GM Crops and Food: Biotechnology in Agriculture and the Food Chain 3:3, 1-9; July/August/September 2012; © 2012 Landes Bioscience

Genetically engineered vegetables expressing proteins from *Bacillus thuringiensis* for insect resistance

Successes, disappointments, challenges and ways to move forward

Anthony M. Shelton

Department of Entomology; Cornell University/New York State Agricultural Experiment Station (NYSAES); Geneva, New York USA

Key words: GM crops, GE crops, Bt, *Bacillus thuringiensis*, insect resistant crops

Genetically engineered (GE) insect-resistant crops that express proteins from *Bacillus thuringiensis* (Bt) have been widely adopted in the two field crops currently commercially available, Bt cotton and Bt corn. However, the development and commercialization of Bt vegetables has lagged in comparison, which is unfortunate since vegetables tend to be insecticide-intensive crops due to high pest pressure and cosmetic standards required for the market. While it is often stated that consumer choice has played a major role in companies avoiding development of Bt vegetables, this concept requires re-evaluation. In market studies in North America when consumers have been provided basic information about Bt genetic engineering, then given a choice between Bt and conventional sweet corn, they have often preferred the former. Likewise, 77% of consumers in a US survey said they would likely purchase foods produced through biotechnology for their ability to reduce pesticide use. Presently, however, the only commercialized Bt vegetable is sweet corn. Perhaps more critical obstacles to Bt vegetables are their relatively smaller acreages and the cost of government biosafety regulations that inadvertently favor large acreage of field crops because companies can obtain a better return on investment. In developing countries, private-public partnerships may provide the vehicle to bring Bt vegetables to market. However, these can be subverted by misinformation from anti-biotech campaigns, as is the case with Bt eggplant in India. Without the use of Bt vegetables as a tool for integrated pest management, farmers and the general public will not be able to realize the substantial environmental and economic benefits that have been well documented with Bt cotton and Bt corn.

Introduction

Genetically engineered (GE) crops have revolutionized agriculture, especially in pest management of weeds, insects and diseases. The commercial production of GE plants expressing proteins from *Bacillus thuringiensis* (Bt plants) is rightly considered another form of host plant resistance,[1] which is a cornerstone in the foundation of integrated pest management.[2] As such, Bt plants have the same benefits (e.g., reduced insecticide use) and the same risks (e.g., evolution of resistant insect strains). Bt plants have captured an increasing market share since their first introduction in 1995. In 2010, 58.6 million hectares were grown to Bt corn and Bt cotton,[3] currently the only commercially available Bt plants. Only a small proportion of the Bt corn crop is for sweet corn, which is the only commercially available Bt vegetable. The benefits of Bt field crops (cotton and corn) have been well documented in terms of reduced insecticide use, reduction in environmental impact and increased profit to growers.[4] While this revolution in insect management in field crops should be applauded, it is unfortunate that these benefits have largely not been realized for vegetables.

Vegetables are essential for well-balanced diets, supplying many essential nutrients not found in staple crops such as rice, wheat and corn. Additionally there is evidence that diets rich in vegetables can lower the risk of heart disease, strokes and several forms of cancer, as well as improve gastrointestinal health and vision.[5] Long-term studies in China have shown that plant-based diets provide increased longevity[6] and that vegetables fight the "Hidden Hunger" of malnutrition. Besides providing benefits to consumers, farmers involved in vegetable production usually earn much higher farm incomes compared with cereal producers, with per capita farm income up to 5-fold higher.[7] Worldwide, the area of arable land devoted to vegetables is expanding annually at 2.8%, a rate that is higher than for other crops.[8]

Vegetables are high value commodities with high cosmetic standards; the main method of insect control has been the frequent use of traditional insecticides.[8] Although statistics for insecticide use worldwide are combined for vegetables and fruits (45% of total insecticide value), if vegetables were conservatively estimated to equal half of this total (22.5%), the insecticide use for vegetables would exceed that for corn (7.6%) plus cotton (14.1%) (**Fig. 1**). The heavy dependence on insecticides in vegetables has

Correspondence to: Anthony M. Shelton; Email: ams5@cornell.edu
Submitted: 12/07/11; Revised: 02/03/12; Accepted: 02/20/12
http://dx.doi.org/

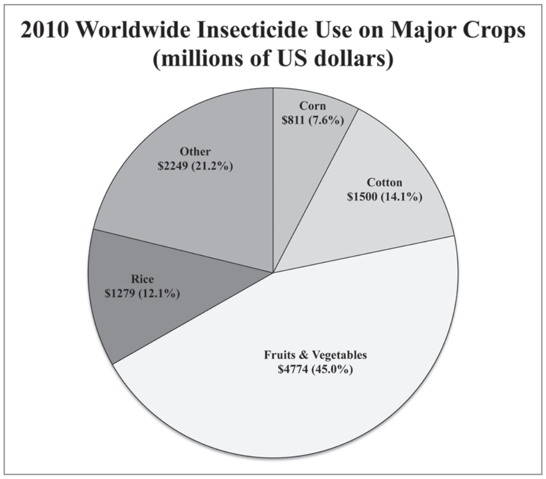

Figure 1. Worldwide insecticide use on major crops, 2010, Cropnosis Limited. Courtesy of R. Hautea and the International Service for the Acquisition of Agri-biotech Applications (ISAAA).

resulted in instances of high insecticide residues on the marketed product, the evolution of resistance in many insect species and negative impacts on human health and the environment.[8]

What is the present status of Bt vegetables globally, and why has their development languished while Bt field crops continue to capture an increasing share of the market? The answers are complex and involve many factors, some unique to GE crops in general, while others are more specific to individual vegetable groups or to individual countries. The purpose of this review is to highlight important issues that have constrained the development and commercialization of Bt vegetables, and to propose actions that could help facilitate their commercialization.

