to that of *B. thuringiensis israelensis (Bti)* but it is more effective in polluted waters. *B. popilliae* isolates are specific pathogens of scarabaeid larvae causing milky disease. They have been successfully applied against Japanese beetle in the USA and in some cases provided long term control over decades (Klein & Jackson, 1992). *Serratia marcescens* has been developed as a control agent against the grass grub *Costelytra zealandica* in N-Zealand (Klein & Jackson, 1992).

Bacillus thuringiensis

Bt is a rod-shaped bacterium closely related with *Bacillus cereus*, which is a common soil bacterium. The main difference between *B. cereus* and Bt is that the latter has plasmids on which toxin genes are located that produce proteinaceous toxins in the form of inclusion bodies or crystals at sporulation. In addition to the inclusion bodies sometimes low-molecular toxic metabolites are excreted called E1-exotoxins that have broad toxic activity against vertebrates. Strains that have these exotoxins are mostly avoided in commercial products, with some exceptions in Finland and Eastern Europe. There are thousands of *Bt* isolates grouped in serotypes or subspecies that are characterised by flagellar antigens. Well known subspecies are *Bt kurstaki* and *Bt azaiwai* with activity against lepidopteran larvae, *Bt israelensis* active against dipteran larvae and *Bt tenebrionis* that shows activity towards some coleopteran larvae. Most of the other serotypes predominantly are active against lepidopteran larvae although some show combined activity against more than one order of insects. In most Bt isolates the crystal contains 3-5 different endotoxins, each with its own specificity for certain receptors in the gut. It depends on the combination of toxins present and their relative expression levels towards which insects a particular isolate will be toxic and effective. So far dozens of different toxins have been described and characterised. Most likely many more will be found over the next few years.

Mode of action

When ingested crystals arrive in the foregut where they dissolve under alkaline conditions into large molecules or protoxins which are digested by proteases present in the gut juice of the insect to form activated toxins also named EB-endotoxins. These toxins bind to receptor sites in the foregut and form small ion-pores in the gut wall changing the semipermeable character of the membrane leading to gut paralysis and shock. The spore itself may play a role by causing septicaemia in the affected insect but this depends very much on the insect concerned.

Biological control

World-wide *Bt* products are yearly being used on several millions of hectares to control lepidopterous pest in agriculture, forestry and stored products. Several hundred tonnes of *Bti* are applied to control mosquito and blackfly larvae, in particular in the third world. *Bt* has 0.5-1% of the world insecticide market with a value of about 100 million dollars. Until a few years ago, nearly all commercial *Bt* products were based on one strain, the HD1 strain of *ssp kurstaki*. This strain combined good activity against some cotton pests with easy production and high yields. Nowadays there is a development towards more diverse products by smaller companies developing *Bt*-isolates tailored for a specific pest or group of pest insects in a specific crop. Nearly always *Bt* is used by inundative application, because *Bt* generally does not build up populations and does not spread as viral disease may do. Only in some contained environments such as a silo with stored products *Bt* causes epizootics that wipe out a population. Normally *Bt* is applied to the food the insect is going to eat. In some cases baits have been used but in general foliar application are the rule. *Bt* persistence on foliage generally does not extend longer than 4-5 days. Major factors reducing persistence are sunlight, rain, microbial degradation and foliar metabolites such as tannins. The costs of *Bt*-production are several dollars per kilogram or litre. If local waste products are used in simple production systems, costs will be considerably less. Generally a quantity of 0.5-1 kg wettable powder per hectare is used containing 50 gram of crystal protein as the active ingredient.

Mass Production

One of the key factors in the success of *Bt* as a microbial insecticide is the production in large amounts at low costs using a variety of simple media such as waste product of fish meal or agricultural industries in large scale fermenters. Cottage industries in developing countries can use solid media such as steamed wheat bran or corn meal(Prior, 1989). A batch culture takes 24-48 hours. Usually 1-5 x 10^9 spores/ml are produced. The normal dosage is about 10^{13}-10^{14} spores/ha. Resistance. A number of reports have been made on the development of resistance towards *Bt*, both in the field and in the laboratory in particular by *Plutella xylostella*. Though a dispute as to the real nature of the resistance is ongoing it shows clearly that development of resistance if *Bt* is going to be expected when *Bt* is applied at a large scale.

