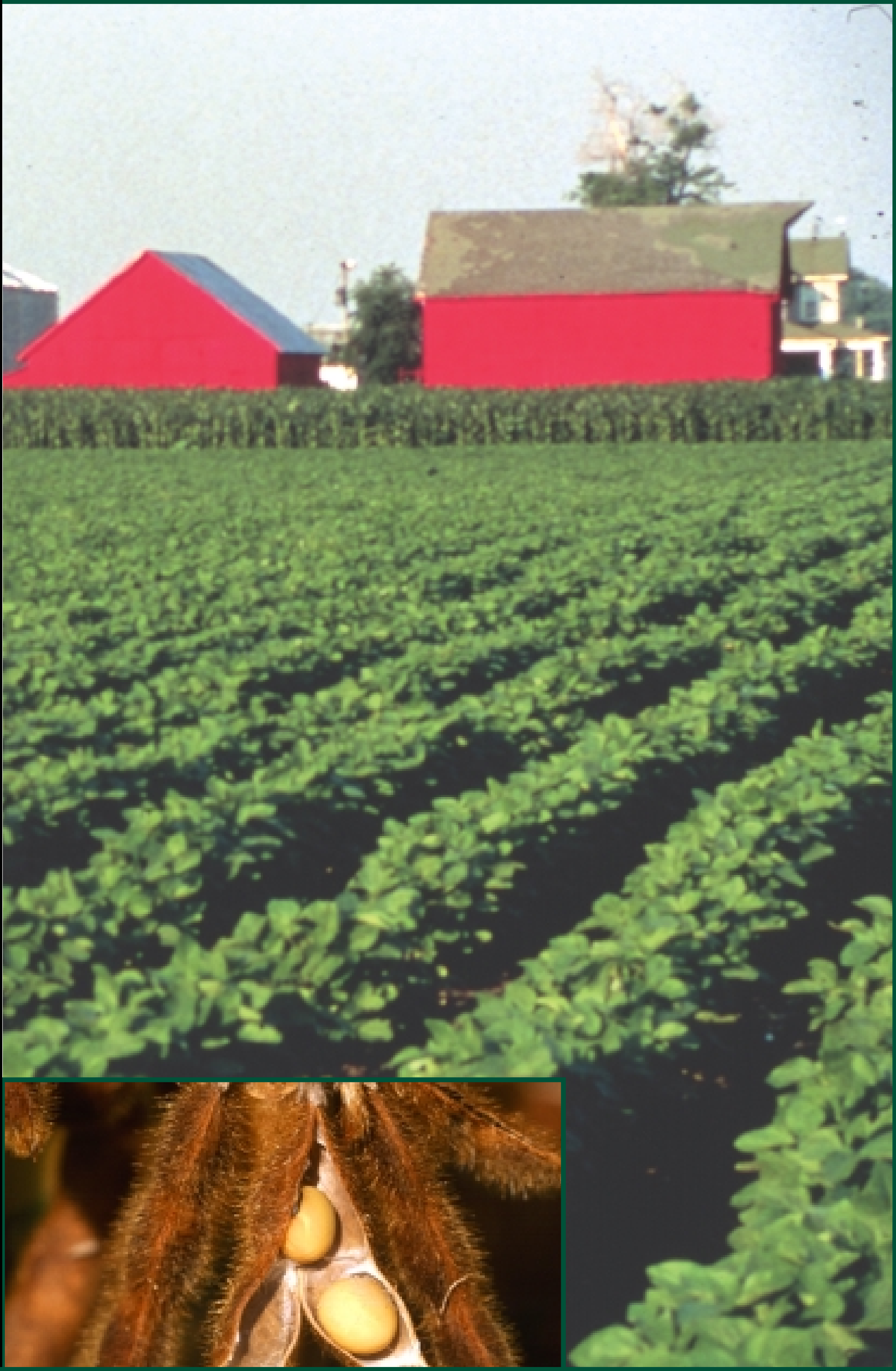
Comparative Environmental Impacts of Biotechnology-derived and Traditional Soybean, Corn, and Cotton Crops



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Foreword

Following a request by the United Soybean Board (USB), the Board of Directors of the Council for Agricultural Science and Technology (CAST) authorized preparation of a report on the environmental impacts of biotechnology-derived soybean, corn, and cotton crops. As directed by the CAST Board of Directors, this report is a comprehensive literature review and not a policy analysis. The information from the scientific literature is placed into context by means of a comparative analysis of the environmental impacts of the three most widely planted, biotechnology-derived commodity crops with the environmental impacts of traditional varieties and cropping practices.

This project was sponsored by the USB who provided funding and identified the initial scope of the report. The USB is a farmer-led organization comprising 61 farmer-directors; it oversees the investments of the soybean checkoff on behalf of all U.S. soybean farmers.

Highly qualified scientists served as the report's authors and reviewers. They included individuals with expertise in agronomy, agricultural economics, entomology, environmental science, plant breeding, plant pathology, soil science, and weed science. The authors prepared an initial draft of the report, which was reviewed by the credited reviewers. The authors revised all subsequent drafts and reviewed the proofs. The CAST Executive Committee, Editorial and Publications Committee, Plant Protection Sciences Workgroup, and Plant and Soil Sciences Workgroup reviewed the report. The CAST staff provided editorial and structural suggestions and published the report. The authors are responsible for the report's scientific content.

On behalf of CAST, we thank the authors, who were compensated through a contribution to their institutions for their efforts in preparing this report. We also thank the reviewers who gave of their time and expertise as a contribution by the scientific community to public understanding of the issue. Additionally, we thank the employers of the reviewers, who made the time of these individuals available at no cost to CAST. Finally, the members of CAST deserve special recognition because the unrestricted contributions they have made in support of CAST have helped finance the preparation and publication of this report.

This report is being distributed widely; recipients include Members of Congress, the White House, the Congressional Research Service, the U.S. Department of Agriculture, the Food and Drug Administration, the Environmental Protection Agency, and media personnel. The report may be reproduced in its entirety without permission. If copied in any manner, credit to the authors, CAST, and USB would be appreciated.

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I. Executive Summary

A comprehensive review of the scientific literature supports the conclusion that overall the currently commercialized biotechnology-derived[1] soybean, corn, and cotton crops yield environmental benefits. Furthermore, a critical analysis of the literature supports the idea that biotechnology-derived soybean, corn, and cotton pose no environmental concerns unique to or different from those historically associated with conventionally developed crop varieties.

Soybean, corn, and cotton farmers in developed and developing nations have rapidly adopted biotechnology-derived commodity crops during the six years of their commercial availability. In 2001, farmers planted biotechnology-derived seed on 46% of global soybean acres, 7% of global corn acres, and 20% of global cotton acres. To date, nearly all of the planted biotechnology-derived crops have introduced tolerance to selected herbicides for weed control or have introduced protection against pest insects. Of the 129.9 million acres (52.6 million hectares) of biotechnology-derived crops planted in 2001, seventy-seven percent were tolerant of specific herbicides (herbicide tolerant), fifteen percent were resistant to selected insect damage (insect resistant), and eight percent were both herbicide tolerant and insect resistant.

The peer-reviewed literature, regulatory assessments, nongovernmental organizations and the popular media have repeatedly raised questions about the environmental safety of biotechnology-derived crops. To answer these questions relative to soybean, corn, and cotton, the scientific literature was reviewed and analyzed to evaluate the environmental impacts of commercially available biotechnology-derived crops in relation to the current agricultural practices for crop and pest management in conventionally bred crops. Nine potential environmental impacts were identified as follows:

1. **Changes in pesticide use patterns** - Does the adoption of biotechnology-derived soybean, corn, and cotton impact the use of pesticides and, if so, do these changes alter farmer practices in ways that affect water quality or soil health?

2. **Soil management and conservation tillage** - Does adoption of biotechnology-derived soybean, corn, and cotton lead to changes in the adoption of no-till and other conservation tillage practices or otherwise impact soil erosion, moisture retention, soil nutrient content, water quality, fossil fuel use, and greenhouse gasses?

3. **Crop weediness** - Have biotechnology-derived soybean, corn, and cotton acquired weediness traits?

4. **Gene flow and outcrossing** - Do biotechnology-derived soybean, corn, and cotton hybridize with local plants or crops and impact the genetic diversity in the areas where the biotechnology-derived soybean, corn, and cotton are planted?

5. **Pest resistance** - Do biotechnology-derived soybean, corn, and cotton possess plant-protectant traits to which pests will become resistant and, if so, is the development of resistance to these traits different than development of resistance to conventional chemical and microbial pesticides? How is the development of resistance being managed?

6. **Pest population shifts** - Do biotechnology-derived soybean, corn, and cotton cause changes in weed or secondary insect pest populations that impact the agricultural system or ecology of the surrounding environment?

7. **Nontarget and beneficial organisms** - Do biotechnology-derived soybean, corn, and cotton with pest protection characteristics have an impact on natural enemies of pests (i.e., predators and parasitoids) or on other organisms in the soil and crop canopy?

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[1] Biotechnology-derived refers to the use of molecular biology and/or recombinant DNA technology, or in vitro gene transfer, to develop products or impart specific capabilities in plants or other living organisms.

8. **Land use efficiency/productivity** - Does the adoption of biotechnology-derived soybean, corn, and cotton impact crop yields or impact the need for cultivating forested or marginal land?

9. **Human exposure** - Do the traits of herbicide tolerance and resistance to pest insects in biotechnology-derived soybean, corn, or cotton pose any new or different safety concerns in comparison to conventionally bred crops with similar traits?

Biotechnology-derived crops provide options and potential solutions for a number of challenges in modern agriculture, but the extent to which they may be viable or the preferred option is dependent on many economic, social, and regional factors. Nevertheless, a number of general conclusions about biotechnology-derived soybean, corn, and cotton are supported by the literature.

- Biotechnology-derived soybean, corn, and cotton provide insect, weed, and disease management options that are consistent with improved environmental stewardship in developed and developing nations.

- Biotechnology-derived crops can provide solutions to environmental and economic problems associated with conventional crops including production security (consistent yields), safety (worker, public, and wildlife), and environmental benefits (soil, water, and ecosystems).

- Although not the only solution for all farming situations, the first commercially available biotechnology-derived crops, planted on over 100 million acres (40.5 million hectares) worldwide, provide benefits through enhanced conservation of soil and water and beneficial insect populations and through improved water and air quality.

- The high adoption rates for commercially available biotechnology-derived crops can be attributed to economic benefits for farmers.

- When biotechnology-derived crops are available to small farmers in developing nations, the farmers can realize environmental benefits and reduce worker exposure to pesticides.

BIOTECHNOLOGY-DERIVED SOYBEAN

- Herbicide-tolerant soybean is the most widely adopted biotechnology-derived crop, planted on 68% of United States' soybean acreage and over 98% of Argentina's soybean acreage in 2001. The United States and Argentina together account for 99% of total herbicide-tolerant soybean production in the world, which represents 46% of the total acreage of soybean planted. Farmers in the United States are projected to plant 74% of soybean acreage to herbicide-tolerant soybean in 2002.

- The major reasons farmers have adopted the herbicide-tolerant soybean so widely are lowered production costs, reduced crop injury, and simplicity and flexibility in weed management.

- Biotechnology-derived herbicide-tolerant soybean has facilitated the adoption of conservation tillage. No-till soybean acreage in the United States has increased by 35% since the introduction of herbicide-tolerant soybean. Similar increases are observed in Argentina, which can be attributed in part to reliable and effective weed control provided by herbicide-tolerant soybean. Use of no-till farming in soybean production results in decreased soil erosion, dust, and pesticide run-off and in increased soil moisture retention and improved air and water quality.

- Biotechnology-derived soybean may lead to increased yield, through improved weed control or the ability to adopt narrow-row spacing, resulting in more efficient land use.

- Cost savings in biotechnology-derived herbicide-tolerant soybean programs have allowed adopters to decrease weed control costs, leading to price cuts of conventional herbicide programs. The result has been weed control cost savings for both adopters and non-adopters.

- Farmers using biotechnology-derived herbicide-tolerant soybean are able to use a herbicide that rapidly dissipates to inactive amounts in soil, has little potential for water contamination as a substitute for herbicides used with conventional soybean varieties, and allows greater flexibility in timing of application.

- Biodiversity is maintained in biotechnology-derived herbicide-tolerant soybean fields. Soil microbes, beneficial insects, and bird populations in conservation tillage biotechnology-derived herbicide-tolerant and conventional soybean fields were similar in number and variety.

- Both conventional and biotechnology-derived soybean production systems require effective management strategies for weed population shifts and to prevent the development of weed resistance to herbicides. Emerging reports on glyphosate-resistant weeds may be a concern in herbicide-tolerant soybean; however, herbicide resistance in weeds is not unique to biotechnology-derived crops.

- Conclusions regarding yield decreases attributed to the biotechnology-derived herbicide-tolerant trait may be inaccurate because the study design included improper comparisons between the biotechnology-derived varieties and conventional varieties.

- Soybean with insect protection properties is also in development and will be useful in climatic regions where insect pressures justify insecticide applications.

BIOTECHNOLOGY-DERIVED CORN

- *Bt* corn can enhance the biodiversity of cornfields because beneficial insects fare better than when conventional cornfields are sprayed with insecticides. Moreover, field studies of biotechnology-derived corn show that populations of beneficial insects are not adversely affected.

- Use of *Bt* corn can decrease farm worker exposure to certified organic *Bt* sprays and chemical insecticides.

- Decrease of naturally occurring mold toxins resulting from use of *Bt* corn can provide direct benefits to people and corn-fed livestock. Insect-protected corn is less vulnerable to mold infestation.

- Yields since the introduction of insect-protected and herbicide-tolerant corn have continued at historically high levels. When European corn borer pressure is high, farmers obtain significant economic benefit from the use of insect-protected corn.

- Herbicide-tolerant corn varieties allow use of herbicides that are less persistent in the environment and reduce the risks of herbicide run-off into surface water. These herbicide-tolerant corn varieties allow for greater flexibility in the timing of application and encourage the application of reduced and no-till soil and soil moisture management practices.

- Insect Resistance Management (IRM) plans have been required, developed, and implemented to prevent or to delay the development of insect resistance to *Bt*.

BIOTECHNOLOGY-DERIVED COTTON

- Herbicide-tolerant cotton enhances the use of herbicides that are less persistent in the environment.

- Herbicide-tolerant cotton is a major factor in promoting reduced and no-till farming practices, which result in improved soil and soil moisture management and reduced energy use.

- Herbicide-tolerant cotton provides greater flexibility for the timing of herbicide applications for effective weed control and less damage to the cotton plants.

- Use of biotechnology-derived cotton in developing nations does not require significant capital investment, changes in cultural practices, or significant training for adoption.

- Rapid adoption of *Bt* cotton in China serves as an example of how, in developing nations, plant-incorporated protectants greatly decrease the volume of pesticides applied and the risks of pesticide run-off while increasing safety and health of agricultural workers.

- *Bt* cotton has been documented to have a positive effect on the number and diversity of beneficial insects in cotton fields in the United States and Australia.

- The introduction of *Bt* cotton in Australia, India, and the United States demonstrates the ability of these varieties to alleviate problems with insect resistance to chemical pesticides. The future production of cotton in these regions was in jeopardy prior to the introduction of *Bt* cotton.

- The ability to add several different genes to control the same pest should delay the time it takes for pesticide resistance to develop.

- *Bt* and herbicide-tolerant cotton decreases production costs to farmers and increases the range of options available for whole-farm management systems.

AUTHORS' RECOMMENDATIONS

1. Given that biotechnology-derived crops can provide positive net environmental benefits, we recommend continued development of agricultural biotechnology to enhance environmental stewardship.

2. Biotechnology provides a tool for management of production risk in agriculture. We recommend evaluating the role of biotechnology-derived crops in the context of whole-farm management.

3. When drawing conclusions regarding the impacts of biotechnology-derived crops on productivity, we recommend that conclusions be based on comparisons involving whole-farm systems.

4. When comparing the consequences of a specific trait, we recommend the following characteristics be held constant: varieties that are genetically identical in all aspects other than the trait(s) being evaluated; the crops be grown during the same time in the same geographic location; and use of identical soil and crop management practices. For example, having observed contradictory and inconsistent data regarding yields in some crops, we recommend better measurement of yield impacts.

5. We recommend evaluating the environmental impacts of biotechnology-derived crops in agricultural regions where the crops may be adopted and in the context of viable, currently available alternatives and practices in agriculture.

6. We recommend large-scale and farm-scale field studies to provide supplemental information to document long-term environmental benefits and safety impacts of adopting biotechnology-derived crops.

7. We recommend continued development of policies for implementation of effective management strategies for insect and weed resistance in both conventional and biotechnology-derived crops. Also, we recommend continued research on management strategies to abate or slow the development of resistance to new and existing pest control tools.

8. Recognizing that gene flow is a natural process that may increase biodiversity, we recommend that research on gene flow between biotechnology-derived and other crops or native plants focus on the environmental and social impacts/consequences of that gene movement.

9. Recognizing the potential for biotechnology-derived corn varieties to help resolve current corn rootworm control problems stemming from the development of insect resistance to both chemical insecticides and crops rotation, we recommend research include consideration of resistance management strategies as well as impacts on soil and other nontarget organisms.

10. Recognizing that enhanced land use efficiency is an important environmental benefit, we recommend continued development of biotechnology-derived hybrids that improve crop yields.

II. Introduction

The application of biotechnology tools to agriculture has allowed scientists to transform plants without the need for sexual compatibility between species, thus establishing the possibility of rapidly producing new crop varieties with traits beneficial to human health and the environment. Plants have been transformed successfully to improve their pest and disease resistance, herbicide tolerance, nutritional qualities, and stress tolerance. The rapid transformation of plants with enhanced traits holds great promise for increased efficiency of land use, a development that can help feed the expanding world population using sustainable growing practices (Mackey and Santerre 2000; Royal Society 1998). The doubling or possible tripling of global food demand by the mid-twenty-first century (Mackey and Santerre 2000) necessitates deployment of appropriate technologies that are culturally acceptable and environmentally sustainable (James and Krattiger 1996; Royal Society 2000).

The deficiency in efficient and adequate food production is greatest in developing countries because they have the largest population growth rates but tend to be in climates with comparatively poor soil and water resources and the greatest pest pressures. The magnitude of the problem is starkly illustrated by the demographics: approximately 4.6 billion people live in developing countries, with a growth rate of 1.9 %, compared with 1.2 billion people who live in the industrial countries, with a growth rate of 0.1 % (James 1997).

Food production increases resulting from the Green Revolution of the 1960s and 1970s have helped to close the gap between food supply and demand. But conventional plant breeding techniques may not be adequate to keep pace with demand for both production increases and improvements in land use and environmental quality. As Nobel Laureate Norman Borlaug has said, "If we grow our food and fiber on the land best suited to farming with the technology we have and what's coming, including proper use of genetic engineering and biotechnology, we will leave untouched vast tracts of land with all of their plant and animal diversity." (Bailey 2000) The international scientific community concurs that conventional technology alone will not support sufficient growth in the nutritional quality (availability of nutrients and micronutrients) and nutritional quantity (caloric input) of food production by 2050, when total world population is estimated to be approximately 11 billion (James and Krattiger 1996). Thus, to ensure both nutritional adequacy and environmental health of the world's poorest people in the twenty-first century, plant biotechnology must be investigated and deployed in both developed and developing countries (Conway and Toenniessen 1999).

The principle goal of this report is to provide a review of the literature addressing the comparative environmental benefits and safety of the three most widely planted biotechnology-derived crops: soybean, corn (commonly referred to as maize), and cotton. To evaluate adequately the potential benefits and safety of agricultural biotechnology the authors provide a comprehensive literature review and place the information in context by comparing recent advances in biotechnology-derived soybean, corn, and cotton with currently available traditional varieties of those same crops. The peer-reviewed literature, regulatory assessments, nongovernmental organizations, and the popular media have raised questions repeatedly about the environmental safety of biotechnology-derived crops. To answer these questions relative to soybean, corn, and cotton, the authors of this report evaluate the environmental impacts of commercially available biotechnology-derived crops in relation to the current agricultural practices for crop and pest management in conventionally bred crops. Nine potential environmental impacts are identified as follows:

1. **Changes in pesticide use patterns** - Does the adoption of biotechnology-derived soybean, corn, and cotton impact the use of pesticides and, if so, do these changes alter farmer practices in ways that affect water quality or soil health?

2. **Soil management and conservation tillage** - Does adoption of biotechnology-derived soybean, corn, and cotton lead to changes in the adoption of no-till and other conservation tillage practices or otherwise impact soil erosion, moisture retention, soil nutrient content, water quality, fossil fuel use, and greenhouse gasses?

3. **Crop weediness** - Do biotechnology-derived soybean, corn, and cotton acquired weediness traits?

4. **Gene flow and outcrossing** - Do biotechnology-derived soybean, corn, and cotton hybridize with local plants or crops and impact the genetic diversity in the areas where the biotechnology-derived soybean, corn, and cotton are planted?

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6. **Pest population shifts** - Do biotechnology-derived soybean, corn, and cotton cause changes in weed or secondary insect pest populations that impact the agricultural system or ecology of the surrounding environment?

7. **Nontarget and beneficial organisms** - Do biotechnology-derived soybean, corn, and cotton with pest protection characteristics have an impact on natural enemies of pests (i.e., predators and parasitoids) or on other organisms in the soil and crop canopy?

8. **Land use efficiency/productivity** - Does the adoption of biotechnology-derived soybean, corn, and cotton impact crop yields or impact the need for cultivating forested or marginal land?

9. **Human exposure** - Do the traits of herbicide tolerance and resistance to pest insects in biotechnology-derived soybean, corn, or cotton pose any new or different safety concerns in comparison with conventionally bred crops that have similar traits?

The literature most pertinent for this comprehensive review was identified through a series of literature database searches and in the course of professional practice (meetings, conferences, and peer-to-peer networking).

Prior to the sections on soybean, corn, and cotton, an overview of modern biotechnology is provided for those readers who are less familiar with the use of modern laboratory methods to transfer traits or characteristics from one living entity to another. Readers also will find a glossary of terms in Appendix I. A list of abbreviations, acronyms, and symbols is available in Appendix II. Crop-specific information and conclusions are addressed in the sections on soybean, corn, and cotton. General conclusions and recommendations based on the authors' analysis of the three crops are addressed in the executive summary.

The literature review has been supported through contributions from the United Soybean Board. Review and comment of the report was provided by individuals from agricultural scientific societies, academic institutions, and nonprofit organizations. A list of reviewers is provided in Appendix III.

III. Overview of Modern Biotechnology

To appreciate the environmental benefits of agricultural biotechnology in the midst of public concerns, one should focus on the traits produced in the context of their environment rather than the techniques for crop improvement. Regardless of the specific trait intended for enhancement, the process of transforming different crop species generally involves common strategies and molecular methods. However, concerns about agricultural biotechnology can be alleviated, in part, by an improved understanding of the biochemical basis for the enhanced traits and the techniques used. Thus, the following section is, in part, a brief historical overview of the biochemical basis and genetic sources of the currently registered and commercial herbicide tolerance and pest-protected traits, and the mechanics of plant transformation.

Herbicide Tolerance

Plant species have long been known to be highly variable in their response to herbicides. For example, grasses are very tolerant to 2,4-D and other growth hormone mimics, but broadleaf plants such as dandelions and grapes are extremely susceptible. Soybean can tolerate trifluralin, but corn growth can be severely stunted. In addition to innate tolerance to specific herbicides, weed populations can evolve resistance to a herbicide, similarly to the development of insect resistance, after repeated selection with the same active ingredient.

During the 1980s, agricultural scientists tried in vain to take advantage of plants' natural variability to herbicide toxicity, and attempts to conventionally breed glyphosate-tolerant crops failed (Kishore, Padgette, and Fraley 1992; Padgett et al. 1995). Such failure is not surprising; after 25 years of glyphosate use, plant resistance in the field had been noted in only several grass species (Heap 2000, 2002) and one broadleaf species (VanGessel 2001). When techniques became available for manipulating molecular genetics, i.e., transferring specific genetic sequences from one organism to another, the stage was set for purposefully making plants resistant to glyphosate and other herbicides.

Three principle mechanisms are responsible in all instances where pest resistance evolves. These include increased ability to detoxify the pesticide; altered biochemical site of interaction with the pesticide (i.e., target site insensitivity); and lack of uptake (or penetration) and translocation of the pesticide (Kishore, Padgette, and Fraley 1992; OECD 1999a; Preston and Mallory-Smith 2001). In the first two mechanisms, a protein or enzyme interacts with the pesticide. Both mechanisms involve altered protein functioning and/or production. In the case of detoxification, the proteins involved are enzymes that possess an enhanced capacity for breaking down the herbicide. Biochemical sites attacked by a herbicide also may be enzymes or, alternatively, receptors that trigger a cascade of physiological reactions. Altered enzymes and receptors of tolerant or resistant plants have less affinity than their "normal" counterparts for binding the herbicide.

Commercial varieties of biotechnology-derived[1] herbicide-tolerant crops depend largely on resistance to the herbicide glyphosate (marketed in one of several Roundup® formulations) or the herbicide glufosinate ammonium (the active ingredient in formulations known as Liberty) (Owen 2000, OECD 1999a). The glyphosate tolerance trait is used in field crops for weed control, but the glufosinate tolerance trait has its practical utility as a selection marker to help produce the commercial insect-protected plants.

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[1] Biotechnology-derived refers to the use of molecular biology and/or recombinant DNA technology, or in vitro gene transfer, to develop products or impart specific capabilities in plants or other living organisms.

Biochemical Basis and Genetic Sources for Glyphosate Resistance

All Roundup Ready® crops contain an enzyme known as EPSPS (5-enolpyruvylshikimate-3-phosphate synthetase) that is less sensitive to the effects of glyphosate. Glyphosate is essentially a phosphorus-containing form of the nonessential amino acid, glycine. EPSPS is found in all plants, fungi, and bacteria but is absent in animals (Padgette et al. 1995). The enzyme is an important catalyst in the biochemical pathway for synthesis of the aromatic amino acids phenylalanine, tryptophan, and tyrosine. Because animals do not contain EPSPS, aromatic amino acids are considered essential; they must be ingested in the diet. Thus, glyphosate is reasonably safe to animals by virtue of the differences between plant and animal biochemistry.

EPSPS is localized in the chloroplasts of plants, the cell organelle responsible for photosynthesis. Glyphosate binds with high affinity to EPSPS, inhibiting its synthetic activity. The cell's inability to produce aromatic amino acids eventually leads to cell death. The tolerant form of EPSPS has a low affinity for binding glyphosate yet it still is capable of synthesizing the amino acids just as efficiently as the glyphosate-susceptible EPSPS (Kishore, Padgette, and Fraley 1992; Padgette et al. 1995).

Some Roundup Ready® (RR) crops (for example, canola and one cultivar of corn, which is not commercially available) also have been transformed to contain an enzyme called glyphosate oxidoreductase, or GOX. GOX, normally found in a common soil bacterium, *Ochrobactrum anthropi* strain LBAA, quickly metabolizes glyphosate into glyoxalate and aminomethylphosphonic acid (AMPA). Glyoxalate is a naturally occurring plant biochemical involved in carbon cycling and AMPA is of no toxicological concern in food (OECD 1999b).

A plant is made resistant to glyphosate by essentially mimicking nature. Whatever the mechanism of herbicide tolerance, genes ultimately determine the characteristics of the proteins. Researchers either search for the genes of an organism that possesses a detoxification mechanism (such as GOX from *O. anthropi*) or add chemical reagents (i.e., mutagens) to plant cells *in vitro* to change the genetic code and produce an "altered" enzyme (i.e., one with less affinity for glyphosate).

All the currently commercialized RR crops, including canola, soybean, cotton, and corn, contain a tolerant EPSPS gene obtained from one or two sources. For soybean, cotton, canola and some corn cultivars, the glyphosate-resistant EPSPS was obtained from a soil bacterium in the genus *Agrobacterium* (strain CP4)

Table III-1. Source of trait genes and ancillary genetic elements in Roundup Read® crops.

	Canola	**Corn**	**Cotton**	**Soybean**
EPSPS[1]	Agrobacterium CP4	Corn (mutagenized)	Agrobacterium CP4	Agrobacterium CP4
CTP[2]	*Arabidopsis*	Sunflower & corn	*Arabidopsis*	petunia
GOX[3]	*Ochrobactrum anthropi* strain LBAA	Not present	Not present	Not present
Promoter Sequence	Figwort mosaic virus	Rice	Cauliflower mosaic virus	Cauliflower mosaic virus
Terminator Sequence	pea	*Agrobacterium tumefaciens*	*Arabidopsis*	*Arabidopsis*
Antibiotic resistance marker gene	Streptomycin (not expressed)	Beta-lactamase (not expressed)	Neomycin phosphotransferase II (not expressed)	Neomycin phosphotransferase II (not expressed)

1EPSPS: 5-enolpyruvylshikimate-3-phosphate shythetase
2CTP: chloroplast transit peptide
3GOX: glyphosate oxidoreductase
5 enolpvruvvlshikimate - 3 phosphate synthetase

(USDA-APHIS 2000; Nida 1996; Padgette et al. 1995) (Table III-1). For one cultivar of corn, the EPSPS gene from corn itself was altered through chemical mutagenesis, which changed several amino acids to make the enzyme insensitive to glyphosate binding (Sidhu et al. 2000).

Biochemical Basis and Genetic Sources for Glufosinate Resistance

Glufosinate ammonium is a mixture of biologically active (designated L) and inactive (designated D) forms of the rare, naturally occurring amino acid, phosphinothricin. L-phosphinothricin is produced by two soil microbes that are classified as actinomycetes (OECD 1999a). Phosphinothricin is an analog (similar in structure) to the nonessential amino acid glutamate. It inhibits the enzyme glutamine synthetase that is the only mechanism plants possess for detoxifying a buildup of ammonia that is produced during amino acid metabolism. Ammonium ions are transferred through the catalytic action of glutamine synthetase to glutamate to produce glutamine. Animals also possess glutamine synthetase but they detoxify ammonia by shuffling it into urea synthesis and subsequent urinary excretion. This difference between animals and plants makes glufosinate reasonably safe to animals.

The enzyme phosphinothricin acetyltransferase (PAT) can rapidly degrade glufosinate. Thus plants producing this enzyme can quickly degrade glufosinate so that it does not accumulate in high enough concentrations to inhibit glutamine synthetase.

The sources of PAT genes are the soil microbes *Streptomyces viridochromogenes* and *S. hygroscopicus.* These actinomycete bacteria naturally occur in the soil and are nonpathogenic to plants and animals. The enzyme inactivates glufosinate by attaching a short carbon chain (acetyl group), producing the nontoxic amino acid, acetyl phosphinothricin. PAT has no ability to acetylate other amino acids (OECD 1999b).

Preparing the Genes for Transfer to Plants

Once discovered, tolerant EPSPS, GOX, or PAT genes can be isolated easily from plant or bacterial cells. Before transfer to other recipient plant cells, however, the genes must be modified to be capable of translation into proteins. Basically, the genes are linked to other pieces of DNA that serve as start and stop signals (promoter and terminator sequences, respectively) for "reading" the herbicide-tolerant gene. Modification of the desired trait gene is accomplished in an intermediate organism or host known as a vector (Dyer 1996).

The most common gene vector is a nonpathogenic strain of the *Escherichia coli* bacterium that commonly inhabits mammalian intestines. The genetics and structure of the *E. coli* chromosome are understood thoroughly. More importantly, *E. coli*, like many other bacteria, contain in addition to their chromosome a smaller piece of double stranded DNA called a plasmid. When plasmids replicate, they can make numerous copies of desirable genes. Thus, *E. coli* can serve as a factory for gene synthesis or cloning, making it an excellent vector for transferring genes from one host to another.

Using variously published and/or proprietary techniques, the molecular biologist piece by piece links to the *E. coli* plasmid the desirable sets of promoter and terminator DNA that will allow translation of the herbicide-tolerant gene into the EPSPS enzyme. These "translator" sequences of DNA come from other plants and their naturally associated viruses (Table III-1). For example, the source of the promoter for soybean and cotton was the cauliflower mosaic virus DNA known as CamV 35S (Nida 1996; Padgette et al. 1995). A rice promoter DNA sequence was used for corn (USDA-APHIS 2000) (Table III-1). A terminator sequence, which signals the end of the gene message, was supplied by attaching part of an *Arabidopsis* gene called nopaline synthetase to the plasmid vector. Neither the promoter nor terminator sequences are translated into a protein product.

Other DNA sequences and/or genes are spliced onto the vector plasmid to aid proper functioning of the herbicide-tolerant EPSPS gene after it is transferred to the plant cells. For example, plant EPSPS is synthesized with a small, attached protein called the chloroplast transporter peptide (CTP). This peptide helps carry the EPSPS from its site of synthesis in the cytoplasm to the chloroplast, the cell organelle responsible for photosynthesis. The peptide is cleaved from the EPSPS at this point to make it a functional enzyme. The source of the CPT DNA is the petunia plant for soybean, the *Arabidopsis* plant for cotton and canola, and a combination of sunflower and corn itself for corn (Nida 1996; Padgette et al. 1996b; USDA-APHIS 2000) (Table III-1).

Not all the *E. coli* cells will contain the right combination of elements on their plasmid. To help select out only the *E. coli* cells containing the plasmids with the right combination of genes, marker sequences of DNA (i.e., selection markers) also are linked to the plasmid. Some common markers are genes for antibiotic resistance (Table III-1). One selection marker is a gene coding for an enzyme (NPTII, neomycin phosphotransferase II) that makes bacteria resistant to neomycin. Another selection marker is a gene (*bla*) coding for the enzyme beta lactamase that breaks down beta-lactam antibiotics. Such antibiotic resistance genes are already widely disseminated among bacteria in the environment (Gebhard and Smalla 1998). When bacteria are exposed to neomycin or the beta-lactam antibiotic, ampicillin, plasmids without the linked EPSPS and NPTII or *bla* gene will die. The remaining living cells will be further cultured to build up large amounts of the vector plasmid. In general, the antibiotic resistance markers are not functional genes in the crops. The specific markers used, even if functional, do not pose concern for antibiotic resistance in human pathogens (Salyers 1999).

GENE TRANSFER TECHNIQUES

The bacterial plasmids can be introduced into plant cells in one of two ways. The oldest way of transferring DNA is to allow the vector bacteria (i.e., the *E. coli*) to transfer its plasmid to a plant parasitic bacterium called *Agrobacterium tumefaciens*. *Agrobacterium tumefaciens* is responsible for crown gall disease (Chilton 2001; USDA-APHIS 2000). The disease is caused by insertion of *A. tumefaciens* plasmid DNA (Ti) into tissue wounds on plants. When used for introduction of trait plasmids into plants, A. *tumefaciens* Ti is disarmed of its disease traits without affecting its natural ability to transfer directly into plant cells. The plasmid DNA transferred from *E. coli* inserts into the Ti plasmid of A. *tumefaciens*. The recipient plant cells are co-cultured with A. *tumefaciens* containing the transformed Ti plasmids, which are then “injected” into the cells of embryonic-like plant tissue known as a callus. Until the mid-1990s, the technique worked well only with dicotyledon plants (canola, cotton, soybean) but not with grasses (wheat, corn). Ishida and colleagues (1996) demonstrated that this barrier no longer exists when they showed that A. *tumefaciens* does work with corn.

Another method for transferring genes is to shoot them into the plant cells using a technique called biolistics (Klein et al. 1987). The *E. coli* cells are broken apart to recover the reconstructed plasmids containing all the trait genetic elements and, typically, the plasmid backbone is removed. The plasmids are coated on miniscule tungsten or gold particles and fired at very high velocity from a gun-like device into a plant callus culture. The particles pass through the cell wall and plasma membrane. The DNA diffuses from the particles and enters into the nucleus of the calli cells where it is incorporated into the genome.

Whether the herbicide tolerant gene-containing plasmids are transferred to plant calli by A. *tumefaciens* or by biolistics, not all of the callus cells will incorporate the DNA successfully. Thus, another round of selection is imposed on the cultured plant tissue. Basically, the plant tissue is exposed to different doses of glyphosate or glufosinate and the tissue showing no signs of toxicity is grown into a whole plant. The resulting plants are allowed to flower, pollinate, and produce seed for further testing.

PLANT-INCORPORATED PROTECTANTS

The techniques used to transform plants for pest-protected traits are the same as those used for herbicide-tolerant plants and will not be repeated in this section. All currently registered commercial cultivars of pest-protected corn are based on insecticidal proteins from one of several subspecies of the microorganism *Bacillus thuringiensis (Bt)*, and the transformed plants are simply known as *Bt* crops. The specific protein that conveys the pest protected trait has no identical physiological or enzymological function in plants, as is characteristic of glyphosate-tolerant plants.

Because of this, the source of the *Bt* traits, their biochemistry, and early attempts to improve the microorganism itself using biotechnological techniques can provide a context for further examining the environmental impacts of *Bt* crops.

Biochemical Basis for Bt Plant-incorporated Protectants

Bacillus thuringiensis (Bt) was first isolated in 1901 from a diseased silkworm moth in Japan. In 1911, E. Berliner isolated a similar microbe from a diseased flour moth in Germany and gave *Bt* its current scientific name (Van Frankenhuyzen 1993). The association of *Bt* with insect pathogenicity suggested its application as an insecticide to control the European corn borer (*Ostrinia nubialis*) in Europe during the late 1920s. Inquiries of the factors responsible for *Bt*'s pathogenicity did not begin until the 1950s and culminated in the late 1980s with an understanding of the molecular basis of its toxic mechanism (Gill, Coweles, and Pietrantonio 1992). However, *Bt* microbial preparations were used to control pests prior to understanding how they worked.

When nutrients are plentiful and pH and temperature are favorable (as in an insect body), *Bt* grows rapidly and reproduces asexually by simple cell division (a.k.a. vegetative growth). As nutrients in their immediate environment become limiting, *Bt* cells produce a spore that only germinates when conditions become favorable again. At the time of sporulation, *Bt* also produces a crystalline proteinaceous inclusion called the parasporal body. When certain insect species incidentally ingest the sporulated *Bt* cells with their parasporal body, the alkaline midgut (i.e., insect digestive tract) solubilizes the crystalline parasporal body releasing protein toxins known collectively as delta-endotoxins (Gill, Coweles, and Pietrantonio 1992). The endotoxins are actually protoxins that must be cleaved by insect midgut proteases into the molecular form that eventually kills the insect.

The toxic protein fragment binds to specific molecular receptors on susceptible insects' midgut cells, causing the membranes to lose their integrity and the gut tissue to swell up (Gill, Coweles, and Pietrantonio 1992). The insect stops feeding and eventually starves to death. A dying insect is probably the most favorable environment for *Bt* growth and reproduction. As the insect body completely decays due to bacterial septicemia, the spores and proteins disperse into the environment where they can be ingested by other unsuspecting insects.

Bt spores and proteins are found ubiquitously in soils, plant foliage, and stored grains, but growth in those environments has not been proven. Indeed, epizootics (i.e., disease outbreaks among animals) of *Bt* among insects are rare if they occur at all. *Bt* spores may be fairly stable in soil after an initial extensive degradation and or predation by other soil microorganisms (Petras and Casida 1985). On plant foliage, the spores and crystal proteins are subject to degradation if exposed to direct sunlight. Thus, the amount of *Bt* available to susceptible insects may be too limited to cause a natural outbreak of disease.

Diversity of Bt Protein Toxins

The first commercial *Bt* products were simply fermentation cultures of isolates having similar host specificity and potency as the original isolates. A product called Sporeine was available in 1938 in France for control of flour moths (Van Frankenhuyzen 1993). The first commercial product in the United States, Thuricide, appeared in 1957.

Bt products deployed in agriculture and forestry prior to the 1970s produced inconsistent results. *Bt* seemed to be pathogenic only to very specific species in the Order Lepidoptera (moths and butterflies). Furthermore, it was only toxic to young larvae. In 1970, a new isolate of *Bt* was discovered that was up to 200 times more active against pests targeted by the existing *Bt* products. This new isolate, which represented a new subspecies, was called *kurstaki* and was given the appellation HD-1. *Bt kurstaki* HD1 became the benchmark for comparing the potency of all future *Bt* isolates.

Further *Bt* isolates were discovered and found to be variably pathogenic to different species of the Order *Lepidoptera*. *Bt* was not considered a general insect pathogen, but during the 1970s a strain toxic to primitive flies of the Order Diptera (mosquitoes and blackflies) was isolated and named subspecies *israelensis*. By 1980, a commercial product was being sold for

Table III-2. Partial Listing of Endotoxins from Different *Bt* Strains and Their Spectrum of Insecticidal Activity

***Bt* STRAIN OR SUBSPECIES**	**INSECT HOST**	**DELTA-ENDOTOXINS [1/]**
kurstaki HD-1	*Lepidoptera; Diptera*	*Cry*1Aa, *Cry*1Ab, *Cry*1Ac, *Cry*2A, *Cry*2B
thuringiensis HD-2 [2/]	*Lepidoptera*	*Cry*1A, *Cry*1B
aizawai	*Lepidoptera*	*Cry*1Aa, *Cry*1Ab, *Cry*1C, *Cry*1D, *Cry*1F
entomocidus	*Lepidoptera*	*Cry*1Aa, *Cry*1B, *Cry*1C
tenebrionis	*Coleoptera*	*Cry*3A
israelensis	*Diptera*	*Cry*4A, *Cry*4b, *Cry*4C, *Cry*4D

1/ All endotoxins are named by the suffix *Cry* which stands for crystalline protein and a number and letter system to designate affinities in toxicity and genetic specificity. *CryI* and *Cry2* are specific toxins for *Lepidoptera*, *Cry3* are specific for *Coleoptera, Cry4* are specific for *Diptera*, and *Cry2A* is also toxic to *Diptera*.

2/ *Bt* thuringiensis HD-2 also produces an exotoxin (extracellularly secreted) that can be toxic to non-insect organisms. This strain is not used commercially in *Bt* products.

Source: Tabashnik 1994

control of mosquito and blackfly larvae; aquatic invertebrates and fish were unaffected by this new strain (Becker and Margalit 1993).

In 1982, a new *Bt* strain named subspecies *tenebrionsis* was isolated from a dead pupa of the yellow mealworm beetle, *Tenebrio molitor* (Order Coleoptera) (Keller and Langenbruch 1993). *Bt tenebrionsis* was particularly pathogenic to beetles in the Family Chrysomelidae (a.k.a. leaf beetles, which includes the Colorado potato beetle, *Leptinotarsa decemlineata*).

Today approximately 280 unique *Bt* strains have been isolated from insects, soils, foliage, and grain dust (http://epunix.biols.susx.ac.uk/Home/Neil_Crickmore/Bt/toxins.html). New strains are differentiated by the characteristics of their crystalline protein, its gene sequence, and its spectrum of insecticidal activity. All the strains have been organized into major groupings depending on their spectrum of insecticidal activity (Table III-2). The various strains contain one or more different endotoxins that are designated by the acronym *Cry* (for crystalline protein) followed by a number and letter (Table III-2).

The tremendous diversity of *Bt* can be explained, in part, by the genetics controlling formation of the parasporal body. The genetic information that codes for the production of the crystalline protein is contained on extrachromosomal circular pieces of DNA called plasmids. The plasmids of any one *Bt* cell can be exchanged with or transferred to other *Bt* cells through a bacterial mating process called conjugation. Indeed, in nature one *Bt* strain may have created new strains by recombination of the DNA between conjugating cells (Lereclus, Delecluse, and Lecadet 1993).

Early Attempts to Improve the Efficacy of Bt through Biotechnology

The specificity of different *Bt* strains for their insect hosts is an advantage in pest control because the probability of adversely affecting nontarget organisms is drastically decreased compared with broad spectrum control methods, such as insecticide use. On the other hand, this specificity coupled with *Bt*'s lack of environmental persistence detracts from its usefulness. *Bt* may be easy to grow in fermentation cultures, but in the environment it must be consumed by a feeding, susceptible insect to ensure its reproduction and dissemination. Thus, to be effective, *Bt* must be sprayed on foliage where it is accessible to insects feeding on leaf surfaces. *Bt* sprays are ineffective if insects are feeding on the bottom surface of the leaf or if they burrow inside the plant tissue. *Bt*'s susceptibility to degradation by sunlight necessitates frequent spraying of crops with high pest infestations. Although *Bt* formulations have been most widely used to control larvae of forest attacking insects, including the spruce budworm and the gypsy moth, agricultural use has been limited in part by the aforementioned problems with rapid degradation in sunlight and inability to control pests that are already boring inside tissue.

The limitations of the use of *Bt* formulations as insecticidal sprays eventually led to biotechnological methods for improving application of the toxin. In the early 1980s, a plasmid containing the toxin gene was transferred successfully into *E. coli* bacteria, making it possible to sequence the gene and develop probes that could be used to screen isolates for DNA sequences associated with the toxin (Van Frankenhuyzen 1993). *E. coli* was the first biotechnology-derived *Bt* toxin-containing organism, but its use was purely for research to understand the gene structure and how gene expression was regulated. Today, *E. coli* is used as a host organism to construct and clone multiple copies of the *Bt* toxin gene and associated DNA sequences like promoters and terminators required for plant cells to read the DNA code. Antibiotic-resistance genes are spliced into the DNA sequences being cloned to provide a mechanism for selecting only *E. coli* cells containing the proper genetic constructs during culturing.

The discovery of the endotoxin genes and their diverse specificity enabled efforts to make *Bt* a broad-spectrum insecticide by combining genes from different subspecies. Gene manipulation without using techniques of genetic engineering (a.k.a. recombinant DNA technology), such as those used with the aforementioned *E. coli* experiments, has been used successfully to bring several different toxic protein genes into a single *Bt* strain (Gelernter and Schwab 1993). Ecogen Inc. has successfully developed *Bt* strains toxic to both Coleoptera and Lepidoptera by using conjugation between two *Bt* strains. Products of higher potency have been similarly produced for controlling lepidopteran forest and vegetable insects.

Another method for introducing the plasmid containing *Bt* toxin into different *Bt* strains is through the use of electroporation (Gelernter and Schwab 1993). When bacterial cells are subjected to an electrical field, pores open up in the membranes allowing the DNA-containing plasmids to enter the cell. The endotoxin *Cry*3A gene (Table III-2), which is active against certain beetles, was transferred to the *Bt israelensis* strain that already contained the lepidopteran active endotoxin *Cry*1Ab gene. The resulting "improved" strain not only had activity against Lepidoptera and Coleoptera as predicted, it also exhibited activity against mosquitoes. The unexpected activity of the manipulated *Bt* strain suggested that the endotoxin proteins could interact synergistically to expand toxicity to insect species not affected by either toxin.

Recombinant DNA technology has been used to improve the stability of *Bt* sprays. Mycogen Corporation engineered the toxic protein gene into the common soil bacterium, *Pseudomonas fluorescens* (Gelernter and Schwab 1993.). The cultured bacteria express the protein but do not produce a spore. The cells are killed and then formulated into a spray containing the encapsulated *Bt* protein. Because the organisms are dead, the regulations concerning release of live biotechnology-derived organisms are not applicable to the product. The encapsulated protein is significantly more resistant to light degradation than the native *Bt* spray formulation. The technique, known as CellCap, has resulted in four different EPA registrations (http://www.epa.gov/oppbppd1/biopesticides/ai/nonviable_microbials.htm).

The utility of *Bt* has been extended to convey three highly desirable properties: stability, ease in delivery, and diversity in pest control. Upon discovery that desirable genes could be cloned into the plasmid DNA of the crown gall bacterium, *Agrobacterium tumefaciens*, a bacterium itself could become the delivery system for a *Bt* toxin. By the late 1980s, tobacco, tomato, potato, and cotton had been transformed to express a *Bt* toxin using the *Agrobacterium* gene transfer system (Ely 1993).

In addition to use of the A. *tumefaciens* transformation system, a unique way of expressing the *Bt* toxic protein in a plant involved cloning the gene into an endophytic bacterial species *Clavibacter xyli*.

Endophytic bacteria invade the vascular system of plants (Gelertner and Schwab 1993). Corn seed is inoculated with the engineered bacteria, which replicate inside the plant and express the toxic protein. Crop Genetics International Corporation developed this novel method of inserting the toxin without transforming the plant genome. The product (trade name, InCide), however, is not currently registered for use in the United States.

Many pest-protected crops currently on the market have been produced through biolistic injection of cloned plasmids with the *Bt* gene constructs directly into plant callus cultures. The constructs contain either the PAT, NPTII, or resistant EPSPS gene as selection markers. The process of selecting successful transformed plants is similar to that for herbicide-tolerant plants. However, herbicide-tolerant Bollgard cotton was developed with utilizing an herbicide tolerance marker. Similarly, though the CP4 EPSPS marker was used during transformation, the event Mon810 did not receive the CP4 EPSPS sequence. Additionally, the tissue from mature plants can be fed to target insect pests to determine if the *Bt* traits are properly expressed throughout the plant tissue.

TESTING TRAIT STABILITY

In the case of both herbicide-tolerant and pest-protected plants, insertions of new genetic characters are tested repeatedly for inheritance characteristics and stability of function. Plants are grown to reproductive stage for several generations under controlled greenhouse and approved field test site conditions in the presence of selection pressure to ensure that the newly acquired trait is stable. Mendelian inheritance is checked along with evidence for dominance. Plants not exhibiting Mendelian inheritance are not pursued. Molecular detection of the inserted gene and associated DNA sequences are used to characterize the number of gene copies in the plant genome. All commercial biotechnology-derived cultivars have exhibited Mendelian segregation and inheritance of the trait gene in a manner consistent with dominant alleles. Depending on the crop, from one to three copies of the inserted genes are present in the genome.