Public Acceptance of Bt Vegetables and Labeling Issues

Certainly, questions about the public perception of GE crops have played a role in biotech companies' strategic plans for producing and marketing Bt vegetables. Likewise, food processors and retailers are important players in determining whether they will use GE crops. These players have been reluctant to be the first in line to produce, process or sell GE vegetables, despite their frequent verbal support of the science behind the products. If retailers perceive that their customers do not want GE vegetables, then companies that could develop them will not do so. Certainly, multinational companies have shown a nervousness about consumer acceptance of GE foods, as opposed to feed, fuel and fiber. This is especially true for GE foods that may be exported to the European Union.[3]

What is meant by public acceptance of a product, how is it measured, and how do companies judge whether to move a specific product into the food chain? Cultural differences are important, but education and advertising also play an important role. The public in developed countries like the United States, where <2% of the general public farms on a commercial scale, is largely unaware of how their food plants were bred and what pest management practices were used to protect them. When I talk with the general public, I find that they are stunned that many of the plant products they regularly consume were created through the common plant breeding practice of mutation breeding, using radiation or mutagenic chemicals. Furthermore, they do not find such descriptions palatable, much less enticing. Being largely naïve about food production, the public can be strongly influenced by marketing messages, whether true or false.

What is the public's perception of GE foods and how might it relate to Bt vegetables? The International Food Information Council (IFIC) (http://www.foodinsight.org) has conducted studies in the US for the past 14 y surveying consumers about their feelings toward GE food products. The 2010 "Consumer Perceptions of Food Technology" Survey was designed to gain insights into consumer perspectives on food technology. A key finding in 2010 was an awareness of sustainable food production and how it might influence consumers' likelihood to purchase foods produced through biotechnology. US consumers were largely familiar with the term "biotechnology"; more than two-thirds of consumers (69%) had read or heard at least "a little" about the concept. However, only about one-third (32%) were somewhat or very favorable toward plant biotechnology, with about three in ten (29%) neither favorable nor unfavorable, and about two in ten (19%) somewhat or very unfavorable toward biotechnology. Interestingly, however, half (51%) of consumers say they were favorable toward farmers using biotechnology to grow more crops that would help meet food demand. Most importantly for Bt vegetables, more than three-quarters (77%) of consumers said they would be likely to purchase foods produced through biotechnology for their ability to reduce pesticide use. However, as of now in the US, the choice to purchase Bt vegetables is limited to sweet corn.

Labeling is another issue often discussed with regard to biotechnology in the food system. The IFIC survey found that consumers are generally satisfied with information currently provided on food labels. Eighty-two percent say they cannot think of anything additional they would like to see on the label. Of the 18% who would like to see additional information on the label, only 3% mentioned anything about biotechnology. However, I suspect that if a well-funded anti-biotech campaign spread misinformation about GE foods, this might change.

In countries like the United States, where food labeling is restricted to nutritional issues, and not how the product was produced, the public is largely unaware of whether a product is GE, contains GE food ingredients, or was created through mutation breeding. Such a regulation has allowed GE products to enter the food chain much more readily than in countries where regulations mandate identification of GE-developed products, such as in the European Union. While the rationale behind labeling is often described as providing consumers with a choice on how their foods were produced and implies that they may gain information about its safety, in reality it does neither. It singles out GE products from other methods of breeding and does not provide any information on how pests were controlled in the absence of the GE technology. Additionally, many consumer labels are so vague as to be unhelpful. For example, if a label states that the product "may contain" an ingredient derived through GE, this provides little concrete information to the consumer. Furthermore, with the nearly ubiquitous presence of GE corn and GE soybean in processed foods in the United States, would the consumer really have a choice with many of the common foods found in grocery stores?

What happens when consumers are given a clear choice between purchasing Bt vegetables and conventional vegetables? Such studies are rare since the only presently available Bt vegetable in the US and Canada is sweet corn. In a study conducted in a farm market in Canada in which bins of sweet corn were labeled as Bt or conventionally produced, with detailed descriptions of what this meant and how insects were controlled, Bt sweet corn outsold the conventional corn 60:40.[9] In this same trial, the majority of consumers interviewed said they were more concerned about pesticides than genetic engineering. In another study in nine participating grocery stores in the Philadelphia area, consumers were given a choice of Bt sweet corn and the same variety without the Bt trait.[10] The two products were labeled and sold side-by-side; sales data indicated a market share of Bt sweet corn of approximately 45%, with store-specific shares varying from 10 to 80%. Over 700 surveys were collected in stores and only 65% of respondents noticed that there were two types of sweet corn for sale despite the labeling and merchandising; 87% spent one minute or less choosing their sweet corn. Only 16% volunteered that the biotechnology trait influenced their purchase decision. About 40% of the sample purchased some Bt sweet corn, with several respondents purchasing some of each. These results also suggest that the costs of a mandatory labeling policy would not be justified by the relatively minor influence the use of biotechnology had on purchasing decisions.

These two studies suggest that other Bt vegetables may have a good chance of being adopted if they are produced and have other qualities that seem more important to the general public, at least in North America, such as color, freshness, taste and other sensory attributes. Whether the public will actually have the opportunity to make such choices depends on other factors that will influence the future availability of Bt vegetables.

Regulatory Issues

Regulatory issues, besides the labeling issues mentioned above, influence the commercialization of Bt vegetables. Following the present worldwide regulatory framework, as described by Matten et al. registration of Bt vegetables requires an assessment of the effects of the insecticidal trait on non-target organisms as well as other potential adverse environmental impacts, such as the evolution of resistance to target pests.

The development of Bt crops will be helped or hindered depending on the philosophy and regulatory process of an individual country.[11] The outcome of each country's regulatory system is dependent on its scientific capacity, financial resources and risk management goals. Thus, there is tremendous variation in each country's ability to commercialize Bt vegetables, or any other GE crop. Absence of well functioning regulatory systems, especially in developing countries, misunderstanding of international agreements and treaties, lack of public sector research to generate data assessing the safety of GE crops, risks to export markets that ban GE crops, and high regulatory costs will all hinder the commercialization of GE crops.[11] This is especially true for small market ("orphan") crops such as Bt vegetables. What is particularly tragic about this situation is that it is in developing countries where these products would provide the most benefit, as is the case with Bt eggplant in India, Bangladesh and the Philippines (see discussion below).

The present worldwide regulatory framework is based on registration of individual transformed lines (i.e., events) that express the trait of interest, such as insect resistance with an insecticidal crystal (Cry) protein from Bt. This situation disadvantages vegetables even more than field crops like cotton and corn. Events serve as the breeding material for producing hybrids or open pollinated lines. For example, MON810 is the Monsanto corn event registered in 1996 that produces Cry1Ab, and has been used as the basis for breeding many cultivars of Bt corn.[12] Since each event must produce its own regulatory dossier of environmental and human health studies, that event must be widely used to recoup registration costs. Selling large quantities of corn seeds, all with MON810 as one of the parent lines, can adequately compensate the producer for the expense of registering MON810. Single Bt events of either corn or cotton can be used for many years to produce the hybrids needed for the market. The advanced breeding techniques in corn also contribute to the ease of moving one event through multiple lines. This results from the massive investment in breeding that is made in commodity crops but less so in specialty crops. Market size is the ultimate driver. Therefore, the return on investment can be positive for these widely grown field crops.

