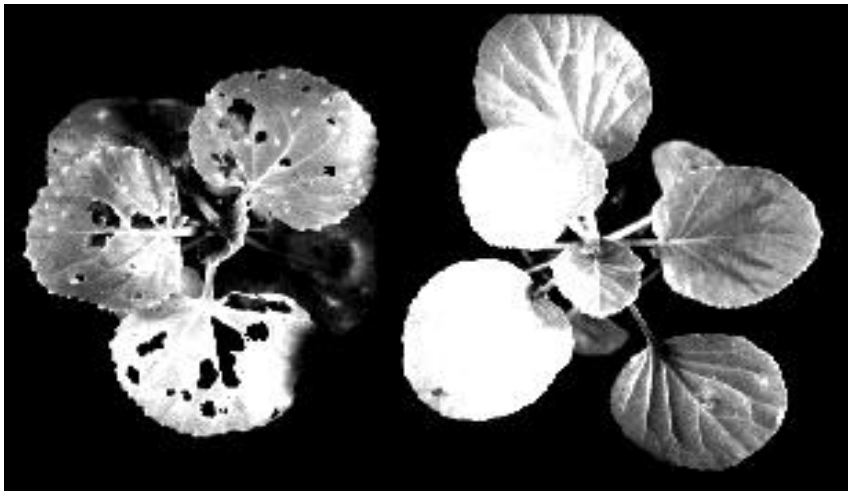
Insect Resistance in Crops:

A Case Study of *Bacillus thuringiensis* (*Bt*) and its Transfer to Developing Countries

Anatole F. Krattiger
Executive Director of ISAAA



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Cover Picture: Left: The diamondback moth prefers wildtype broccoli;
Right: *Bt*-transformed broccoli is resistant to diamondback moth.
Courtesy: T.D. Metz and E.D. Earle (Cornell University).

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Executive Summary

Despite significant increases in per capita agricultural production worldwide over the last decades, the challenge of producing sufficient food supply remains daunting given increasing population growth, reduced availability of water, and limits to agricultural land expansion. Biotechnology applications, if properly integrated into production systems, offer new opportunities to increase production and productivity by using a more sustainable and ecologically friendly agricultural system.

One such near-term biotechnology application is insect resistant crops through the insertion of a gene from *Bacillus thuringiensis* (*Bt*) that produces a protein toxic to certain insects (of *Lepidoptera*, *Coleoptera* and *Diptera* families). A review of the known genes, as well as an outlook on the next wave of insect resistance technology (e.g. smart proteins, VIPs), reveals that *Bt* is merely the beginning of a long series of new and safer technologies to augment productivity, to bring about a more sustainable agriculture, and to protect the environment. With *Bt* alone, there are already over 50 genes with known insecticidal properties. Several of these could be deployed simultaneously (provided they have different modes of action) to increase the level of protection of the crop and possibly reduce the risk of insect populations developing resistance. So far, the efficacy of insect resistant crops through *Bt* has been shown to be comparable to or better than the efficacy of current control methods. One reason is that fewer insecticide applications are required and in some cases a *Bt* crop may not require any insecticide sprays at all. Fewer applications save cost and time, in addition to reducing health risks to workers (a particularly hazardous activity in many developing countries). Ecological benefits should not be underestimated either, since the *Bt* toxins are highly specific against certain insects without affecting predators and other beneficial insects. This is not the case for many insecticides, such as the broad-spectrum pyrethroids.

Bt has been used for a long time as an effective biopesticide in agriculture, yet it represents less than 1% of insecticides used on a global basis. The market of insecticides is US$8.11 billion annually, with 30% of them applied on fruits and vegetables, 23% on cotton and 15% on rice. Asia produces 92% of the world production in rice and nearly US$1 billion is spent on insecticides for that crop in Asia alone. In cotton, more insecticides are applied than in any other crop (US$ 1.9 billion annually). Yet around US$1.2 billion in insecticides on cotton could be substituted with *Bt* biotechnology applications. The development of *Bt* cotton is presented and discussed as a detailed case study on transgenics. In rice, approximately US$400 million is spent on insecticides against the rice stemborer, which could completely be substituted with *Bt* transgenic crops. The total insecticide substitution value for the major crops of cotton, maize, rice, fruit and vegetables is estimated at US$2.69 billion annually.

A review of field trials of transgenic *Bt* crops shows that the first trials took place as early as 1986, but large scale trials were only numerous in OECD countries since the early 1990s. As a consequence, several million acres of *Bt* crops have been planted in the USA in 1996 (cotton, corn/maize, potatoes) and this is expected to increase substantially in 1997, and will include several European countries, Argentina, South Africa and Australia. Few developing countries are near commercialization of the technology which is also reflected by the fact that developing countries conducted less than 3% of the *Bt* field trials worldwide with few having effective biosafety regulatory mechanisms in place. A priority issue for developing countries will be how to gain access to this technology and develop effective and safe deployment strategies.

All commercialized *Bt* crops are by the private sector which is not surprising considering that 410 *Bt*-related patents were issued over the last 11 years: just over half of *Bt* related patents were granted to institutions in North America, 30% to European and Russian organizations, and 18% to companies mainly from Japan; of the total patents, over half are directly relevant to transgenics; and fifty-seven percent of all *Bt* patents have been issued to only eight companies. An analysis of commercialized *Bt* crops and of recent field trials demonstrates that a subset of these eight corporations are the major players in transgenic *Bt* plant technology, viz. Monsanto, Novartis, AgrEvo and Mycogen with their own technologies, and DeKalb Genetics Corporation and Pioneer Hi-Bred International through strategic alliances. The most advanced products include cotton, corn/maize, potato, tomato, canola/rapeseed and tobacco, and approximately 20 corporations are advancing their own products. This large number of companies working on *Bt* (partly under license) demonstrates that the few major players who own enabling technologies are willing to license despite the fact that 23 lawsuits on *Bt* are pending. These are not restricting the technology from being commercialized, but will determine who receives the largest portion of royalties.

Bt crops—if deployed responsibly—offer substantial benefits and have the potential for significant short-term impact. Long-term impact can only be sustained if effective and responsible deployment strategies are adopted to maintain the durability of the *Bt* genes. Such deployment strategies must be aimed at reducing the possibility of long-term impact by preventing resistant insects from mating with other resistant insects, thereby preventing the creation of a resistant population. But the strategies must also be designed to be effective in the event that insect resistance does develop.

Several strategies are presented and discussed (gene strategies, gene promoter, gene expression, field tactics), and a review of adopted procedures shows that a high dose approach (high gene expression) with separate refuge areas has been most widely adopted so far. The strategy still requires the monitoring of fields for early identification of possible resistant insects. This poses formidable challenges because collecting insects at random may not necessarily allow early enough detection of resistance to allow remedial actions to be implemented. Requesting farmers to monitor insect damage to crops has limitations, particularly in developing countries and small-scale agriculture where the extension efforts required for such a system to work are tremendous. This section concludes that the effect of the adopted strategies is still somewhat speculative. Unfortunately, only large-scale deployment will provide the true test for the durability of the genes and the generation of a body of evidence that will allow optimum and safe deployment strategies to be developed.

Finally, critical issues related to the transfer of the technology (e.g. biosafety regulatory obstacles, intellectual property rights and licensing issues) are discussed, with particular reference to their implications for the developing countries. The delivery of new technologies to developing countries, many of which do not have a fully developed private sector seed industry has always been more challenging. With biotechnology applications, some of the constraints imposed by traditional technologies do not apply (for example, biotechnology applications, as opposed to mechanization, is essentially scale-neutral). However, insect resistance with *Bt* presents a particular challenge due to the requirements for managing the deployment of the technology in terms of avoiding insect resistance.

It is concluded that the recent developments in biotechnology demonstrate that *Bt* is merely the beginning of a long series of new and safer technologies to augment productivity, to bring about a more sustainable agriculture, and to protect the environment. With the emergence of an increasingly broad range of possibilities from the point of view of the technology, emphasis must now be placed on the development of transfer and delivery mechanisms to the resource poor farmers who are most dependent on novel solutions for their very livelihood and survival.

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1. Introduction

Today, 800 million people, 200 million of them children, are chronically undernourished in the developing world, and millions more suffer debilitating diseases related to micronutrient deficiencies and contaminated food and water. Everyday, one out of five people in the developing world do not receive sufficient food to meet their daily needs. Paradoxically, over 60 percent of the world's poor live in the largely agrarian countries of South Asia and sub-Saharan Africa where 65% and 79% of the population, respectively, depends on agriculture for their livelihood.

There are numerous ways by which agricultural productivity may be raised in a sustainable way. Many different technologies must be deployed concurrently to counteract the negative impacts of the current degradation of the agro-ecosystem. These include biological fertilizers, soil and water conservation, biodiversity conservation, improved pest control, and changes in land ownership and distribution. Of these measures, biotechnology applications—integrated into traditional systems—probably hold the most promise in augmenting conventional agricultural production and productivity, particularly given the need to increase production sustainably, while protecting the environment and biodiversity and conserving natural resources for future generations.

Biotechnology applications will have short, medium and long-term impacts on agriculture. Some of the essential advantages include: possibilities to increase productivity and food availability through better agronomic performance of new varieties, including resistance to pests; greater stability in farm production and reduced need for expensive inputs; rapid multiplication of disease-free plants; ability to obtain natural plant products using tissue culture; conversion of crop residues to other value-added foods; diagnosis of diseases of plants and livestock; manipulation of reproduction methods increasing the efficiency of breeding; low cost, effective vaccine production in animal husbandry; and in a more general context, the provision of incentives for greater participation by the private sector through investments. Of these, insect resistance through the transfer of a gene for resistance from *Bacillus thuringiensis* (*Bt*) is one of the most advanced biotechnology applications already being commercialized in many parts of the world (along with virus resistance and tolerance to herbicides).

There are an estimated 67,000 pest species worldwide that damage agricultural crops, of which approximately 9,000 species are insects and mites (Ross and Lembi, 1985). It is in this context that the use of *Bt* crops will increase productivity as well as provide important benefits to farmers, the consumer, and the environment. The efficacy of insect resistant crops through *Bt* has been shown in large scale field trials to be comparable to or better than the efficacy of current control methods. One reason is that fewer insecticide applications are required and in some cases a *Bt* crop may not require any insecticide sprays at all. Fewer applications save cost and time, in addition to reducing health risks to workers (a particularly hazardous activity in many developing countries). Ecological benefits should not be underestimated either, since the *Bt* toxins are highly specific against certain insects without affecting predators and other beneficial insects. This is not the case for many insecticides, such as the broad-spectrum pyrethroids.

This paper reviews the technology and application of *Bt* transgenic crops, with particular reference to the potential for replacing traditional pesticides. The development of *Bt* technology is discussed, a case study presented and the status of the technology reviewed. Finally, specific issues related to the transfer of the technology to developing countries are addressed with emphasis on essential management strategies for *Bt* deployment.

2. Overview of Insect Resistance Mechanisms in Crops

Insect resistance in crops has long been a major objective in plant breeding. Yet little on the specific mechanism conferring insect resistance was understood until fairly recently. This is largely because insect resistance in crops is a "non-host specific resistance", meaning that generally certain plant species are not attractive to given species of insects which leads to immunity. In classical breeding, three systems of resistance are of particular relevance:

- morpological barriers to insects (e.g. hairy leaves introduced by the International Potato Center (CIP) through backcrosses to the common potato from wild

Andean potato species which provide mechanical resistance to aphids);

- the presence of insect-repellant or toxic substances (e.g. bitter substances not toxic to insects but that makes insects prefer other plants, if available); and
- toxins that have a repellent effect (e.g. quinonin) or deadly effect (proteolytic enzymes, such as trypsin inhibitors found in peas).

The problem with classical breeding is that the process is slow and often unpredictable in terms of the level and durability of resistance. Hence direct ways of transferring resistance genes has been a primary objective of biotechnology research in plants.

Induced resistance through *Bt* is one of the first modern crop biotechnology applications where products have halready reached the market. However, as Table 1 demonstrates, *Bt* is only one among the several insect control strategies enabled through biotechnology. Pyramiding genes, whereby two or more genes active against a certain insect are transferred into one variety, will become

Table 1: Complementary Systems of Insect Resistance in Crops

System [1]	Gene transfer	Patented [2]	Comment
Substances occurring in Plants			
1. Sugars (glucosinolates, polysaccharides)	No	No	The relative concentration of different sugars determines whether or not certain insects prefer a given plant or species (saccarose is preferred; not used in breeding)
2. Terpenoids	No	No	Large group of substances including pyrethroids, juvabione, pyrethrin I, sesquiterpenoids and phytoecdysteroids.Some terpenoids are common in cotton (i.e. gossypol) and used in classical breeding of to increase insect resistance (restricted to red-flowering cotton). Unfortunately, te substance is undesirable because cotton oil can thus not be used for feeding.
3. Alkaloids and glycoalcaloids	No	No	Includes nicotine and has long been used as biological pesticide produced from tobacco extracts. Also used in classical breeding such as in potato (demissin and α-tomatin). Several *Brassica* species also contain sulfides (glycosyde) which are toxic to some insects and attract others.
4. Flavanoids	No	No	
5. Phenols	No	No	Overexpression of polyphenoloxydase leading to increased production of quinonin which is toxic for insects. Unlikely to be used in genetic engineering due to complex chemistry and possible toxicity to mammals.
6. Protein antimetabolites			(all are secondary mnetabolites)
6.1 Aminoacids and primary storage proteins	No	No	E.g. in wheat, gluten proteins are not digestible by some insects (*Eurygaster integriceps*) and thus confer partial resistance to the insect. The presence of certain aminoacids generally increases (decreases) resistance in rice (peas).
6.2 Lectins (also referred to as plant peptide hormones)	Yes	Yes	Lectins are common in the grains of cereals, particularly during germination. Certain lectins also have antifungal properties. Much work is ongoing with the snowdrop lectin (GNA) and particularly promising for sucking insects (*Homoptera*) which cannot be controlled with *Bt*.
6.3 Protease inhibitors (includes trypsin and chymotypsin inhibitors)	Yes	Yes	Act on exogenous proteolytic enzymes. Trypsin inhibitor has particularly broad insect spectrum and has been demonstrated to have synergistic effects when used in conjunction with *Bt*. Much work is ongoing with the cowpea protease inhibitor (CpTI).
6.4 α-amylase inhibitors	Yes	N/k	Widely occurring in seeds, particularly dicots. For gene transfer, the most effective so far has been the α-amylase inhibitor genes from the common bean.
Substances NOT occurring in Plants			
7. *Bt* endotoxins	Yes	Yes	See text.
8. Secondary metabolites from bacteria	Yes	Yes	E.g. isopentenyltransferase (*ipt* gene) which affects the cytokinin biosynthesis in insects, leading to increased levels of toxins in insects. Such genes from bacteria have been transferred to several crops.
9. Other toxins	Yes	Yes	E.g. spider and wasp toxins have been transferred to plants for experimental purposes. Unlikely to be applied in crops due to effects on mammals of some toxins.
10. Smart proteins	Emerging	N/k	Computer-aided design of novel proteins.

N/k Not known to the author; patent applications have possibly been submitted.
1 Table draws on information from several sources, including Fritzsche *et al.*, 1987; Gatehouse *et al.*, 1992 and 1993; Franck-Oberspach and Keller, 1996.
2 Refers to patents related to transgenic plants only.

increasingly common as genes with novel mechanisms are discovered and developed. Such an approach, as will be discussed below, has many advantages but also disadvantages. Stewart (1995), for example, predicts that ten years from now, most transgenics will have five or more foreign genes against the same insect. To date, however, none have been advanced to a practical level but the efforts have lead to the discovery of a series of new natural insecticidal substances and systems.

Table 1 is not exhaustive and additional mechanisms are constantly being discovered and developed. One such system is the soon to be published discovery of so-called VIPs, proteins that occur in extremely small amounts in *Bt* but, unlike the now well-known δ-endotoxin which *Bt* produces during sporulation, the VIPs are produced during the vegetative phase (see forthcoming volume of *Nature*). These substances have been shown to be toxic to insects and it can be expected that one or several corporations will already have been working on the technology and could soon be field testing the first products.

Some of the examples in Table 1 are relatively advanced systems and patents for the more novel mechanisms have already been applied for or granted. Examples of patents recently issued include the control of insect growth through toxicity of their purine metabolic pathways, use of *Beauveria virulent* with an active ovaricide against *Lepidoptera*, the use of 3-hydroxy-steroid oxidase against *Lepidoptera* and boll weevil, and transformation with a gene for carbonarin anti-insectant metabolites isolated from *Aspergillus carbonarius*.

Possibly the most long-term application in Table 1 are smart proteins and the manipulation of metabolic pathways, such as for the production of azadirachtin, a toxic compound from the Neem tree. The modification of metabolic pathways is already possible and has recently been accomplished for insect resistance. Transformation of corn/maize with the gene encoding limonene synthase resulted in enhanced accumulation of limonene, a natural non-protein compound occurring in fruits and vegetables (Huesing, 1995). Nevertheless, commercial applications should take longer to reach the market and this is only expected to happen in the first years of the 21st century.

The last item in the list, namely smart proteins, or the computer-aided design of proteins, provides one of the most novel ways, and possibly opportunities, to identify proteins with insecticidal action but with hitherto unsuspected modes of action.

Finally, it should be mentioned that there are critical non-crop applications of *Bt* that will become increasingly important in developing countries, such as for the control of mosquitoes. A recent report from Singapore (Liu, 1996) demonstrates the use of *B. sphaericus* in the fight against mosquito larvae (*Culex* and *Anopheles*). Two genes coding for a toxin were inserted into a bacterium, *Asticcacaulis excentricus*, that can easily be grown in cheap media, thus making the insecticide affordable. The bacterium also persists in mosquito breeding places and can be used against the transmission of dengue disease in many parts of the developing world.

3. From *Bt* Biopesticides to Transgenic Crops

3.1 Bt and Biopesticides

Bacillus thuringiensis, commonly known as *Bt*, is a gram-positive bacterium that occurs naturally in the soil around the world. For decades, bacteriologists have known that some strains of *Bt* kill certain insects and that the toxic substance responsible for the insects death is a protein. When certain insects ingest either the bacterium or the protein produced by the bacterium (the protein is called δ-endotoxin), the function of their digestive systems is disrupted, eventually resulting in death. When the dose is high, sudden death occurs. The *Bt* protein is not harmful to mammals, birds or fish, nor to beneficial insects. Mammals, including humans, do not have δ-endotoxin receptors in their guts and all *Bt* proteins tested so far are degraded within 20 seconds in the presence of mammal digestive juices. *Bt* is not effective against all insects; however different *Bt* strains are effective against specific species. The major families of insects that respond to *Bt* are:

- *Lepidoptera* (caterpillars; e.g. European corn borer or cotton bollworm [see Appendix I for Latin names of insect species quoted in the text),
- *Coleoptera* (beetles; e.g. Colorado potato beetles) and
- *Diptera* (flies and mosquitoes).

The use of *Bt* as a biopesticide was discovered in the first decade of this century when larvae of flour moths died suddenly. Research into their deaths led to the discovery that the presence of *Bt* was responsible for the death. However, it took 50 years before *Bt* became a widely used biopesticide with its registration in the USA in 1961. Nevertheless, even today, less than one percent of all pesticides used in the USA each year are *Bt*-based products. The percentage worldwide is estimated to be less than 1%. Over half the *Bt* biopesticides are used in the USA, with a total worldwide market of biopesticides of US$24 million in 1980. This market grew to $107 million in 1989; at current annual growth rates of 11% it will exceed $300 million by the year 2000 (Feitelson *et al*., 1992). Over 90% of the biopesticide sales are one single product type, the *Bt*-based products.

