AGRICULTURAL BIOTECHNOLOGY: a Case Study of Bt Crops

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April 2000

> **"It is humbling and instructive that the most exquisitely specific group of insecticides known originates not from a laboratory, but instead from the common soil bacterium Bacillus thuringiensis. Insecticidal crystal proteins produced by Bt kill insects by binding to and disrupting their midgut membranes. Each of the numerous strains of Bt produces a characteristic set of crystal proteins. Each of these toxins is lethal to certain insects, yet does little or no harm to most other organisms, including people, wildlife and even other insects."**
> **Bruce Tabashnik**
> **Department of Entomology**
> **University of Arizona**
> **1997**

1. INTRODUCTION

Most plant varieties have the ability to resist pests and disease. The mechanisms of resistance can be varied, including structural characteristics of the plant, the production of general metabolites that have toxic properties, or the production of specific toxic substances in response to pest attack. A plant can be completely immune to a pest or it can be partially resistant.

Plant varieties with a greater ability to withstand pests have traditionally been bred from progenitor plants that have high levels of resistance to the target pest. It is now also possible to introduce mechanisms of pest and disease resistance into plants that are not found in the plant kingdom. For example, plants can be genetically engineered to express toxins from invertebrates and microorganisms. These toxins can confer plant resistance to insect attack and disease. Such pesticidal substances can be diverse and can potentially originate from any taxonomic kingdom.

There are a number of types of substances produced in plants that enable plants to resist pest attack and disease. These substances include both those pesticidal substances that would be considered normally a component of a plant and those that would be considered new to a plant. Examples of plant-pesticides that would be considered normally a component of a plant are phytoalexins (plant-produced substances that act against phytopathogenic microorganisms). An example of a plant-pesticide that would not be considered normally a component of a plant is the insecticidal delta endotoxin that is produced in the bacterium, Bacillus thuringiensis.

A range of genetically engineered biopesticides, including pest-resistant plants are currently being developed by the life sciences industry. According to the industry, these biopesticides promise to replace chemical pesticides and thereby reduce the environmental problems associated with the use of certain agro-chemicals over the past 50 years. The industry also claims that biopesticides might be cheaper and easier to use than their chemical counterparts, especially when the toxin is incorporated into the actual plant. There are, however, problems concerning the longer-term biological efficacy of all biopesticides, just as there are with chemical pesticides. Pests are known to develop resistance to all types of pesticides and unless the dynamics of this process are properly studied and understood, *and* the use of pesticides in the field is actually informed by this knowledge and properly controlled, the new biopestides will join their chemical counterparts on the pesticide treadmill, where the industry has to keep inventing new poisons simply in order to maintain the status quo. This outcome is not necessarily contrary to the commercial interests of the life industry and can indeed be economically beneficial to the companies concerned. However, the development of pest resistance to biopesticides will have damaging long term consequences for organic agriculture, the development of sustainable agriculture and low-risk food security programmes. The irresponsible commercialisation of biopestides could also cause irreversible changes in the biosphere. Bt occurs naturally as a soil bacterium throughout the world. Its role in natural ecosystems has not been studied in any detail and remains a mystery but it must be presumed that certain insect populations are naturally reduced or controlled by the presence of Bt. If these insects develop immunity to Bt they will, accordingly, become more abundant. The insects happen to include a number of major crop pests.

Just fifteen years ago, it would have been technically and biologically possible to develop a range of biopestides that would not only have replaced their environmentally damaging chemical equivalents, but could have been used in a sophisticated and sustainable way for the foreseeable future. Bt was the prime candidate for this role but its potential has been all but destroyed by a handful of companies motivated only by short-term economic goals.

This report documents the disaster in order to learn from it, in the hope that the same sorry history is not now repeated with Photorhabdus luminescens (PhL). This bacterium is now being touted around by the genetic engineering industry as the new wonder biopesticide that will replace Bt.

There was no shortage of warnings from scientists, farmers and environmental activists concerning the fate of Bt. In fact the short, disastrous history of the commercialisation of Bt was entirely predictable.

US Environmental Defence Fund

Over ten years ago, pn 9th January 1989, Rebecca Goldberg from the Environmental Defence Fund wrote as follows to the US Environmental Protection Agency:

"Decisions about regulatory policies are often best made a priori rather than in the pressured atmosphere accompanying agency decision-making about particular products......Resistance to chemical pesticides is a pressing problem that continues to grow. Thus it is reasonable to ask whether pests will also evolve resistance to organisms genetically engineered to control them.....The most immediate concern is the evolution of resistance to delta-endotoxins from Bacillus thuringiensis. Bt toxins are environmentally benign compared to most other pesticides and Bt toxin genes are being engineered into a number of plants and bacteria. The evolution of resistance to Bt toxins would jeopardise further use of these genes, and thus the adoption of alternatives to conventional chemical pesticides"

Rural Advancement Fund International

A couple of months later, Hope Shand of the Rural Advancement Fund International wrote an article on Bt in the Journal of Pesticide Reform Vol 9 No 1 pp 18-21 Spring 1989

"If insecticidal Bt 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 bee pushed back to even greater reliance on conventional pesticides."

NovoNordisk 1992

The Danish biotechnology company which invested heavily in the breeding of Bt strains for traditional external use in the 1980s sounded a note of caution regarding the development of Bt plants in 1992 and decided to sell off all its interests in Bt in 1994.

"The successful use of new Bt-based biotechnology products depends on the development and implementation of sound management strategies. In the past, resistance management to chemical insecticides has occurred after resistance reached crisis proportions. With current Bt products, resistance has developed in fields under continuous, intensive use of new Bt-based biotechnology products.....As new biotechnology products based on Bt are developed, the challenge to use these products in a sound manner that maximises their field life becomes critical. Pest control specialists view resistance as the single most important issue in the development of single gene Bt-based products such as single gene transgenic plants. We now have the opportunity to demonstrate the lessons of past use of chemical insecticides by developing sound strategies for using Bt-based biotechnology products in pest management systems in advance of their actual commercialisation and marketing."

Nature

In 1993, Robert May, from the Department of Zoology at the University of Oxford wrote an article in Nature warning that genetically engineered Bt crops could quickly become useless.

"In the heady years after the Second World War it briefly seemed as if DDT and other pesticides would win the age-old human war on insect crop pests and disease vectors. Such dreams overlooked the way the natural world has been shaped by evolutionary challenges and responses - the challenge of chemical pesticides soon evoked a response of resistant genotypes of arthropods. Today, more than 500 species of insects and mites that are pests in crops and orchards have evolved resistance, often to a wide range of different pesticides. Overall, more than one-third of all agricultural production is lost to pests, the same proportion as a century ago. Now a new battle-line is about to be drawn - genetically engineered crops that contain insecticidal toxins will soon be available commercially. But the usefulness of such transgenic crops will be short-lived if insects quickly adapt to the toxins."

Bioworld Today
The editorial in Bioworld Today dated 20 May 1994 drew attention to the widespread myth that insects could not develop immunity to Bt.

"In the never-ending war between farmers and insects the ultimate weapon on the human side is a protein produced by a soil bacterium, Bacillus thuringiensis - Bt for short. Like DDT in decades past, Bt puts down a very wide range of crop pests, but unlike DDT it is environmentally correct, harmless to humans, and apparently defies anti-Bt resistance developed in the insect enemy. Apparently, but not really. Some years ago, watercress growers in Hawaii found that the diamondback moth, which feeds exclusively on cruciform plants - cress, cabbage, cauliflower and the like, was munching on the watercress crop with impunity, even though its leaves had been sprayed with Bt."

New Scientist
The editorial in the New Scientist dated 8 March 1997 accused the genetic engineering industry of irresponsibility.

"It is always a tragedy when a gift from nature is squandered, whether it is a river dead from pollution or a forest laid waste for timber. Lets hope that the biotechnology industry is not about to throw away the enormous potential of a simple bacterium, Bacillus thuringiensis, in its haste to get to market. Properly used, the bacterium could provide a wonderful way to control insect pests and help farmers to gain high yields without synthetic pesticides. Misused, however, and pests will grow resistant to it and its power will be gone for good. Over-eager gene cloners are already rushing in where cautious ecologists fear to tread. The latest research suggests that its time to slow them down."

1.1. PEST CONTROL METHODS

There is nothing new about biological, as opposed to chemical methods for controlling pests. Farmers have used biological methods for the control of pests for generations. In the broadest use of the term, traditional biopesticides control pests by using their natural enemies. More recently it has proved possible to breed or engineer biopesticides for specific purposes.

There are a number of problems with current methods of dealing with agricultural pests:

(a) an increasing need for pest control measures resulting from more and more genetically homogeneous monocultures and an increasing regional division of labour for particular crops which maximise the vectors for the multiplication of pests
(b) increasing environmental problems resulting from the use of agrochemicals - especially water quality
(c) public concern about the implications of agrochemicals for human health - especially agrochemical residues in food

Various solutions have been proposed and are currently being trialed in the field :

(a) integrated pest management (IPM), which involves monitoring and targeting pests in order to reduce agrochemical use by encouraging the specific rather than the routine use of chemical pesticides
(b) integrated crop management (ICM), which aims to reduce pest invasions by planning the juxtaposition of crops and rotations to reduce the vectors for the multiplication of pests
(c) organic agriculture (ORG), which aims to reduce pest invasions using a number of approaches, including increasing the biodiversity of farms (thus minimising the vectors for the multiplication and mobility of pests), companion planting/polycultures, the introduction of pest predators ranging from bacteria to insects, "natural" poisons such as nicotine, and when all else fails, manual control
(d) genetic engineering (GE), which is currently transferring naturally occurring poison-coding genes from bacteria into crops

There is no clear dividing line between any of the four methods of controlling pests described above and to one extent or another, they all face the same systematic biological problem - that pests can develop resistance to chemical and biological pesticides through genetic mutations.

The "ideal" pesticide would be environmentally safe and biologically effective - indefinitely. However, a combination of natural selection and the workings of the market make a mockery of this ideal. Generally speaking, the more biologically effective a pesticide, the more it is used and the quicker the pest becomes resistant. For effective, long-term use, it is thus important both to use and NOT use a pesticide. If pests are not provided with a refuge, we maximise the biological advantage of those individual pests with mutated genetic resistance so that they soon dominate the total population. This is especially the case with insect pests that breed many generations in a single growing season.

No pesticide is effective *per se,* and all pesticides are probably doomed to become more or less totally ineffective if we continue to grow our food in the form of spatial monocultures dominating whole landscapes, and temporal monocultures that never rotate crops from one year to the next. The efficacy and fate of a pesticide is thus determined to a large extent by the way it is used. This is acknowledged in the EU pesticide regulatory legislation, which makes it necessary to report resistance to any pesticide to the responsible authority, these authorities having the power to curtail its use if the resistance is pervasive. However, marketing consents for pesticides are given on the basis of their quality, safety and efficacy at a particular point in time, based usually on data from the most recent field trials. Once in the market, a pesticide can be sold and used within the terms of its marketing consent until its safety or efficacy are called into doubt to such an extent that the terms of the marketing consent are changed. By then it is too late. The current system amounts to a reactive rather than a precautionary approach to the problem of resistance. There are no known cases of resistance to a biopesticide fading away once it has been identified; on the contrary, the resistance always seems to grow, sometimes spreading around the globe in two or three growing seasons.

