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Microbial Insecticides
Special Focus on Bacillus Thuringiensis

> **ISSUE**: Genetic engineers are using the toxic protein of the bacteria, *Bacillus thuringiensis*, to develop insecticidal plants and new, more effective biocontrols for agriculture.
> **IMPACT**: The widespread introduction and use of genetically-modified plants containing the B.t. endotoxin gene may lead to rapid pest adaptation and eventual loss of this effective biological control. Early field tests of plants containing modified B.t. endotoxin gene also raise ecological concerns about the possible impact of B.t. on non-target species and the environment.
> **PARTICIPANTS**: Both small biotech companies and transnational agrichemical corporations are developing B.t.-based bioproducts (see companies listed in appendix)
> **WHEN**: New B.t. products are already available; transgenic plants containing B.t. endotoxin gene are now being field-tested.
> **ECONOMIC STAKES**: Worldwide market for all microbial insecticides could grow to (US) $6-8 billion by end of century.

Microbial insecticides are microbes or microorganisms (mainly bacteria, fungi, viruses) which are used to control insect pests. These organisms often produce toxins that are generally considered harmless to people, animals and the environment.

Biological pest controls have been marketed for several decades with limited commercial success. Since they are typically insect specific, less potent and less persistent than chemical pesticides, microbials have failed to capture even one percent of the annual insecticide market in the U.S. With the advent of biotechnology, however, there is renewed interest and potential for developing new microbial pesticides. According to one industry spokesman, "Biotechnology has done for microbial pesticides what the transistor did for electronics."[1]

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Both economic and environmental pressures are fostering heightened interest in the development of commercial biopesticides. For one thing, a growing number of once highly effective chemicals have become useless because of the alarming increase in the resistance of insects to synthetic pesticides. And the cost of developing new chemical products has soared. It takes 8 - 12 years for a new pesticide to reach the market, and an investment of $35-$40 million.[2] By contrast, commercialization of bioinsecticides requires less than $5 million and only about 3 years.[3] Coupled with growing concerns about the health and environmental problems associated with chemical pesticides, there is a huge, potential market for safe, effective biocontrols. According to biotech industry analysts, the market in the Western world for biopesticides is only $33 to $45 million, but could grow to $6 to $8 billion by the end of the century.[4]

The transition from chemical to biological pest control "is definitely coming," according to William Marshall, president of Pioneer Hi-Bred International's microbial genetics division. Marshall told Chemical Week magazine that, "...in 30 years you won't see chemical pesticides as we know them today."[5]

Bacillus Thuringiensis

An estimated 95% of the commercial biotech research on microbial insecticides focuses on the bacterium, *Bacillus thuringiensis*, a naturally-occuring microbe which lives in the soil and in insects.[6] B.t. endotoxin is a toxic protein produced by the bacteria, *Bacillus thuringiensis*. When certain insects ingest B.t., the protein is turned into a toxin by enzymes in the insect's stomach, causing paralysis and death.

B.t. is not new. It has been used as a commercial biological control in the U.S. since 1970, and is lethal to caterpillars. (B.t. is effective against more than 50 lepidopteran pest species (e.g. larvae of moth and butterflies). Some strains of B.t. kill beetles, others kill flies and mosquitoes.

A long list of small biotech companies and transnational agrichemical corporations are now using genetic engineering to develop a new generation of more potent and effective B.t. products--including genetically-engineered plants containing built-in "insecticidal" genes that produce the B.t. endotoxin. According to one industry observer, companies are scrambling to get on the B.t. bandwagon: "Anyone in ag. chemicals who doesn't have an interest in B.t. is in trouble--they better be on board or they're out of luck."[7]

B.t. and Genetic Engineering

Current research on B.t. focuses not only on novel means of using more potent strains of B.t. to kill a wider variety of insects, but also new ways to deliver the bioinsecticide to the field and insect-resistant plants. The following examples illustrate five different techniques for using the genetically-engineered B.t. endotoxin gene to combat insect pests:

1) **Spores and Crystals** -- This is the conventional way in which B.t. is used as an insecticide (usually sprayed on the crop). When B.t. sporulates, the spores contain the protein which is toxic to insects. The insects are killed when they consume the spores. Using genetic engineering, scientists have modified the bacteria so that it produces ten times more endotoxin.