Two companies, Abbot-Laboratories (since the acquisition in 1995 of Novo-Nordisk's biopesticide business) and Novartis (created through the merger in 1996 of Ciba and Sandoz), dominate the market with approximately 70% of the total production worldwide. The difference is produced by about 30 companies, lead by Phillips Duphar (Lisanski, 1992), with over 100 *Bt* product formulations. Most are based on one *Bt* protein, and several contain as many as 5 different *Bt* toxins, with some newer biopesticides containing recombinant products (e.g. Ecogen's Raven®).

For biopesticide applications, the *Bt* protein is usually used in a formulation containing the spores and crystalline inclusions that are released upon lysis of *Bt* during its growth. The molecular potency of the toxin is 300 times higher than synthetic pyrethroids and the toxin breaks down quickly when exposed to ultraviolet light (e.g. from sunlight). For further information on insecticidal proteins, particularly *Bt,* and their mode of action, see Koziel *et al*., (1993).

This *Bt*-based biopesticides also have several disadvantages (discussed in McGaughey and Whalon, 1992). The production of the biopesticide is relatively expensive; its application requires the use of agricultural machinery; most applications need to be repeated several times per season; sunlight breaks down the active ingredient; and water (rain or dew) washes the protein from the plants, thus limiting the time when insects are exposed to it. Biopesticides therefore must be applied where and when the target insects are feeding. Most of these difficulties are overcome with transgenic insect resistant crops.

3.2 *Bt* Patents and Insect Resistant Crops

With the emergence of biotechnology, the development of insect resistant plants by transferring the gene that produces the *Bt* toxin became possible and this procedure is now well established. First, a strain of *Bt* that is active against the target insect is identified and the gene producing that protein is isolated. Such genes are generally not expressing the *Bt* protein at sufficiently high levels, so truncated versions of the gene are synthesized with altered codon usage and elimination of certain sequences. Plants in the tissue culture phase are then transformed with the *Bt* gene, together with a selectable marker. This transformation can be done with *Agrobacterium tumefaciens*, or with the biolistic approach, whereby a modified gun is used to shoot DNA particles into cells. The selectable marker is used to identify the plants into which the *Bt* gene has been stably inserted into the genome. Commonly used marker genes confer resistance to antibiotics (e.g. kanamycin) or to herbicides, or express certain chemicals for visual identification of transformed cells (e.g. β-glucoronidase or GUS). Once the transformed cells are identified, they are grown into full plants for seed production, testing, multiplication and/or breeding purposes.

The most critical component of the process is to use the gene that is effective against the target insect. Many companies and universities have been working on identifying novel *Bt* genes and have sought appropriate patent protection. An analysis of various *Bt*-related patents issued over the last 11 years in the OECD countries revealed that 410 patents were issued during that period (Table 2). Fifty-seven percent of all patents have been issued to only eight companies:

- Mycogen with 81
- Novartis with 33
- Abbott-Laboratories with 27 (patents mainly related to biopesticides)
- Toa Synthetic Chemicals with 25 (patents mainly related to biopesticides)
- AgrEvo with 22 (AgrEvo acquired most of the *Bt* patents through the purchase of Plant Genetic Systems or PGS in 1996 for nearly US$800 million)
- Ecogen with 19 (patents mainly related to biopesticides)
- Monsanto with 17 (which includes Calgene's patents since Monsanto already owns 53.6% of the company's stocks), and
- Zeneca with 13.

Table 2: List of Institutions Holding Two or More *Bt*-Related Patents
(Patents issued from 1986 to December 1996)

Institution	Country	Total
Abbott-Laboratories	USA	27
Agency for Industrial Science	Japan	2
Agrartudomanyi-Egetem	Hungary	2
Agracetus	USA	3
AgrEvo	Germany	22
Australian National University	Australia	3
BASF	Germany	3
Biotechnica International	USA	2
Cetus	USA	4
CSIRO	Australia	3
Drexel University	USA	2
DuPont	USA	3
Ecogen	USA	19
Fukuoka-Ken	Japan	4
Institut Pasteur	France	11
Kamenek L K	Russia	2
Korea Chem	Korea	2
Kubota	Japan	4
Lubrizol-Genetics	USA	9
Mitsubishi	Japan	3
Monsanto	USA	17
Mycogen	USA	81
National Research Council	Canada	2
NERC	UK	2
Nissan Chemical	Japan	3
Novartis	Switzerland	33
Pioneer Hi-Bred International	USA	2
Repligen	USA	2
Res. Corp. Techol.	USA	2
Shionogi	Japan	2
State Research Institutes	Russia	18
Sumitomo Chemical	Japan	9
Syntro	USA	2
Toa	Japan	25
University of California	USA	4
University of Wyoming	USA	2
USDA	USA	2
Wageningen University	Netherlands	2
Washington Research Foundation	USA	3
Zeneca	UK	13
Others North America		25
Europe and Russia		16
Asia		13
Total		**410**

Compiled from patent offices of various countries. Joint applications of two organizations are listed under the first applicant only and the table does not take into account purchases of patents nor licenses, except those through the purchase of or merger with companies.

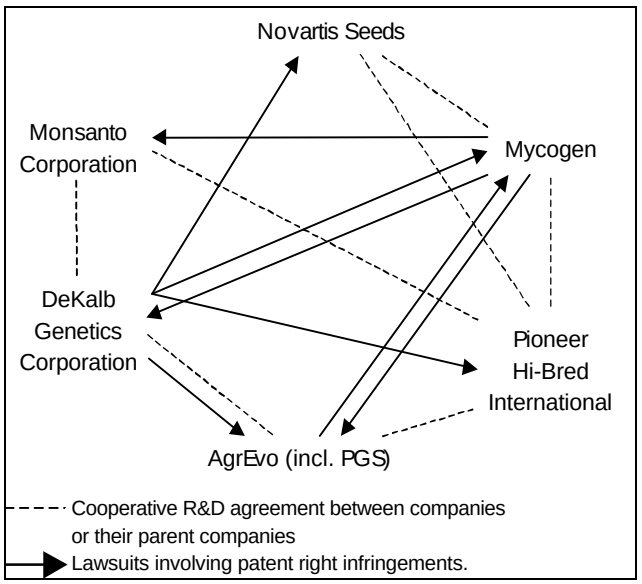
Other organizations holding one patent each are: 3M, USA; AECI, New Zealand; Alko, USA; American Cyanamid, USA; Beijing University of Agriculture, China; Berd-Chem Works, Russia; Berdsk-Fact. Biol. Prep., Russia; Biotechnology Applications, Italy; Boehringer-Mannheim, Germany; Boyce-Thompson Institute, USA; Bristol-Squibb, USA; Canadian Patent Development, Canada; Cantacuzino-Inst., Romania; Chisso, Japan; Cornell University, USA; CRC, Italy; Daiichi Pharmaceuticals, Japan; DeKalb Genetics, USA; Fabriques-Tab.Reunies, France; Finnish National Public Health Institute, Finland; Genexpress, USA; Harvard College, USA; INRA, France; INRS, Canada; Institute of Zoology, Khazakhstan; Kao, Japan; Kurabo, Japan; Leland-Stanford-Jr University, USA; Lim. Technol. Lab., USA; Lynxvale, UK; Marukin-Shoyu, Japan; Meiji Chem, Japan; Michigan State University, USA; Min. Coord. Iniziative, Italy; National University of Singapore, Singapore; O'Brien G T, USA; Plant Cell Research Institute, USA; Rural Development Administration, Korea; Salk Institute of Biological Studies, USA; Serres R A, USA; Silmaran-Tanabal, Japan; Solvay, France; Squibb, USA; Suntory, Japan; Towa Chemical, Japan; Treetech Management, USA; University of Georgia, USA; University of Houston, USA; University of Laval, Canada; University of Memphis, USA; University of Western Ontario, Canada.

It is generally recognized that the greatest concentration of research in this area is with the private companies in North America. Hence, just over half (52%) of *Bt* related patents issued from 1986 to December 1996 were granted to institutions in North America, 30% to European and Russian organizations, and 18% to companies mainly from Japan. Of all the patents, only 17% were issued to public institutions and universities, the overwhelming majority from the USA and Russia. It should be noted, however, that universities in the USA often file joint patents with corporations. The figures presented here do not take into account joint ventures and joint patent applications, for example from a company and university; joint applications were listed in Table 2 under the first applicant only.

Approximately one third of the patents listed in Table 2 are related to *Bt* biopesticides, which applies particularly to Abbott-Laboratories, Ecogen and several Japanese corporations. Many companies, such as Monsanto and Ecogen, or Pioneer Hi-Bred International and Mycogen, have signed collaborative agreements for the development of crops protected against insects (see also Figure 1). Monsanto is developing crops using some of the 10,000 or more *Bt* accessions of Ecogen. Mycogen (which also has an 80% interest in Agrigenetics and recently bought Agriseeds; 45% of Mycogen is also owned by DowElanco) and Pioneer Hi-Bred International Inc. also have a substantial collaboration agreement to develop transgenic crops, among others, worth US$51 million in corn, soybean, sunflower, canola/rapeseed, sorghum with *Bt* genes for insect resistance.

The major players in transgenic *Bt* plant technology therefore are Monsanto, Novartis, AgrEvo and Mycogen with their own technologies, and DeKalb Genetics Corporation and Pioneer Hi-Bred International through strategic alliances. Of these companies, the first three already have several *Bt* products on the market and many more in the pipeline. Other corporations with many *Bt* related patents include Toa Synthetic Chemicals which is a medium size company in Japan that specializes in the synthesis and production of chemicals. It is not

Figure 1: Cooperative R&D Agreements compared with Lawsuits involving Patent Right Infringements



AgrEvo: Patents covering truncated genes and plant cells, tissues and plants containing those genes.

DeKalb: Patent covering fertile, transgenic corn expressing *Bt* proteins. Monsanto owns a large equity stake; yet DeKalb works with AgrEvo to launch LibertyLink™corn hybrids.

Monsanto: Patents covering microorganisms that colonize plants to introduce *Bt*, including *Bt* toxin proteins used to make plants insect resistant.

Mycogen: Two patents covering the transformation with *Bt* toxins into plants where the toxins will be expressed at levels to control pests. Also patents for making *Bt* proteins more plant-like, allowing better expression in plants. Various patents on specific *Bt* genes for different crops. DowElanco owns 45% of equity.

Novartis: Patents covering purified toxins and genes from *Bt*. Also patents covering *Bt* strains and methods of growing *Bt* and expressing insecticidal genes.

Pioneer: Notified to receive patent covering insertion of DNA into corn. License from Monsanto to use of YieldGard™ *Bt* corn. Research agreement with Mycogen to develop/market *Bt* transgenic crops.

Modified and extended after Horstmeier (1997).

known to the author whether or not the company is developing transgenic plants.

It is noteworthy that the six major companies collaborate through various types of cooperative R&D agreements, yet they have also entered into litigation (Figure 1). In fact, litigation in plant biotechnology in general has nearly doubled in 1996. But what is most important is whether or not a company has an enabling patent in a given field. Again, four of the six companies in Figure 1 all have such patents (AgrEvo, Monsanto, Mycogen and Novartis) yet all are interested in the technology gaining acceptance by farmers and by consumers; hence there are a total of 19 major companies who work on advanced products in corn, tomato, potato, canola/ rapeseed, tobacco and cotton. The major *Bt* genes include *cryIA(b)*, *cryIA(c)*, and *cryIII(a)* (Table 3[1]; see also Section 5 for a discussion on the commercialization status). The list in Table 3 is not exhaustive and not all R&D projects are included. What the table does provide is an indication of the crops at a most advanced stage of R&D and the leading players to commercialize *Bt* transgenic crops in the near-term.

[1] It should be noted that many universities, institutes and companies around the world work on multiple aspects of the *Bt* technology, and several will soon be in a position to potentially commercialize or transfer their applications. A survey of such organizations and their main research focus is provided in Appendix II.

This large number of companies working on *Bt* (mainly under license) demonstrates that the law suits will not prevent the technology from being deployed; they will merely determine who receives which portion of royalties. And those with most enabling patents (also called controlling patents) will receive most of the royalties.

3.3 *Bt* Endotoxins and their Genes

Initially, *Bt* toxins were classified into 14 distinct groups and 4 classes (Höfte and Whiteley classification [Höfte and Whiteley, 1989]) based on their host range. These are:

- CryI (active against *Lepidoptera* ["Cry" stands for "crystalline" reflecting the crystalline appearance of the δ-endotoxin; "Cry" is used to denote the protein whereas "*cry*" denotes the respective gene]),
- CryII (*Lepidoptera* and *Diptera*),
- CryIII (*Coleoptera*) and
- CryIV (*Diptera*).

Many more classes have since been added (see Feitelson *et al*., 1992; Crickmore *et al*., 1996). New strains have also been shown to be active against nematodes and other pests. More recent analysis of the molecular sequence reveals that the above classification is not neces-

Table 3: List of Major Corporations Developing Transgenic Crops with *Bt* Genes

Company	*Bt* gene [1]	Major Focus	Status (Field trials/Commercialization) [2]
AgrEvo [3]	*cryIA(b)*	Potato	Small scale
	cryIA(b)	Corn	Small scale
American Cyanamid	*cryIA(c)*	Cotton	Small scale
Cargill	*CBI-Bt*	Corn	Small scale
DeKalb Genetics	*cryIA(b)*	Corn	Large scale
Delta and Pine Land [4]	*cryIA(c)*	Cotton	Commercialized (1996)
DowElanco	*cryIA(c)*	Corn	Small scale
ELM/Asgrow	CBI-*Bt*	Corn	Small scale
Frito Lay	*cryIIIA(a)*	Potato	Small scale
Genetic Enterprises	CBI-*Bt*	Corn	Small scale
Hunt Wesson	*cryIA(b)*	Corn	Small scale
Miles	*cryIA(c)*	Cotton	Small scale
Monsanto [5]	*cryIA(c)*	Cotton	Large scale
	cryIA(c)	Tobaccoo	Small scale
	cryIA(b)	Corn	Commercialized (1996)
	cryIA(b)	Tomato	Large scale
	cryIA(c)	Cotton	Large scale
	cryIIIA(a)	Potato	Commercialized (1995)
	cryIA(a)	Cotton	Commercialized (1995)
	cryIA(c)	Cotton (with herbicide tol.)	Commercialized (1996)
	CBI-*Bt*	Corn	Small scale
Mycogen	*cryIA(b)*	Corn	Commercialized (1996)
	cryIA(b)	Cotton	Large scale
	cryIA(b)	Tomato	Small scale
	cryIA(b)	Canola/Rapeseed	Large scale
Novartis [6]	*cryIA(b)*	Corn	Commercialized (1995)
	cryIA(b)	Tobacco	Small scale
	cryIA(b)	Cotton	Small scale
	cryIA(b)	Tomato	Small scale
	cryIA(b)	Corn	Small scale
Pioneer Hi-Bred International	*cryIA(b)*	Corn	Large scale
Rohm and Haas	*cryIA(b)*	Tobacco	Small scale

1 CBI-*Bt*: Confidential Business Information.
2 Year indicates year of commercialization in the USA.
3 Including PGS.
4 Delta and Pine Land Co. commercializes cotton under license from Monsanto.
5 Includes Calgene where Monsanto owns over half and Holden which was purchased recently for over US$ 1 billion.
6 Ciba and Sandoz (includes Northrup King, Rogers NK and S&G Seeds).

sarily based on homology or evolutionary relationships (Figure 2). A comparison of the nomenclatures is provided in Appendix III for reference.

To date, over 50 Cry proteins are known, of which 28 genes have been described in detail and isolated from 14 different *Bt* subspecies that have been shown to be active against insects (Table 4). Thirty additional proteins have been described in the scientific literature but no insecticidal properties have as yet been identified, although many have been shown to be effective against other biotic pests such as nematodes and mites. Further testing might demonstrate that some of them are effective against insect pests. As will be discussed in Section 6, of particular interest is the identification of genes for the same insect species but with different modes of action (i.e. target sites in the insect gut). In order to broaden that spectrum, research on the manipulation of the *cry* genes is slowly leading to new activities (or binding sites), thus opening new possibilities hitherto unsuspected.

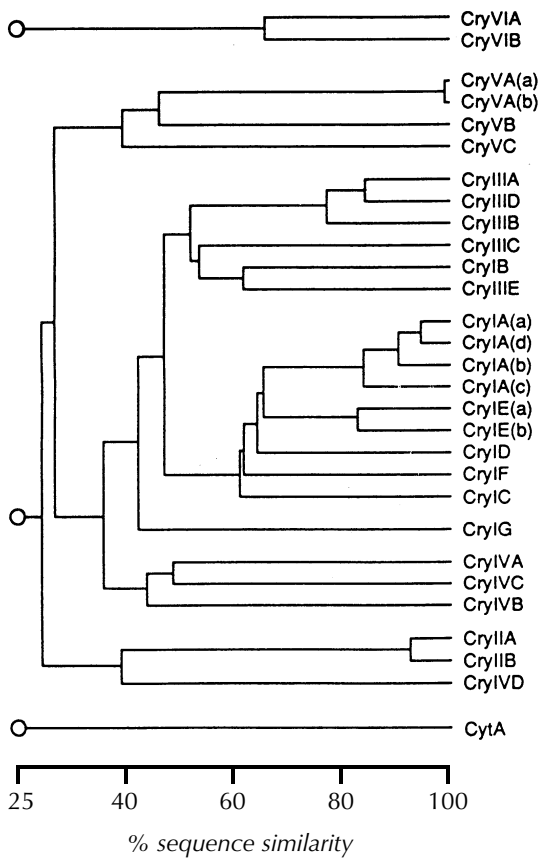
3.4 Case Study on the Development of *Bt* Transgenic Cotton

This section describes how Monsanto developed its first transgenic *Bt* cotton (*Gossypium hirsutum*, although the principles outlined below are also applicable to *G. barbadense*) marketed since 1996 in the USA under the trade name Bollgard™. Some of the material is drawn from

Serdy (1995). The crop already occupied 1.7 million acres in 1996 in the USA and is expected to be approved soon for commercialization in several other countries, in addition to Argentina, Australia and South Africa, where the crop has been approved in late 1996. The insect resistance is from *Bacillus thuringiensis* var. *kurstaki* (*Btk*) which provides resistance primarily against the cotton bollworm, but is also effective against the tobacco budworm, the pink bollworm, the cabbage lopper, the saltmarsh caterpillar and the cotton leaf perforator.

A synthetic *cryIA(c)* gene was produced based on the crystal protein gene sequence of *Btk* strain HD-1. This protein contains nearly identical amino acids portions to the one found in the organism in its natural environment and corresponds to a protein in commercial *Btk* formulations (e.g. Dipel®). A gene promoter (35S) from the Cauliflower Mosaic Virus was added that turns the gene on and produces the RNA leading to the production of the *Bt* protein in the plant (more specifically in the ribozomes of the plant cells). A marker gene was added to the gene construct, the product of which enables the identification of tissue cultured cell lines with stably integrated foreign DNA. The *nptII* gene was used, conferring resistance to the aminoglycoside antibiotics (kanamycin, neomycin, and G-418) which are inactivated after phosphorylation by NPTII. NPTII is produced in minute amounts in plants that contain the marker. NPTII naturally occurs in nature and is present in many microbes on food and feed and within mammal digestive systems (Fraley *et al*., 1986).