The current US and EU regulatory, marketing and intellectual property rights structures not only make it impossible to maximise the full biological and therefore agricultural benefits of biopesticides. They actually guarantee the loss of these biopesticides as useful agricultural tools and thus increase the risks involved in food production.

Opinion now masquerades as fact and vested economic interests frequently masquerade as science. We have decided therefore to separate so far as we can, the science and the politics from one another.

The second part of this paper presents a chronological history of the scientific discoveries concerning Bt, including the emerging problem of insect resistance.

We review the scientific evidence on resistance and the possibility of managing resistance. The consensus amongst entomologists is that all insects targeted by Bt crops will eventually become resistant and the current debate is about how best to delay its onset. Bt has been used by organic farmers as an external spray for over 50 years. Used as a spray, the toxin is effective for 3-5 days. When used in Bt plants the toxin is usually expressed throughout the plant for the whole of the growing season. Although entomologists agree that Bt plants are more likely to result in insect resistance than Bt sprays, key sections of the agrochemical industry, and Novartis in particular, continue to argue that Bt plants are safer than Bt sprays.

Insect resistance to Bt sprays was first reported in the field in 1979 and resistance to Bt plants has been developed in laboratory experiments. Insects bred under laboratory conditions can become several thousand times more resistant to Bt than those naturally occurring in the field. Although all sides of the argument appeal to scientific principles regarding the question of managing insect resistance, we have come to the conclusion that the argument can only be understood as a political conflict between commercial and environmental interests.

If future biopesticides are beset by the same combination of economic (market) and biological (resistance) mechanisms as Bt, the insect resistance problem will simply accelerate. For the moment almost all attention focuses on the search for biological or agricultural solutions to the problem. The notion that it might be the structure of the market that is the main cause of the problem scarcely receives a mention. The notion that it might be easier to sustain biopestides and plan secure food production systems by restructuring the economic rationale of the pesticide sector rather than by trying to constantly re-engineer biopesticides on the pesticide treadmill is what this report explores.

Part 2. The Science

2.1 A Short History of Bt

Bt is a naturally occurring soil bacterium. Little is known about its natural life cycle, distribution or ecology, or its effects on other soil life. It was first detected in 1902 in the dying larvae of Bombyx mori by Ishiwata, who reported his finding in "Pathology of the Silkworm". It was first isolated from the larvae of Ephestia kuehniella by Berliner in 1913 after he noted that it had the capacity to kill certain insects in their grub stage (Z.Angew Entomologie 1915,2, p29).

2.1.1 Early Development

According to an article by Jacobs in the proceedings of the Society of Applied Bacteriology (1950,13 p83) Bt seems to have been used for the first time as a microbial biopesticide against lepidopterous larvae in 1938, thereby giving Bt a role in food production that it has had ever since. The extent to which Bt poisons insect grubs in natural ecosystems has never been studied and remains unknown. This means that it is impossible to predict the consequences of widespread insect resistance to Bt in natural ecosystems, should this occur as a result of its present commercialisation.

The Bt bacterium is easily propagated and can be used as a wettable powder or in a water solution. Bt became commercially available before the Second World War and was used mainly by vegetable growers to eliminate caterpillar infestations. The producers of Bt were typically small family firms operating to a large extent by mail order. Bt was simply one product in the wide armoury of naturally occurring insecticides that were commonly used before DDT ushered in the age of synthetic chemical insecticides during WWII. For the more sophisticated grower, Bt had an advantage over other natural insecticides such as nicotine or pyrethrum (for instance) in that it was fatal only to a small range of insects and left beneficials such as lacewings and ladybirds untouched. In the absence of research on the various Bt strains available, it can be assumed that the products sold on the market consisted of mixtures of strains in unknown proportions. This would have resulted in unexplained differences in the effectiveness of different Bt products, which would presumably have affected growers perceptions of its efficacy. Bt was thus an unreliable product during this period. Some growers (who perhaps by luck had got hold of the strain most effective for their purposes) swore by it; others (who perhaps had the bad luck of getting less appropriate strains or mixtures) regarded it as pretty much useless.

There is no evidence of large scale use of Bt for the first 50 years of its known existence, nor of any research on the various strains of Bt in existence. No one understood why it worked or how it worked, so no one could explain why it sometimes worked effectively, whilst at other times this was not the case. There were too many variables in the field and no one had tested Bt in the laboratory.

There was nothing intrinsically ecological or organic about the use of Bt up to WWII. Indeed, had Bt been more reliably effective, its commercial use might well have expanded to the point of causing resistant insects to dominate their populations. Luckily, Bt was used intermittently and sparingly so it survived as a useful insecticide. It is important to note that the survival of Bt as a useful insecticide was not due to superior knowledge or practice. Its survival was a good accident.

2.1.2. 1940-1960 The Uncritical Age for Synthetic Pesticides

Bt and most interest in it was eclipsed during the uncritical hey-day of synthetic insecticides, lasting from WWII through to the publication of "Silent Spring". For some 20 years, Bt was only of interest to those growers who - for one reason or another - did not want to use the new synthetic insecticides.

During this period, the arguments of those who refused to use the synthetic products were easily marginalised as "unscientific":

- they could give no scientific explanation why their methods worked or sometimes failed to work,
- the evidence we now have of the widespread harmful effects of many synthetic pesticides simply did not exist, - the new synthetic products were reliable, effective and their mode of action understood
- ecological thought existed only at a

philosophical level and had little in the way of a scientific base

After a generation of largely uncritical use, two difficult facts about the synthetic insecticides had to be acknowledged:

- they caused widespread biological destruction and environmental pollution way beyond their insect targets
- constant use caused resistant insects to dominate the target populations, rendering the products useless

The search was on for alternatives.

2.1.3. 1960-1990 Bt becomes Big Business

There are four basic reasons why the agrochemical industry became interested in Bt.

1. The cost of developing new chemical pesticides was soaring to between $35-$40 million whereas the cost of developing a biopesticide was less than $5 million (Bioprocessing Technology, June 1988).
2. The timescale for developing a new chemical pesticide is 8-12 years whereas biopestides can be brought to market in about three years (Bioprocessing Technology, June 1988).
3. Given the acceleration of insect resistance to chemical pesticides, and the time it was taking to bring new ones to market, analysts predicted the possibility of a gap opening, in which some crops would be left with no viable means of chemical defence against insect pests.
4. Scientists are dreamers too. For reasons that no one is now able to explain, it was once widely believed by entomologists that Bt was not only an environmentally friendly biopesticide, but also one which insects could not get around. They were sure that insect resistance would never be a problem.

Bt was described as "the wonder pesticide" and the panacea for all the ills of the pesticide industry even though many insect pests were, and probably always had been naturally resistant to Bt.

A few chemical majors started moving into Bt -
Abbot Laboratories (USA)
American Cyanamid (USA)
BASF (Germany)
Caffaro (USA)
Crop Genetics International Corp (USA)
Ecogen (USA)
DeKalb (USA)
ICI - now Zeneca (UK)
Mycogen (USA)
NovoNordisk (Denmark and USA)
Rohm & Haas (USA)
Sandoz (Switzerland and USA)

Laboratory research on Bt strains and targets started in the 1960s and the market for Bt grew fast in forestry and in vegetable production. Current patterns and volumes of conventional Bt use on some vegetables and fruits in the USA are already high and almost certainly unsustainable. There are four main sub-species of Bt:

Bt aizawai
developed by Sandoz (Novartis)
used against lepidopterous larvae,
produced by Sandoz ("Certan"), NovoNordisk ("Florbac") and Abbott ("Xen Tari")

Bt israelensis
developed by Sandoz (Novartis)
used against mosquito and blackfly larvae
produced by NovoNordisk ("Bactimos") ("Skeetal"), Caffaro ("Bactis"), Abbott ("Gnatrol") ("Vectobac"), Sandoz ("Teknar")

Bt kurstaki
developed by Ecogen and Sandoz
used against lepidoptera larvae in agriculture, horticulture, forestry, also Colorado Beetle
produced by NovoNordisk, Novartis, Ecogen, Abbott, Roussel-Uclaf, Intrachem under 19 different trade names,

Bt tenebrionis
developed by NovoNordisk
used against Colorado Beetle
produced by NovoNordisk ("Novodor")

The statistics on the application of microbial Bt are not complete. In the USA 57 crops were being treated with Bt on 2,037,834 acres by 1992. Six crops were largely dependent on Bt, with over 80% of planted areas being sprayed in some states. See the tables in Annex 4 for more details.

2.1.4. 1980 - 2001? Bt crops

"The scientists studying Bacillus thuringiensis and the companies spending millions of dollars bringing it to market say they are absolutely sure about one thing: Bt, as it is known - a species of microscopic bacterium found in almost every handful of soil the world over - has the potential to replace an entire generation of chemical pesticides.....Even in the past two or three years, as Bt has achieved almost celebrity status amongst those who make a living thinking of better ways to kill bugs, and as scientists have embarked on a world-wide hunt for new strains of the microbe, the questions surrounding Bt have only grown......How on earth, some scientists would like to know, did this seemingly innocuous soil bacterium develop such a powerful and selective weapon against insects? There are theories, of course. But they are all so speculative and sometimes so contradictory that it seems likely Bt will make millions for its manufacturers long before it is fully understood by scientists." (Washington Post 27.11.89)

Some of the companies already investing in Bt sprays moved into genetically engineered Bt plants but on the whole, the Bt market has split into two more or less exclusive groups. The genetic engineering companies with interests in Bt plants include the following:

Agracetus (USA)
Agricultural Genetics Co Ltd (UK)
Agrigenetics Advanced Sciences Co (USA subsidiary of Lubrizon)
Ciba-Geigy (Switzerland, now Novartis)
DeKalb (USA)
Monsanto (USA)
Plant Genetic Systems (Belgium)
Sandoz (Switzerland, now Novartis)

By 1997 these companies were field testing at least 18 different Bt crops, including trees that could be expected to live for 150 years or more:

Alfalfa
Allegheny Service Berry
Apples
Aubergines
Broccoli
Cotton* in commercial production USA '96
Cranberry
Grapes
Maize* in commercial production USA' 96, EU '98
Peanuts
Poplars
Potatoes* in commercial production USA '96
Rice
Spruce
Tobacco
Tomatoes
Walnuts

2.1.5. Beyond Bt

It is generally agreed by entomologists that the biologically useful life of Bt is now limited. The question that most scientists are asking is not *whether* insects will become resistant to Bt, but *when* this will happen and whether there is anything that can be done to delay the process that is now inevitable. Convinced that biopestides will join chemical pesticides on the resistance treadmill, entomologists and the genetic engineering industry have already identified the next panacea as Photorhabdus luminescens (PhL for short). The mere fact that a consensus has developed around the potential use of PhL has self-fulfilling implications. In effect, the market destroys the product. It can be predicted with some certainty that PhL and PhL plants will also have a limited usefulness and that after PhL the industry will be searching for yet another panacea for the problems that they themselves create.