INSECT PATHOGENIC VIRUSES

There are more than 1600 viruses known from over 1100 insect and mite species, mostly belonging to the virus families Rhabdoviruses, Iridoviruses and Parvo viruses, families to which human and plant viruses belong too. There is one family of viruses, the Baculoviruses, that exclusively occurs in insects and a few crustaceans and mites, and that is considered absolutely safe for humans and other vertebrates. Fortunately, this group includes the most useful viruses for biological control. Research on insect viruses is largely focused on this group. This chapter will therefore further focus on baculoviruses (Granados & Federici, 1986). Information on other groups of viruses can be found in Burges (1981), Payne (1986) and Maramorosh & Sherman (1985).

Baculoviruses

Baculoviruses are large, rod-shaped DNA-containing viruses. There are three morphological subgroups, granulosis viruses (GV), nuclear polyhedrosis viruses (NPV) and a small group of non-occluded baculoviruses. Characteristic for the GVs and the NPVs is that the rod shaped virus particles are occluded in proteinaceous occlusion bodies (OBs), often called polyhedral inclusion bodies (PIBs). The protein matrix protects the virus particles from environmental influences and enables these viruses to persist for years under favourable conditions, for instance in the soil. Baculoviruses are mainly found in Lepidoptera and Hymenoptera and in some cases in Diptera, Coleoptera and other groups (Granados & Federici, 1986). Generally only the larval stage is affected. Most baculoviruses are very specific and infect only a single host species, few are known to infect different insect species, such as the *Autographa californica* NPV and the *Mamestra brassicae* NPV. Baculoviruses often cause epizootics, almost wiping out caterpillar populations, usually at the end of the growing season.

Mode of action

In general the virus has to be taken up with food to infect a larva. Under alkaline conditions in the foregut the OBs or PIBs disintegrate and the rod-shaped virus particles are released. They bind to specific receptors on the gut epithelium and infect cells in the gut wall. They replicate in the nucleus of the cell and new virus particles are formed that infect other cells or are occluded into new OBs or PIBs. In Lepidoptera most of the cells in the body of the infected larvae eventually are infected and 30% of the dry weight of a diseased larva finally consists of virus particles. In Hymenoptera the infection is generally confined to the gut only. Death occurs 5-14 days after infection. A single lepidopteran larva produces 10^9 NPV or 10^{11} GV OBs. For infection 1-100 OBs are sufficient and for biocontrol programmes 10^{12} NPV OBs or 10^{14} GV OBs per ha are used. Polyhedra from dead caterpillars contaminate surrounding and lower leaves and thereby form a new source of infection for other caterpillars in their vicinity.

Biological control

The number of commercially successful products is limited in comparison to the number of baculoviruses studied. The first virus developed into a true commercial product was the *Heliothis zea* virus produced and marketed by Sandoz under the name Elcar. Although the product was effective in the field it lost the battle with synthetic pyrethroids also introduced time in the 70s. Sandoz then stopped the production but over the last few years interest in the product was renewed. Another virus that has been used in many countries is the *Cydia pomonella* GV. Several products have been on the market for use in apple orchards. A number of other viruses are or were produced by the USDA for use against caterpillars in forests where chemical pesticides are not allowed and damage thresholds are far higher than in agriculture. The persistence of Baculoviruses is rather short. UV radiation inactivates most virus within two days although a proportion will generally persist much longer and may form sufficient inoculum. Rain does not affect the persistence of virus. Many UV-protectors have been tested in NPV formulations. The general advice is to apply the virus to the underside of the foliage and to spray at dusk. The period of 5-10 days of continued feeding by the caterpillars after virus application causes problems in some crops with low damage thresholds and asks the farmers for patience and confidence.

Mass production

Mass production of baculoviruses is done in the insect host. Large rearing facilities are required. For a hectare dose the virus harvest of 100-1000 infected larvae is required. The virus-infected larvae are usually disintegrated in a mixer producing a crude suspension which can be cleaned and spray-dried to form a powder. Growth of contaminating bacteria during storage and irritating hairs from some host larvae are the main reasons for cleaning the crude suspension. In third world countries in stead of rearing, farmers collect larvae for virus production. These may then be infected on the farm or brought to a regional research station for virus production (Prior, 1989). Production in cell culture is possible but only in small scale fermenters and at high costs. The price of media, the loss of infectivity after in vitro propagation, and scaling up problems with vulnerable insect cells have so far blocked commercial use of cell cultures for virus production.