Figure 2: Amino Acid Sequence Similarity of the *Bt* Endotoxins



Source: Feitelson *et al*. (1992). See also Appendix III for a comparison of the molecular and Höfte and Whiteley nomenclature.

One cotton variety, Coker 312, is easily transformed but most or all other varieties have proven more difficult and less efficient. Monsanto transformed Coker 312 with the *cryIA(c)* gene, crossed it with an "elite" variety, followed by several backcrosses. Although that system is straight forward, the cost is relatively high because of the difficulty in ensuring that the fiber quality and agronomies of the elite variety are maintained. This requires expensive laboratory and genetic tests after each backcross and may cost as much as US$1 million or more, depending on the fiber quality that needs to be maintained.

The first two field trials with *Bt* transgenic cotton were conducted in the USA by Monsanto and by Agrigenetics in 1988 and the first results of these trials were published by Deaton (1991). Standard biosafety regulatory clearance by the Animal Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) was issued for these trials. The biosafety review ensured the safety of the expressed protein, cotton seed and fiber (potential avenues of exposure to humans, animals and non-target organisms), and stipulated certain required environmental precautions. For this and for the dossier for the Food and Drug Administration (FDA; see below), information on the following issues had to be provided:

- background on the host plant (taxonomy of cotton, pollination, geographical distribution, etc.);
- description of the genetically modified trait including molecular characterization (sources of the gene, methods of transformation, description of the gene products, etc.);
- equivalence of genetically modified and non-modified plants (in order to assess any possible pleiotropic effects caused by the insertion of the DNA into the chromosomes of cotton; thus it was shown that no significant differences existed in the major toxicants of cotton, namely gossypol, flavonids, anthocyanin and tannins);

Table 4: *Bt* Endotoxins (Cry) and their Activity against Specific Insect Species

Cry protein [1]	Origin (*Bt* subspecies)	Major Target Insects	
		Order [2]	Common names
CryIA(a)	*kurstaki*	L	Silk worm, Tobacco horn worm, European corn borer
CryIA(b)	*berlineri*	L & D	Tobacco horn worm, Cabbage worm, Mosquito
CryIA(c)	*kurstaki*	L	Tobacco budworm, Cabbage lopper, Cotton bollworm
CryIA(d)	*aizawai*	L	Several *Lepidoptera*
CryIA(e)	*alesti*	L	Tobacco budworm
CryIB	*thuringiensis*	L	Cabbage worm
CryIB(c)	*morrisoni*	L	Several *Lepidoptera*
CryIC	*entomocidus*	L & D	Cotton leaf worm, Mosquito
CryIC(b)	*galleriae*	L	Beet army worm
CryID	*aizawai*	L	Beet army worm, Tobacco horn worm
CryIE	*kenyae*	L	Cotton leaf worm
CryIE(b)	*aizawai*	L	Several *Lepidoptera*
CryIF	*aizawai*	L	European corn borer, Beet army worm
CryIG	*galleriae*	L	Greater wax moth
CryIIA	*kurstaki*	L & D	Gypsy moth, Mosquito
CryIIB	*kurstaki*	L	Gypsy moth, Cabbage lopper, Tobacco horn worm
CryIIC	*shanghai*	L	Tobacco horn worm, Gypsy moth
CryIIIA	*san diego*	C	Colorado potato beetle
CryIIIA(a)	*tenebrionis*	C	Colorado potato beetle
CryIIIB	*tolworthi*	C	Colorado potato beetle
CryIIIC	N/a	C	Spotted cucumber beetle
CryIIID	*kurstaki*	C	N/a
CryIVA	*israelensis*	D	Mosquito (*Aedes* and *Culex*)
CryIVB	*israelensis*	D	Mosquito (*Aedes*)
CryIVC	N/a	D	Mosquito (*Culex*)
CryIVD	N/a	D	Mosquito (*Aedes* and *Culex*)
CryV	N/a	L & C	European corn borer, Spotted cucumber beetle
CryIX	*galleriae*	L	Greater wax moth

Extended after Rajamohan and Dean (1995) and Crickmore *et al. (*1996).

N/a: Not available.

[1] It should be noted that the nomenclature of *cry* genes has recently been modified to take into account recent advances in the understanding of the molecular basis of the genes. Nevertheless, the common nomenclature is that of Höfte and Whiteley (1989) which has been used in this paper (for information on nomenclature, see OSU, 1997; for a comparison of nomenclatures, see Appendix III).

[2] L: *Lepidoptera*; C: *Coleoptera*; D: *Diptera*.

- an assessment of the safety of the expressed proteins, the cotton seed and fiber (an extensive database on the safety of *Btk* already existed from biopesticide studies, but Monsanto, in agreement with the Environment Protection Agency (EPA) of the USA, performed new studies based on the transgenic product. In addition, since cotton seed oil is extensively used for human consumption, the product was also analyzed for mammals. The oil was shown not to contain any detectable amounts of *Bt* protein);
- an assessment of the safety to non-target organisms (this was established through studies of receptor specificity in target insects, and effects on non-target insects such as honey bees, green lacewing, ladybird beetle and parasitic wasp which were all shown not to be affected by the *Bt* toxin; the same applies to birds, rodents and mammals);
- safety to the environment including fate of the gene and expressed protein (includes studies on outcrossing with wild relatives of which two exist in the USA. Since cultivated cotton is an allotetraploid and most wild species diploid, these plants are incompatible and if crosses do occur, the offspring is sterile. The fate of the protein in the environment was shown to be insignificant as it is rapidly degraded in the environment under the influence of UV light); and
- data sustaining the insecticidal efficacy of these plants (field trials have now been carried out for seven years and the transgenic cotton has outper-

formed by an average of 500 pounds per acre or 560 kilograms per ha the sprayed control).

Whereas most of the above information was required for field trials to be authorized, the field trials also served to collect information. Much of that new data was essential in filing a petition to APHIS for deregulation of the *Bt*-transgenic cotton. This deregulation was granted in 1994. The field trials served to generate information on the following:

- evaluate the agronomic characteristics to ensure that the cotton is normal and that besides the insect resistance, no other traits have been modified due to the transformation process, due to pleiotropic effects of the newly introduced genes, or due to possible somaclonal variation as a result of tissue culture and regeneration of the first transformation of Cocker 312. Field evaluations include plant vigor, growth habit, flowering, maturity, and disease susceptibility, among others;
- test the level of insect resistance under varying field conditions and across several years; and
- determine the level of *Bt* expression (which is comparable to or less than the amount of *Bt* protein applied per unit of land using biopesticides). These studies showed that the *Bt* protein is produced throughout the plant at levels ranging from 5.4 to 28.3 micrograms per gram of fresh weight (or µg/g) with lower amounts in the roots (less than 1µg/g or one part per million). The NPTII marker protein was also present in even lower levels.

With the deregulation by APHIS in 1994, field trials with cotton no longer required full biosafety reviews but simple notifications that field trials are planned. The way for commercialization was thus opened, but two additional agencies had to be involved in the USA. The EPA reviewed the data with particular concern for the effect of the toxin on the environment, insects and wildlife, and the potential for and effect of the trait spreading in the environment. Related to this was the EPA's concern with potential development of insect resistance to *Bt*. The latter is probably the aspect of highest concern with *Bt* and Monsanto claims to have studied and tested all plausible proposals for the management of *Bt* transgenic cotton (Serdy, 1995), including high dose expression, refugia for sensitive insects, agronomic practices, monitoring insect resistance, and pyramiding traits. These are discussed in detail in Section 6.

Finally, extensive discussions between Monsanto and the FDA convinced FDA scientists that the new cotton variety is essentially equivalent to the traditional cotton variety, and that no harm could be expected if it is allowed to enter the environment and commerce. With that ruling, the way was opened in 1995 for the commercialization of the *Bt* cotton in the USA.

As to the field performance of *Bt* cotton in 1996, it is generally agreed that overall the crop met expectations in the majority of the fields, although significant problems did develop with the cotton bollworm. The year 1996 was a year with high bollworm pressure in many areas, particularly in the higher altitudes. Thus many farmers applied some insecticides and requested Monsanto to reimburse these additional costs, which the company did after some legal battle.

The fact that problems did develop in the first year of large scale deployment of a transgenic *Bt* crop will have a beneficial impact on the future of this critical application. Farmers and corporations alike have been sensitized that biotechnology applications do not represent a silver bullet but needs to be integrated into production systems. No one will become too complacent and entirely rely on the new transgenics, but all will need to continue to monitor insects as the season progresses.

4. The Potential of *Bt* Transgenic Crops to Substitute for Traditional Insecticide Use

4.1 Crop Losses due to Insects

World crop losses without pesticide use and other non-chemical control strategies have recently been estimated to amount to almost 70% of production, representing a US$400 billion loss (Oerke *et al*., 1994). Despite all efforts to prevent pre- and post-harvest crop losses, pests are destroying over half of all world production (Pimentel, 1996). Recognizing that worldwide pre-harvest losses due to insects, despite the use of insecticides, is in the area of around 15% of total production, and that losses in developing countries are significantly higher (global losses due to insects despite insecticide use is over US$100 billion; in rice alone estimated at around US$45 billion), potential savings from *Bt* crops in developing countries would be substantial. New options to control pest losses must be a

priority. Thus insect resistant crops (with *Bt* or other resistance mechanisms) offer great promise in this area.

Table 5 gives a broad estimate of yield losses due to diseases and due to insects. Average losses for diseases vary from around 10% to over 20%, but individual losses can of course be much higher and sometimes approach 100%. For insects, two types of figures are given: the figures in columns 3-5 represent losses "despite" the use of insecticides. These vary from 5 to 27% on average, representing 300 million MT in losses for the crops given and this loss of production is valued at over US$ 100 billion (average yeild losses due to insects across all crops is estimated to be approximately 15%). The second set of figures in Table 5 (columns 6-8) are losses "in the absence" of insecticides, i.e. losses that would have occurred if no insecticides were used. These losses are on average 7% higher, representing over 500 million MT in losses valued at around US$200 billion. The efficacy, or gain in increased productivity due to insecticides is greatest in vegetables and fruit, and smallest in rice. This is not surprising since it has long been argued that efforts to control leaf folders and stem borers in rice with broad spectrum insecticides cause outbreaks of brown planthoppers because these insecticides kill natural enemies (for a discussion, see Gould, 1994). This point is particularly relevant here because *Bt*-induced resistance to insects in rice, for example, would not target beneficial insects and natural enemies. Hence the value of *Bt*-mediated transgenic resistance is larger than the potential substitution value for insecticides discussed below.

4.2 Potential Substitution Value

Against this background of losses, the worldwide pesticide use in 1994 of US$28 billion seems small. In that year, herbicides accounted for nearly half of all pesticide use, insecticides for almost 30%, and fungicides for 20%, with others accounting for less than 5%. Of the US$8.11 billion insecticides used, around 75% are applied to four groups of crops: fruit and vegetables, cotton, rice and corn/maize (Figure 3). Since rice is predominantly grown in Asia, this crop is the single most important user of insecticides in the developing countries of the Asian-Pacific region with cotton being the single most important user of insecticides worldwide (23%; although a recent estimate puts the figure to 29%; Hearn and Fitt, 1992). The Asian-Pacific region, including Japan, uses more insecticides than Latin America and Africa combined.

Production figures for these four groups of crops underlines their importance in developing countries (Table 6). A high percentage of these crops are grown in developing countries where they occupy a significant place in both agriculture and society. Two-thirds of all cotton is produced in developing countries and half of the worldwide cotton is produced in the developing countries of Asia. Asian developing countries produced half of all fruit and vegetables produced worldwide, 48% of cotton, 91% of rice but only 24% of corn/maize. In addition to the relatively high production totals of these four crop groups in the developing countries of Asia, their importance in terms of acreage and crop distribution is even more significant. This is because productivity for these crops in industrialized countries is higher than in developing countries (with lower yields, more area is required to produce the same amount of harvest). Contrary to many expectations, Africa and Latin America produce a relatively small percentage of the crops, including of corn (where the USA is by far the largest producer).

A breakdown of the insecticide use for major insect pests in the same four crops (Table 7) shows that of the

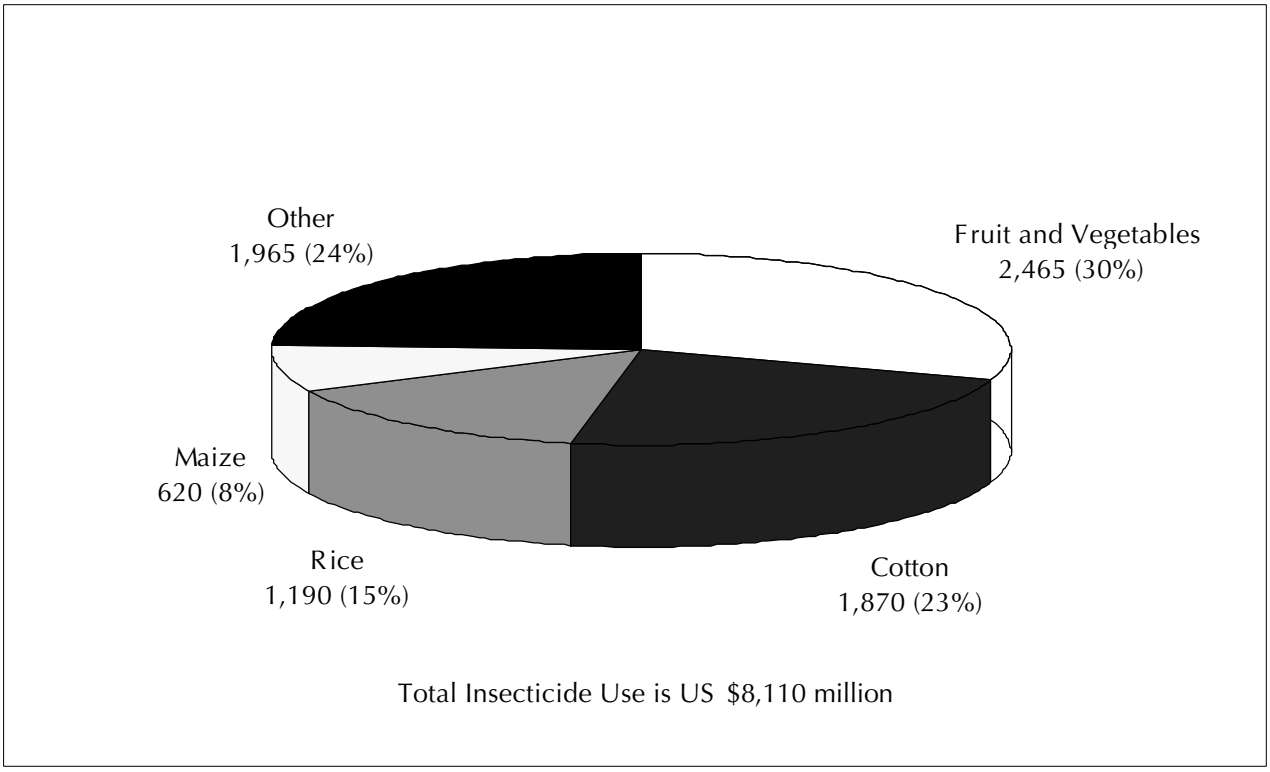
Table 5: Global Losses due to Diseases and Insect Pests
(Based on Production Figures of 1993, in 1996 US$)

	Losses due to	Losses due to Insects (based on Farmgate Values)						Insecticide	Pesticide
	Diseases	Despite Insecticide Use			In the Absence of Insecticide Use			Use	Use [1]
Crop	%	%	million MT	US$ billion	%	million MT	US$ billion	US$ billion	US$ billion
Fruit [2]	16	6	23	20	23	89	76	1.0	3.1
Vegetables	10	9	44	25	15	73	42	1.5	3.5
Cotton	21	N/a	N/a	N/a	35	19	3	1.9	2.1
Rice	9	27	145	45	29	155	48	1.2	2.7
Corn/Maize	9	12	68	8	18	103	12	0.6	2.4

Modified and extended after James *et al*. (1991).
N/a: Not available.

1 Excludes herbicides, includes insecticides.
2 Excludes melon which are included under vegetables.

Figure 3: 1994 Worldwide Insecticide Use on Major Crops
(US$ million)



Data from James (1996).

Table 6: Global Production of Major Crops (1994) [1]

Crop	World (M-MT)	Of which Developing (M-MT)	Developing as Percent of World (%)
Fruit and Vegetables	878	597	68
Vegetables [2]	*486*	*341*	*70*
Fruit	*388*	*254*	*65*
Tree Nuts	*5*	*3*	*60*
Cotton (lint)	19	12	63
Rice	535	519	97
Corn/Maize	570	187	33

	Africa			Asia			Latin America		
Crop	(M-MT)	% World	% Dev.	(M-MT)	% World	% Dev.	(M-MT)	% World	% Dev.
Fruit and Vegetables	86	10	14	434	49	73	119	14	20
Vegetables [2]	*34*	*7*	*10*	*291*	*60*	*85*	*36*	*7*	*11*
Fruit	*52*	*13*	*20*	*141*	*36*	*56*	*83*	*21*	*33*
Tree Nuts	*0.5*	*10*	*17*	*2*	*40*	*67*	*0.4*	*8*	*13*
Cotton (lint)	1	5	8	9	47	75	3	16	25
Rice	16	3	3	485	91	93	4	1	1
Corn/Maize	38	7	20	139	24	74	32	6	17

Based on FAO figures (1995). Note that due to rounding, not all figures add up exactly.
1 M-MT: Million Metric Ton; % World: Percent of World; % Dev.: Percent of Developing Countries.
2 Includes melon.

US$8.11 billion in insecticide use, US$2.69 billion can probably be replaced with transgenic crops containing *Bt*. This represents one third of total insecticide use in these major crops. In cotton, for example, insecticides against bollworms and the *Spodoptera* complex (mainly beet army worm and cotton leaf worm) can be entirely replaced with *Bt* and would result in a US$1.16 billion saving in insecticides alone. The figure considers only the replacement value for the insecticide and does not include other benefits, such as:

- insecticide application costs,
- labor costs,
- human and animal health benefits through reduced hazards,
- environmental benefits and
- indirect benefits through higher levels of beneficial predators.

In addition, control of some insects with *Bt* transgenic crops is significantly better than control by insecticides because insect pests often penetrate into the tissue avoiding contact with insecticides. This is the case for the European corn borer, the foliar insects in corn/maize (*Heliothis* and *Spodoptera*), and the stemborer in rice.