2.2 The Mode of Action of Bt Crystal Proteins

Bt is a soil bacterium noted for its abundant production of insecticidal proteins in the form of a crystal or crystal-complex during sporulation. The insecticidal crystal proteins are called "cry" proteins and the genes encoding them as "cry" genes. Cry proteins have been classified according to the range of insects for which they are poisonous and for their nucleotide sequence. However, this does not result in the categorisation of cry genes into exclusive classes. For example, members of the same protein class can vary significantly in their poisonous effect against insects within a single insect order. Equally, the same protein isolated from different Bt strains can vary in its amino acid sequence, resulting in dramatic differences in their poisonous effects.

More than fifty cry proteins have now been sequenced and the simplified classification shown above is no longer adequate. Classification schemes based solely on sequence data create a different picture of the relationships among the various Bt proteins, and perhaps more accurately reflect the evolutionary relationships between the various cry proteins.

Table. Bt Cry Proteins and their Pest Targets

Gene	sub-type	Crystal Shape	Protein Size (kDa)	Pest Target
Cry1	A(a), A(b), A(c), B, C, D E, F,G	Bipyramidal	130-138	Lepidopteran larvae
Cry2	A, B, C	Cuboid	71, 71, 69	Lepidopteran and Dipteran larvae
Cry3	A, B, B(b)	Flat or Irregular	73, 74, 74	Coleopteran larvae
Cry4	A, B, C, D	Bipyramidal or Round (?)	134, 128, 78, 72	Dipteran larvae
Cry5-CryIX		Various	129, 73, 35, 38	Various

(Hofte and Whiteley, 1989; Microbiol. Rev. 53:242).

The Cry proteins do not become biologically active toxins until they have been dissolved in liquid and activated. Normally this occurs in the highly alkaline mid-gut environment of lepidopteran insects. The toxin is activated by the insects gut enzymes. Most mammalian guts are acidic and do not produce a favourable environment for the Cry toxin. It is generally accepted that the toxin recognises certain receptors on the surface of insect mid-gut epithelial cells. A pore-complex forms through the cell membrane, resulting in the loss of potassium ions which affects the insect's ability to regulate osmotic pressure. Eventually the animal dies due to massive water uptake.

According to DeWald (1995), "crystallography studies with Cry IIIA protein toxin (Li, et al; 1991. Nature 353:815) indicate three structurally distinct domains. Domain I consists of seven alpha-helices and is believed to be involved with membrane interactions and the insertion of the toxin into the insect's mid-gut epithelium and pore formation. Domain II appears as a triangular column of three beta-sheets and is reported to be involved in receptor binding. Domain III consists of anti-parallel beta-strands in a "jellyroll" configuration and, like Domain II, is implicated in insect specificity and stability. It appears that several of the reported cases of insect resistance to specific Cry proteins are due to altered receptor binding specificities. Presently, our knowledge of the various Cry proteins is insufficient to predict how specific protein modifications may affect the efficacy or activity spectrum of a particular protein".

2.3 The Development of Bt Strains

Bt has been developed within two distinct paradigms - the "military" paradigm of the agrochemical industry and the "holistic" paradigm of the organic movement.

2.3.1 The Military Paradigm for the Development of Bt

Bt has been used as an active ingredient in a wide range of biological insecticides for nearly a half century. These products have been used in agriculture and forestry, and for the control of disease vectors such as mosquitoes and blackflies. In agriculture, many of the products have been based on a single strain of Bt, termed HD-1, that was isolated in 1970 at the USDA Cotton Insects Research Laboratory in Brownsville, Texas.

Since the early 1980's, research on Bt has shown that the Cry proteins produced by sporulating cells are encoded on extrachromosomal plasmids. These plasmids are capable of being transferred between strains of Bt by a conjugation-like process. It has also been shown that many strains of Bt have several Cry genes, thus producing either multiple insecticidal crystals or mixed crystals containing several different but related Cry proteins. Bioassays of individual purified Cry proteins have shown that each one has a unique range of target insects in the Lepidoptera (caterpillar), Coleoptera (beetle) or Diptera (fly) groups. Some thirty or more different Cry genes have been cloned and sequenced. By a process of cloning and expression of individual Cry proteins in a common Bt strain background, it is now possible to identify those that are particularly active on various target insects, and to use genetic techniques to construct strains carrying several Cry proteins selected for both optimised activity on the desired insect targets and for the management of the potential for insect resistance.

Bruce Carlton at Ecogen was full of optimism in 1995:
"We have utilised these approaches to construct a number of new Bt strains for different crop and

insect applications. The choice of Bt itself as the expression host has several advantages. First is that Bt is naturally capable of stably maintaining several different ICP (Cry) genes without undergoing loss or gene rearrangement. Second, Bt can express these genes to high levels such that 25-30% of its total protein can be ICP (Cry) protein. Third, we can take advantage of natural Bt plasmids as cloning vectors for constructing new ICP (Cry) combinations, as well as a Bt transposon that encodes both a transposase and a site-specific recombinase. These elements greatly facilitate the construction of new ICP (Cry) combinations that do not contain antibiotic resistance genes or other undesired foreign genes. Thus, the new Bt constructs consist only of Bt DNA, a definite advantage when seeking regulatory approval for conducting large-scale field trials or product registration.''
In fact, Ecogen was granted a blanket approval in 1992 by the Environmental Protection Agency for conducting small-scale field trials of any new recombinant Bt without having to obtain separate approvals by employing exactly this strategy.

According to Carlton, ‘Ecogen's first new product derived by this recombinant technology is called Raven, and was developed as a superior product for control of Colorado potato beetle, as well as caterpillar pests of potato, tomato, and eggplant. This product, the first live Bt derived by recombinant DNA technologies, was approved for registration by the EPA within ten and a half months of submission. The Raven strain contains two different ICP proteins of the beetle-active CryIII group, in addition to two caterpillar-active CryI genes. The two CryIII genes contribute to a much higher productivity in fermentation of this strain as compared to its predecessor strain in the now-discontinued Foil product.''

Carlton argued that ‘the particular combination of genes in the Raven strain is designed to minimise the development of resistance to the product by the Colorado potato beetle, which is recognised as perhaps one of the most active of all insect pests in developing resistance to chemical insecticides. This strategy involves two different approaches.

First is that the two CryIII proteins expressed in the Raven strain have different binding characteristics on potato beetle midgut cell membranes. In studies conducted with researchers at Michigan State University, laboratory-selected potato beetles that are resistant to one of the CryIII proteins showed only minimal resistance to the second CryIII. Thus, in practice, an individual beetle would have to undergo two independent resistance mutations to become resistant to the Raven product.

Second, it was found that when the beetle strain selected for resistance to the one CryIII protein is exposed to a mixture of that CryIII protein and the CryI protein contained in Raven, the CryIII resistance is strongly reduced. This effect is presumably due to some protein-protein interaction that occurs between the two ICPs at the level of midgut binding. Thus, the Raven strain incorporates two different strategies to minimise the likelihood that the principal insect target would develop resistance to the product.

Currently there are two other products under development using the recombinant system described, one (CryMax) for applications on an array of caterpillar pests of vegetables and horticultural crops, and a second (CryStar) specifically aimed at the control of fall armyworm on sweet corn and other vegetables, an insect for which no Bt product is currently available. In the future we expect to continue to develop novel ICPs that have different properties with respect to their modes of action on important insect pests. These activities will come from a combination of new gene discovery efforts and by employing approaches such as protein engineering of selected genes to alter their activities and other physiological properties. We believe that this combined approach will allow us not only to develop new and improved products, but also to effectively manage the potential for insect resistance development by continuing to exploit the ability of Bt to express multiple ICP genes having a diversity of activities."

2.3.2 The Holistic Paradigm for the Development of Bt

According to Kirschenmann, President of Farm Verified Organic Inc, "Organic farmers have traditionally avoided the use of synthetic materials in crop production because, as a rule, they short circuit, rather than enhance, the ecological balances of nature. Synthetic materials, consequently, often create the problems they purport to solve. For example, R. Hindmarsh has pointed out that annual crop losses to insects doubled during the same period of time that insecticide use increased tenfold (The Ecologist, Sept. 1991, pp.198-199)."

Bt sprays have been used successfully in diversified cropping systems as a limited-use pesticide for two generations. Traditionally the first organic line of defence against pests is to maximise the biodiversity of the farm and tghus minimise the vectors available for pests to spread. Applications such as Bt sprays are only used in emergencies and the likelihood of insect resistance emerging under such usage is minimal.

“If Bt is used persistently, pests are likely to build up resistance within a few years. The rule that appears to apply is that the more we homogenise production in the form of genetically uniform monocultures, the greater the risk of pests devastating the crop. And the greater the risk, the more we apply poisons, which in turn results in the insect population becoming resistant.”

“Most farmers operate under very intense financial constraints. They are forced to make field management decisions based on immediate financial constraints and even though they may know that this can or will result in longer term problems for which there are no solutions, they are not economically free to behave otherwise. They are too preoccupied with today's headache to worry much about tomorrow's doom.”

According to Kirschenmann (1995), "farmers in North Dakota were warned by everyone from extension agents to seed sales people, that failing to rotate sunflowers would invite sunflower insect and disease disasters. But sunflowers were a good cash crop that produced much needed revenue, so most farmers raised sunflowers in the same fields at least every other year, and in some instances they continuous-cropped them. Within a few years insect and disease problems became so severe that the cost of pest control forced many farmers to get out of sunflower production. It is not that farmers were stupid or unconvinced of the risks. Short term economics simply took precedence over long term economics."

In a holistic ecological system of complex interactions and reactions it is never possible to predict all the effects of the introduction of a novel organism. According to Kirschenmann, "examples abound. Just recently (New York Times, October 9, 1995) two USDA scientists reported that the infestation of the beet armyworm on Rio Grande Valley and San Angelo, Texas cotton crops may have been caused by heavy applications of malathion designed to eradicate the boll weevil. The malathion, they said, caused "a disruption of the beneficial insect complex that normally suppresses the beet armyworm." Transgenic crops, which introduce instantaneously-created new life forms into the environment, dramatically increase the potential for such disruptions, many of which may be irreversible."

An Example: integrated management of the Colorado Beetle

The September 1992 issue of 'The IPM Practitioner' is devoted entirely to IPM options for controlling the Colorado Potato Beetle. The key to the IPM approach is understanding the biology of the beetle.

The CPB overwinters between 6 and 20 cms beneath the soil in potato fields or in nearby border areas and woodlands. The adults ususally emerge just when the first potatoes are sprouting. They are unable to fly any distance at first and only regenerate their flight muscles if they do not find potato plants within five days. Virtually all overwintered beetles that colonise a non-rotated field originate from that field.

A rotation of maize or wheat with potatoes encourages ladybirds (Coleomegilla maculata) which eat CPB eggs. Maize and wheat also offer a barrier to CPB migration. In one experiment, only a little over 50% of CPBs released into a wheat field reached potato plants just three metres away. The ladybirds which overwinter in wheat fields ate over 50% of the CBM eggs, reducing CBM colonisation of the potato plants by 75%. The surviving CPBs can be concentrated on a trap crop of early potatoes, making physical control easier. Equally, late planting increases the mortality rate of CBM significantly.

