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Biopesticides: An Overview

60 Scientists have known since the 1890s that insects are vulnerable to diseases. However, it was not until the early 1950s that field demonstrations by Steinhouse in California led to the commercial production of biopesticides. The federal government registered the first microbial product in 1948, the bacterium Bacillus popillae, to control the Japanese beetle in turf. Although many entomopathogens of insects have been isolated and described, only a few have any real potential as microbial pesticides. Interest in microbials has accelerated since the late 1960s for several reasons: 1 environmental concerns due to dependence on chemical pesticides and their effect on groundwater pollution, residues on food crops, and nontarget organisms; Z development of resistance to chemicals; 3 interest in integrated pest management; and 4 recent developments in biotechnology; i.e., recombinant DNA technology.

Most entomopathogens must be ingested in order to cause an infection. The exceptions are the fungi, which infect externally and the nematodes which actively seek out and attack their host. Some may question whether nematodes should be considered as an entomopathogen; however, most insect pathologists do include them in this category and they are being actively commercialized for control of soil insect pests. Although some organisms such as bacteria and fungi can be produced in liquid culture, the viruses and microsporidia are still produced in vivo.

The speed of kill by biopesticides is slow as compared to most chemical pesticides. This is a problem in the eyes of the public who have been conditioned to the fast-acting results provided by chemical pesticides. There is a recognized need to educate the public about the mode of action of microbial pesticides and their potential use in integrated pest management systems. Characteristics of the major groups of entomopathogens that are used as biopesticides are discussed in the following sections.

NEMATODES

Nematodes occur naturally in soils and they possess a very wide host range. They are relatively easy to mass produce and apply, however, their persistence in soil is limited to a few weeks. Since they are exempt from Environmental Protection Agency (EPA) registration requirements, they are being actively pursued by industry as a control alternative. Several laboratories are focusing on application technology for using nematodes against soil insects, and they are providing new formulations that include nematodes encapsulated in calcium alginate gels or desiccated species applied with baits. Results from field trials using nematodes have been inconsistent. The soil system as a medium is very complex, consequently moisture, pH, texture, and antagonistic organisms can effect the efficacy of nematodes individually or collectively.

FUNGI

There are about 750 species of fungi that are known parasites or pathogens of arthropod pests in terrestrial and aquatic systems. Fungal epizootics can sometimes decimate populations and their effect can be very dramatic. Fungi are unique in that they infect through the cuticle rather than per os, so they have potential use against insects with piercing/sucking mouthparts.

There are ten genera that are amenable to semisolid fermentation and are being mass-produced by industry and government agencies throughout the world. There is a concerted effort by industry in the U.S. to develop Beauvaria bassiana as a soil biopesticide. It is being used against the pecan weevil in Georgia, the lesser cornstalk borer in Florida, as a prophylactic treatment on cottonwood cuttings in nurseries, and even in gallery injections for pests such as the carpenterworm. Beauvaria bassiana is registered in France by a company called Calliope. Another company in Colorado has recently requested permission from EPA to conduct small field testing of a Brazilian isolate of B. bassiana for control of the fire ant. Unfortunately, as in the case of many microbials, levels causing excellent mortality rates in the lab have not been efficacious in the field. Microhabitat conditions, especially temperature and relative humidity, are critical for germination and infection by fungi and frequently compromise field efficacy.

PROTOZOA

Among the protozoa, the only group considered to have potential as a biopesticide is the microsporidia. Microsporidia generally produce chronic rather than acute disease in insects, consequently their effect on populations is not as dramatic as the epizootics caused by bacteria, fungi, or viruses. However, they do cause debilitating effects on pests such as prolonged development, reduced fecundity, and, in some cases, behavioral changes. Microsporidia are reported to act as a stressor in insect populations, thus predisposing individuals to attack by other organisms such as viruses. Many microsporidia are vertically transmitted transovarially to subsequent generations, which is a desirable characteristic not common to other entomopathogens. They are known to infect over 100 different species of mosquitoes and several major forest defoliators such as the spruce budworm, gypsy moth, and forest tent caterpillar.

One species, Nosema locustae, is registered in the U.S. as a bait formulation for grasshopper control. However, microsporidia, like nematodes and fungi, probably have greater potential when used in inoculative or augmentative releases to effect classical biological control.

BACTERIA

Among the entomopathogens, bacteria and their toxins are the subject of most interest in the field of biotechnology. One species, Bacillus thuringiensis (Bt), is an ideal organism for large-scale commercial production because it can be produced in submerged cultures with standard methods and fermentation equipment. Annual sales of Bt have been estimated at $35-45 million per year representing approximately one percent of the $5 billion in pesticides marketed worldwide.

The commercialization of Bt expanded in the late 1960s with the isolation and development of the HD-1 Kurstaki strain. This strain, which was approximately 15 times more efficacious than other available strains, was accepted as the International Standard and is recommended for use against at least 50 different lepidopteran pests.

