BIOLOGICAL CONTROL
MAKING IT WORK

PART 3: COMMERCIALIZATION OF BIOLOGICAL CONTROL AGENTS

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Biological control of unwanted insects, mites, weeds and plant pathogens can be dealt with in two ways. The first is release of a pest's natural enemy into the environment in order to establish itself permanently in the ecosystem and suppress the pest below the economic threshold level. This strategy has been successfully employed in a number of cases and is used mostly for suppression and control of pests recently imported into a new ecosystem. In most cases, this work is being done either by a government agency or contracted by a government agency to private laboratories. Because biological control using natural enemies is usually a one-time event, it will not be further dealt with in this paper.

The second situation is the control of pests with repeated applications of biological agents. This case is mostly used in agriculture, especially annual crops. The annual agricultural system does not easily lend itself to the establishment of permanent population control because the natural fluctuation of the pest population is too high above the economic threshold. The following comments on commercialization refer only to this type of biological control.

WHERE ARE WE?

Although biological pesticides still account for less than one percent of the total pesticide market, sales of biological pesticides are increasing at a rapid pace, estimated to be between 10 to 25 percent per year. Food safety and other environmental concerns, insecticide resistance, and lack of new chemistries have fueled the growth in biological pesticides. Biologists are being integrated into integrated pest management (IPM) systems which are being used in conjunction or in rotation with chemical pesticides.

Microbial pest control agents including plants genetically engineered to produce pesticidal chemicals represent the largest number of recent registrations obtained by the industry. Presently there are 19 microbial pesticides registered with the Environmental Protection Agency (EPA) (Table 1). Since 1984, EPA has reviewed 44 applications for small-scale field testing of genetically modified microbial pesticides. Since 1986, EPA has reviewed 40 applications for small-scale field testing of transgenic pesticidal plants.

Table 1 EPA registered microbial pesticides

Microorganism	Year Registered	Target Pest
Bacteria		
Bacillus popilliae/B. lentimorbus	1948	Japanese beetle larvae
B. thuringiensis Berliner	1961	Lepidoptera larvae
Agrobacterium radiobacter	1979	Crown gall disease
B. thuringiensis israelensis	1981	Mosquito/blackfly larvae
B. thuringiensis aizawal	1981	Wax moth larvae
Pseudomonas fluorescens	1988	Pythium/Rhizoctonia
B. thuringiensis tenebrionis	1988	Certain beetle larvae
B. thuringiensis san diego	1988	Certain beetle larvae
B. thuringiensis EG 2348	1989	Gypsy moth larvae
B. thuringiensis EG 2371	1989	Lepidoptera larvae
B. thuringiensis EG 2424	1990	Lep/Coleop larvae
Viruses		
Heliothis nuclear Polyhedrosis Virus (NPV)	1975	Heliothis complex
Tussock moth NPV	1976	Douglas fir tussock moth larvae
Gypsy moth NPV	1978	Gypsy moth larvae
Pine sawfly larvae	1983	Pine sawfly larvae
Fungi		
Phytophthora palmivora	1981	Citrus strangler vine
Colletotrichum gloeosporoides	1982	Northern joint vetch
Trichoderma harzianum/ Trichoderma polysporum	1989	Wood rot
Protozoa		
Nosema locustae	1980	Grasshoppers

Bacteria—Although more than 100 bacterial species have been identified as insect pathogens, only certain Bacillus species have enjoyed commercial success.

Of these, *B. thuringiensis* has been most widely exploited. In addition, *B. popilliae* has been sold for many years to home gardeners to control Japanese beetles. *B. sphaericus* has been developed into a commercial product for mosquito control by Abbott Laboratories.

Table 2 summarizes commercial Bt based products. Never before has there been such interest by industry in Bt as evidenced by the proliferation of start-up companies (Ecogen, Mycogen, AGC, PGS, etc.) and large agro-chemical companies developing Bt based products. However, Bt based products still have a number of constraints, which the various companies are addressing through a number of strategies.

Table 2 commercial *b. thuringiensis* based pest control agents

Organism	Target Pests	Commercial Products	Company
B. thuringiensis			
var. *kurstaki*	caterpillars	Dipel	Abbott
		Thuricide/Javelin	Sandoz
		Biobit/Foray	Novo Nordisk
		Bactospeine/ Futura	Duphar
		Cutlass/Condor	Ecogen
		MVP	Mycogen
		Agree	Ciba-Geigy
		Wormbuster	Bactec
var. *israelensis*	Mosquitoes/ Blackflies	Vectobac	Abbott
		Teknar	Sandoz
		Skeetal	Novo Nordisk
		Bactimos	Duphar
	Fungus Gnats	Gnatrol	Abbott
var *tenebrionis*	Colorado Potato Beetle	Novodor	Novo Nordisk
		Trident	Sandoz
		Foil	Ecogen
var. *san diego*		M-One/ M-One Plus	Mycogen
var. *aizawal*	waxmoth	Certan	Sandoz

Narrow host range—Although the narrow host range is seen as an advantage economically, it often limits the market potential for Bt products. To address this issue, Ecogen developed Foil®) a product for controlling European corn borer, a Lepidopterous pest and Colorado potato beetle, a Cleopteran pest on potatoes. Through plasmid curing and transfer, a 150 Md plasmid from a ***kurstaki*** strain and an 88Md plasmid from a ***tenebrionis*** strain were combined into one organism.