INSECT PATHOGENIC FUNGI

More than 750 species of fungi, from all major taxa, have been recognised as insect pathogens (McCoy *et al.*, 1986). Among them a few have been seriously considered as biological control agents. The unique character of fungi as compared to bacteria and viruses is that they penetrate through the insect cuticle. They do not have to be consumed to exert an effect. This character makes them virtually the only group of insect pathogens with potential against insect species sucking phloem or cell contents such as aphids, whiteflies, thrips and mites, important pests in agriculture and horticulture. The main drawback of fungi as direct pest control agents is that many species depend so much on proper environmental conditions. Humidity must be high for prolonged periods of time and temperatures moderate to high. Given the proper conditions the effect of fungi on insect populations can be fast and spectacular.

Deuteromycotina

The most important groups of insect pathogenic fungi presently used in biological control are species of Deuteromycotina (imperfect fungi). Their infective units are condidial spores. *Beauveria bassiana* and *B. brongniarti* are two intensively studied mycopathogens of soil insects that belong to this group of fungi. The first species is frequently found on many species of insects, the latter is particularly associated with scarabid species. Commercial products have been reported from China and the former USSR for control of corn borer and various other lepidopteran pests, and Colorado beetle. In Europe much attention has been given to the control of the cockchafer, *Melolontha melolontha* with *B. bassiana* and *B. brongniarti* but this has not led to commercial development so far. *Metarhizium anisopliae* is common pathogen of many soil insects although it may also infect species living on plants or in trees. The list of host species is long. *M. anisopliae* can be seen as a large group of pathotypes, each with its own specificity. In Brazil this fungus is used for control of spittlebugs in sugarcane. In local communities it is produced on simple media such as rice. In Europe it is regarded as a potential candidate for control of vine weevil in ornamentals strawberries and vineyards. The use against termites and locusts looks promising. Major problems with *M. anisopliae* are the relatively poor spread of spores in soil and its dependence on environmental conditions for infection. *Verticillium lecanii* is associated with aphids, white flies and thrips. Various strains or pathotypes are more or less specific towards each pest insect group. The fungus is often applied on foliage. For successful use high humidity levels for 24-48 hours after application are required. *V. lecanii* was developed into commercial products to control whitefly, aphids and thrips in glasshouse crops in Europe. The fungus is either sprayed with conventional sprayers or applied at planting by dipping transplants in a spore suspension. Care must be taken to actually hit the target organisms with spores. The use of *V. lecanii* takes advantage of the relatively high humidity levels inside a glass house and the possibilities to manipulate the climate. The fungus is produced in a fermentor and spores are harvested. Several other Deuteromycotinae contain promise. *Nomuraea rileyi* is a potent pathogen of lepidoptera, in particular noctuid larvae. A fungus effective against whitefly larvae at lower humidity levels is Aschersonia aleyrodis. *Hirsutella thompsonii* is an effective against the citrus rust mite.

Zygomycotina

The most important family for biological control is that of the Entomophthorales (Samson *et al.*, 1988; Zimmerman, 1986). They often cause spectacular epizootics wiping out complete populations of aphids. Infected insects often look spectacular. From all the intersegmental parts of the infected animals sporophores radiate outward. Killed insects often stick to the vegetation. Spores are actively discharged into the air after the death of the host. Important genera are Entomophthora, Conidiobolus, Erynia, and Zoophthora with activity against aphids, flies, and caterpillars. No products have be en developed so far because it is difficult to produce infective conidia or hyphal bodies that will quickly develop into conidia after application. Earlier attempts to produce and use resting spores have failed. The family contains many species which are yet undescribed. In recent years there is a trend to modify the environment in order to stimulate the effect of naturally occurring entomophthorales.

Mode of action

The infective stage is the spore, which attaches to the insect cuticle. Spores of Entomophthorales stick to the insect's cuticle. The spores germinate and the cuticle is penetrated by the germ tube. The process of adhesion and penetration includes mechanical and enzymatic processes. In the haemocoel fungal growth takes place as mycelium, hyphal bodies or wall-less protoplasts. The immune response of the insect has to be overcome. The infection process ends with complete colonisation of all host tissue, usually within a few days. Finally infective spores are produced and released. For winter and dry period survival several insect pathogenic fungi produce resting spores inside the dead host body.