2) **Bioencapsulation** -- Mycogen has pioneered a new biopesticide delivery system system called MCap, which encapsulates the B.t. endotoxin inside a dead cell. The endotoxin gene is moved into a Pseudomonas bacteria. The bacteria is then treated so that the cells containing the bioinsecticide are killed, but the endotoxin is encapsulated and "fixed" inside. Within the capsule, the B.t. endotoxin is protected from degradation by ultraviolet light, and therefore persists in the field longer than conventional B.t. products. Even though MCap is produced from a genetically engineered organism, the product was rapidly approved by the U.S. Environmental Protection Agency (EPA) because the organism is dead when applied to crops.

3) **Epiphytes (microbes that colonize the roots or leaves of plants)** -- Since many insects feed on the roots of plants, Monsanto developed a technique which uses the B.t. endotoxin gene to provide natural protection against soil-dwelling insects that feed on roots. Since B.t. does not naturally colonize plant roots, however, Monsanto scientists moved the B.t. endotoxin gene into a root colonizing bacteria (Pseudomonas). If approved for commercial sale, these microbes would be applied to the seed, either by the seed manufacturer or by the farmer, before planting.

4) **Endophytes (microbes that live inside plant tissue)** -- Crop Genetics International is currently field testing a B.t.-derived insecticide which is designed to kill corn earworms on corn plants. The company has genetically-engineered an endophyte (Clavibacter xyli bacterium) to contain the B.t. endotoxin gene. When inoculated into corn seed, the genetically-engineered endophyte multiplies and eventually colonizes the entire corn plant. If successful, the toxin produced by the B.t. gene in the endophyte will kill the corn borer when it feeds on the corn plant.

5) **Transgenic Plants** -- Scientists have moved the B.t. endotoxin gene into the cells of tomato, potato, cotton, corn and tobacco plants, thus producing transgenic plants which contain the insecticidal B.t. gene. Companies such as Rohm & Haas, Monsanto and Sandoz have begun field-testing transgenic plants.

"New" B.t.

Recent discoveries of new varieties of B.t. suggest that naturally-ocurring microorganisms found in the soil may provide a treasure chest of microbes with untapped, unknown potential for agriculture.

In 1987, two scientists at the U.S. Department of Agriculture (USDA) announced the discovery of 72 new varieties of B.t. Since only about 24 varieties of B.t. were previously known, the identification of new B.t. germplasm could radically change B.t. history.[9] Using a new technique which isolates B.t. from the soil, the USDA scientists combed through soil samples collected from the U.S. and around the world--including Iceland and Tibet. According to Dr. Russell Travers, "We've observed that some environments, like the Mediterranean, are richer in B.t. than others."[10] But the most potent B.t. strain was found near the airport outside of Baltimore, Maryland, USA.

The discovery of new B.t. increases the chances that future insecticides may come from the soil, rather than the laboratory. Several of the newly discovered B.t. strains are considered 20 times as effective as present commercial strains. Some of the "super strains" may, in fact, be potent enough to compete with synthetic pesticides.[11] In addition, new strains are effective against beetles, thus broadening the potential use of B.t.-derived bioinsecticides.[12]

Patenting B.t.

Even though B.t. is a natural component of many soils, newly found B.t. varieties are all available for patenting and/or commercial licensing. Patents are now pending on three of the new B.t. varieties.

The question arises, should microorganisms become subject to patent protection and commercial exploitation when freely extracted from the soil? If new, naturally-ocurring insecticide genes are derived from Mediterranean soil samples, who "owns" these genes and who should be compensated for their use?

As the raw material for the biotechnology industry, the global debate over ownership and control of microorganisms is likely to intensify. It is these very issues that recently prompted the United Nations FAO Commission on Plant Genetic Resources to consider extending its mandate beyond plant genetic resources to include the broader issues of biological diversity.

Resistance to B.t.

At least 18 U.S. and European companies are pursuing research on a variety of potential products incorporating the B.t. endotoxin gene for use as a microbial insecticide (see appendix).

Crops targeted include tobacco, tomato, corn, cotton, potatoes, sunflowers, citrus, and more.

The good news is that there is a great deal of commercial interest in the development of biological pest controls. The bad news is that scientists are already questioning the long-term efficacy of genetically-engineered biopesticides because of the development of toxin-resistant insects.