Once inheritance patterns and stability of the trait are confirmed under controlled conditions, seeds are planted in the field in numerous locations for several years. Plants are compared with their original non-transformed cultivars, which are called parental lines. Selection pressure under field conditions is applied to the transformed cultivars. For example, herbicide-tolerant crops are sprayed with various rates and frequencies of the target herbicide. Pest-protected plants are tested for trait stability under field conditions by artificially infesting plants with target pests and by allowing natural infestations to develop. Plant growth parameters and yields are monitored to ensure similarity in production with the parental and isogenic lines grown at the same time under the same soil and management practices.

IV. Soybean

INTRODUCTION

Herbicide-tolerant soybean is the most extensively planted biotechnology-derived crop in the world, accounting for 46% of total soybean acreage and 63% of total genetically modified crop acreage in 2001 (James 2001b). Other biotechnology-derived traits for improved pest management have been and continue to be developed. As with any new technology, especially one that could be released on vast areas worldwide, consideration of the potential environmental impacts of biotechnology-derived crops is desirable. Indeed, regulatory agencies worldwide assess these potential impacts before commercialization. This chapter provides a review of the scientific literature regarding the potential environmental impacts of biotechnology-derived soybean varieties in comparison with those of conventionally bred varieties. A similar review was recently conducted by a European group, examining the agronomic and environmental impacts of glyphosate-tolerant soybean in the United States (Hin, Schenkelaars, and Pak 2001). That study examined the patterns of adoption, yields, pesticide use and toxicity, farm level economic impacts, reasons for adoption, weed resistance, weed shifts, energy consumption and carbon dioxide production, and weed management alternatives. The conclusions of that report regarding the impact of glyphosate-tolerant soybean are similar to those reported here.

Soybean is one of the largest acreage crops in the world, grown on approximately 178 millions acres (72 million hectares) worldwide (USDA–NASS 2001d). Total crop value is estimated at $32.5 billion/year [M. Ash, 2001, USDA ERS, Personal communication]. The crop is valued for its unique chemical composition, useful in both food and feed. As with all crops, farmers strive to increase yields and quality; therefore, plant breeders endeavor to increase yield potential and to improve quality traits and pest control characteristics. Before the advent of modern agricultural science, farmers would regularly select the plants with the best characteristics for replanting. More recently, scientists have achieved significant varietal improvements through breeding, including induced mutation. In the past several decades, biotechnology has been used to achieve improved crop characteristics.

Biotechnology extends conventional breeding efforts to develop improved pest management traits. Pest management traits developed through both conventional and genetic engineering techniques include herbicide tolerance, insect resistance, nematode resistance, and disease resistance.

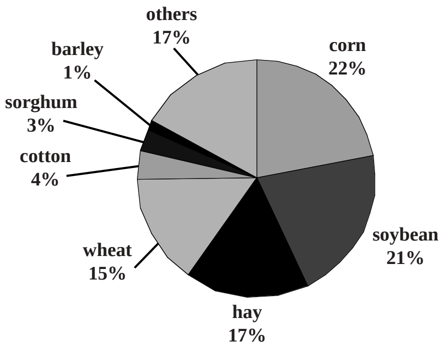
Potential environmental impacts that might be associated with these traits include changes in pesticide use patterns, tillage practices, air quality, water quality, weediness, outcrossing, pest resistance, pest population shifts, nontarget impacts, land use efficiency, and human exposure. All these potential impacts of biotechnology-derived soybean in comparison with those of other soybean are considered in this document. Finally, an overview of the economic issues surrounding biotechnology-derived soybean is provided.

Background

Soybean (*Glycine max*), a member of the Leguminosae family, is indigenous to East Asia (Oerke 1994) and first was cultivated in China 5,000 years ago. The crop was introduced into North America in the eighteenth century (Hymowitz and Harlan 1983) and originally was used as a forage crop, or was grown with corn to increase soil nitrogen. Successful use of soybean as an oilseed crop was first achieved in Europe in the early 1900s, and use in the United States followed (Smith and Huyser 1987). Before World War II, the United States imported 40% of its edible fats and oils. As war broke out and foreign supplies were cut, processors turned to domestic farmers for soybean oil production (Smith and Huyser 1987). Soybean meal had already become an accepted ingredient of livestock and poultry feed by the 1920s (Paul et al. 1989).

Discovery of the valuable uses of soybean products, in combination with decreased imports, led to increased acreage devoted to soybean through the early decades of the twentieth century. With yield and

Figure IV-1. U.S. soybean area harvested by crop 2000



Source: USDA-NASS 2001d

quality improvements realized through breeding efforts, the development of modern crop production techniques, and expanding markets, soybean acreage continued to increase throughout the century, in the United States and abroad. Soybean currently is the second largest acreage crop (Figure IV-1) and the third largest cash crop in the United States (USDA–NASS 2001d) and the fourth largest acreage crop in the world, after coarse grains, wheat, and rice, and before cotton [M. Ash, 2001, USDA ERS, Personal communication].

Soybean's unique chemical composition makes it a valuable agricultural commodity. The soybean seed consists of fats, proteins, carbohydrates, minerals, and vitamins. Soybean seed contains 20% oil, an amount second only to that in peanut (48%) among food legumes, and also contains 40% protein, the highest amount of all legumes and cereals (Liu 1997). Soybean accounted for 29% of the vegetable oil market and 67% of meal production worldwide in 2000 (ASA 2001b).

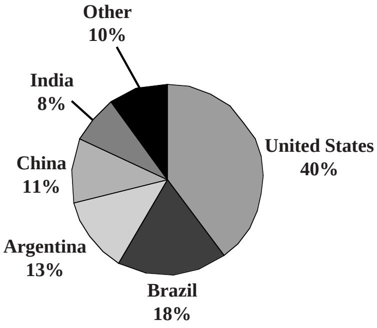
Soybean is a dominant source of oil and protein, with a multitude of uses in both human food and animal feeds. Soybean oil is rich in polyunsaturated fatty acids and contains no cholesterol. Soybean oil has numerous applications in bakery products, pharmaceuticals, lubricants, pesticides, and cosmetics. Soybean oil is used in food products such as margarine, salad dressings, shortening, and cooking oils. Lecithin, extracted from soybean oil, is commercially important as an emulsifier in foods such as candy bars, peanut butter, and certain baked goods (ASA 2001b). Soybean oil also is used in the production of a petroleum-substitute called biodiesel. Biodiesel is environmentally more favorable as it is less chemically complex than gasoline or diesel fuel and burns with fewer pollutants.

Soybean protein is used in a variety of foods and feeds. Soybean contains eight essential amino acids that are necessary for human nutrition and are not produced naturally in the body. Soybean protein is used in livestock and poultry animal feed and in pet foods such as fish food. In addition, soybean protein is used in pharmaceuticals, textiles, and paints, and possesses numerous industrial applications for emulsification, dispersion, stabilization, anti-foaming, and adhesion (ASA 2001b; Smith and Huyser 1987).

World Soybean Industry

In terms of total value, soybean is the seventh largest commodity in the world (World Bank 2000)[1]. The United States is the world's largest soybean producer. During the 1999/2000 growing season, the United States had 72.4 million acres (a.) [29.3 million hectares (ha)] under cultivation, or 41% of total world acreage, followed by Brazil with 33.1 million a. (13.4 million ha) or 18% of total world acreage. Other major soybean-producing countries are Argentina (13%), China (11%), and India (8%) (Figure IV-2; USDA–NASS 2001d). The United States, Brazil, and Argentina produced 79.1, 41.5, and 27.0 million metric tons (t) of soybean, respectively, in the 2000/2001 season (USDA–ERS 2001b). Total world crop value was $32.5 billion [M. Ash, 2001, USDA ERS, Personal communication]. Total U.S. soybean crop

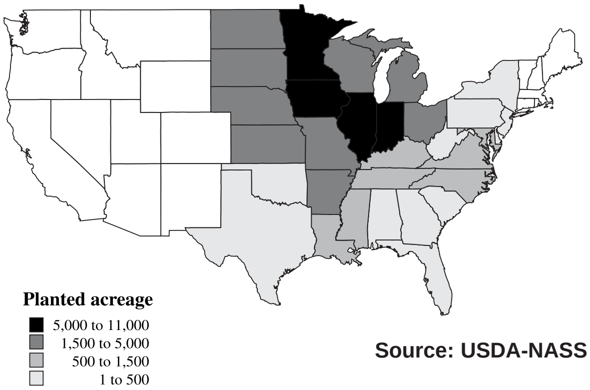
Figure IV-2. World soybean production 1999/2000



Source: USDA-NASS 2001d

value was $12.4 billion in 2001 (USDA–NASS 2002a). The United States exports approximately one-third of the soybean it produces. China is the largest market for U.S. soybean, accounting for 17% of total U.S. soybean exports. Other major markets include Japan (14% of U.S. exports), Mexico (13%), and the Netherlands (9%) (USDA–NASS 2001d). Brazil and Argentina export approximately 28% and 24% of their total production, respectively (USDA–ERS 2001b).

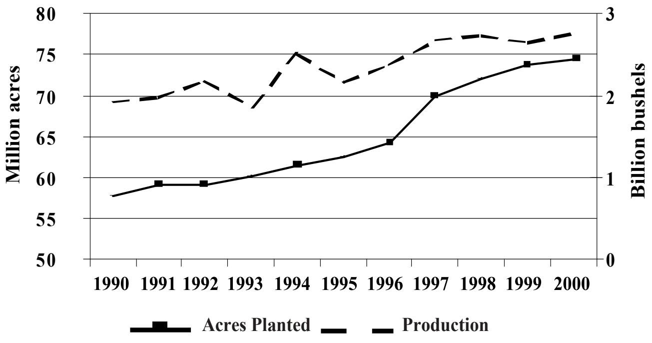
Figure IV-3. U.S. soybean production



Source: USDA-NASS 2001a

United States soybean production is concentrated in the midwestern states. Iowa and Illinois together account for nearly 30% of total soybean acreage in the United States. Significant production also is located in the southern Mississippi River Valley, the southern coastal plains, and along the Eastern Seaboard (Figure IV-3; USDA–NASS 2001d). Brazil's major soybean producing areas are located in two distinct regions, the South and the Center-West (USDA–ERS 2001b). Nearly all soybean production in Argentina is located in the northeastern one-third of the country (USDA–ERS 2001b).

Figure IV-4. U.S. soybean production 1990-2000



Source: USDA-NASS 2002b

World soybean acreage has increased in recent years. In the United States, strong soybean prices relative to those of other crops, absence of acreage set-aside programs, and declining production costs have contributed to the expansion of soybean acreage (Figure IV-4; USDA–ERS 1998). In addition, the adoption of moisture-saving "no-till" practices has made soybean a crop of choice in western states, where acreage has increased the most dramatically (Marking 1999a). Since 1990, soybean production in Brazil and Argentina has doubled. Economic and political reforms during the early and mid 1990s, combined with improvements in transportation and marketing infrastructure, underlie these increases in output (USDA–ERS 2001b). The introduction of biotechnology-derived herbicide-tolerant soybean, used in conjunction with "no-till" or low-till production systems, also is credited for the expansion of soybean in Argentina (James 2001a).

SOYBEAN IMPROVEMENT FOR PEST MANAGEMENT TRAITS

Soybean Pest Problems

Economically important pest problems in soybean include weeds, insects, fungi, nematodes, and viruses. Weeds are considered the number one problem in all major soybean-producing countries and can decrease yields by as much as 50% (Oerke 1994). Weed problems in the United States include common cocklebur (*Xanthium strumarium*), jimsonweed (*Datura stramonium*), velvetleaf (*Abutilon indica*), morningglories (*Ipomoea,* spp.), Johnsongrass (*Sorghum halapense*), and foxtails (*Setaria* spp.) while Johnsongrass, crabgrass (*Digitaria* spp.), barnyardgrasses (*Echinocloa* spp.), fierce thornapple (*Datura ferox)*, pigweeds (*Amaranthus* spp.), and spurred anoda *(Anoda cristata)* are problematic in Argentina (Barrentine 1989; Oerke 1994; Stoller and Woolley 1985). The principal weeds of soybean in Brazil are morningglories, pigweeds, sidas (*Sida* spp.), purslane (*Portulaca oleracea*), goosegrass (*Eleusine indica*), crabgrass, Beggar's tick (*Bidens pilosa*), and small flower galinsoga (*Galinsoga parviflora)*. More than 90% of the acreage in the United States and over 70% of the soybean acreage in Argentina and Brazil are treated with herbicides, which emphasizes the importance of weeds and their control worldwide in the prevention of soybean yield losses (Oerke 1994).

The incidence and severity of insects and fungi is higher in Argentina and Brazil than in the United States, mainly because of tropical climates that favor pest infestations. Insect pressure is generally greatest in the southern United States because of increased survival of insect pests, multiple generations per year facilitated by the warm climate, and because of the area’s proximity to the tropics, where some insect pest populations overwinter. In the southern United States, the most damaging defoliating insects are velvetbean caterpillar (*Anticarsia gemmatalis*) and soybean looper (*Pseudoplusia includens*) (Higley and Boethel 1994). The larval stages of these pests feed on leaf tissue, and heavy infestations can defoliate entire fields. In addition to lepidopteran pests mentioned as problems in the United States, other prominent insect pests in Brazil and Argentina include *Nezara viridula (*green stink bug*), Piezodorus guildinii* (soybean stink bug), and *Euschistus heros* (neotropical brown stink bug) (Oerke 1994). Root and stem rot, *Diaporthe phaseolorum*, and *Colletotrichum* species are among the most damaging soybean diseases in U.S. soybean production. *Diaporthe phaseolorum* var. *sojae*, *Rhizoctonia solani*, *Fusarium* species, and *Sclerotinia sclerotiorum* infest soybean in Brazil (Oerke 1994).

Soybean cyst nematode and root knot nematodes are the principle nematode species causing economic yield losses in the United States (Oerke 1994). Root knot nematodes are the primary nematode species in South America (Oerke 1994).

The bean pod mottle virus (BPMV) is a widespread problem in the soybean growing areas of the southern and southeastern United States. Over the past 2 to 3 yr, it has become an increasingly serious production problem in the North Central and Northern Great Plains states. Bean pod mottle virus causes a mottling disease of soybean and may occur in conjunction with the soybean mosaic virus (SMV). These two viruses interact synergistically, resulting in more severe yield losses than caused by either virus alone (Reddy et al. 2001). Bean pod mottle virus alone may decrease yields by 10 to 17%, but in association with SMV may result in losses exceeding 60% (Sinclair and Backman 1989). The BPMV not only decreases soybean yield, but also predisposes soybean to *Phomopsis* seed infection, a major cause of poor seed quality in soybean (Stuckey, Ghabrial, and Reicosky 1982).

Crop Development Techniques

Development of agronomically acceptable, high-yielding, pest-tolerant cultivars allows effective management of soybean pests while potentially decreasing dependence on chemical control options. Traditional methods of soybean improvement include selection in combination with hybridization or mutation breeding. These methods have been used since the early nineteenth and twentieth centuries, respectively (Figure IV-5).

Figure IV-5. Timeline of developments in crop improvement

2,000 BC	**Cultivation**
19th Century	**Selective cross breeding**
Early 20th Century	**Mutagensis and selection**
Mid 20th Century	**Cell culture**
1930s	**Somaclonal variation**
1940s	**Embryo rescue**
1950s	**Polyembryogenesis**
1970s	**Anther culture**
1980	**Recombinant DNA**
1980s	**Marker assisted selection**
1990s	**Genomics**
2000	**Bioinformatics**

Source: Newell-McGloughlin, 2001.

Conventional breeding involves manual crossing of sexually compatible species. Plants are selected for breeding after screening for a particular trait of interest. Desirable traits from different soybean varieties may be introduced into a single variety using conventional breeding methods. A germplasm collection of approximately 18,000 different soybean lines, mostly from Asia where soybean originated, is maintained in Stoneville, Mississippi, as a source of inherent resistance when a new pest problem arises (Barnes 2000; Hartwig 1987). Within this base of genetic characteristics, soybean varieties vary in many characteristics, including the ability to withstand pest infestations. But despite the large number of accessions, the genetic base of commercially available soybean varieties is narrow, and most soybean cultivars in use are derived

Table IV-1. Sources for genetic elements of biotechnology-derived soybean

	Glyphosate-tolerant[a]	Insect-resistant (*Bt*)[b]	Bean pod mottle virus-resistant[c]
Trait gene	EPSPS from Agrobacterium CP4	*Cry*1Ab and *Cry* 1Ac from *Bacillus thuringiensis*	Bean pod mottle virus derived capsid polyprotein
Promoter sequence	Cauliflower Mosaic Virus	Cauliflower Mosaic Virus	Cauliflower Mosaic Virus; Figwort mosaic virus; peanut chlorotic streak virus
Terminator sequence	Arabidopsis	Agrobacterium	Tobacco
Marker gene	Neomycin phosphotransferase II	Hygromycin phosphotransferase	Hygromycin phosphotransferase

Source: a. Padgette 1996a.
b. Parrot et al. 1994; Stweart et al. 1996; Su et al. 1999
c. Di et al; Reddy et al. 2001

from only a few parental lines. This narrow base limits the development of soybean for such traits as pest resistance using conventional plant breeding methods (Christou et al. 1990). Limited levels of herbicide tolerance, insect resistance, and other pest control characteristics have been developed by means of conventional breeding.

Mutation breeding also relies upon the genetics of existing soybean lines. Through exposure to chemical and physical mutagens, the soybean genome is rearranged. In this manner, the production of desirable traits can result, although at low frequencies; most induced mutations have detrimental affects. Herbicide tolerance has also been developed through mutation breeding, for example a soybean resistant to sulfonylurea-containing herbicide was developed through mutation breeding.

Modern biotechnology holds much promise for the continued improvement of soybean through introduction of traits that may not be available in the current soybean germplasm. Thus far, modern biotechnology has been used successfully in the development of herbicide-tolerant, insect-resistant, and disease-resistant soybean varieties although only herbicide-tolerant soybean is the only commercialized biotechnology-derived soybean with an improved pest control trait. The sources of genetic elements for the construction of plasmids that conferred resistance to different soybean pests or herbicides are presented in Table IV-1.

TRAITS IN SOYBEAN

Herbicide Tolerance

The tolerance of soybean to herbicides that would normally damage the crop has been developed through conventional breeding, mutation breeding, and genetic engineering. Variability in the natural tolerance of soybean to commercially available herbicides such as 2,4–DB, metribuzin, and glyphosate has been recognized (Barrentine et al. 1982; Hartwig 1987). This tolerance may be improved through screening and breeding processes. Soybean varieties such as “Tracy” and “Tracy-M” were developed for tolerance to 2,4–DB and metribuzin, respectively, using conventional selection and hybridization methods (Hartwig 1987). Efforts to screen soybean germplasm for commercial levels of tolerance to glyphosate have not been successful (Kishore, Padgette, and Fraley 1992).

Mutagenesis was used successfully to create sulfonylurea tolerant soybean (STS) (Sebastian et al. 1989). The process involved soaking 450,000 soybean seeds in the chemical mutagen (ethyl methanesulfonate) and then screening and selecting the plants for sulfonylurea tolerance. The result was one plant that exhibited elevated tolerance to sulfonylurea herbicides such as chlorimuron and thifensulfuron. In a comparison of the tolerance of STS and other soybean to herbicide application, STS soybean sustained only 3% injury, while the other soybean sustained up to 65% injury (Sebastian et al. 1989).

Soybean varieties have been developed through genetic engineering to express tolerance to two herbicides: glyphosate (Roundup™) and glufosinate (Liberty™). Glyphosate-tolerant soybean (Roundup Ready™), developed by Monsanto, is tolerant to the nonselective herbicide glyphosate (Roundup™). Glufosinate-tolerant soybean (Liberty Link™) was developed by Aventis to be tolerant of another nonselective herbicide, glufosinate (Liberty™).

The development of soybean tolerant to glyphosate began in the early 1980s. Multistate field trials were conducted in Illinois, Indiana, and Iowa in 1988 and the first generation of seed for the commercially approved event was field-tested in 1991 (Padgette et al. 1996a). Genetically modified glyphosate-tolerant soybean was approved by U.S. regulatory agencies in 1994 and was first made available for commercial production in 1996.

Glyphosate inhibits 5-enolpyruvylshikimate-3-phosphate synthetase (EPSPS), which is the key enzyme in the synthesis of aromatic amino acids such as tyrosine, phenylalanine, and tryptophan. These amino acids are essential for crucial plant processes such as protein synthesis, cell wall formation, defense against pathogens and insects, production of hormones, and production of compounds required in energy transduction such as plastoquinone (Duke 1988). A gene for glyphosate-tolerant EPSPS was isolated from the soil bacterium *Agrobacterium* sp. strain CP4 and was introduced into the soybean genome by means of the particle acceleration method (Barry et al. 1992; Padgette et al. 1996a). Introduction of the glyphosate-tolerant EPSPS into soybean by genetic modification techniques conferred glyphosate tolerance in soybean. Upon glyphosate treatment, soybean expressing glyphosate-tolerant EPSPS remains unaffected because of the continued action of the introduced EPSPS that meets the plant's need for aromatic amino acids (Steinrucken and Amrhein 1980).

Glufosinate-tolerant soybean has not been commercialized [Sweet, K., 2002, Aventis, Personal communication]. Glufosinate-tolerant soybean was first field tested in 1990 (USDA–APHIS 1996b). U.S. regulatory approval for glufosinate-tolerant soybean was obtained in 1998 (USCEQ/OSTP 2001).

Glufosinate inhibits the biosynthetic enzyme glutamine synthetase, which is involved in general nitrogen metabolism in plants, including the assimilation of ammonia accumulated as a result of photorespiration and nitrate reduction. The herbicidal activity of glufosinate is based on the resulting accumulation of ammonia in cells, the cessation of photorespiration and photosynthesis, and the disruption of chloroplast (Vasil 1996). Multiple lines of glufosinate-tolerant soybean were described in AgrEvo's (now Aventis Crop Science) petition to the U.S. Department of Agriculture (USDA). All lines produce phosphinothricin acetyl transferase (PAT), an enzyme that detoxifies the herbicide. The *bar* gene that was used in the creation of glufosinate-tolerant soybean lines was a modified version of a *bar* gene from the soil bacterium *Streptomyces viridochromogenes* (USDA–APHIS 1996b).

Insect-Resistant

Insect resistance in soybean has been achieved through the use of conventional breeding and genetic engineering. Marker assisted breeding also has facilitated identification and selection of natural insect resistance. Natural insect resistance in soybean has been identified in several exotic Japanese soybean lines towards Mexican bean beetles, bean leaf beetles, striped blister beetles, and stink bugs (Clark et al. 1972; Gilman et al. 1982; Hartwig 1987; Van Duyn, Turnipseed, and Maxwell 1971). The development of elite, insect-resistant, high-yielding soybean varieties by means of conventional plant breeding techniques has proven difficult due to the genetic complexity of insect resistance and the lack of cost effective methods for evaluating insect resistance in breeding programs (All, Boerma, and Todd 1989; Rowan et al. 1991). Four cultivars developed through conventional breeding methods possessing moderate insect tolerance have been released commercially. But these cultivars had agronomically unacceptable characteristics such as inferior yields, late maturity, and lodging susceptibility (Walker et al. 2000).

Using genes from the soil bacterium *Bacillus thuringiensis* (*Bt*), genetic engineering has been used to introduce insect resistance to soybean. University of Georgia researchers made the first report of the successful expression of an insect-resistant gene from *Bt* in soybean, by means of the *Cry*1Ab gene (Walker et al. 2000). Subsequently, soybean lines were developed to express another *Bt* protein, *Cry*1Ac, by two separate groups, at the University of Georgia (Walker et al. 2000) and at Monsanto (Marking 2001). Field tests showed that *Cry*1Ac *Bt* soybean had significant resistance to velvetbean caterpillar and lesser cornstalk borer (*Elasmopalpus lignosellus*), and less pronounced resistance to corn earworm (*Heliothis zea*) and soybean looper. These insects are the major insect pests of soybean.

The availability of resistance from various sources allows the "stacking" of insect resistance traits for more broad-spectrum insect control. Stacking may be used to combine different types of native insect resistance and/or resistance developed using genetic engineering through the use of conventional breeding or any combination. Indeed, to develop insecticidal soybean with a broad spectrum of control, *Bt* soybean is being stacked with the natural insecticidal resistance from exotic Japanese soybean lines mentioned earlier (Walker et al., in press).

Insect-resistant *Bt* soybean is still in development and has not been commercialized. Field testing began in 1997 and has been conducted in several areas of the United States (ISB 2002). Monsanto's version of the *Cry*1Ac *Bt* soybean is also being field tested in Brazil and Argentina (CTNBio 2002; Marking 2001), where insects are more of a problem than in the United States.

Nematode Resistance

Nematode-resistant soybean varieties have been developed through conventional breeding and genetic engineering. As early as 1957, the lack of effective and economical chemical options for nematode control in soybean increased the pressure to screen and to identify nematode-tolerant soybean genotypes (Caviness and Riggs 1976; Hartwig 1987). Research efforts by the USDA led to the development of such soybean varieties as "Pickett," "Franklin," "Centennial," and "Govan," which were resistant to cyst and root knot nematodes using conventional selection and breeding techniques (Bernard and Shannon 1980; Brim and Ross 1966; Hartwig and Epps 1997; Hartwig, Musen, and Maxwell 1978). Recently, an eight-year phenotypic screening project at Purdue University led to the development of a germplasm, CystX, which possesses the strongest known genetic resistance to soybean cyst nematode (Marking 2000). CystX was found to possess resistance to more than 150 populations of soybean cyst nematode. CystX resistance was made available in a limited release for the 2001 and 2002 growing seasons. More extensive marketing will be facilitated by the stacking of CystX with glyphosate tolerance [C. Sylvester, 2002, Midland Genetics Group, Personal communication].

Soybean resistance to cyst nematode also is being developed through biotechnology approaches using marker-assisted selection (Bell-Johnson et al. 1998). Research is in progress at the Universities of Illinois, Georgia, and North Carolina to identify and to characterize genes for the enhanced or over expression of proteinase inhibitors that are toxic to nematodes, through the use of marker-assisted selection. Proteinase inhibitors interfere with protein digestion in nematodes, eventually leading to mortality. But all this research is at a very preliminary stage [C. N. Lambert, 2002, University of Illinois, Personal communication].

Other Soybean Pest Control Traits

Traditional crop improvement techniques have been used in the development of soybean varieties that can tolerate diseases such as Phytophthora root rot, downey mildew, bacterial pustule, target spot, and wild fire (Hartwig 1974; Hartwig, Musen, and Maxwell 1978; Hartwig and Epps 1977).

Modern biotechnology methods are being used to address another significant soybean disease, bean pod mottle virus (BPMV). Management of BPMV through conventional breeding methods has not been possible, because none of the known soybean genotypes possess resistance to BPMV. A biotechnology-derived soybean cultivar resistant to BPMV was first developed by Di et al. (1996) by transformation with BPMV coat protein precursor gene mediated via *Agrobacterium*. The transgene used in this study, however, was not stable and resulted in the loss of resistance in subsequent plant generations. More recently, Reddy et al. (2001) created a viable BPMV-resistant soybean plant through particle bombardment. The BPMV-resistant soybean plants showed little or no symptoms in response to inoculation with a virulent strain of BPMV. The stability of gene expression over multiple generations of this newly developed soybean line is still being tested.

Permits have been obtained from the USDA's Animal and Plant Health Inspection Service to evaluate the field performance and broad-spectrum resistance to different strains of the BPMV-resistant soybean in the 2002 growing season [S. Ghabrial, 2001, University of Kentucky, Personal communication]. If field resistance is stable, BPMV-resistance can be incorporated into commercial varieties using plant breeding.

ADOPTION OF BIOTECHNOLOGY-DERIVED SOYBEAN

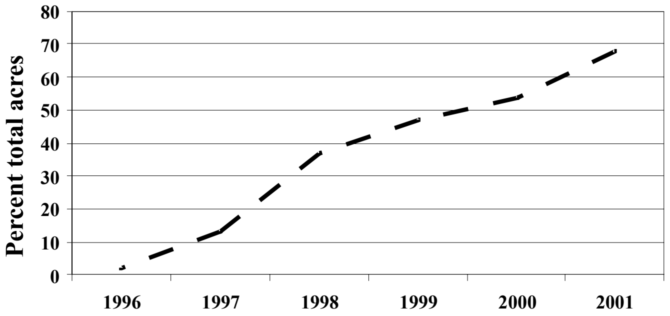
Glyphosate-tolerant soybean is the only commercially available biotechnology-derived soybean with an improved pest control trait. Insect-resistant soybean could be available in the next few years, while other pest control traits are expected further down the road. The reasons for the adoption of biotechnology-derived soybean are explored next.

Herbicide Tolerance

The commercial adoption of glyphosate-tolerant soybean may be the most rapid case of technology diffusion in the history of agriculture. Grown on 82.3 million a. (33.3 million ha) worldwide in 2001, glyphosate-tolerant soybean accounted for 46% of the total global soybean acreage (James 2001b).

Glyphosate-tolerant soybean has received regulatory approval for planting in Argentina, Canada, Japan, Mexico, the United States, and Uruguay (Nickson and Head 1999). First available in the United States and Argentina in 1996, adoption in those two countries account for 99% of total herbicide-tolerant soybean acreage in the world. The United States has the largest area planted to glyphosate-tolerant soybean, planted on 68% of the total 75.4 million acres (30.5 million ha) of soybean in 2001 (USDA-NASS 2001a, Figure IV-6). Adoption is expected to climb to 74% in the United States for the 2002 growing season (USDA-NASS 2002c). Currently, there are over 1000 glyphosate-tolerant soybean varieties available in the United States from more than 200 seed companies (Anderson 2001; Lawton 1999). Argentina has the highest adoption rate of glyphosate-tolerant soybean, planting 98% of its 27.7 million a. (11.2 million ha) to these varieties in 2001 (James 2001b). This is the highest adoption rate for any biotechnology-derived crop in the world.

Figure IV-6. U.S. adoption of glyphosate-tolerant soybean



Source: Marshall
USDA-NASS 2001a

The popularity of glyphosate-tolerant soybean is due to advantages of the technology over conventional weed control practices. It is necessary to understand the limitations of conventional weed control options to appreciate why farmers have embraced glyphosate-tolerant soybean. The major reasons farmers have adopted the herbicide-tolerant soybean so widely are lowered production costs, reduced crop injury, and simplicity and flexibility in weed management.

Weed Control in Non-biotechnology-derived Soybean

Weed control is one of the biggest challenges for soybean farmers because poorly controlled weeds drastically decrease crop yield and quality. Common cocklebur, jimsonweed, and velvetleaf are among the worst broadleaf weeds that infest soybean fields. Full season infestation of these weeds can result in soybean yield losses ranging from 12 to 80%, depending on the competing weed species and their density (Barrentine 1989; Stoller and Woolley 1985).

United States soybean farmers began switching from the use of tillage to control weeds to herbicides in the late 1950s. By the mid-1960s, about 30% of U.S. soybean acreage was treated with herbicides (USDA-

ARS 1965). Herbicide usage in soybean grew rapidly in the late 1960s and early 1970s and reached more than 90% acreage treated by 1982 (USDA–ERS 1975, 1983). Throughout the 1990s, soybean farmers have consistently treated more than 95% of soybean acreage with herbicides (USDA–NASS 1992, 1993, 1994, 1995, 1996, 1997, 1998, 1999, 2000, 2001b).

Herbicides are applied at various points of the growing season, during field preparation, planting, or crop growth. A traditional soybean weed control program includes either a preplant incorporated herbicide application before planting or a preemergence (PRE) herbicide application at planting or before crop emergence, followed by one or more postemergence (POST) applications after the crop has emerged. Alternatively, a farmer might make only POST treatments. Preplant incorporated and PRE herbicides are applied directly to the soil. The efficacy of soil-applied herbicides depends greatly on rainfall, with poor weed control resulting from extremely low or high rainfall conditions after herbicide applications. Moreover, the decision to make a soil-applied herbicide treatment, and the selection of herbicide, involves guesswork because these decisions are made anticipating the weed species that may emerge. Commonly used soil applied soybean herbicides are chlorimuron, metolachlor, metribuzin, pendimethalin, and trifluralin.

Postemergence herbicides are applied after the crop emerges from the soil, which provides farmers the opportunity to determine which weed species are emerging with their crop and enables them to better focus their weed management strategies on particular weed problems. Achieving weed control within 4 to 8 weeks after soybean emergence is crucial to avoid significant yield losses (Barrentine 1974; Eaton, Russ, and Feltner 1976). Typically, POST applications are made during this crucial period, and weed resurgence thereafter is decreased because of canopying soybean plants, which shade the ground and compete with emerging weeds (Yelverton and Coble 1991). Some commonly used POST soybean herbicides include acifluorfen, 2,4–DB, basagran, bentazon, clethodim, cloransulam, fluazifop, fomesafen, imazethapyr, quizalofop, and sethoxydim.

Soybean, as one of the world's largest acreage crops, is a major market for pesticides in general and for herbicides in particular. Currently, there are at least 70 registrations for herbicides for weed management in soybean, plus numerous mixtures (Crop Protection Reference 2002). Even with all these choices, several deficiencies persist in soybean weed control. These deficiencies include potential for crop injury, development of herbicide-resistant weeds, carryover of herbicides, narrow spectrum of control, and antagonism between broadleaf and grass herbicide tank mixes.

Herbicides are effective if they have selective toxicity to weeds without also being toxic to the crop. Many commonly used soybean herbicides, however, can cause injury to the soybean crop (Kapusta, Jackson, and Mason 1986; Wax, Bernard, and Hayes 1973). Crop injury symptoms resulting from some commonly used herbicides include stunted growth, yellowing of leaves, reddening of leaf veins, and speckling, bronzing, or burning of leaves. Although these symptoms do not always decrease yield, they sometimes delay canopy closure, which increases weed competition with the crop (Padgette et al. 1996a). But short season soybean crops, such as those planted in rotation with another crop during the same season (i.e., a double-crop), may suffer yield losses due to insufficient time to recover from this injury (Vidrine, Reynolds, and Griffin 1993). Application rates for some herbicides are intentionally kept low to decrease the possibility of crop injury. To achieve adequate weed control at low rates, weeds must be relatively small at the time of treatment. In some instances, low herbicide rates may result in incomplete weed control in heavily infested sites (Rawlinson and Martin 1998).

Another limitation to conventional programs is the development of herbicide-resistant weeds. The imidazolinone, sulfonylurea, and sulfonamide herbicides all have the same mode of action, inhibiting the acetolactate synthase (ALS) enzyme (Ahrens 1994). ALS-inhibiting herbicides such as imazethapyr and chlorimuron are used widely in soybean (USDA–NASS 1996, 1997, 1998, 1999, 2000, 2001b). In various areas of the Midwest, several ALS-resistant weed populations have developed, e.g., waterhemp, shattercane, cocklebur, and kochia (Hartzler 1997; Rawlinson and Martin 1998), that limit the effectiveness of these compounds.

Some conventional herbicides used in soybean have residual deleterious effects on rotation crops due to persistence of the herbicide in soil. This is called carryover. Carryover of an herbicide with residual activity from one planting to the next may cause economically significant levels of damage in subsequent plantings of sensitive crops other than soybean. Residues of some herbicides can remain in the soil for a year or more, and so farmers must pay close attention to the herbicide history of a field when making planting decisions. For example, guidelines specify a waiting period of 26 months (mo) after imazethapyr application before planting potato or flax and 18 mo before planting sweet corn, cotton, sunflower, oats, safflower, or sorghum (Crop Protection Reference 2002).

Finally, many soybean herbicides control only certain weeds at specific growth stages. Herbicides generally control either grass or broadleaf weeds, but seldom both. Farmers usually have several species of weeds in any particular field and would normally need to control both grass and broadleaf weeds. A combination of herbicides would therefore be necessary. But tank mixing herbicides to minimize the number of trips over the field can result in antagonism between the compounds, whereby efficacy of the grass herbicide is diminished. In this case, two passes over the field or increased rates of the grass herbicide may be necessary to avoid this antagonism.

Weed Control in Biotechnology-derived Herbicide-tolerant Soybean

The primary reason that farmers have switched from conventional weed management programs to glyphosate-tolerant soybean is the simplicity of the program: instead of using multiple herbicides to achieve adequate weed control, farmers can rely on one herbicide to control a broad spectrum of weeds without crop injury. In addition, glyphosate has no crop rotation restrictions and may be used to control weeds that have developed resistance to other herbicides.

Glyphosate is a highly effective, nonselective, broad-spectrum POST herbicide. It is effective against a wide spectrum of annual and perennial grasses and broadleaf weeds. By planting glyphosate-tolerant soybean, farmers are able to apply glyphosate directly over the crop and take advantage of this wide spectrum of weed control. Glyphosate-tolerant soybean facilitates the use of a single herbicide rather than a combination of several narrow spectrum herbicides. Control of both annual and perennial broadleaf and grass weeds can be obtained in most situations with POST applications of glyphosate alone, with no need for a tankmix partner. Glyphosate also is more effective than other herbicides against generally larger weeds, thus giving farmers more flexibility in timing herbicide treatments. This flexibility in timing allows farmers to wait for sub-optimal spraying weather, such as several days of high winds, to pass before applying the herbicide treatment. A glyphosate-tolerant soybean-based POST weed control program is less restricted by weed species, weed size, tank-mix partners, and adjuvant type than the conventional soil-applied herbicide programs are (Webster, Bryant, and Earnest 1999). Owen (2000) suggested that control of perennial weed problems such as hemp dogbane was improved in the Midwest with the adoption of glyphosate-tolerant soybean. This simplicity in weed control is the reason most often cited by farmers for the adoption of glyphosate-tolerant soybean (Owen 1997a).

Glyphosate can be applied at any stage of growth in soybean, with little or no crop injury (Tharp, Schabenberger, and Kells 1999). Further, glyphosate has no carryover restrictions, because it is degraded rapidly in the soil, which allows farmers the added flexibility of planting any crop in rotation with soybean.

Finally, improved weed control decreases the level of weed seed contamination of the harvested crop. Weed seed contamination is problematic because farmers receive decreased prices proportional to the level of weed seed contamination. Culpepper et al. (2000) found that weed seed contamination was at least 50% greater in a total PRE weed program using conventional herbicides than in POST programs where glyphosate-containing herbicides were used with glyphosate-tolerant soybean.

Insect Resistant

Insect-resistant *Bt* soybean is still under development and could be available in the next few years. Once *Bt* soybean is commercialized, adoption will depend on the level of pest pressure in different areas and on its efficacy and price compared to conventional insect control.

Chemical control remains the only consistently effective tactic for control of mid- or late- season outbreaks of insect pests on soybean (Higley and Boethel 1994). Chemical control of soybean looper is difficult, however, because it has developed resistance to a variety of conventional insecticides, including organophosphates, carbamates, and pyrethroids that have been used to manage it on the several crops it infests. Insecticide use is low in most soybean producing regions of the United States, with less than 1% of soybean acreage nationwide treated with insecticides. But approximately one-third of the soybean acreage in several Southeastern and Delta states is treated regularly.

Adoption of *Bt* soybean will occur where farmers find advantages over conventional insect control practices. For instance, *Bt* soybean may provide better control of pests such as the lesser cornstalk borer, that are difficult to control using foliar insecticides because of their burrowing nature. Soybean with innate insect protection properties will be useful in climatic regions where insect pressures justify insecticide applications.

Nematode Resistance

Crop rotation is effective in controlling soybean cyst nematode (SCN) because few crops are susceptible to SCN. Growing a nonhost for two years is generally adequate to allow a susceptible soybean cultivar to be grown. A resistant soybean cultivar may occasionally be used in place of a nonhost crop. But continuous or frequent use of resistant soybean cultivars results in SCN race shifts, and “resistance-breaking” types (i.e., races) increase. Nematicides are rarely profitable for controlling nematodes in soybean. Several soybean cultivars are tolerant to root knot nematode species (Sinclair and Backman 1989).

Adoption of biotechnology-derived nematode-resistant soybean varieties, when they might become available, will depend on efficacy, cost, and spectrum of control compared to other management practices, such as crop rotation and conventionally developed SCN-resistant varieties.

Virus Resistance

Bean pod mottle virus is transmitted efficiently in nature, within and between soybean fields, by several species of chrysomelid beetles. Cultural control practices are recommended for control of BPMV, including planting nonhost plants between soybean fields and controlling broadleaf weeds in areas next to soybean fields (Sinclair and Backman 1989). Like nematode-resistant soybean varieties, biotechnology-derived virus-resistant varieties will be adopted depending on the efficacy, cost, and spectrum of control compared with those of current management practices.

ENVIRONMENTAL IMPACTS OF CONVENTIONAL AND BIOTECHNOLOGY-DERIVED SOYBEAN

Changes in Pesticide Use Patterns

With the advent of biotechnology-derived crops, pesticide use patterns have changed, in some instances quite dramatically, with accompanying environmental impacts. Assessing these changes, however, is not as straightforward as it may seem. Measuring shifts in pesticide use attributable solely to the introduction of biotechnology-derived crops is a challenge as many factors also influence farmer decisions, not the least of which are changes in weather patterns and natural variability in pest pressure. While the benefits of reductions in pesticide use may be clearer, assessing the benefits of the substitution of one pesticide for another is more complicated, raising complex issues surrounding relative toxicity.

Here the evidence on changes in herbicide use since the introduction of glyphosate-tolerant soybean is reviewed, and potential changes in pesticide use for those traits not yet commercialized are considered.

Glyphosate-tolerant Soybean

Since the commercial introduction of glyphosate-tolerant soybean in the United States in 1996, dramatic changes have been observed in the mix of herbicides being used for weed control in soybean crops. While the total amount of herbicides that are used per acre has changed little, the number of herbicide applications made to soybean has decreased. No information on herbicide use trends was available for countries other than the United States; therefore, the following discussion is limited to observed trends in herbicide use in the United States.

Since the introduction of glyphosate-tolerant soybean, the use of most traditional herbicides in soybean crops has decreased while the use of glyphosate has increased (Table IV-2). Glyphosate was used on 20% of soybean acreage in 1995, as a burndown before planting, or as a spot treatment during the growing season, applied directly to weeds in the field. By 2000, glyphosate-treated acreage had increased to 62% of soybean acreage (Figure IV-7). Glyphosate has displaced the use of many competing herbicides. Imazethapyr was the most commonly used herbicide in soybean, applied to 44% of acreage in 1995. Since the commercialization of glyphosate-tolerant soybean in 1996, however, imazethapyr usage steadily decreased to just 12% of soybean acreage by 2000. Similar trends were noted with the other soybean herbicides such as chlorimuron, pendimethalin, and trifluralin.

Because of differences in average application rates between different herbicide active ingredients, the net effect of substituting glyphosate for other herbicides may be either an increase or decrease in the total pounds of active ingredient (ai) used. Table IV-3 shows the relative importance of each herbicide active ingredient from 1995 to 2000. Between 1995 and 2000, herbicide application rates rose, peaking in 1997, then declined (Figure IV-8). Average soybean herbicide application rates, in terms of active ingredient per acre, have increased slightly since the introduction of glyphosate-tolerant soybean. In 1995, the year before glyphosate-tolerant soybean were commercialized, the average soybean herbicide application rate was 1.00 pound active ingredient per acre (lb-ai/a). By 2000, farmers applied 1.01 lb-ai/a. on average, including both conventional and glyphosate-tolerant soybean (Carpenter and Gianessi 2002). In a USDA Economic Research Service analysis of data from a 1997 survey of farmers, herbicide use by adopters of glyphosate-tolerant soybean was found to be slightly higher than by nonadopters, controlling for differences in production practices including tillage (Lin, Price, and Fernandez-Cornejo 2001). In two of five regions, including the Midwest, herbicide use declined while in three other regions, use increased. Overall, it was estimated that herbicide use (measured in pounds of active ingredient per acre) increased 3% as a result of adoption of glyphosate-tolerant soybean (Lin, Price and Fernandez-Cornejo 2001).

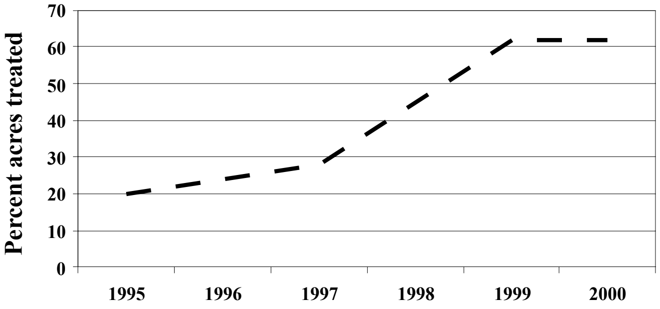
Table IV-2. Major herbicides used in U.S. soybean production from 1995-2000

Herbicide	1995	1996	1997	1998	1999	2000
	Percentage					
2,4-D	4	5	3	3	2	2
Acifluorfen	3	2	2	2	1	1
Alachlor	7	9	6	3	3	2
Bentazon	8	8	6	4	3	2
Clomazone	2	2	3	3	1	1
Dimethenamid	1	1	1	2	<1	<1
Fomesafen	1	1	1	1	1	1
Glyphosate	11	15	19	40	55	56
Imazethapyr	2	2	2	1	1	1
Metolachlor	13	7	11	6	5	4
Metribuzin	3	3	2	1	1	1
Pendimethalin	23	23	2	16	12	10
Sulfosate	0	0	<1	1	1	4
Trifluralin	15	17	16	14	11	13

Source: Derived from USDA-NASS 1996, 1997, 1998, 1999, 2000, 2001b

An independent analysis of similar data from a 1998 farmer survey showed the average application rate for glyphosate-tolerant soybean was 1.22 lb-ai/a., compared to 1.08 lb-ai/a. for other soybean (Benbrook

Figure IV-7. Glyphosate use trend in U.S. soybean



Source: USDA-NASS 1996, 1997, 1998, 1999, 2000, 2001b

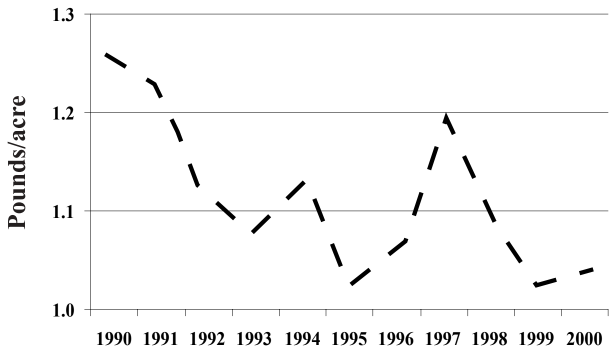
Table IV-3. Herbicide use trends in U.S. soybean, 1995-2000

Herbicide	1995	1996	1997	1998	1999	2000
	Percentage of acres treated					
2,4-D	10	13	8	7	5	5
2,4-DB	1	<1	1	<1	<1	0
Acifluorfen	12	11	12	7	3	3
Alachlor	4	5	3	2	2	1
Bentazon	12	11	11	7	4	2
Chlorimuron	16	14	13	12	12	10
Clethodim	5	7	4	4	5	4
Clomazone	4	3	5	4	1	<1
Cloransulam	0	0	0	1	5	4
Dimethenamid	1	1	1	1	<1	<1
Ethalfluralin	1	1	<1	0	<1	<1
Fenoxaprop	6	4	6	4	4	4
Fluazifop	10	7	7	5	4	5
Flumetsulam	2	2	4	2	2	2
Flumiclorac	0	2	1	<1	<1	<1
Fomesafen	4	5	6	6	4	7
Glyphosate	20	25	28	46	62	62
Imazamox	0	0	0	7	3	6
Imazaquin	15	15	13	8	5	4
Imazethapyr	44	43	38	17	16	12
Lactofen	5	8	4	2	2	2
Linuron	2	1	1	<1	<1	<1
Metolachlor	7	5	7	4	4	2
Metribuzin	11	9	10	6	5	4
Paraquat	2	1	2	1	1	<1
Pendimethalin	26	27	25	18	14	11
Quizalofop	6	7	4	3	1	<1
Sulfentrazone	0	0	0	3	4	4
Sulfosate	0	0	1	1	<1	4
Thifensulfuron	12	10	9	5	5	6
Trifluralin	20	22	21	16	14	14

Source: USDA-NASS 1996, 1997, 1998, 1999, 2000, 2001b

2001). But this comparison did not control for confounding factors such as differences in tillage practices. Glyphosate-tolerant soybean is commonly used in conservation tillage programs, which rely more on herbicide use to control weeds, in the place of mechanical tillage. At least some of the difference in application rates between glyphosate- tolerant soybean acreage and other soybean acreage is associated with increased adoption of conservation tillage practices such as "no-till" by U.S. farmers who plant glyphosate-tolerant soybean, which is discussed in more detail below.