Ecogen, Ciba-Geigy and Sandoz-Repligen scientists have used various molecular techniques (electroporation, transconjugation, etc.) to develop products combining genes to increase the activity on key Lepidopteran pests such as armyworm ***Spodoptera*** and cotton bollworm ***Heliothis spp.*** In addition, fusions of baculovirus with Bt ***tenebrionis*** genes and Bt ***israelensis*** with Bt ***kurstaki*** genes are being used to expand the host range. These products are in various stages of development and commercialization.

Several companies have amassed large collections of Bt isolates from soil, plants, insects, grain dust and other environmental niches, with the objective of finding strains with enhanced activity on key pests and new activity on pests such as corn rootworm. After bioassays of 7000 isolates against ***Diabrotica undecimpunctata,*** Ecogen reported to have discovered a weakly active strain. ICI (Imperial Chemical Industries) also reported a similar discovery.

Poor residual activity—It is well known that Bt lasts only a few days on plant foliage under typical field conditions due to UV degradation, rainfall, etc. Mycogen uses ***Pseudomonas*** as a delivery system for Bt genes (***kurstaki*** for MVP®)and ***san diego*** for M-One Plus®)). The Bt bearing ***Pseudomonas*** is killed (to avoid regulatory hurdles for registering recombinant microorganisms) and sprayed on the crop like other Bt products. The Pseudomonad wall is reported to protect the Bt protein from environmental degradation, thus providing longer residual activity.

The USDA research laboratory in Peoria, Illinois has developed a starch encapsulation method for protecting Bt and viruses in the field. Field trials of experimental products are being conducted.

Novo Nordisk's Foray®>for gypsy moth control has a unique formulation designed specifically for aerial applications to forest canopies. The formulation has unique rainfast, palatibility, and settling properties.

Inconsistent control on cryptic insects—Because Bt must be ingested to act on the insect, there are a number of fruit feeding insects that are difficult to kill under field situations. For example, the cotton bollworm takes only a few bites of cotton tissues before entering the square. A number of companies have chosen to engineer Bt gene(s) directly into the plant or into plant colonizing microorganisms to address this problem. Monsanto, PGS, Agracetus, ICI, Sandoz, Calgene and Ciba-Geigy all have plant genetic engineering programs for insect control.

Crop Genetics International has transferred a Bt gene into an endophyte, ***Clavlbacter xyli,*** for control of European corn borer, a stem and ear feeding pest. Because of the difficulty in controlling this insect and low cost of corn, insecticides are not widely used on this insect. CGI aims to tap into this market potential with InCide® one of the few recombinant microorganisms approved for outdoor field tests.

Although there could be a number of disadvantages to plant genetic engineering Bt genes, such as resistance development and public acceptance of engineered foods, companies see several advantages in addition to increased efficacy. These are increased potential for a proprietary position, improved residual activity, and reduction in chemical pesticides. In addition, field testing transgenic plants, which is regulated through APHIS, has been considerably easier than field testing transgenic microorganisms through the EPA.

Weak intellectual property protection—An additional constraint to the commercialization of Bt and all other biological agents is the state of our intellectual property protection. Biological control in the future, if it is to go beyond the small niche it occupies today, will need investment by private industry and government agencies. Private industry is only willing to make those investments if it can enjoy protection from competitors granted through the patent process. However, while the patent process is very clear and very well understood for chemical pest control agents, it is not clear for biological agents, and a large backlog in processing biotechnology patent applications has not helped industry gain the confidence that it will have a guaranteed return on investment.

VIRUSES

There are currently five insect viruses registered in the United States (Table 1). Commercialization of viruses has lagged behind Bt primarily because of the lack of an economical *in vitro* production system. Large agrochemical companies (with the exception of Sandoz's former entry into Elcar® production) have avoided developing *in vivo* rearing production systems. Progress being made in *in vitro* production system is largely being driven forward by pharmaceutical applications for baculovirus expression of proteins. One company, American Cyanamid, is developing *in vitro* production for an insect (gypsy moth) virus.

Espro, a small company based in Maryland, is producing GypChek® for the U.S. Forest Service. Espro has developed an economical *in vivo* production system for this virus, and hopes to expand into other viruses for control of codling moth, *Heliothis,* and *Spodoptera.*

Lou Falcon, from the University of California at Berkeley, is producing codling moth virus for sale under the Experimental Use Permit (EUP) to fruit growers in California and Washington. Registration is expected in 1991, and the virus will then be available for licensing to a company for large scale commercialization. This virus is already registered and produced in a number of European countries.