4.3 Current Licensing Costs of *Bt* Technology

For obvious reasons, the use of *Bt* transgenic crops may also add expenses due to the costs of developing the technology. With crops such as rice, where the International Rice Research Institute (IRRI) in the Philippines is developing *Bt* rice with a gene donated by Ciba of Switzerland (now Novartis), the germplasm may be made freely available to a selected list of national programs and other interested parties since the development costs

Table 7: Global Value of Current Annual Insect Control Costs and Potential to Substitute with *Bt* Technology for Selected Major Crops and Insects (1994 values and 1996 US$)

Crop	Insects (Groups)	Insect Control Cost (US$ million)	Substitution Value [1] (US$ million)	Major Benefiting Countries [2] Africa	Asia	Latin America
Fruit and Vegetables	Sucking (Scale, aphids, leafhoppers)	807		Essentially all of Africa	Essentially all of Asia	Essentially all of Latin America
	Mites	652				
	Foliar (leafminers and other *Lepidoptera)*	953	953			
	Soil (e.g. root worm)	52				
	Subtotal	2,465	891			
Cotton	Bollworms (*Heliothis, Pectinophora, Earias*)	827	827	Egypt	China	Brazil
	Spodoptera (inc. complex)	334	334	Zimbabwe	India	Paraguay
	Aphid & other sucking	477		Turkey	Indonesia	Peru
	Boll Weevil	207		Sudan	Thailand	Argentina
	Soil (e.g. Cotton root worm)	25			Pakistan	
	Subtotal	1,870	1,161			
Rice	Hoppers	548		Nigeria	China	Brazil
	Stemborer	422	422	India	SierraLeone	Colombia
	Leafroller, folders, other	81		Bangladesh	Ghana	Peru
	Rice Water Weevil	139		Philippines	Cameroon	Bolivia
	Subtotal	1,190	422	Thailand		
Corn/ Maize	Soil Insects	430		Zimbabwe	India	Brazil
	Foliar (*Heliothis*, *Spodoptera*)	95	95	Nigeria	China	Mexico
	Borer, Earworm	63	63	Tanzania	Indonesia	Argentina
	Sucking (Aphids, leafhoppers, stink bugs)	32		Ethiopia	Vietnam	Costa Rica Paraguay
	Subtotal	620	158			Bolivia, Peru
Other		1,965				
Total		**8,110**	**2,694**			

Modified and extended after James *et al*. (1991).

[1] Substitution value for insecticide cost only. Does not include insecticide application costs, labor costs, human and animal health benefits through reduced hazards, environmental benefits and indirect benefits through higher levels of beneficial predators.

[2] The list of major countries potentially benefiting from the technology is not exclusive, but includes the major countries based on the acreage of the respective crops and insects occurring in those countries which that can be targeted with *Bt*.

have been sponsored by the international community through the CGIAR (Consultative Group on International Agricultural Research), by the Rockefeller Foundation's considerable investment into the Rice Biotechnology Program (Toenniessen, 1995), or by other institutes of the CGIAR (such as CIMMYT where work on corn with *cryIA(b)* and *cryIA(c)* has been going on for some time [see Wilcox and Bergvinson, 1997]). Many varieties of several crop species, however, will be developed by the private sector which must recover the investment costs through the sale of the seed. The research and development costs are substantial as exemplified in the different steps involved based on the case study on cotton (Section 3.4 above). As with many new technologies, however, a company may not necessarily demand a premium for the transformed variety but opt to keep the prices identical to a non-transformed variety as a way of boosting market share.

In the USA, where *Bt* transgenic crops have reached the market in 1996, companies have so far opted to charge higher prices for the transgenics. Cotton varieties, for example, are sold under contract to farmers and Monsanto entered into approximately 7,000 such contracts in the USA in 1996 for 1.7 million acres of cotton (around 0.7 million ha). Each farmer paid US$32/acre (US$80/ha) in royalties for the *Bt* cotton for an estimated saving of US$60-120/acre (US$140-280/ha) on about 10 insecticide sprays. This provided farmers, according to Monsanto, to a net saving of "at least" US$33 per acre (US$70 per ha).

With potatoes, the case is more complex since potatoes for planting command variable prices depending on the growing region, variety, and distance from the seed producer. In this case, Nature Mark™ Potatoes produces the first generation of transgenic planting material (nuclear seed) and licenses that first generation to seed producers. They multiply the varieties for two generations and then sell them as certified seed to farmers at normal prices plus a premium for the *Bt* technology. That premium essentially is what Nature Mark™ Potatoes receives from the seed producers (estimated to be between US$1.0 and US$4.5 per 100 pounds [US$2-10/100 kg] of planting material depending on the geographic location and variety). Considering that planting requires on average 1,600 pounds per acre (1.8 tons/ha), the premium per acre is US$16-72 (US$40-180/ha), compared to the chemical control costs of US$30-120 per acre (US$75-300/ha) in the USA.

In summary, *Bt* transgenic crops may be replaced for over US$2.69 billion in insecticide use in addition to offering environmental benefits, provided they are properly managed. This figure does not take into account the yield losses due to insects when no insecticides are used (or when they are misused leading to human health hazards), nor the relative use of insecticides in industrial and developing countries, and by large and small-scale farmers. For small-scale farmers, mainly subsistence farmers, the real issue is not the average loss, but the variation of the loss from year to year. In this context, *Bt* crops may provide an advantage if deployed regularly and on a large scale, but this may also create new problems, namely the emergence of insects resistant to *Bt* which is further discussed in Section 6.

5. Current Status of Field Trials with, and Commercialization of *Bt* Crops

The advent of modern biotechnology in the early 1970s offered new possibilities for using *Bt*. In 1971 the first gene was transferred from one species to another but it took more than a decade for gene transfer to become routine in plants and more specifically in the economically important cereal crops. Developments were fast, with all major crops now transformed, leading to a series of field trials with genetically modified organisms (GMOs).

The commercial and environmental interests in their technology is because biotechnology offers the possibility of delivering the active protein of *Bt* directly into plants. Furthermore, the protein can be targeted to be expressed in certain parts of the plant or in the entire plant. As there are no obvious physiological disadvantages with plants producing the *Bt* endotoxin, transgenic plants expressing the *Bt* protein suffer no yield disadvantage.

Since the mid-1980s, when transgenic crops were first produced for field release, governments of the Organization for Economic Cooperation and Development (OECD) began to develop regulatory mechanisms, particularly in the USA, the United Kingdom, France and the European Union (EU; see Krattiger and Rosemarin, 1994). Part of the success of the plant biotechnology program in many OECD countries is due to the safe and effective regulatory systems implemented by these governments. The success was spurred by the comprehensive—and costly—safety pro-

grams carried out by industry. This has led to an excellent public and scientific acceptance of the regulatory system in the USA and elsewhere. The safety data generated by industry is generally considered appropriate to protect human health and the environment from potential harm from these new crop products. Without public acceptance, the biotechnology industry would not have been able to achieve so much progress over the past decade. The lack of effective biosafety regulatory mechanisms in most parts of Africa and Southeast Asia is thus a major constraint for similar biotechnology development in these regions.

The first field trials with transgenic crops were conducted in France and the USA in 1986 with tobacco. Since then, over 70 crop species have been genetically modified and 56 different crops field tested (for a recent review, see James and Krattiger, 1996). The first such crop to be commercialized was tobacco in China in the early 1990s, and the USA followed in May 1994 when it authorized the growing and commercialization of the delayed-ripening McGregor™ tomato (Calgene's FlavrSavr™ tomato).

The first transgenic *Bt* plant was tobacco resistant to the Tobacco horn worm produced in the early-1980's by Plant Genetic Systems (reported in *Nature* by Vaeck *et* al., 1987) and many more crops soon followed (see Fischhoff *et al*., 1987). As early as 1986, PGS field tested *Bt* tobacco both in France and the USA (Table 8) and Monsanto tested tomato with *Bt,* herbicide resistance (Roundup™) and virus resistance in 1987 (in Jerseyville, Illinois, by David Fischhoff and Roger Beachy at the time at Monsanto), and several small-scale tests of the same crop followed the soon thereafter (G. Barton, Monsanto—Personal Communication). Larger field testing of *Bt* crops, however, took off in 1988 by Agrigenetics (tomato), Monsanto (cotton) and Novartis (tobacco) (Table 8), and by the end of 1990, nearly 20 trials took place in the USA alone.

In other OECD countries, besides the early tobacco trial in 1986 in France, field trials with *Bt* plants followed much later. Canada and the Netherlands were the first in 1991 to authorize field trials with potatoes and only 10 trials with three crops were conducted by the end of 1992.

With the exception of Mexico, developing countries also begun much later than the USA with the testing of *Bt* technology. By the end of 1992, twelve trials with *Bt* crops took place, most notably in Latin America and China (Table 8). To date, 3,647 field trials of transgenic crops have been conducted world wide and eight crops constituted one third of the trials: corn/maize with 1,024 field trials, canola/rapeseed (665), potato (362), tomato (353), soybean

Table 8: The first *Bt* Field Trials with Transgenic Crops

USA

Institution	Crop	Submission[1]	Trial Year
PGS	Tobacco	N/a	1986
Monsanto	Tomato	N/a	1987
Monsanto	Tomato	11/25/87	1988
Agrigenetics	Tomato	1/29/88	1988
Novartis	Tobacco	2/5/88	1988
Monsanto	Tomato	2/10/88	1989
Monsanto	Tomato	11/9/88	1989
Rohm and Haas	Tobacco	11/28/88	1989
Agracetus	Cotton	12/16/88	1989
Monsanto	Tomato	1/30/89	1989
Calgene	Tobacco	3/15/89	1989
Monsanto	Cotton	5/30/89	1989
UC Davis	Walnut	8/8/89	1990
Monsanto	Tomato	10/5/89	1990
Monsanto	Tomato	10/5/89	1990
Novartis	Tobacco	11/22/89	1990
Novartis	Cotton	12/5/89	1990
Rohm and Haas	Tobacco	12/28/89	1990

Other OECD (Europe, Canada, Australia)

Country	Institution	Crop	Trial Year
France	PGS	Tobacco	1988
Canada	Monsanto	Potato	1991
Netherlands	Hettema ZK	Potato	1991
Australia	CSIRO	Cotton	1992
Belgium	AgrEvo (PGS)	Potato	1992
Canada	Monsanto	Potato	1992
Canada	Monsanto	Potato	1992
Canada	Monsanto	Potato	1992
France	Novartis	Maize	1992
France	INRA	Maize	1992
Italy	Novartis	Maize	1992

Developing Countries (Latin America)

Country	Crop	Trial Year
Mexico	Tomato	1988
Cuba	Tobacco	1990
Argentina	Corn	1991
Bolivia	Cotton	1991
Cuba	Tobacco	1991
China	Tomato	1992
Belize	Cotton	1992
Costa Rica	Corn	1992
Costa Rica	Cotton	1992
Cuba	Sugarcane	1992
Argentina	Corn	1992
Mexico	Tomato	1992

Source: James and Krattiger (1996) and USDA/APHIS (1997), OECD (1997), W. de Greef and G. Barton, Personal Communications.

1 Refers to date of submission to USDA/APHIS. Trials marked N/a were submitted prior to the formal regulations and approved by a special USDA committee.

(278), cotton (224), tobacco (161) and melon and squash (92). All eight crops have already been commercialized. In addition, alfalfa, cantaloupe, carnations, flax, rice, sugarbeet and sunflower are near commercialization, or have also been commercialized.

Field trials with insect resistance during the period 1986 to 1995 represent 18% of all trials, or 738 field trials. Herbicide tolerance has been the trait with the highest number of trials (1,450 representing 35% of all trials). The data presented by James and Krattiger (1996) indicate that for the ten year period 1986 to 1995, the industrialized countries of the USA, Canada, the EU and Asia accounted for 3,320 of the total of 3,647 trials, equivalent to 91% of the trials worldwide. The balance of 9% were conducted in the developing countries of Latin America (5%), Asia (2%,) Africa (1%), with 1% in the countries of Eastern Europe/ Russia.

As of early 1997, over 70 transgenic crops have been approved for commercialization in nine countries plus the EU (see also James and Krattiger, 1996). Many are approved for growing and human consumption, particularly in the USA and Canada, whereas some are for import and human consumption of the product. In addition, over 10 crops are pending approval. Of the 80 crops or so approved or pending approval in eight countries, 21 are *Bt* transgenic crops dominated by corn/maize (11) and followed by potato (5) and cotton (5) (Table 9). The developing countries that commercialized transgenic crops are Mexico and South Africa.

In the USA, *Bt* transgenic crops already occupy over 3 million acres (over 1.2 million ha): 1-2 million acres of Monsanto's corn/maize and 0.5 million acres of Novartis' corn/maize, 1.7 million acres in transgenic cotton by Monsanto, and over 50,000 acres of seed potatoes by Nature Mark™ Potatoes (a subsidiary of Monsanto). The acreage for 1997 is expected to be over 20 million acreas for all crops.

In summary, about two thirds of the genes incorporated in the newly commercialized transgenic crops confer either herbicide tolerance, insect resistance or virus resistance. The potential impact of biotechnology in the near-term on global food production and reduced insecticide use will be substantial with the insect resistant crops offering many new opportunities in crop protection. One of the most critical aspects of the use of *Bt* transgenic crops will be the deployment of the resistance genes. These crops require deployment management similar to other resistance genes if they are to be durable. These aspects are addressed in the next section.

Table 9: Commercialization Status of *Bt* Transgenic Crops

Country/ Crop	Company [1]	Gene [2]	Trade name [3]
Argentina			
Corn/maize	Novartis 96	*cryIA(b)*	Maximizer™
Corn/maize	Monsanto 96	*cryIA(b)*	YieldGard™
Corn/maize	Novartis 96	*cryIA(b)*	Maximizer™
Australia			
Cotton	Monsanto 96	*cryIA(c)*	Bollgard™
Canada			
Corn/maize	Mycogen 96	*cryIA(b)*	NatureGard™
Potato	Monsanto 96	*cryIIIA(a)*	NewLeaf™
Corn/maize [4]	Novartis 97	*cryIA(b)*	
Corn/maize	Monsanto 97	*cryIA(b)*	Maximizer™
EU			
Corn/maize	Novartis 96	*cryIA(b)*	
Japan			
Corn/maize	Novartis 96	*cryIA(b)*	
Potato	Monsanto 96	*cryIIIA(a)*	
Mexico			
Cotton [5]	Monsanto 96	*cryIA(c)*	
Potato [5]	Monsanto 96	*cryIIIA(a)*	
USA			
Corn/maize	Novartis 95	*cryIA(b)*	Maximizer™ *0.5*
Corn/maize	Novartis 96	*cryIA(c)*	
Corn/maize	Monsanto 96	*cryIA(b)*	YieldGard™ *1-2*
Cotton	Monsanto 95	*cryIA(c)*	Bollgard™ *1.7*
Cotton	Monsanto 95	*cryIA(a)*	
Potato	Monsanto 95	*cryIIIA(a)*	NewLeaf™ *0.05*
Potato [6]	Monsanto 96	*cryIIIA(a)*	NewLeaf Plus™
South Africa			
Cotton	Delta & Pine Land 96	*cryIA(c)*	

Extended from James and Krattiger (1996).

1 Number indicates year approved for sale.
2 All have the 35S promoter, and as a marker gene the following have been used: EPSPS & GOX for Monsanto's corn, NPTII for Monsanto's cotton, phosphinothricin acetyltransferase for Mycogen's corn, and NPTII for Monsanto's potatoes.
3 Number in italics indicates estimated 1996 acreage in million.
4 With herbicide resistance.
5 Import of product only.
6 With virus resistance (VR).

6. Management Strategies of *Bt* Deployment

6.1 Overview and Introduction

Bt has the potential to impact significantly in the near-term on productivity and sustainability and represents a replacement for a significant quantity of the conventional pesticides currently applied to crops. Yet the long-term impact can only be sustained if effective and responsible deployment schemes can maintain the durability of the *Bt* genes. In the USA, the management of transgenic resistance has to be carefully planned and in many cases requires approval by the EPA. Little conceptual or scientific work, however, has been done in developing countries compared to the USA or Canada where large scale releases have already occurred. For example, with Monsanto's Bollgard™ cotton, individual licensing agreements are drawn up for each farmer and the company inspects (or sends representatives) to inspect some of the fields. This is possible in the USA where most farms exceed 1000 acres (400 ha). However, the number of farmers is small compared to the many small scale farmers in developing countries, which raises many questions regarding how to manage deployment and verify its implementation. The problem is further aggravated by the general reluctance of farmers to change their agronomic practices without appropriate demonstration plots and extension work. Much must be done in the area of managing the deployment of *Bt* crops to minimize the possibility of *Bt* resistance developing in insects.

The purpose of this Section is to briefly review the deployment strategies adopted so far in the USA and describe alternative options that have been proposed. These fall into four "tactics" (Table 10) but are not mutually exclusive. For example, within *Gene Strategies,* one or several genes against the same insect may be deployed, these may be expressed constitutively or tissue-specific (*Gene Promoter*), and may be producing a high dose or a low dose of the endotoxin (*Gene Expression)*, and finally, these may be deployed in different ways (*Field Tactics*), as mixtures or as rotations. These tactics (classified according to the technology) lead to several strategies in the field and are discussed below.

It should be noted that the issue of managing deployment to reduce insect resistance is not unique to transgenics and has been at the core of integrated pest management (IPM). Even with *Bt* biopesticides, insect resistance has occurred with the Diamond back moth[1] in many parts of the world and with the Indian meal moth (for review of such resistances, see Tabashnik *et al*., 1991; Tabashnik, 1994). In laboratory research, insects exposed to high doses of *Bt* over many generations have also developed resistance. Many of these induced resistances are recessive, few are additive or recessive, and none so far has been dominant. Consequently, deployment management strategies have focused on ensuring that sufficient populations of susceptible insects are present to mate with possible resistant ones, ensuring that the frequency of the resistance allele is not fixed in the population.

6.2 Refugia and Mixtures

The fundamental purpose of the deployment strategy of resistance genes is to reduce the possibility of insect resistance. Sound strategies also anticipate that resistant insects do develop and incorporate measures that would be appropriate for such situations.

The most promising and currently practical strategy is that of using refuges (Strategy A, Table 11). The strategy calls

Table 10: Tactics Available for the Deployment of Insect Resistance Genes in Plants

Gene Strategies	Single gene Multiple genes (e.g. pyramid) Chimeric genes
Gene Promoter	Constituitive Tissue-specific Inducible (e.g. wounding)
Gene Expression	High Dose Low Dose Mixtures
Field Tactics	Uniform single gene Mixtures of Genes Gene rotation Mosaic planting Refuges (spatial, temporal)

Source: McGaughey and Whalon (1992).

[1] It is noteworthy that the diamondback moth is a particularly fast evolving insect and was the first insect of agricultural importance to develop resistance to DDT; since then, it developed resistance to almost every insecticide used to control the insect. This property, however, also makes it an ideal insect with which to study the predictions of field resistance with *Bt* crops (see for example Tang and Shelton, 1995).

Table 11: Complementary *Bt* Deployment Strategies

	Strategy	Objective
A	**Refugia** of non-transgenics and mixtures of plants with high level expression and plants with no *Bt*	A refuge enables insects to breed and thus a steady supply of wild type insects (or non-resistant ones) are provided which would be the most likely ones to mate with potentially resistant insects. This would reduce the chances of an increase in the frequency of resistance genes.
B	**High levels of expression** of a single toxin in all plants	Aimed at killing the highest possible percentage of insects and generally implemented in conjunction with refugia.
C	**Low levels of expression** of a single toxin in all plants	Sublethal dose would reduce fertility and growth of insect populations and also make the affected insects prone to predators and parasites.
D	**Multiple gene deployment** or pyramiding of genes	Reduces the likelihood of resistance development since multiple mutations would have to occur concurrently in individual insects.
E	**Targeted expression** of *Bt* in certain parts of the crops or at given times in the plant development	Aimed at reducing the time period of insect exposure to a toxin by expressing it only in vulnerable parts of the plant or both in a certain part of the plant and at a particularly critical time in the development of the plant.