Vegetables are inherently different. Within a particular species such as tomato, *Solanum lycopersicum*, there is a tremendous variation in plant type. Slicing tomatoes are used primarily for fresh eating, plum tomatoes for sauce and paste, and cherry and grape tomatoes for salads. Each tomato type (as well as cultivar) is the product of specific parental lines that contribute specific characteristics such as fruit shape and color, as well as plant growth habit (e.g., determinate, indeterminate, etc.). Thus, registering individual Bt events for different tomato types is more complex and expensive and less likely to have an adequate return on investment than a large acre field crop. This situation is repeated in most other vegetables and disadvantages the commercialization of Bt vegetables compared with Bt field corn and Bt cotton. Tomato may the exception since it is often grown in large blocks of land, has a substantial farm gate value, and is subject to high losses by Lepidoptera. If the economics for Bt tomatoes prove to be favorable, then it is likely that they will be commercialized despite the existing event-based regulation.

However, strong arguments can be made that event-based registration is an inefficient, costly and scientifically unjustified policy that has the consequence of limiting the availability of Bt crops, especially Bt vegetables. The transformation of a plant line to express a Bt protein is now routine, and even the diverse parental lines needed in tomato breeding programs could be met if the regulatory process were modified to have less emphasis on the specific event and more on the safety of the Bt protein. If a Cry1A protein is expressed in different events, what scientific rationale warrants that registration of each event require testing of Cry1A against a suite of non-target organisms or be assessed for toxicity and allergenicity to humans? From a scientific standpoint, it would make more sense if the emphasis of regulation were on the Cry1A protein rather than the event that expressed the protein. For example, once a particular Cry protein is approved based on studies of toxicity, allergenicity, ecotoxicoloy and effects on non-target organisms then, no matter what crop the Cry protein is expressed in, additional studies for these aspects should not be required. Some other studies on the particular event, however, would be required and these would include expression levels of the Cry protein in the plant (needed for insecticide resistance management programs) and other agronomic studies (e.g., yield and field performance). However, the major biosafety emphasis should be on the Cry protein itself. In the US, the Animal Plant Health Inspection Service (APHIS) is considering an alternative of a "trait-based approach" in which GE plants would be regulated based on the engineered genes in the plant (the genotype) and the traits resulting from those genes (the phenotype). This approach would allow plants that have the same transgene and phenotype as a previously deregulated GE plant to be considered "familiar" and thereby not trigger regulation.[13] Such a regulation would also allow APHIS to concentrate its resources on those organisms that exhibit novel phenotypes.

Based on the familiarity and safety of the currently used Cry proteins, regulatory agencies not only in the US but worldwide should move toward a registration that emphasizes the Cry protein regardless of what crop it is expressed in. This would greatly facilitate the development of much needed insect-resistant crops, such as Bt vegetables.

Bt Potatoes

Depending on the region of the world, potatoes are considered either a field crop or a vegetable. They will be discussed here as a vegetable because of their unique historical role in the United States where they are considered a vegetable. Grafius and Douches[14] provide an interesting history of Bt potatoes and the following is a summary of their publication. Potato cultivars expressing the *Bacillus thuringiensis* var *tenebrionis* Cry 3A toxin for resistance to the Colorado potato beetle, *Leptinotarsa decemlineata* Say (cv NewLeaf™, Monsanto Corp.,) were the first GE food crop approved for human consumption, and were the first GM crop to be commercially produced in the USA (1995). In 1997 the release of the Bt cry3A potatoes combined with either resistance to potato virus Y, or to potato leaf roll virus, were referred to as NewLeaf™ Y and NewLeaf™ Plus, respectively.[15] When NewLeaf™ cultivars were introduced in 1995, 1,500 acres were grown commercially; as seed stocks increased, the commercial acreage reached 50,000 acres. Market success of NewLeaf™, NewLeaf™ Y and NewLeaf™ Plus potatoes could be attributed to the difficulty in controlling *L. decemlineata*, and also high pest populations of aphids and associated virus problems due to mild winters in the Pacific Northwest.[16] With NewLeaf™ potatoes, growers were able to reduce insecticide costs. NewLeaf™ Plus was grown mainly in the Pacific Northwest. The added virus resistance benefited seed producers and commercial growers benefited from reduced need for insecticides. However, these benefits were short lived.

Growers had concerns about the mandated refuge of a non-Bt potato for resistance management, something that was not required for traditional insecticides, and also the requirement that they could not save potato seed for future plantings. There were also concerns about the pricing system of Monsanto, which had a sliding scale depending on where the potatoes would be planted and the corresponding pest pressure. Another factor was the registration of a new insecticide, imidicloprid, which when applied in-furrow, gave excellent control of *L. decemlineata* and provided control of other pests such as aphids and leafhoppers at no additional cost.[16] Imidacloprid offered growers a conventional alternative for *L. decemlineata* control without being limited by varietal selection. But the final blow to Bt potatoes was the anti-biotech campaign aimed at consumers. In response, the processing industry became concerned that their market share could be negatively impacted in Europe and Japan. Finally, McDonald's Corporation's decision to ban GM crops from its restaurant chain had a major impact on the market. With such forces against growth of this technology and the fact that at its peak only 3% of the US potato acreage was Bt potatoes,[17] Bt potatoes did not have a viable future. Nature Mark, which marketed the product for Monsanto, dissolved after the 2001 season.

It appears that management of the recalcitrant *L. decemlineata* will continue to rely on insecticides for the foreseeable future, an ironic outcome for those interested in reducing the use of traditional pesticides. Meanwhile, research efforts are continuing on use of another gene, *Bt cry1Ia1*, for controlling the potato tuberworm, *Phthorimaea operculella* [Zeller], a particularly devastating insect in many parts of the world. This project is headed by Michigan State University, with funding by the US Agency for International Development, and the developers hope to bring Bt potatoes to farmers in South Africa and Egypt.[14] The failure to commercialize the product as of February 2012 has been quite complex with political and financial issues rather than efficacy or other science-based issues with the technology. Whether it will eventually suffer the same fate as NewLeaf™ potatoes did in North America remains to be seen.