In addition to a lack of large-scale economical *in vitro* production methods, insect viruses have many of the commercial weaknesses as Bt. Baculoviruses have an even narrower host spectrum and take longer to kill than Bt. Like Bt, they must be ingested and also rapidly degrade on plant foliage. A number of approaches are being developed to address these weaknesses, with much of this research being conducted at universities. Several groups are exploring insertion of insecticidal protein genes (Bt, juvenile hormone, insect specific neuropeptides) into the viral genome in order to reduce the time to kill the insect. In addition, gene deletions (Louis Miller, University of Georgia) and viral proteins (Robert Granados, Boyce Thompson Institute) have been shown to increase the unit activity of certain viruses. Researchers at the Boyce Thompson Institute performed the first outdoor field test of a genetically altered virus.

FUNGI

Fungi have been developed into very few commercial products. Although many fungi are known to be very effective against insects in laboratory tests, efficacy in field situations has not met expectations. To address the problem, EcoScience, a Massachusetts based company, has developed bait and trapping techniques for delivering well-known, but effective insect pathogens such as *Metarhizium* and *Beauveria* against flies and cockroaches. Products based on these bait traps should be available in the early 1900s.

Fungi for agriculture have not been exploited due to lack of formulation and delivery technology that provides protection against desiccation, wind, rain, UV light, etc.

PROTOZOA

The only commercial product based on protozoans is *Nosema locustae* for grasshopper control. Protozoa are not viewed by the industry as having commercial potential because of the chronic nature of the activity and requirement for *in vivo* production.

ENTOMOGENOUS NEMATODES

Two companies have invested considerable resources into development of nematode based products. These are Biosys (Palo Alto, CA) and BioEnterprises (Hobart, Tasmania). Biosys sells BioSafetS) a home and garden product for catalog sales and for cranberry weevil control and BioVector®) for citrus weevil control. A grub product is in development with Ciba-Geigy. Biosys markets *Steinernema* spp. produced in liquid fermentation at a fermentation plant in Alberta Canada.

BioEnterprises, owned by Biotechnology Australia and Hoechst sells Heterohabdltid nematodes produced in solid culture for home and garden and nursery applications in Australia and Europe. Products for U.S. turf markets are being developed in conjunction with ChemLawn.

Development of nematode products has been slow, although considerable progress in commercialization has occurred in the last five years. Limitations continue to be economic production, shelf life and quality control.

FUNGICIDES

A number of companies were developing biofungicides in the early to mid-1980s. Much of the development centered around plant colonizing bacteria, such as *Pseudomonas,* which could be engineered to improve fungicidal activity, contain new pesticidal genes or improve plant colonizing characteristics.

Very few companies are still involved in this type of biofungicide research and development. Expectations for the technology were higher than could be delivered in the short time frame planned for commercial development of the products. In addition, recombinant microbial pesticides encountered considerable regulatory and public acceptance hurdles (ice-minus, for example) making continued development too costly for many companies.

Despite the setbacks, there is a core of university and USDA researchers that are pushing forward the biofungicide area. In addition, W. R. Grace, Kodak and Gustafson have commercialized biofungicides for greenhouse use and as seed treatments.

HERBICIDES

Sandoz, Mycogen and Crop Genetics International have active programs in bioherbicide development. Dow-Elanco is commercializing bioherbicide products in Canada. Bioherbicides have not had as much attention as bioinsecticides because of the availability of inexpensive, relatively safe and very effective chemical herbicides. This will continue to be the situation until the market situation changes.

WHERE ARE WE GOING?

Biological pesticides will continue to increase in importance relative to chemical pesticides, but will not replace the need for chemical pesticides. Biological insecticides will remain the most important segment, followed by biofungicides and then bioherbicides.

Biological pesticides have been increasing in efficacy while becoming competitive in price with chemical pesticides. This trend will continue, increasing the grower acceptance of biological products. In the next decade, we will see a mix of naturally occurring, genetically modified (developed through conjugation, plasmid curing, classical mutation, etc.), genetically biologicals, transgenic pest resistant plants and chemicals integrated in pest management systems.

HOW CAN WE GET THERE?

For biologicals, whether naturally occurring or engineered, to reach their full potential, there are some key areas for continued focus:

1— Clear, consistent federal regulation of microbial pesticides in the existing federal regulatory framework.

2— Regulatory focus on the pesticide product, not the process by which it was produced.

3— State legislation consistent with federal regulation.

4— Public understanding and acceptance of products developed through non-traditional methods (recombinant DNA techniques).

5— Adequate state and federal funding for basic university research (mechanism of action of Bt and viruses, physiology of entomogenous nematodes, Bt resistance monitoring and management strategies).

6— Economic incentives for development of scale up technologies for insect viruses, predators, parasites, fungi and nematodes in order to make the products more attractive to industry.

7— Increased research and development of formulation and application technology of microbial products.

8— Increased grower education of the differences between chemicals and biological products (necessity for increased scouting, timing, attention to application, etc.).

9— Clear intellectual property protection policy by the Patent Office, expedited treatment of patent applications, and a guaranteed lifetime of those patents in a way that industry can recoup its money.

Note: Points 1-3 and 6 are addressed in the President's Council on Competitiveness: Report on National Biotechnology Policy (February, 1991). This is a very positive sign for the future commercialization of microbial and recombinant plant products.