Different control methods are appropriate to each of the four stages in the life cycle of the beetle:

Table IPM Management of Colorado Potato Beetle

Season	Stage in Life Cycle	Method
winter	hibernating adults	crop rotation, tillage
spring	emergence of overwintering adults	trap crop, flame, mulch & remove, barrier (wheat)
late spring	adults and first eggs	vacuum, flame, mulch, nursery for predators: Coleomegilla maculata, Chrysoperia rufilabris, Edovum puttleri, neem antifeedant
early summer	1st generation young larvae	mass release of predators, mulch
	1st generation larvae	Bt spray, cryolite, cyromazine
summer	1st generation pupae	Beauveria bassiana (fungus), nematodes
	1st generation adults	spiders (mulch), vacuum
late summer	egg laying adults	neem, release predators
	migrating adults	Bt, vacuum, Beauveria bassiana
autumn	overwintering adults	trap crop, flame

According to Jeff Waage, Director of the International Institute of Biological Control at Imperial College, London, Bt could have had a good future if used within a system of integrated pest management.

"As a product, Bt is valuable in IPM systems because it is much less harmful to predators and parasites than broad spectrum chemical pesticides. Therefore it can be substituted for chemical products in "insecticide treadmill" situations and will allow the recovery of natural enemy populations. Like many biopesticides, it is often less effective on its own than a highly potent chemical product. However, in an IPM system, where it is used only when needed and it conserves natural enemies its impact is augmented by the action of those natural enemies and can be both more economical and sustainable.

However, present product registration and evaluation systems often neglect this, favouring through various procedures and protocols the development of "this is all you need" products.

A second problem facing Bt is the risk of resistance. Where Bt is used as a single technology solution, like its chemical predecessors, it is sprayed regularly and a range of insect pests are now developing resistance.

Bts third problem is that it lacks the most desirable property of a biological control agent: its ability to reproduce and perpetuate itself in crops. A key advantage of biological agents relative to chemical pesticides is their capacity to both kill pests (functional response) and reproduce at the expense of the pest (numerical response) thereby giving some control in future pest generations. Bt is not adapted to persist in the crop environment and its commercial development has focussed less on preserving its ability to reproduce and spread, but more on maximising the efffect of its insect-killing toxin. In other words, iits commercial development has focused on using it like a chemical pesticide and not as a living biological control agent. This is true of most biopesticide development today..... It also reflects the fact that the multinational agrochemical industries which have dominated biopesticide development have traditional skills which are limited to the production and marketing of pesticide-like products.’’

According to Waage, “Engineering genes for Bt toxins into plants is an ingenious method of delivering these toxins to pests which might naturally avoid them, such as insects which feed inside plants. From an IPM perspective, this technology has more similarities to plant resistance breeding than biopesticide development.... Biotechnology for plant protection is still in its early days. So far, it has been focussed conservatively on improving conventional pest control approaches, biological pesticides and vertical resistance in crops to pests, in order to make better, single-technology solutions to insect pest problems which will outcompete current, non-engineered products. IPM promotes a more diversified approach which will limit over-reliance on any specific technology and the consequences of this, such as resistance development. It promotes greater reliance on exploiting living, self-renewing processes in pest control, such as the action of natural enemies of pests." (Waage.J in

Biotechnology & Development Monitor No 32 pp 19-21 1997).

2.4 The Development of Resistance to Bt

At least four species of insect have evolved resistance to Bt in the field and over ten species have evolved resistance under laboratory conditions. The latter figure is more a reflection of the number of experiments that have been carried out than the number of insects that could develop resistance. By presenting the crucial scientific findings in chronological order we show how the scientific research on Bt has generated a roller-coaster of undulating optimism and pessimism in the genetic engineering industry. With hindsight, it is easy to see that more account was taken of the optimistic findings than the pessimistic ones.

The resistance problem started in 1979 when **R. Kinsinger** published his paper on **"Susceptibility of populations of Indian Meal Moth and Almond Moth to Bt"** in the Journal of Economic Entomology Vol 72 pp 346-349. His laboratory studies showed a 42 fold increase in resistance to Bt. However, this study was largely forgotten and is infrequently cited. Moreover, when cited, the two moths have been treated as special cases since they live in bins of harvested crops which tend to be more heavily dosed with insecticides than insects in the field.

Two years later, **D. Briese** cast doubt on Kinsinger's findings in his chapter entitled **"Resistance of insect species to microbial pathogens"** in Davidson.E (ed) Pathogenesis of Invertebrate Microbial Diseases, Allenheld, NJ 1981 Briese summarised all the failed attempts to breed Bt resistant insects in the laboratory. This study led to the belief that insects in the field (as opposed to insects in bins) could not or would not become resistant to Bt.

However, four years later **W. McGaughey** published his paper on **"Insect resistance to the biological pesticide Bt"** in Science 229 pp193-195 1985. He reported on his laboratory selection showing a 27 fold increase in Indian meal moth and almond moth resistance after 2 generations, and a 97 fold increase after 15 generations. The study confirmed the Kinsinger findings but added nothing new to the debate other than to confirm that continued exposure to Bt led to increased insect resistance.

A year later, **A.Chang** published **'Defense Reaction of Mid-Gut Epithelial Cells in the Rice Moth Larva Infected with Bt"** in the Journal of Invertebrate Pathology 47 pp333-339 1986. This research identified the mid-gut reaction as the method by which the larvae defended itself from the Bt toxin. The study provoked the hypothesis that the development of Bt resistance was a specific reaction that could possibly be circumvented.

The statistical basis for generalising from laboratory experiments to the field was then questioned by **R. Roush** in **"Ecological Genetics of Insecticide and Acaracide Resistance"** which was published in the Annual Review of Entomology No 32 pp 361-380 1987. Roush analysed the laboratory results on resistance and concluded that laboratory insect populations are normally derived from small samples of the total gene pool of the insect and these normally develop polygenic resistance mechanisms which will not necessarily occur in the field. This study encouraged the belief that resistance in the field could not be predicted by cases of resistance developed in the laboratory. The importance of laboratory evidence was thus played down.

The enormous natural diversity of Bt strains was revealed by **J. Morrison** in **"Soil Yields 72 New Varieties of a Natural Pest Control"** published in Agricultural Research Vol 14 1988. Morrison reported the results of a world-wide trawl for Bt varieties which increased the number known from 24 to 96. It was found that the Mediterranean region had the greatest density of Bt in the soil. Bt was found at such high altitudes in Tibet that no known host organisms managed to exist there. Seventy two new Bt varieties were identified, some of them 20 times more toxic than existing commercial varieties. The findings led to the optimistic assertion that naturally occurring Bt strains could provide the basis of a comprehensive insecticide resource for the foreseeable future.

There was further cause for optimism when **G.Georghiu** published his famous book on **"The Occurrence of Resistance to Pesticides" FAO Rome 1988** Although the book is mostly a documentation of innsect resistance to insecticidces it also includes a summary of unsuccessful attempts to produce Bt resistant insects in the laboratory. This led to renewed hopes that the resistance of the Indian meal moth and almond moth to Bt were exceptions rather than the demonstrations of a rule.

As the commercialisation of conventional Bt got underway, **David Ferro** argued that Bt sprays were compatible with systems for integrated pest management in **"Toxicity of a new strain of Bt to Colorado Potato Beetle** in the Journal of Economic Entomology Vol 82 No 3 1989. He argued that Bt san diego was compatible with IPM systems because it killed the Colorado beetle but left its predator ladybirds unharmed. The results of this study have been thrown into doubt by more recent research indicating the opposite phenomenon.

The same year **Ferro** argued for caution within the industry. The industry magazine **AGROW** (No 85 21/4/1989) began to question whether Bt was really unassailable in **" Bt - potential for pest resistance?"** The report of an Agbiotech Meeting held in Virginia hadDr Ferro noting "that any attempts to increase the effectiveness of Bt products will also increase the competitive pressure for resistance to develop. He therefore urged companies not to go too far in improving their Bt products." Whilst Ferro put his finger on the central contradiction, his recommendation was rather like asking sprinters not to run too fast in the Olympic Games.

Doubts about Bt increased when **T.Stone** published the **"Selection of tobacco budworm to a genetically engineered P. fluorescens containing the delta-endotoxin Btk"** in the Journal of Invertebrate Pathology No 53 pp 228-234 1989. This was the first report of laboratory selection of insects resistant to Bt as opposed to the reports of increasing resistance amongst insects that were already resistant. After 3 generations there was a three-fold increase in resistance and after 14 generations this had increased to 24-fold. This study severely dampened the optimism that had been generated by the Briese (1981) and Georghiu (1988) studies but it did not answer the argument made by Roush (1987) that laboratory studies were not necessarily a good predictor of behaviour in the field.

Later the same year **Stone** sent the Bt industry into a spin with a presentation of laboratory evidence on **"Insect resistance to Bt delta-endotoxins"** at the International Symposium of Molecular Insect Science, 22-27/10/1989, inTucson, Arizona. This was the first report of cross-resistance. Helicoptera virescens resistant to the Cry1Ab protein showed varying levels of resistance to Bt aizawai, Bt colmeri, Bt darmstadiensis, Bt entomocidus, Bt kurstaki and Bt thuringiensis. The study shocked entomologists and genetic engineers. The results could not be explained by existing models and they marked the end of the optimistic scenario in which an infinite variety of Bt subspecies could be used to constantly replace those Bt subspecies to which insects had become resistant. The industry set up a working group on Bt resistance in response to this study.

J. Van Rie was amongst the first entomologists to start searching for viable resistance management strategeies. In "**Mechanism of Insect Resistance to the Microbial Insecticide Bt"** published in Science Vol 24 on 5/1/1990 he argued that: "Strategies for resistance management are needed to extend the lifetime of chemical insecticides. It is equally important to implement such strategies with Bt to maintain its usefulness as a safe and environmentally sound insect control agent." Van Rie argued for the 'pyramid' approach to genetically engineered Bt plants. This entails the insertion of several Bt Cry proteins at the same time so that if one does not work the other one does. The initial laboratory results were sufficiently encouraging to justify the announcement of a whole new strategy against insect resistance. The argument for "pyramiding" toxic genes is still propagated by industry representatives in various parts of the world despite the fact that subsequence evidence on the development of cross-resistance and multiple resistance sharply reduces the number of combinations of Cry proteins that can be used simultaneously. The strategy has been rendered worthless by several factors which are considered in more detail below:

- many Cry proteins are only poisonous to specific insect species
- almost all crops have more than one economically important insect pest
- the same insect pest can eat several different crops in the same region
- insects can develop multiple resistance to several Cry proteins

From 1990 onwards the scientific literature contains any number of papers reporting the increase in insect resistance to Bt over several generations under laboratory conditions. The study by **D. Miller** on the "**Development of a strain of Colorado Potato Beetle resistant to the delta-endotoxin of Bt"** in the WRCC Newsletter

No 2 p 25 1990 is an average example of a study showing showing a 67-fold increase in resistance after 10 generations. Studies of insect resistance to Bt had become standard material for an American Phd in entomology.