Sweet Corn

Sweet corn has been the most successful Bt vegetable to date, but its history has also not been a smooth ride. First introduced into the North American market in 1998 by Novartis Seeds, it was based on event Bt 11, which expresses Cry1Ab and had already been registered for field corn in 1996.[12] (Such piggy-backing on an event registered for field corn substantially reduces registration costs for sweet corn.) By 1999, Bt sweet corn was grown on over 30,000 acres in the US.[18] However, like Bt potatoes, it also became caught up in the anti-biotech fervor and in 2000 was grown on less than 3,000 acres. It was not possible to obtain figures for area grown to Bt sweet corn after 2003, but at that time it had risen to over 5,000 acres and was marketed by Syngenta Seeds.

As reported by Shelton et al.[8] studies in New York have shown Cry1Ab to be very effective against the European corn borer, *Ostrinia nubilalis* (Hübner), providing 100% clean ears when no other lepidopteran species were present and >97% when the two noctuids, *Spodoptera frugiperda* (Smith) (fall armyworm) and *Helicoverpa zea* (Boddie) (corn earworm), were also present.[19] Studies in other states have shown that Bt sweet corn provided consistently excellent control of the lepidopteran pest complex.[20-24] However, under very high pressure by *H. zea*, supplemental sprays of synthetic insecticides often are required. By using appropriately timed insecticide applications with Bt sweet corn varieties, fresh market sweet corn growers in North Carolina have been able to extend their production later into the season, when populations of *H. zea* and *S. frugiperda* are generally too high to control satisfactorily with insecticide applications alone. Even when two insecticide sprays are required on Bt11 sweet corn (e.g., for late season control of *H. zea*), an economic assessment in Virginia found a gain of $1,777/ha for fresh-market Bt sweet corn vs. non-Bt sweet corn sprayed up to six times with pyrethroid insecticides.[24]

As with Bt cotton and Bt field corn, there is a trend to using multiple Bt toxins in sweet corn to enhance performance across a range of species. Thus, trials conducted in Maryland and Minnesota under high *H. zea* pressure have indicated superior control, compared with Bt11, with sweet corn expressing both Cry1Ab endotoxin (Bt11 event) and the vegetative insecticidal protein VIP3A (MIR 162 event).[25] In 2010 and 2011, multiple location trials were conducted in the US with a Monsanto product identified as MON QHB9004 (derived from event 89034) that expresses Cry1A.105 and Cry2Ab2. In New York in 2010, this product provided ≥99% clean ears even under very high pressure *H. zea*, without the use of foliar sprays of a commonly used pyrethroid insecticide, lambda cyhalothrin (Warrior II). This is in stark contrast to the non-Bt variety 'Obsession', which had only 18% clean ears even with 8 sprays of Warrior II (**Table 1**). Monsanto received registration in 2011 for this product under the trade name of Obsession II. Growers may rapidly adopt this product, and similar dual Bt gene products, because of its increased efficacy against *H. zea*, which is becoming more problematic in the northern US due to its increased abundance and its earlier arrival compared with historical trends. However, a stumbling block may be an emerging noctuid lepidopteran pest, the western bean cutworm (*Striacosta albcosta* (Smith)), which is not susceptible to Cry1A or Cry2A. It may be useful to pyramid another protein, Cry1F, to obtain control of this emerging pest in sweet corn, as has been done in field corn. Again, this might lead to another example of piggy-backing on technology developed for a field crop.

Unlike Bt potatoes that are primarily marketed as processed food (e.g., fries and chips) in the US, the value of fresh market sweet corn in 2009 was much greater than processed sweet corn ($835 vs. 335 million).[26] Not having a major consumer target, such as was the case with McDonald's and Bt potatoes, makes it less likely that pressure from anti-biotech forces would derail Bt sweet corn. It seems that the future of Bt sweet corn is promising, and two other Bt vegetables may share a similar future, although they too have had a rough start.

Table 1. Undamaged ears (%) of sweet corn vs. frequency of Warrior II application

	Applications of Warrior II[a]		
Variety	0	4	8
Obsession II[b] (Bt)	100.0 ± 0.0[a]	99.0 ± 1.0[a]	100.0 ± 0.0[a]
Obsession (non-Bt)	6.0 ± 3.5[b]	10.0 ± 2.0[b]	18.0 ± 10.9[b]

Means (±SE) followed by the same letter within a column are not significantly different (Fishers LSD means separation test, p > 0.05). [a]Sweet corn was treated from tassel to harvest using a ground sprayer. [b]Obsession II is the isoline of Obsession but expresses Cry IAb and Cry2Ab and was commercialized in the US in 2010.

Crucifers

As summarized by Shelton et al.[8] Cry1 Bt genes have been introduced into several Brassica species, conferring resistance to the diamondback moth, *Plutella xylostella* (L.), the main insect pest of crucifers worldwide,[27] as well as other Lepidoptera. Our program's early impetus for using Bt brassicas was as a research tool to study insecticide resistance management (IRM) strategies, since *P. xylostella* was the only insect to have evolved resistance to foliar sprays of Bt in the field.[28,29] Through a collaborative program with E.D. Earle, also at Cornell, Bt crucifers of different types were produced and studies were undertaken using this model system. Results over the past 15 y have led to the following key findings that have implications for commercialized Bt crops and those yet to come. Studies have confirmed the importance of refuges in maintaining susceptible alleles in the population;[30,31] demonstrated the superiority of using dual gene (pyramided) Bt plants compared with introducing single gene plants in a mosaic or sequential fashion;[32] demonstrated the increased speed of resistance evolution to dual gene Bt plants if they are grown in association with single Bt gene plants that express one of the same proteins;[33] demonstrated the potential usefulness of inducible promoters in plants for creating a refuge in time or space;[34] and demonstrated the lack of toxicity by a Cry1 toxin to a hymenopteran endoparasitoid.[35] While these findings from this model insect-Bt plant system have been helpful for understanding IRM and biological control in current commercialized Bt crops (corn and cotton), they also prepared the way for the introduction of commercialized Bt brassica vegetables.