Production

Entomophagous fungi can be produced in two ways, by simple methods in plastic bags using sterile rice, grain or other products as medium, or in liquid fermentation on well-defined media (Prior, 1989). Either way large quantities of spores are required to get good control under field conditions, generally in the order of 10^{14} spores per hectare or more. To produce such quantities at least 100 kg of grain or rice may be needed.

INSECT PATHOGENIC NEMATODES

Nematodes from some 10 families have been reported as entomopathogenic. Research on the use of nematodes for biological control focuses on three families: Steinernematidae, Heterorhabditidae and Mermithidae. The latter group has primarily been used for control of mosquito larvae, but problems in field efficacy and mass production have reduced the interest. Research and commercial development focuses on the genera *Steinernema* and

Heterorhabditis, characterised by their symbiotic association with bacteria of the genus *Xenorhabdus* and *Photorhabdus*. *Steinernema* species occur more commonly in the soil and have a longer persistence after application than *Heterorhabditis* species but the latter are more mobile and have superior host searching activity. *Heterorhabditis* species are more difficult to produce, mainly because of their more specific relation with the symbiont *Photorhabdus*.

Mode of action

The infective stage of the nematodes is a second or third instar juvenile that is free-living in the soil. The juveniles of most species have a size of 500-800 μm and carry the symbiotic bacteria in their gut. The juveniles actively search for host insects reacting to carbon dioxide and other excretion products of potential hosts. The juveniles enter the host through one of the natural openings i.e. mouth, anus or spiracula. *Heterorhabditis* species are also reported to pass through the cuticle. Once inside the haemocoel the nematodes release their symbiont. Then a battle starts between the immune system of the insect and the invading nematodes and he symbiotic bacteria as well as other micro-organisms that entered the haemocoel through the hole made by the nematode. The nematodes excrete metabolites that repress the immune system of the host so that *Xenorhabdus* or *Photorhabdus* can develop. The symbiotic bacteria excrete toxins that kill the insect within one or two days and produce antibiotics that repress and kill the other micro-organisms invading the haemocoel. In most cases the symbionts colonises the whole cadaver and the nematodes begin to develop and feed on the bacteria. After two to three weeks the whole cadaver is exploited and two to three generations of nematodes have developed. A normal sized caterpillar will then yield some 100,000 juveniles that each again carry their symbiotic bacteria with them. Each species is associated with a specific symbiont species.

Biological control

Nematodes are usually applied against soil insects although some successful attempts have been made using them against tree-boring insect larvae, and spraying them onto foliage against caterpillars. The prime target for use of nematodes, in particular *Heterorhabditis*, are vine weevil larvae in pot plants, strawberries and cranberries. For grass grub control the nematodes can be sprayed on top of the soil. Entomophagous nematodes are compatible with several chemical pesticides (Rovesti & Deseo, 1991). Usually a dose of 0.3 to 1 million nematodes per sq. meter are required to give adequate control. At a price of 0.1-0.5 US$ per million the costs of treating a hectare are high. This limits the use of these nematodes to high cash crops such as pot plants or cranberries. The survival of the nematodes is rather short, although small numbers can still be found even years after application. The vast majority disappear within a week or two. Soil humidity and soil structure are important for treatment efficacy and nematode survival. In sandy soil nematode mobility and control efficacy are generally better than in loamy soil. The temperature range preferred by most nematode species is between 12 and 25° C. Although nematodes will spread and multiply after release and should show potential for inoculative release and long term control they are generally applied inundatively.

Mass production

Mass production of nematodes can be done on insect larvae but most commercial production is done using either Bedding's method or liquid fermentation (Gaugler & Kaya,1990). Bedding's method consists of mixing chicken or liver parts with oil and sponge in a container. After development of the *Xenorhabdus* bacteria the nematodes can be added. Development of juveniles takes several weeks. The liquid fermentation methods use the same principle of first growing *Xenorhabdus* and later adding the nematodes. Up to 300.000 juveniles per ml can be produced.

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