Figure IV-8. Soybean herbicide application rates 1990-2000



Source: Carpenter and Gianessi 2002

Despite the slight increases in herbicide amounts recorded since the introduction of glyphosate-tolerant soybean, there have been large reductions in the number of herbicide applications made in soybean. Between 1995 and 2000, the number of soybean herbicide application acres² decreased by 22 million, or 13%, while the total number of soybean acres increased by 19% in the United States (Carpenter and Gianessi 2002). This trend is thought to be due to the broad spectrum of weed control provided by glyphosate, which can substitute for the use of mixes of two or more conventional herbicides. The decrease in herbicide application acres demonstrates farmers are using fewer active ingredients and making fewer trips over each field, which translates into ease of management. Based on 1998 survey data, glyphosate-tolerant soybean farmers applied an average of 1.4 active ingredients per acre while other soybean farmers applied an average of 2.8 active ingredients per acre (Benbrook 2001).

Herbicide Drift

Herbicide drift refers to the movement of an herbicide from the intended treated area into neighboring areas. Off-target movement of herbicides can have detrimental effects on sensitive plants in neighboring areas. Herbicide drift is a risk for all herbicides, especially those that are applied foliarly because the potential for exposure resulting in damage to nearby plants and crops increases after those plants have emerged. The potential for glyphosate drift from glyphosate-tolerant soybean fields is no different in this regard. Given the intensive use of herbicides in agriculture, increased potential for glyphosate drift from glyphosate-tolerant soybean is minor. Applications of glyphosate later in the season to control tall weeds in glyphosate-tolerant soybean may necessitate that the spray boom be adjusted higher over the soybean canopy. As a result, drift potential increases. Additionally, temperatures are typically higher later in the growing season, which further increases the potential for glyphosate drift due to inversions. Multiple in-crop applications and labeled use of glyphosate in the later stages of crop growth increase its potential to drift (Owen 1997b).

Complaints of herbicide drift by Iowa farmers were more frequent in 1998 than in other years, following the introduction and widespread planting of glyphosate-tolerant crops (Owen 1998). Although these complaints were related to glyphosate-tolerant crops, windy conditions and poor decisions made by private and commercial applicators were cited to be the reasons for these drift complaints.

Regardless of whether the herbicide applied contains glyphosate or other active ingredients, farmers and commercial herbicide applicators should exercise caution and common sense to prevent herbicide drift. Proper selection of application method, application timing, herbicide formulation, nozzle selection, and spray pressure can alleviate potential problems with drift from conventional and glyphosate-tolerant soybean fields (Hartzler 1998). Increasing droplet size through the selection of type and spray pressure is one measure that can decrease the potential for drift and potential for herbicide damage to nontarget plants or crops. Some conventional herbicides require small droplets for optimum performance. Droplet size has little effect on weed control with glyphosate, as it is readily translocated (Dexter 1993).

Water Quality

Environmental contamination with agrochemicals has long been a concern in agricultural production. Agrochemicals including herbicides, and their breakdown products, have been detected in surface and groundwater. Since the introduction of biotechnology-derived soybean, pesticide use patterns have changed and as a result, impacts on water quality are bound to change based on the characteristics of the pesticide used. Farmers using biotechnology-derived herbicide-tolerant soybean switch to using a herbicide that rapidly reaches inactive levels in soil and has little potential for water contamination, as a substitute for herbicides used with other soybean varieties.

There is an increasing trend toward total POST herbicide programs in soybean because of groundwater and carryover concerns. Preplant incorporated and PRE herbicide applications are, by their nature, prophylactic treatments and are applied at higher rates than needed for weed control. Potential impacts of currently used PRE herbicides on the quality of ground- and surface water are a concern in soybean production. Several herbicides commonly used in soybean production have formal EPA groundwater advisories, including alachlor, metolachlor, and metribuzin. Although the amount of herbicide that may be carried off a field during a rainfall is normally small (less than 2% in terms of total amount applied), the yearly flow-weighted average herbicide concentrations in affected waterways frequently exceed drinking water standards (Shipitalo and Malone 2000). As a result, there is growing pressure to decrease the use of PRE herbicides. Postemergence herbicides such as glyphosate are less subject to transport in runoff because they are foliar-applied.

In general, the fate of all herbicides in the environment is related to their retention, degradation (length of persistence), and transport in air, water, and soil (Moorman and Keller 1996). The retention of herbicides in soil depends on adsorption. Adsorption, a reversible process, refers to the binding of herbicide to soil particles. The sorbed portion of the herbicide (usually 20 to 70% of the applied herbicide) is generally unavailable for leaching, degradation, or plant uptake. Sorption capacities, usually measured as k_{α} values, are lower for other soybean herbicides than for glyphosate. A sorption value that ranges from 100 to 200 indicates that the herbicide is not tightly sorbed and is prone to movement in soil (Moorman and Keller 1996). For example, the sorption values for traditional herbicides such as those containing chlorimuron, metribuzin, and metolachlor are 110, 60, and 200 milliliter (mL)/gram (g), respectively, indicating that all these herbicides are sorbed weakly to soil.

On the other hand, the sorption value for glyphosate is 24,000 mL/g, which suggests that glyphosate is sorbed tightly and rapidly to soil (Ahrens 1994). Because glyphosate is strongly adsorbed to soil by forming complexes with exchangeable metals adsorbed to clays, glyphosate is not readily available for leaching or runoff losses (McBride 1991; McBride and Kung 1989; Sprankle, Meggitt, and Penner 1975). As a result, glyphosate run-off from the application site in a glyphosate-tolerant soybean production system would be much lower when compared to herbicides used in a non-herbicide-tolerant soybean system.

Length of persistence of herbicides in soil depends on the concentration and rate of degradation by biotic and abiotic processes and is usually measured by their half-life periods. Half-life of a herbicide refers to the length of time it takes for a herbicide in soil to degrade to one half of its original concentration. In general, herbicides with a half-life of greater than 50 days (d) are potentially more mobile in soil (Moorman and Keller 1996). Microbial degradation of glyphosate is rapid compared to herbicides used in other soybean such as chlorimuron (Ahrens 1994). As a result, persistence of glyphosate in soil is shorter than herbicides used in other soybean. The half-life for glyphosate is lower than that for the herbicides used in other soybean. Smith and Aubin (1993) estimated the half-life of glyphosate to be less than 40 days, depending on the mineralization rate (i.e., the amount of herbicide degraded per day). By comparison, the half-life of imazethapyr and metolachlor, the most widely used soybean herbicides before introduction of glyphosate-tolerant soybean, were 90 and 150 days, respectively (Ahrens 1994; Heimlich et al. 2000; Table IV-4). Because glyphosate persists for a shorter period of time than other soybean herbicides, glyphosate was detected less frequently and in lower quantities in runoff water in several studies, as discussed below.

Concentrations and losses of glyphosate from watersheds in a glyphosate-tolerant corn-soybean rotation were lower than those of conventional PRE herbicides despite the fact that rainfall followed soon after glyphosate application (Shipitalo and Malone 2000). In addition, soybean herbicides, metribuzin and alachlor, that have groundwater advisories, were detected through October and January of the following year, whereas the last detection of glyphosate in runoff was within a few days of application.

Table IV-4. Persistence of common soybean herbicides in soil

Herbicide	Half-life (days)
2,4-D	10
Acifluorfen	14 - 60
Alachlor	21
Bentazon	20
Chlorimuron	40
Glyphosate	40
Imazethapyr	90
Metolachlor	90 - 150
Metribuzin	30
Pendimethalin	45 - 54
Trifluralin	45 -120

Source: Ahrens 1994

Glyphosate entering surface waters was 40% lower than metribuzin, a PRE herbicide used in other soybean (Zaranyika, Wyse, and Koskinen 1991) because glyphosate is more tightly sorbed to soil. A limited potential exists for the transport of glyphosate from plant and soil surfaces in runoff by comparison to other soybean herbicides; only 7 of 247 groundwater samples contained glyphosate, and none of these concentrations exceeded maximum contamination levels (MCL, i.e., the highest concentration or level of a contaminant that is allowed in drinking water, as established by the United States Environmental Protection Agency) (Jacoby et al. 1992).

Glyphosate may be found occasionally in surface water; however, it occurs at low levels, is seasonal, and dissipates rapidly compared to other soybean herbicides. Established MCLs for glyphosate in water are 700 micrograms per liter (µg/L) in the United States and 280 µg/L in Canada. In a study by Shipitalo and Malone (2000), flow-weighted glyphosate concentrations year-round were well below this level in all watersheds from soybean-corn rotation. Monitoring for glyphosate has confirmed that residue levels in furface water remail relatively low, despite widespread use of glyphosate. (Wauchope et al. 2001). Also, negligible detections of glyphosate in groundwater were reported in Italy (Sartorato and Zanin 1999).

Wood (2001) found that glyphosate used to control roadside weeds was never detected in samples taken from the road shoulder, the drainage ditch, or the water stream in a natural rainfall study. The study was designed to assess whether the use of glyphosate, sulfometuron methyl, or a premix of diuron plus bromacil could contribute to the load of herbicides carried by Oregon streams. In contrast, the other herbicides were recorded in significant quantities (Wood 2001).

In an analysis of the environmental profile of glyphosate compared to conventional herbicides, it was concluded that glyphosate has a relatively favorable environmental profile. Most herbicides were found to have relatively low risks to the environment, although some (bentazon, metribuzin, sethoxydim, and thifensulfuron) were found to have a high risk of leaching to groundwater. However, it was cautioned that the use of glyphosate in the spring and fall may lead to leaching into groundwater and that there were associated low risks to water organisms. It was noted that the use of sulfonylurea-tolerant soybean may result in a reduction in the total amount of herbicides used in soybean, due to the low use rate, but that the environmental profile of sulfonylureas is less favorable than glyphosate (Hin, Schenkelaars, and Pak 2001).

Thus, soil behavioral characteristics of glyphosate make it an environmentally favorable herbicide compared to the available conventional herbicide options in soybean. Water quality would be significantly improved with the use of glyphosate in glyphosate-tolerant soybean because of its tight and rapid soil sorption, rapid degradation by microorganisms, and significantly lower half-life compared to competing soybean herbicides.

Insect-resistant Soybean

Since *Bt* soybean is still in the development stages, and not yet commercialized, only projections can be made as to how insecticide use patterns in soybean may be affected by its introduction. The most commonly used insecticides for control of the four target insect pests in the United States are thiodicarb, methyl parathion, lambdacyhalothrin, spinosad, tralomethrin, methomyl, and esfenvalerate. It has been estimated that *Bt* soybean usage in the southern Delta region of the United States could result in decreased insecticide use of 295,000 lb/yr, which represents 67% of total soybean insecticide use in the five states of Alabama, Georgia, Louisiana, Mississippi, and South Carolina (Gianessi et al. 2002).

Approximately 80% of the insecticides used in soybean production in Argentina and Brazil are targeted to control velvetbean caterpillar (*Anticarsia gemmatalis*) and other pests (Aragon, Molinari, and Lorenzatti de Diez 1997). Insect-resistant *Bt* soybean could replace much of the use of insecticides towards this pest in those areas.

Nematode Resistance

Pesticides are not commonly used for nematode control in soybean, as the benefits in increased yields are rarely great enough to justify the expense of the treatment. Therefore, pesticide use is not expected to be altered with the introduction of biotechnology-derived nematode-resistant soybean varieties.

Disease Resistance

Similarly, pesticide use is unlikely to be affected by the introduction of biotechnology-derived disease-resistant soybean, as pesticides are generally not used for disease control in soybean.

Changes in Tillage Practices with Glyphosate-Tolerant Soybean

Biotechnology-derived herbicide-tolerant soybean has facilitated increased adoption of conservation tillage. The most environmentally-beneficial of the various conservation tillage practices is “no-till.” Use of “no-till” farming in soybean production results in reductions in soil erosion, pesticide run-off, and dust, and in increasing soil moisture retention and improved air and water quality.

In 1995, one year before the commercialization of glyphosate-tolerant soybean, approximately 27% of the total full-season soybean acres in the United States were under “no-till” production (Table IV-5). With the increasing glyphosate-tolerant soybean acreage, “no-till” acres also are on the rise. By 2000, about 31% of the total soybean acreage in the United States was planted using no-tillage production practices (CTIC 2001). This represents a 35% increase in the "no-till" soybean acreage since the introduction of glyphosate-tolerant soybean. Surveys showed that farmers that are already using no-tillage are leaving more residues on the soil than prior to introduction of glyphosate-tolerant soybean (ASA 2001a).

A recent survey by the American Soybean Association (ASA) of 452 farmers from 19 states in southern and midwestern regions of the United States attributed the increase in no-tillage soybean acreage to glyphosate-tolerant soybean (ASA 2001a). The survey indicated that "no-till" soybean acres have more than doubled (111% increase) and number of acres using reduced-tillage techniques have increased over one-fourth. About 53% of the soybean farmers made fewer trips and 73% left more crop residues on the soil surface by using glyphosate-tolerant soybean.

Both reports by CTIC and ASA suggested that no-tillage soybean acreage increased dramatically subsequent to the introduction of glyphosate-tolerant soybean. But CTIC reported a 35% increase whereas ASA reported a 111% increase in "no-till" soybean acreage in the United States. Sampling methodology used by ASA accounts for some of these discrepancies. The ASA survey sampled farmers that planted more than 200 acres of soybean in midwestern and southern states whereas, the numbers reported by CTIC were on a national basis and encompassed all crops.

Adoption of no-tillage has increased in other countries such as Argentina, which can be attributed in part to reliable and effective weed control provided by herbicide-tolerant soybeans. "No-till" soybean acreage in Argentina increased by 57% between 1996 and 1999, totaling more than 11.1 million acres (4.5 million ha) by 1999 (James 2001a). The high adoption rates of glyphosate-tolerant soybean in Argentina also were attributed to the benefits of producing soybean in a "no-till" situation (James 2001a).

Table IV-5. No-till full-season soybean acreage in the U.S., 1995-2000[1]

U.S. soybean acreage	1995	1996	1997	1998	2000
	Million acres				
Total	58.8	60.6	65.1	66.6	70.0
No-till	15.9	16.2	17.9	19.0	21.5
No-till as a % of total	27	27	28	29	31

[1]Data not available for 1999
Source: CTIC 2000

Traditionally, mechanical tillage has been used to control existing weeds and to prepare seedbeds before seeding soybean, as well as to control weeds during the growing season. In a conventional-tillage system, soybean fields are usually plowed in the fall, disked before planting, and then cultivated once or twice during the growing season to control weeds. Although this method controls weeds, it also leaves the ground exposed to wind and water erosion, which carries fertile soil and agricultural chemicals into nearby streams and rivers.

The no-tillage system involves controlling the existing weed vegetation with herbicides such as glyphosate or paraquat and planting the crop into the stubble of previously grown crops and dead weeds without plowing the field. In general, more than 30% of the crop residues will be left on the ground in conservation tillage. Adoption of glyphosate-tolerant soybean technology has promoted a shift toward increased no-tillage production because glyphosate use is compatible with "no-till" production systems and has been a herbicide choice for farmers for controlling weeds in decreased or "no-till" situations. No-tillage production often eliminates the use of PRE herbicides that need incorporation into the soil and cultivations needed for late-season weed control.

The "no-till" system offers numerous advantages in the management of runoff of topsoil, water, pesticides and soil nutrients resulting in decreased sedimentation, soil erosion, groundwater contamination; improved water infiltration, soil tilth, and fuel savings; and conservation of nutrients, and organic matter. Other benefits include decreased machinery wear and soil compaction, and an increase in beneficial soil dwelling insect and bird populations (Kay 1995; Towery 2002-in press). All these benefits will be derived from the use of glyphosate-tolerant soybean since its use is compatible with no-tillage practices.

Surface runoff of water from conventionally tilled fields can pollute rivers and streams by carrying sedi-

Soybean grown in conservation tillage.
Photo by Dr. Wayne Parrott, University of Georgia

ments and by increasing levels of bacteria. Surface water quality will be improved in "no-till" soybean production as crop and weed residues hold soil particles along with associated nutrients and pesticides on the field. Comparison studies show that crop residues in "no-till" systems resulted in 70% less herbicide runoff, 93% less erosion, and 69% less water runoff than conventional tillage (Baker and Laflen 1979; Hebblethewaite 1995).

An Iowa study suggests that production costs could be decreased 10 to 20% in "no-till" compared with conventional tillage (Mitchell 1997). The number of trips across the field in conventional tillage will be at least three to four (two to three tillage operations and planting) in comparison with one to two for "no-till" (one trip for herbicide application and another for planting) (CTIC 2000). A decrease in number of tillage operations and number of trips across the field translates to decreased fuel usage and decreased carbon dioxide (CO_2) emissions from the motorized farm equipment. Consequently, global warming may be delayed with the adoption of glyphosate-tolerant soybean in conjunction with "no-till" farming practices.

Intensive tillage releases soil carbon into the atmosphere where it combines with other gases that contribute to global warming. By contrast, decreased tillage conserves carbon due to improved sequestration in organic matter (Reicosky 1995; Reicosky and Lindstrom 1995) and decreases CO_2 emissions (CTIC 2000; Kern and Johnson 1993). Reicosky and Lindstrom (1995) recorded a 5-times greater loss of the carbon dioxide from soil with one pass of a moldboard plow than in no-tilled plots as measured over a 19-day period. More organic matter was oxidized in 19 days in plowed fields than was produced all year, which accounted for the steady decline in organic matter content in tilled plots. Organic matter has increased by as much as 1800 lb/a./yr in long-term "no-till" studies (Reicosky 1995).

Greenhouse gas emissions will be decreased 88% from farm operations using glyphosate-tolerant soybean planted in a no-tillage system compared to other soybean planted in conservation tillage. A review on global warming potential from greenhouse gases in intensive agriculture showed that global warming potential from conventional tillage practices is 88% greater than no-tillage systems (Robertson, Paul, and Harwood 2000). This substantial reduction of global warming potential in no-tillage systems was attributed to increased carbon storage in "no-till" soils and reductions in fuel consumption for "no-till". Accumulation of carbon in "no-till" system was 30 grams per centimeter squared (g/cm^2), while conventional tillage accumulated 0 g/cm^2.

Barnes (2000) predicted that by 2020, approximately 37 million tons of topsoil would be preserved by planting glyphosate-tolerant soybean in no-tillage. Barnes further predicted that reduction in tillage operations saves approximately 9 gallons (gal.) of fuel per hectare, which translates to a decrease of 400,000 tons of carbon entering the atmosphere by 2020. Crop residues that are left in no-tillage farming will allow better soybean root system development. Similar to Barnes (2000), Kern and Johnson (1993) estimated that conversion from conventional tillage to no-tillage in row crops would save the equivalent of 14.5 gal. per hectare of diesel fuel. For every gallon of fuel saved, 3.72 lb of CO_2 emissions are decreased.

The adoption of glyphosate-tolerant soybean, which is compatible with conservation tillage practices, is expected to decrease agricultural dust significantly. Dust from agricultural fields resulting from tillage operations and burning crop residues is a major air quality concern.

"No-till" glyphosate-tolerant soybean may provide more favorable wildlife habitat compared to other soybean in conservation tillage. Farmers have observed increased return of insect-feeding birds and other wildlife to glyphosate-tolerant soybean fields compared to other soybean fields, mainly due to "no-till" production practices in those glyphosate-tolerant soybean fields. No-tillage systems provide food and shelter at crucial times for wildlife such as pheasants and ducks (Barnes 2000; CTIC 2000). Researchers at North Carolina State University suggested that quail chicks acquired their minimum daily insect requirement in only 4.2 hours (hr) in "no-till" fields compared with 22 hr in conventional tillage fields (Palmer, Lane, and Bromley 2001). Beneficial microbial populations, including microbes that degrade pesticides, are often found in higher numbers in "no-till" soils than in tilled soils (Doran 1980). Numbers of beneficial predatory arthropods, earthworms, and small mammals were consistently higher in "no-till" fields than in conventional-tillage fields (Brust and House 1988; House and Parmelee 1985; Warburton and Klimstra 1984).

Before the introduction of glyphosate-tolerant soybean, farmers had to deal with increased infestations of perennial and large-seeded weed species that are favored in no-tillage systems. But perennial and heavy weed infestations can be controlled more efficiently in "no-till" now, because glyphosate is a more effective herbicide compared to other soybean herbicides on those weeds.

Some insect and disease problems may be higher in "no-till" production; however, soybean farmers are embracing no-tillage because environmental and economical benefits surpass challenges. Incidence of *Fusarium*, *Phytophthora*, and *Rhizoctonia* was lower on soybean in no-tillage compared to conventional tillage (Unger and McCalla 1980). Defoliating insects such as Mexican bean beetle (Dietz et al. 1976) and redlegged grasshopper (Sloderbeck and Edwards 1979), however, are more prevalent in "no-till" soybean compared to conventional tillage.

Crop Weediness

Crop seed that is left on the soil surface after harvest may remain viable and germinate the following year in the rotational crops. These plants are out of place, act as weeds, and are termed volunteers. Similar to weeds, crop volunteers compete with the cultivated crop for resources such as sunlight, water, nutrients, and space.

Following crop harvest, soybean (conventional or glyphosate-tolerant) may germinate as a volunteer weed in succeeding crops such as corn or wheat. Overwintering of soybean is rare due to lack of innate dormancy (Padgette et al. 1996a). Thus, any seed that might remain in the field after harvest is likely to germinate, emerge, and be killed by frost or field preparation the following year. Similar to volunteer traditional soybean, if volunteer glyphosate-tolerant soybean behaves as a weed in the succeeding crop, mechanical methods or herbicides with an alternate mode of action can be used to control it.

Similar to disturbed habitats (i.e., crop fields), the probability for glyphosate-tolerant soybean to behave as a weed in natural ecosystems will be no different than the probability for other soybean because cultivated crops are unlikely to become weed problems, as most crops are noncompetitive under natural conditions without human intervention (Riches and Valverde 2002).

There are no indications in the scientific literature that glyphosate-tolerant soybean developed through biotechnology possess weediness traits and could turn into an aggressive weed. Based on a literature review, Madsen (1994) concluded that herbicide-tolerant crop plants could become weeds if they already possess traits for weediness and if only one herbicide is used consecutively in several crops for a longer period of time. Weediness for the purposes of this part of the discussion is an attribute, which causes a crop to act as a weed due to the addition of a gene. If the competitive ability of a crop improves in natural or agricultural ecosystems due to addition of gene(s), weediness is said to be present in that crop. Soybean, whether glyphosate-tolerant or conventional, do not possess any weediness traits. Additionally, the adaptive advantage of glyphosate-tolerant soybean will be in the presence of glyphosate only. Thus, the chances for glyphosate-tolerant soybean to behave as a weed are negligible. In general, herbicide tolerance was found to confer less feral fitness advantage than disease or insect resistance (Kareiva, Parker, and Pascual 1996).

Crawley et al. (2001) conducted the first extensive weediness and fitness studies on biotechnology-derived crops in the United Kingdom. In this 10-year study conducted in 12 different habitats that included exposure to various stresses such as herbivory and pathogen attacks, genetically modified oilseed rape, corn, potato, and sugarbeet were similar in their invasiveness and persistence compared with their non-transformed, conventional counterparts. Fredshavn and Poulsen (1996) also reported similar results in field trials conducted in the absence of herbicide selection pressure using glufosinate-tolerant oilseed rape and glyphosate-tolerant sugarbeet.

In summary, glyphosate-tolerant soybean is unlikely to become a self propagating weed in either disturbed or nondisturbed habitats. Voluntary soybean, whether in a glyphosate-tolerant or a conventional system, can be controlled easily with available weed management solutions.

Outcrossing

Outcrossing, also referred to as gene flow, refers to the transfer of genetic material from one crop to another or from a crop to a weed. Gene flow, a regularly occurring phenomenon, has occurred since crop domestication began and is not a concern reserved strictly for biotechnology-derived crops. Gene flow between plants may occur if the source and recipient plants are grown in close proximity to each other, flower at the same time, and are sexually compatible for interbreeding.

Crop to Crop

Gene flow may occur between biotechnology-derived and conventional crops, if the crop in question is open-pollinated and if both crops are grown adjacent to each other. To prevent gene flow between biotechnology-derived and conventional crops and to produce genetically pure seed, both crops should be grown physically isolated from each other (isolation distance). Isolation distances usually vary between crops based on the type of pollination. Isolation distances for crops such as soybean, wheat, barley, dry bean, and tobacco are usually small and not an issue because these crops are predominantly self-pollinated. On the other hand, when growing open-pollinated crops such as corn and canola, isolation distances need to be strictly followed. If gene flow occurs between biotechnology-derived crops and their conventional counterparts and the trait is fitness enhancing, the frequency of the trait increases in the environment and could lead to the increase in potential for gene flow between crop and related weeds. If the trait is not fitness enhancing, frequency would not be expected to increase.

Crop to Weed

Gene flow between crop and weed is a complicated process. It is dependent on several factors such as flowering synchrony, extent of compatibility, abundance and method of pollen spread (pollen vectors such as wind and insects), distance of pollen movement, and environmental conditions pertinent to cross-pollination.

Many agricultural crops grown in the United States are not native species, having been imported from their native habitat specifically for cultivation. Closely related weed species, therefore, are largely absent. As a result, lateral gene transfer generally is not conceived to be a risk in the United Sates for crops such as corn, wheat, soybean, alfalfa, cotton, barley, dry bean, and tobacco (Regulatory Considerations: Engineered Plants 1987).

The implications of gene flow from a crop to a weed depend on many factors, but generally surround the potential problems associated with altering a plant’s fitness. In the presence of selection pressure, the crop-weed hybrids may or may not have a greater adaptive advantage as compared to their parents. If the adaptive advantage is greater, hybrids may be more competitive (increased weediness). If the adaptive advantage is lower, the hybrids may not survive the intensity of crop production and could become extinct (Ellstrand, Prentice, and Hancock 1999).

Another implication of gene flow is that if crop-weed hybrids were formed and seeds were dormant (a trait often found in weeds), hybrids germinate over an extended period of time and some hybrids may establish with the weed at a similar time. As a result, hybrids may continue to cross-pollinate over a period of time leading to a stable introgression (Jorgensen et al. 1996).

Several technologies have been suggested to contain gene flow and to decrease the risk of escape of herbicide-resistance genes to weeds (Rices and Valverde 2002). Seed protection technology (euphemistically referred to as "terminator" technology) in which the viability of the progeny seed is genetically controlled was proposed but did not receive much support due to public objection. Other approaches proposed to mitigate gene flow that are still in experimentation include chloroplast transformation of herbicide-resistance traits and use of tandem constructs. In a tandem construct, the herbicide-resistance gene is tightly linked to another gene that codes for deleterious traits in weeds, but not to crop. Availability and use of these approaches reduces the potential risks associated with gene flow between crop and its wild relatives and could lead to increased adoption of herbicide-tolerant crops.

Glyphosate-tolerant Soybean

The genus Glycine comprises two subgenera, Glycine and Soja (Hancock, Grumet, and Hokanson 1996). Glycine consists of more than ten perennial species that are distributed in the Philippines, Taiwan, Australia, and South Pacific Islands (Skvortzow 1972). Soja consists of three annual species—*max*, *soja*, and *gracilis*—that are spread throughout Asia. Of these three, *max* is the cultivated form of soybean, *soja* is the wild form, and *gracilis* is the weedy soybean (Kwon, Im, and Kim 1972). Some members of the wild perennial species of subgenus Glycine may be found in U.S. territories in the Pacific. But there are no known reports of successful natural hybridization between the cultivated soybean and the wild perennial species (Hood and Allen 1980; Ladizinsky, Newell, and Hymowitz 1979).

Soybean is a predominantly self-pollinated crop. The anthers mature in the bud and shed their pollen directly onto the stigma of the same flower before flower opening, ensuring a high degree of self-pollination. Hybridization in soybean, aided by honeybees, can occur with an estimated frequency of up to 1%; however, soybean is not a preferred plant for honeybees (Erickson 1984). The limited potential for cross-pollination in soybean, however, also is evident in certified seed regulations for Foundation seeds, the most stringent category in the Certified Seed Regulations, which permits zero distance between different soybean cultivars in the fields. Thus, the probability for the transfer of glyphosate-resistance trait from glyphosate-tolerant soybean to wild relatives of other soybean is very small.

The transfer of glyphosate resistance from soybean to its weedy relatives is not considered a risk in the United States and Latin America (which account for about 83% of the total soybean acreage worldwide) because there are no sexually compatible relatives of soybean growing wild in the Americas. Although wild soybean plants are grown in research plots, there are no reports of their escape to unmanaged habitats.

In areas where soybean weedy relatives naturally occur such as the Philippines, China, Taiwan, Australia, and South Pacific, the risk of transfer of glyphosate resistance is *de minimus* because soybean is self-pollinated. The chances of the spread of glyphosate resistance in these regions are further decreased by other limiting factors as mentioned above such as flowering synchrony between soybean and its relatives; extent of sexual compatibility; abundance, method, and distance of pollen spread, and environmental conditions pertinent to cross-pollination.

No reports on transfer of glyphosate resistance trait from glyphosate-tolerant soybean to its weedy relatives have been reported in the scientific literature.

Insect-resistant Soybean

Insect-resistant soybean has not been commercialized, to date. Recognizing that the probability of transfer of insect resistance from biotechnology-derived soybean to weedy relatives is low as discussed above, measures could be implemented to decrease gene flow when wild relatives are in proximity to the cultivated soybean. If it were deemed necessary, prevention measures to limit the movement of insect-resistance trait to weeds and other related plant species could include use of buffer areas, pollen traps, pollen vector barriers, isolation distances and molecular markers to assist in monitoring.

Pest Resistance

Pest population developing resistance to pesticides is an important problem confronting crop production today. Pest resistance to pesticides decreases the ability of farmers to control economically damaging pests. Reports indicate that more than 200 weeds, 500 species of insects and mites, and 150 plant pathogens are resistant to pesticides that once controlled them (Bellinger 1996). Repeated applications of the same pesticide at low doses is more likely to result in the development of pest resistance than high dose applications. A commonly advocated measure to avoid or delay resistance development in pests is to use pesticides that have different chemical modes of action in rotation. So long as the resistance does not lead to cross-resistance to other related pesticide compounds, development of resistance to a specific pesticide is a manageable problem.

Herbicide-resistant Weeds

Herbicide resistance is a genetic phenomenon in which weed populations survive a particular herbicide treatment that, under normal use conditions, would effectively control the same population. Emerging reports on glyphosate-resistant weeds may be a concern in herbicide-tolerant soybean. However, herbicide resistance in weeds is not unique to biotechnology-derived crops.

The intensive use of herbicides in conventional agriculture has led to the development of resistant weeds (Holt, Holtum, and Powles 1993). Applying the same herbicide in the same field year after year can select for resistant weeds. The resistant weeds set seed and may eventually dominate the population. As a result, resistant weeds are not effectively controlled by the selecting herbicide. Herbicide resistance is an example of evolution at an accelerated pace and an illustration of the "survival of the fittest" principle.

Lebaron (1991) reported that by 1991 there were more than 109 weed biotypes demonstrating resistance to selected herbicides, with over half of them to one class of herbicides, the triazines. Triazine resistance in weeds was first reported in the 1970s. Subsequently, weeds resistant to various herbicide families such as phenoxy, benzonitriles, ureas, and bipyridyliums also were reported. In recent years, weeds such as kochia, Russian thistle, common waterhemp, palmer amaranth, common cocklebur, shattercane, and common sunflower have developed resistance to ALS inhibitors such as imidazolinones and sulfonylureas (Peterson 1998). The sulfonylureas and the imidazolinones are reported to be particularly prone to the rapid evolution of resistant weeds. About 14 weed species including common cocklebur, which is a common soybean weed, have exhibited resistance to imidazolinone herbicides (Goldburg 1992; Hartzler 1997). To date, more than 250 cases of herbicide-resistant weed populations for almost all classes of herbicides have been documented (Heap 2002).

While crop characteristics are not important, the properties of the herbicide and the weed play an important role in the selection of resistant weeds. The evolution of weed resistance to herbicides is usually accelerated with the use of herbicides with long residual activity, a single target site and specific mode of action, broad spectrum of activity, and frequent applications with no rotation to other herbicides or cultural practices. Resistance development in weeds to herbicides also depends on the fitness and average lifespan of weed species in the soil seedbank.

While glyphosate is the only herbicide on the market with inhibitory activity on the target enzyme EPSPS, glyphosate has no residual activity and is degraded quickly in the soil by microorganisms. Thus, glyphosate is a low-risk herbicide for resistance development in weeds. But increased use of glyphosate has increased selection pressure, which led to the sporadic development of resistant weeds (Gressel, Ransom, and Hassan 1996; Powles et al. 1998; VanGessel 2001). Before the introduction of biotechnology-derived soybean, glyphosate was used in combination or in sequence with other herbicides that decreased the pressure for selection of glyphosate-resistant weeds (VanGessel 2001).

Based on the label recommendations, glyphosate can be applied as a pre-plant burndown herbicide followed by one or two in-crop applications in biotechnology-derived herbicide-tolerant soybean. Thus, weed population could be treated up to three times in one season, and the label allows use up to 6.72 kilograms per hectare (kg/ha) applied during a season (Shaner 2000).

Hartzler (1997) reported that the likelihood of herbicides used in other soybean such as those in ALS-family (example, imazethapyr) to select for resistant weed biotypes is much greater than with other herbicides. Resistance was noted in several weed species such as waterhemp, common cocklebur, powell amaranth, ragweed (common and giant), and kochia to ALS-herbicides used in other soybean such as imazethapyr throughout the United States. In Brazil, wild poinsettia and hairy beggarticks developed resistance to ALS-inhibiting herbicides, while in Argentina, pigweed evolved resistance to imazethapyr and chlorimuron used in other soybean (Riches and Valverde 2002). Resistance was selected for very quickly, in many situations after only two applications of an ALS-inhibiting herbicide in other soybean. Cross-resistance to other herbicides was also reported in cocklebur, which makes weed management even more difficult.

On the other hand, development of resistance in weeds to glyphosate has been slower than conventional herbicides. Only three sporadic cases of glyphosate-resistance in weeds have been documented. These include rigid ryegrass in Australia (Powles et al. 1998), goosegrass in Indonesia (Lee and Ngin 2000), and marestail in Delaware (VanGessel 2001). Resistance in rigid ryegrass and goosegrass resulted from the use of glyphosate in conventional cropping systems before the introduction of biotechnology-derived soybean while glyphosate resistant marestail appeared after the commercial introduction of glyphosate-tolerant soybean.

Rigid ryegrass in Australia exhibited a seven- to elevenfold resistance to glyphosate following a 15-year continuous glyphosate use (Powles et al. 1998). Glyphosate-resistant goosegrass (5X more tolerant to glyphosate than susceptible plants) appeared after 10 yr of use and resulted from an altered binding site of the EPSP synthetase enzyme. Glyphosate-resistant marestail in Delaware exhibited an eight- to thirteenfold greater resistance than the susceptible population within a 3-year glyphosate usage in biotechnology-derived glyphosate-tolerant soybean (VanGessel 2001).

The appearance of weed resistance to herbicides used in both other soybean and glyphosate-tolerant soybean suggest that weed resistance problems in biotechnology-derived crops are no different than in conventional crops. Instead, weed resistance to herbicides is dependent on the nature of the herbicide and not the nature of the crop. This further emphasizes the importance of integrated weed management and careful use of herbicides to preserve their efficacy.

Diversifying weed control tactics can delay resistance development and aid in controlling herbicide-resistant weeds. For example, glyphosate-tolerant soybean can be grown in rotation with STS or other soybean. Glyphosate-resistant weeds are resistant to glyphosate only and can be killed by the routine herbicide programs used in either STS or other soybean. Mixing herbicides with different modes of action would also provide farmers a choice in managing the glyphosate-resistant weeds.

No information is available in the scientific literature on the fitness of glyphosate-resistant weeds. Fitness (ability to survive and reproduce) is a major factor affecting the establishment and spread of the herbicide-resistant weeds. Whether the herbicide-resistance trait in the weeds (in conventional or glyphosate-tolerant soybean production systems) increases the fitness or confers a selective advantage is an important environmental concern. The positive fitness value or the selective advantage of herbicide-resistant weeds is restricted to agricultural habitats where glyphosate is applied. If decreased fitness occurs, glyphosate-resistant weeds are not expected to persist for long in the soybean agroecosystem.

Overall, similar to other soybean systems, weeds may develop resistance to herbicides used in glyphosate-tolerant soybean. But resistance development can be delayed and managed using herbicide mixtures and crop and herbicide rotations.

Insect Resistance to *Bacillus thuringiensis*

The evolution of pest resistance to insecticides has been a major problem since long before the development and introduction of biotechnology-derived crops. Over the past several decades, soybean looper has become the most costly pest to control in soybean because this insect has developed resistance to most major classes of insecticides used against it such as carbamates, cyclodienes, organophosphates, and pyrethroids (Mascarenhas and Boethel 1997; Mascarenhas et al. 1995).

Insect pests have developed resistance to one or more *Cry* proteins from *Bacillus* species under both field and laboratory conditions in several locations around the world in conventional crops treated with microbial *Bt* sprays (Frutos, Rang, and Royer 1999; Tabashnik 1994). Resistance development due to *Bt* in microbial sprays is different from biotechnology-derived soybean in which there is a consistently high dose of *Bt* toxin expressed. The problem may be compounded if a large proportion of these crops is planted in a geographic region expressing the same transgenes. However, *Bt* soybean is still in development and it is not clear if selection pressure will be higher or similar to other crops. Also, no studies have been found which demonstrate that soybean insect pests have developed resistance to *Cry* proteins.

Insect-resistant *Bt* soybean has the potential to provide significant control of soybean insects. Long-term effect will be sustained if effective insect resistance management strategies are adopted to facilitate the durability of the resistance. These strategies must be aimed at preventing the mating of the resistant insects with nonresistant insects and insects resistant to other forms of C*ry* proteins. Use of resistance management strategies used in other *Bt* crops such as tissue- and time-specific promoters, gene pyramiding, and refuges can decrease the rate of insect adaptation in *Bt* soybean.

The use of tissue- or time-specific promoters may aid in the expression of *Bt* toxin in selective plant parts and at only selected periods when insect infestations are likely to occur. This would decrease exposure of insects to toxins and could help delay evolution of resistant insect populations in *Bt* soybean (Gould 1998).

In many situations, multiple *Bt* strains have been identified for the control of *Bt*-sensitive pests (McGaughey 1994). Use of these multiple strains – in other words, stacking/pyramiding multiple resistance genes – could increase the level and breadth of pest control and could slow the development of resistance. Co-expression of compatible *Cry* proteins was found to be effective in cotton pest control (Stewart et al. 2001). A variation on this strategy was investigated in soybean by Walker et al. (in press), who combined a *Cry*1Ac transgene with resistant alleles at two quantitative trait loci from the plant introduction, PI 229358. In other *Bt* crops such as cotton and corn, a “high dose plant” is suggested to delay resistance (Andow and Hutchinson 1998). A high dose plant is one that expresses the toxin at 25 times the dose required to kill 99% of a susceptible insect population. This concentration would be high enough to kill most insects that are heterozygous for resistance. This approach promotes mating opportunities between resistant and susceptible insects from nearby non*Bt* soybean refuge. A potential problem with the “high dose” concept is that soybean has multiple pests, and an expression level that is acutely toxic to the primary pest may not control other less sensitive pests. Another limitation to refuges is the possible lack of stewardship by farmers as in a case of *Bt* cotton in Brazil and Argentina. Refuge requirements and recommendations set forth by the U.S. Environmental Protection Agency (EPA) have been reported to be complied with by *Bt* corn farmers in the United States (Agricultural Biotechnology Stewardship Technical Committee 2002). The effectiveness, likelihood of adoption, and farmer feasibility should be carefully considered when designing the refuge options for managing insect resistance in soybean.

Viral Recombinations

The potential to create more virulent forms of plant viruses through recombination between viral transgenes and an infecting virus has been mentioned as a possible risk with virus-resistant soybean. The issue of recombinant viruses was first brought to light by Schoelz and Wintermantel (1993). The key argument against it is that multiple infections occur all the time in the wild. A virus infecting an infected conventional crop cell offers identical recombination opportunities as a virus infecting a biotechnology-derived cell. Virus-resistant soybean is still in the development stage.

Pest Population Shifts

Crop production and pest management practices used by farmers select certain pests to survive and kill others. As a result, pest populations will change in crop fields over time in response to these management practices or selection pressures. This section addresses the impact of management practices in biotechnology-derived soybean on weed population shifts and implications of these shifts.

Weeds

Weed shift refers to a species composition change due to the systematic elimination within an agroecosystem of those species that are well controlled by the herbicide and proliferation of those species that are naturally more tolerant of the herbicide (Erickson and Lemaux 2000). Repeated use of herbicides with the same modes of action could lead to weed shifts because the application of a single herbicide creates a favorable niche for weeds not completely controlled by the herbicide.

Shifts in relative frequency of weeds may not necessarily occur in response to permanent selection pressures. Rather, a short-term response caused by tillage, cropping practices, or herbicide applications may cause weed species shifts. A number of studies have demonstrated the impact of agricultural practices or weed management techniques on the composition of weed flora (Chauvel, Gasquez, and Darmency 1989; Cussans 1976; Derksen et al. 1993, 1995; Liebman and Dyck 1993).

When one herbicide is used extensively, certain weeds are likely to increase in frequency and will require supplemental management tactics. A Brazilian study noted that weed species shifted to morning glory species with metribuzin, morning glory species and Florida beggarweed with flumetsulam and imazaquin in other soybean (Bezutte et al. 2002). In the United States, shift occurred from *Amaranthus retroflexus* to *A. rudis* and *A. palmeri* due to the use of ALS herbicides in other soybean (Sweat et al. 1998). Pike, Hill, and McGlammery (1998) suggested that herbicide mixtures have provided more reliable weed control than the use of single ALS products and had less impact on weed shifts.

As with herbicides used on other soybean, continuous use of glyphosate in glyphosate-tolerant soybean could exert increased selection pressure and could lead to shifts in weed populations that were not controlled by glyphosate. Those weeds that have some natural tolerance to glyphosate and those that are left untreated due to germination and emergence patterns may be more likely to increase in frequency and require supplemental management (Shaner 2000).

In response to widespread use of glyphosate-tolerant soybean in Iowa, common waterhemp and velvetleaf, which are not effectively controlled by glyphosate, are becoming increasingly problematic (Owen 1997a). A Kansas study showed that ivyleaf morning glory and large crabgrass that were not controlled by glyphosate dominated the weed community in a corn-soybean rotation using glyphosate applications for weed control (Marshall, Al-Khatib, and Maddux 2000). In a similar study, Coble and Warren (1997) showed that morning glory species increased in abundance with the continuous 3-year use of glyphosate compared with other herbicide programs. Other weed species that exhibit some tolerance to glyphosate and pose problems in glyphosate-tolerant soybean include velvetleaf (Hartzler and Battles 2001), nutsedge, marestail, hemp sesbania, and common waterhemp (Shaner 2000). Inconsistent waterhemp control with glyphosate in Iowa was researched further and was attributed to weather (cold temperatures and drought at the time of glyphosate application) and insufficient coverage (Hartzler 2002).

As with weed problems that increase with the use of herbicides in other soybean production, weed problems due to the use of glyphosate in glyphosate-tolerant soybean can be managed. Farmers will either need to use other herbicides that have a higher level of activity on the problem weeds or increase the rate of glyphosate. For example, morning glory control, which has become difficult with glyphosate can be improved by addition of other herbicides such as imazethapyr or chlorimuron.

Though glyphosate cannot provide 100% control of problem weeds, it may stunt them. As a result, fitness of these weed species may be decreased to an extent they are no longer competitive with the crop. Hartzler and Battles (2001) reported that velvetleaf which survived glyphosate applications in a glyphosate-tolerant cropping system possessed decreased fitness and had minimal effect on soybean yield. This suggests that problem weeds such as velvetleaf and morning glory in a glyphosate-tolerant soybean system are not a major concern since yield impacts from these treated weeds are negligible.

A possible solution to slow the development of weed population shifts is use of crop rotation with biotechnology-derived crops tolerant to a different herbicide or use of conventional weed management programs. Another alternative is to stack different herbicide tolerances into one variety so that different herbicides can be used in rotation.

An implication of weed population shifts is a change in weed diversity of agroecosystems. Weed diversity in crop fields plays important ecological roles such as enhancement of biological insect pest control, better soil cover decreasing erosion, attracting beneficial insects, and food supply for birds and small mammals. Effective weed removal caused by the use of broad-spectrum herbicides such as glyphosate may result in weed diversity different from other herbicides used in other soybean.

According to a long-term study, weed diversity in a corn-soybean rotation that used glyphosate-tolerant weed management options was reported to be no different from conventional herbicide programs (VanGessel 2002). This suggests that though levels of weed control obtained from conventional herbicide programs and glyphosate differ, weed diversity did not differ between the two programs. Thus, impact of glyphosate-tolerant soybean on agroecosystems should be no different than other soybean.

Currently, there is no consensus on the value of weed community diversity (Clements, Weise, and Swanton 1994). In one school of thought, researchers postulate that low weed diversity will result in a less stable agroecosystem, which provides optimal conditions for unhampered growth of weeds, insects, and diseases because many ecological niches are not filled by other organisms (Altieri 1998). In another school of thought, researchers postulate the opposite–that increased vegetation complexity may lead to increased problems with phytophagous insects (Crepps and Ehler 1983; Murdoch 1975).

Nontarget Impacts

Nontarget effects are defined as unwanted or negative effects of crops or their accompanying farming practice on organisms living in or around the agricultural field that are not intended to be impacted (Kinderlerer 2001). Studies pertaining to nontarget effects of ecological risk assessment include mostly the impacts on birds, fish, aquatic vertebrates, soil-dwelling invertebrates, and other beneficial insects, such as bees.

Glyphosate-tolerant Soybean

A simple change in crop management practices such as tillage, crop rotation, or alteration in the amounts of nutrients or pesticides used affects the size, composition, and functions of the micro-ecosystems around the crop plants. The impact herbicides may have on soil microbes and other organisms in one soil may be completely different from the impact on another soil based on different physical, chemical, and mineralogical characteristics.

Soil microorganisms are a diverse community that catalyzes many processes important to soil fertility and plant growth, such as nutrient cycling from soils and fertilizers. Soil organisms interact with each other through predatory, competitive, antagonistic, or mutualistic interactions (Angle 1994).

The enzyme EPSPS is present in all plants and microorganisms and thus ordinarily is present in food and feed derived from plant sources (Schulz, Kruper, and Amheim 1985). Because of the ubiquitous nature of EPSPS, all organisms that feed on plants or microbes have been exposed to EPSPS protein. Thus, impacts on microorganisms before and after the adoption of glyphosate-tolerant soybean are expected to be similar.

According to Anderson (1978), herbicides generally appear to have no adverse effect on soil bacterial populations except at concentrations that well exceed normal use rates. Anderson (1978) further pointed out that soil fungi and actinomycetes are not as susceptible to herbicides and insecticides as they are to fungicides. But Duah-Yentumi and Johnson (1986) suggested that although herbicides such as MCPA and simazine caused no detectable effects to soil microflora, repeated paraquat applications significantly lowered soil microbial biomass. In a similar study, field applications of 2,4–D, picloram, and glyphosate had no effects on soil microbial biomass and activity in conventional systems (Wardle and Parkinson 1990).

Heterotrophic soil organisms acquire carbon and nitrogen for growth and maintenance by decomposing plant residues and other organic materials added to the soil including glyphosate. In a study to evaluate the effect of quantity and frequency of glyphosate application, which has escalated with the advent of glyphosate-tolerant crops, Haney et al. (2000) reported that glyphosate significantly simulated microbial activity as measured by carbon and nitrogen mineralization, but did not affect soil microbial biomass. Microbes degraded glyphosate rapidly, even at high application rates, without adversely affecting their activity. This suggests that use of glyphosate in glyphosate-tolerant soybean should have no adverse effects on soil microbial populations.

Nitrogen fixation by symbiotic bacteria provides soybean with 30 to 70% of its total nitrogen requirement (Thurlow and Hiltbold 1985). Although glyphosate-tolerant soybean contains resistant EPSPS, the nitrogen-fixing bacteria in soybean root nodules, *Bradyrhizobium japonicum*, does not contain resistant enzymes. As a result, glyphosate applications may affect the relationship and, ultimately, nitrogen fixation. In a study to evaluate the interaction, it was found that early glyphosate applications delayed nitrogen fixation and decreased soybean biomass and nitrogen accumulation and that these effects were more pronounced when moisture conditions were limiting (King, Purcell, and Vories 2001). In a similar study, Reddy, Hoagland, and Zablotowicz (2000) suggested that glyphosate (at both 1.12 and 2.24 kg/ha) decreased nodule leghaemoglobin content by 6 to 18%, nodule mass and number by 39 and 30%, respectively, and chlorophyll content. Use of glyphosate-tolerant soybean may thus decrease soil fertility. But more large-scale field studies are needed to verify this finding.

Glyphosate-tolerant soybean had no negative impacts on beneficial insect populations (Jasinski et al. 2001). No differences for any of the 14 natural enemy categories were noted when untreated glyphosate-tolerant and other soybean fields were compared in an Ohio sweep net field study. In fact, eight of fifteen beneficial insect categories were more numerous in glyphosate-tolerant soybean fields, including both generalists and specialists. Comparisons between two sample collection methods, sweep nets and sticky traps, revealed that multicolored Asian ladybird beetle, parasitic hymenoptera *C, maculata*, hoover flies, and soldier beetles were more common in glyphosate-tolerant soybean than in other soybean. Similar to conventional herbicides, glyphosate applied at varied rates did not affect insect populations of acarina, coleoptera, and hymenoptera in a "no-till" glyphosate-tolerant soybean in a Brazil field study (Ferri and Eltz 1998).