It was the ability of the high-expressing pyramided Bt plants to delay evolution of Bt resistance in *P. xylostella* populations that led to the formation of a private-public partnership called the Collaboration on Insect Management for Brassicas in Asia and Africa (CIMBAA) in 2003.[8,36] The original partnership involved Nunhems, a major vegetable breeding company located in The Netherlands, and the following public partners: the Asian Vegetable Research and Development Center in Taiwan (AVRDC); the Centre for Environmental Stress and Adaptation Research at the University of Melbourne in Australia; Cornell

University in the US; and the Natural Resources Institute, University of Greenwich-UK. The initial goal of CIMBAA was to make the dual-Bt technology available in varieties that are optimally adapted to growing conditions for cabbage and cauliflower in India, and then other countries in Asia. It was decided that the CIMBAA plants would use *Cry1Ba2* and *Cry1Ca4* genes, because they had been shown to be effective against *P. xylostella*, and cross-resistance between the two toxins was not detected.[37] Additionally, studies had shown that resistance to Cry1C in *P. xylostella* is polygenic,[38] making it more difficult for the insect to evolve resistance. Because the material would be transferred to brassica breeders, it was important that the integrity of the material be maintained. Therefore, Nunhems focused on placing the two genes so closely linked on the chromosome that they would not be separated in conventional breeding programs, thus ensuring that lines originating from them would contain the pyramided genes.

Laboratory and greenhouse studies in 2006 with CIMBAA breeding lines showed potential, and cabbage and cauliflower elite events showed high levels of resistance under field conditions in 2008 and 2009, not only to *P. xylostella* but also to other lepidopterous pests, including *Hellula undalis* (F.), *Crocidolomia binotalis* (L.) and *Diacrisia obliqua* Walker. However, the elite events displayed lesser control of *Spodoptera litura* F. and *Helicoverpa armigera* (Hübner), so that other integrated pest management (IPM) interventions would be needed. Baseline studies of susceptibility to these two toxins were conducted in 2006–7 in India and other regions where CIMBAA plants were intended to be commercialized.[8] To help lay the groundwork for commercialization of CIMBAA plants, an article was published[36] which provided worldwide justification based on biological, sociological and economic considerations. However, in May 2010, Nunhems informed all CIMBAA partners that they no longer intended to continue with the development of Bt crucifers.

The reasons for the demise of CIMBAA are varied but point again to the difficulty of bringing Bt vegetables to market, despite their clear need. The proposed activities of CIMBAA were comprehensive and included not only the technical development of the plants by Nunhems, but also safety assessments of the proteins, environmental risk assessment of the plants, product registration in different countries, communication to growers and the public, pre- and post-release studies, building the IPM context for the plants, and stewardship of the final product. Likewise, the number of parties was large and included government and non-government agencies in India and other countries. As a private-public partnership, the goals and obligations of each partner were complex. The original idea was that the public sector would develop and field test the plants and that the public sector would have a major role in developing the information needed for the regulatory dossier, developing the IPM context for the plants, and distributing the breeding material to breeders in developing countries to create lines suitable for their local needs. Funding for the public sector was very limited and hampered CIMBAA's efforts. Furthermore, the private sector became concerned about its burgeoning multi-million dollar effort on Bt crucifers in an Indian market that was already debating whether a more advanced product (Bt eggplant) would ever make it to market (see below). While costs and organizational factors played a role in dissolving CIMBAA, perhaps the final blow was the question of liability. There were no clear guidelines or precedents in the Indian regulatory system to help either party know exactly what this liability meant, and Bayer, the parent company of Nunhems, insisted that the government of India or the public sector of CIMBAA bear liability for the product. Neither party agreed to do this. While there is still on-going research on Bt crucifers in India and other parts of the world, including China, I am not aware of any plans for commercialization in the near future.

Eggplant

The Bt eggplant (brinjal) story in India was summarized by Shelton.[39] Eggplant, *Solanum melongena*, is a widely cultivated vegetable in India grown on 560,000 ha in 2008.[40] Eggplant, along with tomato and onion, is the second most important vegetable in India and is considered the most affordable, so is consumed in a wide variety of dishes popular throughout India. As with most solanaceous vegetables, eggplant has a diverse pest complex of insects and diseases, but the most serious is the fruit and shoot borer (FSB), *Leucinodes orbonalis*. As is typical with this insect family, preventing injury relies on the difficult task of using well-timed insecticide applications before the eggs hatch into larvae that bore into the stems or fruits of the plant. In India FSB causes yield losses of 60–70% even after repeated insecticidal sprays, resulting not only in significant crop losses but hazards to human health and the environment.[40] About 30% of the eggplant area is grown to hybrids, while open pollinated varieties (OPV), which are less expensive and geared for resource-poor farmers, account for the rest.[41] In a survey conducted in Maharashtra in 2005, growers sprayed an average of 27 times, although some sprayed more than twice this during the season with most of the sprays occurring during flowering and ripening to prevent damage by FSB.[41] In many cases, this resulted in daily spraying to the fruit even at harvest, high insecticide residue levels, a potential hazard to consumers and earned eggplant the moniker of a "pesticide bomb." A similar situation occurs in Bangladesh where 8 million farmers grow eggplant, and in the Philippines where eggplant is grown on 20,000 ha (http://www.absp2.cornell.edu/projects/project.cfm?productid = 2).

The transformation of Bt eggplant was first begun in 2000 by the Maharashtra Hybrid Seed Company (Mahyco) in India, under a partnership with Monsanto and using its *cry1Ac* gene, which had already been widely used in Bt cotton in India. Control of FSB by Bt eggplant was demonstrated in greenhouse trials and, in late 2003, a partnership was developed with Mahyco, Cornell University and the United States Agency for International Development (USAID) under the Agricultural Biotechnology Support Program II (ABSPII) (http://www.absp2.cornell.edu/projects/project.cfm?productid=2). Each group shares in the responsibility to bring Bt eggplant to market, but what is unique is that the partners proposed two market channels: a "pro-poor" for the distribution of OPV lines created by local agricultural universities, and the "normal" channel through which the higher priced hybrid varieties would be sold and Mahyco would recover some of its investment.

As with any GM crop, the Indian regulatory system requires a number of studies, all of which were completed, including: toxicity, allergenicity, animal feeding, pollen flow, food equivalency, non-target organisms, large scale field trials as well as studies on the socio-economic impact of Bt eggplant. Additionally, an insecticide resistance management program and product stewardship program were required (many of these reports can be seen on the website, http://www.envfor.nic.in/divisions/csurv/geac/information_brinjal.htm).