Modified from Gould (1995a, 1995b).

for reducing the possibility of long-term impact by preventing resistant insects from mating with other resistant insects, thereby preventing the creation of a resistant population. This is achieved by ensuring that there are always plenty of susceptible insects nearby for the few resistant ones to mate with. A steady source of susceptible insects can be achieved without significant damage to the crop. This is done through refuge areas that are provided in two possible ways:

- only part of the field is planted with the transgenic crop and another part as close as possible is maintained as an unimproved, conventionally treated area; or
- only part of the field is planted with the transgenic crop and another part (significantly smaller than above) as close as possible is maintained as an unimproved, totally untreated area.

In both cases, the non-transgenic fields will generate insects to largely outnumber any possibly resistant ones. It is hoped that this will prevent the mating of resistant insects, which would lead to the establishment of the resistance gene in the population. Mixtures of resistant and non-transgenic lines in the same field have been proposed but this option, also referred to as mosaic, has generally been discarded by industry as unviable.

6.3 High Dose and Low Dose Approaches

The basic principle of the high level expression approach (Strategy B; Table 11), which should also be incorporated into the mixture and refuge approach discussed above, is to deploy plants with high levels of expression of the toxin (over LC99) with the expectation that it would take a long time for insects to overcome the toxin. It assumes that most or all resistance is recessive or at worst additive, and that most resistance carriers would be heterozygous. The strategy also anticipates that even resistant homozygotes would be killed by the high level of toxin as the toxin would reduce the insect's fitness. For that model to work, without the concurrent use of refuge areas, many assumptions must be met (see Gould, 1994). The predictions become complex when multiple insects and multiple crops are deployed in a region.

The strategy of low levels of expression (Strategy C; Table 11) makes the insect vulnerable to predators and parasites. This option, however, has been discarded by companies since a considerable level of damage would still be inflicted on the crop which would not be acceptable from a commercial point of view.

6.4 Multiple Gene Deployment

A viable complementary strategy that will emerge in the near future and that is best adopted concurrently with Strategy A and B is the deployment of multiple resistance, or pyramiding of resistance genes (Strategy D; Table 11). This strategy requires more than one resistance gene with different modes of action (or binding sites in the case of *Bt*) to be available for a given insect species. It could be achieved either with additional *cry* genes or with novel methods of insect resistance (see Section 2), but requires the use of refuges (for a detailed discussion, see Roush, 1994a, 1994b; Gould, 1995a, 1997). One reason why this strategy should be adopted in conjunction with refugia and high dose expression of the toxin is that some insect

resistances, as demonstrated in the laboratory, may evolve for two genes at the same time (called cross-resistance).

6.5 Targeted Expression

Targeted expression (Strategy E; Table 11) is also complementary to strategies A and B and will become possible in the near future. A toxin gene is expressed only specifically in a certain vulnerable part of the plant (e.g. stem in the case of corn/maize borer), or is expressed both in a certain part of the plant as well as at a particularly critical time in the development of the plant (e.g. flowering). This strategy would allow plenty of susceptible insects to breed normally, thus increasing their predator and parasitic populations, while at the same time be prevented from causing damage in the critical plant parts or life cycles. Much has been achieved in this direction over the past years with progress in the understanding of gene regulation.

6.6 Subsection Conclusions

Most of the complementary strategies presented here might be possible in the near future but for the moment, deployment strategies already implemented show that a high dose strategy with refuges has been adopted in all five cases (Table 12). These are all in the USA where five *Bt* transgenic products are already on the market. The exact refuge area depends on the crop and on the selected treatment of the refuge area. In cotton, for example, the farmer may choose to either sow 20% of unprotected cotton (non-transgenic) as a refuge using conventional insect controls if desired or 4% of unprotected cotton (non-transgenic) as a total refuge using no insect control whatsoever.

The 20% unprotected mark was initially set as a compromise between entomologists, population geneticists, corporate managers and the EPA. Interestingly, recent work with the diamondback moth by Anthony M. Shelton and colleagues at Cornell University shows that a refuge of 20% is indeed an acceptable optimum. For the 1997 growing season, corporations in the USA tend to favor this approach compared to the refuge of 4% with totally untreated plants. Shelton's work also demonstrated that mixtures do not perform as well as separate refuge areas, partly because larvae can easily migrate from a resistant plant to an adjacent non-resistant plant. Nevertheless, he believes that mixed seed might be the most viable option in developing countries where separate refuge areas might not be as well controlled and enforced.

The monitoring of insects for early identification of possible resistant ones is critical in all the strategies outlined above. This poses formidable challenges because collecting insects at random may not necessarily allow early enough detection of resistance to allow remedial actions to be implemented. Requesting farmers to monitor insect damage to crops has limitations, particularly in developing countries and small-scale agriculture where the extension efforts required for such a system to work are tremendous. In addition, in certain crops damage by one insect may look similar or identical to damage from other insect species. Also, much damage in cotton is done by insect larvae where species identification is extremely difficult. This renders such a monitoring system impractical. Finally, remedial actions may require the cooperation of many different groups if the insect is highly mobile and affecting an entire region.

The effect of the strategies proposed or adopted, whether or not in conjunction with various other management practices, such as IPM, is still somewhat speculative and based on extrapolation from scientific experiments and predictions based on prior experiences. Unfortunately, only large-scale deployment will provide the true test for the durability of the genes and the generation of a body of evidence that will allow optimum and safe deployment strategies to be developed.

Table 12: Minimum Refuge Areas for Different Transgenic Crops to Prevent the Likelihood of Insect Resistance

Product [1]	Crop	Measure
Bollgard™	Cotton	25% of unprotected cotton (non-transgenic) as a refuge using conventional insect controls if desired or 4% of unprotected cotton (non-transgenic) as a total refuge using no insect control whatsoever
NatureGard™	Corn	High-dose strategy with levels exceeding the LC99 for European corn borer; also propose to plant 5-50% of unprotected corn (non-transgenic)
NewLeaf™	Potato	20% of unprotected potatoes (non-transgenic) as a refuge using conventional insect control if desired
Maximizer™	Corn	High-dose strategy with levels exceeding the LC99 for European corn borer; also propose to plant 5-50% of unprotected corn (non-transgenic)
YieldGard™	Corn	5% of unprotected corn (non-transgenic) as a total refuge using no insect control whatsoever

1 Bollgard™, YieldGard™ and NewLeaf™ are trademarks of Monsanto, Maximizer™ of Novartis and NatureGard™ of Mycogen.

7. Issues Related to the Transfer of *Bt* Technology

7.1 Introduction

The development of *Bt* crop biotechnology required substantial financial resources, and most of these resources were invested in the North for the development of products primarily useful to the North. There are several examples of developing countries developing their own transgenics (e.g. Mexico, China, Cuba, and Thailand to name but a few) in addition to successful biotechnology transfer projects. However, developing countries wishing to access agricultural biotechnology applications, particularly recombinant products, often cannot due to several constraints. Similarly, private sector corporations wishing to test, share and market recombinant products in developing countries, are precluded from doing so. Several essential factors mitigate against the transfer of biotechnology to developing countries:

- biotechnology R&D is expensive to undertake and requires significant long term investments of a minimal critical mass to be cost-effective;
- much of the current R&D in biotechnology is conducted in the industrialized world and aimed at targets in these countries;
- in industrialized countries the private sector is the major investor in biotechnology and the majority of the products are proprietary and expensive;
- developing countries lack foreign currency and financial resources to acquire the technology;
- there is overall no policy consensus among industrial and developing countries for utilizing the primary genetic resources that are mainly in developing countries;
- with few exceptions, developing countries do not have legislation covering intellectual property rights, let alone the institutional capacity and resources, and their economies cannot support the acquisition of high value-added biotechnology products; and
- few developing countries have the necessary legislation and institutional capacity is in place that will allow for the responsible testing, release and introduction of rDNA technology.

This section will address some of the most critical aspects regarding accessing transgenic technology, namely R&D capacity, biosafety and food safety regulatory issues, and intellectual property rights (IPRs) and licensing aspects. It should be noted that the *Bt* deployment management issues are equally important potential obstacles to a safe transfer of the technology and these have been discussed in Section 6 above.

Several international programs specifically aim at building scientific capacity in agri-biotechnology. One is the Rockefeller Foundation's Rice Biotechnology Program (Toenniessen, 1995) which awards many Ph.D. and post-doctoral fellowships to young scientists. The first wave of these scientists are now returning to their countries. Other centers providing career training are the centers of the CGIAR, as well as the International Center for Genetic Engineering and Biotechnology (ICGEB) in New Delhi. Much more needs to be done, however, so that emerging national private sector activities can be strengthened with experienced personnel. This is a two-way process with increased private sector activities also encouraging more people to pursue career studies in areas where there is potential employment.

7.2 Research and Development Capacity

The most important resource on earth is people. Because of the need to feed this population plant biology is an essential research area that will yield returns and contribute to agriculture in the future (IFPRI, 1995). Most of the capacity in biotechnology research resides in the industrial countries, whereas the principal need and constraints for increased productivity are in the developing countries, where only 6% of all the world's scientists reside. In many parts of the world, particularly in Southeast Asia, there is a decreasing enrollment for both undergraduate and graduate students in the agricultural sciences (APO, 1994). These disciplines will be most important as biotechnology commercialization accelerates, and the traditional agricultural-education programs will need to change rapidly to incorporate more training in biotechnology. This was identified by APO, which serves as a regional adviser for productivity information in Asia, as a major road block affecting R&D in the biotechnology area. Other priority areas include financial investments, the development of a critical mass of trained people, linking research and development with the private sector, and identifying public-private joint venture opportunities including possibilities for licensing technologies.

7.3 Regulatory Issues (Biosafety and Food Safety)

Biotechnology has been viewed by many political leaders, policy-makers and leading scientists in developing countries as a priority for nearly a decade. Nevertheless, the development of biosafety regulatory mechanisms has been slow (this has also been the case in many industrialized countries, and a few OECD countries still lack regulatory mechanisms today). Effective biosafety regulations are critical for the safe and effective introduction of transgenic crops, and are a prerequisite for the transfer of the technology to developing countries. In Asia, only China, India, the Philippines and Thailand have regulatory mechanisms in place, with Indonesia and Malaysia having regulatory laws in the final drafting phase. Industrialized countries in Asia with regulations include Australia, Japan and New Zealand. Other developing countries with regulatory mechanisms in place include Argentina, Brazil, Chile, Costa Rica, Cuba and Mexico in Latin America; and South Africa and Egypt in Africa. Countries in an advanced stage of development are Bolivia, Colombia, Indonesia, Kenya, Malaysia, Nigeria, Venezuela and Zimbabwe.

Thailand does not already have a law, yet it does have a functioning regulatory committee, and several crops have been tested in small field trials with the fields covered by nets. Recent requests from Monsanto for field testing its *Bt* cotton (Bollgard™) and for the FlavrSavr™ tomatoes of Calgene have been approved for net-house trials. However, private corporations are not alone in wishing to test their material. Indonesia is preparing to test *Bt* corn/maize developed through the Agricultural Biotechnology for Sustainable Productivity project (ABSP) of the United States Agency for International Development (USAID) involving collaboration with ICI Seeds (owned by Zeneca) for resistance to the Asian corn borer. IRRI in the Philippines also conducted greenhouse evaluation with their *Bt* rice for resistance to stem borers, and the Asian Vegetable Development Research Center (AVRDC) in Taiwan is currently evaluating the possibility of testing *Bt* technology for the control of Diamond back moth. Finally, the International Potato Center (CIP) has several potato accessions with the *Bt* gene and is evaluating possibilities for field testing them in Asia and elsewhere, pending formal biosafety approval.

Several international organizations, such as the United Nations Industrial Development Organization (UNIDO), the Stockholm Environment Institute, through its now defunct Biotechnology Advisory Commission, ABSP and ISAAA, have been providing and will continue to provide assistance to developing countries in the development of biosafety capacity and regulatory mechanisms. However, much is still required before the majority of the developing countries will have regulations in place. Additional training in biosafety is urgently required, combined with short-term internships during which members of review commissions have an opportunity to participate in existing regulatory agency institutions. Even when regulations are approved, a commission needs to be established to review applications. Individuals will have to assume responsibility and make decisions. Biosafety workshops are an essential component in the development of institutional capacity, however there can be no substitute for hands-on training for an extended period of time within an agency that reviews proposals, or within a company that prepares applications and conducts field trials.

Food safety is perhaps a more complex scientific issue than biosafety. However, as products are evaluated in one country, there is conceptually no reason not to authorize the same products in another country. The OECD is working on a code of mutual recognition of such decisions, although sovereign governments would still need to be notified of the transgenic food or feed products to be imported. In developing countries, however, there is an overwhelming absence of food safety review systems for natural pathogens and additives. There is often limited technical expertise available and few initiatives are underway to remedy this situation. It would be most unfortunate, if after overcoming so many technical complexities of biotechnology, the commercialization of biotech food products throughout the world were to be delayed by the absence of pragmatic food safety review systems.

There are only a few initiatives aimed at building capacity in this area in developing countries. One is the codex alimentarus, a joint initiative by the World Health Organization, the Food and Agriculture Organization (FAO) and others, but food safety of transgenic products will only be addressed late in 1996. Another initiative is the ISAAA Food Safety Initiative prompted by a request from Mexico. Mexico began to address the commercialization of biotechnology products, in part because it has it's own virus resistant potatoes utilizing a Monsanto technology transferred and produced under the aegis of ISAAA. Having its own technology to commercialize provides a strong motivating factor for the government to develop food safety procedures, since no sales can take place without them.

Industry also has a stake in the rapid adoption of workable and standardized food safety regulations. These are a prerequisite for the production and commercial export of bioengineered food products to developing countries.

ISAAA is addressing this urgent need through the food safety initiative (ISAAA, 1995) comprising a worldwide action plan to focus initially on those developing countries that have assigned high priority to biotechnology and are prepared to play a leadership role in their geographic region.

7.4 Intellectual Property Rights (IPRs) and Licensing Issues

Plant Breeders' Rights (PBR) provide patent-like rights for plant varieties. A royalty must be paid for varieties when and where protected, and where protection does not exist, no royalty payments are owed. Under this system, only improved varieties are subject to charges with the original materials remaining available in the public domain without additional cost. Because private companies in industrialized countries are realizing value from genetic resources originating from developing countries, the system has been the focus of much debate over the last two decades (see review by Lesser, 1991). This debate increased in 1985 when seeds were declared to be a patentable subject matter in the USA, extending the scope of opportunities for private biotechnology companies to create value for genetic materials. This subsection will review and discuss the current status and presents some avenues for progress (much is drawn from Krattiger and Lesser, 1995).

With the Uruguay Round of the General Agreement of Tariffs and Trade (GATT, now the Word Trade Organization, WTO) adopted, a new aspect was introduced, namely Trade Related Aspects of Intellectual Property Rights (TRIPs) (GATT, 1993). Signatory countries (which include most countries with the exception of Vietnam and a few others) are mandated to adopt a full compendium of IPRs within 10 years. Those pertaining to plant varieties are the PBRs. Because protection is applicable only in those countries where it has been sought and granted, protection is non-existent in countries where it is not allowed. To date, approximately 50 countries do not admit "plant and animal varieties" for patent protection (WIPO, 1990). Similarly, only one developing country, South Africa, is a member of the International Convention for the Protection of New Varieties of Plants (UPOV) although several countries (Chile, Argentina, Zimbabwe, among others) have national laws.

The current Status of PBR Legislation in the Asian and Pacific Region is that Australia, Japan and New Zealand are presently members of UPOV. All adhere to the 1978 text, but each is preparing modifications necessary to accede to the 1991 convention; Australia is most advanced in that regard. Taiwan has a national PBR law with many parallels to UPOV 1978. India, Pakistan and the Philippines have advanced drafts of laws in 1995 with intent to conform to the 1978 Act of the Convention. They all submitted their laws by January 1, 1996, which was the closing date of that Act. Indonesia, Malaysia and the People's Republic of China have working groups preparing legislation. Thailand also has a committee working on legislation, but the status of that legislation is not clear.

Overall, this indicates very rapid progress on PBR. UPOV had only one developing country member during its first 30 years of existence. Within a few years that number will increase considerably, a result of GATT/TRIPs as well as changing world views toward the role of private firms in the seed sector. Numerous additional countries are less advanced in the drafting process, but they are less technically advanced and probably provide a smaller market for private sector varieties. In many respects, adopting a law is the easiest part of implementing it. Here too the private sector's expertise can be of assistance in the establishment of efficient and effective national offices. Countries must decide if they want to follow the approach of limited examination (e.g. the approach of the USA) or the model that involves public trials at multiple sites (e.g. the EU model). Generally, fees do not cover the full cost of those activities. An intermediary approach is that of Canada, where applicants are required to propagate the varieties themselves at their own expense and supply the data to the PBR Office.

The TRIPs agreement set the basis for a rapidly changing environment as it requires signatory states, including some 70 developing countries, to provide for added protection ("contracting parties shall provide for the protection of plant varieties by patents and/or by an effective *siu generis* system" [GATT, 1993]). The next 5 to 7 years will see some changes although even with legislation, restrictions will remain, as much scope is in the interpretation of the new terminology. In all likelihood, patents for most life forms (except micro-organisms) will be prohibited in at least some countries as will biotechnology processes, even when applied to living organisms. In sum, developing countries opposed to IPR may, under TRIPs, have to make relatively modest changes, but none in the immediate future.

Although empirical justification of patent systems is difficult (since it is impossible to determine what would have happened if no system were in place), IPR systems, or the

lack thereof, are directly related to technological access. Sometimes developing countries reject the concept of IPR on the grounds of not having sufficient R&D capacity. Under that perspective, IPR only raises by the amount of the royalty the cost of acquiring creations from abroad, particularly from industrialized countries. As justification, low patenting rates by developing country nationals, usually below five percent of all patents, is cited. That position overlooks another aspect of IPRs: facilitating access to technologies. Even in cases like seeds where the technology can be acquired at the seed store, private companies are likely to delay movement to markets where no protection is available. The cost associated with the delay in access, perhaps several years of using a less productive variety while the new seed appears on the market, is acquired, and the important trait transferred to locally adapted varieties, must be counted against the royalty fee, making the choice less clear in many cases (see Lesser, 1991).

The primary issue is the unwillingness of companies to license valuable technologies in countries with inadequate PBR protection (from the perspective of the provider). Of particular concern is the absence of patent-like protection for plants in some 39 developing countries. However, as PBRs protect the entire plant, little protection is provided for valuable genes; for this, stronger protection (patents) must be sought. If a gene were not protected through patents, it could generally be transferred among varieties through conventional breeding practices. This is also the case with the 1991 UPOV, although the concept of dependency was introduced. This is a complex area and not addressed here.

Another related complexity involves the licensing of materials which contain technologies of several owners. For example, in the production of *Bt* plants the gene itself may belong to one company; and the transformation and regeneration protocol, promoters and plasmids to several other companies. The companies appear to place low priority on resolving these matters because they are time-consuming and offer limited revenue potential. With many products now in the commercialization stage, management time is a scarce factor for those companies. At the same time, they are understandably unwilling to make agreements which could affect their core markets. Some private companies are willing to donate technologies for use by small (essentially non-commercial) farmers, raising the ancillary problem of identifying clear demarcations among the various categories of users. Questions such as: How could one portion of growers be charged royalties, the others not; how can market segments be demarcated (for example one product such as sweetpotato grown by large farmers for export while small scale farmers may produce it for home use and local sales). These issues can delay the biotechnology transfer process, which is not in the interest of companies or developing countries.