No adverse effects on earthworms were reported with the use of glyphosate. Glyphosate was ranked as zero on a scale of zero (nontoxic) to four (extremely toxic) based on its toxicity to earthworms (Edwards and Bohlen 1996). Herbicide concentration needed to cause mortality in 50% of the earthworms (also referred to as LC_{50} value) is greater than 5,000 parts per million (ppm) (Ahrens 1994). In contrast, LC_{50} values for soybean herbicides such as metolachlor and trifluralin were greater than 140 and 27 ppm, respectively, suggesting that these herbicides are more toxic to earthworms compared to glyphosate (Ahrens 1994). Earthworms are an important component of soybean ecosystem because they recycle organic matter in the soil.

In bird-feeding studies using bobwhite quail, no differences were recorded in food consumption, body weight, or behavior between birds fed with glyphosate-tolerant soybean or other soybean (Canadian Food Inspection 2001; Monsanto 2000). Birds may feed on the soybean seed that is left on the fields after harvest.

Crop residues left on soil surface in "no-till" production attract insect eating birds for nesting and egg laying. To study the effects of the introduction of genetically-modified crops, Watkinson et al. (2000) used sugarbeet to model British cropping systems. Based on their computer model, Watkinson et al. predicted that weed populations would be decreased to low levels or practically eradicated, depending on the form of management. Consequently, local use of fields by weed seed eating birds such as skylarks were predicted to decrease with decreasing weed seed availability associated with biotechnology-derived crops. The results from this model are opposite to farmers' observations that birds are more drawn to nest in "no-till" glyphosate-tolerant soybean fields than in conventional tillage soybean fields. A major criticism to Watkinson's finding is that weed seed eating birds do not commonly forage in plowed fields of either conventional or biotechnology-derived crops outside the United Kingdom.

Biodiversity is maintained in biotechnology-derived herbicide-tolerant soybean fields. Soil microbes, beneficial insects, and bird populations in conservation tillage biotechnology-derived herbicide-tolerant and other soybean fields are similar in number and variety.

Insect-resistant Soybean

No impacts have been reported yet on nontarget organisms from insect-resistant soybean since *Bt* soybean is not commercialized. The potential nontarget impacts associated with *Bt* soybean may be expected to be similar to those of *Bt* corn or *Bt* cotton; however, they must be characterized on a case-by-case basis. Most *Cry* proteins reported to date generally have a narrow target spectrum, being toxic to only lepidopteran, dipteran, or coleopteran insects (Betz, Hammond, and Fuchs 2000).

The knowledge base of the long-term impacts of *Bt* soybean on soil organisms will be enhanced if research evaluates such issues as secretion of *Cry* proteins through roots, length of persistence and accumulation of *Cry* proteins in soil under field conditions, and horizontal and vertical movement of *Cry* proteins to ground- and surface waters. Furthermore, the impacts should be considered in a risk-benefit context by comparing the effects of *Bt* soybean with the effects of other insecticides used to control the target pest.

Soybean roots secrete proteins and other chemicals all the time. *Cry* proteins, similar to other proteins, adsorb to clay particles in the soil. Adsorbed proteins are not biologically active. The amount of proteins a given soil can adsorb depends on the cation exchange capacity of the soil. Thus, the adsorption varies between soil types. A significant concentration of *Cry* proteins was reported to decline rapidly in about 14 days (Palm et al. 1996). As a result, the impact of *Cry* proteins on nontarget organisms is expected to be negligible.

Nematode-resistant Soybean

Biotechnology-derived nematode-resistant soybean is early in the developmental process and field affects on nontarget species are yet to be determined.

Virus-Resistant Soybean

Similarly, biotechnology-derived virus-resistant soybean is still in the developmental process and field effects on nontarget species are yet to be determined.

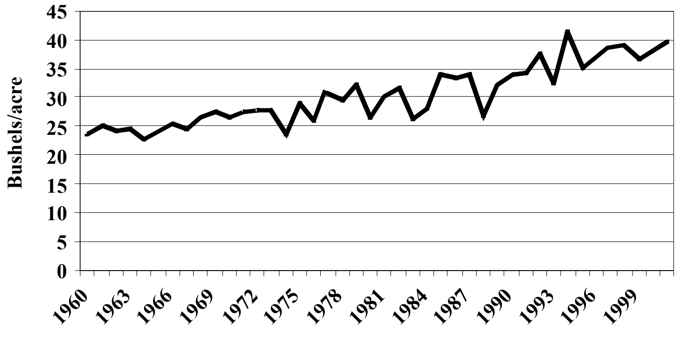
Land Use Efficiency

Glyphosate Tolerance

Land use efficiency refers to the potential to increase yields, i.e., to produce more output on less land. The introduction of glyphosate-tolerant soybean and associated glyphosate use may aid in improving land use efficiency if farmers are able to increase yields over other soybean farmers. Yields may vary due to the inherent yield potential of glyphosate-tolerant varieties, level of weed control, crop injury related to herbicide use, susceptibility to disease and other crop stresses, the ability to adopt narrow row spacing, and impact on nontarget pests. If yields improve due to biotechnology-derived soybean, pressure to convert additional land to agricultural production may be alleviated or certainly minimized. Pengue (2000) raised the concern that decreased cost of soybean production as a result of biotechnology methods will expand soybean production into agronomically marginal but ecologically sensitive areas. But it could be argued that increased yield per acre should decrease the likelihood that ecologically sensitive areas would need to be cultivated.

Evidence exists to support arguments that the introduction of glyphosate-tolerant soybean has increased or decreased yields. But the trend in soybean yields is continually increasing through 2001 (Figure IV-9), a year in which 68% of total soybean acreage was planted with glyphosate tolerant soybean varieties. This would seem to suggest that glyphosate-tolerant soybean is unlikely to have caused decreased yields, and may have contributed to increased yields.

Figure IV-9. U.S. soybean yields 1960-2001



Source: USDA-NASS 2002

Yield Potential

Conclusions regarding yield reductions attributed to the biotechnology-derived herbicide tolerant trait may be inaccurate because the study design included improper comparisons between the biotechnology-derived varieties and conventional varieties. Herbicide tolerance does not change the inherent yield potential of soybean varieties bred to incorporate the trait. But observed differences in yield potential of glyphosate tolerant soybean varieties compared to conventional varieties in the first years of commercialization have led to further investigation into the causes of these differences. Much of the speculation on yield differences was based on university run variety trials. Variety trials assess yield potential, among other variety performance characteristics, in experiments conducted under weed free conditions.

Trials conducted in eight northern U.S. states in 1998 and 1999 indicated average differences in yield potential between glyphosate-tolerant soybean varieties and conventional varieties of 4% and 3%, respectively (Carpenter 2001; Oplinger, Martinka and Schmitz 1998). Similar results were reported for southern states in 1998 (Minor 1998). Benbrook also conducted an analysis of the 1998 variety trials. His report reviews the results reported by Oplinger, then extends his analysis of trials conducted in Minnesota and Wisconsin, comparing the yields of the highest-yielding conventional variety and the highest-yielding glyphosate-tolerant variety by maturity group for entries from 10 seed companies. The conventional varieties achieved yields 10% higher on average than their glyphosate-tolerant counterparts (Benbrook 1999). However, the selection of Minnesota and Wisconsin for in-depth analysis is misleading, as one would reach different conclusions if two other states had been chosen. For instance, in Illinois and Michigan, Roundup Ready varieties outyielded conventional varieties in 198 variety trials in several areas (Oplinger, et al. 1998; Carpenter 2001). These differences generally are believed to be due to the differences in the agronomic background of the varieties in which the glyphosate-tolerant trait was available. Several years of backcrossing is necessary to introduce the biotechnology-derived trait into the highest yielding varieties using conventional breeding. As the glyphosate-tolerant trait is introduced into the highest yielding varieties, it is expected that this lag will disappear. The narrowing yield difference from variety trials conducted in 1998 and 1999 would support this expectation (Carpenter 2001).

Some have attributed yield differences to the glyphosate tolerance gene or the gene insertion process (Elmore et al. 2001). Researchers in Nebraska attempted to isolate this effect by comparing yields of glyphosate-tolerant and conventional “sister lines” of similar genetic backgrounds. Yields of the glyphosate-tolerant varieties were 5% lower than those of their conventional “sisters,” which was interpreted as evidence of yield “drag” (Elmore et al. 2001). But the “sister lines” were not genetically identical, so this difference may be due to other genetic differences.

Weed Control and Crop Injury

None of the studies on yield potential, however, consider differences in weed control or crop injury associated with glyphosate-tolerant compared to conventional weed control programs. Weed control trials are also regularly conducted by university researchers to compare weed control programs in terms of control of particular weed species, crop injury, and yields. It is difficult to generalize about the results from the weed control studies although there appears to be no resounding yield advantage or disadvantage in the glyphosate-tolerant programs compared to conventional programs. Some have speculated that farmers might have adopted glyphosate-tolerant weed control programs on fields with particularly heavy or difficult to control weed problems (Duffy 2001). If this is so, farmers may be achieving improved weed control, and higher yields, than possible with conventional programs, in those fields.

Plant Diseases

Susceptibility to plant diseases impacts the potential ability of soybean to produce economic yield, and thereby decreases land use efficiency. Potential differences in the incidence and severity of plant diseases in glyphosate-tolerant soybean compared to conventional varieties have been investigated. If these differences translate into differences in yields, land use efficiency would be affected. Differences in disease incidence may be due to the effects of herbicides on soil-borne pathogen populations, effects of herbicides on the susceptibility of the plant, or innate disease susceptibility of the soybean variety.

Most herbicides can impact soil-borne plant pathogens, either increasing or decreasing the incidence and severity of plant disease (Sanogo, Yang, and Lundeen 2001). The impact of glyphosate on soil-borne plant pathogens has been studied primarily in relation to its use as a burndown treatment before the introduction of glyphosate-tolerant soybean. These effects can be either direct or indirect. Direct effects are the result of the stimulation or inhibition of soil microbes by the herbicide, while indirect effects include the impact of herbicide treated weeds on soil microbial populations.

Direct effects of herbicides on soil microbes depend on the availability of the herbicide in the soil, which is affected by exudation and adsorption. Glyphosate may be available in root exudates from glyphosate-treated weeds and may stimulate pathogen invasion of the weeds. But the concentration of glyphosate in soil solution is very low because of strong adsorption to soil colloids and organic matter. Research indicates that glyphosate can either increase or decrease populations of soil microbes. Glyphosate application rapidly stimulated soil microbial activity but did not affect soil microbial biomass in a laboratory study (Haney et al. 2000). Glyphosate was degraded by microbes even at high application rates, without adversely affecting microbial activity (Haney et al. 2000). Growth of Pythium and Fusarium was stimulated or inhibited by glyphosate in another laboratory study, depending on concentration (Kawate et al. 1992).

Indirect effects of herbicides, through the interactions between herbicide-treated vegetation and pathogens, may be greater than the direct effects of glyphosate in soil. Residues of herbicide-treated weeds provide moisture and nutrients in the soil, which may lead to increased pathogen populations (Kawate et al. 1997). Fusarium and Pythium populations increased in soils with glyphosate-treated weeds, in greenhouse studies (Kawate et al. 1997). In a field study, Fusarium populations were increased after glyphosate treatment of the weed cover. But crops subsequently sown in treated fields were not detrimentally affected by glyphosate treatment of these weeds (Levesque, Rahe, and Eaves 1987). The incidence of Rhizoctonia root rot was more severe, and yields were lower in fields where intervals between glyphosate treatment and crop planting were shorter, which was attributed to the greater availability of nutrients for pathogen populations (Smiley, Ogg, and Cook 1992).

Researchers in Missouri reported that *Fusarium* colonization of glyphosate-tolerant soybean ranged from a 50% decrease to a 500% increase in the soil rhizosphere after glyphosate applications or glyphosate in combination with conventional herbicides compared to glyphosate-tolerant soybean treated with conventional herbicides. Conventional herbicides (pendimethalin + imazaquin) applied to glyphosate-tolerant soybean did not result in higher incidence of *Fusarium* on soybean roots than in untreated controls. But soybean yields were not affected by herbicide treatment (Kremer et al. 2001).

Apart from the effects of herbicides on soil microbial populations, the susceptibility of the soybean plant impacts the incidence of disease. The glyphosate-tolerant soybean variety that was distributed by Monsanto to seed companies for use in breeding programs was highly susceptible to sudden death syndrome (*Fusarium solani*), but the marketed varieties of glyphosate tolerant varieties are many generations removed from that variety. Pioneer Hi-Bred, a major seed supplier, stated that there has been enough crossing away from the original donor variety that its susceptibility should no longer be relevant (Lane 1998).Two studies directly addressed the susceptibility of glyphosate-tolerant soybean varieties to disease in comparison to conventional varieties (Lee, Penner, and Hammerschmidt 2000; Sanogo, Yang, and Lundeen 2001).

Three-year field experiments were conducted to assess the development of sudden death syndrome in three soybean cultivars following foliar application of four herbicides commonly applied to soybean: one glyphosate tolerant, one glyphosate tolerant with resistance to sudden death syndrome, and one conventional. There was an increase in the disease levels under application of acifluorfen, glyphosate, and imazethapyr compared with nontreated or lactofen-treated plants. The results obtained indicate that the response of glyphosate-tolerant soybean to sudden death syndrome is not different from the response of other soybean to this disease following application of the selected herbicides, and the resistance of soybean to sudden death syndrome was not changed with application of glyphosate (Sanogo, Yang, and Lundeen 2001).

Researchers in Michigan found no difference in the susceptibility of near isogenic glyphosate-tolerant and other soybean varieties to white mold (*Sclerotinia sclerotiorum*) after reports of increased disease by some Michigan farmers (Lee, Penner, and Hammerschmidt 2000). Further, glyphosate and adjuvants did not increase soybean susceptibility to white mold or hinder yield (Lee, Penner, and Hammerschmidt 2000). Researchers concluded that factors other than the glyphosate tolerance trait or glyphosate treatment were responsible for farmers' observation of increased white mold in glyphosate-tolerant soybean. Such factors might include the trend towards planting in narrow rows, which might create conditions more suitable for white mold development or susceptibility in the genetic backgrounds of the varieties in which the glyphosate-tolerant trait has been made available (Lee, Penner, and Hammerschmidt 2000).

Heat Stress

The susceptibility of glyphosate-tolerant soybean to heat stress was investigated in response to farmer reports in Georgia. Heat stress can result in stem splitting and consequent yield losses. Initial studies found that the particular varieties of glyphosate-tolerant soybean varieties that were tested tended to be more susceptible to heat stress than the conventional varieties to which they were compared. Stem-splitting was in the range of 90 to 100% for glyphosate-tolerant soybean compared to 40 to 70% for conventional varieties, which was attributed to elevated lignin content in the glyphosate-tolerant soybean varieties (Gertz, Vencill, and Hill 1999). But it is likely that these differences are due to inherent differences in heat stress susceptibility of the background variety rather than the glyphosate resistance trait. The study did not use isogenic or sister lines. Therefore, the glyphosate-tolerant and conventional varieties differed in traits besides herbicide tolerance. In addition, the reported differences were only marginally significant (at a 10% level).

Narrow Row Spacing

Adoption of narrow row spacing may improve yields and therefore enhance land use efficiency. Evidence suggests that soybean planted in narrow rows intercept sunlight more efficiently than in wide rows, thereby resulting in increased yields. Yield and weed control in glyphosate-tolerant soybean planted in narrow rows was greater than in wide rows (Nelson and Renner 1999), similar to other soybean (Mickelson and Renner 1997; Nelson and Renner 1998; Patterson et al. 1988; Yelverton and Coble 1991).

Soybean that is not herbicide-tolerant is generally planted in wide rows to allow tillage for late-season weed control, because use of herbicides late in the season would cause crop injury. However, late-season applications of glyphosate in glyphosate-tolerant soybean fields do not result in crop injury and thus allow farmers to eliminate late season cultivation and facilitate the adoption of narrow row plantings as is commonly practiced in conjunction with conservation tillage practices.

In 2001, 35% more soybean acreage was planted in narrow rows compared to1996 in midwestern and southern states (ASA 2001a). Factors contributing to this trend include more rapid canopy development, suppression of later emerging weeds, and more uniform soybean root distribution (Burnside 1979; Carey and DeFelice 1991; Nelson and Renner 1999). By planting soybean in narrow rows, farmers may be able to forego an herbicide application needed to control late-season weeds. The size of the weed seedbank that farmers have to deal with the following season and intensity of weed management will be considerably decreased due to narrow row planting made possible through the use of glyphosate-tolerant soybean.

Nontarget Pests

Nontarget pests are those pests that are not the direct targets of pest control, yet are affected by a particular pest control practice, potentially affecting yields. The dynamics of soybean canopy-inhabiting insects may change with changes in weed diversity and community structure that result from the use of glyphosate in glyphosate-tolerant soybean. Weed management systems that included conventional herbicides allowed for more weed escapes and were found to have higher densities of tarnished plant bugs, grasshopper nymphs, insidious flower bugs, and damsel bugs. Weed management systems that used glyphosate and glufosinate-tolerant soybean resulted in decreased weed diversity and were preferred by only potato leafhoppers (Buckelew et al. 2000). Buckelew et al. (2000) further concluded that effect of herbicide-tolerant biotechnology-derived soybean varieties is no different than other soybean. But weed management systems can affect soybean insect populations. Thus, weed management with glyphosate in glyphosate-tolerant soybean could lead to decreased pest infestations compared to other soybean, where weed species would be more diverse.

Human Exposure to Glyphosate-Tolerant Soybean

The potential human health impacts evaluated include allergenicity, toxicity, nutritional content, and the development of resistance to orally administered antibiotics. Biotechnology-derived soybean is compared to other soybean with regard to these characteristics. In general, these characteristics are assessed for the newly introduced protein, as well as for the whole food.

Allergenicity assessments begin by considering the allergenicity of the host plant. Soybean is among the most common food allergens. Other common allergens are cow's milk, eggs, peanuts, and wheat. Therefore, the composition and levels of these endogenous allergenic proteins were evaluated to assess whether the levels of these proteins had been altered in the genetic engineering process, making the biotechnology-derived soybean more problematic than their conventional counterparts for allergy sufferers. Known allergenic proteins of soybean were found to be unchanged compared to the parental variety and other commercial varieties, based on an evaluation of protein extracts from nontoasted, defatted soybean flakes and soy flour (Burks and Fuchs 1996).

The assessment of the potential allergenicity of the introduced CP4 EPSPS protein analyzed the amino acid similarity to known allergens. The analysis performed indicated that there were no significant stretches of homology between CP4 EPSPS and any of the allergens in the database used (Harrison et al. 1996). Next, the protein characteristics were compared to the characteristics common to allergenic proteins (Table IV-6). CP4 EPSPS shares one characteristic of allergenic proteins, that of molecular weight, but does not share any of the other characteristics. The CP4 EPSPS protein was examined for glycosylation and shown not to be glycosylated (Harrison et al. 1996). In simulated digestion tests, CP4 EPSPS degraded in 15 seconds, similar to the amount of time required for many common plant proteins (Harrison et al. 1996).

Toxicity of biotechnology-derived herbicide-tolerant soybean in comparison to other soybean was also assessed, considering toxicity of the introduced protein as well as the whole food. Soybean naturally produces toxic substances. For example, naturally occurring soy lectin can cause nausea, vomiting, and diarrhea if not removed and destroyed by proper soaking and cooking (FDA 1992). The composition of glyphosate-tolerant soybean was compared to its parent, traditional soybean. Antinutrients that were measured included trypsin inhibitor, lectins, isoflavones, stachyose, raffinose, and phytate. In

Table IV-6. Comparison of known allergen and CP4 EPSPS characteristics.

Characteristic	Allergens	CP4 EPSPS
Molecular weight 10 — 70 kdal	Yes	Yes
Glycosylated	Yes	No
Stable to digestion	Yes	No
Stable to processing	Yes	No
Similar to known allergens	NA	No
Similar to soybean proteins	NA	Yes
Prevalent protein in food	Yes	No

Source: Fuchs 1996.

analyses of seed composition, no significant differences between glyphosate-tolerant soybean and other soybean were found in the levels of any of these antinutrients (Padgette et al. 1996b). These components were also compared for defatted toasted soybean meal, commonly used in animal feed. Again, levels of all antinutrients in glyphosate-tolerant soybean were similar to those of other soybean, with the exception of lectins, which were below detection limits (Padgette et al. 1996b).

The toxicity of CP4 EPSPS was assessed in an acute mouse gavage study. Mice were fed a dosage of CP4 EPSPS intended to represent 1,000 times the anticipated consumption level of food products potentially containing the CP4 EPSPS protein. No ill effects were observed in terms of body weight, cumulative body weight, or food consumption (Harrison et al. 1996). The analysis of amino acid similarity comparing CP4 EPSPS to known allergens also compared CP4 EPSPS to known toxic proteins in the same database (Harrison et al. 1996). That analysis indicated that there were no significant stretches of homology between CP4 EPSPS and any of the toxins to which it was compared (Harrison et al. 1996).

Toxicity issues related to the use of glyphosate-tolerant soybean include the toxicity of glyphosate in comparison to conventional herbicides. Human exposure to glyphosate may be direct, for applicators working with the herbicide, or indirect, for consumers through residues in food. The enzyme EPSPS, on which glyphosate has inhibitory activity, is present only in plant species and thus mammalian toxicity is limited. Glyphosate is classified by the EPA as a category E pesticide, indicating evidence of noncarcinogenicity to humans (Griffin, Reynolds, and Jordan 1994). The use of glyphosate in glyphosate-tolerant soybean replaces the use of other soybean herbicides that are 3.4 to 16.8 times more toxic to humans (Heimlich et al. 2000).

Potential differences in nutritional value of glyphosate-tolerant soybean have been compared to other soybean. In comparisons of protein, fat, fiber, ash, carbohydrates, amino acids, and fatty acids, no differences were found between glyphosate-tolerant soybean, both treated and untreated with glyphosate, and other soybean (Padgette et al. 1996b; Taylor et al. 1999). Additional studies were conducted to evaluate levels of isoflavones in glyphosate-tolerant soybean compared to other soybean. Isoflavones are the direct products of the pathway by which glyphosate is effective, and which has been altered in the creation of glyphosate-tolerant soybean. Isoflavones in soybean have been reported to possess health enhancing characteristics, including the reduction of several types of cancer, including breast and prostate cancer (Taylor et al. 1999). Some studies have also shown deleterious effects in animals fed soybean meal (Taylor et al. 1999). Levels of isoflavones in glyphosate-tolerant soybean, both treated and untreated with glyphosate, were found to be similar (Padgette et al. 1996b; Taylor et al. 1999).

In 1999, Lappe et al. claimed that glyphosate-tolerant soybean possess decreased levels of isoflavones. This study was criticized based on the fact that isoflavone levels in soybean are highly variable and are greatly influenced by several factors such as environment, varieties, slope of the field, and the time and year when the crop is grown (Eldridge and Kwolek 1983). In studies using other soybean, wide variability in isoflavone levels was documented among Brazilian varieties (three- to eightfold) (Carrao-Panizzi and Kitamura 1995). Similarly, two- to fivefold differences were noted in levels of isoflavones between conventional American and Japanese varieties (Wang and Murphy 1994). Thus, variability is expected in levels of isoflavones in biotechnology-derived soybean similar to other soybean.

The potential for transfer of antibiotic resistance genes, used as selection markers in the genetic engineering process, into pathogenic bacterial populations, and the consequences of any such transfer, have also been considered for biotechnology-derived crops in general. Transfer of antibiotic resistance genes into bacteria would be most likely to occur in the field where the crop is grown, or in the guts of animals that ingest the crop. Genetic sequences, including the antibiotic resistance markers, may become free and present as the crop degrades, releasing deoxyribonucleic acid (DNA). The persistence and availability of free DNA to be taken up by competent bacteria are well documented (Smalla et al. 2000). The transfer of antibiotic resistance from transgenic sugarbeet plants to bacteria has been demonstrated under laboratory conditions (Gebhard and Smalla 1998). But the probability of bacteria gaining increased fitness through the acquisition of an antibiotic resistance gene from a biotechnology-derived crop is remote (Thomson 2001). Further, the particular antibiotic resistance marker used in the development of glyphosate-tolerant soybean confers resistance to kanamycin/neomycin antibiotics, which are infrequently used (Thomson 2001; USFDA 1998).

ECONOMICS OF BIOTECHNOLOGY-DERIVED SOYBEAN

Economic advantage is another key indicator and a useful index for comparing glyphosate-tolerant soybean to conventional weed control programs. The unprecedented high rate of acceptance and adoption of glyphosate-tolerant soybean in industrial and developing countries demonstrates the economic advantage of glyphosate-tolerant soybean.

A major impact of the introduction of glyphosate-tolerant soybean has been a reduction in weed control costs for soybean farmers. In the first few years that glyphosate-tolerant soybean was available, many farmers were able to decrease their weed control costs by adopting a glyphosate-tolerant soybean weed control program, which was relatively inexpensive compared to many conventional weed control programs. The costs of conventional weed control programs were higher than the cost of glyphosate-tolerant soybean because lower seed costs for the conventional systems were more than offset by higher herbicide costs (Persley and Siedow 1999; Roberts, Pendergrass, and Hayes1999; Sartorato and Zanin 1999). Manufacturers of conventional herbicides soon reacted to their declining market share by decreasing the prices of conventional herbicides, such as imazethapyr and chlorimuron, by as much as 40%. The result has been a reduction in weed control costs for both adopters and nonadopters of glyphosate tolerant soybean. It is estimated that in 2000, U.S. soybean farmers spent $307 million less on weed control than in 1995 (Carpenter and Gianessi 2002).

Findings regarding cost savings in Argentina were even more dramatic. In 1998/1999, Argentinean farmers saved $35 per hectare, or $214 million countrywide, on herbicide costs alone using glyphosate-tolerant soybean (James 2001a). A second study, conducted in Argentina during the 1999/2000 season, found that glyphosate-tolerant soybean had an economic advantage of $55.64 per hectare, or $356 million aggregate, over all glyphosate-tolerant soybean acreage (James 2001a).

The impact on farmers' net returns also show that glyphosate-tolerant soybean farmers do at least as well as conventional farmers. Surveys conducted in Iowa indicate that glyphosate-tolerant soybean farmers achieved lower yields than other soybean farmers in 1998 and 1999 (Duffy and Ernst 1999; Duffy 2001). But lower yields were offset by weed control savings, including herbicide costs. The result was similar net returns for glyphosate-tolerant soybean farmers compared to other soybean farmers. Others have found that glyphosate-tolerant soybean results in higher returns than conventional programs (McKinley et al. 1999; Reddy and Whiting 2000).

Three aggregate studies have been conducted that consider the impact of the technology on yields and costs, for U.S. farmers as a whole. An early assessment of the benefits of glyphosate tolerant soybean estimated a total benefit to U.S. farmers of between $126 million and $800 million in 1997, depending on the assumed elasticity of supply, and using assumptions based on USDA survey data (Falck-Zepeda, Traxler, and Nelson 2000). But these estimates were based on differences between adopters and non-adopters of the technology from the USDA survey data, which may be due to factors other than the adoption of herbicide-tolerant varieties (Gianessi and Carpenter 2000).

Another model of the impact of the introduction of glyphosate-tolerant soybean was developed by researchers at Iowa State University (Moschini, Lapan, and Sobolevsky 2000). Several scenarios were considered, including varying assumptions about adoption patterns, market structure for the technology, and yield changes. In the scenario most closely depicting what would be expected for crop year 1999-2000, an adoption rate of 55% of U.S. soybean acreage is assumed, and farmers are assumed to realize a $20/ha cost savings and no difference in yields compared to conventional farmers. Under this scenario, the change in producers' surplus was estimated at $256 million.

A USDA analysis of differences in returns for adopters compared to nonadopters of the technology in 1997 estimated that U.S. glyphosate-tolerant soybean farmers benefited by $60 million in aggregate, including differences in yields and weed control costs (Lin, Price, and Fernandez-Cornejo 2001).

Farmers who adopt glyphosate-tolerant soybean, as with any technological innovation, may realize other impacts on farm management that extend beyond soybean production and, therefore, are not accounted for in most economic models. For example, most farmers have diversified operations, including the production of more than one crop, and perhaps also engage in animal agriculture, or off-farm work. Farmers who adopt glyphosate-tolerant soybean report that they are able to decrease the amount of time they spend in their fields, which gives them the flexibility to devote their time to total farm management and other activities.

CONCLUSIONS

The issues that challenge biotechnology-derived soybean also confront other soybean. Biotechnology-derived soybean offers solutions to some of the problems posed by other soybean. Assessing the benefits and risks of biotechnology-derived soybean in comparison with those of other soybean offers meaningful insights into how the issues can be addressed and the problems corrected. The environmental benefits associated with the biotechnology-derived soybean exceed the environmental benefits of other soybean cropping systems. As with other soybean, challenges raised with weed and insect resistance in biotechnology-derived soybean can be managed effectively. Biotechnology-derived soybean offers tremendous potential for pest management with significant positive environmental impacts compared to other soybean, thereby increasing the sustainability of soybean agriculture.

[1] Other major commodities, in order of value, include crude oil, coal, rice, wheat, maize, and hardwood logs.

[2] Total “application acres” is the number of applications per treated acre, times the number of treated acres, for each active ingredient, summed over all active ingredients.

V. Corn

INTRODUCTION

Corn (*Zea mays*) is an annually cropped grass that is seeded directly into tilled or untilled soil. Evolution of corn over thousands of years has resulted in multiple biotypes that can be grown over diverse geographical areas ranging from the tropics to the north temperate zone and from sea level to 12,000 feet altitude (Brown et al. 1984). The seedling to seed lifecycle requires from six weeks to thirteen months. In the temperate regions, field corn (the designated terminology for corn destined for grain and silage production) is sowed in April and May and usually harvested by October. In tropical regions, planting and harvesting will occur earlier. The majority (50-60%) of corn is used for animal feed, but substantial amounts are used for ethanol production and human consumption (high fructose corn syrup, corn flour, starch, and corn oil).

WORLD CORN INDUSTRY

Corn is grown worldwide on an estimated 140 million hectares (ha) (346 million acres) (James 2001). The major farmers include the United States (43% of world production), China (18%), the European Union (mainly France, 7%), Brazil (6%), Mexico (3%), Argentina (3%), and India (2%) (USDA 2001a).

In the United States, 32.2 million ha (79.5 million a.) of corn intended for grain and silage production were planted during crop year 2000, and 31.8 million ha (78.6 million a.) were harvested (USDA 2001b). Of the harvested total, 29.4 million ha (72.7 million a.) were used for grain. Fresh and processed sweet corn, which are directly consumed by humans, accounted for a total of 227,000 ha (623,000 a.) in the United States during crop year 2000 (USDA 2001c).

CORN IMPROVEMENT FOR PEST MANAGEMENT AND QUALITY TRAITS

Crop Development Techniques

Historical Crop Development Techniques

The progenitor species of corn has been hypothesized to be teosinte (*Zea mays* spp. *parviglumis*, formerly classified as *Zea mexicana*), a grass native to Mexico (Benz 2001). Through thousands of years of artificial selection of seed, teosinte has evolved to today's recognizable corn crop, although the major changes in branching and seed number may have taken as little as several hundred years of selection. Another hypothesis, however, suggests that pod corn, a little used variety of *Zea mays*, is the progenitor species (Brown et al. 1984). Nevertheless modern landraces of corn, unlike teosinte, have lost the ability for self-dispersal of seeds. Of the 300 races of corn that have been described from all of Latin America, perhaps 150 are truly distinct landraces and have been collected from this region. Certain of these landraces were ancestors of the varieties currently grown in the United States.

Two early postcolonial landraces of corn in the United States, the late-maturing Virginia Gourdseed and the early-maturing Northeastern Flints, were crossed circa the early 1800s to yield a hybrid of recognized superior qualities to the parental races (Brown et al. 1984). The cross resulting in this early hybrid was repeated many times as settlers moved from the eastern coastal plains across the continental United States, and it was the progenitor of the currently grown yellow dent corns.

In the early 1900s, G. H. Shull of the Carnegie Institute proposed the development of inbred lines. Inbreds are landraces that are self-pollinated by placing tassels from a corn plant on its own silks (Bauman and Crane 1985). In six or seven generations, parental lines can be produced that are uniform and able to transmit their characteristics consistently to the next generation. Each generation of an inbred line theoretically has one-half of the genetic variation of the previous generation. The inbreds are crossed with one another to select a superior performing hybrid possessing much greater genetic variation than either of the parents. For example, hybrids may have contributed to 60% of the per acre increases in corn yields since commercial introduction before the 1930s and widespread planting by the 1940s.

To maintain the distinct characters of hybrids, inbred lines must be continually grown using spatial isolation techniques and controlled pollination. Between the 1930s and 1950s, hybrid seed was produced from inbreds in fields that were separated from other fields by approximately 660 ft (200 m). The female and male single-cross parents (inbreds) were planted in alternating blocks of six and two rows, respectively (Bauman and Crane 1985). Female rows were detassled prior to silk emergence, and male pollen was then allowed to pollinate the female parents.

Because inbreds had poor vigor and yielded poorly, their early development was slow. In 1922 double crosses were introduced to speed seed production. A superior hybrid from a single cross of two inbreds was selected for crossing with another hybrid. The resulting double-crossed hybrids could then be selected for superior yield and designated for seed production (Bauman and Crane 1985). However, by the 1960s selection of new inbreds from crosses of the best inbreds of previous generations (known as recycling) had produced inbreds with sufficient seed yield and stability to serve as seed parents for commercial production of single-cross hybrids (Kannenberg 1999). Thus, it became commercially feasible to use the seed of single crosses for seed production again, and today most hybrids are produced by single crosses owing to their greater uniformity.

Single-cross hybrids were more expensive than open-pollinated varieties but their superior production qualities outweighed the expense. Early development of hybrids was carried out by the U.S. Department of Agriculture and university breeding programs; accessions were considered public domain. By the early 1940s, however, seed companies started their own corn breeding programs that developed proprietary hybrids (Bauman and Crane 1985; Kannenberg 1999). Thus, privatization of seed resources has been an ongoing development in U.S. agriculture for at least 60 years.

The dramatic change from open-pollinated corn to the almost exclusive use of hybrids is easy to understand from the viewpoint of agronomic efficiency. Certified hybrid corn seed is higher yielding, and more tolerant to drought, diseases, and insects than open-pollinated varieties. Furthermore, hybrid seed has better standability, thereby aiding mechanical picking. Given these superior agronomic characteristics, farmers abandoned seed saving in favor of planting certified seed whose production qualities are guaranteed.

Application of Biotechnology to Corn Improvement

All currently registered and commercialized biotechnology-derived corn cultivars possessing traits for herbicide tolerance and pest protection are first developed by cloning the traits on *Escherichia coli* plasmids. The traits are assembled in cassettes for functional expression using bacterial antibiotic resistance selection markers and DNA accessory elements. The protein products of the bacterial antibiotic resistance gene are not expressed in any of the commercial corn cultivars owing to lack of proper promoter sequences (EPA 2001a; Sidhu et al. 2000).

Cloned plasmids (minus the plasmid backbone) are biolistically inserted into corn callus cultures and the recipient tissues with the properly expressed traits are selected with the addition of herbicide to the culture medium. Glyphosate-tolerant corn can be selected with glyphosate.

Successful transformation of corn using biotechnological techniques has shortened development time for new traits from tens of years to several years or less. Testing and regulatory approval adds several years to the time required for commercialization of the enhanced cultivars.

TRAITS IN CORN

Herbicide Tolerance

Tolerance to imidazolinone herbicides has been bred into commercial corn cultivars using recurrent selection of embryogenic corn callus cultures following treatment with the herbicides (Dyer 1996). The trait

Table V-1.
Genetic characteristics of pest-protected and herbicide-tolerant corn plants

Event (Trait)	Genes (source)	Promoter and other Sequences	Terminator	Form (number of copies)
MON832 (Glyphosate resistance)	Glyphosate oxidoreductase, GOX (Ochrobactrum anthropi)	CaMV 35S		3
MON832 (Glyphosate resistance) HT	EPSPS (Agrobacterium sp. strain CP4)	Enhanced CaMV 35S, maize HSP70 intron	nos (nopaline synthase 3 polyadenylation signal from *A. tumefaciens*	1
MON832 (Functionality of EPSPS)	Chloroplast Transit Peptide (CTP I from *Arabidopsis thaliana* SSU1A gene; CTP 2 from *A. thaliana* EPSPS gene)			
MON832 (Selection marker)	Neo (neomycin phosphotransferase II) (*E. coli* K12)	Bacterial promoter		1 complete; 1 rearranged; not expressed in plant tissues because of presence of bacterial promoter
GA21 (Glyphosate resistance)	EPSPS (from *Z. mays*)	Rice actin I promoter and intron sequences	nos (nopaline synthase 3' polyadenylation signal from *A. tumefaciens)*	Modified by in vitro mutagenesis; single insertion site with 3 complete copies of EPSPS cassette plus 3 incomplete copies
GA21 (Functionality of EPSPS)	CTP (chloroplast transit peptide)	Ribulose-1,5 – bisphosphate carboxylase oxygenase (RuBisCo) derived CTP from corn and sunflower		
***Bt* 176** (Insect protection)	*Cry*1Ab (*Bacillus thuringiensis* subsp. kurstaki, *Bt*k)	Gene copy 1: maize phosphoenolpyruvate carboxylase gene and CaMV35S terminator; Gene copy 2: calcium-dependent protein kinase gene and CaMV 35S	CaMV 35S poly A signal	2

Table V-1. (continued)
Genetic characteristics of pest-protected and herbicide-tolerant corn plants

Event (Trait)	Genes (source)	Promoter and other Sequences	Terminator	Form (number of copies)
TC1507 (Insect protection)	*Cry*1Fa2 (*Cry*1F delta-endotxin from *Bacillus thuringiensis* var. *aizawai*) from ORF25		3' polyadenylation signal from *A. tumefasciens*)	1 functional; 1-2 partial
TC1507 *Cry*1F (Glufosinate resistance)	pat (phosphinothrincin N-acety ltransferase (AT) (*S. viridochromogenes*)	CaMV 35S	CaMV 35S 3' polyadenylation signal	1, functional
NK603 (Glyphosate resistance)	EPSPS (*Agrobacterium* sp. strain CP4)	P-ract1/ract1 intron containing rice actin 1 promoter	nopaline synthase (nos) 3' -polyadenylation signal from *A. tumefasciens* 1	1 CP4 EPSPS gene modified for plant preferred codons
NK603 (Glyphosate resistance)	EPSPS (*Agrobacterium* sp. strain CP4)	enhanced CaMV 355, maize HSP70 intron	nopaline synthase (nos) 3' -polyadenylation signal from *A. tumefasciens*	1 CP4 EPSPS gene modified for lant preferred codons
NK603 (Functionality of EPSPS enzyme)	Chloroplast Transit Peptide CTP2 (from *A. thaliana* EPSPS gene)			

(continued on following page)

was expressed in regenerated plants as possible unlinked, semidominant alleles. The lines were backcrossed to inbred lines to develop 14 commercial imidazolinone-resistant lines.

Several biotechnology-derived glyphosate-resistant corn hybrids have been developed. Each plant cultivar in which a desired trait was successfully incorporated into the genome is called an event and is designated by a letter code. For hybrid events known as MON802 and MON832, which have not been commercialized, the source of EPSPS was *Agrobacterium* (strain CP4). However, for the commercially available hybrid known as GA21, the source was corn's own cloned gene that had been mutagenized in vitro (i.e., in cell culture) (Sidhu et al. 2000) (Table V-1). This technique involves changing the DNA bases of cultured plant cells by adding mutagenic chemical reagents. Resulting changes in DNA bases could slightly affect the amino acid composition of the host (i.e., corn) enzyme. Normally, mutagenesis will produce nonfunctional enzymes, but in some cases a few changes in amino acid sequence can still produce a functional enzyme. With the mutagenized corn line, the resulting EPSPS was 99.3% similar to the non-mutagenized EPSPS and still functional (i.e., it produced the aromatic amino acids), but it was resistant to the effects of glyphosate (Sidhu et al. 2000). The development of RR corn using a mutant version of its own EPSPS gene followed research nearly a decade earlier where petunia EPSPS was successfully altered and then reintroduced into the plant to effect tolerance to glyphosate (Kishore, Padgette and Fraley 1992; USDA-APHIS 2000).

Insect-Resistant Corn

Traditional Breeding of Corn for Insect Control

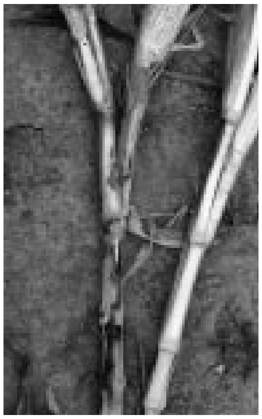
Most commercial corn hybrids have some degree of resistance to whorl-stage feeding by European corn borer (ECB) larvae (Mason et al. 1996; Steffey et al. 1999). Hybrids have been introduced for resistance to first generation ECB, and to a lesser extent resistance

Table V-1. (continued)
Genetic characteristics of pest-protected and herbicide-tolerant corn plants

Event (Trait)	Genes (source)	Promoter and other Sequences	Terminator	Number of copies (form)
***Bt* 176** (Selection marker)	bar (phosphinothricin) N-acetyltransferase (PAT) from *S. hygroscopicus*)	CaMV 35S	CaMV 35S poly A signal	
***Bt* 176** (Selection marker)	bla (beta-lactamase)	Bacterial promoter		Not expressed because of bacterial promoter
***Bt*11** (Glufosinate resistance)	pat (phosphinothrincin) N-acety ltransferase (AT) (*S. viridochromogenes*)[1]	CaMV 35S; IVS 2 intron from the maize alcohol dehydrogenase gene	nopaline synthase (nos) 3' -polyadenylation signal from *A. tumefasciens*	1
***Bt*11** (Insect protection)	*Cry*1Ab (delta-endotoxin) (Btk HD-1) (*S. viridochromogenes*)	CAMV 35S; IVS 6 intron from the maize alcohol dehydrogenase gene	nopaline synthase (nos) 3' -polyadenylation signal from *A. tumefasciens*	1
MON810 (Insect protection)	*Cry*1Ab (*Bacillus thuringiensis* subsp. kurstaki)	Enhanced CaMV 35S; maize HSP70 intron	None (lost through 3' truncation during integration)	1, Truncated
MON802 (Glyphosate resistance)	EPSPS (Agrobacterium sp. strain CP4)	Enhanced CaMV 35S; HSP70 intron; gox cassette		1
MON802 (Glyphosate resistance)	goxv247 (glyphosate oxidoreductase) (Ochrobactrum anthropi)			3
MON802 (Functionality of EPSPS enzyme)	Chloroplast Transit Peptide (from A. thaliana SSUaA gene, CTP1			
MON802 (Insect protection)	*Cry*1Ab delta-endotoxin from *Btk*	Enhanced CaMV 35S	nopaline synthase (nos) 3' -polyadenylation signal from *A. tumefasciens*	1, full length 2, truncated
MON802 (Selection marker)	neo (neomycin phosphotransferase II (*E. coli* K12)			2

[1] Streptomyces,viridochromogenes, the source of the pat gene is ubiquitous in the soil. The microorganism displays slight antimicrobial activity, is inhibited by streptomycin, and has not exhibited any adverse effects on humans, animals, or plants.

to second generation ECB (Barry and Darrah 1991). Inheritance of resistance for whorl feeding (first generation resistance) is believed controlled by a combination of 6 genes. Resistance to sheath collar feeding is conferred by the interaction of 7 genes. A chemical called DIMBOA, which is lethal to ECB larvae, is produced by young corn plants at high concentrations



Damage by stalk-boring insects causes reduced yields, increased costs, and harvesting losses from insect damaged corn plants. *Bt* corn (right) and non-*Bt* corn (left)

Photo by Dr. Galen Dively, University of Maryland

during the whorl stage. Corn germplasm with resistance to ECB also may provide some resistance to other corn insects (Wilson et al. 1995). Development of second generation feeding resistance has lagged behind first generation resistant hybrids. Yet, second generation feeding is more economically damaging than first generation feeding because densities of second-generation ECB often tend to be larger than densities of first-generation ECB, and stalk breakage and dropped ears have a direct impact on efficiency of combine harvesting.

Through traditional breeding, corn plants can tolerate significant feeding by corn rootworms (Levine and Oloumi-Sadeghi 1991). This tolerance is due to a large root system that is able to regenerate more roots after damage. Branson et al. (1983) reported actual resistance to western corn rootworm larvae in three experimental hybrids compared with a commercial variety.

Because of concern for cosmetic damage to ears in fresh market sweet corn and the presence of insect parts in processed sweet corn, much traditional breeding has focused on sweet corn. In sweet corn, one of the most important pests is corn earworm, *Helicoverpa zea*. The inbred corn “Zapalote Chico” is known for its resistance to corn earworm (Wiseman and Bondari 1995). Natural resistance to corn earworm is due to husk cover and chemicals in the silks such as the flavone, maysin (Butron et al. 2000). Although maize germplasm with resistance in the whorl stage to the larval feeding by fall armyworm, *Spodoptera frugiperda,* and to the southwestern corn borer, *Diatraea grandiosella,* has been identified and released, little progress has been made in identifying resistance to ear damage by these insects (Williams et al. 1998). Wilson, Wiseman and Snook (1995) investigated corn accessions in the National Plant Germplasm System with pure red pericarp color. Results of the diet testing with corn silks identified four plant introduction accessions that were similar to the resistant control, “Zapalote Chico.” One accession had low levels of maysin in the silks but was resistant. Wiseman and Isenhour (1994) evaluated 27 commercial sweet corn hybrids, but found no resistance to corn earworm in the field. One hybrid did have antibiotic silks, but the same hybrid had a loose husk, which renders it susceptible to insect damage in the field.

Wiseman and Snook (1996) discovered that several commercial corn hybrids exhibited high antibiosis against larvae of corn earworm. The antibiotic compound isoorientin is present in silks of some inbreds at a concentration that is harmful to the larvae (Widstrom and Snook 1998). Wiseman and Carpenter (1998) concluded that the growth inhibition factor in popcorn introductions containing maysin or isoorientin in silks is not a feeding deterrent, but rather an anti-nutritive factor for corn earworm. Pertinently, antibiosis does not kill larvae outright but inhibits their development and is measured as a reduction in weight gain.

Guo et al. (1999) successfully transferred antibiosis to corn earworm from dent corn containing high silk maysin to sweet corn. Williams and Davis (2000) report that two corn germplasms, MP713 and MP714, were released as sources of resistance to leaf feeding by southwestern corn borer and fall armyworm. Abel et al. (2000) evaluated experimental lines of maize for resistance to corn earworm, fall armyworm, southwestern corn borer, and sugarcane borer, *Diatraea saccharalis*. They found several experimental lines that could provide plant breeders with new sources of resistance to lepidopteran insects.

In summary, breeders have attempted to enhance naturally occurring toxins in corn to reduce feeding damage by the principal insect pests of field and sweet corn, the corn earworm and the European corn borer. The compounds function largely through antibiosis mechanisms whereby development is inhibited owing to an anti-nutritive effect. While experimental cultivars have been produced, none currently has sufficient feeding resistance to prevent economic damage.

Biotechnology-derived Insect Resistance

The currently registered and commercialized insect resistance characteristics are all based on transformation of corn to express the *Bt* toxin proteins, *Cry*1Ab and *Cry*1F (Table V-1). Targeted insects controlled in corn include the European corn borer (ECB), the corn earworm (CEW), and the Southwestern corn borer (SWCB). Registered but not yet commercialized traits against ECB include a *Bt* protein called *Cry*1F. The *Cry*1F event (Herculex 1) should provide a broader spectrum of activity than other commercially available *Bt* corn hybrids. In addition to ECB, Herculex 1 should provide excellent control against fall armyworm, SWCB and black cutworm. Similar to other *Bt* events, *Cry*1F has moderate efficacy against CEW (Agbios 2001).

Bt proteins from class 3B are under development for control of the various species of corn rootworms (CRW). Monsanto has petitioned government regulators in the United States and Japan for regulatory approval to commercialize event MON863 (USDA-APHIS 2002). The event was transformed using similar techniques already discussed for other herbicide-tolerant and pest-protected corn cultivars.

Disease Resistance

Traditional breeding has been the primary means for pathogen and disease control in field and sweet corn production (Ullstrup 1978; White 1999). Over 60 pathogens (viruses, bacteria, mollicutes, and fungi) infect the roots, ears, stalks, or leaves of corn (Hooker 1978; White 1999). As many as a million potential corn hybrids are tested each year by the corn seed industry in the United States (White 1999). Few, if any, will ever reach commercial markets. Those that do must demonstrate improvements in yield and disease resistance compared with existing hybrids.