Field trials have been conducted since 2003–4. Krishna and Qaim[42] analyzed these trials and surveyed eggplant farmers in the Central/South and East regions of India, and estimated that the farmers' gross margins in these areas would increase by Rs. 16,299/acre ($361) and Rs. 19,744/acre ($437), respectively. They noted that yields of Bt hybrids were double those of non-Bt counterparts and, nationally, they estimated the aggregate economic surplus gains of Bt hybrids could be around $108 million/year, with consumers capturing a large share of these gains. Furthermore, farmers would realize an additional $3–4 million per year in health benefits from the reduced insecticide sprays, but that this is "only a small fraction of the technology's environmental and health externalities."[42]

On 14 October 2009, the Genetic Engineering Approval Committee (GEAC) of India approved the commercial cultivation of transgenic Bt eggplant. However, on 9 February 2010, Mr. Jairam Ramesh, the Minister of the Environment and Forests of the Indian Government, imposed a moratorium on Bt eggplant until "such times independent scientific studies establish, to the satisfaction of both the public and professionals, the safety of the product from the point of view of its long-term impact on human health and environment…" (http://moef.nic.in/downloads/public-information/Annex_BT.pdf). It appears that Mr. Ramesh's action was due to pressure by Greenpeace and other anti-biotechnology NGOs (non-government organization) that sent thousands of faxes and emails urging him to ban the cultivation of Bt eggplant, as well as his public hearing meetings which were often disrupted by anti-biotechnology activists. Why are some NGOs against Bt eggplant? Greenpeace, perhaps the world's largest and most powerful NGO on environmental issues, has taken an antibiotech stand in agriculture (http://www.greenpeace.org/international/en/campaigns/agriculture/). However, a careful reading of the information they present indicates their position is based on anti-globalization and anti-capitalization issues, rather than the biology and ecology of biotech crops in agriculture. They fail to report the positive benefits of pesticide reduction through the use of GE crops and have used fear and misinformation to undermine the use of GE plants. An analysis of their tactics is described by Davidson's article[43] on GE virus-resistant papaya in which she exposes their tactics and documents how Greenpeace uses their opposition to GE crops as a major fundraising message.

It is clear to me that Mr. Ramesh's moratorium decision was based on political pressure rather than on the body of scientific evidence. Subsequently, the scientific questions on which he supposedly based his decision have been addressed, and six institutions representing India's top scientists (three academies of science and the academies of agriculture, engineering and medicine) have given their joint and unanimous backing of Bt eggplant (http://news.sciencemag.org/scienceinsider/2010/09/indias-scientific-leadership-endorses.html). In a particularly critical review of Ramesh's decision, C.K. Rao, from the Foundation for Biotechnology Awareness and Education in India, points out the biological, agronomic, political and cultural errors on which the Minister based his decision, including the claim that India is the center of origin for eggplant and that Bt eggplant would disrupt the biodiversity (www.whybiotech.com/resources/tps/Moratorium_on_Bt_Brinjal.pdf). As Kolady[41] points out, the moratorium was conveniently invoked based on the precautionary principle, yet resulted in continued over-use of "deadly" insecticides.

On the other hand, field trials of Bt eggplant are continuing in Bangladesh and the Philippines and ABSPII is optimistic for approval in these countries. In conversations I had with the Minister of the Environment and the Minister of Agriculture from Bangladesh in the summer of 2011, they indicated verbal support for Bt eggplant and advised that anti-biotech NGOs have little presence in Bangladesh, so would not be able to have the impact that Greenpeace and other anti-biotech NGOs did in India where it is rumored they spent $100 M on efforts to derail Bt eggplant. One has to ask whether approval of Bt eggplant in Bangladesh would result in illegal plantings in neighboring India. It should be remembered that illegal plantings of "stealth" Bt cotton seeds occurred in India prior to their approval by the Indian government; the only reason approval was granted was due to the threat of a farmer strike if not approved.[44] According to discussions with members of ABSPII, indications are that the Philippines are on track to approve Bt eggplant within a couple of years.

Conclusions

The first Bt vegetable was developed by Fischhoff et al. who engineered tomato plants resistant to tobacco hornworm (*Manduca sexta)* and the tomato fruitworm (*Heliothis virescens*). Since that time, many other Bt vegetables have been developed but only potatoes and sweet corn have been commercialized, and only sweet corn remains on the market. Meanwhile the area planted to Bt field crops (cotton and corn) continues to increase; in 2010 they were grown on 58.6 Mha globally.[3]

Why are there such differences? As discussed above, there are a complex of issues, but it appears to me that two are the major impediments, and they are interrelated. The first is the present regulatory system that requires event-based registration, rather than the more scientifically justified registration of the expressed Bt protein. The present system results in very high costs without any increased safety. The second is the complexity of vegetable breeding for so many different vegetable types and the rapid turnover of commercial cultivars that results in an unfavorable economy of scale for most vegetables. However, this is inherent in vegetable breeding regardless of whether the crop is GE or not. A Bt gene can be inserted into vegetable breeding material routinely and inexpensively nowadays, so the real problem gets back to the event-based registration. If Cry1Ab were cleared for all tomato types (and other plant families), then Cry1Ab could become common in tomato breeding material. Thus, event-based registration appears to be the major hurdle for Bt vegetables.

While consumer preference is often suggested as a major reason for consumers not purchasing GE crops, in truth, consumers have had little opportunity to make such a choice. Either the GE products are not available to them (e.g., Bt tomatoes) or the products they purchase (e.g., cereals) are not labeled, so they are unaware of ingredients from GE crops. The studies by Powell et al. and James et al.[10] suggest that, when consumers are provided information about choosing Bt sweet corn, it sells as well as, if not better than, conventionally grown sweet corn. The 2010 report by IFIC also suggests that consumers would not avoid Bt vegetables if they were available and had other characteristics that were more important for the majority of consumers: freshness, color, taste, etc. Whether consumers will be able to eventually make that choice for Bt vegetables is unclear. For now, consumers in the United States, Canada, the Philippines and a few other countries can choose only one Bt vegetable, sweet corn. It should be noted that even this crop was commercialized only after its event (Bt 11) had already been registered and commercialized in field corn. The same piggy-back situation occurred with Obsession II, a new dual Bt gene sweet corn, which was based on an event already registered in field corn.