Over the years, the acceptance and use of Bt products has been hindered by their inconsistent performance in the field. This in turn has been reflected by the emergence and departure of several Bt producers in the past five to ten years.

The interest in and development of Bt related research has exploded in recent years, due mainly to the isolation of new strains, the emergence of genetic engineering, and our interest in the delta endotoxin, which is produced in the fermentation process. Bacillus thuringiensis is a spore-forming bacterium that, when cultured under the appropriate conditions, forms a crystalline parasporal inclusion body called a crystal protein which contains the delta endotoxin. Usually one crystal is produced per cell, although in some strains there are up to three crystals per cell. One strain, Bt var. israelensis, exhibits a high level of insecticidal activity for mosquito and black fly larvae. It has been used successfully against both pests in Africa, Germany, and in abatement districts in the United States and is extremely important in public health programs. Other strains have been isolated and recently registered by EPA that are active against Coleoptera (Bt var. tenebrionis and var. San Diego). There is a flurry of commercial interest to develop and evaluate these strains against the Colorado potato beetle, the elm leaf beetle, the yellow mealworm, and other coleopteran pests.

A tremendous amount of effort is going into the research and deveopment of Bt. For example, certain strains can be induced to produce 25 to 30 times the normal amount of endotoxin by modifying the culture media or temperature; other strains have been developed with toxic proteins that decompose more slowly in the environment. A combination of strains has been found to be synergistic against hard to kill species; sprayable, starch-encapsulated formulations are now being developed for use against the corn borer. These formulations protect Bt from ultraviolet radiation and can also be used to incorporate phagostimulants. Both these processes have been known to enhance persistence and effectiveness in the field for up to 12 days.

One commercial biotechnology firm has developed a novel insecticidal delivery system for the delta endotoxin, called MCAP®. The toxin is microencapsulated within a nonviable cell of Pseudomonas fluorescence, which is a soil inhabiting, plant colonizing, nonpathogenic microbe. This process seems to enhance field persistence. Genetic engineering technology is also capable of producing recombinant organisms of P. fluorescence and Escherichia coli.that express the delta endotoxin of Bt. Some foresee the day when many major insect pests will have a tailor-made Bt product available for use against it.

INSECT-RESISTANT TRANSGENIC PLANTS

Molecular biologists using gene insertion techniques have produced a third approach to pest control—plants that produce insecticidal or antifeedant proteins continuously in the field. The first prototype products, tobacco and tomato plants that produce delta endotoxins of Bt to control larvae of the hornworms, have already undergone one or two seasons of field trials. Field tests of genetically engineered cotton have been approved by APHIS (the Animal and Plant Health Inspection Service). The agency is currently reviewing applications for field trials using soybeans, alfalfa and potatoes. Based on experience with traditionally bred crop lines, it is projected that the first genetically engineered insect resistant seed will reach the marketplace between 1992 and 1995.

There are both advantages and disadvantages associated with the development of transgenic plants. From the grower's perspective, there would be a reduction in application costs, equipment, chemicals, and the application itself. The protection would be effective independent of weather conditions and there would be better plant coverage, especially of those tough-to-reach plant parts. All of this would translate into greater profits. From industry's perspective, the cost to discover, develop, register, and produce a new chemical is estimated at about $25 million and up. Conversely, the cost of a new crop variety has been estimated to be closer to $ 1 to $2 million. From the environmentalist's perspective, there is no concern about spray drift, groundwater contamination, and effects on nontarget organisms because the endotoxins are part of the plant tissue. Documenting the safety to humans will be easier, since an inserted gene would be fully characterized and there would be no need for residue analysis or toxicology.

Regarding disadvantages, there is concern that resistance may develop sooner since the toxin will be exposed continuously to the target pest; some evidence of this has been reported when Bt was used against the Indian meal moth in storage bins. Some regulatory uncertainties still exist that could make the burden of registration potentially prohibitive. Theoretically, a modified crop could be considered a pesticide by EPA, a food additive by the Food and Drug Administration (FDA), and a plant pest by APHIS.

Currently, there are some limitations concerning the insertion of genes into major crops, particularly grains. There are also some questions regarding patent availability and whether or not an invention or investment could be protected.

VIRUSES

More than twenty groups of viruses are known to be pathogenic for insects. Of these, the most interest has been directed toward nuclear polyhedrosis viruses (NPVs), and, to a lesser degree, the granulosis viruses. These viruses belong to the family Baculoviridae, and are referred to as baculoviruses. The virions, or infectious agents, are cylindrical and enclosed within an inclusion body that is polyhedral in shape, thus they are called polyhedral inclusion bodies or PIBs. These inclusion bodies are similar to a bacterial spore or a fungal conidium in that they are resistant to desiccation, very stable, and thus can be stored in a viable state for many years.