Most hybrids are highly resistant to only a few diseases but moderately resistant to many. Resistance is based on either cytoplasmic or nuclear genetic material in the hybrid. In 1970 in the United States, corn cytoplasm became infamous for contributing to disease susceptibility. For several decades before 1970, a maternally inherited cytoplasm providing male sterility, *cms-T*, came into increasing use as a major aid to hybrid seed corn production because it eliminated the laborious and expensive detasseling procedures. In 1970, a major epidemic of southern corn leaf blight was caused by a new race of the pathogen that was highly virulent in *cms-T* corn. The seed corn industry continues to investigate ways to safely use cytoplasmic male sterility (Duvik and Noble 1978).

Traditional breeding can involve the use of genes from plant species other than *Zea mays*. Annual and perennial teosinte (*Zea diploperennis*) and a few species of *Tripsacum* have been crossed with corn to improve disease resistance and other traits (Bergquist 1979; Hoisington et al. 1999).

Some companies are exploring the use of biotechnology to develop disease resistance in corn, but no hybrids have yet been commercialized. Murry et al. (1993) described the development of a biotechnology-derived corn modified with a virus coat-protein gene that provided resistance to two plant viruses. Inoculations of maize dwarf potyvirus or a mixture of maize dwarf potyvirus and maize chlorotic mottle machlovirus that caused symptoms in conventional sweet corn did not cause disease symptoms in biotechnology-derived plants.

ADOPTION OF BIOTECHNOLOGY-DERIVED CORN

Herbicide Tolerance

Herbicide Tolerance and Weed Control in Conventional Corn Systems

In the United States, over 95% of the corn acreage is treated annually with one or more herbicides to control annual and perennial grass and broadleaf weeds (USDA-NASS 2000). The purpose of controlling weeds is to decrease the competition with the crop for nutrients, water, and light. Weeds are very competitive with corn because they tend to have very rapid early growth and extensive vegetative structures. Furthermore, some weeds can tolerate lower soil temperatures and moisture better than corn, and thus these species will become better established as competitors.

Before the development of efficacious broad-spectrum chemical herbicides, cultivation was the primary mechanism of weed control. Extensive cultivation of soils subject to heavy spring rains leads to significant erosion of topsoil and degradation of soil productivity. With the advent of grass and broadleaf selective herbicides, however, reduced- and no-tillage practices allowed direct seeding of corn without extensive disturbance of the planting bed. In general, weeds have produced an extremely large overwintering seedbank that will continue to germinate for many years. Thus, herbicides have been universally adopted in corn production because of their efficiency for managing weeds that are inevitably present.

Generally, herbicides registered for corn are used pre-planting and/or post-planting but before emergence of the corn crop (Iowa State University Extension 2000). Thus, they are used during a time of year subject to the heaviest rainfalls and when the soil is least covered with residue from the previous crop that could impede runoff. Depending on the herbicide, pre-plant herbicides generally are either incorporated into the soil during or immediately after application or left on the soil surface.

Over the last decade new herbicides were commercialized that encouraged a shift from the use of pre-plant and/or pre-emergence herbicides to postemergence herbicides that could be safely sprayed in the crop. Corn is innately tolerant to many of these herbicides but prescriptions on corn size must be followed to avoid crop injury.

Despite the shift to postemergence herbicides, pre-plant and pre-emergence herbicides that are applied directly to soil remain the most widely used herbicides. Atrazine, acetochlor, and metolachlor were the most used active ingredients; they were applied to 68, 25, and 28%, respectively, of crop year 2000 corn acres (USDA-NASS 2001) (Table V-2).

The most widely used postemergence herbicides during crop year 2000 were dicamba (28% of acreage), nicosulfuran (15%), and pirimisulfuron (9%). Some products are designated for both pre-emergence and postemergence use (e.g., flumetsulam mixed with clopyralid, 10% of corn acres) (USDA-NASS 2001).

Table V-2. Major herbicides used over the last ten years in U.S. corn production (Data from the NASS Databases for crop years 1991, 1995, and 2000).

	1991			1995			2000		
Herbicide	Pounds x1000	Area Applied %	Pounds /Acre per crop yr	Pounds x1000	Area Applied %	Pounds /Acre per crop yr	Pounds x1000	Area Applied %	Pounds /Acre per crop yr
Acetochlor				23,312	18	2.01	31,442	25	1.73
Alachlor	37,174	27	2.01	8,718	8	1.64	4,748	4	1.74
Atrazine	52,060	66	1.14	45735	65	1.1	53,594	68	1.07
Glyphosate	1,156	2	0.93	2358	6	0.64	4,438	9	0.7
Metolachlor	38,792	30	1.91	35,075	29	1.86	14,232	12	1.67
Metribuzin				85	1	0.12	190	2	0.13
Nicosulfuron	76	4	0.03	224	13	0.03	199	15	0.02
Primisulfuron	29	1	0.03	42	3	0.02	140	9	0.02
S-metolachlor							15,383	16	1.33
Total herbicides x 1000	189,469			166,860			152,776		
Acres harvested	68,822			65,210			72,732		

Herbicides have been very successfully employed for weed control in corn, but a number of problems have occurred despite their applicability for reduced- and no-tillage soil management. First, the commonly used pre-plant/pre-emergence products—atrazine, alachlor (which has been substantially replaced by acetochlor), and metolachlor—frequently have been detected in surface water samples throughout the world. To a lesser extent, triazine and chloroacetamide herbicides have been detected in shallow groundwater and drinking water wells. Second, the newer sulfonylurea (SU) herbicides such as nicosulfruon and pirimisulfuron are used at comparatively low per acre rates, but corn is more at risk for injury under certain conditions of use: namely, recommendations prohibit using the product if the crop exceeds a specific height or leaf stage. Third, phytotoxicity can result when an organophosphate (OP) soil insecticide is used at planting time and an SU herbicide is used post-emergence. The OP soil insecticides synergize the SU herbicides by inhibiting their rapid oxidative metabolism in corn; thus the target site, acetolactate synthase, is more susceptible to inhibition (Baerg et al. 1996). Because OP soil insecticides remain the most frequently used pesticides for corn rootworm control, the potential for negative interactions is not trivial. Thus, the low per-acre use rates of the newer herbicides are beneficial, but these compounds also pose some new complexities in weed control.

Postemergence use of glyphosate on biotechnology-derived glyphosate-resistant corn has some advantages over the use of other postemergence products. For example, it can be used from the time of plant emergence until the crop is 30 inches tall. Many sulfonylurea herbicides can be applied post emergence only until the crop is generally 12 inches or less. Thus, a farmer using glyphosate-resistant corn can delay herbicide treatment until after numerous weeds have germinated and then burn them down with an approved Roundup product. However, many studies have shown that delaying removal of early weeds often decreases crop yields.

Biotechnology-derived Herbicide Tolerance

Adoption of herbicide-tolerant corn has been slower than the adoption of pest-protected corn. In 1998 and 1999, an estimated 8 and 7%, respectively, of the corn acreage was planted to glyphosate-tolerant corn (Dexter 2000; Fernandez-Cornejo and McBride 2000). However, in crop year 2000, only 4% of corn was estimated to be glyphosate-tolerant in the United States (Benbrook 2001), and worldwide the percentage was estimated to be 2.5% (James 2001). Owen (2000) suggested reasons for the lack of adoption of biotechnology-derived herbicide-tolerant corn in the United States may include good alternative weed management programs, questionable yield potential, and potential phytotoxicity problems. Additionally, lack of farmer adoption of biotechnology-derived herbicide-tolerant corn in the United States is, in part, because most U.S. corn processors refuse to accept them due to their pending approval status in the European Union and other regions of the world.

Insect-Resistant Corn

Historical Control of Corn Insect Pests

The European corn borer (ECB) (*Ostrinia nubilalis*) and the corn rootworm complex (western corn rootworm, *Dibrotica virgifera virgifera*, northern corn rootworm, *Diabrotica barberi*, and southern corn rootworm, *Diabrotica undecimpunctata howardi*) are the most economically damaging insect species attacking corn in the midwestern and southern United States. The corn earworm (CEW) (*Helicoverpa zea*) is a sporadic pest of field corn in the Midwest, but a principle pest in the southern United States and generally in sweet corn. The Southwestern corn borer (*Diatraea grandiosella*) is economically damaging in the irrigated western regions of the Corn Belt, southwestern states, and southern Corn Belt (Missouri bootheel, southern Illinois/Indiana, and western Tennessee). The following discussion will focus on the CEW in sweet corn, and the ECB and CRW in field corn.

Traditional Control of Insects in Sweet Corn

Among vegetable crops, sweet corn ranks near the top in market value and acreage harvested, but it also has traditionally been intensively treated with insecticides (Guo et al. 1999). Owing to the importance of undamaged ears to marketability and consumer acceptance, CEW, ECB, and other insect pests require management in sweet corn fields. Larvae of both species will feed on developing ears and can be found as late instars[1] when sweet corn matures. In the northern United States, 12-14 insecticide applications may be required for economic control (Hoffmann et al. 1996), but in the southern United States as many as 25-40 insecticide applications may be made in a single season to control ear-feeding insects (Guo et al. 1999). During crop year 2000, about 84% of the estimated 200,000 acres (80,940 ha) of fresh market sweet corn were treated with nearly 500,000 pounds of insecticides (USDA-NASS 2001c). The OP insecticides (chlorpyrifos), pyrethroids (cyfluthrin, cyahalthrin, permethrin), and carbamates (methomyl, thiodicarb) are the most frequently used corn insecticides. About 73% of the 400,000 acres (161,880 ha) of processing sweet corn are treated with an insecticide, but the intensity of pesticide use is much less, as evidenced by a total of only 95,000 pounds applied during crop year 2000 (USDA-NASS 2001c).

Formulations of a microbial insecticide containing the bacterium *Bacillus thuringiensis* (microbial *Bt* sprays) are registered for control of lepidopteran pests on sweet corn, but their use has been limited to about 1% of sweet corn acres. An average of 2.6 *Bt* spray applications are used per season. Compared to conventional insecticides, *Bt* sprays are often ineffective in providing the high level of control needed for marketing fresh sweet corn. However, research has shown that aerial applications of combinations of pyrethroid or OP insecticides with formulations of *Bt* (e.g., Dipel or MPV) can give effective control at lower insecticide rates (Bartels and Hutchison 1995).

Traditional Control of Insects in Field Corn

European Corn Borer Biology, Damage, and Control

In late May and early June, the ECB moth lays on average 15-30 eggs per egg mass on the undersides of corn leaves near the midrib (Steffey and Gray 2001). “Each mated female [first generation] is capable of depositing an average of two egg masses per night for 10 nights . . . Each second- or subsequent-generation female will lay about 400 eggs during her life.” (Mason et al. 1996). Early-planted corn is more attractive than late-planted corn for egg laying by ECB females in the spring (first generation) and is more conducive for survival of the first generation. Late-planted corn is more attractive than early-planted corn for egg laying by ECB females in the summer (second generation). After the eggs hatch, the larvae feed their way through the leaf to the whorl, resulting in shot holes in the leaves. The fourth and fifth larval instars bore in the stalks where they pupate. The moths that emerge are the culmination of the first generation. These moths mate, and females lay eggs to begin the second generation. After mating, the female lays eggs on the undersides of leaves between the ear zone and the tassel. Newly hatched larvae feed on leaf collar tissue and accumulating pollen in the collar area. As the larvae mature they tunnel into the stalks, ear shanks, and ears. Feeding damage of ECB results in disruption of the plant’s water flow and nutrients to the developing corn ear (Steffey and Gray 2001). Tunneling in the stalks causes breakage or lodging (tipping over) of the plant. Tunneling also can cause ear drop, and damaged kernels become conducive to growth of fungi, producing biologically active compounds called mycotoxins under certain conditions of temperature and moisture.

The second-generation ECB larvae mature and overwinter in the stalks in the Midwest. Third- and even fourth-generation larvae overwinter in the southern states. In early May, the larvae pupate, and the adults emerge in late May and June. In more southern parts of the Corn Belt, the second-generation larvae may complete their development to start a third generation.

Normally, deep tillage of the stalks in the fall and shredding can reduce the overwintering survival of larvae. Minimal or no-tillage increases the probability of survival. However, whether ECB is effectively controlled by tillage will depend on soil management practices in adjacent fields as emerging adults from non-plowed, non-shredded fields will move to other fields to lay eggs (Steffey and Gray 2001.)

ECB populations fluctuate significantly from year to year, so economic returns for controlling this pest will vary according to the intensity of the population (James 2001; Steffey et al. 1999). Earlier experimental studies on artificial infestations of corn plants with egg masses indicated an average yield loss of 1.7% per egg mass per plant in the early stages of plant development and 3.0% losses in the later stages (Lynch et al. 1980). Recent research from Canada indicated 5% average yield reductions of untreated corn under natural ECB infestations (Baute, Sears, and Schaafsma 2002). Overall, economic value of yield losses are estimated to be at least one billion dollars when ECB is not managed (Mason et al. 1996; Ostlie, Hutchison, and Hellmich 1997).

The insecticides permethrin, chlorpyrifos, and methyl parathion are recommended for controlling ECB (2000 Illinois Agricultural Pest Management Handbook), but comparatively few treatments are made across the Corn Belt. Estimates of treated acreage range from about 1-2% over the last five years in Iowa (Obrycki et al. 2001) to 25 % in the southern United States (including Arkansas, Louisiana, Mississippi, Texas, and Oklahoma) (Shelton, Hutchison, and Hellmich 2002). Owing to production of irrigated corn, eastern Colorado, western Kansas, and Nebraska are also high insecticide use states (Ostlie, Hutchison and Hellmich 1997). The variability in insecticide use across corn production regions reflects farmer responses to the consistently higher densities of the Southwestern corn borer and predictably greater prevalence of ECB, especially in irrigated corn.

Although economic damage by ECB is widely recognized as occurring, most farmers in the Midwest do not treat for this pest. Reasons for this benign neglect include (Ostlie, Hutchison, and Hellmich 1997):

- larval damage is hidden;
- heavy infestations are unpredictable;
- scouting multiple times each summer takes time and requires skill;
- insecticides are expensive and raise health or environmental concerns; and
- benefits of European corn borer management are uncertain.

Older research has examined the use of a *Bt* spray for controlling ECB (Lynch et al. 1980). Sprays gave erratic results but granular and foam formulation significantly decreased tunneling. Although results were not compared with conventional insecticide treatments, only a 12-33% reduction in tunneling was achieved with applied *Bt* formulations.

Corn Rootworm Biology, Damage, and Control

Three species of corn rootworms (CRW) are economically damaging on corn in the United States: the western corn rootworm (WCR), the northern corn rootworm (NCR), and the Southern corn rootworm (SCR). Adult beetles lay their eggs in soil at the base of corn plants during most of August. Larvae of the WCR and NCR overwinter as eggs in the Midwestern Corn Belt. SCR will fly into the Corn Belt from southern areas and lay eggs during the concurrent cropping season. Larvae hatch in late May and early June. They bore into tender roots and then make feeding channels along the root toward the base of the stalk, causing severe necrosis and die-back (root pruning). As a result, plant nutrient uptake is adversely affected, and standability is severely reduced. Lack of sufficient root support causes lodging, and during high winds, plants can tilt over, reducing significantly machine harvestability.

After passing through three instars, the larvae pupate in the soil. Adults emerge in mid-July and feed on silks. Adults are not often economically damaging, but when their densities are high, they can cause sufficient silk damage to reduce pollination.

Adult CRW are mobile and will leave the field where they emerged and seek out other fields, especially those fields that have been planted later and thus are in an earlier stage of development. However, significant egg laying also occurs in the field where the adults have emerged. WCR and NCR larvae have very few host plants other than corn; thus, most egg laying occurs in corn fields. Taking advantage of this behavior, the key cultural practice for managing CRW populations has been annual crop rotations between corn and soybean.

If corn will be planted into last season’s corn fields, then soil insecticides are recommended to control larval feeding damage. However, extension scientists also recommend that cornfields be scouted for adult beetle the previous season in August to determine how intense the next larval population is likely to be.

Most soil insecticides are applied in 18-cm bands over or in the seed furrow during planting time. The insecticides are formulated as granules and are covered by the soil with a drag chain or harrow. Use of soil insecticides (in terms of acreage treated) has dropped by over 75% since the 1970s, largely as a result of widespread adoption of crop rotation. However, this decreased soil insecticide use is a trend that is shifting since populations of corn rootworm (CRW) resistant to control through crop rotation are increasing.

The most commonly used insecticides throughout most of the 1990s have been the organophosphates chlorpyrifos and terbufos, but their use in crop year 2000 was only 6% and 3% of the total corn acreage, respectively (USDA-NASS 2001). Over the last few years, however, the pyrethroids tefluthrin and lambda-cyhalothrin have gained increased use (7% and 2% respectively). Pyrethroids are used at average rates of 0.1 pounds active ingredient per acre (lb AI/a) compared to the 1 lb AI/a typical of OP insecticides. Thus, the total mass of soil insecticides has dropped from nearly 21 million pounds used in 1991 to 10.6 million pounds in 2000.

Biotechnology-Derived Resistance to Insects

During crop year 2000, the worldwide acreage of *Bt* corn represented about 6% of total area planted (346 million a., 140 million ha) (James 2001). In the United States, two sets of estimates exist for delineating the adoption rate of *Bt* crops. The USDA Economic Research Service (ERS) has estimated that peak planting of *Bt* corn occurred during 1999 (25% of total corn acreage) (USDA-ERS 2002). In 2000 and 2001, however, acreage planted to *Bt* corn dropped to 18%. To derive its estimates, the USDA-ERS used the database in the National Agricultural Statistics Survey (NASS); thus, the estimates are based on farmer surveys in the corn producing states.

In contrast to the methods used by the USDA-ERS, the EPA requires that registrants provide sales data (EPA 2001a). As a result, the EPA estimated that *Bt* corn was planted on approximately 25% of the total corn acreage (79 million a., 32 million ha) during 2000.

Corn Earworm and Modern

Biotechnology-derived Corn

Lynch et al. (1999a) evaluated *Bt* sweet corn hybrids containing a gene for the *Cry*1Ab toxin for resistance to the corn earworm, *Helicoverpa zea*, and fall armyworm, *Spodoptera frugiperda*. Laboratory tests revealed that all *Bt* sweet corn hybrids were highly resistant to leaf and silk feeding by young corn earworm larvae. Ear damage in the field to the *Bt* sweet corn hybrids caused by corn earworm was negligible. Most *Bt* sweet corn hybrids were moderately resistant to leaf and silk feeding by the fall armyworm. Wiseman et al. (1999) performed a laboratory bioassay to evaluate *Bt* sweet corn hybrids for resistance against corn earworm and fall armyworm. They concluded that the *Bt* sweet corn hybrids containing a *Cry*1Ab gene were highly resistant to leaf, silk, and kernel feeding by corn earworm and highly resistant to leaf and silk feeding by fall armyworm. Lynch et al. (1999b) evaluated *Bt* sweet corn containing a modified *Cry*1Ab gene and a minimal number of applications of methomyl for management of injury to ears by corn earworm and fall armyworm. Injury to ears on the *Bt* sweet corn was not eliminated, but was minimal and confined to a few kernels at the ear tip. Lynch et al. (1999b) determined that use of the *Bt* sweet corn could eliminate 5 insecticide applications in Georgia. Burkness et al. (2001) drew similar conclusions in Minnesota.

Williams et al. (1998) incorporated husks and silks from *Bt* corn hybrids in laboratory-diet bioassays to determine the effects of the *Bt* corn toxin on larval survival and growth of three pests. Southwestern corn borer (*Diatraea grandiosella*) larvae that fed on diets containing husks of *Bt* corn did not survive. Corn earworm larvae fed on diets containing husks from *Bt* corn died, and those that fed on diets containing silks harvested from *Bt* corn exhibited decreased survival and growth. Fall armyworm larvae were least susceptible to the *Bt* toxin. The bioassays indicated that *Bt* toxin in husks and silks could decrease insect damage in ears. Pilcher et al. (1997a, b) evaluated *Bt* field corn for control of corn earworm and other pests. They found that corn earworm survived on and caused damage to *Bt* corn ears and the number of larvae living on each plant was no different on *Bt* corn and non-*Bt* corn. Buntin et al. (2001) observed that *Bt* field corn consistently decreased whorl infestation and damage to low levels and also decreased ear infestations and larval numbers per ear. However, larval establishment did occur on many ears of resistant plants, but once established in ears, larvae of both species developed more slowly and caused much less kernel damage on *Bt* corn.

Bt sweet corn (*Cry*1Ab, tradename: Attribute) was registered for commercialization in the United States in 1998. Despite its positive environmental profile, *Bt* sweet corn has not been readily adopted (less than 30,000 a. (12,141 ha) planted of the estimated total of 740,000 a. (299,478 ha) (EPA 2001a)). One explanation for the low adoption rate is uncertainty about the willingness of food processors or local markets to purchase *Bt* sweet corn (EPA 2001a; Shelton, Zhao, Roush 2002). Indeed, Gerber Baby Foods, a company previously owned by the registrant of *Bt* sweet corn (Syngenta Crop Protection), issued a policy that would eliminate biotechnology-derived ingredients not because of safety issues, but because of potential impact on consumer buying habits.

ENVIRONMENTAL IMPACTS OF TRADITIONAL AND BIOTECHNOLOGY-DERIVED CORN

Changes in Pesticide Use

Herbicide-Tolerant Corn

Before the development of biotechnology-derived herbicide-tolerant crops, glyphosate was used on approximately 2% of the corn acres, primarily as a pre-plant burndown. Glyphosate is especially useful for controlling early sprouting weeds in no-tillage systems. The adoption of herbicide-tolerant lines such as Roundup Ready® (RR) corn has increased glyphosate use to 9% (Table V-2). However, a comparison of herbicide use that was estimated in the USDA-NASS database among the years before adoption of glyphosate-tolerant corn and crop year 2000, shows that overall herbicide amounts are down despite the increase in acreage harvested (Table V-2). A 43% reduction of herbicide use in corn has been projected should Europe adopt herbicide-tolerant varieties (Phipps and Park 2002).

Atrazine use was greater in crop year 2000 than in crop year 1995, but glyphosate-tolerant corn has potential advantages for reducing its use if farmers adopted the ideal strategy of two glyphosate treatments without using a pre- or postemergence soil applied herbicide. At this time, acreage of biotechnology-derived herbicide-tolerant corn may be too small to accurately gauge changes in pre-emergence herbicide use. The difficulty is compounded by the adoption of other types of herbicide tolerant corn in the United States (e.g., imidazolinone resistant varieties) that still benefit from applications of atrazine.

Insect-resistant corn

Because many farmers do not use insecticides to control the European corn borer (ECB), large changes in insecticide use are not expected (EPA 2001a). Rather, yield increases are anticipated to be the greatest benefit. Nevertheless, even a small decrease (1 to 2%) in insecticide use would be significant considering that 15 to 20 million a. are planted to *Bt* corn (EPA 2001a).

Disagreement exists over interpretation of the impact of *Bt* corn adoption on current pesticide use data. EPA's own analysis indicated a reduction of about 3.9 million a. (1.6 million ha) treatments with insecticides for all pests in field corn (EPA 2001a). One analyst, however, concluded that insecticide applications targeting the ECB directly rose from 4% of acres treated in 1995 to 5% in 2000 (Benbrook 2001). In contrast, other analysts concluded that insecticide use attributed to control of ECB dropped by 1.5% between 1995 and 1999 (Carpenter and Gianessi 2001).

Changes in Soil Management and Tillage Practices

Over the last 30 years, corn farmers have essentially moved from moldboard plowing in the fall to reduced tillage or no-tillage. In many cases, farmers do not use reduced tillage until the spring. The move to conservation tillage, especially no-tillage, was well under way before the adoption of herbicide-tolerant crops. The low adoption rate of glyphosate-tolerant corn suggests that the technology at present has not affected the adoption of tillage practices.

Gene Flow/Outcrossing

One of the ecological concerns regarding planting of biotechnology-bred plants is the possible introgression of biotechnology-derived characters into wild-type relatives and subsequent unintended consequences that could lead to a loss of biodiversity. Feral ancestors of modern crops are highly valued as sources of possible stress tolerance and pest resistance that can be used to improve their cultivated descendants. The other concern is that the biotechnology-derived character will outcross with either the wild type and/or the non-genetically transformed cultivar, which will then have a selective advantage that elevates them to weed status. For example, an insect-protected character might reduce natural incidence of herbivory, and the feral population would expand at the expense of other more susceptible surrounding species. One possibility is a herbicide resistance character that could make impossible the control of volunteer biotechnology-derived plants in crop-rotated fields. Alternatively, biotechnology-derived plants that escape by seed dispersal outside of the cultivated field will become densely populated and difficult-to-control weeds themselves.

Presently, the aforementioned concerns that may pertain to HT (herbicide tolerant) and *Bt* corn in the United States are hypotheses without any foundation, for several reasons. Maize has no feral ancestor of concern in the United States. Indeed, the ancestral progenitor has been hypothesized to be *Zea mays* subsp. *parvaglumis*, commonly known as teosinte (Benz 2001). The several varieties of teosinte have a geographical area largely restricted to Mexico and other Mesoamerican countries. Thus, compatible species for gene flow are not present in the United States with one recent exception. *Zea mays* can outcross with Eastern gammagrass, *Tripsacum dactyloides*, a native U.S. plant grown as a new crop on limited acreage in the United States (NRC 2002). The USDA Animal and Plant Health Inspection Service determined that hybrids between *Tripsacum* sp. and *Zea mays* were often sterile or at least had reduced fecundity.

The hypothesis that escape or volunteer corn plants will have a selective advantage and become weeds has been empirically dismissed by the results of an intermediate term experiment conducted in the U.K. (Crawley et al. 2001). Arable crops, including glyphosate-resistant corn, did not increase in fitness or abundance outside of cropped fields. *Bt* corn was not tested, but results similar to the glyphosate-resistant corn were observed for *Bt* potatoes. Successful introgression of the *Bt* trait into *Tripsacum*, if it were to occur, does not automatically mean that the recipient population will rise to weed status owing to release from insect herbivory. Such a hypothesis can be answered by a priori examination of *Bt*-susceptible nontarget insect herbivores, but perhaps more importantly, it could also be answered by defining the important mortality and reproductive factors for species of concern. Thus the NRC report on environmental effects of biotechnology-derived plants (NRC 2002) stated that "risk assessment cannot depend on general characteristics such as the amount of new genetic information introduced but must focus on the ecology of the specific introduced organism (or both the donor and recipient in the case of biotechnology-derived organisms) and the characteristics of the accessible environment into which the organism will be released."

One important factor to consider about the propensity among *Zea mays* varieties for gene flow, introgression, and the subsequent establishment of a population with a new trait is the potential for seed dispersal and distance of pollen movement. Modern corn, as opposed to its ancestor, teosinte, has lost the ability to disperse its own seed. Modern corn can fertilize itself, but outcrossing is common and pollen moves sufficiently across several rows to warrant isolation in hybrid breeding programs. For example, 660 feet is the distance recommended for separation of pure varieties used to produce hybrids from other fields of corn (Bauman and Crane 1985). Because U.S. farmers purchase certified hybrid seed each year, introgression between different cultivars, which is certainly possible given the range of pollen movement and typical sizes of Corn Belt farms, is unlikely to be important (Jarvis and Hodgkin 1999).

Mexico is in the process of developing regulations regarding biotechnology-derived corn. In the interim, Mexico has halted the planting of biotechnology-derived corn seed while assessing the potential impacts to the biodiversity of Mexican maize landraces and their ancestral forebear, teosinte. A recently published communication in the journal *Nature* by Quist and Chapela (2001) asserted, however, that biotechnology-derived DNA introgression has already occurred in native landraces. Quist and Chapela concluded they found evidence of CaMV 35S in five of seven landrace samples collected from an isolated region near Oaxaca, Mexico. CaMV 35S is the promoter DNA segment introduced into the transgene plasmid cassette used to create events *Bt*11 and MON810. The conclusions of biotechnology-derived DNA "contamination" were solely based on the use of two consecutive PCRs (polymerase chain reactions) to detect a piece of the CaMV 35S promoter DNA. Based on many generations of crossing descendants of these events, the gene construct is known to be stable, but the authors used a technique called inverse PCR to indicate that the CaMV 35S DNA introgressed into the native landrace genome at multiple regions and also broke into smaller fragments. Quist and Chapela (2001) also indicated that one corn sample tested positive for the *Bt Cry*1Ab gene, but no DNA evidence was shown to prove that the *Bt* gene was actually present, let alone functional.

Within several weeks after the release of the Quist and Chapela report, CIMMYT (International Maize and Wheat Improvement Center), a public research foundation whose mission includes, but is not limited to, preservation of maize biodiversity and crop improvement, issued a press release of their foundation's own results in a search for biotechnology-derived DNA introgressions (CIMMYT 2001). None of the 43 Oaxacan landraces in CIMMYTs gene bank or a new collection of 43 different varieties had detectable levels of CaMV 35S promoter.

The editorial board of the journal *Transgenic Research* issued an editorial critiquing the paper by Quist and Chapela (2001) (Christou 2002). Two recent communications to *Nature* also concluded that the techniques used by Quist and Chapela were deficient and their conclusions on DNA introgression stemmed from a misinterpretation of their data (Kaplinsky et al. 2002; Metz and Futterer 2002). In short, the work of Quist and Chapela was negatively critiqued solely for its major flaws in experimental execution. The editorial board of *Transgenic Research* recommended that all claims of introgressed biotechnology-derived DNA should be supported by growing out the F1 hybrid and re-doing the molecular tests along with examining obvious effects on plant morphology. Quist and Chapela presented new evidence using Southern blotting to show hybridization of DNA from landrace corn extracts and CaMV 35S probes (Quist and Chapela 2002). However, data were still lacking to determine if conclusions regarding introgression into the F1 plants are still valid. As to the scientific merit of the original article, the journal *Nature* concluded the following in an editorial note on April 4, 2002: "*Nature* has concluded that the evidence available is not sufficient to justify the publication of the original paper. As the authors nevertheless wish to stand by the available evidence for their conclusions, we feel it best simply to make these circumstances clear, to publish the criticisms, the authors' response and new data, and to allow our readers to judge the science for themselves."

Notably, the editorial board of *Transgenic Research* emphasized that landraces from Oaxaca, Mexico, are *Zea mays*, not teosinte varieties. Thus they began their critique of the *Nature* paper by stating wind pollination would inevitably lead to gene flow between domesticated crop varieties and their wild ancestors when grown in close proximity to each other. However, recent literature about the origin of maize and likelihood of introgressions with teosinte suggest a lot of uncertainty about whether introgressions are even occurring between cultivated corn in Mexico and the teosintes (Kato Y 1997), or whether such introgressions can become fixed without selection pressure (Martinez-Soriano and Leal-Klevezas 2000).

The striking evolutionary divergence in inflorescence morphology of domesticated maize and teosintes also suggests genetic isolation after the initial characteristics of consumable corn were fixed. Recent research shows that one gene, tb1, largely controls the difference in inflorescence morphology. In cultivated maize, the transcribed region tb1 has maintained its polymorphic character as has teosinte, but its nontranscribed regulatory region has only 3% of the genetic variation found in teosinte (Wang et al. 1999). Given the fact that at minimum several hundred years of artificial selection were required to fix the changes in the nonregulatory region of tb1, it is difficult to support a hypothesis that a transgene coding for a pest resistance character would change biological diversity in teosinte or native landraces in the absence of intense selection pressure.

The publicity over the alleged introgression of "foreign" genes into native landraces of corn has been fueled by concerns that such phenomena would reduce biodiversity. However, the current production and seed selection systems in Mexico suggest that such concerns are without merit. Mexican farmers have long been exchanging seeds from local varieties with each other to improve productivity (and genetic diversity) of their corn (Louette 1997). The difference between Mexican and U.S. seed corn production practices boils down to open pollination vs. hybridization. In the United States, inbred seed lines (i.e., corn varieties that are allowed to pollinate only themselves) are crossed each year to produce superior performing (and more genetically diverse) hybrids. U.S. farmers pay a premium for hybrid corn bought every year from seed companies. Hybrid corn has a certified genetic makeup, and it consistently yields well under the environmental conditions in which it was developed. In Mexico, farmers grow their own seed from varieties that are open-pollinated. In other words, they grow varieties that are subject to cross pollination (i.e., gene flow) from similar varieties or non-local varieties.

Indeed, studies of farmer practices in Mexico show that there are many distinct varieties of corn grown in fields with close proximity to one another. In the region of Cuzalapa on the western Pacific coast of Mexico, 26 distinct varieties were grown in a 59,280 acre (24,000 ha) watershed containing 2470 a. (1000 ha) of corn (Louette 1997). For example, 53% of the corn in the watershed was produced from an individual farmer's own seed planted in previous years. The rest of the corn was produced from seed exchanged with other farmers in the same watershed (36%) or from seed outside the region (11%). One of the non-local varieties was identified as an improved cultivar of hybrid corn from the United States.

Because Mexican farmers make no attempt to segregate different varieties, cross pollination has been occurring (estimated at 38% probability for outcrossing in the Cuzalapa region) (Louette 1997). About one-third of local corn varieties may already have introgressed genes from non-local and improved varieties (Gonzalez and Goodman 1997). Consequently, a continuum of morphological traits and genetic characteristics exists among all the major local varieties (Louette 1997). In other words, within a region abrupt shifts from one morphological trait to another were absent. For example, seeds were not necessarily all one color (white, blue, or yellow) in one field, but there were a lot of mixtures (a.k.a., heterozygosity). Yet, despite the tremendous amount of gene flow from non-local to locally adapted and selected cultivars, the varieties survived intact as recognizable entities.

Given that a plethora of genes are moving among distinct local varieties and non-local varieties all the time without loss of biodiversity, the ecological effects of gene flow in the context of the local habitat, not the origin of the DNA, should be the real focus of concern. A quote from Martinez-Soriano and Leal-Klevezas (2000) referring to ecological risks of pest-protected plants in Mexico is revealing: "The main concern regarding the possible effects on the native maize and relatives has little if any scientific basis; it is more related to cultural factors rather than biological ones". Thus, future studies should shift their focus to consideration of whether hybridization among any corn cultivar confers selective advantages, including increased yield, in the context of the environment that the plant is growing in and the typical agricultural practices.

Pest Resistance

Weed Resistance

The overwhelming majority of the herbicide-tolerant crops worldwide are glyphosate-tolerant, raising concerns that repeated use of glyphosate may produce resistant weeds. However, herbicide-resistant weeds had become an issue long before the advent of biotechnology-derived crop technology. In the world today, over 250 weed biotypes have developed resistance to one or more herbicides, and about 80 resistant biotypes have been found in the United States (Heap 2000, 2002).

Like insects, weeds can develop resistance when continually selected by a single herbicide or group of herbicides having the same mechanism of toxic action (also called mode of action). Weeds develop resistance in one of two ways. First, a few individuals in a population may possess a gene that enhances metabolic detoxification reactions, thereby breaking down the herbicide fast enough to avoid its phytotoxicity. The second more prevalent method is occurrence of some individuals with a gene that alters the herbicide's biochemical target site (usually an enzyme), making the plant resistant to injury. In either case, if these infrequent individuals escape control and successfully go to seed, comparatively more individuals may occur in the population during the next growing cycle. Eventually, this may lead to most of the population being resistant to that specific herbicide or class of herbicides with the same mode of action.

An individual gene for a given plant species that produces an insensitive target site causes most cases of herbicide resistance. The vast majority of weed resistance has developed to herbicides that specifically inhibit the synthesis of amino acids or photosynthesis. For example, herbicides that inhibit the enzyme acetolactate synthase (ALS) kill plants by shutting down branched-chain amino acid synthesis. Sulfonylurea and imidazolinone herbicides are two distinctly different chemical classes that inhibit ALS. Animals lack ALS and the ability to synthesize branched-chain amino acids, so ALS-inhibiting herbicides are of very low hazard to animals. More than 60 biotypes of weeds worldwide have developed resistance to the effects of ALS inhibitors. The triazine herbicides are one chemical class of several that can inhibit photosynthesis at a specific reaction center, photosystem II, which resides in the plant cell chloroplast, the chlorophyll-containing organelles that make a plant green. At least seventy-eight weed biotypes have developed resistance to herbicides inhibiting photosystem II.

At least nine weed species have developed resistance to ALS inhibitors (sulfonylurea and imidazolinone herbicides) in corn fields in the United States and Canada (Owen 2001). At least 36 weed species have developed resistance to ALS inhibitors and photosystem II inhibitors (triazine herbicides) in Europe (Owen 2001). In many cases resistant weeds exhibited multiple resistance to different herbicides of the same class and cross resistance to herbicides from different classes.

After more than 25 years of commercial use before the development of biotechnology-derived herbicide tolerance, glyphosate-resistant weeds had only been documented for two species, annual rigid ryegrass (*Lolium rigidum*) and goosegrass (*Eleusine indica*) (Hartzler 1998, 1999). Glyphosate-resistant ryegrass has been confirmed in Australia and California (wheat production), and resistant goosegrass was observed in Malaysia (oil palm production). In both cases, resistance occurred after 10-15 years of intensive glyphosate use (greater than 2 applications per site per season).

To date, one reported incidence of resistant weeds has been related to the introduction of a herbicide-resistant crop. Horseweed (*Conyza canadensis*), a dicot weed in the Asteraceae family first evolved resistance to the herbicide glyphosate in 2000 in Delaware following several years' use in "no-till" soybean (VanGessel 2001). Similar cases of glyphosate resistance in marestail are also reported from New Jersey, Maryland, and Tennessee.

Presently, adoption of herbicide-tolerant corn has been on a limited number of acres. Thus, selection pressure with glyphosate has not been sufficient to cause any notable cases of resistance strictly due to adoption of biotechnology-derived corn.

In summary, with or without biotechnology-derived crops, resistance development always remains a threat if chemical control is not carefully managed and integrated with nonchemical methods. A consortium of agrochemical manufacturers have formed the Herbicide Resistance Action Committee (HRAC) to provide information about resistance cases and strategies for management (HRAC 2002). USEPA and PMRA (Pest Management Regulatory Agency of Canada) have worked closely with the HRAC and WSSA (Weed Science Society of America) to address herbicide resistance. USEPA and Canada have developed voluntary resistance management guidelines based on rotation of mode herbicide of action for all agricultural use of pesticides (Canada PMRA 1999; EPA 2001; Matten et al. 1996)

Insect Resistance

Development of Resistance to Conventional Insecticides

The European corn borer is a sporadic pest in some corn growing regions, some years. Its first generation in the Corn Belt can lead to yield losses that can be managed using traditionally bred resistant cultivars. However, its second generation is not manageable by traditionally bred resistant cultivars, so farmers may manage second generation ECB with insecticides. With the exception of some geographical locations, insecticide treatments tend to be infrequent, and therefore, development of insecticide resistance has not been a concern. In contrast to traditional management of ECB, most insecticides used in corn production have been historically applied directly to the soil to control one of several species of corn rootworm (CRW) larvae. Insecticides are most likely to be used when corn is grown annually rather than rotated with other crops like soybean. Coincidentally, insecticide resistance was first observed in the 1960s when most corn was not rotated with soybean and the persistent, bioaccumulating cyclodiene insecticides were used commercially.

Following farmer reports of ineffective control of CRW with aldrin in Nebraska during 1959-1961, Ball and Weekman (1962) were the first to report aldrin and heptachlor resistance based on topical toxicity studies with adult western corn rootworms (WCR). Their report was followed by observations of resistance in an isolated northern corn rootworm (NCR) population in Illinois (Bigger 1963). By 1963, aldrin resistance in NCR and WCR populations was reported in insects collected in Minnesota, Ohio, S. Dakota, and Kansas (Ball and Weekman 1963; Blair, Triplehorn, and Ware 1963; Burkhardt 1963; Howe, Ortman, and George 1963). Hamilton (1965) noted a gradient of increasing aldrin resistance in a southerly direction from certain Iowa populations of CRW exhibiting high resistance. This observation implied that resistant beetles were dispersing in prevailing northwesterly winds. Patel and Apple (1966) also noted a southerly dispersion of resistant NCR from populations in Wisconsin. Blair and Davidson (1966) concluded that it took approximately 10-12 years of aldrin use before high levels of resistance developed in CRW populations in Ohio.

Reports of cyclodiene resistance in CRW were based solely on toxicity testing with the adult stage. Although the larvae were the target stage, the hypothesis of resistance was accepted because control failures occurred in areas of intense insecticide use. Furthermore, WCR adults collected from areas of annual corn monocultures with extensive insecticide use were several hundred-fold less susceptible to cyclodienes than adults collected from areas of crop rotation with low pesticide inputs. Hamilton (1966) was the first to show that larvae were more tolerant than adults to the cyclodienes. His research complicated the resistance hypothesis by showing that level of adult susceptibility was dependent on beetle emergence date. Nevertheless, many extension personnel recommended against the use of the cyclodiene soil insecticides to control CRW on the basis of resistance.

As the organophosphorus (OP) and carbamate (CB) insecticides replaced the cyclodienes, Ball (1968, 1969) devoted much effort to monitoring the susceptibility of adults collected annually from the same fields. Ball (1968) noted a decrease in susceptibility to diazinon and phorate in WCR over a five-year period, but no control failures had been reported. Similar observations were noted for carbaryl (Ball 1969). By 1973, however, Ball (1973) reported a reversion in WCR susceptibility to diazinon.

In the early 1970s, farmers were reporting poor or inconsistent control with the use of bufencarb (Bux) soil insecticide (Ball 1977; Kuhlman 1974), although only a five-fold decrease in adult susceptibility was measured between 1963 and 1970 (Ball 1977). By 1975, there appeared to be a reversion to susceptible levels (Ball 1977). By 1975, carbofuran was reportedly failing to control CRW feeding damage (Felsot, Maddox and Bruce 1981; Kuhlman 1976). Chio et al. (1978) noted increases in LC_{50} values over "primitive" levels for carbofuran, phorate, terbufos, and fonofos in adult CRW from Illinois. A 6- to 12-fold difference in susceptibility was noted for populations assayed in the 1970s compared with those collected in 1980. If resistance had developed to bufencarb and carbofuran, the magnitude of differences between adult populations was very small compared with that observed with the cyclodienes. Meanwhile, poor efficacy was noted for all registered products during the 1980s.

Although development of CRW resistance to cyclodienes had been readily accepted as the prevailing hypothesis to explain the problems in insecticide efficacy, development of resistance to OP and CB insecticides was less certain. Part of the problem was the difficulty in bioassaying larvae. Both the WCR and NCR are diapausing insects, making difficult the maintenance of a vigorous colony. Collection of larvae from the field is very time consuming, and large numbers of similar age are required to properly construct dose-response functions. Additional problems include determination of larval mortality (if assayed in soil), obtaining larvae within a desired weight range, time required to treat larvae vs. adults, and mixed populations of WCR and NCR (Ball et al. 1975). The 1977 report by Walgenbach and Sutter of a decrease in susceptibility of WCR larvae between 1975 and 1976 was essentially the last concerted effort to determine if resistance had developed to OP and CB soil insecticides. Walgenbach and Sutter (1977) concluded that adult topical assays had little value for determining whether resistance was developing to the OP and CB insecticides. Although reports of inadequate CRW control with soil insecticides continued into the 1980s, Felsot et al. (1985) observed that variability in percentage control of WCR feeding damage was not correlated with measurement of adult susceptibility.

Development of Enhanced Biodegradation of Soil Insecticides

Although it was never proven that CRW larvae developed resistance to the OP and CB soil insecticides, small changes in their susceptibility could have been important. The soil insecticides are applied at planting, which may be anywhere from 30-60 days before the first hatch of larvae. Given the normal dissipation rate of OP and CB insecticides in soil, concentrations would tend to be approximately 50% of applied amounts after 60 days. Such levels would be approaching the LC95 level or below for WCR larvae in soil. Thus, even a two-fold change in susceptibility of larvae might make soil insecticide efficacy more risky.

With soil insecticides, not only is bioactivity dependent on insect susceptibility, but extent of sorption and desorption from soil (and associated physical and chemical factors influencing sorption) also are important in determining control efficacy (Felsot and Lew 1989). Along with sorption potential, rate of degradation in soil would be important. The cyclodienes were extremely persistent, with one application reportedly capable of controlling feeding damage four years later. In contrast, OP and CBs are easily biodegradable. Nevertheless, these compounds worked well to control feeding damage when first introduced.

A report from Philippine rice cultures led to a different line of inquiry about the failure of the soil insecticides to adequately control CRW larvae. The OP insecticide diazinon applied to rice paddies failed to control the brown planthopper, yet resistance could not be proven. However, the problem was elucidated by the finding of a very rapid microbial degradation of diazinon in rice paddies repeatedly treated for several years (Sethunathan 1971).

The phenomenon of very rapid (or accelerated) pesticide degradation following repeated use in soil became known as enhanced biodegradation. Enhanced biodegradation developed as a result of microbial adaptation characterized by either subsequent enzyme induction or population increases, presumably because the pesticide served as a carbon or nitrogen source.

Enhanced biodegradation of carbofuran and several OPs was found to be applicable to repeated use in soils of the Corn Belt (reviewed by Felsot 1989). Felsot et al. (1982, 1985) showed that control of CRW feeding damage tended to occur when carbofuran persistence was unusually short in fields with a history of using the compound. The phenomenon was not isolated to corn, however, and it was subsequently reported for other crops in Canada and Europe (Harris, Morris, and Stevenson 1988; Suett and Walker1988).

Development of Resistance to Adult Control Strategies

Although management of CRW is overwhelmingly dominated by the use of soil insecticides, the practice is more or less prophylactic where corn is continuously grown without crop rotation. In other words, insecticide is used before egg hatching and the actual size of larval populations is not easily monitored. However, economic thresholds based on the number of adult beetles counted in a field during the previous season were recommended by the Cooperative Extension Service.

With the focus on monitoring adult beetles and the realization that CRW had become resistant to cyclodienes, a strategy of reducing adult populations to decrease potential for egg laying evolved. In addition to reducing potential larval feeding damage in subsequent growing seasons, adult control had the advantage of reducing feeding by adults on corn silks and the consequent reduction in pollination. Thus, in the 1970s Sevin, a formulation of carbaryl, was occasionally sprayed on fields to reduce egg-laying potential of the emerging adult population. Adult control with sprays of microencapsulated methyl parathion was practiced for many years in south central Nebraska (Zhu et al. 2001). However, control failures likely due to OP resistance were reported (Meinke et al. 1998). Furthermore, isolated populations also appear resistant to carbaryl (Scharf et al. 1999).

During the 1980s, Metcalf et al. (1982) discovered the adult feeding stimulant properties of curcurbitacins and began to incorporate them into baits with carbaryl for adult CRW control. Further proprietary research led to the development of an adulticide bait called Slam that contained the feeding stimulant and carbaryl (Hoffmann et al. 1995). The USDA helped implement an area-wide management program using Slam with carbaryl as the insecticide. However, over the last few years, CRW adults have developed moderate levels of resistance to carbaryl after using Slam for four years (Zhu 2001), placing the area-wide program in jeopardy.

Development of Resistance to Cultural Practices

Integrated pest management (IPM) strategies have always relied on cultural practices where possible as an environmentally sound and efficient technique for controlling insect pests. Because CRW larvae cannot complete their development successfully on soybean roots, crop rotation of corn with beans has been recommended by Corn Belt entomologists for at least 30 years. The practice generally works because few female beetles normally moved to and laid eggs in soybean fields.

However, the viability of crop rotation as a cultural control practice has lost its effectiveness due to two different phenomena in different parts of the Corn Belt. First, it was discovered nearly 20 years ago that NCR, which will diapause during winter, had the capability of prolonging diapause for at least two years. Thus, NCR eggs laid in a corn field destined for rotation to soybean in the following growing season could remain in the soil but not hatch until corn was planted again (phenomenon reviewed by Levine and Oloumi-Sadeghi 1991). Thus, root damage to so-called first year corn (i.e., corn grown the season following a soybean crop) was noted in areas of the Corn Belt where the NCR had historically been most abundant (Levine and Oloumi-Sadeghi 1991).

More recently, especially in central Illinois and Indiana, damage to corn grown the season after soybean was particularly evident. Researchers discovered that WCR was laying eggs in soybean fields in unusually high densities, suggesting that WCR was adapting to corn-soybean rotations (Levine et al. in press; Onstad et al. 1999; Onstad et al. 2001b). Thus, many corn farmers who weaned themselves from the use of soil insecticides in corn planted after soybean, who reaped the yield benefits of crop rotation, are losing successful control of CRW with environmentally sound management practices. The introduction of biotechnology-derived CRW-resistant varieties may provide new, environmentally sound alternatives when they become commercially available.

Potential for Development of Insect Resistance to Bt -Protected Corn and Its Management

Pest resistance to a highly effective management practice is inevitable if the technology (i.e., rotation, insecticide application, new hybrid) is used long enough over a large geographic area. This phenomenon is described for the adaptation of pathogen races to corn breeding (below) and for the adaptation of western corn rootworm to crop rotation by Onstad et al. (2001b). Although no insects resistant to *Bt* corn have been observed in the field, several laboratory studies have shown that potential resistance alleles are present in the gene pool of the ECB (Andow et al. 1998, 2000; Bolin, Hutchison, and Andow 1999; Chaufaux et al. 2001; Huang et al. 1999; Huang, Higgins, and Buschman 1999; Huang, Buschman, and Higgins 1999). After extensive field applications of a microbial insecticide containing *Bacillus thuringiensis*, the diamondback moth developed resistance, but it is not a pest of corn (Tabashnik et al. 1998).