8. Conclusions and the Future of Insect Resistant Crops in Developing Countries

Monsanto's *Bt* cotton research and development cost have not been released but it is estimated to exceed US$100-150 million. In 1984, Monsanto invested US$166 million (in 1996 dollars) into a plant biotechnology building and the cost for the regulatory approvals of the first transgenics were high, not to mention the significant budgets that must be available for the legal departments in any biotechnology company. The second and following transgenic cotton varieties will cost much less, but over the next few years the R&D costs are still expected to be several million US dollars per variety. In addition to the high research costs, the development of the first biotechnology products required substantial investments, particularly in regulatory issues and marketing strategies. This aspect is best illustrated with the commercialization of the first transgenic crop, the FlavrSavr™ tomato by Calgene. The company elected to create a new trademark, McGregor™ Tomato and provide consumer information on the transgenic product. It had to have the tomatoes grown under contract and develop direct distribution and marketing systems. This was not without difficulty and partly explains why the product, having been launched in 1994, is still not widely available in the USA and will take many more years before reaching developing countries.

The review of the development and current status of *Bt* technology demonstrates the considerable research that went into the technology, and the critical steps involved in the development of a marketable and useful product. The delivery of new technologies to developing countries, many of which do not have a fully developed private sector seed industry has always been more challenging. With biotechnology applications, some of the constraints imposed by traditional technologies do not apply (for example, biotechnology applications, as opposed to mechanization, is essentially scale-neutral). However, insect

resistance with *Bt* presents a particular obstacle due to the requirements for managing the deployment of the technology in terms of avoiding insect resistance. The potential for the technology to replace traditional toxic chemical insecticides is also great for the resource poor farmer who does not have access to the capital expenditures of pesticides. In such cases, the technology offers to increase yields and productivity without the need for any additional external input.

How can the technology be transferred to areas with the greatest need? Several complementary options exist, and the first and foremost for seed companies in developing and industrialized countries alike is licensing. The development of the technology itself is based largely on licensing and strategic alliances, and companies specializing in the development of biotechnology have had to invest in plant breeding and seed companies, or forge strategic alliances or joint ventures, or create new companies. The latter has been one of the dominant strategies of Monsanto with the creation of a subsidiary to handle its transgenic potato product development and marketing. Alternatives are the licensing of the technology. Again, Monsanto licensed its *Bt* and herbicide technology in cotton to Delta and Pine Land Co. for the commercialization of the product worldwide, except in the USA where Monsanto is directly marketing the product. Many other companies also license their biotechnology applications for specific crops.

One of the reasons why such alliances and licenses abound is the fact that countries in which companies have their major operations all honor strong IPRs (e.g. South Africa, Argentina), making contracts enforceable. Likewise, effective biosafety regulatory mechanisms are in place in those countries. In Asia, as discussed in Section 7, the situation is diverse but overall, the major investments in agriculture have been made by the public sector (James, 1996), nevertheless with a rapid growth of the private seed industry.

In India, for example, since 1989 when foreign seed companies were allowed to establish operations, the private seed business has been increasing steadily with seed sales around US$280 million in 1993. It is expected that the commercial seed market by both the public and private sector (foreign and national companies) will exceed US$1.4 billion by the year 2000. One of the reasons for this growth has been the availability of new technologies that enable the protection of non-hybrid crops. Male sterility in canola/rapeseed, developed by Plant Genetic Systems (PGS; now owned by AgrEvo), has been commercialized in India and allows hybrid seed to be developed competitively. This technology is being considered critical by companies in order to safeguard their technologies and ensure returns of their investments.

In addition to licensing agreements, the building of partnerships between companies in the North and the South, or between public institutions where appropriate, is possible, but this will require more time. The foremost ingredient is trust and workable models, as there are many uncertainties.

A third option for transfer is donation of the technology, and ISAAA's experience indicates that some companies are willing to do so, i.e. Monsanto, Novartis, and Pioneer Hi-Bred International. With ISAAA's focus on crops grown predominately by small scale farmers, such as potatoes in Mexico, cassava, sweetpotatoes and open pollinated corn/maize in Africa, donation of technology overcomes the lose-lose situation where the private sector cannot deploy the technology and the developing countries cannot access it. These projects further enable countries to build their biotechnology capacity, including the required biosafety regulatory mechanisms, and allow the pragmatic development of new distribution and technology management strategies. In addition, pilot projects provide developing countries with the opportunity to test the technology and decide for themselves what is best and in their national interests.

ISAAA's approach will continue to include efforts for the transfer of biotechnologies from the private sector to developing countries. In the longer term, South-South transfers are possible and even anticipated with the first such project underway (virus resistant potatoes from Mexico to Kenya). Finally, the particular focus of ISAAA's activities, namely efforts directed toward biotechnologies that offer clear benefits to the small and resource-poor farmers as well as to the environment, will enable the building of trust between the players and eventually open up new channels for sharing the benefits that biotechnology has to offer the world. ISAAA's approach is based on the premise that effective pilot projects must be needs driven, meaning that technology transfer projects must respond to national priorities. It is through the needs driven approach that optimal commitment to successful projects and to the technology can be stimulated. This needs driven approach allows the countries to see and reap directly the benefits of the new technologies. As a consequence, a self-interest develops to invest in the development of regulatory structures and other means to foment biotechnology at the national level.

ISAAA dedicates much of its resources to developing such projects by brokering agreements that fall outside the traditional IPR framework. The reason is, as mentioned above, that a large percentage of developing countries do not permit patents on plants and animals. As a consequence, in order to develop new channels of technology transfer, it is imperative to develop new systems initially based on trust and with time ISAAA believes that such model agreements will become modus operandi. Considering the private sector's understandable reluctance to donate expensive technology for free, ISAAA invests much of its resources to building trust in the private sector by gradually increasing the complexity of transfer agreements. These projects, it is hoped, will enable the private sector to gain confidence in sharing its technology, including insect resistance through *Bt*, enable developing countries to test the technologies and develop appropriate deployment management strategies, and reap the benefits the technology has to offer farmers and for a safer environment for all.

These recent developments in biotechnology, more specifically insect resistance in crops, demonstrate that *Bt* is merely the beginning of a long series of new and safer technologies to augment productivity, to bring about a more sustainable agriculture, and to protect the environment. With the emergence of a wide range of possibilities from the point of view of the technology, emphasis must now be placed on the development of transfer and delivery mechanisms to the resource poor farmers who are most dependent on novel solutions for their very livelihood and long-term survival.

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Appendix I: List of Latin Names for Cited Insect Species

Beet armyworm	*Spodoptera exigua*
Cabbage caterpillar	*Pieris rapae*
Cabbage looper	*Trichoplusia ni*
Cabbage worm	*Pieris brassicae*
Codling moth	*Cydia pomonella*
Colorado potato beetle	*Leptinotarsa decemlineata*
Corn Earworm	*Helicoverpa zea*
Cotton bollworm	*Heliothis, Pectinophora, Earias*
Cotton leaf perforator	*Bucculatrix thurberiella*
Cotton leaf worm	*Spodoptera littoralis*
Cupreous chaffer beetle	*Scarabaeid* spp.
Diamondback moth	*Plutella xylostella*
European corn borer	*Ostrinia nubilalis*
Greater wax moth	*Galleria mellonella*
Green lacewing	*Chrysopa carnea*
Gypsy moth	*Lymantria dispar*
Hessian fly	*Mayetiola destructor*
Honey bee	*Apis mellifera*
Indian meal moth	*Plodia interpunctella*
Ladybird beetle	*Hippodamia convergens*
Mosquitoes	*Aedes aegyti, Culex pipiens*
Navel organgeworm	*Amyelois transitella*
Parasitic wasp	*Nasonia vitripennis*
Pink bollworm	*Pectinophora gossypiella*
Porina moth	*Wiseana* spp.
Saltmarsh caterpillar	*Estigmene acrea*
Silk worm	*Bombyx mori*
Spotted cucumber beetle	*Diabrotica undecimpunctata*
Stemborer (yellow, in rice)	*Scirpophaga incertulas*
Sugarcane borer	*Eldana saccharina*
Tobacco budworm	*Heliothis virescens*
Tobacco horn worm	*Manduca sexta*

Appendix II: List of Major Research Centers Working on Various Aspects of *Bt*

Bioremediation..... 32

Characterization..... 32

Culture..... 33

Expression 34

Formulation..... 36

Gene Identification..... 37

Transgenics 37

Various..... 39

Note that the classification is in some cases arbitrary and many research projects are overlapping.
Based on a review of published articles from 1993 to 1996.

Bioremediation

Institution	Country	Major *Bt* Research	Major Pest	Major Crops	Notes
Shimadzu	Japan	*Bt* culture			For application in biodegradable plastic manufacture
University of Houston	USA	*Bt* for bioremediation			A patented new method for bioremediation consists in suing a ketone degrading *Bt* which is genetically engineered
University of Maryland	USA	*Bt cerus*			Useful for bioremediation and selected from nitroglycerin (NG)
University of Maryland	USA	Bioremediation using *Bt*			Denitration of glycerol by *Bt*
University of Milan	Italy	*Bt* biodegradation			Several strains identified for biodegradation of herbicides
University of Maryland	USA	*Bt* bioremediation			*Bt* and *B. cerus* enzymes help with bioremediation of nitrate ester waste

Characterization

Institution	Country	Major *Bt* Research	Major Pest	Major Crops	Notes
BIOTRO-IGEPAM CIRAD-CA	France	*Bt* subsp. *cameroun*	Several	Several	H32 strain has been characterized
Cambridge University	UK	*Bt* characterization (CryIA(b), CryIIA, CryIIB)	Several	Several	YD 226 strain characterized and related to one *cry1A(b)*, one *cryIIA* and two *cryIIB* genes. Controls housefly and *Lepidoptera* (*Pieris* and *Spodoptera* spp.)
CPRO	Netherlands	*Bt* Cry protein analysis CryIA(b)	Several	Several	Insecticidal activity due to domain II of the three domains of the toxic moiety
Hawaii University	USA	CryIB, CryIE, CryIF cross-resistance	*Plutella xylostella*	Vegetables	Extremely high levels of cross resistance were conferred across classes of CryI toxins in *P. xylostella* in laboratory tests
Horticultural Food Research Institute	New Zealand	*Bt* isolation and toxicity studies	Several	Several	*Bt* isolates for the control of *coleoptera, diptera,* and *lepidoptera* have been identified
Horticultural Food Research Institute	New Zealand	*Bt CryV* screening & characterization	*Epiphyas potvittana*	Several	Identified genes cloned into *E. coli*. Lysates of *E. coli* transformants were toxic to insects
Institute Pasteur	France	*Bt serovar medelin* ICP characterization	Mosquitoes	*Diptera* infested crops	Crystal proteins from *Bt serovar medelin* collected from Colombia characterized and tested for mosquito control
Institute Pasteur	France	*Bt* sequencing	*Anopheles aegypti, Anopheles stephensi*	*Diptera* infested crops	New nucleotides sequences for *Bt* identified
Institute Pasteur	France	*Bt* characterization	*Diptera*	*Diptera* infested crops	A patent for DNA sequence which contributes to control of Diptera larvae
Institution	Country	Major *Bt* Research	Major Pest	Major Crops	Notes
Jadavpur University	India	*Bt israelensis* mutant characterization	Mosquitoes	*Diptera* infested crops	Sporulation and cp production are not related
Korea-Institute of Science and Technology	Korea	CryV gene characterization	*Lepidoptera* (*Plutella xylostella* and *Bombyx mori*) and *Coleoptera*	*Coleoptera* and *Lepidoptera* infested crops	*CryVI* protein was toxic to *P. xylostella*
Kubota University	Japan	*Bt serovar japonensis* characterization	*Scarabaeid* beetles	Crops infested with *Scarabaeid* beetles	Characterization of larvicidal protein from *Bt* serovar *japonensis* specific to beetle control
Kyushu University	Japan	*Bt* (flagellar serotype-44)	Mosquitoes	*Diptera/Lepidoptera* infested crops	Several *Bt* strains have been characterized for control of mosquitoes and *Lepidoptera*. These strains should be useful for biological control of pests
Madurai-Kamaraj University	India	Mosquito specific *Bt* strain characterization	Mosquitoes	*Diptera* infested crops	10 *Bt* isolates characterized and found effective for mosquito control. Strains similar to type strain *kyushuensis*
Mie University	Japan	*Bt* mating study	Ecological	Ecological	Improvement of rumen bacterium to increase its cellulose degradation activity
Montana University	USA	*Bt* molecular characterization *Bt* subsp *dermastadiensis*	*Lepidotera* and *Diptera*	*Lepidoptera* and *Diptera* infested crops	*Bt* subsp. *dermastadiensis* cp's have been characterized for further host range studies
National Defense Medical Center	China	Mosquitocidal *Bt israelensis*	Mosquitoes	*Diptera* infested crops	Mosquitocidal *Bt* cp expressed in *Streptomyces lividans*, purification and characterization of cp for use in mosquito control
National Polytechnic Institute	Mexico	*Bt* characterization	Several	Several	A novel strain of *Bt* characterized
Petrozovodsk University	Russia	*Bt* enzyme purification and characterization	Several	Several	A chitinase was isolated from a submerged culture of *Bt*.
Purdue University	USA	CryIA isolation and characterization	*Lepidoptera*	Several	*Bt*k strain HDI with 3 *cryIA* protoxin gene studied and *cryIA(b)* was maintained in the population by cell mating
Research Institute of Genetic Selection and Industrial Microorganisms	Russia	*Bt* 4KH synthesis	Colorado potato beetle	Potatoes	*Bt* 4KH that synthesizes an endotoxin for control of Colorado potato beetle is reported

Institution	Country	Major *Bt* Research	Major Pest	Major Crops	Notes
Taiwan University	Taiwan	*cry* gene identification	Several	Several	Several *cry* type genes identified and characterized
University of Georgia	USA	CryIC iodination and binding	*Spodoptera exigua*	Vegetables	*CryIC* toxin developed with removal of SDS and renaturation
University of Montreal	Canada	*Bt*. characterization	Mosquitoes and black fly	*Diptera* infested crops	Several mutants of *Bt* var *israelensis* HD 500 obtained for diptera control
University of New South Wales	Australia	*Bt sphaericus*	Mosquitoes	*Diptera* infested crops	New screening techniques for cost effectiveness are discussed
University of the Middle East	Turkey	Plasmid patterns of *Bt*	Several	Several	*Bt* 81 characterized and its plasmid determined
University of the Middle East	Turkey	*Bt* biochemical and molecular characterization	Several	Several	Molecular characterization of *Bt* mutants
Washington State University	USA	*Bt* ICP activity	Several	Pea, Alfalfa	CryIII containing *rhizobia* to reduce feeding damage by nodule feeding insects

Culture

Institution	Country	Major *Bt* Research	Major Pest	Major Crops	Notes
Acadmia Sinica	China	*Bt* var *israelensis* culture	Mosquitoes	Diptera infested crops	Culture medium developed for mass culture of *Bt* var. israelensis
Advanced Institute of Sci. & Technol.	Korea	*Bt*k culture	Several	Several	Poduction of *Bt*k HD1 strain using a fermenter to improve spore concentration
Agri-Food Canada	Canada	*Bt* mass culture	Several	Several	Cultural conditions for production of *Bt* developed
Anna-Madras University	India	*Bt* culture	Several	Several	*Bt* subsp *galleriae* grown in continous cultures with 2-1 chemostats
Biorational Resources	Australia	*Bt israelensis*	Mosquitoes	*Diptera* infested crops	Factors influencing in predicting the success of *Bt. i.* are identified
Bombay University	India	*Bt* toxin production	Mosquitoes	*Diptera* infested crops	Defatted mustard seed meal based media gave good results for growing of *Bti* and Bs.
CINDEFI	Argentina	*Bt* var. *israelensis* culture	Mosquitoes	*Diptera* infested crops	A simple fed batch culture system designed for *Bt*. var. israelensis
CINDEFI	Argentina	*Bt* batch culture	Mosquitoes	*Diptera* infested crops	Batch culture of *Bt* var. israelensis
CNRS/INRA	France	*Bt* production of ICP	Several	Several	*Bt spoa* constructed and used to produce ICP which are stable and safe
Industrial Research Ltd.	New Zealand	*Bt*	Several	Several	Process development for biological control agent production - culture, medium design, process scale up and downstream processing
INRS	Canada	*Bt kurstaki* mass culture	Several	Several	Waste water treatment plants used for mass culture of *Bt*
Jiangsu-Agricultural College	China	*Bt* culture	Several	Several	Flask culture method developed
Karmanek	Russia	*Bt* culture	Several	Several	A patent for culturing *Bt* is described
Kurabo	Japan	Polypheonol glycoside production from several *Bt* strains			A patent awarded for polyphenol glyoside synthase preparation from *B. subtilis*, *Bt*, *B. licheniformis* or *B. amyloliquefaciens*
Lynxvale	UK	*Bt* Crystal protein production	Several	Several	A patent has been assigned for use of *Bt* cp gene in *Lactococcus* sp
Middle East Technological University	Turkey	*cryI* gene product culture	Several	Several	Luria broth or Yousten's synthetic medium used for growth of ICP from *cryI* gene
Nagoya University	Japan	*Bt. subtilis* batch culture	Mosquitoes	*Diptera* infested crops	Production of recombinant protein highest in soybean peptone containing medium
National Polytechnic Institute	Mexico	*Bt* culture	Several	Several	Biomass yield under steady state conditions promotes continuos culture
National University Tsing-Hua	Taiwan	*Bt darmastadiensis* (HD-199)	Several	Several	High cell density fed batch cultures of *Bt* for *thuringiensis* in production
National University Tsing-Hua	Taiwan	Mass culture	Several	Several	Mass culture in an air lift modified tank fermentor
National University Tsing-Hua	Taiwan	*Bt thuringiensis* production	Several	Several	*Bt* substrain darmastadiensis HD 199 grown in airlift fermenter
National University Tsing-Hua	Taiwan	Batch culture of *Bt*	Several	Several	A new batch culture for *Bt* subsp. *darmastadiensis* has been developed
Research Inst. of Appl. Microbiology	Russia	*Bt* mass culture	Several	Several	A patent assigned for production of exotoxin for protecting plants from harmful plants
Research Inst. of Appl. Microbiology	Russia	*Bt* strain G7 culture	Fungi	Fungal infections	production of bacteriocin activity against *B. cerus* and *Bt*. active against 11 fungi and antifungal activity caused by a secreted factor
Research Inst. of Appl. Microbiology	Russia	*Bt* delta-endotoxin production	Several	Several	Production of multiple delta endotoxins by *Bt*
Research Inst. of Appl. Microbiology	Russia	*Bt* 98-1 production	Several	Several	A patent assigned for a new beta-endotoxin protein from strain 98-1. Gives increased yield of exotoxin for use in biocontrol
Research Inst. of Appl. Microbiology	Russia	*Bt* culture for subsp. *morrisoni*	*Coleoptera*	*Coleoptera* infested crops	Culture methods have been developed
Research Inst. of Appl. Microbiology	Russia	*Bt* biomass production	Several	Several	A new patented method for micro-organism biomass of *Bt* using fermentation has been developed