Disease caused by viruses are usually fatal. Death of larvae usually occurs three to six days after the first symptoms appear, however this may be delayed for several days under varying meteorological conditions in the field. Epizootics caused by viruses, especially in forest insects, are dramatic and frequently cause total collapse of populations. Unfortunately, these epizootics usually occur only after very dense, defoliating populations are stressed by lack of suitable host foliage; by this time the damage and impact caused by the pest population has already been realized.

The first virus registered in the U.S. was Elcar® (1975) for control of Heliothis sp. on cotton, however since then, commercialization of viruses has been at a standstill. Subsequently, the federal government was involved in the development and registration of three forest insect viruses. The reluctance of industry to develop and register viruses can be attributed to their host specificity and the lack of predictable and expanding markets for viral products. On the other hand, more than 150 commercial laboratories are using baculoviruses as an expression vector system to manufacture proteins. Viruses can be engineered to produce massive amounts of protein in a short period of time. Some recent development in the use of baculoviruses are listed below:

—The University of California has recently obtained an experimental use permit to evaluate codling moth granulosis virus on pear, apple, and walnut. This pilot production project is a joint venture between a nonprofit grower's cooperative, IR-4, and the California Legislature.

—In many Third World countries, conditions are ideal for developing baculoviruses, because inexpensive labor is abundant; producing viruses in live insects is a very labor-intensive industry. Most of these countries do not have the hard currency available to import chemical pesticides or even commercially produced Bt. There are several good examples where government sponsored farmer cooperatives are producing baculoviruses for the control of agricultural pests. These include the viruses for the velvet bean caterpillar in Brazil, alfalfa looper in Guatemala, beet army worm in Thailand, and the cotton Ieafworm in Egypt.

—Field efficacy of the gypsy moth virus, Gypchek, was improved substantially by the addition of an inexpensive sunscreen to the tank mix. The product, Orzan LS®, is a lignosulfonate and a waste by-product of the pulping industry.

—Agricultural Research Service scientists, in collaboration with industry, have successfully produced quantities of the gypsy moth NPV in a new fat body cell culture system. This could be a major breakthrough in the commercialization of this virus product.

The potential role of biotechnology in the development of baculoviruses is unlimited. Recombinant DNA technology offers many new avenues to improve the pathogenicity and effectiveness of baculoviruses. From 1986 to 1988, scientists in England obtained permits to release a genetically altered baculovirus in a screen-contained small-scale field tests. They inserted an innocuous genetic marker to follow the fate of the virus in the environment and distinguish it from naturally occurring viruses in the field.

In 1989, scientists at the Boyce Thompson Institute for Plant Research received EPA approval to release a genetically disabled isolate of Autographa californica virus, (cabbage looper) into field plots in order to follow its survival and spread under natural conditions; in this case the polyhedrin gene has been deleted. Using genetic engineering, it may be possible to improve viral pesticides by inserting toxin or hormone genes to improve direct toxicity, alter behavior, or arrest development in target pest populations.

REGULATORY ISSUES

Microorganisms intended for use as pesticides are subject to the Federal Insecticide, Rodenticide and Fungicide Act (F1FRA). The guidelines for the registration of biorational pesticides, referred to as Subdivision M guidelines, were issued by EPA in 1982. A revision of FIFRA guidelines which has been pending since 1986, has just been released by EPA and is now available. Some of the requirements for Tier-1 testing have been relaxed, which is certainly good news for those who are trying to register microbial products.

A statement of policy on microbial products of biotechnology and nonindigenous microorganisms was issued in the Federal Register in June of 1986. Microbials are distinguished from conventional chemical pesticides by their unique mode of action, their low use volume, and their target species specificity. Each new variety or strain of microbial pesticide must be evaluated and may be subject to additional data requirements. Genetically engineered organisms used as pesticides will be subject to additional data on a case-by-case basis depending on the organism, the patent organism, and the proposed use pattern.

The major categories of data required will still include product chemistry, wildlife and aquatic toxicology, and environmental fate. Satisfying these data requirements will be expensive and time-consuming. The recent development of new strains of Bt and increased submissions of recombinant products by new biotechnology companies has put tremendous strain on the EPA and has slowed the processing of new registration and experimental use permits. Obviously, the goal of regulatory oversight should be to ensure safety while minimizing unnecessary or counterproductive regulatory burdens.

CONCLUSION

Microbial pesticides can play an important role in pest management systems, either as a principal or supplementary control tactic. However, they are not a panacea and should not be considered as such. There is a need to continue the isolation and evaluation of new and more virulent strains of microorganisms for potential use as microbial pesticides. Along these lines, the introduction of new exotic organisms against native pest insects should be pursued and insect pathologists need to be personally involved in foreign exploration for these organisms. Although there have been a few successful applications of this classical approach to biological control, the approach has been underutilized.

The success in using microbial pesticides has been compromised by a lack of research and development in the area of formulation and aerial application technology; methodologies being used were developed years ago for applying chemical pesticides. The most promising microbial products will fail unless we learn how to apply them properly and enhance their persistence on foliage.