Insect resistance management (IRM) strategies prevent or delay the development of resistance by insects to *Bt* plants. The regulatory aspects of the implementation of formal management plans were addressed by a committee of the National Research Council of the National Academy of Sciences and by the USEPA (EPA 1998, 1999, 2001; NRC 2000). Onstad and Guse (1999) and Hurley, Babcock, and Hellmich (2001) have demonstrated the economic value of maintaining ECB susceptibility with IRM.

Three major approaches have been proposed: toxin pyramiding, tissue-specific or inducible promoters, and high-dose refuge strategy. The efficacy of each approach, as discussed below, has not been proven (Frutos, Rang, and Royer 1999; Gould 1998; Maagd, Bosch, and Stiekema 1999; Roush et al. 1998). One approach to IRM is toxin pyramiding which uses multiple toxin genes with different modes of action (Roush et al. 1998). A hybrid can be developed to express two forms of *Bt* toxin that would bind to different insect midgut receptors. Alternatively, pyramiding could include both a *Bt* gene and another completely unrelated toxin gene. One advantage of this approach is that it would be transparent to the farmer. However, it is difficult to establish conclusively that modes of action are adequately different for resistance management.

Another approach to IRM is the use of tissue-specific or inducible promoters that decrease or prevent expression of the toxin gene in parts of plants with decreased biological and/or economic importance, as well as the control of timing of expression of the gene at a desirable developmental phase, when plants are more susceptible to damage caused by pests. This approach may decrease the selection pressure over time because the expression of toxin is limited to specific tissues at specific times, diminishing the exposure of pests and other insects to the toxin. Williams et al. (1998) demonstrated how tissue-specific toxin concentrations could affect three pest species. However, Onstad and Gould (1998b) showed that declining toxin concentrations over time may be worse for resistance management in corn than a constant high dose throughout the season.

For *Bt* corn, the current IRM approach is the high dose/refuge strategy. Spatial refuges are combined with hybrids producing an extremely high dose of toxin (Gould 1998, 2000; Ostlie, Hutchison, and Hellmich 1997). This method relies on four principles: that *Bt* corn tissues produce a high dose of toxin; that inheritance of resistance is functionally recessive; that random mating occurs between susceptible and resistant insects so that heterozygotes (not resistant homozygotes) are produced; and that the initial frequency for the resistant gene is very low, less than one in one thousand in the total population. The objective of this method is to decrease the likelihood that susceptible insects that harbor “silent” resistance genes in heterozygotes will contribute resistance genes to future generations of resistant heterozygotes.

The EPA’s FIFRA Scientific Advisory Panel (2001b) and Gould (1998) defined a high-dose hybrid as a plant-incorporated protectant that produces more than 25 times the toxin dose necessary to kill 99% of susceptible target insects that are exposed to the toxin (EPA SAP 1998; Gould 1998). This dose is supposed to kill all or most of the functionally recessive heterozygous individuals that would develop in the *Bt* cornfield. The high-dose strategy appears to be feasible. However, Onstad and Gould (1998b) pointed out that the production of *Bt* toxin in some *Bt* corn was dynamic due to corn senescence, particularly after flowering. This decreased toxin concentration observed in some *Bt* hybrids (primarily Event 176), combined with late season infestation of *Bt* crop fields by pests, could conceivably accelerate the development of insect resistance. Furthermore, Onstad et al. (2001a) used a mathematical model to show the importance of toxin dose in biotechnology-derived corn for WCR management. Susceptible insects exposed to hybrids having intermediate doses of *Bt* toxin may develop resistance ten times faster than insect populations exposed to high-dose hybrids.

Three other key issues are the inheritance of resistant genes, the initial gene frequency, and the mixing of the resistant and susceptible insects. Huang et al. (1999) demonstrated that resistance was inherited as an incompletely dominant autosomal gene in ECB fed *Bacillus thuringiensis* microbial insecticide incorporated into a laboratory diet. When ECB are exposed to the high doses of toxin expressed by *Bt* corn in commercial use, resistance may be functionally recessive, but limited evidence has been obtained to empirically validate this hypothesis. Huang has observed no survival of young “resistant” larvae feeding on high-dose, commercially available *Bt* corn hybrids (Huang, 2002. Personal communication). Andow et al. (1998, 2000) and Chaufaux et al. (2001) estimated only low rates of resistant-gene frequencies in several different populations of ECB. Bourguet et al. (2000a,b) and Glover et al. (1991) observed significant gene flow between ECB populations infesting several areas. In the north central Corn Belt, Showers et al. (2001) demonstrated that ECB can disperse significantly from corn fields.

For *Bt* field-corn grown in regions without *Bt* cotton, the USEPA-approved IRM plan for corn borers requires at least 20% of corn planted on a farm to be traditionally bred without the *Bt* toxin. This refuge may or may not be sprayed with a chemical insecticide, and it must be located within 1/2 mile of the *Bt* corn field (1/4 mile or closer is preferred). In-field non-*Bt* refuge strips may be used, but they must be 4 rows wide (EPA 2001). The size of the refuge and its proximity to *Bt* corn were first proposed by a national committee of scientists with expertise on management and biology of the ECB (Ostlie et al. 1997). Although infrequent spraying of refuges once every 4 years will not likely decrease the effectiveness of this IRM plan, spraying once each year in refuges in regions that have greater pest pressure from corn borers and other pests may lead to decreased effectiveness, particularly where much *Bt* corn is grown (Onstad in press). Compared to potential resistance development of ECB with an unsprayed 20% refuge, resistance develops 2-4 times faster when the refuge is sprayed every year.

To delay resistance development in ECB, the refuge corn should not be planted as a seed mixture of non-*Bt* and *Bt* corn seed in the same field as a single-toxin, *Bt* corn hybrid (Davis and Onstad 2000; Ostlie, Hutchison, and Hellmich 1997). Onstad and Gould (1998b) showed that seed mixtures are much riskier than within-field block or row-strip refuges or adjacent-field refuges for ECB. Such observations support the EPA-approved IRM tactic of four rows or larger in-field refuge strips.

To date, Shelton et al. (2000) and Tang et al. (2001) have published the sole experimental field and greenhouse studies that tested the high dose/refuge strategy. The experiments were conducted using *Bt* broccoli and diamondback moth as a model system. The researchers concluded that refuges are useful, but sprayed refuges are less efficient than non-sprayed refuges. Although specific pest-crop combinations may require specialized management practices (Shelton et al. 2000), the research thus far suggests the results are applicable to other pests.

Monitoring plans also are important components of IRM. The USEPA and industry need to know when and where resistance to *Bt* corn develops so that adequate remediation or modifications to IRM requirements can occur (EPA 2001). More sensitive monitoring methods have been created by several groups (Andow et al. 1998; Marcon et al. 2000; Venette, Hutchison, and Andow 2000a). Other researchers have been monitoring corn borer populations over large geographic areas including Minnesota in the USA and regions of Spain (Gonzalez-Nunez, Ortego, and Castanera 2000; Venette, Luhman, and Hutchison 2000b). The USEPA has required industry to institute a program of large-scale monitoring for changes in *Bt* susceptibility in ECB, SWCB, and CEW (EPA 2001). Insect resistance management for *Bt* sweet-corn is much different from the IRM plan for field corn in the United States. The USEPA has concluded that harvesting of ears and destruction of the stalks within one month (less than 14 days is preferred) of harvest is sufficient to prevent the evolution of resistance. This is because lepidopteran larvae cannot mature to adulthood and survive under these field conditions (Lynch et al. 1999a).

Venette et al. (2000c) used an in-field screen using late-planted *Bt* sweet corn to detect corn earworm with potential resistance to *Bt* and to monitor changes in the frequency of these individuals in Minnesota. From 1997-1999, they found 0 larvae per 4,200 ears, 131 larvae on 19,744 ears, and 33 larvae on 36,120 ears of *Bt* sweet corn, respectively. Frequencies of late-instar larvae surviving on *Bt* sweet corn increased from 3.01×10^{-4} in 1997 to 5.84×10^{-3} in 1998 and declined to 2.3×10^{-3} in 1999. Because corn earworm does not overwinter in Minnesota, different source populations in the southern United States with differing susceptibilities to *Bt* may account for the fluctuations in frequency estimates. Alternatively, movement of susceptible late-stage instar larvae from non-*Bt* plants could account for larvae found on *Bt* sweet corn. There is a range of baseline susceptibility to *Cry*1Ab and *Cry*1Ac. For “resistance” to occur there must be a shift in the population from susceptible to resistant. There is no evidence that such a shift has occurred. Careful management of *Bt* cotton and *Bt* corn is required in the southern states to prevent an increase in the resistant population.

Disease Resistance Evolution

The many pathogen races identified in the Compendium of Corn Diseases (White 1999) are due to the evolution of pathogen species to traditionally bred corn. A pathogen race is distinguishable from other races within the same species when it is virulent on a corn hybrid generally resistant to all other races. All pathogens are under some selection pressure to evolve virulence to overcome resistance in traditionally bred corn, and many pathogens can currently cause disease on some commercially available hybrids.

Development of formal resistance-management plans to prevent the adaptation of pathogens to traditionally bred corn has not been required. The primary approach to the evolution of pathogen virulence in new races is the continual breeding and testing of new hybrids, so that new corn hybrids will be available every few years. However, as Agrios (1997) points out, corn grown in the United States is highly vulnerable to new pathogen races because of the overall genetic uniformity of most hybrids. In other words, by growing genetically similar corn hybrids over large areas throughout the country, farmers are selecting for and contributing to the evolution of new pathogen races. The breeding for cytoplasmic male sterility is a clear case in which traditional breeding and genetic uniformity increased the susceptibility of corn plants to a disease (southern corn leaf blight, White 1999).

Population Shifts

Insects

Control of insect pests with broad-spectrum pesticides has historically been associated with population shifts of pest species (Stern et al. 1959). The shifts are actually changes in the economic importance of secondary pests that were not previously the targets of management. Secondary pests were already in the agroecosystem but they arise in abundance when populations of their specific natural enemies (e.g., specific parsitoids) and generalist predators are reduced by application of a broad-spectrum insecticide. The advent of reduced risk pesticides has increased the probability of using more insecticide selectivity and lessening the impact of insecticides on natural enemies (see Felsot 2001 for reduced risk concept discussion). Screening programs can differentiate the toxicity of new compounds between pests and natural enemies (for example, see James and Coyle 2001).

Pest-protected plants, especially the *Bt* incorporated hybrids, can act like selective insecticides because natural enemies are essentially protected from exposure to the toxin. Thus, if secondary pests arise in importance mainly because of significant reduction in their natural enemies following an insecticide spray, then it is unlikely that widescale planting of *Bt* corn would cause other corn herbivores to become economically important. This hypothesis does not discount the potential of changes in physical factors, e.g., weather, in influencing population abundance of secondary pests, but in those cases any rise in their importance would not be attributable directly to the planting of *Bt* corn. Indeed, if abundance of the primary pests is significantly lowered by *Bt* corn, then generalist predators may become even more apt to seek out the secondary pests owing to comparatively fewer food sources. This hypothesis has been tested in *Bt* cotton and *Bt* potato and to a lesser extent similar behavior has been observed in generalist predator populations in *Bt* corn fields. The use of *Bt* protection instead of broad-spectrum insecticides preserves generalist predator populations that, in turn, can reduce damage by secondary predators. Additional research is needed to examine the effects of predation on secondary pest populations in *Bt* corn.

Weeds

Herbicide-resistant (HR) corn was only registered in 1998 and it is still planted on few acres compared to HR soybean. Thus, any discussion of shifts in weed species and/or population abundance due directly to the use of HR corn are hypothetical but based on knowledge of population shifts in other crops (e.g., soybean) or in cornfields managed by conventional herbicide programs and tillage systems. For example, shifts in weed composition to species comparatively tolerant to glyphosate have been noted in HR soybean (Owen 2000; Shaner 2000). Although development of resistance in response to repeated selection by glyphosate has some probability of changing weed pressures, lack of knowledge about the ecology and biology of weed/crop interactions, and by implication appropriate management, may be more important for causing weed shifts not directly due to selection resistance (Owen 2000).

Crop management system has a major influence on weed species composition and abundance that is independent of specific herbicide use. For example, continuous no-tillage soil management has tremendous benefits for soil and water conservation, but species composition, temporal pattern of seedling emergence, and total weed biomass are different than in reduced or complete tillage systems (Halford et al. 2001). On the other hand, some have suggested that changes in farm management systems due to the type and frequencies of herbicide use in HR crops could result in species shifts on field margins and adjacent areas because of the removal of perennials and subsequent invasion of annuals (Riches and Valverde 2002).

However, considering that postemergence herbicides are commercially available for annual weeds suggests that such concerns are probably neutral rather than detrimental in effect.

Cover crops, in addition to no-tillage, have been advised for soil moisture conservation and weed suppression (Tharp and Kells 2001). But under low-input management systems (i.e., no-tillage and a nonresidual pre-plant herbicide), crop-covered cornfields have a higher weed diversity than conventionally managed systems (plowing and residual pre-emergence herbicide) (Barberi and Mazzoncini 2001). Furthermore, whether certain weed species become dominant or not depends on the type of cover crop. For example, redroot pigweed, common lambsquarters, and black nightshade were regularly associated with a rye cover cropped low-input management system in comparison to a clover and corn stubble system (Barberi and Mazzoncini 2001).

In summary, weed shifts are inevitable independently of whether HR corn is used. The consensus among researchers is that no-tillage and cover cropping systems themselves are critical influential factors on weed population ecology.

Nontarget Species

Natural Enemies (Parasitoids and Predators)

Several reports clearly express the variability in the toxicity and harm to nontarget insects when exposed to a *Bt* toxin targeted against a pest (Flexner, Lighthard, and Croft 1986; Peacock, Schweitzer, and Dubois 1998; Obrycki et al. 2001; Zangerl et al. 2001) regardless of whether the *Bt* is formulated as a sprayable pesticide or the toxic protein has been engineered into a plant as a pest-protected character. Two conclusions emerge from the various reports on *Bt* toxicity: (1) Toxicity is age-specific, so the larval stage or stages that will be exposed in corn habitats must be known. (2) Toxicity is species-specific; knowledge of the insect family and even genus can tell us little about the potential for toxicity and harm to a given species. The extraordinary diversity of insects makes impossible any inclination to test all life stages of all species of a given order of insects that inhabit or live near corn fields. Thus, testing must be prioritized to identify and manage risk to an acceptable level. The dose of toxin in the corn tissue and the probability of encountering it must also be considered in any risk assessment.

The direct effect of insect-resistant biotechnology-derived plants on nontarget arthropods occurs by the contact or consumption of biotechnology-derived plant tissues or products of expression (i.e., pollen, nectar, sap, and exudate). Because no natural enemies of corn pests are Lepidoptera, the direct harm to natural enemies is not an important issue with regard to the *Bt* corn hybrids produced for corn borer control. However, a variety of beetles are important natural enemies (predators in the families Carabidae and Coccinellidae) in corn habitats, so the same testing for potential nontarget effects with biotechnology-derived potato modified to kill the Colorado potato beetle is applicable to new corn hybrids produced to kill corn rootworms, which are also beetles (Dogan et al. 1996; Riddick and Barbosa 1998; Riddick et al. 1998). If the predators do not eat corn tissue in addition to insects, then the risk of harm to these natural enemies and other beetles will be less than for those that do directly consume plant tissue.

Any corn hybrid that harms pest insects can alter the behavior or population numbers of nontarget natural enemies of the pests. Beneficial predators and parasites, most of which are insects or mites, can be indirectly affected by (1) the depletion of host/prey, (2) changes in prey and predator/parasite behavior, or (3) the consumption of the toxin that accumulated in the host/prey when they fed on the corn (Schuler et al.1999). However, all of these can also result from other effective management tactics, such as chemical insecticide use or application of biological control agents. Thus, these consequences of pest management are not unique to host plant resistance or to biotechnology-derived crops. The effects will be greater for specialist natural enemies that exclusively consume insects that damage *Bt* corn than for generalist predators that have a wide host range in addition to corn pests. A key point is that natural enemy populations are likely to decline to levels observed under natural, wild conditions once successful management of the pest(s) (i.e., their specific host) occurs.

Over a 2-year period, Wold et al. (2001) assessed the impact of *Bt* sweet corn on several beneficial insects, including predatory coccinellids (ladybird beetles), chrysopids (green and brown lacewings), and anthocorids (e.g., minute pirate bugs). Both fields and cages of *Bt* corn had significantly lower *Coleomegilla maculata* (Coccinellidae) densities than non-*Bt* corn. However, the researchers did not observe significant within-year differences in the overall density of beneficial insect populations or additional differences in species diversity of beneficial insects between *Bt* and non-*Bt* cornfields.

Two field studies describe the effects of *Bt* corn on natural enemies in Italy. Lozzia (1999) observed no significant differences in ground beetle (Carabidae) diversity and populations between *Bt* corn and non-*Bt* cornfields over a 2-year period. Similar conclusions were drawn for an earlier, single-season study (Lozzia and Rigamonti 1998)

Over a single season, Orr and Landis (1997) observed oviposition, predation, and parasitism of the ECB in *Bt* corn and non-*Bt* corn fields. Both kinds of fields had the same number, distribution, and size of corn borer egg masses. Egg predation was slightly, but not significantly, higher in the *Bt* corn. Parasitism of eggs was not significantly different between the *Bt* and non-*Bt* corn fields. Percentage of eggs within masses that hatched was lower in *Bt* corn than in non-*Bt* corn. Densities of predators and parasitism of ECB larvae by the parasitoids *Eriborus terebrans* [*Diadegma terebrans*] and *Macrocentrus grandii* were not significantly different between fields.

Jasinski et al. (2001) surveyed 6 *Bt* corn, 1 glyphosate-resistant corn, and 5 non-biotechnology-derived cornfields on a weekly basis during one growing season. They observed a significantly higher density of *Orius* predators at one *Bt* cornfield. No-spot ladybird beetles (*Cycloneda munda*), green lacewing (*Chrysoperla carnea*) adults, and mites were more numerous in biotechnology-derived cornfields. The remaining 11 categories of beneficial insects were higher in non-biotechnology-derived cornfields. More than 2000 parasitic wasps were collected in non-biotechnology-derived cornfields, approximately 5% more than in biotechnology-derived cornfields.

Al-Deeb, Wilde, and Higgins (2001) studied *Orius insidiosus* (Anthocoridae), a predator of ECB, over a single season in the field and in several laboratory experiments. They found few significant differences in predator densities between *Bt* corn and non-*Bt* cornfields. No significant differences were observed in the laboratory studies of mortality and development time of larvae fed *Bt* corn silks (no direct effects) or prey that had consumed *Bt* corn tissue (no indirect effects). Zwahlen et al. (2000) also did not observe indirect effects on *Orius majusculus*, a predator that fed on thrips eating *Bt* corn in a laboratory study.

In small field plots over two years, Pilcher et al. (1997b) observed no detrimental effects of *Bt* corn on predators. They drew the same conclusion regarding *Bt* corn pollen fed to *Orius insidiosus*, *Chrysoperla carnea*, and *Coleomegilla maculata* under laboratory conditions.

Hilbeck et al. (1998a) performed laboratory feeding experiments with *Bt* corn-fed pest insects on the predator *Chrysoperla carnea*, the green lacewing. Two prey species were studied, ECB and *Spodoptera littoralis*. Mortality of lacewing larvae reared on *Bt* corn-fed prey differed depending on predator developmental stage. For example, first and second instars suffered 26 and 42% mortality, respectively, when fed on *Bt* exposed prey. Mortality of the corresponding lacewing instars fed non-*Bt*-exposed prey was 9.4 and 21%, respectively. On the other hand, mortality of third instar fed on non-*Bt*-exposed prey (6.3%) did not differ significantly from those instars fed on *Bt*-exposed prey (10%). The development time of lacewing larvae was longer when *Bt* corn-fed ECB was given to predators, but not for *S. littoralis*. Although the differences in development time were statistically significant, they differed by a day or less for the various instars. Hilbeck et al. (1998a) concluded that the prolonged development time of lacewing larvae reared on *Bt* corn-fed ECB was probably due to a combined effect of direct toxin exposure and nutritional deficiency caused by sick prey. The applicability of this study to the field is somewhat ambiguous because the researchers did not offer a choice in prey (either different types of prey or non-exposed prey) for the lacewings as would normally occur.

Hilbeck et al. (1998b) further explored the relationship between *Bt* corn and *C. carnea* in diet feeding studies. *Cry*1Ab protein was "synthesized" in *E. coli* cells and then extracted and added to an artificial diet medium developed for the lacewing. Thus, the lacewing was directly exposed to the toxin at a single dose of 100 µg/mL. They concluded that mortality of *Bt* exposed lacewing larvae (57%) was significantly higher than that of larvae reared on artificial diets that do not contain *Bt* (30%). Thus, some of the additional mortality recorded by Hilbeck et al. (1998a) in the former study is likely due to consumption of *Bt* inside of the prey cadavers.

The relatively high mortality observed by Hilbeck et al. (1998b) in the control treatment suggested that poor nutritional quality of the diet may have exacerbated mortality when the toxin was present. Pertinently, when green lacewings were offered a nutritious diet of insect eggs during their earliest developmental period, and then switched to an artificial diet during later development, mortality was much lower—27% for *Bt* toxin diets vs. 17% for control diets. Although these results still suggest some detrimental effect of the *Bt* toxin on green lacewings, only second instar larvae were significantly affected.

Hilbeck et al. (1999) confirmed the results of their earlier studies (Hilbeck et al. 1998a,b) by feeding prey (ECB and *Spodoptera*) on meridic diets containing different concentrations of *Cry*1Ab (0, 25, 50, 100 µg/g) and then allowing lacewings to feed on the prey. Mortality between *Bt*-exposed lacewing larvae fed on *Bt*-exposed ECB was significantly greater than mortality of lacewings from the control treatment. However, this difference was observed for lacewings exposed to *Bt*-fed Spodoptera only at the highest dose of 100 µg/g. While the Hilbeck et al. (1999) study appears more definitive, its utility in predicting the likelihood of effects under field conditions is doubtful because the doses do not reflect the levels of *Cry*1Ab in plant tissue. For example, *Cry*1Ab levels in event MON810 average 10.3 µg/g in the leaves and 4.7 µg/g in the whole plant. Considering that Hilbeck et al.(1999) showed a dose-response effect for *Cry*1Ab, the levels of actual toxin in the plant must be taken into consideration before concluding that lacewings will be adversely affected in the field.

Other studies lead to conclusions obverse to those of Hilbeck et al. (1998a,b). For example, Lozzia et al. (1998) observed no effects on the green lacewing, *C. carnea*, when it was reared on *Rhopalosiphum padi*, an aphid feeding on *Bt* corn. Direct analysis of phloem sap from *Bt* corn using ELISA (enzyme linked immunosorbent assay) failed to detect any *Cry*1Ab protein (Raps et al. 2001). The protein appears to reside in the cells, because extraction of pooled leaf samples resulted in a positive detection, indicating release from ruptured cells. Honeydew from *R. padi* that fed on *Bt* corn contained no measurable toxin protein, nor did the whole insect. On the other hand, the herbivorous insect species *Spodoptera littoralis* and its feces contained the toxin after feeding on *Bt* corn. Considering that aphids are preyed on by lady bird beetles (Coccinellidae), important predators in numerous agroecosystems, the likelihood of indirect adverse effects from *Bt* corn is nil (Raps 2001).

Head et al. (2001a) used ELISA to measure whole body concentrations of *Cry*1Ab in the corn leaf aphid (CLA, *Rhopalosiphum maidis*), ECB, CEW, and black cutworm (*Agrotis ipsilon*) after feeding on diet fortified with toxin. The body burdens of toxin in the tested insects were 10-100 times lower than the concentrations in the diet. When the CLA and ECB fed on *Bt* containing diet with a minimum of 20 µg/g toxin or higher, levels in the body were bioactive when tested in an early instar ECB feeding bioassay. When fed on *Bt* corn plants (as opposed to artificial diet), however, no significant bioactivity was found within the tissues of the prey insects when tested in the ECB bioassay. Thus, incorporation of toxin and subsequent bioavailability to prey is much lower when pests feed on *Bt* incorporated into tissue than when they feed on the toxin mixed into an artificial diet.

In conclusion, current field studies do not indicate any adverse effect on generalist feeders (predators). Natural enemy populations fluctuate in response to the dynamics of their prey or host populations. If a pest management technique is successful at reducing pest populations, then natural enemies that exhibit host specificity (such as is common for many parasitoids) are likely to have reduced populations also. Several lab studies suggest secondary (or tritrophic) effects on predators, but these have relied on artificial diets fortified with toxin levels far above what occurs in *Bt* corn tissues. Aphid pests seem to pick up no toxin protein and thus pose no risk to their predators. Herbivores feeding on leaf tissue pick up substantial amounts of toxin, but one study shows that the levels are not likely to have much biological activity.

Nontargets Other Than Pest Natural Enemies

Impact of Conventional Pesticides

Thousands of experimental studies have demonstrated the hazards to nontarget organisms of chemical insecticides belonging to the chlorinated cyclodiene, organophosphate, carbamate, and pyrethroid classes. Currently registered insecticides for ECB and corn rootworm control belong to one of these classes. The likelihood of effects under commercial field conditions has always been presumed to be occurring based on the numerous hazard studies conducted under experimentally controlled laboratory, greenhouse, and small plot-field studies (Paoletti and Pimentel 2000). However, field studies of bird populations following realistic application scenarios of granular-formulated insecticides in corn fields have not always found the adverse effects predicted from the plethora of experimental hazard assessments (Buck et al. 1996; Johnson, Krueger, and Balcomb 1993). Similarly, aquatic mesocosm studies that simulate pond conditions do not strongly support adverse effects of organophosphate insecticides (OP) at levels likely to be found in streams of the Corn Belt (Giddings et al. 1996; Van Den Brink et al. 1996). On the other hand, mesocosm studies suggest that insecticides such as pyrethroids, which have supplanted much of the OP insecticide use in cotton and corn, can adversely affect macroinvertebrate and fish populations, at least temporarily, at levels simulating field application rates and runoff (Fairchild et al. 1992).

Despite the ambiguous conclusions drawn from studies trying to link experimentally observed hazards of conventional insecticides with likelihood of adverse effects in the field, the EPA has noted during the reregistration process of all OP insecticides that exposure to aquatic and terrestrial organisms exceeds levels of concern. Wildlife and fish kills following OP insecticide applications are documented in the Reregistration Eligibility Decision Documents (REDs) for products registered on corn. As a result, the EPA is negotiating with manufacturers to change labels and implement no-spray buffer zones to decrease the risk of adverse effects (USEPA 2001).

Impact of Bt Biopesticide Formulations

Sprays of formulations containing the spores of *Bacillus thuringiensis* kurstaki (*Bt*k) have been used extensively in forestry and to a lesser extent in agricultural production. The many studies summarized by Glare and O'Callaghan (2000) demonstrate the safety of various *Bt* toxins for vertebrates, particularly mammals that may be directly exposed to biopesticides. Risks of direct adverse effects (i.e., acute toxicity) to mammals, birds, and fish are thus believed to be nil. On the other hand, any broadcast spray, even sprays of *Bt*k, could drastically lower the Lepidopteran fauna with possible secondary effects on foraging animals. Indeed, a field experiment with *Bt*k sprays revealed that spruce grouse chicks in sprayed plots gained significantly less weight than birds not exposed to the sprayed plots (Norton et al. 2001).

Although reduction in insect food sources has been hypothesized to cause some effect on population dynamics of insectivorous vertebrate species, long-term consequences would depend on the frequency of sprays and the time period of study. For example, the serovar named *Bacillus thuringiensis* israelensis (*Bt*i) is sprayed in wetlands to control mosquito larvae. Its use in Minnesota wetlands did not affect insect fauna the first year of study (Hershey et al. 1998). However, in subsequent years, insect numbers and diversity severely declined relative to untreated wetlands in the same area. Although bird populations also decreased, the link to the secondary effects of *Bt*i treatment was very weak (Niemi et al. 1999).

Nontarget butterfly populations in arboreal habitats have been decreased by applications of *Bt*k for gypsy moth and spruce budworm control (Wagner et al. 1996; Whaley, Anhold, and Schaalje 1998). In one study, drift of the spray to 3000 meters downwind deposited at levels toxic to Satyrid butterflies (Whaley, Anhold, and Schaalje 1998). Another study showed that *Bt*k spray deposits were still toxic to early instar swallowtail butterflies (genus *Papilio*) 30 days after application (Johnson et al. 1995). Nontarget Lepidoptera abundance and diversity were decreased for two years in a stand of oak sprayed once with *Bt*k (Miller 1990). Concerns were voiced about potential adverse effects on overwintering monarch butterflies if *Bt*k was planned for use in Mexican forests to control pest Lepidoptera (Brower 1986).

In summary, effects on nontarget invertebrates and vertebrates have not only been observed with conventional pesticides, but similar adverse effects also can occur following applications of *Bt*k biopesticide formulations. The effects on bird populations are indirect due to a reduction of food resources. The effects on nontarget butterflies is direct, and toxic residues of *Bt*k can drift long distances and persist for at least one month following application. Thus, *Bt*k sprays seem to have a broad spectrum of biological activity, which may impact a broader spectrum of lepidoptera (USEPA 1998).

Impact of Bt Corn on Nontarget Organisms

In contrast to the potential broad-spectrum effects of *Bt*k sprays and conventional pesticides, *Bt* corn was not expected by the EPA to have any unreasonable adverse effects on invertebrates. During the early registrations of *Bt* corn, the EPA was aware of the nontarget effects of *Bt* sprays on Lepidoptera species, but the corn agroecosystem was not perceived to be at risk because *Bt* protein was essentially “encapsulated” in the corn plant and only a few target pests were susceptible. However, the publication by Losey, Rayor, and Carter (1999) hypothesized that *Bt* corn pollen could severely inhibit development of monarch butterfly larvae. That study has been roundly criticized as flawed in its conclusions because it was based on a poorly controlled lab experiment (Shelton and Sears 2001).

A study by Hansen and Obrycki (2000) was interpreted as lending credence to the hypothesis generated by Losey, Rayor, and Carter (1999). Hansen and Obrycki (2000) showed that within field deposition of *Bt* corn (event 176) pollen on potted milkweed plants decreased survivorship of early instar monarch larvae by 20% when they were allowed to feed on small circular disks punched from the plants. The location of the plants, within the field or at the edge, was not correlated with increased mortality. Laboratory bioassays with *Bt* corn events 176 and *Bt*11 showed that pollen deposited at an estimated density of 135 grains/cm^2 on milkweed leaf disks decreased survivorship of early instar monarchs. Ironically, survivorship of larvae on leaf punches treated with *Bt*11 at a density of 1300 grains/cm^2 did not differ from survivorship of larvae on non-*Bt* treated leaf punches. Hansen and Obrycki (2000) also conducted a laboratory bioassay using 14 grains/cm^2, but they did not mention the results of that experiment in their report. Unnoticed in public reactions to this study was the unusual absence of a linear dose and effect response, and the fact that event *Bt*11 corn pollen seemed to have a different potency than event *Bt*176 corn. Other criticisms of the study were the failure to provide larvae with a feeding choice between pollen-dusted and undusted leaves, the appearance of anthers in the pollen used to treat leaves with *Bt*11 corn, and the unusually high value for *Cry*1Ab in the *Bt*11 corn (Shelton and Sears 2001).

Hansen and Obrycki (2000) cited within their report the experiments by Wraight et al. (2000) with the black swallowtail butterfly. Although in a different family than the monarch, these butterflies also are common in the Corn Belt and feed on hosts in close proximity to corn fields. Wraight et al. (2000) demonstrated no effect on swallowtail larvae exposed in the field to event MON810 *Bt* corn pollen. Yet under laboratory (Wraight et al. 2000) and field conditions (Zangerl et al. 2001), event *Bt*176 corn pollen exhibited toxicity. Thus, Wraight et al. (2000) gave credence to a developing hypothesis that different *Bt* corn events had different potencies likely due to differences in protein expression in pollen. Furthermore, their study pointed out that there may be a range of susceptible nontarget species. Scriber (2001) recently reported that other swallowtail species (Eastern tiger and the spice bush swallowtail) were not significantly affected by very high deposition (1400-3600 grains/cm^2) of pollen from *Bt* sweet corn with event *Bt*11, which is the only event found in commercialized *Bt* sweet corn.

In none of the studies reported by the year 2000 did researchers look carefully at the likelihood of exposure by butterflies and thus they failed to examine the risk faced by nontarget Lepidoptera. Tschenn et al. (2001) examined exposure from the perspective of the likelihood that monarchs would lay eggs on milkweed in corn fields. Releasing mated monarch butterflies into flight chambers, the researchers observed that monarch adults laid eggs preferentially on potted milkweeds not dusted with pollen. When monarchs did lay eggs on milkweed, most of the eggs were laid near the top one-third of the plant and on the ventral side of the leaf. The source of pollen, *Bt* or non-*Bt*, was not a significant factor influencing oviposition. Monarchs also preferred to lay eggs when milkweed plants were isolated rather than in patches surrounded by corn. Although the Tschenn et al. (2001) study was conducted in a greenhouse, the observations were consistent with other reports of lepidopteran behavior being influenced by surrounding plants (Cromartie 1975).

Uncertainty surrounding the potential effects of *Bt* corn has moved closer to resolution with the publication in the Proceedings of the National Academy of Sciences of a group of six papers that taken together represent a comprehensive risk assessment for adverse effects on the monarch butterfly. Much of the work reported in these papers addresses questions raised by a US EPA December 1999 data call-in (DCI) notice for information regarding whether *Bt* corn pollen poses a significant risk to monarch butterflies. The papers represent the culmination of a multi-state collaboration of entomologists and ecologists that worked on different pieces of the problem. The series of papers starts with determination of the LC_{50} for the various *Bt* crystalline proteins incorporated into the diet (Hellmich et al. 2001). The *Cry*1Ab proteins characteristic of *Bt* corn lines were very toxic to first instar monarch larvae. However, second and third instar larvae were 10-30 times less susceptible. Susceptibility to pollen varied, however, depending upon the cultivar source. As hinted in the studies of Hansen and Obrycki (2000) and Wraight et al. (2000), *Bt*176 corn was very toxic at relatively low pollen densities. On the other hand, *Bt*11, MON810, and TC1507 (*Cry*1F) were not toxic even at pollen densities over 1600 grains/cm^2. Pertinently, *Bt*11 corn pollen contaminated with the larger pieces of anther exhibited toxicity at fairly low pollen densities. The anthers were described as artifacts from the pollen preparation process and thought not to be significant sources of exposure in the field. Differences in protein expression levels in the *Bt*11 and MON810 lines (0.09 µg/g *Cry*1Ab/g) from the *Bt*176 line (7.1 µg/g) explained differences in relative susceptibility. Anthers were apparently a high protein expression tissue.

The absence of toxicity at high pollen densities for the most prevalently used *Bt*11 and MON810 lines raised the issue of the distribution of pollen densities on milkweed leaves both within and outside of the field. Deposition of pollen on potted milkweed in four different states was studied by Pleasants et al. (2001). Mean pollen densities ranged from 10-426 grains/cm^2. More importantly, 95% of the pollen density was less than 600 grains/cm^2, and nearly 80% was less than 300 grains/cm^2 in the field. Pollen density one meter from the field edge averaged 35 grains/cm^2. Pleasants et al. estimated that rain could remove from 54-86% of the pollen on leaves. Thus, the density of pollen at the 95th percentile was still nearly three times lower than the pollen level causing no effects in the Hellmich et al. (2001) toxicity assays.

Monarch larvae were placed on potted, transplanted, or naturally occurring milkweed in a field study involving four states (Stanley-Horn et al. 2001). Larvae were observed in the field for up to 22 days. Monarch larvae placed in *Bt*11 or MON810 corn did not differ in survivorship or development from larvae placed in non-*Bt* isogenic lines. However, in non-*Bt* sweet corn that was sprayed with the pyrethroid cyhalothrin, monarch larval numbers were significantly decreased in comparison to larval numbers found in the unsprayed *Bt* corn cultivar.

The final question surrounding likelihood of adverse effects of *Bt* corn on monarchs concerned the contribution of agricultural fields to the total estimated monarch population and whether monarch egg laying and the appearance of the early instars overlapped with corn anthesis (pollen shed). Oberhauser et al. (2001) coordinated a survey of milkweed and monarch densities in habitats in four regions of the monarch breeding range. Milkweed growing in agricultural fields in Iowa potentially contributed nearly two orders of magnitude more butterflies than non-agricultural regions. This astounding figure could partly be accounted for by the magnitude of cultivated land in some regions of the Corn Belt. The potential overlap between the peak of the migratory monarch generation and pollen shed varied by region. For example, overlap was estimated to average 15% in Iowa but 40% in Minnesota/Wisconsin. The significance of overlap in Iowa, which had a much better database than the other sites, suggested that only approximately 3% of the monarchs emerging in Iowa over the summer would be exposed to *Bt* pollen.

Sears et al. (2001) used the data collected by Hellmich et al. (2001), Pleasants et al. (2001), Stanley-Horn et al. (2001), and Oberhauser et al. (2001) to conduct a probabilistic risk assessment that characterized the likelihood monarch larvae would be exposed to hazardous levels of *Bt* pollen. In short, for *Bt*11 and MON810 corn, Sears et al. (2001) estimated that monarch larvae would experience a 1% or less inhibition in growth rate at a level of exposure theoretically representing the 99.9th percentile of pollen density distribution. The relationship between likelihood of exposure and growth inhibition would constitute a probability of an effect of only 0.1%. Given the assumption that the market for *Bt* corn would saturate to 80% of corn acres (i.e., the total market reflecting the necessity to allow a non-*Bt* refuge of 20%), the estimated contribution of each state's land area to the monarch population, and the probability of a toxic effect, Sears et al. (2001) calculated that the risk of any adverse effect would be significantly less than 1%. For example, in Iowa the overall risk of an adverse impact on monarch populations is barely 0.05%. In other words, less than 0.05% of the total monarch population in Iowa would be at risk of an adverse effect.

The analysis of Sears et al. (2001) was the first time that probabilistic risk assessment (PRA) was applied to a biotechnology-derived crop protection technology. PRA is now being used more extensively for pesticide ecological risk assessment (reviewed by Solomon, Giesy, and Jones 2000). The EPA has endorsed the development and use of PRA as a goal for more realistically characterizing the risk of adverse effects in the environment (ECOFRAM 1999). A probabilistic risk assessment essentially uses the entire distribution of available empirical data and estimates, and thus its outcome of estimated risk for an effect will change if the input data change appreciably. Pertinently, *Bt*176 corn, which presents the most risk of adverse effects on monarchs, never occupied more than 2% of the biotechnology-derived corn planted – its registration expired in 2001 and existing stocks will be phased out through 2003. Thus, the PRA conducted by Sears et al. is conservative in that it assumed no further plantings of *Bt*176 corn and any new cultivars would have no greater toxicity than the *Bt*11 and MON810 events. Sears et al. concluded that any unresolved questions related to chronic toxicity and sublethal effects not directly measured thus far may be moot because overall exposure of monarch larvae to *Bt* pollen is low (EPA 2001a,c).

Nontarget Impacts—Water

Impacts of biotechnology-derived corn on water resources should be viewed from several perspectives: (1) potential movement of the *Bt* toxin to water resources with subsequent exposure of nontarget organisms; (2) anticipated changes in herbicide use in HT corn that can potentially affect loads of herbicide residues in water resources; and (3) changes in insecticide use projected for *Bt* corn resistant to corn borers and rootworms that can potentially affect aquatic organisms.

Potential for Movement of Cry1Ab Protein

For the *Cry*1Ab toxin in commercial varieties of *Bt* corn to be of potential concern for water contamination, a case must be made that the protein exists freely in the soil. A series of papers from the laboratory of Professor G. Stotzky at NYU (hereinafter referred to as the NYU studies) has definitively shown that the *Cry*1Ab protein is exuded from the roots of various events of *Bt* corn (*Bt*11 and MON810) and their associated hybrids (Saxena and Stotzky 2000; Saxena et al. 2002a). However, the NYU studies also show that a great proportion of the protein is sorbed to kaolinite and montmorillonite clays naturally in and/or added to the soil. In the sorbed state the proteins appear resistant to microbial degradation by proteases (Crecchio and Stotzky 1998, 2001).

Equilibrium between sorption on clays and dissolution into soil water would be expected, and thus potential for movement through soil exists. This hypothesis was tested in soil column experiments that were leached with water for different times after addition of purified protein extracted from a Dipel (*Bt*k) microbial spray formulation (Saxena et al. 2002b). The protein was detected in leachates from a 15-cm column containing soil at its moisture holding capacity and subject to leaching cycles of 5 mL water over a 24-hr period. When the soil column was leached just 10 minutes after adding the protein, 75% of the protein could be detected in the leachate using an immunoassay based on the Western blot technique. Protein was found throughout various sections of the soil column, and it was biologically active both when extracted from the soil as well as in the leachates. Pertinently, biological activity was detected by adding either soil extracts or leachate directly to *Manduca sexta* (tobacco hornworm; Family Sphingidae) diet and then allowing a period of feeding for up to seven days. Nevertheless, whether immunoassay or bioassay was used as the detection method for *Cry*1Ab, leaching potential was curtailed when increasing amounts of kaolinite or montmorillonite were added to the soil. Time of incubation in the column also allayed leaching because after 12 and 24 hours, no toxin was detected in leachates.

*Cry*1Ab protein was also detected in leachates of rhizosphere soil from test tubes in which several *Bt* corn hybrids were grown. The bioactivity of detected toxin (limit of detection was ~10 ppb) was comparatively low in rhizosphere leachates compared to the levels found when *Cry*1Ab isolated protein was leached. *Cry*1Ab was also detected by bioassay and immunoassay in soil with *Bt* corn plant biomass that had pre-incubated with soil (1% wt/wt loading rate) for either 300 or 350 days.

The experiments of the NYU researchers show that the *Cry*1Ab protein has the potential for leaching, but its relevance to actual field conditions is obscure because of the soil water regime the columns were subjected to. In the experiment with the *Cry*1Ab protein, 5 mL of water were added twice within the first hour after protein addition to a 16-cm column containing 50 g of soil already at its moisture holding capacity. Thus, the soil was essentially primed to produce leachate. Most importantly, however, translocation of the protein ceased after 12 hours. Even more importantly, the experiments conducted with *Bt* plant rhizospheres or degraded *Bt* plant biomass did not examine translocation of the released *Cry*1Ab through a column of unexposed soil as would occur in the field. Indeed, the addition of 10 mL of water four times within one hour to 50 g of soil already at moisture holding capacity was not too dissimilar from directly extracting the protein from soil. The NYU researchers routinely extract 0.5 g soil with 0.5 mL of buffer using vortexing. Thus, detection of protein in leachates of soils exposed to *Bt* plant material is not unexpected.

The experiments of the NYU researchers can be interpreted as supporting a hypothesis for potential movement of *Cry*1Ab into water resources. However, under field conditions, such an occurrence, if detectable, will have negligible impact. Earlier NYU research showed that *Cry*1Ab protein was completely degraded (i.e., all biological activity was lost) when roots were placed in a hydroponic solution and incubated for 25 days (Saxena and Stotzky 2000). Thus, even if the protein moves into surface water, it will likely desorb into the water column and then become inactivated by either sunlight or microbial proteases. Finally, research with bacteria and viruses has shown that these organisms are translocated vertically in soil columns, as well as subject to runoff processes, so movement of proteins from plants would not be expected to behave any differently if they have any persistence in the soil.

Impact on Herbicide Contamination of Water

Herbicides can be found in water resources, especially surface water, throughout the United States. While herbicide detections have been particularly prevalent in the watersheds and reservoirs of the midwestern Corn Belt, the level of herbicides detected continues to decrease as farmers increasingly implement more effective management practices such as reduced tillage and buffer strips. Current herbicide use in corn production is dominated by atrazine (68% of corn acres treated in crop year 2000), metolachlor (28%; combination of S-metolachlor and racemic mixture), and acetochlor (25%). During crop year 1994, acetochlor began to replace alachlor, which is in the same class of chloroacetamide herbicides. Prior to 1994, alachlor was sprayed on 27% of the corn acreage (Table V-2). Historically, atrazine and alachlor were the most frequently detected herbicides in all aquatic systems. Acetochlor has been found in a number of sampling programs. Atrazine concentrations are nearly always higher than alachlor concentrations owing to its higher use rate. With the advent of crops resistant to glyphosate or glufosinate, potential reduction in detections of the most frequently used herbicides has been hypothesized. This hypothesis, if it proves to be true, would be beneficial to drinking water facilities' efforts in insuring that finished drinking water supplies remain safe.

HT corn, especially corn resistant to glyphosate, has been on the market only since 1998, so it is too soon to have enough data to properly test the hypothesis that herbicide concentrations in water supplies would decline. Whether the herbicide residues decline will be highly dependent on how farmers change their herbicide use practices. For example, the ideal program would be to eschew a pre-plant or pre-emergence application of atrazine or acetochlor in favor of a burn-down application with glyphosate (none at all) and one or two postemergence applications of either glufosinate or glyphosate. Although total usage on a per acre basis of herbicide may or may not decrease, glufosinate and glyphosate are much less prone to runoff in water when compared to the runoff potential of atrazine, alachlor, acetochlor, and metolachlor (Wauchope et al. 2001).

Thus far, the hypothesis of reductions in concentrations of herbicides in water systems has been tested in two ways that have yielded encouraging albeit preliminary results. First, as is consistent with EPA registration eligibility decision making based on pesticide risk assessments, a probabilistic pesticide runoff and water behavior model was run to simulate impacts of adoption of HT corn in comparison with conventional corn (Wauchope et al. 2001). The herbicide use parameters modeled were either conventional treatments with early postemergence applications of atrazine and alachlor and no postemergence or Liberty Link™ and Roundup Ready™ corn treated early postemergence with glufosinate or glyphosate, respectively, or with the aforementioned herbicides and reduced rates of atrazine and alachlor. The HT corn also received a late postemergence application of glufosinate or glyphosate. Assuming spray drift may have occurred (a benchmark 6.75% of applied was assumed to drift), the estimated concentrations of atrazine and alachlor in the simulated watershed were reduced from 160-180% above the maximum contaminant level (MCL) to 0.12-0.45% of the MCL. Without spray drift, modeled reductions were 2-4 times greater.

Monitoring programs have not focused specifically on the impacts of HT corn adoption at this time. However, under the auspices of the Acetochlor Registration Partnership program (Hackett et al. 1999), drinking water supplies have been monitored in several Illinois watersheds where HT corn has been adopted. Concentrations (annualized time-weighted means) of acetochlor, atrazine, and metolachlor during 1999 and 2000 were the lowest they have been in three selected watersheds since the introduction of acetochlor in 1994 (Wauchope et al. 2001). The results are still preliminary but trends in water quality will be revealed as monitoring continues over the next five years and geographic locations of HT crops within watersheds become better characterized.

Impact on Insecticide Contamination of Water and Effects on Aquatic Organisms

By virtue of its massive acreage, corn production accounts for the highest uses of insecticides. Because most of the corn insecticides are applied for corn rootworm control in the early spring prior to crop emergence and coincident with intense rainfall events, the potential exists for runoff to aquatic systems and transient episodes of acute toxicity to aquatic invertebrates. Although adoption of *Bt* corn is still too new to have much study on impacts of insecticide contamination, modeling has been employed to estimate insecticide loadings relative to potential ecological impacts should there be a 50% and an 80% reduction in insecticide use (Estes et al. 2001). The modeling exercise was based on use of GENEEC software that is also employed by the EPA during registration to estimate the risk of ecological effects of pesticides in aquatic systems. The modeling scenario assumes a 1 ha pond, 2 meters deep receives drift from an application to an adjacent 10 ha corn field. Runoff occurs from a rainfall event every two days.

The output of the GENEEC modeling exercise is a probability distribution of ratios of the EEC (expected environmental concentration based modeling) after 96 hours to the LC_{50} for the most vulnerable species (usually the macro crustacean *Daphnia* spp.). When the ratio is greater than 1, then the EEC exceeds the LC_{50} and the likelihood of adverse effects is higher than when the ratio is less than 1. A modeled 50% reduction in insecticide use on *Bt* corn reduced the probability of exceeding the benchmark ratio of 1 from 54% to 24%. A further reduction in insecticide use of 80% reduced the probability of exceedance to less than 10%.

Soil Ecology

The soil abounds with a bewildering diversity of organisms interwoven in a food web that is indispensable to fertility. So when soil microbiologists from

New York University (NYU) reported that *Bt* corn roots exude the *Bt* toxin (Saxena, Flores, and Stotzky 1999), attention focused on possible threats of biotechnology-derived crops on the soil ecosystem.

In the NYU studies, *Bt* corn seeds were germinated on an agar and transferred to a nutrient solution for 25 days of growth. The nutrient solution tested positively for *Bt* toxin, and the analyses were confirmed by bioassays with tobacco hornworm larvae (*Manduca sexta*) that were fed the root exudates. When soil in test tubes with the growing corn seedlings was extracted and bioassayed, most of the hornworms also died.