Institution	Country	Major *Bt* Research	Major Pest	Major Crops	Notes
Technical University Graz	Austria	*Bt* var. *israelensis* mass culture	Mosquitoes	*Diptera* infested crops	Mass rearing based on a monod system
University California, Davis	USA	*CryIVD-CryIA(c)* improved	Several	Several	Improved production of *CryIVD* using *Cry1A(c)* promoters
University New South Wales	New Zealand	*Bt* production	Several	Several	Sonication increases cell and spore counts of *Bt* israelensis 1897 by 1.5 and 2.3 times
University of Illinois	USA	*Bt* culture	Several	Several	Effective microbial fermentation
University of Illinois	USA	*Bt* fermentation technology	Several	Several	A protype neural network-based supervisory control system for *Bt* fermentations
University of Missouri	USA	*Bt*.k HD1	*Lepidoptera*	*Lepidoptera* infested crops	Arcas culture medium modified and this resulted in higher yield and should be useful for large scale biological control agent production
University of Missouri	USA/ India	High density cultivation of *Bt*	*Lepidoptera*	*Lepidoptera* infested crops	Strategies for high density cultivation of HD 1 strain developed
Yantai University	China	*Bt*K HD1	several *Lepidoptera*	*Lepidoptera* infested crops	Fermentative culture in a malt sprout root based culture medium has been developed

Expression

Institution	Country	Major *Bt* Research	Major Pest	Major Crops	Notes
Agriculture Canada	Canada	Nucleotide sequencing and vector development	Several	Several	Proteins encoded by *Bt* studied
All Russian Science Research Institute of Agricultural Biotechnology	Russia	CryIIA, CryIA(a) construction of hybrid protein	Colorado potato beetle	Potato	Immunoblotting revealed hybrid proteins of expected size. Cell extracts most toxic to colorado potato beetle
Auburn University	USA	*CryIIA* expression	*Lepidoptera, Diptera*	Several	CryIIA of *Bt*k strain NRD-12 cloned into E. coli. The ICP's produced resulted in insecticide activity against lepidoptera and diptera
Beijing University	China	*B.cerus/Bt*k	*Lepidoptera*	*Lepidoptera* infested crops	A patented method for genetic engineering and cloning
Beijing University	China	Cloning, expression in *B. cerus, B. brevis, B. subtilis*	*Heliothis assulta, H. armigera, Ostrinia furnacalis*	Cereals/Vegetables/Cotton	*Bt* gene clone TH48 with the 6.6kb gene inserted via a shuttle vector into wild *B. cerus* and others to increase the toxin production. Best electorporation conditions were found resulting in highest transformation efficiency
Ben-Gurion University	Israel	*CryIVA, CryIVD* cloning	Mosquitoes	*Diptera* infested crops	Larvicidal activity of these *Bt* strains to mosquitoes was 7 fold higher than CryIVA alone
Bhabha At. Research Center	India	CryIA(c) host range expression	Potato tuber moth	Potatoes	Cry IA(c) expressed in *E. coli.* A toxin gene other than *cryIA(c)* responsible for toxic effect on potato tuber moth larvae. *cryIA(c)* effective against *Heliothis armigera*
Biotechnology Introduction Center	Russia	*Bt.k* strain VKPM	Several	Several	A new *Bt* strain has been obtained via chemical mutagenesis with ethylmethane sulfonate and can be cultured in media
Cambridge University	UK	*Cry1C, Bt aizawai* cloning and expression	Mosquitoes	*Diptera* infested crops	Cp gene cloned and expressed in *E. coli*
Cambridge University	UK	*CytB* for *Bt*k cloning and characterization	Several	Several	*CytB* inclusions developed and are more protease resistant than *CytA*
Cape Town University	South Africa	*CryIA(c)* gene expression and construction	Sugarcane borer	Sugarcane	New bioinsectidal strain of *P. fluorscens* with *CryIA(c)* for control of sugarcane borer
CNRS	France	*Bt* genes: sigE, sigK expression	Several	Several	*cryIA* expression in several mutants of *Bt* for bio control
CNRS	France	*cryIIIA* expression	*Diptera*	*Diptera* infested crops	*CryIIA* toxin gene is not dependent on a sporulation specific sigma factor
Cornell University	USA	*Bt* expression/ resistance management-*CryIA(c)*	Diamondback moth	Broccoli	Transgenic broccoli with CryIA(c) developed and tested. *Bt* gene under the control of a light inducible promoter.
Cornell University.	USA	*Bt* gene expression	Several	Several	CytA gene from *Bt*i is expressed selectively in a plant pistil.
CPRO	Netherlands	*Bt* cloning and expression *cryIC* and *cryIE*	Several	Several	*Bt* toxins have been engineered with increased specificity for insect gut proteases
CPRO	Netherlands	Recombinant *Bt* ICP- *cryIC* and *cryIE*	Several	Several	*In vivo* recombination used to develop new ICP's for control of resistant insects
Crop Genetics Institute	USA	*cryIA(c)* cloning, expression in *Clavibacter* subsp. *cynodontis*	European corn borer	Maize	*cryIA(c)* gene cloned in a plasmid and introduced into *Clavibacter cynodontis* by electroporation. Recombinant strains produce protoxin toxic to European corn borer

Institution	Country	Major *Bt* Research	Major Pest	Major Crops	Notes
CSIC University	Denmark	*Bt* containment and release			*Bt* linked with suicide function
CSIRO	Australia	Recombinant *Bt baculovirus*	*Heliothis armigera*	Cotton, Vegetables, Legumes	A patent has been awarded for development of an insect baculo virus; a recombinant *Bt* cp sequence for control of insect pests
Drexel University	USA	Expression in plants	Several	Roses, orchid, legumes	A patent assigned for DNA sequence and expression in rose, orchids or legume plant cell culture
Indian Agricultural Research Institute	India	*Bt* HD1	*Helicoverpa armigera*	Grain legumes	HD1 ICP gene of *cryI(a)* has been cloned
Indian Agricultural Research Institute	India	Codon usage for *Bt*	Vegetable pests (*Lepidoptera*)	*Brassica*	Codon usage studies done here will help in understanding *Bt* ICP expression in oilseed and *Brassica*
INRA	France	*Bt* cloning and expression	Several	Several	*Bt* gene effective for coleoptera expressed in a shuttle vector and introduced into *E. coli* with *cryIIA* gene. The plasmids then introduced into *Bt*k HD-1. The final strain had comparable activity to many coleoptera
Institute of Applied Micorbiology	Russia	*Bt* recombinant research	*Coleoptera* and *Lepidoptera*	*Lepidoptera* and *Coleoptera* infested crops	Recombinant strains of *Bt* with higher exotoxin for control of insects developed
Institute of Applied Micorbiology	Russia	CryIAb7, CryIG, CryIX cloning and expression	Several	Several	Cloning and expression *Bt* subsp. *galleriae* has been performed in *E. coli*. High homology in *CryIG* and *CryIX* 3' terminal parts
Institute of Applied Microbiology	Russia	*Bt* CryIII Expression	Colorado Potato Beetle	Potato	*Bt* active aganist Colorado Potato Beetle identified
Institute Pasteur	France	*cryIIIA* toxin expression	*Coleoptera*	Coleoptera infested crops	*cryIIA* transcripts were increased to enhance its effect on control of coleoptera
Institute Pasteur	France	*B.subtilis, B.sphaericus, B.megaterium* recombinant antigen production	Several	Several	A patent awarded for a new vector with stable controllable replication in *Bt* strains for insect biological control or antigen production
Institute Pasteur	France	*cryIVA* and *cryIVB* genes expression	Mosquitoes	*Diptera* infested crops	Proteins toxic to larvae of mosquitoes. In combination, activity was higher
Institute Pasteur	France	*cryIIIA* expression	Several	Several	A patent for a DNA sequence for *cryIIIA* gene is reported
Institute Pasteur	France	*cryIVB, cryIVD* expression and cloning by plasmid	Mosquitoes	*Diptera* infested crops	Genes encoding for *cryIVB* and *cryIVD* of *Bt* var *israelensis* cloned on a stable shuttle vector and transfered to *B. sphaericus* by electroporation.
Institute Pasteur	France	Recombinant *Bt*	Mosquitoes	*Diptera* infested crops	A patent has been assigned for a new *Bt* Crystal protein with mosquito larvicide
Institute Pasteur	France	*Bt* subsp *jegathesan cryIIB* protein	Mosquitoes	*Diptera* infested crops	A *cryIIB* gene has been cloned and may be useful in developing novel biological control agent strains
Korea Research Institute of Biosciences and Biotechnology	South Korea	*cryIK*	*Artogeia rapae*	Vegetables	A novel crystal protein gene *cryIK* was cloned and sequenced from *Bt* subsp. *morrisoni* BF 190
Kubota University	Japan	*Bt* gene construction/expression	Several	Several	Deletion mutants help in ICP production specific to certain insect pests
Kyoto University	Japan	*Bt* cloning, characterization	Mosquitoes	*Diptera* infested crops	Methods for cloning/characterization of *Bt*i and Bs have been developed
Leicester University	UK	Production of recombinant proteins in plants	Several	Several	Production of recombinant proteins (*Bt*) in plants k
Memphis-State University	USA	Mosquitocidal *Bt*	Mosquitoes and other *Diptera*	*Diptera* infested crops	cloning of mosquitocidal *Bt* for use as a insecticide
National Institute of Occupational Health	Denmark	*Bt* subsp. *israelensis* nucleotide sequencing	Several	Several	Complete DNA sequence of *Bt* subsp *israelensis*
National Taiwan University	Taiwan	*cry* gene isolation and expression in transgenic plants	Several	Several	Cry gene cloned into *E. coli* and also transferred into plants through Ti plasmid mediated transformation. Low level of expression
National University of Singapore	Singapore	*Bt* expression control sequences	Mosquitoes	*Diptera* infested crops	Expression control sequences used to drive *Bt* subsp. *israelensis* cp gene in *Caulobacter crescentus* for use use as a recombinant bio control agent
National University of Singapore	Singapore	Mosquitocidal *Bt*	Mosquitoes	*Diptera* infested crops	Mosquitocidal toxin genes from *Bt*i and *B. sphaericus* expressed in buoyant strain for mosquito control
NERC	UK	Genetic engineering of viral insecticides	Several	Several	*Bt* ICP gene introduced into a baculo virus for insect control. Safety tests completed. Effective against pests and applicable for bio control of insects
Ohio-State University	USA	*Cry(b)* cloning and site-directed mutagenesis	*Manduca sexta*	*Lepidoptera*	Single amino acid changes in *Cry(b)* affect irreversible binding to *M. sexta* midgut membrane vesicles
Oxford University	UK	DNA sequence, Vector- Expression	*Autographa californica*	Several	A patent assigned for recombinant protein production in transgenic insect or insect cell culture
Public Health Institute	Finland	*Bt* expression	Several	Several	A patent for expression system for enhanced secretion of exoproteins
Purdue University	USA	*Bt* survival	Several	Several	PT genes were manipulated to enhance *Bt* survival and to increase its efficiency to gain access to nutrients available in larval hemolymph

Institution	Country	Major *Bt* Research	Major Pest	Major Crops	Notes
Research Inst. of Appl. Microbiology	Russia	*CryIII(a)* expression	Colorado potato beetle	Potato	Cry III(a) gene expressed in *Pseudomonas putida* cells
Research Corporation of Technology	USA	*Bt* subsp *israelensis* host range	Mosquitoes	*Diptera* infested crops	A patent has been awarded for the genetic modification of *Bt* that produces a protoxin by expression of a foreign chomosal PT gene
Research Institute of Genetic Selection and Industrial Microorganisms	Russia	*Bt*-design of vectors	Several	Several	Several plasmid vectors with genes for *Bt* ICPs have been cloned and studied
Rutgers University	USA	Cloning of *Bt* ICP gene into *Clavibacter xyli*	Several	Several	*CryIA(a)* cloned and introduced into *Clavibacter* using a plasmid as a vector. Recombinant plasmid transformed via electroporation in *E. coli* was transformed via the same plasmid. Good protein expression in both cases
Rutgers University	USA	Vector development, cloning, expression	*Lepidoptera*	Several	Plasmid from *Clavibacter cynodontis* mapped, a stable shuttle vector constructed, *Bt* ICP genes cloned into vector and used for expression
Scripps Research Institute	USA	*Bt* expression in *Pseudomonas fluroscens*	*Heliothis armigera, Spodoptera litura*	Several	ICP gene from *Bt* introduced into *P. fluorscens* by protoplast fusion
Simaran-Tanabal	Japan	*B. sphaericus* expression	Mosquitoes	*Diptera* infested crops	A patent awarded for expressing an ICP gene form *Bt* or *B. sphaericus* in *Caulobacter*. Transformed bacteria useful in control of mosquitoes
Tokyo University of Agriculture	Japan	Cloning of *CryIA(a)*	Several	Several	A new *CryIA9(a)* from *Bt* strain FU-2-7 with improved insecticide activity has been developed
Tokyo University of Agriculture	Japan	*Bt* serovar *japonensis* cloning, expression	*Scarabaeid* beetles	Several	Cloning, heterologus expression and localization of a novel *Bt* serovar *japanese* strain toxic to scarbaeid beetles
University Federal do Rio de Janeiro	Brazil	*Bt* var. *morrisoni* genetic engineering-*CryIVB*	Several	Several	*Psedomonas flurescens* modified with the *CryIVB* gene and its survival and root colonization studied in Brazil.
University Madurai-Kamaraj	India	*CryIA(a)* expression in *B. megaterium*	*Lepidoptera*	Cotton	CryIA(a) gene expressed in cotton leaf colonizing *B. megateirum* and ICP produced on cotton leaf surfaces for 30 days were effective in controlling the *Lepidopteran* insect pests
University of Buenos Aires	Argentina	*B. sphaericus subtilis*	Mosquitoes and flies	*Diptera* infested crops	ICP are expressed in vector plasmids for application as an insect biological control agent
University of California	USA	*CryIVD* and *cytA* expression	Mosquitoes	*Diptera* infested crops	Protein gene cloning yields new mosquito biological control agents
University of New Jersey State	USA	*Bt* expression, cloning	Several	Several crops & livestock	Gene manipulation yields *Bt* effective against nematodes
University of New South Wales	Australia	Cloning	Mosquito	*Diptera* infested crops	Several *Bt* genes cloned into *Synechococcus* and *Synechocystis*
University of Toronto	Canada	*CryIVB* gene expression	Several	Several	*CryIVB* was maximized in *Cyanobacterium (Synechococcus* sp.) This acted as a natural food source for mosquitoes providing control
University of Wyoming	USA	*Bt* israelensis gene expression	Mosquitoes	*Diptera*	A gene encoding mosquitocidal cp was used to transform *Cyanobactrium*. The cp produced was toxic to mosquitoes
University of Wyoming	USA	*CryA(b), Bt* "receptors" Expression	Several	Several	*Bt* expression in host insect cells, use in therapy and pesticide screening
Wageningen University	Netherlands	*Bt* gene transfer	Several	Several	Conjugal gene transfer with special emphasis in soil have been developed. Marker genes, expression in other spp. such as *E. coli, Agrobacterium, Pseudomonas* and detection in soil by selective plating, PCR developed
Wageningen University	The Netherlands	*cryIA(b)*	*Lepidoptera*	*Lepidoptera*	Baculovirus insecticides can now express tailored *Bt Cry1A(b)* Crystal protein which can be used as a biological control agent
Wageningen University	Netherlands	*Bt* expression	Several	Several	*Bt* expression in plants; patented technology
Wageningen University, Sandoz	Netherlands	*Cry1A(b)* expression	*Lepidoptera*	Several	Superior toxic *CryIA(b)* developed and expressed
Washington Research Foundation	USA	*Bt* subsp. *thompsoini* gene cloning	Several	Several	Ppatent awarded for isolation of cp gene form *Bt* subsp. *Thompsoni*; expression in *E. coli* for potential use as an insecticide

Formulation

Institution	Country	Major *Bt* Research	Major Pest	Major Crops	Notes
Bologna-Stud University	Italy	*Bt* sprays	Gypsy moth	Trees	Control of gypsy moth is related to concentration and spray droplet density of *Bt*
Kamnek K.	Russia	*Bt* cytostatic preparation	Several	Several	Patent issued for cytostatic preparation of *Bt* var. gallerie in insect bio-control
Massachusetts University	USA	*Bt* for post harvest use	*Lepidoptera*	Several	Research on post harvest
O'Brien	USA	*Btk* in fowl houses	Darkling beetle		A patent awarded for the use of *Bt*k in fowl houses to control beetles
Research Inst. of Industr. Microbiology	Russia	*Bt* H14, VKM B-2547	Mosquitoes	*Diptera*	A new preparation for an inexpensive control of mosquito

Gene Identification

Institution	Country	Major *Bt* Research	Major Pest	Major Crops	Notes
Cambridge University	UK	*CryIC* mutagenesis	*Diptera*	*Diptera*	Mutant toxins generated by PCR. Both non-toxic and toxic mutants to Mosquitoes identified
Geneexpress	USA	*Bt* survival	Several	Several	A patent awarded for *Bt* ICP gene which is pesticidal, and is environmental pollutant degrading
Institute of Zoology	Kazakhstan	*Bt* application	Locust and mosquito	Several	A new strain of *Bt* VKPM B-5383 as an insecticide for control of locusts/mosquitoes has been developed
Kyoto University	Japan	*Bt* var. *israelensis in vivo* and *in vitro* analysis	Mosquitoes	*Diptera*	Mosquitocidal proteins were delineated in *CryIVB* gene
Kyushu University	Japan	CytB protein in *Bt* serovar *kyushuensis*	Mosquitoes	*Diptera*	Parasporal inclusions from *Bt kyushuensis* purified these are toxic to mosqito larvae
National Polytechnic Institute	Mexico	*Bt* subsp. *kenyae* (H4a-4c)	Diamond back moth	Vegetables	A new type of insecticide parasporal body is reported
National Research Council	Canada	*Bt* purification and screening	Several	Several	A patent for isolation, quantifying, purification of poor-toxins of *Bt* reported
Ohio State University	USA	*CryIA(a)* and AI identification	*Lepidoptera, Bombyx mori*	Several	Deletions by loop-out mutations produced toxic and non toxic ICP's for *Bombyx mori*
Oslo University	Norway	*B. cerus*, *CryIA*	Several	Several	Genetic diversity analyzed, *B. cerus* and *Bt* could be considered as one species
Research Corporation of Technology	USA	Nematocidal *Bt* strains	Nematodes	Many crops and vegetables	Patent awarded for New *Bt* strains CR-371 collected from Costa Rica are active aganist several nematodes
Research Institute of Genetic Selection and Industrial Microorganisms	Russia	*Bt* strain identification with toxicity to insects	*Lepidoptera* and *Diptera*	*Lepidoptera* and *Diptera*	*Bt* strains specific to insects have been identified
Research Institute of Industrial Microbiology	Russia	*Bt* strain identification	Colorado potato beetle	Potato	A patent given for a new strain of *Bt* producing sigma endotoxin Crystal
Stockholm University	Sweden	*Bt* collection and distribution	Several	Several	50 colonies were isolated from Swedish soils. Forest soils more rich in *Bt*. Wide diversity representing different biochemical groups
Talca University	Chile	*Bt* strain characterization	South American tomato moth and Corn earworm	Tomato, Potato, Maize	*Bt* strains specific to certain insects have been identified
UNAM	Mexico	*Bt* ICP mutant	Several	Several	*Bt* ICP mutant with depressed expression of the terminal oxidase resulted in improved insecticide production
Universidad Nacional Autonoma de M	Mexico	*CryI, CryIII*	Several	Several	Specific PCR primers directed to identify *CryIII* genes have been developed
Universidad Nacional Autonoma de M	Mexico	*Bt* ICP analysis by PCR	*Lepidoptera*	Several	PCR developed for rapid and accurate identification of *CryI* producing family of *Bt*
University of California	USA	*Bt* subsp. *jegathesan*	Mosquitoes	*Diptera*	A new mosquitocidal *Bt* strain has been isolated
University of Laval	France	*CryIA*	Several	Several	A patent for PCR detection of *Btk*
USDA-ARS Northern Crop Science Laboratory	USA	*Bt. i.*	Sunflower seed weevils	Sunflower	Identification of *Bt* strain active against adult red sunflower seed weevils and cloning of *Bti* crystal protein gene
Washington University	USA	*CryIB*	*Lepidoptera* and *Coleoptera*	*Lepidoptera* and *Coleoptera*	*CryIB* ICP's with dual specificity to *Coleoptera* and *Lepidoptera* are reported

Transgenics

Institution	Country	Major *Bt* Research	Major Pest	Major Crops	Notes
Beijing University	China	*Bt* transgenic plants	*Lepidoptera*	*Solanaceae*	Transgenic tobacco with chimeric *Bt* ICP for control of tobacco hornworm developed
CEA	France	Transgenic plants, risk assessment	Several	Several	Developing insect resistant transgenic plants with the *Bt* cp gene
Central China Normal University	China	*CryIA*	Cabbage caterpillar	*Rutabaga*	Transgenic rutabaga with resistance to cabbage caterpillar have been developed
Cornell University	USA	*CryIA*	Diamond back moth	Broccoli, cabbage	Transgenic Broccoli and Cabbage with *Cry* gene developed
Cornell University	USA	*Bt* toxin expression	Diamond back moth	Cabbage, broccoli	Transgenic cabbage and broccoli developed with *Bt* cp genes had 100% mortality of Diamond back moth larvae
Cornell University	USA	*CryIA(c)* expression	Diamond back moth	Cabbage, Broccoli	Transgenic plants with *CryIA(c)* under the control of a light regulated promoter developed for cabbage and broccoli. Plants grown in light gave 100% mortality of first instar larvae of Diamond back moth.