In India there was hope that Bt eggplant would have a much smoother path to commercialization than it has had, and that it would become the first Bt food crop grown there. The need for the crop is undeniable because of the high pesticide load non-Bt eggplant incurs, the direct financial benefit to growers, and the increased safety to consumers and farm workers.[42] But India is an incredibly complex society whose regulations can be disrupted by politics and special interest from the outside. In time, Bt eggplant will be grown in India to help feed its 1.1 billion people, the majority of whom are poor farmers. But the question is whether it will come from "stealth" seeds, as was the case with Bt cotton,[44] or whether it will come through a well-functioning Indian regulatory system.

Acknowledgements

I am grateful to H. Collins for her editing and the following people for their helpful suggestions: S. Davidson, P. Griffiths, K. Raman, N. Storer, F. Shotkoski, K. Vijayraghavan and J. Wolt.

References

1. Kennedy GG. Integration of insect-resistant genetically modified crops within IPM programs. In: Romeis J, Shelton AM, Kennedy GG, Eds. Integration of Insect-Resistant, Genetically Modified Crops within IPM Programs. Dordrecht, The Netherlands: Springer 2008; 1-26.
2. Naranjo SE, Ruberson JR, Sharma HC, Wilson L, Wu K. The present and future role of insect-resistant genetically modified cotton in IPM. In: Romeis J, Shelton AM, Kennedy GG, Eds. Integration of Insect-Resistant, Genetically Modified Crops within IPM Programs. Dordrecht, The Netherlands: Springer 2008; 159-94.
3. James C. Global status of commercialized biotech/GM crops: ISAAA Brief No. 42. ISAAA: Ithaca NY 2010.
4. Brookes G, Barfoot P. Global impact of biotech crops: environmental effects 1996–2008. AgBioForum 2010; 13:76-94; http://www.agbioforum.org/v13n1/v13n1a06-brookes.htm.
5. Harvard School of Public Health (HSPH). Fruits and vegetables. 2007; www.hsph.harvard.edu/nutrition-source/fruits.html (accessed 7 November 2011).
6. Campbell TC, Campbell TM. The China Study: The Most Comprehensive Study of Nutrition Ever Conducted and the Startling Implications for Diet, Weight Loss and Long-Term Health. Dallas, TX: BenBella Books 2004.
7. Lumpkin TA, Weinberger K, Moore S. Increasing income through fruit and vegetable production: opportunities and challenges. CGIAR Priorities: Science for the Poor. Draft note. Consultative Group on International Agriculture Research Science Forum, Marrakech, Morocco 2005.
8. Shelton AM, Romeis J, Kennedy GG. IPM and GM, insect-protected plants: thoughts for the future. In: Romeis J, Shelton AM, Kennedy GG, Eds. Integration of Insect-Resistant, Genetically Modified Crops within IPM Programs. Dordrecht, The Netherlands: Springer 2008; 419-30.
9. Powell DA, Blaine K, Morris S. Agronomic and consumer considerations for Bt and conventional sweet-corn. Br Food J 2003; 105:700-13; http://dx.doi.org/10.1108/00070700310506254.
10. James JS, Fleischer SJ, Johnson DH, Schwab BD, Lord J. Consumer purchases of biotech sweet corn: results from a market experiment. American Agricultural Economics Association Annual Meeting, Providence RI 2005; http://purl.umn.edu/19213.
11. Matten SR, Head GP, Quemada HD. How government regulations can help or hinder the integration of Bt crops within IPM programs. In: Romeis J, Shelton AM, Kennedy GG, Eds. Integration of Insect-Resistant, Genetically Modified Crops within IPM Programs. Dordrecht, The Netherlands: Springer 2008; 27-40.
12. Hellmich RL, Albajes R, Bergvinson D, Prasifka JR, Wang ZY, Weiss MJ. The present and future role of insect-resistant genetically modified maize in IPM. In: Romeis J, Shelton AM, Kennedy GG, Eds. Integration of Insect-Resistant, Genetically Modified Crops within IPM Programs. Dordrecht, The Netherlands: Springer 2008; 87-118.
13. USDA. Animal and plant health inspection service. Introduction of genetically engineered organisms. Draft programmatic environmental impact statement-July 2007.
14. Grafius EJ, Douches DS. The present and future role of insect-resistant genetically modified potato cultivars in IPM. In: Romeis J, Shelton AM, Kennedy GG, Eds. Integration of Insect-Resistant, Genetically Modified Crops within IPM Programs. Dordrecht, The Netherlands: Springer 2008; 195-221.
15. Thomas PE, Kaniewski WK, Lawson EC. Reduced field spread of potato leafroll virus in potatoes transformed with the potato leafroll virus coat protein gene. Plant Dis 1997; 81:1447-53; http://dx.doi.org/10.1094/PDIS.1997.81.12.1447.
16. Thornton M. The rise and fall of NewLeaf™ potatoes. NABC Report 15: Biotechnology: Science and Society at a Crossroad 2003; 235-43. http://nabc.cals.cornell.edu/pubs/nabc_15/chapters/Thornton.pdf (accessed November 7 2011).
17. Guenthner JF. Consumer acceptance of genetically modified potatoes. Am J Potato Res 2002; 79:309-16; http://dx.doi.org/10.1007/BF02870167.
18. Plaisted D. Slow, uncertain, but steady rise of biotech sweet corn. Program Symposium: Biotech Vegetables for Insect and Insect-Vectored Disease Management, ESA Annual Meeting, Cincinnati, Ohio 2003.
19. Musser FR, Shelton AM. Bt sweet corn and selective insecticides: impacts on pests and predators. J Econ Entomol 2003; 96:71-80; PMID:12650347; http://dx.doi.org/10.1603/0022-0493-96.1.71.
20. Lynch RE, Wiseman BR, Sumner H, Plaisted D, Warnick D. Management of corn earworm and fall armyworm (Lepidoptera: Noctuidae) injury on a sweet corn hybrid expressing a cryIA(b) gene. J Econ Entomol 1999; 92:1217-22.
21. Sorenson KA, Holloway CW. Corn earworm and European corn borer control with Bt treated corn, Brunswick and Sampson Co., NC 1998. Arthropod Management Tests 1999; 24:429-30.
22. Burkness EC, Hutchison WD, Bolin PC, Bartels DW, Warnock DF, Davis DW. Field efficacy of sweet corn hybrids expressing a *Bacillus thuringiensis* toxin for management of *Ostrinia nubilalis* (Lepidoptera: Crambidae) and *Helicoverpa zea* (Lepidoptera: Noctuidae). J Econ Entomol 2001; 94:197-203; PMID:11233113; http://dx.doi.org/10.1603/0022-0493-94.1.197.
23. Hassell R, Shepard BM. Insect populations on *Bacillus thuringiensis* transgenic sweet corn. J Entomol Sci 2002; 37:285-92.
24. Speese J III, Kuhar TP, Bratsch AD, Nault BA, Barlow VM, Cordero RJ, et al. Efficacy and economics of fresh-market Bt transgenic sweet corn in Virginia. Crop Prot 2005; 24:57-64; http://dx.doi.org/10.1016/j.cropro.2004.06.008.
25. Burkness EC, Dively G, Patton T, Morey AC, Hutchison WD. Novel Vip3A *Bacillus thuringiensis* (Bt) maize approaches high-dose efficacy against *Helicoverpa zea* (Lepidoptera: Noctuidae) under field conditions: Implications for resistance management. GM Crops 2010; 1:337-43; PMID:21844691; http://dx.doi.org/10.4161/gmcr.1.5.14765.
26. National Agricultural Statistics Service (NASS). Vegetables 2010 summary. NASS, USDA 2011; http://usda.mannlib.cornell.edu/usda/current/VegeSumm/VegeSumm-01-27-2011.pdf.
27. Talekar NS, Shelton AM. Biology, ecology and management of the diamondback moth. Annu Rev Entomol 1993; 38:275-301; http://dx.doi.org/10.1146/annurev.en.38.010193.001423.