People once believed that B.t. was immune to resistance. But in recent years there have been documented cases of insect resistance to conventional B.t. products. Dr. William McGaughey of the U.S. Grain Marketing Research Laboratory in Kansas (USA) reported partial resistance in the stored grain pest, Indian-meal moth.[13]

Scientists now agree that genetically-engineered biopesticides, like their chemical counterparts, will suffer from insect resistance. According to Bio/Technology Magazine, "Mathematical models of selection pressure predict that if engineered anti-pest plants become a permanent part of the environment, insect resistance would develop rapidly."[14]

Current research and development on plants engineered to contain the B.t. endotoxin gene indicate that, in the near future, insect-resistant seeds may be widely introduced. One major market for an effective B.t. toxin, for example, is corn. The European corn borer is the largest uncontrolled insect in the United States; farmers in the U.S. and Western Europe spend about $350 million annually on conventional chemical sprays that are only 50% effective against this caterpillar.[15] If scientists succeed in developing transgenic corn plants containing the B.t. endotoxin gene, corn farmers throughout the U.S. and Europe could be routinely planting "insecticidal corn plants." The problem with the "prophylactic control approach"[16] is that the selective pressure for adaptation would be intense, and the European corn borer would likely develop resistance to B.t. rapidly.

N.C. State University entomologist, Dr. Fred Gould, warns that: "If pesticidal plants are developed and used in a way that leads to rapid pest adaptation, the efficacy of these plants will be lost and agriculture will be pushed back to reliance on conventional pesticides with their inherent problem."[17]

There are a number of strategies which could be adopted to curtail the rapid rate of insect resistance to B.t. Dr. Gould suggests that genetic engineers will someday have the ability to produce crops that express insect-resistance genes only at times and places where they are required. Another approach involves the use of seed mixtures. If only half of the seeds in a field contained genes for B.t.-endotoxin production, for example, the rate of the pest adaptation could be cut by two-thirds or more.[18]

Industry Response

Will companies seeking a much-needed biotech breakthrough and short-term profit heed the warning of scientists and take steps to insure long-term conservation of pest resistance genes? The recent formation of a U.S.-based "Industry Working Group on B.t." suggests that industry recognizes insect resistance to B.t. as a serious problem and a threat to their multi-million dollar research programs.

In 1988, researchers at Monsanto Co. conducted laboratory studies on insect resistance to genetically-engineered B.t. endotoxin. Their results indicate that, in the laboratory, resistance to B.t. develops rapidly.

The "Industry Working Group on B.t." was initiated by Monsanto and now has 27 member companies--18 of which are actively involved in B.t. research.[19] Their goal is to coordinate future industry and university research on B.t. resistance, formulate strategies to maintain effectiveness of B.t., and develop technical guidelines for implementing those strategies.

Ecological Concerns

Throughout the U.S. and Europe there is intense debate about risks associated with the deliberate release of genetically-engineered microorganisms into the environment. Will these organisms survive? Will they multiply? Will they transfer their inserted genetic characteristics to other organisms? Will they be transported to new or unintended sites? These questions and others cannot be answered with scientific certainty.

Although Bacillus thuringiensis is generally regarded as an environmentally safe microbe, the environmental release of altered microbes containing the B.t. endotoxin gene raises many of the same questions.

In 1986, the Environmental Protection Agency denied approval for Monsanto's application to field-test a B.t.-producing bacteria designed to colonize plant roots and kill soil-dwelling insects. One of the concerns involved the potential for harm to beneficial insects that are relatives of these pest insects, such as butterflies, which are important pollinators.

In 1988, Crop Genetics International (Hanover, Maryland, USA) conducted small-scale field tests of corn plants innoculated with a microbe modified to express the Bt endotoxin gene. Since the altered microbe lives only in the plant's vascular system, the company was confident that environmental risk was minimized. However, the company's own data revealed that the altered bacteria containing the Bt endotoxin gene had been found in flea beetles during field tests.[20] Unexpectedly, the B.t. endotoxin gene was transmitted from the plant to an insect feeding on the corn plant.

Could the flea beetle then transmit the B.t. endotoxin gene to another plant or insect?

USDA scientist, Phyllis Martin, describes one possible scenario:[21] "The concern is that if the flea beetle then feeds on other plants, such as a weed species--a weed which is normally controlled by caterpillars, for example, the weed species incorporating the Bt endotoxin gene might no longer be controlled by caterpillars. Or what if a monarch butterfly (or other beneficial insect) were to feed on such a weed and become an unintended target of the insecticidal gene?"

At this point, these concerns are largely theoretical, but they illustrate potential problems associated with widespread release of genetically altered microbes--even those which are considered relatively benign.