Institution	Country	Major *Bt* Research	Major Pest	Major Crops	Notes
Cornell University	USA	Transformation	Diamond back moth	Broccoli, Cabbage	Transformation method for cabbage, broccoli using *Agrobacterium tumefaciens* has been developed
CPRO-DLO	Netherlands	*CryIA(b), CryIc* production in transgenic plants	Spodoptera exigua, Heliothis virescens, Manduca sexta	Vegetables, cotton	Translational fusion helps broaden the insect resistance of transgenic plants
CSIRO	Australia	*Bt* expression, Cotton	Lepidoptera	Cotton	Deployment strategies for Transgenic cotton
Federal Institute of Technology, Institute of Plant Sciences	Switzerland	*CryIA(c)* promoters	Lepidoptera	Rice	A protoplast transformation system in rice developed. *CryIA(b)* gene driven by the CaMV 35S promoter
Federal Institute of Technology, Institute of Plant Sciences	Switzerland	*Cry1A(b)* transgenic rice	Lepidoptera	Rice	Rice has been transformed with *CryIA(b)* gene for resistance to yellow stem borer
Georgia University	USA	*CryIA(c)*	Tobacco budworm, Corn earworm	Soybean	Transformed Soybean with *CryIA(c)* using microprojectile bombardment
Georgia University	USA	Transformation of peanut with *Cry1A(c)*	*Lepidoptera*	Peanut	Transgenic insect resistant peanut with *Cry1A(c)* gene developed using microprojectile bombardment
Georgia University	USA	*Bt* transformation *CryIA(b)*	*Lepidoptera*	Soybean	Soybean with *CryIA(b)* gene have been developed via particle bombardment
Horticultural Research	New Zealand	Transgenic clover	Porina moth	White clover	White clover transformed and tested for insect resistance
INRA	France	Transformation of Poplar with *Bt* ICP gene	*Chrysomela populi* and *Chrysomela tremulae*	Poplar	Gene transfer and plant propagation system developed for 2 polar clones
Michigan State University	USA	*Bt (Cry IA(c)* transformation, potatoes	Lepidoptera	Potatoes	Transgenic potato plants with *CryIA(c)* gene for the control of Potato tuber moth and European corn borer developed. 10% mortality for Potato tuber moth and European corn borer larvae were significantly less capable of surviving in transgenics
Michigan State University	USA	*Bt* transformation of plants	Lepidoptera	Juneberry	A simple transformation system using adventious shoot multiplication of june berry developed for insect resistance
Michigan State University	USA	*Bt* transformation using *CryIa(c)*	*Lepidoptera*	Rice	Basmati rice has been transformed for insect resistance with *CryIa(c)* gene
National Laboratory of Molecular Plant Genetics	China	*Bt* transformation in cabbage using *CryIA(c)* gene	Lepidoptera (Diamond back moth)	Cabbage	Transgenic cabbage with *CryIA(c)* developed, plants resistant to neonate larvae of Diamond back moth
New Jersey State University	USA	*CryIIIB*	Colorado potato beetle	Eggplant	Transgenic eggplant developed but did not demonstrate any significant resistance to the early instar larvae of Colorado potato beetle
Ohio State University	USA	Transformation of sweet gum using *Bt* cp genes	Several	Sweet gum	Sweet gum was transformed with the *Bt* toxin gene and confirmed via genome DNA blots and GUS activity
Oregon State University	USA	Poplar transformation with *Bt* ICP genes	*Lepidoptera*	Poplar	*Agrobacterium* mediated transfer used in hybrid poplar to introduce a ICP *Bt* gene for insect resistance
Oregon State University	USA	*Bt. tenenbrionis* endotoxin - transgenic plants	Colorado potato beetle	Potato	Fate of *Bt* toxin in transgenic plants and its effect on soil microflora determined
Osmania University	India	Transformation	*Heliothis* sp.	Cotton	Transformation of cotton cultivars in India
Russian Academy of Science	Russia	*Bt*	late blight, black leg, insect pests	Potato	Transgenic resistant potatoes developed
Russian Academy of Science	Russia	Transgenic potato plants with *Bt*	Colorado potato beetle	Potatoes	Potato cultivars Desiree, Resy, Tep, Granat have been transformed with the *Bt* ICP gene. These plants showed incomplete protection from the larvae
Seres RA	USA	*Bt* ICP gene transformation of cranberry	Lepidoptera	Cranberry	A patent awarded for the development of micoroprojectile bombardment technology for developing cranberry with the *Bt* ICP gene into plants for insect resistance
Tokyo University	Japan	Transfer of *Bt* ICP gene into *Azospirillum*	Several	Several	ICP gene from *Cry I(a)* has been transferred via conjugation into *Azospirillum* for use in insect biological control
University of California	USA	*CryIa(c)* expression	Cydia pomonella	Walnut	Walnut transformed with *CryIA* protein genes. 12 clones showed high resistance
University of California	USA	*CryIA(c)* for transgenic walnut	Codling moth, Navel organgeworm, Indian meal moth	Walnut	Transformation with pWB139 using the gene *Cry1A(c)* was ineffective in protecting walnut embryos from damage by *Lepidopteran* larvae
University of California	USA	*CryIA(c)* transgenic plant construction	Codling moth	Walnut	Transformed embryos of Walnut showed a mortality of 0-39% control of codling moth larvae
University of California	USA	*Bt* transgenic plants	Laspeyresia pomonella	Apple, walnut, hawthorn, pear	Control of plant pests using transgenic plants. Patented

Institution	Country	Major *Bt* Research	Major Pest	Major Crops	Notes
University of California	USA	Transgenic apples and strawberries *CryIA(c)*	Lepidoptera	Apples/Strawberries	Transgenic apples, strawberries with *CryIA(c)* have been developed and field tested.
University of California	USA	*Bt* transformation of woody crops	Several	Several woody crops	3 types of transformation have been used to produce transgenic trees with *Bt* cp genes
University of Ottawa	Canada	*CryIA(b, CryIA(c))*	*Lepidoptera*	Maize	Modified *Cry1A(b)* and *Cry1A(c)* expressed in maize endosperm
University of Wisconsin	USA	Transgenic plants, reporter genes	*Lepidoptera*	Cranberry	Transgenic cranberry with a gene encoding beta-glucuronidase developed
Wageningen University	Netherlands	*Cry1A(b)* and *Cry1C* Transgenics	*S. exigua*, *Heliothis virescens*, *Maduca sexta*	Tobacco, tomato	Resistance management strategies developed for transgenic tobacco, tomato

Various

Institution	Country	Major *Bt* Research	Major Pest	Major Crops	Notes
Bar Illan University	Israel	*Btt* ICPs their fate in soil	Several	Potato, tobacco, tomato	Fate of *Bt* in soil was studied over a 6 mth period. ELISA was used for detection
Hokkaido University	Japan	*CryV* Toxicology	*S. litura, P. xylostella*	Vegetables, cotton	Insecticidal activity of *CryV* gene demonstrated
Horticulture Research International	UK	Mapping and DNA sequence of *Bt* plasmid	Several	Several	Plasmid from *Bt* var. *israelensis* isolated the identified locus useful in *Bt* vector construction
Institut Pasteur	France	CryIVD toxicity	Mosquitoes	*Diptera*	A gene encoding *CryIVD* cp characterized and cloned in a vector and the ICPs produced were toxic to *Diptera* (mosquito larvae)
Man Tech-Environmental Technology	USA	*Bt* persistence in soil			Detection methods for Cp protein in soils developed. The half life of cp protein in soild was 2-10 days
Man Tech-Environmental Technology	USA	*Btk* effect on soil microorganisms	*Lepidoptera*	Cotton	Microbial populations in the presence of *Bt*k were studied in soil by placing different lines of cotton genetically engineered to produce *Btk*(CP)
Mie University	Japan	*Bt* transconjugation with *Ruminococcus albus*			New host vector systems involving filter mating of *Bt* enhance the rate and extent of forage degradation in ruminants
National University, Seoul	Korea	*Bt* NTO423 *CryIVD*	*Diptera* and *Spodoptera exigua*	*Diptera* and *Lepidoptera*	Improved toxicity of *CryIVD* as biological control agent to *Diptera*
New Jersey State University	USA	Plastid transformation-*CryIA(c)*	*Lepidodptera*	*Lepidoptera* infested crops	Efficient containment from plastid encoded *Cry(c)* gene
New York University	USA	*Bt* monitoring	*Lepidoptera, Coleoptera*	*Lepidoptera* and *Coleoptera* s	Dot blot enzyme linked immunosorbent assay for monitoring the fate of *Bt* ICPs have been developed
New York University	USA	Insecticidal *Btt, Btk*	*Manduca sexta, Leptinotarsa decemlineata*	Potatoes, tobacco	Persistence of *Btt* and *Bt*k indicate that toxins bind to clays. Thus non-target larvae could be susceptible to toxins, and toxin accumulation could result in selection toxin-resistant target species
Research Institute of Genetic Selection of Industrial Microorganisms	Russia	*Bt*- vector construction and use	Several	Several	A patent assigned for plasmid rAU135a vector- *Btk* Crystal protein gene cloning and expression by plasmid for use as insect biological control agent
State Institute of Quality Control and Agricultural Production, Wageningen	Netherlands	*CryIA(b)* food safety			Toxicology testing with *CryIA(b)* done using tomatoes as a model crop
Universidad Nacional Autonoma de M	Mexico	*Bt*-ICP purification	Several	Several	A two phase for partioning of *Btk* HD1 strain developed. The procedure is inexpensive, appropriate for small as well as large scale purification
University of California	USA	*CytA* crystal from *Bt*	Several	Several	A 20 Kilodation protein preserves cell viability and promotes *CytA* crystal formation in *Bt*

Appendix III: Nomenclatures of *Bt* Endotoxins (Genes)

Ordered by "Molecular" Classification

"Molecular"Classification	Höfte and Whiteley[1]
cry1Aa1	cryIA(a)
cry1Aa2	cryIA(a)
cry1Aa3	cryIA(a)
cry1Aa4	cryIA(a)
cry1Aa5	cryIA(a)
cry1Aa6	cryIA(a)
cry1Ab1	cryIA(b)
cry1Ab2	cryIA(b)
cry1Ab3	cryIA(b)
cry1Ab4	cryIA(b)
cry1Ab5	cryIA(b)
cry1Ab6	cryIA(b)
cry1Ab7	cryIA(b)
cry1Ab8	cryIA(b)
cry1Ab9	cryIA(b)
cry1Ac1	cryIA(c)
cry1Ac2	cryIA(c)
cry1Ac3	cryIA(c)
cry1Ac4	cryIA(c)
cry1Ac5	cryIA(c)
cry1Ac6	cryIA(c)
cry1Ad1	cryIA(d)
cry1Ae1	cryIA(e)
cry1Ba1	cryIB
cry1Ba2	
cry1Bb1	ET5
cry1Bc2	cryIb(c)
cry1Ca1	cryIC
cry1Ca2	cryIC
cry1Ca3	cryIC
cry1Ca4	cryIC
cry1Ca5	cryIC
cry1Ca6	cryIC
cry1Ca7	cryIC
cry1Cb1	cryIC(b)
cry1Da1	cryID
cry1Db1	prtB
cry1Ea1	cry1E
cry1Ea2	cry1E
cry1Ea3	cry1E
cry1Eb1	cryIE(b)
cry1Fa1	cryIF
cry1Fa2	cryIF
cry1Ga1	prtD
cry1Ha1	prtA
cry1Hb1	
cry1Ia1	cryV
cry1Ia2	cryV
cry1Ia3	cryV
cry1Ia4	cryV
cry1Ib1	cryV
cry1Ja1	ET4
cry1Jb1	ET1
cry1Ka1	
cry2Aa1	cryIIA
cry2Aa2	cryIIA
cry2Aa3	cryIIA
cry2Ab1	cryIIB
cry2Ab2	cryIIB
cry2Ac1	cryIIC
cry3Aa1	cryIIIA
cry3Aa2	cryIIIA
cry3Aa3	cryIIIA
cry3Aa4	cryIIIA
cry3Aa5	cryIIIA
cry3Aa6	cryIIIA
cry3Ba1	cryIIIB
cry3Ba2	cryIIIB
cry3Bb1	cryIIIBb
cry3Bb2	cryIIIC(b)
cry4Aa1	cryIVA
cry4Aa2	cryIVA
cry4Ba1	cryIVB
cry4Ba2	cryIVB
cry4Ba3	cryIVB
cry4Ba4	cryIVB
cry5Aa1	cryVA(a)
cry5Ab1	cryVA(b)
cry5Ba1	PS86Q3
cry6Aa1	cryVIA
cry6Ba1	cryVIB
cry7Aa1	cryIIIC
cry7Ab1	cryIIICb
cry7Ab2	cryIIICc
cry8Aa1	cryIIIE
cry8Ba1	cryIIIG
cry8Ca1	cryIIIF
cry9Aa1	cryIG
cry9Aa2	cryIG
cry9Ba1	cryIX
cry9Ca1	cryIH
cry9Da1	N141
cry10Aa1	cryIVC
cry11Aa1	cryIVD
cry11Aa2	cryIVD
cry11Ba1	Jeg80
cry12Aa1	cryVB
cry13Aa1	cryVC
cry14Aa1	cryVD
cry15Aa1	34kDa
cry16Aa1	cbm71
cry17Aa1	cbm72
cyt1Aa1	cytA
cyt1Aa2	cytA
cyt1Aa3	cytA
cyt1Aa4	cytA
cyt1Ab1	cytM
cyt1Ba	
cyt2Aa1	cytB
	"cytB"
	cryC35
	vip3A(a)
	vip3A(b)

Source: OSU, 1997.

1 Höfte and Whiteley did not assign names to all proteins listed here since many were not known at the time.

Ordered by Höfte and Whiteley Classification

Höfte and Whiteley	**"Molecular" Classification**
"cytB"	
34kDa	cry15Aa1
cbm71	cry16Aa1
cbm72	cry17Aa1
cry1E	cry1Ea1
cry1E	cry1Ea2
cry1E	cry1Ea3
cryC35	
cryIA(a)	cry1Aa1
cryIA(a)	cry1Aa2
cryIA(a)	cry1Aa3
cryIA(a)	cry1Aa4
cryIA(a)	cry1Aa5
cryIA(a)	cry1Aa6
cryIA(b)	cry1Ab1
cryIA(b)	cry1Ab2
cryIA(b)	cry1Ab3
cryIA(b)	cry1Ab4
cryIA(b)	cry1Ab5
cryIA(b)	cry1Ab6
cryIA(b)	cry1Ab7
cryIA(b)	cry1Ab8
cryIA(b)	cry1Ab9
cryIA(c)	cry1Ac1
cryIA(c)	cry1Ac2
cryIA(c)	cry1Ac3
cryIA(c)	cry1Ac4
cryIA(c)	cry1Ac5
cryIA(c)	cry1Ac6
cryIA(d)	cry1Ad1
cryIA(e)	cry1Ae1
cryIB	cry1Ba1
cryIb(c)	cry1Bc2
cryIC	cry1Ca1
cryIC	cry1Ca2
cryIC	cry1Ca3
cryIC	cry1Ca4
cryIC	cry1Ca5
cryIC	cry1Ca6
cryIC	cry1Ca7
cryIC(b)	cry1Cb1
cryID	cry1Da1
cryIE(b)	cry1Eb1
cryIF	cry1Fa1
cryIF	cry1Fa2
cryIG	cry9Aa1
cryIG	cry9Aa2
cryIH	cry9Ca1
cryIIA	cry2Aa1
cryIIA	cry2Aa2
cryIIA	cry2Aa3
cryIIB	cry2Ab1
cryIIB	cry2Ab2
cryIIC	cry2Ac1
cryIIIA	cry3Aa1
cryIIIA	cry3Aa2
cryIIIA	cry3Aa3

Höfte and Whiteley	"Molecular" Classification
CryIIIA	cry3Aa4
CryIIIA	cry3Aa5
CryIIIA	cry3Aa6
cryIIIB	cry3Ba1
cryIIIB	cry3Ba2
cryIIIBb	cry3Bb1
cryIIIC	cry7Aa1
cryIIIC(b)	cry3Bb2
cryIIICb	cry7Ab1
cryIIICc	cry7Ab2
cryIIIE	cry8Aa1
cryIIIF	cry8Ca1
cryIIIG	cry8Ba1
cryIVA	cry4Aa1
cryIVA	cry4Aa2
cryIVB	cry4Ba1
cryIVB	cry4Ba2
cryIVB	cry4Ba3
cryIVB	cry4Ba4
cryIVC	cry10Aa1
cryIVD	cry11Aa1
cryIVD	cry11Aa2
cryIX	cry9Ba1
cryV	cry1Ia1
cryV	cry1Ia2
cryV	cry1Ia3
cryV	cry1Ia4
cryV	cry1Ib1
cryVA(a)	cry5Aa1
cryVA(b)	cry5Ab1
cryVB	cry12Aa1
cryVC	cry13Aa1
cryVD	cry14Aa1
cryVIA	cry6Aa1
cryVIB	cry6Ba1
cytA	cyt1Aa1
cytA	cyt1Aa2
cytA	cyt1Aa3
cytA	cyt1Aa4
cytB	cyt2Aa1
cytM	cyt1Ab1
ET1	cry1Jb1
ET4	cry1Ja1
ET5	cry1Bb1
Jeg80	cry11Ba1
N141	cry9Da1
prtA	cry1Ha1
prtB	cry1Db1
prtD	cry1Ga1
PS86Q3	cry5Ba1
vip3A(a)	
vip3A(b)	
	cry1Ba2
	cry1Hb1
	cry1Ka1
	cyt1Ba