28. Tabashnik BE, Cushing NL, Finson N, Johnson MW. Field development of resistance to *Bacillus thuringiensis* in diamondback moth. J Econ Entomol 1990; 83:1671-6.
29. Shelton AM, Robertson JL, Tang JD, Perez C, Eigenbrode SD, Preisler HK, et al. Resistance of diamondback moth to *Bacillus thuringiensis* subspecies in the field. J Econ Entomol 1993; 86:697-705.
30. Shelton AM, Tang JD, Roush RT, Metz TD, Earle ED. Field tests on managing resistance to Bt-engineered plants. Nat Biotechnol 2000; 18:339-42; PMID:10700153; http://dx.doi.org/10.1038/73804.
31. Tang JD, Collins HL, Metz TD, Earle ED, Zhao JZ, Roush RT, et al. Greenhouse tests on resistance management of Bt transgenic plants using refuge strategies. J Econ Entomol 2001; 94:240-7; PMID:11233120; http://dx.doi.org/10.1603/0022-0493-94.1.240.
32. Zhao JZ, Cao J, Li Y, Collins HL, Roush RT, Earle ED, et al. Transgenic plants expressing two *Bacillus thuringiensis* toxins delay insect resistance evolution. Nat Biotechnol 2003; 21:1493-7; PMID:14608363; http://dx.doi.org/10.1038/nbt907.
33. Zhao JZ, Cao J, Collins HL, Bates SL, Roush RT, Earle ED, et al. Concurrent use of transgenic plants expressing a single and two *Bacillus thuringiensis* genes speeds insect adaptation to pyramided plants. Proc Natl Acad Sci USA 2005; 102:8426-30; PMID:15939892; http://dx.doi.org/10.1073/pnas.0409324102.
34. Bates SL, Cao J, Zhao JZ, Earle ED, Roush RT, Shelton AM. Evaluation of a chemically inducible promoter for developing a within-plant refuge for resistance management. J Econ Entomol 2005; 98:2188-94; PMID:16539150; http://dx.doi.org/10.1603/0022-0493-98.6.2188.
35. Chen M, Zhao JZ, Collins HL, Earle ED, Cao J, Shelton AM. A critical assessment of the effects of Bt transgenic plants on parasitoids. PLoS One 2008; 3:2284; http://dx.doi.org/10.1371/journal.pone.0002284; PMID:18523682.
36. Grzywacz D, Rossbach A, Rauf A, Russell D, Srinivasan R, Shelton AM. Current control methods for diamondback moth and prospects for improved management with lepidopteran-resistant Bt vegetable brassicas in Asia and Africa. Crop Prot 2010; 29:68-79; http://dx.doi.org/10.1016/j.cropro.2009.08.009.
37. Zhao JZ, Li Y, Collins HL, Cao J, Earle ED, Shelton AM. Different cross-resistance patterns in the diamondback moth (Lepidoptera: Plutellidae) resistant to *Bacillus thuringiensis* toxin Cry1C. J Econ Entomol 2001; 94:1547-52; PMID:11777062; http://dx.doi.org/10.1603/0022-0493-94.6.1547.
38. Zhao JZ, Collins HL, Tang JD, Cao J, Earle ED, Roush RT, et al. Development and characterization of diamondback moth resistance to transgenic broccoli expressing high levels of Cry1C. Appl Environ Microbiol 2000; 66:3784-9; PMID:10966391; http://dx.doi.org/10.1128/AEM.66.9.3784-9.2000.
39. Shelton AM. The long road to commercialization of Bt brinjal (eggplant) in India. Crop Prot 2010; 29:412-4; http://dx.doi.org/10.1016/j.cropro.2010.02.016.
40. Choudhary B, Gaur K. The development of and regulation of Bt brinjal in India. ISAAA Brief No. 38. ISAAA: Ithaca NY 2009.
41. Kolady DE. Are we missing something in the debate on Bt eggplant in India? Asian Biotechnology and Development Review 2011; 13:39-51.
42. Krishna VV, Qaim M. Potential impacts of Bt eggplant on economic surplus and farmers' health in India. Agric Econ 2008; 38:167-80.
43. Davidson SN. Forbidden fruit: transgenic papaya in Thailand. Plant Physiol 2008; 147:487-93; PMID:18524879; http://dx.doi.org/10.1104/pp.108.116913.
44. Herring R. Stealth seeds. J Dev Stud 1997; 43:130-57; http://dx.doi.org/10.1080/00220380601055601.
45. Fischhoff DA, Bowdish KS, Perlak FJ, Marrone PG, McCormick SM, Niedermeyer JG, et al. Insect tolerant transgenic tomato plants. Nat Biotechnol 1987; 5:807-13; http://dx.doi.org/10.1038/nbt0887-807.