Conclusion

The development of safe, effective biocontrols for agriculture is a welcome alternative to synthetic pesticides. But despite the potential benefits, it is clear that insecticidal plants are no panacea for chemical-intensive agriculture. If insecticidal B.t. genes are widely introduced in commercial, homogeneous cultivars, pests will adapt to them and this valuable natural resource will be squandered. A safe and effective biological insecticide could be rendered ineffective and potentially damaging because of over-use or mis-use. Ironically, agriculture could be pushed back to even greater reliance on conventional pesticides.

1 A.D. Stern of Mycogen, quoted in Agricultural Genetics Report, June, 1987, p. 3.
2 Bioprocessing Technology, June, 1988, p. 2.
3 Ibid.
4 "Biopesticides: An $8 Billion Market Potential", Chemical Week, May 4, 1988, p. 35.
5 Ibid.
6 Genetic Engineering and Biotechnology Monitor, UNIDO, July-September, 1986, p. 40.
7 Dr. Russell Travers, Group Manager for R&D for Biocontrol Division, Novo Laboratories, telephone conversation, November 30, 1988.
8 Gould, Fred. "Ecological Considerations in Releasing Genetically Engineered Organisms", presentation before N.C. Biotech. Ctr., Advisory Committee on Biotech. in Agriculture, 28 July 1988.
9 Morrison, Jessica, "Soil Yields 72 New Varieties of a Natural Pest Control," Agricultural Research, January, 1988, p. 14.
10 Telephone conversation with Dr. Russell Travers, Group Manager for R&D, Biocontrol Division, Novo Laboratories, November, 30, 1988.
11 Morrison, Jessica, "Soil Yields 72 New Varieties of a Natural Pest Control", Agricultural Research, January, 1988, p. 14.
12 Agricultural Genetics Report, October, 1987, p.1.
13 McGaughey, William, H., "Insect Resistance to the Biological Insecticide Bacillus thuringiensis", Science, 12 July 1985, p.193.
14 Knight, Pamela, "Biological Controls Squash Insect Pests", Bio/Technology, Vol. 6, October, 1988, p.1137.
15 Agricultural Genetics Report, June, 1987, p.4.
16 Gould, Fred, "Pesticidal Transgenic Plants and the 1990 Farm Bill," from Proceedings of the Annapolis Conference on Transgenic Plants, 7-9 September 1988, p.2.
17 Ibid.
18 Gould, Fred, "Evolutionary Biology and Genetically Engineered Crops", BioScience, Vol. 38, No.1, January, 1988, p.
19 All information on the industry working group comes from personal conversation with Pamela Morrone, Monsanto, January 5, 1989.
20 Sun, Marjorie, "Preparing Ground for Biotech Tests" in Science, 28 October 1988, p. 504 and letter from Crop Genetics Intl. to US-EPA Re: APHIS Permit 87-355-01, 6 September 1988.
21 Personal conversation with Dr. Phyllis Martin, December, 1988.

Bacillus Thuringiensis

Abbott Laboratories (North Chicago, IL, USA)

Advanced Genetic Sciences (Oakland, CA, USA)

Agracetus (Middleton, WI, USA) -- working on transgenic cotton plants containing BT endotoxin gene

Agricultural Genetics Co, Ltd. (Cambridge, UK)

Agrigenetics Advanced Sciences Co. (Madison, WI, USA)--sub. of Lubrizol Corp.

American Cyanamid (Wayne, NJ, USA)-- company has agreement w/ Ecogen, BT insecticides for rice, veg. crops and stored grain.

Crop Genetics Intl. Corp. (Hanover, MD, USA)-- using BT to target European Corn Borer

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ICI plc (London, UK)

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Mycogen (San Diego, CA, USA)-- BT derived insecticide M-One for control of potato beetle, potential $100 million world markt; agreements w/ Lubrizol and Kubota on BT.

Novo Laboratories (Danbury, CT, USA) -- subsidiary of Novo Industri (Denmark)

Plant Genetic Systems NV (Ghent, Belgium) -- agreement w/ Rohm & Haas, BT for cotton, tobacco

Repligen Corp.(Cambridge, MA, USA) -- agreement w/ Sandoz (company partially owned by Sandoz)

Rohm & Haas (Philadelphia, PA, USA)-- agreement w/ Plant Genetic Systems on BT

Sandoz, Ltd. (Basel, Switzerland)-- agreement w/ Repligen; and Sandoz Crop Protection Div. (USA)

Source: Rural Advancement Fund International (Information derived from published materials and industry sources).

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Survey of Companies Developing Bioinsecticides with Bacillus Thuringiensis

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