The first of several bombshells from the American entomologist **Bruce Tabashnik** was published as **''Field Development of resistance to Bt in diamondback** moth"in the Journal of Economic Entomology 83 pp1671-1676 1990. Diamondback moths were found to be largely resistant to watercress sprayed with Btk in Hawaii. According to Tabashnik: "The lack of previous reports of substantial field resistance to Bt led many to conclude that such resistance was unlikely, particularly in defoliating crop pests........Expression of Bt toxin genes in crop plants and other related advances in technology are likely to intensify selection for resistance to Bt." This was the first study proving that Bt resistance could develop in the field since the 1979 study by Kinsinger. It finally resulted in entomologists and genetic engineers realising that insects would develop resistance to Bt just as they did to chemical insecticides.

After the Tabashnik study put resistance firmly on the map, laboratory experiments were designed to elucidate the dimensions of this resistance. In 1991 S.Sims reported on the **''Genetic basis of tobacco budworm resistance to an engineered Photorhabdus fluorescens expressing Btk''** in the Journal of Invertebrate Pathology No 57 pp. 206-210 1991. He showed that tobacco budworm with an initial Bt resistance 69 fold greater than normal fell to 13 fold greater after 5 generations of non-exposure to Bt. These findings by Sims indicated that occasional use of Bt, or the use of Bt within a rotation of different insecticides, might overcome the resistance problem, since insects showed much reduced resistance after a few generations. However, instead of leading researchers into making some potentially fruitful IPM/ICM field studies, the Sims study led mainly to a host of laboratory experiments designed simply to indicate whether insect resistance would continue to reduce back to zero with subsequent generations, thus giving some hope for its sustainable use. (It is worth noting in passing that PhL was at this time being used as a host to Bt rather than as an insecticide in its own right.)

Still working on conventional uses of Bt, **David Ferro** reported on a study of the conditions under which sprays are most effective in **''Colorado Potato Beetle Larval Mortality: operative effect of Bt san diego''**published in the Journal of Economic Entomology Vol 84 No 3, 1991. His study showed that timing and temperature were crucial. Larvae could not survive more than 6-8 hours of Bt.sd. when the temperature exceeded 24 centigrade, indicating however, that this Bt might be ineffective in the northern range of the beetle. There are many studies of the conditions under which Bt sprays are most effective and they mostly point up very specific conditions which are difficult to replicate in the field. These problems have in turn been used to justify the production of Bt plants on the grounds that they are more effective and require less skill from the farmer. Bt plants are of course more effective for killing borer insects, most of which are well protected from Bt sprays by the fact that they live inside the plant.

In 1991 **J. Ferre** drew further attention to the problem of cross-resistance to Bt in **''Resistance to the Bt bioinsecticide in a field population of P. xylostella''** published in Proceedings of the National Academy of Sciences Vol 88 pp 5119-5123 1991. He showed that Diamondback moths resistant to Cry1Ab toxins were also susceptible to Cry1B and Cry1C toxins. The previous study of cross-resistance by Stone in 1989 dealt with Bt sub-species which each produce either a number of different Cry toxins, and/or the same Cry toxins in different proportions. Ferre pinned down the multiple resistance to particular Cry toxins, which made it possible to speculate as to whether Bt genes could be designed to contain particular Cry toxins and whether these could then be specifically combined as pyramids of multiple toxins, or used in planned rotations of different toxins from one year to the next.

By 1992 a clear schism was developing between entomologists and the industry. Whilst the industry continued to carry out field trials one Bt crop after another, the entomologists were increasingly sounding a note of alarm. Perhaps with some justification, the industry tended to treat these interventions as a way of fund-raising pet research projects. M. Caprio published an article on **''Arresting Resistance''**in Bio/technology Vol 10 May 1992 in which he said that "it will be important to evaluate genetic variation for resistance, and the potential magnitude of resistance in target and non-target pests exposed to Bt, as well as the patterns of cross-resistance among Bt toxins. Until these data are available, it is prudent to treat susceptibility to Bt as a limited resource, and concerted efforts should be made to delay the evolution of resistance." However, Caprio's plea for the time to get the science right and plan strategies to deal with insect resistance to Bt was not compatible with the rush to get Bt products into a highly competitive market where the winner was likely to get all.

The work reported by Chang in 1986 indicated that resistance to Bt was associated with changes in the mid-gut. Other work had indicated that resistance to Bt was still limited to specific strains and it was thought that resistance was inherited as a partially or fully recessive trait. This had led to optimistic plans for the management of resistance. However, work reported by **Fred Gould** in **"Broad spectrum resistance to Bt toxins in Heliothis virescens (Bollworm)"** in Proceedings of the National Academy of Science Vol 89 pp7986-7990 September 1992 called all of this into doubt. Gould showed that a laboratory strain of the bollworm developed cross-resistance in response to a Cry1A(c) protein, including resistance to very different forms of Bt. The resistance was not associated with any changes in the mid-gut and the trait was inherited as an additive trait when larvae are treated with large doses of Cry1A(c) protein. The study confirmed the general scientific ignorance of the resistance mechanisms at work, and called into doubt the possibility of planning precise strategies for delaying insect resistance, just at a time when other scientists were beginning to declare the possibility of understanding and planning for cross-resistance.

The situation became further confused with **David Ferro's** mathematical modelling of the development of resistance in dynamic insect populations. He reported on the **"Potential for Resistance to Bt in the Colorado Potato Beetle"** in the American Entomologist Vol 39 No 1 1993. This study concluded that plants expressing LOW levels of Bt toxin which slowed the rate of larval development might produce the best long-term control of the Colorado Beetle. The study thus undermined the growing scientist-Monsanto consensus that highly toxic Bt plants grown with an insect refuge would provide the best possible insect control, plus the best possible management of insect resistance. It was, however, music to the ears of Novartis, which happened to be stuck with a Bt maize expressing low levels of toxin during the latter part of the season which did not fit well with the high toxin/refuge strategy for managing resistance.

Fred Gould shifted ground and was one of the first entomologists to argue that although the high toxin/refuge strategy might not be the panacea it was once thought to be, it nevertheless offered the best trade-off between ecological and economic interests and was thus the most likely strategy that the genetic engineering industry would accept. The entomologists were by now trying to find an accommodation with the economic interests of the industry. In **"Insect Resistance to Bt Toxins - can it be delayed?"** published in the Proceedings of the 2nd Canberra Bt Meeting 1993, Gould reviewed evidence for various strategies, concluding that the high dose/high refuge strategy was the most realistic strategy to follow. "Executing this strategy properly in local areas will require local research on pest/natural enemy population dynamics as well as education of local crop managers so that they can make informed decisions........Only solid local ecological research will allow us to determine how to put the least selective pressure on the pest while lowering its numbers and damage to acceptable levels." This paper marked the entry of the entomologists into the economic world of the genetic engineering industry. Science now became mixed with "economic realism", resulting in the political concept of "a trade-off between economic and ecological interests". Gould the entomologist began here to look at the problem from a commercial point of view. At the same time he argued, with justification, that the only way of managing insect resistance was by devising local strategies based on local ecological research, something which he knew was prohibitively expensive and could never be financed by the genetic engineering industry.

Gould's demand for local management resistance strategies was thoroughly supported by the study reported by **T.Stone** on the **"Geographic Susceptibility of H. virescens and H. zea to Bt"** published in the Journal of Economic Entomology Vol 86 No 4 1993. Insects collected from 12 states in the USA showed significant differences in susceptibility to Bt. If Gould's views on the need for local research on local conditions needed empirical justification, Stone provided it in plenty, thus questioning the desirability and efficacy of using the same Bt spray or Bt plant even against a single insect species. The same Bt product could end up working as a LOW level toxin in one place and as a HIGH dose toxin somewhere else, depending on the susceptibility of the insects.

Graham Head is one of the many entomologists who have moved between academia and industry. In 1998 he was working for Monsanto but the research he reported in 1994 was not particularly convenient for the company. In the **"Quantitative Genetics of Behavioural and Physiological Resistance to Insecticides in Diamondback Moth and the Colorado Potato Beetle"**published in Resistant Pest Management Vol 6 No 1 1994 he showed that the Colorado Beetles which are most responsive to Bt and non-Bt plants are also the ones that are most resistant to Bt. "Larvae moving onto transgenic foliage in a mixture are more likely to return to the non-transgenic foliage and survive if they are more resistant, making the seed mixture strategy potentially counter-productive." Head provided an argument against using seed mixtures containing Bt and non-Bt plants, and against Bt plants that did not uniformly express Bt. His findings were damaging to the random refuge strategy if the larvae moved between the refuge and the Bt crop, but the refuge strategy should still work if it were only the adult beetles that moved between the refuge and the Bt crop. The implication was that the spatial design and juxtaposition of refuges and Bt crops would thus determine the effectiveness of a refuge strategy. almost all refuge strategies from this time on advocated block planting of Bt and non-Bt crops alongside one another rather than mixing them in the same rows.

Mark Whalon introduced a further complication in **"Bt Resistant Colorado Potato Beetle and Transgenic Plants"** published in Biocontrol Science and Technology Vol 4 pp 555-561 1994. He argued that "given the use of Bt products as conventional insecticides has increased sharply, it is likely that a degree of selection by Bt may have occurred in field populations by the time the transgenic plants are introduced...... Several models suggest that providing alternative hosts (seed mixtures or refugia) can significantly slow the development of resistance, resulting in a portion of the population that is not selected, which supplies susceptible genes to the next generation. However, if the Colorado potato beetles do not have the genetic capacity to survive high Bt expression levels in transgenic plants, seed mixtures may be counter-productive." The reality is probably more complicated than Whalons theoretical model. If Bt potatoes are planted where there are already populations of beetles resistant to certain Bt sprays containing particular Cry toxins, the best strategy would be to plant Bt potatoes containing different Cry toxins which have not (yet) succumbed to multiple resistance. If this were not possible, the next best strategy would be to plant potatoes expressing high levels of toxin in the hope that the already resistant beetles could niot survive such an onslaught of poison. However, if this strategy failed, the Bt potato would become ineffective in just a few weeks.

Whalon eventually concluded that there were no answers to his questions. In **"Insect Resistance to Bt"** he wrote that "the development of genetically engineered plant with Bt toxins is not without controversy in our society, but it now appears that transgenic plants containing Bt will be approved in the same manner as genetically altered conventional Bt products. How conventional and genetically engineered plants with Bt toxins will exert selection pressure on pest populations individually and together is not understood." The dilemma outlined in commenting on Whalons first paper above is the subject of this contribution, which concludes that there is no scientific basis for predicting the outcome where both Bt crops and Bt sprays are used.