These root exudate experiments followed earlier studies by the NYU scientists that showed *Bt* endotoxin proteins extracted from *Bt* spray products were rapidly adsorbed by natural soils, pure clays, and extracted humic acids. Adsorption rendered the *Bt* proteins resistant to microbial degradation, but they maintained their toxicity (Crecchio and Stotzky 1998; Koskella and Stotzky 1997; Tapp, Calamai, and Stotzky 1994). The persistence of the *Bt* proteins varied among soil types, but the retention of their biological activity generally decreased over time (Tapp and Stotzky 1998).

In contrast to the results of the NYU studies, earlier studies in the UK showed a rapid loss of toxicity of the whole crystalline endotoxin when extracted from soil and bioassayed against white sulfur butterflies (*Pieris brassicae*) (West 1984; West and Burges 1985). Information submitted to the EPA for registration of biotechnology-derived *Bt* corn plants showed rapid loss of *Bt* toxin incorporated as plant material into the soil (USEPA 2000). *Bt* toxin from biotechnology-derived cotton foliage was incorporated into soil with a fast initial loss of extractable protein in several soils (Palm et al. 1996). However, the authors noted that in some soils 35% of the added toxin could still be recovered after several months, suggesting a binding effect to soil constituents as reported by the NYU researchers.

Other studies suggest that toxin proteins in *Bt* corn tissue have shorter soil persistence than added toxin as noted previously. For example, *Cry*IAb protein added to soil as a component of biotechnology-derived corn tissue had an estimated DT50[2] of 1.6 days and a DT90 of 15 days. Without soil contact, the corresponding dissipation times were 25.6 and 40.7 days (Sims and Holden 1996). The recently registered *Cry*1F as expressed in *Bt* corn degrades in soil with most of the degradation occurring in one day (Herman et al. 2001). *Cry*1Ac, the toxin found in *Bt* cotton, did not accumulate in soil nor result in detectable biological activity after 3-6 consecutive years of cropping (Head et al. 2002). Because of the similarity of *Cry*1Ac degradation rate with that of *Cry*1Ab toxin, researchers have concluded that no accumulation of toxin is likely following years of cropping with *Bt* corn.

Whether *Bt* endotoxin persists in soil or is adsorbed is probably ecologically irrelevant unless susceptible species are directly feeding on the adsorbed fraction. In the aforementioned NYU studies, soil extracts were essentially forced upon insect larvae that do not eat soil particles. No contact activity should occur because the *Bt* toxin is only toxic when directly ingested by susceptible insects. Thus, the relevant question is how do ecologically important soil organisms react to the presence of plant-incorporated *Bt* toxins.

Table V-3. Comparison of Bt toxin no observable effects concentration (NOEC) in soil relative to the estimated environmental concentrations (EEC)

Biotechnology-derived *Bt* Corn	EEC (mg/kg soil)	Earthworm NOEC (mg protein/kg soil)	Springtail NOEC (mg protein/kg soil)
Monsanto YieldGard; *Cry*1Ab	NA	>200	>200
Novartis; *Cry*1Ab	0.00042	"non-toxic"	0.08
Dekalb; *Cry*1Ac	9.8	>98	>98
Aventis (AgrEvo) StarLink; *Cry*9c	0.11	>1.84	>180

Sources: USEPA 2000b; USEPA 2001a, c

The hypothesis of adverse effects advocated by the NYU researchers can be tested on earthworms, springtails (Collembola) or other organisms ingesting soil and bits of organic matter (i.e., detritivores). The registered *Bt* biotechnology-derived plants have been ground up in soil and bioassayed with earthworms and springtails that are considered the indicator species for adverse ecological effects in soil. Comparisons of the no-observable-effect concentrations (NOECs) for toxicity with the estimated environmental concentrations from incorporating biotechnology-derived plant material into soil indicates the likelihood of an effect is very low (USEPA 2000b; USEPA 2001a,c) (Table V-3). The NYU researchers themselves concluded that the adsorbed *Bt* toxin is not likely to be ecologically relevant (Saxena and Stotzky 2001). When they added *Bt* corn biomass to soil or grew *Bt* corn in soil, they observed no effect on earthworms, nematodes, protozoa, bacteria, or fungi.

Considering that soil fertility depends directly on decomposition of dead plant material (a.k.a. litter), tests should be conducted with decomposition of *Bt*-containing plant material. One study monitored the decomposition of *Bt* corn plants (a Northrup King accession) by the woodlice, *Porcellio scaber* (Crustacea: Isopoda) (Escher et al. 2000). No significant effects in comparison to the non-*Bt* corn plant control were found in decomposition, colonization by microorganisms or weight gain and reproduction of the woodlice. In another study, earthworms were unaffected by *Bt* corn plant litter, and decomposition rates did not differ significantly from the rate of non-*Bt* plant litter (Zwahlen et al. 2001).

Thus far, the evidence indicates that soil invertebrates are not affected by *Cry*1Ab toxin either in plant tissue or when added directly to the soil. Perhaps such a conclusion is inevitable considering that *Bt* spores and their associated toxic crystalline proteins are naturally abundant in soil (Martin 1994) Thus, soil- and detritus-ingesting organisms may already be frequently exposed to the toxic protein. Pertinently, the known susceptible invertebrates are not soil-dwelling, but rather plant-dwelling and aquatic insects (Martin 1994).

Resistance to Crop Diseases

Several naturally occurring species of mold fungi infest seeds and secrete bioactive secondary metabolites called mycotoxins. Fumonisins are mycotoxins secreted by *Fusarium* sp. that are contained in mold spores and also secreted directly into plant tissue (Pribela and Sinkova 1995). The molds may be commonly found on growing grain crops and on stored seed.

One environmental impact of fumonisin was suggested in a study of the distribution of mycotoxin contamination in Costa Rican maize. Seed germination potential and levels of fumonisins were negatively correlated (Danielsen and Jensen 1998). Because the seeds suffered insect and mechanical damage, the association of fumonisins with poor germination may have been coincidental. Nevertheless, *Fusarium* species are common contaminants of maize and infection may be associated with yield reduction (Munkvold and Desjardins 1997; Nelson, Desjardins, and Plattner 1993). Furthermore plants may be infected without showing any symptoms (Nelson, Desjardins, and Plattner 1993; Sobek and Munkvold 1999).

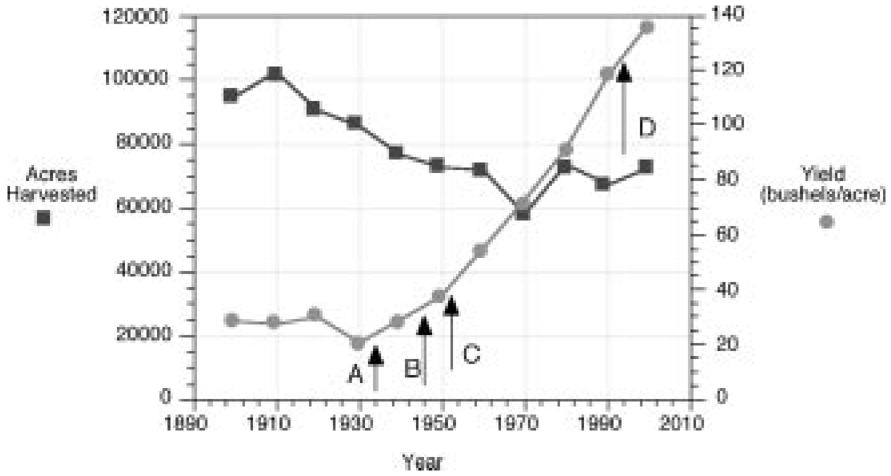
Fusarium infections and mycotoxin contamination are correlated with both mechanical and insect damage to the grain. Insect control in *Bt* corn has the potential to reduce contamination and adverse effects on seed quality. Indeed, several studies have shown that *Fusarium* infections and fumonisin content are significantly lower in *Bt* corn than in non-*Bt* corn under natural and artificial infestations of ECB (Bakan et al. 2002; Dowd 2000, 2001; Munkvold, Hellmich, and Rice 1999).

Land Use Efficiency

Corn production in the United States and other industrialized countries has successfully used land resources efficiently to maximize production. Historical tracking of acres planted and harvested, yield, and productivity shows that the land base devoted to corn production has not changed appreciably in 100 years despite an exponential increase in population since the 1940s (Figure V-1) (USDA 2001b). More importantly, productivity, as measured by bushels harvested per acre, has grown exponentially as new technologies have been implemented. For example, with the introductions of hybrid corn, mineralized fertilizers, and soil insecticides, yields correspondingly increased (Figure V-1).

The introduction of insect-protected corn did not occur until 1996, so discerning further positive trends in corn productivity is difficult at present. However, the yields of corn continue at high levels since the introduction of biotechnology-derived varieties (Figure V-2). Corn yields fluctuated widely during the crop years before 1996. Since the introduction of biotechnology-derived varieties, yields have been comparatively stable and at their highest average levels when compared with the period from1990 to 1994 (131.8 vs. 119.6 bushels per acre) (Table V-4).

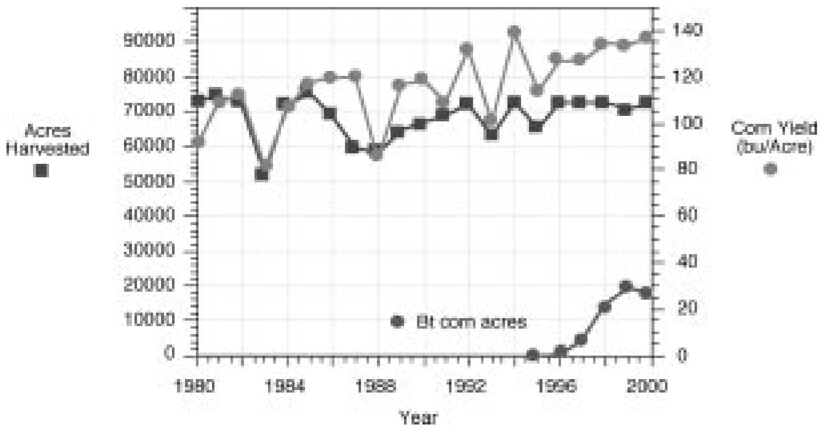
Figure V-1. Relationship between yield and acres harvested during the last 100 years.



The approximate year of adoption of new techologies are represented by the arrow.
A = introduction of hybrids; B = introduction of mineralized fertilizers;
C = introduction of soil insecticides for control of corn rootworms;
D = introduction of transgenic pest-protected and herbicide tolerant plants

Source: Yields and acres based on USDA 2001b; introduction of transgenic crops based on James 2001; introduction of other technologies based on graphic in Hayes and Laws 1991).

Figure V-2. Relationship between yield, acres harvested, and yearly use of *Bt* corn during the crop years 1998 through 2000.



Source: Based on USDA 2001b and James 2001

Yield will vary depending on yearly and regional meteorological conditions, fertilizer input, insect density, insect damage, and weed density and competition. Fertilizer input did increase by 4 pounds per acre between the periods 1996-1997 and 1998-2000. Thus, the specific influence of pest-protected and herbicide-resistant corn on agronomic productivity cannot be definitively concluded until the accumulation of many more years of yield trend data.

Energy Use and Global Warming

Modern corn production is highly dependent on fossil fuels for manufacturing and operating equipment, irrigating fields, drying harvested crops, and manufacturing and transporting fertilizers (Pimentel and Pimentel 1996). Burning of fossil fuels directly or indirectly for agricultural use converts extracted forms of carbon (i.e., oil, gas, and coal) to CO_2 that resides in the planet's atmosphere and there acts as a greenhouse gas, contributing to global warming and local air pollution.

Table V-4. Average harvest (x1000), yield (bushels/acre), production (bushels x 1000), and fertilizer use (lbs per acre) for five year intervals between crop years 1980 and 2000 and the first two and latest three years of Bt-protected corn use.

Years	Average Acres Harvested 1/	Average Yield/Acre 1/	Average Production 1/	Nitrogen Use Per Acre 2/	Phosphate Use Per Acre 2/	Potash Use Per Acre 2/
1980-1984	68,716	100.2	6,967,901			
1985-1989	65,331	111.6	7,338,630			
1990-1994	68,660	119.6	8,254,748	128	58	79
1995-1999	70,720	127.1	9,005,747	132	55	81
1996-1997	72,658	127	9,219,695	130	57	81
1998-2000	71,936	135.1	9,719,218	134	55	81

1/ USDA 2001b
2/ USDA-NASS 2001d

Any technology that decreases modern corn production's dependence on fossil fuels will help mitigate this problem. For example, modern biotechnology-derived corn developed for pest protection may decrease synthetic insecticide use. Based on an assessment of the environmental benefits of *Bt* crops for the period 1998-2000, the USEPA (2001) concluded that the public and the environment significantly benefit from the use of certain *Bt* biotechnology-derived crops. One major benefit is the reduction in the use of hazardous insecticides, particularly in sweet corn fields. The USEPA predicted that environmental benefits will increase as adoption of *Bt* crops increases (USEPA 2001). Because synthetic chemical insecticides require fossil fuel in their production, transportation, and application (Pimentel and Pimentel 1996), energy use and CO_2 in the atmosphere should decline.

Recently, reported research indicates that *Bt* corn tissues contain more lignin than non-*Bt* tissues of the same hybrid. The researchers speculate that *Bt* corn tissue would decompose comparatively more slowly (Saxena and Stotzky 2002a). If true, such a change in rate might tie up more carbon, which would reduce the flux of carbon dioxide to the atmosphere. To further investigate this hypothesis, research with direct measurements of CO_2 is recommended.

Human Health

Overview of Concerns about Human Health and Safety Assessment Strategies

General questions about the impact on human health of crops produced through biotechnology arise out of concerns for potential epistatic and pleiotropic effects of random gene insertions. In other words, the locations of the inserted DNA sequences in the plant genome raise the possibility of adverse affects or alterations of the expression of other genes or traits. Single genes may potentially affect the expression of other unrelated genes (epistasis), whereas the protein produced by a single gene can have effects on multiple plant traits (pleiotropy). Some of the envisioned problems include poor agronomic performance and susceptibility of crops to disease. Every attempt is made by the breeder to select against these potentially problematic traits. The human health concerns fall into one of four categories: pathogenic properties of "foreign" DNA; allergenic or toxic properties of introduced characters; production of new compounds and/or change in composition of nutrients or antinutrients; and spread of antibiotic resistance due to use of marker genes. Concerns raised over potential adverse effects from both short- and long-term consumption of genetically altered foods are not specific to any one crop. However, each can only be addressed on a crop-by-crop basis.

Following its 1992 "new" plant variety foods policy (FDA 1992), the FDA began to examine the possible consequences of epistatic and pleiotropic effects in herbicide-resistant and pest-protected crops (Maryanski 1995). Unintended gene effects can be examined directly and indirectly. Direct tests include studies of the expression and inheritance of the new genes in the recipient crops. Indirect tests include studies of plant agronomic performance, toxicity, allergenicity, and nutritional equivalence.

Whether a biotechnology-derived character is pesticidal, i.e., directly controls a pest (e.g., *Bt* proteins), or represents a novel food only, similar kinds of safety data must be submitted to all of the appropriate regulatory agencies (USDA-APHIS, FDA, and EPA) before commercialization. Molecular probing and inheritance tests are designed to determine the copy number (or number of insertion points) of the gene construct. The number of insertion points (gene copies) is important, because a high number of random insertions would have a greater probability of causing unpredictable epistatic or pleiotropic effects, assuming the plant survived the genetic engineering event in the first place. If there are only one or two insertion points in the genome, and the plant can be bred through several generations of fertile seed production, then the likelihood of unintended genetic effects is remote.

Inheritance tests are designed to determine the stability of heritance and whether the trait segregates as a dominant or semi-dominant character. Backcrossing biotechnology-derived lines with conventional cultivars is used to determine whether genes are inherited according to the rules of Mendelian genetics. The sta-

Table V-5. No Observable Effect Level (NOEL) for mortality following exposure of rats to purified Bt toxic protein[1]

Registered Transgene	NOEL (mg/kg) (Based on LD50 acute testing)	Digestibility in Simulated Gastric Medium	Homology to Known Food Allergens
MON810 CryIA(b)	>4000	Rapidly degraded (<2 minutes in gastric fluid)	None
Bt11	>4000	Rapidly degraded (<2 minutes)	None
Cry1F	>5050	Rapidly degraded (<5 min)	None
Bt176 CryIA(b)	>3280	Rapidly degraded (<2 min)	None
Bt Cry9C	>3760	Stable	None
Bt spray	>5050	Degraded	None

1/ All information from the EPA Biopesticide Safety Sheets available for each registration at www.epa.gov/oppbppd1/biopesticides/ai/plant_pesticides.htm

bility of a trait is tested by growing the transformed crop over multiple generations in geographically disperse locations throughout the United States. In each generation, plant morphology, disease susceptibility, and yield are examined.

Two kinds of toxicity data are obtained—the lethal dose (usually estimated as the dose at which 50% of the test subjects die and abbreviated as LD50) following a single or short-term (acute) exposure, and a NOAEL (No Observable Adverse Effect Level) following multiple doses in the diet over different intervals of time (subchronic dietary toxicity). Rodents are primarily used for these studies, but chickens and cows are also used because they are the primary consumers of field corn (Table V-5). Because of the high amounts of material needed for acute lethality tests, novel proteins (e.g., *Bt* protein) are "synthesized" in *E. coli* hosts rather than extracted from the plants. Nevertheless, the protein is biochemically characterized to ensure equivalence between the two sources. For the dietary studies, the transformed plants may be ground up and incorporated into the normal diet. Alternatively, novel proteins can be added directly to the diet.

Pleiotropic effects can be teased out by examining nutrient and secondary metabolite equivalence between the biotechnology-derived and isogenic crop cultivars. The concept of substantial nutritional equivalence between new food varieties and their conventional counterparts is a principle adopted internationally by the World Health Organization (WHO), the United Nations Food and Agricultural Organization (FAO), and the Organisation for Economic Cooperation and Development (OECD) (Kuiper, Kleter, and Noordam 2000; Sidhu et al. 2000). The principle asserts that if a new food or feed derived from conventional breeding or genetic engineering is substantially equivalent in standard nutritional parameters to its conventional counterpart, then the new food should be considered equally safe.

Although it is difficult to obtain human sera to run routine allergenicity studies against biotechnology-derived protein products, principles for deducing human food allergens have been well elucidated (Lehrer 2000; Metcalfe et al. 1996). Food allergens are nearly always proteins of sizes ranging from 10-80 kDa, are acid stable (pH~2), heat stable, and may be glycosylated. Novel proteins can be subjected to simulated gastric conditions in vitro (pH~2 in the presence of pepsin enzyme), heat stability tests, and amino acid sequence homology tests. If a protein is not glycosylated, breaks down in simulated gastric environments within seconds, is labile to thermal degradation, and has a series of no more than eight amino acid acids that are homologous to known food allergens, then it is usually considered of no allergenic threat.

Several overviews of safety assessment strategies and procedures for biotechnology-derived crops have been published (Kuiper and Kleter 2000). General safety assessments for a number of commercial biotechnology-derived cultivars have recently been published (e.g., Gay 2001; Pedersen, Eriksen, and Knudsen 2001; Penninks, Knippels, and Houben 2001). *Bt* plant-incorporated protectants safety assessments are available from the EPA Biopesticides internet site (www.epa.gov/biopesticides). In the general papers that review the various strategies for assessment (Kuiper 2001), several examples of noncommercial biotechnology-derived traits are used to illustrate the potential for manifestation of unintended effects. Thus far, no unintended pleiotropic effects have been reported for any commercially planted biotechnology-derived crop cultivars.

Pathogenic Potential of Biotechnology-derived Elements

The genetic elements used to "construct" new characters in plants do not pose any extraordinary safety problem, especially when compared with conventional breeding methods (Conner and Jacobs1999). Thus, the focus of human safety should be on the nature of the gene product or protein.

Nevertheless, the safety of the commonly used promoter sequence, CaMV 35s has been questioned (Ho, Ryan, and Cummins 1999; 2000a,b). Ho, Ryan, and Cummins (2000a) have suggested, for example, that the promoter is inherently unstable and may activate dormant integrated viral sequences upon recombination with other parts of the plant chromosome. On the other hand, Hull and Covey (2000) have contrarily argued that the CaMV 35S is naturally occurring (mainly in plants of the family Cruciferae), and the necessary homologies with animal viruses are absent. Therefore, the promoter is unlikely under realistic conditions to pose any risk. Thus, notwithstanding the controversy over the commonly used CaMV 35S promoter sequence that is present in all commercial corn biotechnology-derived cultivars except GA21, the following discussion will focus on various toxicological aspects of the gene products, including the likelihood of risks from antibiotic resistance.

Toxicity and Allergenicity Issues of Herbicide-Resistant Traits in Corn

In general, the majority of herbicide-resistant crops rely on an altered EPSPS enzyme from a bacterium to confer glyphosate resistance, but any plant already possesses one of a myriad of forms of the same enzyme. The difference between the plant enzyme and the bacterial source is essentially changes in the amino acid sequence of the protein but not its molecular weight, nor its normal physiological functions (Harrison et al. 1996). The modified EPSPS of event GA21 has three amino acids that are different among the total of 445 amino acid in the unmodified enzyme (European Commission 2002). The amino acid sequence changes greatly decrease the tendency of glyphosate to bind to the enzyme, but do not completely negate binding at very high doses of glyphosate. Thus, the biochemical kinetics of the enzyme-glyphosate complex has been transformed in corn, but not the functionality toward native amino acids. Given that variations in EPSPS protein among

Table V-6. Concentration of protein (microgram per gram of wet plant tissue) in various corn tissues at plant maturity and estimated grams of protein per acre of corn.

Registered Transgene	Whole Plant	Leaf	Roots	Pollen	Grain	Grams *Bt* protein per acre (based on whole plant)
	Micrograms transgene per gram plant tissue (g/g)					
*Cry*IA(b) MON810 (YieldGard)	3.65-4.65	7.93-10.34	NA	0.09	0.19-0.39	188
*Cry*1A(b) *Bt*11 (YieldGard)	NA	3.3	NA	0.09	8.2	259 g
*Cry*1F (Herculex)	NA	1.52-2.63	NA	32	1.7-3.4	
*Cry*IA(b) *Bt*176**	0.6	4.4	<0.008*	7.1	<0.005 *	24
*Cry*9C Starlink***	4.7	9.5	5.6	0.1	4.0	191
Bt spray 0.81 kg/acre	NA	NA	NA	NA	NA	14*

Note that for Bt1, protein expressed as ng/mg plant protein; thus, not directly comparable with other cultivars. According to EPA, assumption of 89,300 pounds per acre at maturity
1) Limit of detection for roots was 0.008 g protein per gram tissue and for grain 0.005 g/g
2) Assumed a maximum rate of spray application of 0.81 kg/acre with a formulation containing 1.7% delta-endotoxin (Bernhard and Utz 1993).
****- registration expired in 2001 with a phase out of existing stocks through 2003.**
*****- registration voluntarily canceled in 2000.**
Table references:
Bernhard, K., and R. Uts. 1993. Production of Bacillus thuringiensis insecticides for experimental and commercial uses. Pp. 255-267 in Bacillus thuringiensis, an Environmental Biopesticide: Theory and Practice, P.F. Entwistle, J.S. Cory, M. J. Bailey, and S. Higgs, ed. John Wiley & Sons, NY.
USEPA 2001. BRAD
All information on protein concentrations from EPA Biopesticide site–the registration documents

different food sources are also due to differences in amino acid sequence (Harrison et al. 1996), it is unlikely that humans would experience any adverse biochemical or physiological effects; thus the EPSPS enzyme itself should pose no special hazard. Furthermore, the variation in amino acids composition between the plant and bacteria is likely no greater than the variation found between plants.

The EPSPS enzyme is thermally labile, rapidly digested in a gastric environment, not glycosylated, and possesses no amino acid sequences homologous to known food allergens. Thus, bacterial and plant sources of EPSPS are not of allergenic concern.

A proximate analysis of biotechnology-derived corn lines has been conducted and all nutrients were within the ranges found for conventionally derived corn (Sidhu et al. 2000). RR corn that constituted 50-60% of the diet weight was fed to broiler chickens from 2 days old to 40 days. No differences were found in growth, feed efficiency, and fat pad weights between chickens fed glyphosate-resistant corn and the parental conventional control grain (Sidhu et al. 2000). The anti-nutrient phytate was analyzed in a glufosinate-resistant line produced by AgrEvo (Novak and Haslberger 2000). No significant differences in concentration were found with the conventional bred counterpart. Secondary metabolite levels (including furfural, raffinose, phytic acid, p-coumaric acid, ferulic acid, trypsin inhibitor, and vitamin E) as well as essential metals (copper, iron, magnesium, manganese, potassium, and zinc) in event GA21 were not significantly different than the levels in the unmodified cultivar (European Commission 2002).

In summary, the conclusion of the European Commission safety review of event GA21 HT corn is representative of hybrids possessing a modified EPSPS enzyme. "Having reviewed all the information provided by the petitioner and in the light of current published scientific information it is concluded that from the point of view of consumer health, maize grain from maize line GA21 and derived products that are the subject of this application are as safe as grain and derived products from conventional maize lines" (European Commission 2002).

Plants engineered to resist the herbicide glufosinate are essentially manufacturing a novel enzyme, phosphinothricin acetyltransferase (PAT). However, the gene for this enzyme is obtained from the bacterium *Streptomyces viridochromogenes*, a naturally occurring soil species that is not pathogenic to invertebrates or vertebrates (OECD 1999). The enzyme is highly specific for acetylation of the herbicide glufosinate that leads to its inactivation. PAT has not been observed to aceylate any nutrient amino acids. PAT has been fed to rodents at high doses, and the estimated LD50 was greater than 5000 mg/kg (OECD 1999). In simulated gastric juice PAT is completely hydrolyzed into smaller fragments and amino acids within one minute. Thus, it is not considered to pose any risk of allergenicity.

Herbicide Safety

Because herbicide-resistant crops rely on applications of a herbicide not likely to have been routinely used during production of the conventionally bred cultivars, questions of chemical safety are raised. Both glyphosate and glufosinate are considered the safest herbicides on the market by European regulators (Kuiper, Kleter, and Noordam 2000). The EPA registered both compounds and the Agency raised no concerns about human health, ecological effects, or worker exposure.

A mammalian hazard and dietary exposure assessment of glyphosate has been published in the peer-reviewed literature (Williams, Kroes and Munro 2000). All levels of exposure from the diet or spray drift were significantly below the established reference dose.

An ecological hazard assessment of glyphosate was also published in the peer-reviewed literature (Giesy, Dobson, and Solomon 2000). Compared with levels of glyphosate actually measured in water and soil, hazards to aquatic and terrestrial organism were extremely low.

Recently, the European Commission's Health & Consumer Protection Directorate-General completed a review of glyphosate safety with respect to its inclusion in a category known as Appendix I (European Commission 2002). Appendix I chemicals have comprehensive databases, which allow a confident determination of safety to be made. In its re-assessment of glyphosate, the following conclusions were stated.

- "With particular regard to residues, the review has established that the residues arising from the proposed uses, consequent on application consistent with good plant protection practice, have no harmful effects on human or animal health."
- "The review has also concluded that under the proposed and supported conditions of use there are no unacceptable effects on the environment, as provided for in Article 4 (1) (b) (iv) and (v) of Directive 91/414/EEC, provided that certain conditions are taken into account as detailed in section 6 of this report."

Section 6 of the EC report mandated that member countries take appropriate precautions to protect groundwater, a provision common to nearly all pesticide product labels in the United States.

Although the PAT trait is used for selection of successful recombination events during development of several of the *Bt* corn cultivars, the plant also resists the effects of the herbicide glufosinate that is formulated as the product Liberty and is registered for use on field corn. Like glyphosate, glufosinate has similarly low toxicity to humans (EPA 1999a).

Toxicity Issues Related to Bt Traits in Corn

Insect-protected corn based on the engineered *Bt* traits is fundamentally different from glyphosate-resistant plants because a new plant protein is produced. The protein is a truncated version of the exact protein that occurs in the serovar[3] *Bt* kurstaki (*Bt*k). Thus, the place to start with questions about the safety of *Bt* biotechnology-derived crops is with *Bt*k itself. Formulated fermentation cultures of *Bt* can be sprayed on foliage to control selected insects because the ubiquitous bacterium synthesizes a toxic protein, known as the delta-endotoxin, every time it stops growing and produces a spore. *Bt* is but one kind of microbial pesticide, and over many years data about its safety were collected. In 1998, the EPA finally compiled the data into a RED that covers all *Bt* products not produced by genetic engineering (USEPA 1998). Based on nearly forty years of commercial use of microbial pesticides formulated with *Bt*k, EPA concluded that the data overwhelmingly support the safety of *Bt* to humans and nontarget organisms. As a result of finding a reasonable certainty of no harm, the EPA re-registered all *Bt* formulations and waived all requirements for a food tolerance and submission of further studies. Certain *Bt* spray formulations are some of the few insecticides that are certified as acceptable for organic agriculture (WSDA 2000).

Several comprehensive reviews of the toxicological properties of *Bt* serovars have been published in the peer-reviewed literature (McClintock, Schaffer, and Sjoblad 1995; Siegel and Shadduck 1989; Siegel 2001; Siegel, Shadduck, and Szabo 1987). These reviews cover acute toxicity, infectivity, and allergenicity from animal studies and they discuss the few available epidemiological studies motivated by large-scale *Bt* spray programs. In short, the insecticidal proteins produced by the various strains of *Bt* are only toxic to comparatively few species of insects by virtue of the coincidence of the insects' specialized gut physiology and shape of the proteins themselves. All other organisms, lacking these unique factors, tolerate *Bt* exposure without exhibiting symptoms of injury. Indeed, high doses of *Bt* that are fed, injected, or placed in the air of laboratory rats are essentially non-toxic.

Exhibiting similarity to studies with *Bt*k and the delta-endotoxin, the plant-produced *Cry*1Ab proteins that are fed to rats at high doses cause no measurable toxic effects (Table V-5). Controversy surrounds the potential for non-protein chemicals to cause adverse effects after long-term (i.e., chronic) daily exposure, but toxic proteins are only of concern for acute effects in response to an immediate exposure (Sjobold et al. 1992). Furthermore, toxic proteins are bioactive at low doses. Thus, the high dose feeding studies with *Bt* proteins would be able to detect toxic proteins if they existed.

One key element in determining the likelihood for any toxicity from consuming corn grain directly, as opposed to consuming meat or dairy products from animals that have fed on *Bt* corn, is to elucidate the levels of the *Bt* protein in various corn tissues. The levels of *Bt* protein in currently registered biotechnology-derived corn hybrids range from non-detectable quantities (less than 0.005 micrograms per gram of plant tissue, µg/g) to 4 µg/g (Table V-6). Based on the highest amount of protein present in grain, a human 2-year-old child would have to consume 27.5 pounds of popcorn in a day to reach a human-equivalent dose of 5000 milligrams of *Bt* protein per kilogram of rat body weight (mg/kg).

All *Bt* corn grain has been fed to livestock as a major fraction of their diet by the various *Bt* crop registrants. No effects were reported to the EPA as part of the reassessment of the risks and benefits of *Bt* plant-incorporated protectants (USEPA 2001). These studies strongly suggest that production of secondary toxic products owing to pleiotropic effects has not occurred over the many generations of *Bt* corn. Further evidence of lack of pleiotropic effects comes from an examination of the conventional corn borer-resistant trait resulting from plant production of DIMBOA. No differences in DIMBOA production were seen in event *Bt*176 corn and its isogenic line (Pedersen, Eriksen, and Knudsen 2001). No evidence of any increase in chymotrypsin-inhibitory activity was found compared with a conventional line (Novak and Haslberger 2000). Finally, proximal analyses of general nutritional parameters proved substantial equivalence between *Bt* corn and isogenic lines.

Allergenicity Issues Related to Bt Traits in Corn

Allergic reactions to proteins are not uncommon in the population, although allergy has a well-defined etiology (i.e., biochemical cause) that is quite distinct from toxicity. The kinds of proteins that cause problems are well known (Lehrer 2000). First, an allergic response should be distinguished from toxicity. Toxicity is the cascade of reactions resulting from exposure to a dose of chemical sufficient to cause direct cellular or tissue injury or otherwise inhibit normal physiological processes. Allergic responses, on the other hand, are immune system reactions resulting from stimulation of a specific group of antibodies known as IgE.

All *Bt* proteins from currently registered cultivars have been subjected to the scientifically accepted battery of tests for judging allergenic potential. With the exception of the *Cry*9C-containing hybrid of corn, all biotechnology-derived *Bt* proteins are rapidly degraded by the stomach environment and are unstable when heated (Table V-5). The *Cry*9C protein is stable to simulated stomach conditions, but it is not glycosylated and has not caused any adverse effects characteristic of immune system responses in mammalian toxicity studies. Furthermore, no part of its structure resembles known allergenic proteins.

A few epidemiological studies have investigated complaints of allergic-like (or immune response) symptoms following spraying of *Bt*k products on forests and wooded urban lands (reviewed in Siegel 2001). Thus far, no public health investigations have been able to confirm that reported allergy symptoms were due to the *Bt* sprays.

It has been suggested that other ingredients in the formulations containing both vegetative cells and parasporal bodies may trigger immune reactions rather than the *Bt*k *cry*stalline protein itself. For example, a study of vegetable farm workers exposed to Javelin, a *Bt*k formulation certified for organic agricultural production, showed early signs of immunological reactions (Bernstein et al. 1999). The unique experimental design of the farm worker study allowed the authors to confidently conclude, “results of this investigation should partially allay recent concerns about the occurrence of possible adverse health effects in consumers after exposure to biotechnology-derived foods.” Furthermore, “it is unlikely that consumers would develop allergic sensitivity after oral exposure to biotechnology-derived foods (e.g., tomatoes, potatoes) that currently contain the gene encoding this [*Cry*] protein.”

THE CASE OF *CRY*9C AND ALLEGED ALLERGENIC REACTIONS

When the EPA registered the *Cry*9C protein as expressed in the crop and the DNA needed for its production (commercialized as StarLink), initial approval was for corn designated only for animal feed or industrial uses such as production of ethanol. Co-mingling of the corn with commodity corn grain designated for food processing resulted in the detection of the protein in consumer foods. One reason that the StarLink *Bt corn* was not registered for use in human food was because of the stability of the protein in the *in vitro* gastric digestibility test, although it lacked any homologies with known food allergens. The registrant of StarLink, Aventis Crop Science, voluntarily cancelled the *Cry*9C registration in 2000 (USEPA 2000c).

The Centers for Disease Control (CDC) conducted an epidemiological and immunoreactivity study to determine if the alleged allergic reactions following consumption of corn products perceived to contain StarLink were indeed associated with *Cry*9C. The FDA, which routinely receives complaints about adverse reactions from eating foods, turned its subject files over to the CDC for determination of suitability for follow-up. The CDC chose subjects as cases if they had eaten a corn product within 12 hours of showing symptoms. The subjects also had to have exhibited symptoms characteristic of food allergy. Of the 51 cases received for review, only 21 met the criteria for a possible relationship between an adverse reaction and consuming a corn product. Sera were collected from these individuals to test for IgE antibodies specific for *Cry*9C protein. Such a test would indicate hypersensitivity to the *Cry*9C protein.

The CDC found no evidence that any of the 21 subjects had IgE antibodies reactive to the *Cry*9C protein. The CDC cited one 1993 research article that suggested that food allergies do not necessarily occur with detectable IgE responses. However, in the 1999 study of workers exposed to *Bt*k sprays, IgE antibodies were induced. Furthermore, most known food allergy cases involve reaction with IgE (Penninks, Knippels, and Houben 2001).

An expert panel determined that *Cry*9C in StarLink represents a low risk. Despite the panel's determination that *Cry*9C represents a medium-likelihood of being allergenic, the potential for exposure to *Cry*9C in StarLink is very low as StarLink is no longer registered for planting and levels in existing commodity corn stocks are diminishing (USEPA 2001b).

Other Genetic Elements in *Bt* Corn

Other genetic elements that are in *Bt corn* include the CaMV 35S promoter and the PAT gene for phosphinothricin resistance (Table V-1). Health concerns about the use of the CaMV 35S promoter were discussed previously, but after six years of commercial cultivation thus far, this unexpressed piece of DNA shows no evidence of causing any effects *in vivo*. The safety of PAT was discussed in the herbicide-resistant corn section.

Exposure to *Bt* Proteins May Be Ancient

As the number of acres farmed using certified organic practices increases along with the acreage of *Bt* biotechnology-derived crops, more people would be expected to be exposed to *Bt* proteins. Pertinent to the argument over exposure to novel proteins in *Bt* crops is the realization that with or without biotechnology-derived transformations, people are already exposed to *Bt* proteins. Indeed, exposure may be unavoidable as well as ancient. Studies of the natural ecology of *Bt* show it is abundant on the surfaces of foliage of numerous plant species, and its presence in the soil may result from washoff with rainfall (Martin and Travers 1989; Smith and Couche 1991). Even more curious is the occurrence of *Bt* in stored grain that has not been specifically sprayed. Stored grain is commonly infested by moths and beetles that may be susceptible to naturally occurring *Bt*. Exposure to *Bt* by organisms feeding on the grain is confirmed by finding *Bt* spores in the feces of birds and rodents collected from the feed mill (Meadows et al. 1992). Indeed, birds and rodents have been suggested as possible spreaders of the *Bt* spores. A recent study from Canada indicated that *Bt* can be detected occasionally on produce in grocery stores before known aerial applications of spray formulations (Capital Health 1999).

Antibiotic Resistance

Concerns have been raised that antibiotic resistance marker genes in biotechnology-derived crop tissues may be taken up by bacteria, making them resistant in turn. Such concern is fueled by current problems of the frequent occurrence of antibiotic-resistant strains of medically important pathogenic bacteria. The likelihood of transfer and subsequent transformation of bacteria by biotechnology-derived antibiotic resistance marker genes has been directly tested (Gebhart and Smalla 1998). The literature has recently been critically analyzed and published (Smalla et al. 2000). The consensus is that transfer of selection antibiotic resistance markers occurs at an extremely low frequency if at all in soil, but it may occur in the digestive system, albeit still at low frequency. However, an analysis of the bla gene, which was used as a selection marker gene in *Bt*176 corn, concluded that it is very unlikely to be transferred and become established in intestinal *E. coli* (Salyers 1999).

The synthesis of the plasmid construct used to make event GA21 HR corn relied on an ampicillin resistance gene (bla) during cloning in *E. coli*. However, tests showed that this gene was not transcribed in corn and therefore no risk exists that the antibiotic trait will be horizontally transferred to microorganisms (European Commission 2002).

Smalla et al. (2000) pointed out that antibiotic resistance is already ubiquitous in the environment and biotechnology-derived crops would not change this incidence. In fact, they argued that the proper focus of concern should be on the widespread, seemingly indiscriminate use of antibiotics in human therapeutics rather than on the use of antibiotic marker genes in biotechnology-derived crops.

Potential Reduction in Mycotoxin Exposure

Several naturally occurring species of mold fungi contribute to the contamination of stored grain or food stocks by secreting bioactive secondary metabolites called mycotoxins. The two groups of mycotoxins of major health concern include the aflatoxins, which are secreted by *Aspergillus* sp., and the fumonisins, which are secreted by *Fusarium* sp. (Park et al. 2001; Pribela and Sinkova 1995). The mycotoxins are contained in mold spores, but they are also secreted directly into the food tissue (Pribela and Sinkova 1995). The molds commonly secrete mycotoxins when food is in storage, but they also can be found on growing grain crops.

Mycotoxins may be acutely toxic if concentrations are high enough. For example, the aflatoxins LD50 range from 0.5 mg/kg in ducks to 60 mg/kg in mice (Park et al. 2001). To put the magnitude of aflatoxins acute toxicity in perspective, if they were pesticides they would carry the signal word “danger” and be accompanied by a picture of a skull and crossbones. Aflatoxins are liver toxins, but they also are considered potentially carcinogenic at levels close to what humans generally consume (Park et al. 2001).

Insect feeding is one of the main pathways by which mold infests grain
Photo by Dr. Galen Dively, University of Maryland

Fumonisins have been associated with a wide array of pathologies in animals including equine leucoencephalomalacia, porcine pulmonary edema, hepatocarcinogenicity in rats, liver toxicity in poultry, and acute congestive heart failure in primates (Park et al. 2001). Fumonisins also have been associated with high incidences of human esophageal cancer.

Mycotoxin contamination is prevalent on corn grains worldwide. The United Nations Food and Agriculture Organization estimates that an average of 25% of worldwide grain is contaminated with mycotoxin-producing mold each year (Pribela and Sinkova 1995; Shephard et al. 1996; Tanaka et al. 1988). Insect feeding and mycotoxin presence are associated and result from the opportunistic infection by the molds in damaged plant and kernel tissue. Early studies showed that corn insects transferred *Aspergillus* sp. spores from the silk region of the ear into the region of developing kernels with subsequent formation of aflatoxins in seed (Fennell et al. 1978). European corn borers (*Ostrinia nubilalis*) can carry spores of *Fusarium moniliforme* from the leaves to the developing kernels and thereby increase the incidence of kernel rot due to a *Fusarium* infection and symptomless infections (Sobek and Munkvold 1999). Ear damage by caterpillar or bird feeding leads to secondary fungal infestations by sap beetles that also enhance mycotoxin contamination (Dowd 1995; Rodriguez-del-Bosque, Leos-Martinez, and Dowd 1998).

Specialty corn, such as seed corn or high starch corn, is likely to be routinely treated for ear damaging pests including the ECB and the corn ear worm. Thus, the use of malathion or chlorpyrifos was tested to determine what effect controlling these pests would have on mycotoxin contamination (Dowd et al. 1998, 1999, 2000). Insecticide treatments did decrease mycotoxigenic fungi and fumonisins. However, insecticide sprays also significantly decreased the abundance of ladybird beetles, important insect predators (Dowd et al. 1998, 2000). A special flour granular formulation of malathion gave good control of ear feeding damage and decreased mold incidence without affecting predators (Dowd et al. 2000).

The association between insect damage, infection by mycotoxigenic fungi, and mycotoxin contamination stimulated interest in determining whether *Bt* corn was effective at reducing mycotoxin contamination. Both artificial and natural infestations of ECB were used to determine the effects on fumonisin levels in ears of different *Bt* corn varieties in comparison with non-*Bt* corn. Because many farmers do not use insecticides for ECB control and essentially accept some damage and yield loss, the experiments did not compare mold and mycotoxin incidence to sprayed corn. All studies published thus far have shown significant decreases in mycotoxin contamination in *Bt* corn compared with the related isogenic cultivars (Bakan et al. 2002; Dowd 2000, 2001; Munkvold, Hellmich, and Rice 1999).

Contamination of grain by mycotoxins has received close scrutiny in Europe (Bakan et al. 2001; Castella, Bragulat and Cabanes 1999; Scudamore and Patel 2000; Visconti and Doko 1994), especially because concentrations less than1 microgram fumonisin per gram of grain have been proposed as a health protective standard (Bakan et al. 2002). The potential of *Bt* corn in Europe to reduce mycotoxin contamination has been studied in five field locations (three in France and two in Spain) where the biotechnology-derived event MON810 and its isogenic cultivar were planted side by side (Bakan et al. 2002). *Bt* corn (MON810) had 10 times less insect infestation (ECB and pink stem borer, *Sesmia nonagriodes*) and 2-5 times less incidence of *Fusarium* infection in kernels than was found in the isogenic corn. These large differences in *Fusarium* spp. biomass were confirmed by ergosterol analysis, which showed similar significant differences between the cultivars. Consequently, fumonisin content in *Bt* corn was up to 10 times less than in the non-*Bt* corn. In three of the fields, trichothecenes were less in the *Bt* corn than in the non-*Bt* corn. Most importantly, the level of fumonisins in *Bt* corn were well below the proposed health safety limit of 1 ppm, whereas some of the samples of non-*Bt* corn were nearly 10 times higher than this level.

Once seeds are contaminated with mycotoxins, they are difficult to decontaminate. Fumonisins are more stable than aflatoxins (Park et al. 2001). Decreased tillage practices tend to leave stalks closer to the soil surface, yet these can serve as a very long reservoir of *Fusarium* contamination to subsequent corn crops (Cotten and Munkvold 1998). Both insecticides and *Bt* corn cultivars can decrease the ECB damage that is associated with significant levels of *Fusarium* infection (Munkvold, Hellmich, and Showers 1997), and by implication the comparatively higher levels of mycotoxin contamination. The magnitude of the problem with contaminated feed was illustrated in an Iowa study that found fumonisins in all maize feed samples. Additionally, the mycotoxins fusaproliferin and beauvericin were discovered for the first time in North America (Munkvold et al. 1998).

The FDA has propsed maximum contaminant levels for aflatoxins in animal feed and human foods, including milk. Prevention from contamination is the best management practice, and preventing contamination of livestock feed would have great benefits for keeping aflatoxins out of milk products. The reluctance of the majority of corn farmers to control ECB with insecticides suggests that adoption of *Bt* corn may have a direct health benefit by lowering levels of mycotoxins in livestock feed and directly consumed corn products.

ECONOMICS OF BIOTECHNOLOGY-DERIVED CORN

Economic impacts of biotechnology-derived corn depend on an interaction between yields, costs of production, and market prices. Yields in turn depend in part on the density of the damaging pest assuming all other conditions like crop and soil management practices are held constant. Farmers expect weeds to be pests every year as well as throughout the growing season. Regardless of the crop, control of weeds will yield very positive net returns per dollar expended for weed control. Yet, herbicide-tolerant corn has been adopted on a very small percentage of corn planted worldwide (~2.5% of total acreage, James 2001a,b) and in the United States (~7% of total acreage, USDA-ERS 2002). One explanation for this low adoption rate is the long time availability of excellent weed control programs and the availability of non-biotechnology derived corn that is tolerant of the soybean herbicides in the imidazolinone class (Baldwin 2000). Additionally, biotechnology-derived HT corn approvals are pending in the European Union and other regions, so farmers in the United States have developed a program to channel grain unapproved for these export markets while these approvals are pending.

In contrast to the herbicide-tolerant trait, *Bt* corn was planted on 18% (USDA-ERS 2002) to 25% (USEPA 2001a) of U.S. corn acreage during crop year 2000. As will be shown below, *Bt* corn frequently results in higher yields per acre than non-*Bt* corn that is either sprayed or unsprayed with insecticides for ECB. Despite the yield increases, significant net economic benefits are returned to farmers only when borer populations are high. Certain regions of some states, however, have historically and consistently high levels of ECB (e.g., Iowa, Minnesota, Nebraska, and South Dakota) or Southwestern corn borer (Kansas, Colorado, New Mexico, Texas, and Oklahoma) (USEPA 2001a). Adoption of *Bt* corn in these states is more certain to yield a positive economic return. As a result, selected areas of these states have adopted *Bt* corn on about 25% to 50% of producing acres (USEPA 2001a).

In addition to considering annual variability in insect population density and economic risk in adopting *Bt* corn, indirect costs, such as soil erosion and degradation of water quality by pesticide residues, should be considered as an economic issue. However, indirect costs are more difficult to quantify. For example, atrazine is heavily used on corn, in part because it is effective and inexpensive, but it also contaminates water supplies at the highest levels among herbicides. While farmers can limit input expenses by using atrazine as a pre-emergence soil-applied spray, costs are borne by local public water utility districts that must meet regulatory standards for pesticide contamination under mandates of the Safe Drinking Water Act. However, if biotechnology-derived corn leads to less use of atrazine and more use of glyphosate, a herbicide significantly less likely to cause water contamination (Wauchope et al. 2001), then external costs of corn production will be correspondingly minimized. Thus, in addition to the favorable economic benefits returned to a farmer when *Bt* corn is planted in areas of high pest population densities (as elucidated in the following section), benefits can accrue to communities when reductions in use of persistent herbicides accompany adoption of herbicide-tolerant crops.

Herbicide-Tolerant Corn

Presently, herbicide-tolerant corn has been planted on too few acres (7% of the U.S. total during 2001; USDA-ERS 2002) to make any definitive assessments of possible economic impacts.

Nevertheless, some field plot studies suggest positive net returns when glyphosate is used pre- and/or postemergence. In Michigan, both early and late postemergence use of glyphosate alone gave similar grain yields as the use of a nicosulfuron plus dicambra treatment and a hand-weeded treatment (Tharp and Kells 1999). The comparable yields were obtained even at the lowest rate of glyphosate (0.21 kg/ha; 0.19 lbs/acre) and without cultivation. If such reductions in herbicide rates and fuel use are proven to be the case in other areas of the United States, net returns for herbicide-resistant corn should be positive.

Studies of weed control and net income in glyphosate and non-herbicide-resistant corn at university field stations in Missouri and Illinois suggest a more general applicability of the observations in Michigan. Pre-emergence applications of acetochlor followed by early postemergence applications of glyphosate returned a net income of nearly $900 per hectare ($364 per acre) that was not significantly different than the income returned from postemergence use of atrazine (Johnson et al. 2000). More importantly, an early postemergence treatment with glyphosate followed by a glyphosate treatment after weed re-growth netted $860 per hectare ($348 per acre). A conventional metolachlor plus atrazine pre-emergence treatment netted only $700 per hectare ($283 per acre) despite not having to account for a $15 per acre technology fee. The differences among the treatments reflected significant differences in yield. Similarly, studies in Kentucky illustrated the close dependence of net returns on corn yield but also showed that net returns for glyphosate-resistant corn did not differ significantly from the conventional treatment employing pre-emergence atrazine (Ferrell and Witt 2000).