Richard Roush experimented with Whalon's dilemma and reported his findings in**"Managing Pests and their Resistance to Bt - can transgenic crops be better than sprays?"** published in Biocontrol Science and Technology 1994. He showed that a strain of Colorado Beetle could survive Bt sprays but could not survive on Bt potato plants, even those expressing very small quantities of Bt. He argued that "this suggests that more mechanisms are available for resistance to sprays than to transgenic plants." Although the problem that Whalon grappled with was partly answered by Roush, the implications of his experiment depend on the reasons for the findings. Unfortunately there is no single explanation. The paper does not tell us whether the Bt spray and the Bt potato used in the experiment both contained the same Cry toxin(s) in the same proportions. If the answer is yes then the results are interesting, but if the answer is no, the results are neither interesting nor surprising.

Bruce Tabashnik produced a seminal review of the state of the art, or rather, the state of ignorance in "**Evolution of Resistance to Bt"**published in the Annual Review of Entomology Vol 39 pp 47-79

1994. "Results from laboratory selection experiments show that evolution of resistance to Bt is possible in moths, beetles, mosquitoes and other flies. Pests can develop resistance to a variety of Bt strains and toxins, even when many toxins are used simultaneously.......Knowledge of how different management tactics affect rates of resistance development is sorely needed........Until the tactics of managing resistance to Bt are rigorously evaluated, we must admit and accept the consequences of our ignorance. Theoretically one could use Bt extensively, perhaps in combinations or high doses, and somehow avoid resistance, but virtually no experimental evidence supports these approaches. Because intensive use of Bt could potentially produce rapid and widespread resistance, the burden of proof rests heavily on those who advocate attempts to overwhelm pests with Bt......Although genetic variation for resistance may be somewhat less for Bt than for conventional insecticides, the limited use and low persistence of Bt are probably the primary reasons for the scarcity of resistance to Bt in the field so far. The surest way to conserve the efficacy of Bt is to use it judiciously in conjunction with other controls." Tabashnik warned against what has now happened.

Faced with the growing list of insoluble problems associated with resistance management strategies, **T. Watson** came up with a straightforward military plan for the eradication of the pink bollworm on cotton. In a letter to R. Lavis dated 20/9/95 he proposed the following:
"As I see it this (Bt) technology can be utilised in either of two ways: management of pink bollworm or eradication. The former would be more applicable to an individual grower or community effort while the latter will need a total industry commitment.... Based upon my experience in all aspects of pink bollworm research I'll offer the following thoughts for consideration by the ACGA group who will be considering the eradication option.

1. It will require a two year commitment.
2. Cultural control must still form the basis for the eradication program.
3. All acreage must be planted with Bt-transgenic cotton
4. A planting window (by zones) and a mandatory termination date (by zones) should be strictly adhered to. This would reduce the likelihood of pink bollworm surviving in late season bolls when the expression would accelerate the development of resistant populations.
5. Utilise a good pink bollworm pheromone monitoring program.
6. Continue using your PCA's to manage all pests and monitor for pink bollworm.
7. There should be no need for conventional insecticides against the pink bollworm and some other lepidoptera pests.

There is indecision and controversy about how to deploy Bt-transgenic cotton to prevent the development of resistance. The present strategies being proposed are in variance with the plan I have proposed above. Most plans include a refugia whereby some pink bollworm will survive in non-Bt cotton. This is a logical plan for resistance management but incompatible with an eradication effort."

The eradication option was eventually rejected as unworkable. There was the danger that if 100% of US cotton acreage was planted with Bt plants, the pink bollworm would survive in the form of a 100% resistant insect. Either way, the result would not have been commercially advantageous to Monsanto, the sole developer of Bt cotton.

In 1995 Tabashnik threw further doubt on the usefulness of simple models of insect resistance and emphasised how little was really known about resistance to Bt. In **"Diamondback Moth resistance to Bt"** (email edition 1995) he wrote that "many field populations of diamondback moth (DBM), Plutella xylostella, have evolved resistance to Bacillus thuringiensis kurstaki (Btk) (Tabashnik 1994). Resistance to Btk in DBM did not cause cross-resistance to Cry1C, a major toxin in Bacillus thuringiensis aizawai (Bta). In laboratory selection studies, several insects have evolved resistance to Cry1C. Although low-level resistance to Bta was found in some field populations of DBM 1995, no previous cases of resistance to Cry1C have been reported from the field. Recently, we found that a Btk -resistant DBM field population in Hawaii evolved 20-fold resistance to Cry1C toxin less than two years after Bta products were used.... Our results suggest that Btk-resistant DBM populations can evolve resistance to Cry1C in the field in less than two years. In the NO population, resistance to Cry1C apparently evolved faster than to Bta. Several factors might cause the difference: (1) spores in Bta (2) toxins in Bta other than Cry1C, (3) Bta materials other than spores and toxins, or (4) formulation ingredients. The difference in resistance of DBM between Cry1C toxin and a Bta spore-crystal formulation suggests that spore-crystal formulations may be more durable than single toxins. Our data, however, do not address the more difficult issue of whether it is best to combine toxins or to deploy them sequentially."

In previous reports the DBM resistance to Bt was unstable and the insect again became susceptible to Bt after about 10 generations. However, in **"Stable resistance to Bt in Plutella xylostella (DBM)"** published in Resistant Pest Management Vol 7 No 1 1995 **Richard Roush** reported a stable, high level of resistance to Btk even in the absence of further selection. According to Roush, "this presents tremendous problems for developing resistance management strategies." These results confounded a lot of previous studies, though it is possible that there were crucial differences in the patterns and persistence of resistance to Bt between different species. If the results produced by Roush were to be replicated with other insect pests, there would be little possibility of managing insect resistance by planning rotations of different insecticides over time AFTER resistance had set in. In other words, ICM and IPM strategies could only be expected to work if Bt was not massively commercialised either as an exterrnal spray or asd an engineered genetic construct. If multiple resistance were also to stabilise, the future of Bt as a useful insecticide will be rather short.

U.Rahardja threw further light on the stability of insect resistance in **"Inheritance of Resistance to Bt tenebronis Cry111A endotoxin in Colorado Potato Beetle"** published in the Journal of Economic Entomology Vol 88 No 1 1995. He showed that resistance was conferred by incompletely dominant genes. Without exposure to Bt, resistance started to decline from 200 fold after 5 generations and went down to 48 fold after 10 generations. The resistance was then maintained after 12 generations up to the end of the experiment which studied 17 generations. Rahardja thus added detail to the Roush (1995) contribution, by showing that after an initial decline in resistance, subsequent generations maintained a high level of resistance even in the absence of any contact with Bt. Once resistance has set in, it appears to be permanent.

The contribution by **J. Tang** marked the beginning of a new-found pessimism regarding the future of Bt In **"Consequences of Shared toxins in Strains of Bt for resistance in Diamondback Moth"** in Resistant Pest Management Vol 7 No 1 1995 he showed that the use of Bta where resistance to Btk has developed may provide control via the Cry1C and/or Cry1D toxins, but at some cost to resistance management because resistance to Cry1A was maintained. This cost was exacerbated by the fact that resistance to Cry1A toxins could stabilise, thus making it impossible to use Btk even occasionally. The contribution by Tang marked a new phase in Bt research, where the entomologists started asking desperate questions in the search for some way of managing resistance to Bt. Every short term solution has turned out to have a long term cost and the significance of this research is that it begins to attach more importance to short term solutions than long term costs. In other words, the paradigm had now shifted from asking how Bt could be managed as a useful pesticide into the future, to a much more specific question which simply asked what we can get out of Bt whilst it still works.

Reporting on a field study in **"Stable Resistance to Bt in Plutella xylostella"** published in Resistant Pest Management Vol 7 No 1 1995, **J. Tang** showed that resistance in diamondback moths from the field was more than 1500-fold higher than normal. This declined significantly over three generations and then stabilised at between 150 and 300 fold for the next seven generations. This suggested that there could be multiple resistance alleles even when the same gene is involved. Tang had shown that the basic models no longer sufficed to explain the empirical variance.

In 1995 **Fred Gould** reported on laboratory experiments in the **"Selection and Genetic Analysis of a H. virescens Strain with High Levels of Resistance to BtToxins"** published in the Journal of Economic Entomology Vol 88 No 6 1995. Tobacco budworms were fed a diet containing the Cry1Ac Bt protein. After 19 generations the survivors developed a resistance more than 500 times the normal. Further selection led to larvae which were 10,000 times more resistant than the normal population. These larvae were resistant to Cry1Aa, Cry1Ab and Cry1F as well as the Cry1Ac protein and were partly resistant to Cry1B, Cry1C and Cry11A proteins. Perhaps with hindsight it will be this paper by Gould that marks the beginning of the end of Bt. The experiment showed that insect resistance to a toxin could climb to 10,000 times the normal and could also convey resistance to seven other toxins. If the companies currently marketing Bt crops end up dominating the market - as seems quite likely in the USA - then there is every reason to expect these laboratory results to be replicated in the field, simply because the selection pressure that was created in the laboratory will have been replicated in the fields.

J. Muller-Cohn bucked the trend in 1996 by questioning once again whether resistance to Bt was stable. In **"Spodoptera littoralis Resistance to Cry1C and Cross-Resistance to Other Bt Crystal Toxins"** published in theJournal of Economic Entomology Vol 89 No 4 1996 he reported that after 14 generations of exposure to Cry1C the spodoptera grubs were from 10-500 fold more resistant and showed partial cross-resistance to Cry1D, Cry1E and Cry1Ab toxins. Resistance declined from 500 fold to 74 fold after one generation without selection pressure and fell to 11 fold after 8 generations.

In 1996 **J. Zhao** reported the inevitable consequences of a massive military style application of Bt on cotton in **"Resistance monitoring of H. armigera to Bt in North China"**published in Resistant Pest Management Vol 8 No 2 1996. Resistance of the cotton bollworm to all chemical insecticides from 1992-95 led to the application of Bt, (which has often been used for the very first time under the threat of total crop failure.) One thousand tons of Bt (5% of the total Chinese production) were applied to 160,000 hectares of cotton in 1994 in the form of 4-6 sprays over the season. The research concluded that "the potential of H. armigera resistance to Bt toxins is most threatening to the use of both Bt formulations and transgenic Bt cotton.".

The same conclusion was reached by **J.Shen** in **"Early Detection of Resistance to Bt in H. armigera in China"** also published in Resistant Pest Management Vol 8 No 1 1996. He studied field resistance after Bt was sprayed up to 4 times per season on cotton. "It is absolutely necessary to restrict the use of conventional Bt to a maximum of 2 sprays per season in north China as a precaution against Bt resistance development in H. armigera." There is, however, no evidence that restricting Bt use to two sprays per season will really slow down the increase in insect resistance.

Meanwhile, **Y.Liu** reported the development of **"Field-evolved Resistance to Bt Toxin Cry1C in Diamondback Moth"** in the Journal of Economic Entomology Vol 89 No 4 1996. The study confirmed the spread of resistance to the Cry1C toxin which was previously effective against insects resistant to Cry1Ab found in Btk. Another weapon in the Bt armoury had thus fallen to the diamondback moth, confirming an acceleration in the phenomenon of multiple resistance.

In the name of sociological realism **D. Alstad argued,** controversially that the most useful application of Bt was simply to use it to control insects now and forget about refuge options. In **"Implementing Management of Insect Resistance to Transgenic Crops"** published in AgBiotech News Vol 8 No 10 October 1996, he proposed that any trade-off between pest control now and delaying resistance in the future should focus on the now because growers would not willingly adopt refuge strategies and they would therefore not work. His proposal was tempered by an apparently happy correlation between the preferential behaviour of corn-borers and the economic interests of farmers. See the explanation and reply from Ives below.

In response to Andow, **A. Ives** published the **"Evolution of Insect resistance to Bt-transformed Plants"** in Science Vol 273 6/9/1996. He argued that "a critical feature of corn-borer natural history is its preferential migration into the most mature stands during the first of its two annual generations. Alstad and Andow state that resistance evolution can be delayed by using Bt toxic plants in the preferred crop, thus creating a "trap crop". Because preference biased migration concentrates insect densities it increases density-dependent mortality and reduces insect abundance. The improvement Alstad and Andow attribute to the "trap-crop" strategy is actually caused by preference-biased migration itself........Changing the distribution of toxic plants among fields is not a silver bullet to combat resistance evolution."

In 1997 **Fred Gould** showed that the statistical assumptions regarding the naturally occurring resistance to Bt in the field had been hopelessly optimistic. In **"Initial frequency of alleles for resistance to Bt toxins in field populations of Heliothus** virescens"published in Proceedings of the National Academy of Sciences pp 3519-3523 April 1997 he pointed out that the rate at which a pest adapts to a toxin depends on the initial frequency of resistant genes in normal field populations. Because there was no empirical information on this frequency, all predictions regarding the spread of resistance had been based on speculative assumptions. His field research found that the natural frequency of resistant alleles was 1.5x10-1, higher than had been expected. "This high initial frequency underscores the need for caution in deploying transgenic cotton to control insect pests." When statistical assumptions are based on guesswork they seem always to err on the side of optimism. When the assumptions are eventually tested against empirical realities, the truth is often rather sobering. Gould showed that the statistical likelihood of resistance setting in the field was very much higher than had been hoped.

In the same issue of the same publication **Bruce Tabashnik** contributed an article about **"Seeking the root of insect resistance to transgenic plants"**(pp 3488-3490). He wrote that "excitement about the prospects for Bt-expressing transgenic plants and increasing knowledge about the genetics and mechanisms of resistance to Bt must be tempered with an admission of ignorance. Although many tactics have been proposed for delaying insect resistance to transgenic plants, none have been tested rigorously in the field. Nothing will be gained and much will be lost if we pretend to know more about resistance management than we really do. A lesson in the pitfalls of overzealous promotion occurred last summer when some growers found that Bt cotton did not adequately control the bollworm Helicoverpa zea." In fact, previously published data from Monsanto had shown that the Cry1Ac protein in the Bt cotton was effective against Heliothis virescens but not effective against Helicoverpa zea. This did not stop Monsanto, the manufacturers, from stupidly and dishonestly calling their product 'Bollgard' which led most cotton growers to think that the Bt cotton controlled the cotton bollworm, Helicoverpa zea rather than the tobacco budworm, Heliothis virescens. Had Monsanto marketed their Bt cotton as 'Budgard' there might have been less panic and less confusion.

No doubt under instructions to come up with a solution to the Bt disaster with cotton in Northern China in 1994 (see above) **J.Zhao** sought a solution in gene pyramiding. In **"Gene pyramiding: an effective strategy of resistance management for H. armigera and Bt"** published in Resistant Pest Management Vol 9 No2 1997 he explored the use of multiple toxin genes in tobacco plants, concluding that this approach could delay resistance development. Whilst it is possible that Bt pyramiding works better against some insects than against others, the contradictory conclusions drawn by Zhao and Liu simply show that much more research is needed.

In Hawaii farmers had different problems. **Y.Liu** reported on the **"Genetic basis of diamondback moth resistance to Bt toxin Cry1C"** in Resistant Pest Management Vol 9 No 2 1997. A field population of diamondback moths in Hawaii was found to have at least one recessive mutation conferring resistance to Cry1A toxin as well as genes that confered partially dominant resistance to Cry1C toxin. The dominance of resistance could vary amongst Bt toxins for a single population and the given toxin could vary among populations from different locations. This casted doubt on the effectiveness of high dose/refuge strategies which work best when resistance is recessive. Planned rotations using Cry1A then Cry1C would be a more effective insecticide strategy than use of both at once - yet another argument against pyramiding.

In December 1997 **Bruce Tabashnik** wrote **"One gene in diamondback moth confers resistance to four Bt toxins"**in Proceedings of the National Academy of Sciences Vol 94 and demolished the pyramid strategy for countering insect resistance in the very title of his contribution. He pointed out that any attempt to delay resistance to Bt by using two or more Bt toxins assumed that independent mutations were required to counter each toxin. It was also generally assumed that resistant alleles were rare in target populations. His research showed that a single autosomal recessive gene conferred extremely high resistance to no less than four different Bt toxins - Cry1Aa, Cry1Ab, Cry1Ac and Cry1F. "The finding that 21% of the individuals from a susceptible strain were heterozygous for the multiple toxin resistant gene implies that the resistance allele frequency was 10 times higher than the most widely cited estimate of the upper limit for the initial frequency of resistant alleles in susceptible populations. These findings suggest that pests may evolve resistance to some groups of toxins much faster than previously expected." This was another nail in the Bt coffin, showing that scientists had consistently underestimated the number of resistant insects in the field, and wrongly assumed that separate mutations were required for resistance to each Bt toxin. It was becoming clear that much of the original hype about what Bt could do was based on little more than wishful thinking aided by corporate propaganda machines.

Also in 1997 **U.DiCosty** returned to the dilemma posed by Whalon in 1994. The **''Selection of Colorado Potato Beetle Resistant to Cry3A on Transgenic Potato Plants''** published in Resistant Pest Management Vol 9 No1 1997, showed that "laboratory selected, highly resistant beetles could survive on Bt transgenic plants for a short period of time. If alternative host plants were encountered after selection, beetles could survive. Conversely, successive generational exposure to transgenic plants resulted in 100% mortality of this resistant strain within three generations." This article gave rise to the hope that Bt plants could be effective where the use of conventional Bt sprays had already resulted in insect resistance. However, it also questioned the effectiveness of refuge strategies.

The supposedly 'green' or 'ecological' image of Bt as the great alternative to chemical pesticides was rather tarnished by **O. Sarnthoy** in **''Cross-resistance of Bt resistant population of diamondback moth''** published in Resistant Pest Management Vol 9 No 2 1997. He recommended the use of conventional insecticides and a range of different Cry toxins to retard or reverse the development of resistance to Delfin, a Bt product produced by Novartis. The moral is rather simple: the more successful a company is at dominating the market with its pesticide product, the less time its product is likely to be biologically effective. Where the trade-off lies between massive short-term sales and moderate long term sales is a question of economics rather than entomology, though the consequences are clearly ecological.

In 1999 Y. Liu and B. Tabashnik brought the highdose/big refuge strategy for delaying insect resistance into question with a short contribution in Nature (Vol 400 p 519) which reported that insects feeding on Bt plants matured later than insects fed a normal diet. This cast doubt on the degree to which resistant and non-resistant insects might be able to breed. Interbreeding of the insects feeding on the Bt crop and the insects feeding on the refuge is necessary for the delaying strategy to work.

2.5 The Problem of Identifying Resistance in the Field

If a target insect is found eating a Bt crop there are at least four possible explanations of the phenomenon.. According to Purdue University Extension entomologist Larry Bledsoe, some crop damage is to be expected even in genetically modified crops. "No bag of Bt seed is pure. No quality control can manipulate the amount of control in each plant," he says. "If a farmer was in a field and found a couple of plants being chewed up by corn borers, that would be normal."

1. Typically seed lots of Bt-maize contain a small amount - less than 4 percent - of plants that produce little or none of the protein. "This means that a few plants aren't expressing the Bt gene. That's to be expected," Bledsoe says.
2. Another explanation for finding corn borer caterpillars in resistant corn may lie in where the Bt-corn is planted. "If you plant next to a field with no resistance, some of those corn borers are going to come into the resistant field and feed along the edges for a while before they are killed," Bledsoe says.
3. A third reason for corn borers in resistant corn is that the amount of resistance in the plants isn't consistent through the growing season. "There's a slow loss of resistance in the plant," Bledsoe says. "It's very strong at the beginning of the season, but later in the season the amount of resistance drops."
4. The fourth reason why a farmer might find corn borer caterpillars in the corn, and it is that a new strain of Bt resistant corn borers have evolved in that area. There have been more than 500 examplesof insects that have developed resistance to various chemical insecticides, and widespread overuse of genetically enhanced crops could cause the same thing to happen with those control methods.To date there are no known incidents of corn borer developing widespread resistance to Bt crops in the field, but scientists know that it is possible, because resistant corn-borers have been bred in the laboratory.

2.6 Strategies for the Management of Resistance

Entomologists and population geneticists have been experimenting with methods designed to slow down the evolution of pesticide resistance in Bt for the past seven years. According to McGaughey and Whalon (1992) there are at least

15 possible tactics for slowing down insect resistance to Bt:

A. Gene Strategies
  1. single gene
  2. multiple gene
  3. chimeric genes

B. Gene Promoters
  4. constitutive
  5. tissue-specific
  6. inducible

C. Gene expressions
  7. high dose
  8. low dose
  9. mixture

D. Field tactics
  10. uniform single gene
  11. mixture of genes
  12. gene rotation or sequencing
  13. mosaic planting
  14. spatial refuge
  15. temporal refuge

"The possible tactics for resistance management include many options. None offer clear advantages in all environments and with all pests, except, perhaps, tactics that encourage survival or immigration of susceptible genotypes. Regardless of the approach used, resistance management becomes very complex where tactics must be co-ordinated against a pest on more than one crop or against more than one pest species."

According to Gould (1995) there are now at least five different strategies being proposed:

"(1) Constitutive expression of high levels of single toxins in all plants
(2) Constitutive expression of high levels of two or more toxins in all plants
(3) Spatial or temporal mixtures of plants having high levels of constitutive expression of one or more toxins with other plants having no toxin expression
(4) Low levels of expression of single toxins interacting with the pests' natural enemies
(5) Targeted Bt gene expression."

The first two strategies rely on making the toxin so poisonous that the pest is less likely to develop the genetic potential to overcome it. However, it is already known that certain pests targeted with Bt have the ability to develop extremely high levels of resistance and that once this resistance has developed it can transfer to other Bt strains, rendering several of them useless. It is also known that the Novartis Bt maize now being grown in the USA, Germany, France and Spain is unable to express high levels of toxin in all plants, expresses the toxin with considerable variability over time and at different rates in different parts of the maize plant.

The second strategy only works if the genes controlling resistance are rare in natural populations, and resistance to one toxin does not confer resistance to the second or third toxin. It is now known that neither of these requirements is met by studies with the diamondback moth. In fact, multiple toxin approaches could actually accelerate the evolution of resistance to a whole group of toxins.

The third strategy relies on plants that express high levels of one or more toxins (strategies 1 and 2) that are spatially or temporally mixed with plants that do not express any toxin. The supposed advantage of this strategy is that resistant insects are likely to mate with non-resistant insects coming from the refuges of non-Bt plants. To date, laboratory studies have shown that the offspring of resistant and non-resistant insects can not survive high doses of Bt toxins. However, this is presumably because the Bt resistant insects do not possess a dominant Bt resistant gene. As and when such a gene is encountered - and Tabashnik think that it can only be a matter of time - strategies 1, 2 and 3 will no longer work for that particular insect. In the meantime, according to Gould, "The higher the number of susceptible insects produced in refuges, the more likely they are to mate with resistant individuals that develop on the Bt producing plants. If there are enough of these susceptible insects, almost all of the resistant insects will mate with them instead of mating with other resistant individuals. This should result in a dramatic decrease in the rate at which resistant individuals take over the population." Strategies 1, 2 and 3 all require Bt plants to express a dose of Bt toxin sufficiently high to kill insects with intermediate levels of resistance. However, some of the Bt crops, and particularly the Novartis Bt-maize fall far short of this standard and according to Gould (1995) "are likely to undermine this strategy."

The third strategy is the only one that has ever been required by law. In the USA, the EPA has made the planting of a refuge a licensing condition for growing Bt cotton. Farmers can choose between a 4% non-treated (organic) refuge and a 25% refuge that can be treated with any insecticide other than the Cry protein expressed by the Bt crop. It follows that the resistance management strategy only works if the insecticides used in the refuge are ineffective. The refugia strategy thus contains an internal contradiction. Those who err on the side of precaution argue for the biggest refuge. But the bigger the refuge, the more important it is to control the pest within the refuge by other means, otherwise there is no advantage in planting the Bt crop. This dilemma is inherent in the biology of the system and cannot be resolved.

The fourth strategy relies on a very low level of toxin which does not kill the pests so much as debilitate them, thus rendering them more vulnerable to parasites or predators. Whilst this approach has demonstrated ecological efficacy, it has not been developed by the agrochemical industry because it can only be successfully marketed to those farmers with a sophisticated understanding of the ecological environment of agriculture - the organic farmers plus the IPM/ICM practitioners.

The fifth strategy is relies on the fact that most crops do not need comprehensive protection of the whole plant all of the time. There are many pests that feed throughout the life of a plant but only cause economic damage towards harvesting time. According to Gould (1995), "in such cases advanced techniques in molecular biology could turn on the genes for toxin production only in later stages of plant development. This would decrease the exposure of the pest to the toxin, and thus decrease the rate of resistance development." However, the art of engineering Bt plants has a long way to go before it is likely to achieve this degree of sophistication, and it is likely to prove workable only for single pest species.

In practice, the five strategies can be reduced to two:

- the high toxin(s)/refuge strategy
- the low toxin marginal use strategy

2.7 Secondary Effects of Bioinsecticide Plants

There are some studies on the secondary effects of Bt and these are summarised below.

1988 Sun.M
Preparing Ground for Biotech Tests
in Science 29/10/1988 p 504
Maize was inoculated with a microbe modified to express a Bt endotoxin. The microbe lives in the vascular structure of the plant but the microbe containing the BT toxin was later found in flea beetles that had fed on the maize.

1993 James.R
Btk Affects a Beneficial Insect, the Cinnabar Moth
in Journal of Economic Entomology Vol 86 No 2 1993
Field and laboratory experiments indicate that Bt used to control the western spruce budworm can also kill the cinnabar moth which is used to control tansy ragwort, a forest weed.

1994 Swadener.C
Bt
in Journal of Pesticide Reform Vol 14 No3 pp 13-18
"Bt has impacts on a number of beneficial species. For example, studies of a wasp that is a parasite of the meal moth found that treatment with Bt reduced the number of eggs produced by the parasitic wasp, and the percentage of those eggs that hatched. Production and hatchability of eggs of a predatory bug were also decreased. On collards, aphis-eating flies in the family Syrphidae were reduced by Dipel (Bt) treatment. Both Bt tenbebrionis and Dipel have caused mortality of the cinnabar moth, used for the biological control of the weed tansy ragwort. Finally, Bt israelensis has caused mortality of a moth (Sybclita obliteralis) that helps controlo aquatic weeds in Florida." A variety of studies have shown that Bt applications can reduce populations of many different caterpillers and larvae beside those of target insects. This in turn is shown to affect insectivore bird populations. Bt israelensis decreases the weight of tadpoles and delays their metamorphosis and in the form of Vectobac it is acutely toxic to fathead minnows, though this may because of other ingredients in the product besides Bt.

1994 Palm.C
Quantification in soil of Btk endotoxin from transgenic plants
in Molecular Ecology Vol 3 pp 145-151 1994
Development of procedure to determine the fate and persistence of transgenic Btk toxin in both laboratory and field to provide information on the consequences of exposure of non-target soil organisms to transgenic pesticidal proteins.

1997 Hawkes.N
Ladybirds harmed in transgenic crop test
New Scientist 23/10/97
Scientists in Scotland have urged caution in the introduction of genetically modified crops after

discovering that they could harm ladybirds.

Nick Birch and a team from the Scottish Crop Research Institute in Dundee found that female ladybirds that ate aphids that had fed on genetically modified potatoes laid fewer eggs and lived only half as long as the average. The team tested a potato plant that had been modified to produce a natural insecticide that discouraged aphids from feeding on them.

The team found that the modified potatoes did indeed suffer reduced attack but the cut, of 50 per cent, was insufficient on its own, so it was important that ladybirds also did their work. The potatoes were not Bt plants and it is not yet known whether Bt plants can indirectly affect insect beneficials in the same way.

1998 Greenpeace
Ge-maize contaminates Conventional Crops
Amsterdam/Hamburg, October 12, 1998
Greenpeace today published new evidence which shows that Novartis genetically engineered (GE) maize has cross-pollinated an adjacent field of conventional maize in Germany. The samples analysed were taken next to a field of GE-maize in the region of Baden-Württemberg, in southern Germany. Greenpeace marked the GE-field with a giant X in an action a month ago. The neighbouring farmer only learnt during the Greenpeace action that the maize growing less than a meter away from his field wasgenetically engineered. Neither Novartis, nor the German authorities have released informationabout the fields of transgenic maize nor did they warn neighbouring farmers. HoweverNovartis, in a special contract with growers of its GE-Maize, did mention that a safetyzone of 200 meters is necessary to avoid cross-pollination.
Maize cobs up to 10 meters away from the GE-field were taken by the Freiburger Institut für Umweltchemie e.V. and analysed by Gene-Scan for the foreign DNA of the Novartis' maize. Analysis indicates that the rate of cross-pollination was around 5% at the field border, 0,2% at 5 meters and 0,1% at 10 meters distance.

1998 Hilbeck.A
Impact of Bt maize on Populations of Beneficial Insects
unpublished paper from Swiss Federal Research Station for Agroecology and Agriculture
Lacewings are an important predator of many insect pests killed by Bt and they are bred for use in organic and IPM strategies. The research showed 60%-65% mortality amongst lacewing larvae fed on lepidopteran larvae reared on Bt maize.

2.8 Position Statement by the Entomological Society of America

"Transgenic plants that produce insecticidal substances are, and should continue to be subjected to careful testing to ensure safety and minimise environmental risks.

Insect-resistant crop plants should be deployed in accordance with scientifically based resistance-management plans to prevent the evolution of genetically adapted insect strains.

The use of insect-resistant plants is not equally appropriate for all crops in all agricutural systems; therefore a case-by-case scientific analysis of risks and benefits should be conducted before commercial use.

To prevent evolution of resistance to transgenic plants, resistance prevention tactics should be devised before pest-resistant crops are widely and intensively deployed.

Transgenic plants, especially those that produce toxins throughout the season, could bring with the difficult challenges not experienced with externally applied insecticides.

Transgenic control of a pest in one crop couldlimit the food source of important natural enemies and lead to increased pest problems.

Management strategies should be developed that take advantage of the ability to manipulate the spatial arrangement of transgenic plants, thereby incorporating refuges for susceptible insects into the cropping environment.

The farm-level implementation of resistance management will face practical and social obstacles.

The wisdom of using a specific insect-resistant crop should be evaluated relative to the long-term goals of reducing pesticide use and fostering sustainable crop production systems."

Entomologists are agreed that Bt plants are much more likely to result in Bt resistant insects than Bt sprays. Faced with the fact that the widespread marketing of Bt crops would inevitably foreshorten its life as a biologically useful bioinsecticide, the industry started to consider military rather than economic options. Throughout 1995 the US agrochemical industry, entomologists and cotton farmers seriously discussed the possibility of totally eradicating the pink bollworm using Bt cotton. The strategy was eventually deemed too risky and attention returned to resistance delaying tactics.

The US classifies Bt crops as pesticides so they fall under the terms of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) and are dealt with by the Environmental Protection Agency (EPA) in Washington. As early as 1987 the EPA sponsored a conference on the questions raised by Bt plants. In November 1994 the EPA stated that it had 'begun to analyse the regulatory and non-regulatory tools it could use to address resistance to all pesticides, including plant pesticides'. In January 1998 the EPA published a white paper on Bt plant pesticide resistance management. By then the EPA had been forced to consider the complexities of cross resistance between Bt cotton and Bt maize. Cotton monocultures are decimated by three pests in the USA, whilst maize can be decimated by five pests. One pest - Helicoverpa zea - attacks both cotton and maize. This led the EPA to ignore free market economics and limit the sale of the Novartis Bt 11and the Monsanto 810 to 40,000 hectares each across the cotton belt, and to ban the DeKalb 418 maize from the cotton belt altogether. Meanwhile the Novartis Bt 176 was allowed a free run of the cotton belt because neither the silk nor the kernel are capable of producing more than a tiny trace of Bt toxin. However, this deficiency is precisely the reason why the Bt 176 fails to control the second generation european corn borers and thus negates the EPA policy for delaying insect resistance. In other words, the EPA only permits the sale of the Novartis Bt 176 in the cotton belt because it does not work!

The EPA, the agrochemical industry and academic entomologists currently believe that the best chance of delaying the onset of insect resistance to Bt plants is to grow highly toxic plants alongside suitable insect refuges of non Bt plants. For some years, almost all research has focussed on the size of the refuges and the problems when Bt cotton and Bt maize are grown in the same locality. Entomologists favour large refuges whilst the industry wants to keep them as small as possible. Meanwhile, recently published research is showing that the EPA policy is probably wishful thinking. It seems that the grubs that are resistant to Bt crops are sexually retarded and are thus less likely to mate with non-resistant insects than the resistance management plans call for.

Of all the Bt crops now developed, only the Novartis Bt 176 maize has been legally commercialised in Europe. In 1998 it was grown in France, Germany and Spain and in 1999 it has been grown in Spain and Portugal.

It is now widely accepted in the USA that Bt 176 is a deficient product and it is probably only a matter of time before the EPA is forced to ban it from the US market. Interestingly, the Bt 176 deficiency is actually used as a marketing feature by Novartis and Northrup King in Spain.