In summary, net returns from use of glyphosate only or glyphosate plus pre-emergence conventional herbicide treatment are positive, providing increased yield while lowering herbicide costs. At this time, farmers have not adopted herbicide-resistant corn despite early reports of favorable net returns. One major factor limiting adoption may be the many effective choices farmers already have for weed control in corn, especially under conservation tillage. Pending approvals for commercialization in the EU and other regions, market barriers to the adoption of biotechnology-derived HT corn exist.

Bt Field Corn

Because *Bt* corn was used on about 18% of corn acres during 2001 (USDA-ERS 2002), its economic impacts bear examination over a larger corn production area than did herbicide-resistant corn. Currently registered and commercialized *Bt* field corn hybrids are planted mainly to control the European corn borer (ECB) and Southwestern corn borer (SWCB). *Bt* sweet corn was developed to control the ECB and corn earworm (CEW). Although it is registered, it has not been marketed or adopted by farmers. *Bt* corn for control of corn rootworms (CRW) is being field tested under an experimental use permit (USEPA 2001), and a petition for registration has been submitted. Thus far, economic impacts of *Bt* field corn have been based on simple models with deterministic or semi-probabilistic input parameters or on field experiments conducted at many locations.

With two generations per growing season over a large part of its range, the ECB can be a sporadic economic pest whose populations in a specific field are difficult to forecast (Ostlie, Hutchison, Hellmich 1997). Economic injury levels (EIL, the pest infestation level where costs of control are equal to or less than the value of pest damage) for ECB infestations will vary depending on crop use (e.g., as grain, silage, or seed), the costs of control measures (including scouting, pesticide, premium for hybrid trait), and the prevailing market price. For example, the EIL for silage corn in Wisconsin (based on loss of biomass as the endpoint) ranged from a low of 9% of plants infested at a control cost of $10 per acre to 18% infested plants when costs doubled (Myers and Wedberg 1999). One generalized rule of thumb is a 5-10% yield reduction per ECB-infested plant for first generation feeding damage (Hyde et al. 2001).

In much of the U.S. and Canadian Corn Belt, farmers do not scout or treat fields for ECB. As a result of this general acceptance and lack of awareness of ECB feeding damage, aggregate economic impact from lost yield potential has been estimated to be about one to two billion dollars per year (Hyde et al. 2001; Ostlie, Hutchison, Hellmich 1997). This aggregate loss estimate translates to a per acre loss of $6.57 and $12.90 for first and second generation ECB, respectively (Ostlie, Hutchison, Hellmich 1997).

Aggregate estimates of net losses are based on regional estimates of the impact on potential yield of endemic (i.e., “low”) and outbreak (i.e., “high”) populations of ECB taking into account yields, production costs (including seed technology fees), and price. For example, an examination of the historical ECB populations in Minnesota between 1988 and 1995 resulted in a $17.24 benefit for *Bt* corn that is well above the seed technology fee of $7-10 per acre (Ostlie, Hutchison, Hellmich 1997). Current seed technology fees charged by seed companies range from $6 to $8 per acre. Of course such modeling analyses must make certain assumptions, and the Minnesota calculations used the following variables:

- 95% control of ECB with *Bt* corn compared to 80% and 67% for first and second generation ECB, respectively, with insecticide sprays;
- Physiological losses of 5.5% and 2.8% for first and second generation tunneling, respectively;
- Yield, 123 bushels per acre;
- Market price (average 1991-1995), $2.23 per bushel;
- No yield disadvantage due to *Bt* hybrid agronomic characteristics.

A different set of assumptions was made for corn production in Kentucky to model per acre economic returns from *Bt* corn in comparison to either no control or insecticide spray rescue treatments (Bessin 2001). The *Bt* technology fee was assumed to be $14 extra per bag of seed, and borers per plant varied from 0 to 2. Corn prices varied from $1.50 to $3.00 per bushel. If no management of ECB was implemented, then the breakeven point would be exceeded even when the corn selling price was $1.50 per bushel and ECB density was 0.5 borers per plant (potential savings of $0.58 per acre). Because rescue insecticide treatments have to account for scouting and chemical costs, the breakeven point for *Bt* corn was just under 0.25 borers per plant at a market price of $1.50 per bushel (potential savings of $0.52 per are). The yield was assumed to be 144 bushel per acre for both the *Bt* corn and insecticide treatment scenarios. When corn market price was $2.00 per bushel and ECB infestation rate was 2 borers per plant, the per acre savings exceeded the breakeven point by $22.81.

As part of its decision to re-register *Bt* corn events MON810 and *Bt*11, EPA conducted an economic benefits analysis using a partial budgeting approach (USEPA 2001a). Two ECB infestation levels were assumed—low impact (resulting in a 5.4 bushel per acre increase over unprotected corn) and high impact (resulting in a 10.8 bushel per acre increase). With a technology fee of $8 per acre, the per-acre benefit was $2.11 and $12.21 for low and high ECB pressure, respectively. In the aggregate, farmers could receive a benefit of approximately $38 million and $219 million for low and high ECB infestation levels, respectively.

The projections of benefits from planting *Bt* corn largely rely on deterministic variables. For example, the ECB density is fixed at one or another discrete level over the entire Corn Belt or a state. A semi-probabilistic analysis has been applied to determining rates of return in Indiana, Illinois, Iowa, and Kansas (Hyde et al. 2001; Martin and Hyde 2001). In one study, infestation levels were varied according to location in the state (Martin and Hyde 2001). For example, the infestation levels in different parts of Indiana were categorized by percentage of plants infested: less than 20% ("low"), 20-30% ("medium"), or 30-40% ("high"). In contrast, the medium and high infestation levels in Iowa had 20-39% and 40-60%, respectively, of plants with at least one borer. Dollar value per acre for *Bt* corn was varied by assuming that a farmer was either risk neutral or risk averse. The "high" category of infestation levels always yielded net positive values per acre.

In a similar study, the probabilistic approach was carried further by consideration of percentiles of farmers who planted prior to a specific date. Date of planting was overlaid on number of ECB larvae per plant (0-3) and generation number (1-3). The model was applied specifically to ECB infestations in Indiana and was compared to a scouting and insecticide spraying program. Furthermore, a yield drag or penalty for planting non-optimized hybrids was applied to decrement return values. At a $16.20 seed premium per acre and an infestation probability of 25% (i.e., the equivalent of an economically damaging infestation one out of four years), the value of *Bt* corn exceeded non-*Bt* corn by $4.65 per acre. Seed premiums have dropped dramatically since commercial introduction; thus the advantage in per acre value would be even greater today.

The latter study (Hyde et al. 2001) addressed the need to allow refuges, but it indicated that the costs would be minimal and only the number of acres planted would be affected. Another study showed that planting the refuge in strips within a field would cost less than planting a block or U-type refuge. Regardless of which method was chosen the increased labor costs were only $0.038-0.075 per acre for the strip planting and $0.15-0.30 per acre for the block or U-type planting (Hyde et al. 2000).

The various studies that have projected yields and economic returns based on deterministic or semi-probabilistic input variables illustrate that outcome changes dramatically from negative to positive by altering input assumptions. Farmers need to base decisions on historical field data, and thus studies in commercial fields are needed to further improve forecasting of the economic returns on a more local level. A few available studies have focused on commercial farms.

During crop year 1991, 16 of 18 commercial fields across 10 Iowa counties had yield losses exceeding four bushels per acre that translated to an equivalent economic loss of about $10 per acre (Rice 1997). This study relied on a comparison of insecticide treatments for first generation ECB with untreated portions of the field. Because insecticide treatments are recognized to only give partial control (~50% in the Iowa study), the actual loss was likely greater. Thus, this study, which randomly chose fields without attention to ECB population density, suggested that under actual field conditions, *Bt* corn is likely to at least break even after considering the cost of the technology fee (assuming $7-10 per acre). If ECB pressure is sufficient, then higher rates of return will accrue from fields planted with *Bt* corn than from fields with either no treatment or insecticide treatments alone.

A cross sectional study of 128 non-*Bt* commercial corn fields and 46 *Bt* corn fields was conducted in Iowa during crop year 2000 (Duffy 2001). This study is distinctly different than the previously described Iowa study because side-by-side comparisons of treatments were not conducted. Average yields for the *Bt* corn and non-*Bt* corn fields were 152 and 149 bushels per acre, respectively, and the market price was $2.06 per bushel. Owing to higher seed costs ($33.05 for *Bt* corn vs. $28.74 for non-*Bt* corn) and fertilizer costs ($53.30 vs. $48.67), net return per acre for *Bt* corn was less (-$28.28 per acre) than for non-*Bt* corn (-$25.02 per acre). In this study, the comparatively high yield of the non-*Bt* corn in comparison with studies showing damage potential in relation to ECB infestation levels suggests that the regional ECB populations were below the economic injury levels. Nevertheless, the non-*Bt* corn fields did not post a positive per acre return even in the absence of any extra expenses for ECB control. The study points to an important internalized economic factor, however. Namely, farmers will plant *Bt*-corn as an insurance policy, knowing that ECB populations are unpredictable and have historically been very economically damaging (Duffy 2001).

*Bt*11 hybrids and their isolines were planted in strips in 43 commercial cornfields in Ontario, Canada, during 1996 and 1997 (Baute, Sears, and Shaafsma 2002). The area covered represented different heat unit regions and ECB biotypes with one or two-generation ECB per year. Damage was categorized by length of stalk tunnels caused by ECB feeding. Although all fields were predicted to have yield advantages, the economic return to *Bt* corn was only positive when tunneling damage in non-*Bt* corn was at least 5 cm, the seed premium was at most $8.78 per acre, and the market price was at least $1.88 per bushel. Among the commercial fields studied, only 55% of the farmers experienced a positive return. Tunneling damage greater than 6 cm occurred only 33% of the time, which is equivalent to a positive return on seed investment of once in every three years. At this probability of infestation, the value advantage of *Bt* corn would have to be $18.71 per acre, which would only occur with greater than16 cm of tunneling (probability of 7%).

Other studies based partly on field-collected data show similar lack of positive returns when ECB damage is light and the obverse in the years when ECB damage is comparatively heavy. In a six-year study of data aggregated for each state based on available data from ECB infestation monitoring and variety trial yields, a net gain from using *Bt* corn occurred in three years (Benbrook 2001b). However, the study outputs appeared to be sensitive not only to infestation levels and market price, but also to the seed premium, which varied among suppliers by nearly an order of magnitude. Pertinently, in the three years of the study that showed a net loss for *Bt* corn adoption, price per bushel was below $2.00. An important output missing from the six-year study is a net profit (or loss) indication for individual states rather than an aggregate for all corn-producing states. Such an accounting would be important for states like Colorado, Kansas, Nebraska, and Texas that have significantly above average plantings of *Bt* corn. These states would more likely experience both a yield advantage and net positive profit because they frequently have economically damaging levels of SWCB and ECB (Sloderbeck et al. 2000; USEPA 2001a).

In summary, projections of net economic returns owing to the adoption of *Bt* corn indicate that under low and high ECB infestation levels, yields tend to be greater than in fields planted with non-*Bt* corn. Rates of return for *Bt* corn when ECB infestation levels are at outbreak levels are greater than for scouting and insecticide spraying, largely because of costs incurred for scouting and chemicals in combination with a control efficacy of only 80% or less. Some attempt to bring a more probabilistic analysis to the economic projection models has been accomplished and applied for ECB infestation scenarios in several states. Because the economic projection models are very sensitive to the assumed input variables, studies in a diversity of commercial fields should be conducted to help validate the model projections. Under actual field conditions, the few studies published show economic returns are very sensitive to infestation levels and market price of corn. Regardless of infestation levels of ECB, some states, especially those with irrigated production, nearly always have economically damaging levels of SWCB and thus will benefit the greatest from routine planting of *Bt* corn.

Bt Sweet Corn

The EPA re-registered *Bt* corn event *Bt*11, which is targeted for control of corn earworm (CEW) and ECB in sweet corn. Although registered, farmers have yet to adopt fresh market *Bt* sweet corn. Nevertheless, EPA conducted a benefits analysis to project economic returns as well as environmental benefits from reduced pesticide spraying (USEPA 2001a). Among the states growing both processed and fresh sweet corn, the number of insecticide applications range from 1 per season (Oregon) to 17 per season (Florida). Thus, substantial savings are expected if insecticide applications for CEW can be totally averted. Based on a technology fee of $30 per acre, EPA calculated aggregate net per-acre benefit to *Bt* sweet corn adopters of $3.55 for process corn and $5.75 for fresh corn. Approximately 5 insecticide treatments were eliminated from the *Bt* corn pest management program.

A case study focusing only on Florida fresh sweet corn production forecasted a 79% reduction in insecticide use and a corresponding $3.9 million per year increase in production value (Gianessi and Silvers 2001). The change in production costs was estimated to provide $1.3 million in net savings in insect control. On a per acre basis, farmers in Florida (38,900 acres of sweet corn) would save $33.

In conclusion, sweet corn production is affected by cosmetic insect damage as well as potential yield losses. Thus, insecticide use, mostly for CEW control, will occur routinely several times a year on average. *Bt* sweet corn farmers will have definitive net positive economic returns that are less variable than the net returns to field corn farmers who are trying to manage the more sporadic ECB infestations.

[1] The term instar refers to any of various immature life stages of an insect or other arthropod.

[2] DT50soil or soil “half-life” ($t_{1/2}$) is equal to the length of time it takes for a pesticide active ingredient to transform (break down, degrade) to 50% of its initial concentration in soil. The soil half-life is highly dependent upon environmental conditions such as soil pH and climate.

[3] Serovar refers to a serological variety of a microbial species characterized by its antigenic properties.

VI. Cotton

INTRODUCTION

Cotton with herbicide-tolerant and insect-resistant traits is the third most extensively planted biotechnology-derived crop in the world, accounting for 13% of total cotton acreage and 20% of total genetically modified crop acreage in 2001. Forty-two percent of the total biotechnology-derived cotton crop was herbicide-tolerant, 25% was *Bt*, and 33% was stacked *Bt*/herbicide-tolerant (James 2001b). As with any new technology, especially one that could be released on vast areas worldwide, consideration of the potential environmental impacts of biotechnology-derived crops is desirable. Indeed, regulatory agencies worldwide assess these potential impacts before commercialization. This document provides a review of the scientific literature regarding the potential environmental impacts of biotechnology-derived cotton varieties in comparison with those of traditionally bred varieties.

Cotton is an important crop in the United States and internationally. A natural fiber, cotton is a renewable resource, used extensively for thousands of years primarily to provide people with textile products. No other natural fiber compares to cotton in terms of abundant supply and the quantity and quality of products that cotton provides. The synthetic fiber industry endeavors to find substitutes for cotton, and has been successful for some applications; however, cotton remains a major raw material of the worldwide textile market. In 2000 cotton supplied 77% (43,539 million pounds) of the natural fiber market and 33% of the total fiber market (133,144 million pounds) which included both synthetic and other natural fibers. (Fiber Economics Bureau 2001).

The cotton plant is indigenous to many parts of the world. Early ancestors of modern cotton varieties were found in tropical and warm temperate regions. Cotton continues to be cultivated in similar climates. Worldwide, cotton production is an important component of the agricultural economies of approximately 80 countries (Table VI-1). However, over 80% of the world's cotton production in market year 2001/02 is projected to come from eight countries: China, the United States, India, Pakistan, Uzbekistan, Turkey, Brazil, and Australia, with over two-thirds of the total world production from the top three producers – China, the United States, and India (USDA-FAS 2001a). The U.S. cotton industry claims direct impacts on the national economy on the order of $120 billion annually (National Cotton Council 2001b).

Table VI-1: World Cotton Production By Country, Global Share, and 1000 480-lb Bales (Market Year 2001/2002)

Country	1000-480lb Bales	Global Share (%)	Country	1000-480lb Bales	Global Share (%)	Country	1000-480lb Bales	Global Share (%)
China	23500	24.28	Tanzania	350	0.36	Bolivia	23	0.02
United States	20175	20.84	Togo	275	0.28	Ecuador	21	0.02
India	12200	12.60	Burma	270	0.28	Angola	20	0.02
Pakistan	8300	8.57	Sudan	250	0.26	Kenya	20	0.02
Uzbekistan	4700	4.855	Peru	184	0.19	Zaire	15	0.02
Turkey	4050	4.18	Zambia	170	0.18	Indonesia	14	0.01
Brazil	3300	3.41	Azerbaijan	145	0.15	Nicaragua	10	0.01
Australia	3100	3.20	Kyrgyzstan, Rep.	140	0.14	Tunisia	10	0.01
Greece	1900	1.96	Colombia	135	0.14	Philippines	7	0.01
Syria	1600	1.65	South Africa	130	0.13	Somalia	7	0.01
Egypt	1250	1.291	Uganda	120	0.12	Haiti	5	0.01
Mali	1100	1.14	Mozambique	110	0.11	North Korea	5	0.01
Turkmenistan	900	0.93	Israel	100	0.10	Niger	5	0.01
Burkina	750	0.77	Afghanistan	85	0.09	Sri Lanka	5	0.01
Cote d'Ivoire	750	0.77	Ghana	70	0.07	Cuba	4	0.004
Benin	625	0.65	Ethiopia	70	0.07	Honduras	4	0.004
Tajikistan	600	0.62	Yemen	65	0.06	Dominican Rep.	3	0.003
Zimbabwe	600	0.62	Bangladesh	60	0.06	Guatemala	3	0.003
Argentina	550	0.57	Madagascar	60	0.06	Albania	1	0.001
Kazakhstan	550	0.57	Cen. African Rep	50	0.05	Cyprus	1	0.001
Iran	500	0.52	Venezuela	50	0.05	El Salvador	1	0.001
Spain	475	0.49	Vietnam	50	0.05	Italy	1	0.001
Mexico	440	0.45	Thailand	44	0.05	South Korea	1	0.001
Cameroon	435	0.45	Senegal	40	0.04	Morocco	1	0.001
Nigeria	430	0.44	Guinea	37	0.04	Yugoslavia	1	0.001
Paraguay	400	0.41	Iraq	32	0.03	Costa Rica	1	0.001
Chad	350	0.36	Malawi	30	0.03			
						Total		**96871**

Source: USDA-FAS 2001

Approximately 5.4 billion pounds of cotton are consumed annually by the U.S. textile industry and approximately two to three billion additional pounds are grown for export markets worldwide (National Cotton Council 2001a; USDA-FAS 2001a).

In 2001, 19.6 million metric tons (mmt) of cotton lint were produced worldwide. In addition, the co-products of cotton lint – cottonseed meal and cottonseed oil – amounted to 13.7 mmt and 4.2 mmt, respectively. Cottonseed meal is used in livestock feed and cottonseed oil is used predominantly as cooking oil for human consumption. Cotton lint is by far the most important cotton product, with an annual value of over $20 billion on a worldwide basis. Cotton lint is used predominantly in textile production. The contributions of cotton co-products were notable: the value of cottonseed meal in 2001 was approximately $1.6 billion and the value of oil was approximately $2.8 billion (UNFAO 2001).

Cotton requires high levels of production inputs. Production cost estimates for major U.S. field crops show that cotton is second only to rice in per acre costs of production (USDA-ERS 2001). Cotton costs are in the range of $520 per acre compared with $380 per acre corn, and $260 per acre soybean. The major differences include costs of chemicals, for which cotton costs of approximately $60 per acre are more than double those of corn or soybean. Cotton also is a heavy user of nitrogen fertilizers. Cotton fertilizer costs are approximately $30 per acre, compared with $40 per acre for corn and less than $10 per acre for soybean. Only rice requires heavier fertilizer application than corn and cotton. The same pattern of costs is observed worldwide. Cotton is grown in warm climates where insect pests thrive, and chemical pesticides are the predominant control measures in developing and developed nations. Therefore, public health and environmental issues pertaining to chemical pest control in cotton are not restricted to high-input production systems in developed countries. In fact, because of a less sophisticated approach to pesticide handling, pre-existing health challenges in potentially exposed populations, and underdeveloped regulatory systems in developing nations, the negative impacts of chemical use are more prevalent in developing countries (WHO 2001).

WORLD COTTON INDUSTRY

Although no starting date of domestication is given, cotton was considered a major crop in four parts of the Ancient World. These ancient cotton types and the regions in which they were grown are *Gossypium hirsutum* grown in Mesoamerica; *Gossypium barbadense* grown in the Andes and Amazonia; *Gossypium herbaceum* grown in West Africa and the Sahel; and *Gossypium arboreum* grown in India (Diamond 1997). These four types or species of the genus *Gossypium* have been domesticated and are now commonly known as cotton. They are the only cultivated cotton species grown primarily for their seed hairs also referred to as lint, which are used in textiles (Fryxell 1984; USDA-APHIS 1996). Though the seeds of all *Gossypium* species have epidermal hairs, most of the wild species of cotton are lintless (Fryxell 1979). The wild species have only one type of hair, but the seed hairs of the cultivated species are differentiated into two types, namely, fuzz and lint (Fryxell 1963). Thus, although there are approximately 39 species of *Gossypium* worldwide, only the four types with seed hairs are cultivated (Fryxell 1984; Museums Online South Africa 2001).

The four cultivated species have origins of domestication throughout the world. *G. herbaceum* is native to sub-Saharan Africa and Arabia where it grows in semi-desert and savanna areas as a perennial shrub. This species was domesticated probably in Ethiopia and southern Arabia, with cultivation spreading to Persia, Afghanistan, Turkey, North Africa, Spain, Ukraine, Turkestan, and China. It was domesticated to produce cultivars that grew as annuals. *G. arboreum*, a Pakistani-Indian cotton, native to Northwest India and Pakistan, was used in the production of cotton textiles. Some cultivars of this line are tall perennial shrubs and others are short annuals. *G. barbadense*, South American cotton, was probably widespread on both coasts of South America nearly 5000 years ago. Wild species of South American cotton are now only found in Ecuador. Domestication is believed to have begun around 2500 B.C., because cotton bolls found in archaeological sites dated to that time show characteristics intermediate between wild and modern domestic forms. *Maya*, *Aztec*, and smaller tribes used *G. hirustum*, a Mexican cotton, for textiles. When moved into southern North America, cultivars were developed to produce annual forms capable of growing in long summer days outside the tropics. Evidence in Arizona shows that cotton was grown in that area by 100A.D. (Museums Online South Africa 2001).

The genetics of cotton consists of at least seven genomes found in the genus, designated A,B,C,D,E,F, and G (Endrizzi 1984). The A genome is restricted in diploids to two species *G. arboreum* and *G. herbaceum* in the Old World, and the D genome is restricted in diploids to some species of the New World such as *G. thurberi*. The most important cottons, however, are *G. hirsutum* and *G. barbadense*, which are both allotetraploids[1] of New World origin of possible cross between Old World A genomes and

New World D genomes. *G. tomentosum*, native of Hawaii, which in its wild form had been an invasive species growing near the ocean, has been crossed with *G. hirsutum* in breeding programs and is considered a New World allotetraploid. Cultivated cottons were developed from these New World allotetraploids (Fryxell 1979).

G. barbadense cotton appeared in the United States about 1790 from the Bahamas and is known as the Sea Island type (McGowan 1961). Sea Island cotton is of the species *G. vitifolium*. (Max Planck Institut für Züchtungsforschung 2001). Known today as *G. barbadense,* Sea Island cotton was originally a short-fibered type; however selections over 4000 years of domestication produced the long-fibered domesticated strain. Fiber quality and length surpass the original form.

COTTON IMPROVEMENT FOR PEST MANAGEMENT

Insect Pest Management

Principal insect pests are those that attack the bolls or the flower buds and thereby have impact on fiber yields. Other pests attack the leaves, stems, and the sown seeds (Gianessi and Carpenter 1999). Bohmfalk et al. (1996) distinguish two groups of insect pests of cotton based on different criteria. These are key insect pests and occasional pests. Key insects are those that are serious, perennially occurring, persistent pests that dominate control practices because in the absence of their control severe economic damage will ensue. For the eastern part of the Cotton Belt, these are the boll weevil (*Anthonomous grandis grandis* Boheman), bollworm (*Helicoverpa zea* [Boddie]) and tobacco budworm (*Heliothis virescens* [Fabricius]). The second group is considered occasional pests whose populations become economically damaging only infrequently. These are the beet armyworm (*Spodoptera exigua* [Hubner]), cotton fleahopper (*Pseudatomoscelis seriatus*), brown cotton leafworm (*Acontia dacia* [Drucel]), cabbage looper (*Trichoplusia ni* [Hubner], cotton aphid (*Aphis gossypii* Glover), cotton leafworm (*Alabama argillacea* [Hubner]), cotton leafperforator (*Bucculatrix thurberiella* Busck), cotton square borer (*Strymon melinus* [Hubner]), cotton stainer (*Dysdercus suterellus* [Herrick-Schaffer]), cutworms (*Agrotic* ssp.), fall armyworm (*Spodoptera frugiperda* [J.E. Smith], tarnished plant bug (*Lygus lineolaris* [P. de Beav.]), omnivorous leafroller (*Platynota stultana* [Walsingham]), pink bollworm (*Pectinophora gossypiella* [Saunders]), saltmarsh caterpillar (*Estigmene acrea* [Drury]), spider mites (*Tetranychus* spp.), stink bugs (*Acrosternum hilare* [Say] and *Chlorochroa ligata* [Say]), thrips (*Thrips* spp. and *Frankliniella* spp.), whiteflies (*Bemesia tabaci* [Gennadius]), and Yellow-striped armyworm (*Spodoptera ornithogalli* [Guenee]). Boll weevil eradication and the introduction of biotechnology-derived cotton have had major impacts on the ranking of importance of traditional cotton insect pests.

Pest problems vary from region to region. For example, in several states across the Cotton Belt the boll weevil has been eradicated, whereas in other states, such as California, boll weevil was never established. Western farmers face different pest problems than farmers in other regions. The bollworm and tobacco budworm are not severe problems in California or Arizona, but farmers in Arizona, New Mexico, the Imperial Valley of California, and the Rio Grande Valley in Texas are subject to infestations from pink bollworm, which is not found in other producing areas. Plant bugs and cotton fleahoppers cause more economic damage in Arizona and California than in other areas (Williams 1999). For an in-depth discussion of the biology, life cycle, infestation evidence, and nature of damage of cotton insect pests see Bohmfalk et al. (1996).

Beneficial insects or native biological control agents are important in controlling insect pests of cotton, and these predators and parasitoids provide an enormous economic benefit to cotton farmers (Bohmfalk et al. 1996). They naturally enter cotton fields, multiply, and provide economic benefit through the consumption of insect pests. There are over 600 beneficial insects and other predator species recorded in cotton fields (Bohmfalk et al. 1996). Among the most important are minute pirate bug (*Orius* spp.), bigeyed bug (*Geocoris* spp.), ant (*Solenopsis* spp.), lady beetle (*Hippodamia* spp.), cotton fleahopper (*Pseudatomoscelis seriatus* [Reuter]), green lacewing (*Chrysopa* spp.), damsel bug (*Nabis* spp.), Assassin bug (*Zelus* and *Sinea* spp.), and the spiders striped lynx (*Oxyopes salticus* [Hentz]), celer crab spider

(*Misumenops celer* [Hentz]), winter spider (*Chiracanthium inclusum* [Hentz]), star-bellied orb weaver (*Acanthepeira stellata* [Walckenaer]), grey dotted spider (*Aysha gracilis* [Hentz]), black and white jumping spider (*Phidippus audax* [Hentz]), long jawed orb weaver (*Tetragnatha laboriosa* [Hentz]), and ridgefaced crab spider (*Misumenoides formosipes* [Walckenaer]). It is interesting to note that the cotton fleahopper was listed as a cotton insect pest because its feeding causes small squares to be shed. However, it is a predator on bollworm and tobacco budworm eggs because it feeds on about one bollworm or budworm egg per day.

It is due to the economic losses and the increasing costs of pest control methods, both explicit and implicit, combined with the research and development of plant genetic transformation technology with economic incentives, that pest-resistant plants have been developed (Benedict and Altman 2001; Edge et al. 2001). Modern biotechnology has redefined pest management. With the progress of biotechnology research, pest-resistant cotton varieties have been developed that enable cotton producers to use biotechnology-derived plant-incorporated protectants as part of their integrated pest management systems. This development has allowed farmers to decrease negative environmental impacts significantly, increase profitability, and enhance their quality of life (Edge et al. 2001).

Bollgard I®/Ingard®

Bollgard® cotton (trademark Monsanto Company), also known and sold as Ingard® cotton in Australia (Fitt 2000), was developed through a strategic alliance between Monsanto and the dominant U.S. cotton seed firm Delta and Pineland (D&PL) (Falck-Zepeda, Traxler, and Nelson 2000). It became widely commercialized in 1996 in the United States with an estimated 1.8 million a. (0.7 million ha) planted, thus comprising 14% of U.S. cotton acreage (Adamczky et al. 2001; Cohen 1999; Falck-Zepeda, Traxler, and Nelson 2000; Gianessi and Carpenter 1999; James and Krattiger 1996). The original goal was to provide cotton farmers an economic, environmentally friendly, and efficacious means to control specific cotton insect pests (Sciumbato and Hurst 2001).

Bollgard varieties containing the *Cry*1Ac delta-endotoxin have provided a wide range of control of lepidopteran pests. Bollgard cotton has provided excellent control of tobacco budworm and pink bollworm with good control of bollworm in the United States (Liu et al 2001; Ridge, Turnipseed, and Sullivan 2000). Bollgard cotton has provided only fair to poor control of the secondary lepidopteran pests including soybean looper, fall armyworm, and beet armyworm (Adamczyk et al. 2001; Ridge, Turnipseed, and Sullivan 2000).

The germplasm for the first biotechnology-derived cotton varieties was provided by D&PL: DP5415, a mid-season variety, and DP5690, a full-season variety. These two varieties were popular in the mid-South and Southeast (Falck-Zepeda, Traxler, and Nelson 2000). The Bollgard gene was developed by Monsanto from a soil microorganism, *Bacillus thuringiensis* Kurstaki produces a protein that is toxic to several lepidopteran species when ingested. Target pests were the bollworm, pink bollworm, and tobacco budworm (ICAC Recorder 2000b). Initially, *Bt* cotton was developed from a now obsolete variety known as Coker 312. Later, Monsanto chose D&PL as its seed partner to develop elite parent lines with improved high-yielding characteristics and back-crossed to replace the traits of the Coker line (Falck-Zepeda, Traxler, and Nelson 2000).

Cry1Ac

Many private and public sector biotechnology laboratories worldwide have screened tens of thousands of protein samples against cotton insects since biotechnology research on cotton began in the 1980s (ICAC Recorder 2000b). The largest source of protein mixtures came from the fermentation of *Bt* and non-*Bt* microbes. Numerous proteins (delta-endotoxins) from *Bt* proved to be effective against bollworm, pink bollworm, and tobacco budworm, but *Cry*1Ac was found to be better than the others. The *Cry* proteins are toxic to certain larval Lepidoptera, Coleoptera, and Diptera, making them economically valuable pest control agents (Crickmore et al. 1998; Hofte and Whitely 1998, 1999, 2000; Perlak et al. 1990). Xia and Guo (2001) further report the stability of *Bt* genes in biotechnology-derived cotton developed in China and the United States. For diversity and classification of crystal proteins see Hofte and Whiteley (1989) and Krattiger (1997). *Bt* toxins were classified into 14 distinct groups and there are now at least 34 classes based on host range. The most widely studied and used for commercial insecticidal products include the classes *Cry*I, which are active against Lepidoptera; *Cry*II active against Lepidoptera and Diptera; *Cry*III active against Coleoptera; and *Cry*IV active against Diptera. It is worth noting that "*Cry*" stands for "crystalline" reflecting the appearance of the delta-endotoxin (Krattiger 1997).

The crystal and spore preparations of *Bt* ssp. *Kurstaki* such as Dipel and Thurcide are effective against approximately 55 Lepidopteran pest species and have been used as commercial insecticides for many years. At least 11 distinct genes encoding Lepidopteran-specific proteins were cloned and four expressed at insecticidal levels in biotechnology-derived plants by 1989 (Macintosh et al. 1990a). By 1998 at least ten genes encoding *Bt* toxins had been introduced into plants. These proteins are *Cry*1Aa, *Cry*1Ab, *Cry*1Ac, *Cry*1Ba, *Cry*1Ca, *Cry*1H, *Cry*2Aa, *Cry*3A, *Cry*6A, and *Cry*9c (Schuler et al. 1998). Researchers have demonstrated the ability to use modern biotechnology methods to introduce the *Cry*1Ac protein into a number of crops (i.e., apple, broccoli, cabbage, grape, oilseed rape, peanut, rice, soybean, tobacco, tomato, and walnut) which have not been developed for commercialization (Schuler et al. 1998).

Cry1Ac Protein as a Plant-incorporated Protectant

The *Cry*1Ac as expressed in biotechnology-derived cotton (Bollgard , technology) was developed to control lepidopteran pests such as the pink bollworm, bollworm, and tobacco budworm (Edge et al. 2001; ICAC Recorder 2000b; Perlack et al. 1990). The mode of action of the *Cry* proteins, in particular the *Cry*1Ac protein, is described as a multi-component process. All the *Bt* insecticidal proteins are toxic only after ingestion by the susceptible insect and must be solubilized in the insect gut, then cleaved by proteases to yield an active fragment (Cohen 1999; English and Slatin 1992). However, according to Cohen (1999), toxins produced in plants are already solubilized, and therefore only the DNA coding for the active fragment is used in plant transformation. For a comprehensive review of the mode of action of *Bt* with other bacterial toxins see English and Slatin (1992), and of the specificity and efficacy of *Bt* proteins see Macintosh et al. (1990b).

Moore (2001) summarizes studies of the efficacy of Bollgard cotton, indicating that *Bt* cotton control on tobacco budworm is 95%, bollworm pre-bloom is 90%, bollworm blooming is 70%, pink bollworm is 99%, cabbage looper is 95%, beet armyworm is 25%, fall armyworm is 20% or less, saltmarsh caterpillar is 85% or more, cotton leaf perforator is 85% or more, and the European Corn Borer (ECB) is 85% or more. By comparison, Gould (1998) reports that tests of *Bt* cotton cultivars indicated that the *Cry*1Ac provided 100% mortality of tobacco budworm, 75-90% mortality of susceptible bollworm relative to non-biotechnology-derived cultivars, and that the *Bt* toxin level in commercial cotton cultivars acted as a high-dose for the pink bollworm. Gore et al. (2001) conducted an experimental study in Louisiana to determine if differences in bollworm larval behavior occur in Bollgard cotton plants compared with non-Bollgard plants. They report that Bollgard provides satisfactory control against low to moderate bollworm densities, but that insecticide applications are needed to prevent economic injury when high population densities exist for several days. Furthermore, Gore et al. (2001) indicate that Bollgard cotton provides excellent control of the tobacco budworm and pink bollworm. The data from the study suggest that current scouting protocols and action levels to initiate insecticide treatments for bollworms on non-Bollgard cotton are not appropriate for Bollgard cotton. The importance of this experiment is to further refine action thresholds for bollworms on Bollgard cotton.

Bt cotton (biotechnology-derived cotton containing the gene encoding for the *Bt* protein) has demonstrated its effectiveness against feeding damage by major cotton pests. After ten years of development and two years in commercialization, *Bt* cotton provided effective control and decreased insecticide usage (Peferoen 1997). Gianessi and Carpenter (1999) report that decreased insecticide use occurred for nine insecticides, which primarily targeted bollworm and budworm, whereas use of two insecticides increased after the introduction of *Bt* cotton. They caution that the decrease of insecticide treatments and amount of insecticide used for tobacco budworm, bollworm, and pink bollworm may be attributed to other things besides the adoption of *Bt* cotton. Additionally, Mahaffey et al. (1994); Greene and Turnipseed (1996); Turnipseed and Greene (1996); Bacheler and Mott (1996); Mahaffey et al. (1995); Roof, DuRant, and Walker (1997); and Bacheler, Mott, and Morrison

(1997) report efficacy of Bollgard cotton on various lepidopteran pests and the importance of other insect pests requiring pesticides for control. These studies demonstrate that *Bt* cotton provides effective control against the principal cotton insect pests for which it is marketed; however, other pests, stink bugs in particular, may require chemical control. Adamczyk et al. (2000) determine the susceptibility of fall armyworm to *Bt* cotton, noting that the host plant of the armyworm significantly affects susceptibility to *Bt* cotton.

Bollgard II®

Bollgard II is the second generation of insect control for cotton developed by Monsanto. It contains an additional *Bt* gene (*Cry*2Ab) that increases Bollgard's insect control spectrum to include high level control of beet armyworm, fall armyworm, and cabbage and soybean loopers. The second generation Monsanto genotypes, designated Bollgard II, produce two proteins that are active against bollworm (Jackson et al. 2001). Bollgard II was produced by particle bombardment to add the *Cry*2Ab gene to DP50B that previously had *Cry*1Ac inserted using modern biotechnology methods (Rahn et al. 2001). The two-gene *Cry*1Ac and *Cry*2Ab endotoxin line has demonstrated a ten-fold improved efficacy over the single-gene lines against pink bollworm large larvae infestations (Marchosky et al. 2001). As of this writing, registration of Bollgard II is pending and thus Bollgard II is not yet commercialized. In field and greenhouse trials in North Carolina the Bollgard II genotypes significantly decreased numbers of susceptible and *Cry*1Ac-tolerant bollworm larvae below that of the Bollgard cottons (Jackson et al. 2000, 2001). These results were obtained from field and greenhouse trials evaluating the efficacy of Bollgard and Bollgard II cottons and the traditional sister line by measuring bollworm numbers, fruit damage, and yield under traditional chemical treated and untreated systems.

Additionally, the two genes reduce the likelihood of insect resistance developing and thus provide better insect resistance management (Gould 1998; Rahn et al. 2001; Rouch et al. 1998). The combination of these two genes improves insect control efficacy and increases the spectrum of control. Bollgard I with the *Cry*1Ac gene has provided excellent control of tobacco budworm and pink bollworm; however, it provides only good control of bollworm and fair to poor control of other pests. With fair to poor control, use of *Cry*1Ac must be supplemented with chemical insecticides to provide adequate control of bollworm and other pests (Ridge, Turnipseed, and Sullivan 2000; Stone and Sims 1993). The *Cry*2Ab gene improves the spectrum of insect control to include the fall armyworm, beet armyworm, cabbage looper, and the soybean looper (Voth et al. 2001). North Carolina field trials verified that Bollgard I and Bollgard II cottons could sustain significant fruit damage and yield losses making it necessary for additional insecticide sprays for bollworm control (Burd, Bradley, and van Duyn 1999; Jackson et al. 2001; Lambert, Bradley, and van Duyn 1996, 1997; Mahaffey et al. 1994, 1995). Jackson et al. (2001) and Greenplate (1999) suggest that this was caused by less than optimal levels of *Cry*1Ac endotoxin over the course of a season. The bollworm population receives a sublethal dose of *Cry*1Ac and therefore additional control by spraying is warranted. These data confirm that Bollgard I produces only a moderate dose for control of bollworm, thus creating a need for further technological developments. Catchot (2001), Norman and Sparks (2001), Jost (2001), Rahn et al. (2001), Pitts (2001), Jackson et al. (2001), Ridge, Turnipseed, and Sullivan (2001), Penn et al. (2001), Lorenz et al. (2001), and Marsh, Tiner, and Weybright (2001) report results of insect control efficacy field trials of Bollgard II. Results of these studies demonstrate that Bollgard II provides better control of lepidopteran pests than Bollgard. Voth (2001) suggests that Bollgard I will likely be phased out of commercial distribution when Bollgard II becomes available.

Disease Management

Diseases have always been a problem wherever cotton is grown. The major diseases of cotton are seedling diseases (seed-rot, root-rot, and damping off), fusarium wilt, boll rots, leaf spots, and verticillium wilt. Seedling diseases are caused by *Rhizoctonia* (fungus), *Phythium* (fungus), *Thielaviopsis spp*. (fungus), and several other fungi and bacteria. Fusarium wilt is caused by *Fusarium oxysporum* and *F. vasinfectum* (fungus). Boll rots are caused by several fungi and bacteria. Leaf spots are caused by *Ascochyta* (fungus), *Cercospora* (fungus), *Alternaria spp* (fungus), and some bacteria. Verticillium wilt is caused by *Verticillium albo-atrum* (fungus) (Norman 2000).

Controlling these diseases requires fungicide treatments for seedling diseases and leaf spots; reducing nematode population with nematicides (fusarium wilt); avoiding excessive nitrogen rates (boll rots); use of plant-resistant varieties (fusarium wilt, verticillium wilt, leaf spots); insecticides to decrease insects that damage bolls (boll rots); and practicing overall good farming practices (Norman 2000). Feng et al. (2001) report a study to determine the effects of a fusarium resistance gene on agronomic traits of cotton. Results indicated that the resistance gene Fw2 has no deleterious impact on agronomic traits and fiber quality. Nelson et al. (1998) discuss cotton virus diseases and varieties resistant to leaf curl available in Pakistan. Although leaf curl is a problem sporadically in the western hemisphere, it does not result in an economically significant level of damage. However, in Pakistan leaf curl has been a severe problem resulting in economically significant damage warranting management efforts since 1991.

Control of plant diseases has not been a focus of biotechnological development in cotton. However, there is evidence associating herbicide use and development of plant pathogen activity (Berner, Berggren, and Snow 1991; Huang 1993; Kawate et al. 1997). Therefore, impacts of plant diseases must also be a factor in the management of biotechnology-derived cropping systems.

MULTIPLE ACTION BIOTECHNOLOGY-DERIVED COTTON

Inserting multiple genes that trigger the plant to make proteins with different modes of pesticidal action in one cell enables the plant to express more than one desirable trait as a result of biotechnology (Europa 2001). These can be input traits or output traits. Currently, the most common cotton product expressing multiple traits (also referred to as stacked traits) is both herbicide-tolerant and resistant to insect damage. Because stacked genes may improve crop quality and productivity, Penn (2000) suggests that it is likely that more crops with stacked traits will be appearing in the marketplace in the near future. Cotton stacked with both Bollgard insect resistance and Roundup Ready® herbicide tolerance has been available commercially in the United States since 1997.

In the two types of stacked trait cotton commercially available, the herbicide tolerance comes from one of two types of herbicide-tolerant cotton varieties: one with tolerance to bromoxynil and one with tolerance to glyphosate. And the insect resistance trait in both stacked trait cotton varieties comes from *Bacillus thuringiensis* (*Bt*). Ethredge, Nunnery, and Briggs (2001) have begun field studies of the stacked varieties in Southwest Georgia. Early results of their studies show that the new, stacked varieties produce good results. More information for application to local conditions is needed to guide farmers in the use of these new multi-trait varieties.

OTHER VARIETIES AND TRAITS

Most biotechnology-derived varieties developed and commercialized to date have been modified with genes for herbicide or insect resistance. However, there have been numerous field trials that have included more agronomic traits such as enhanced growth rate and increased yields (Dunwell 2000). The improvement of genetic understanding of various characters has improved significantly. There have been no biotechnology-derived cotton varieties with improvements in the percentage of oil content. Instead, the focus has been on improvements in fiber quality and yield (ICAC Recorder 2000b). Biotechnology-derived gossypol-free cotton has been developed and commercialized. This is advantageous because gossypol is a toxic pigment found in parts of the cotton plant, especially on the cotyledons, and represents about half the intra-glandular pigments in cotton. In excessive quantities, it is toxic to animals other than cud-chewing animals, thus limiting the use of cottonseed.

Seed company scientists report that fiber quality characteristics are developed using a backcrossing procedure with the recurrent parent for new Roundup Ready® varieties with disease tolerance and better yield performance (Albers, Kerby, and Lege 2001; Albers, McGowen, and Williams 2001). McCall and Robinson (2001), Barfield (2001), and Calhoun (2001) describe new Roundup Ready® varieties backcrossed into recurrent parent traditional varieties. The new varieties were released by Stoneville Pedigreed Seed Company in 2001. They all perform as well or better than traditional varieties.

DEVELOPMENTS IN TRADITIONAL VARIETIES

Biotechnology-derived commercialized cotton varieties are derived through backcrosses to traditional germplasm. This, plus the continued demand for traditional varieties by farmers, underpins the development of traditional varieties (Lege, Leske, and Burdett 2001). Cotton varieties are being developed that typically are concerned with improved fiber quality and better yields. These characteristics are integrated into seasonal varieties to provide for the opportunity to have longer growing seasons. For instance, Delta & Pine Land has developed two new mid-full season picker varieties giving the opportunity to provide better yields by taking advantage of full-season weather (Lege, Leske, and Burdett. 2001). These varieties provide farmers with effective tools to take advantage of full-season environments and minimize risks associated with weather and other environmental conditions.

Production objectives for all cottons include increasing fiber production (yield), early ripening, ease of mechanical harvesting, and improvements in pest resistance. Other breeding objectives include longer fiber length, fineness, elasticity to enhance the quality of the fiber, and increased oil content and decreased gossypol content in the seeds to improve the characteristics of cotton co-products (Max Planck Institut für Züchtungsforschung 2001).

Regional Variations

Regional variations in both traditional and biotechnology-derived cotton varieties are produced primarily due to weather and other growing conditions. These regional differences also determine the degree to which insects and weeds are problematic and which control methods are economically viable.

There are three principal groups of cotton of commercial importance (Cotton Incorporated 2001). *Gossypium hirsutum* is native to Mexico and Central America and developed extensively in the U.S., accounting for more than 95% of U.S. production. This is also known as upland cotton and varies in fiber length from 7/8 to 1 5/16 inches. *Gossypium barbadense* constitutes the balance of United States production and is of South American origin. Its fiber varies in length from 1 1/4 to 1 9/16 inches. It is known in the United States as Pima cotton, but is also referred to as Extra Long Staple (ELS) and Egyptian cotton. The last group is composed of both *Gossypium herbaceum* and *Gossypium arboreum*. It has a shorter fiber length of 1/2 to 1 inch and is native to India and East Asia (Cotton Incorporated 2001).

Regional variations are best described by maturity groups. There are three maturity groups used to classify cotton: 1) short season or more determinate plants, 2) medium season varieties, and 3) long or full season varieties, which are more indeterminate in nature. However, according to Silvertooth (1998), classification of cotton varieties into these categories is not always straightforward. Mid-season and full season varieties are popular in the midsouth and southeast. Pima cotton is popular in the Pima Belt (California, Arizona, New Mexico, and Texas). Two new types have been developed for the San Joaquin Valley and Pima Belt (Olvey et al. 2001).

A cold-tolerant Upland cotton was tested for early planting (Green and Roberts 2001). Other examples of short season varieties are DPL 20 and Suregrow 125. Mid-season varieties are DPL 50, DPL 51, Stoneville 453, DPL 5409, and Stoneville 474. Full season varieties include DPL 77, Pima S-7, DPL 90, DPL 5816, and Pima S-6 (James 2000; Mississippi State Extension Service 1998). For information on cotton variety trends see Cotton Council International (2000) and Mississippi State Extension Service (1998). Texas Agricultural Extension Service (1998) is a good source of background information describing the characteristics of different cotton varieties and varieties used in breeding including modern biotechnology.

India grows four species of cultivated cotton — *Gossypium hirsutum*, *Gossypium barbadense*, *Gossypium arboreum*, and *Gossypium herbaceum* — plus hybrids between them (Ecottonindia 2001). They estimate that over 80 varieties are grown over a large area with diverse agro-climatic conditions and farming practices, but there are efforts to decrease the number of hybrids. Twenty-six of the major hybrid varieties account for approximately 80% of the total production of cotton in India.

Until 1986, Australian farmers grew varieties of cotton predominantly developed for optimal growing performance in the United States (CSIRO 2001a). These varieties were susceptible to a number of pests and diseases and had low fiber strength (CSIRO 2001b). These deficiencies led Australia to develop its own breeding program and older varieties have been replaced by upland cotton varieties bred by the Commonwealth Scientific and Industrial Research Organization (CSIRO). These new varieties are better adapted to local conditions in Australia (CSIRO 2001a, b) and are grown in Northern New South Wales and Southern Queensland, on approximately 553,000 ha in the 1998-1999 season. Fifteen percent of the 1998-1999 Australian cotton crop is biotechnology-derived cotton (Adelaide University 2000). Advantages of the CSIRO varieties include 6-25 % higher yield, up to 30% longer fiber length, and up to 30% stronger fiber (CSIRO 2001b). Other developments by CSIRO, using traditional plant breeding techniques, include the creation of Okra leaf cotton varieties, which provide better tolerance to pests such as mites and *Heliothis*. These varieties also produce high yields and high seedling vigor. To address bacterial blight in cotton, CSIRO has developed disease-free cotton through breeding to rid the cotton plant of disease causing lesions on plant leaves as well as rotting cotton bolls. There is also a verticillium-resistant type available, and others from combinations of the above (CSIRO 2001b). In the future CSIRO envisions applications of modern biotechnology methods to further improve the varieties they have developed using traditional breeding.

For a comprehensive breakdown of cotton varieties planted, both biotechnology-derived and traditional, in the cotton producing states of the United States see National Cotton Council (2001).

Fiber Characteristics

Upland fiber characteristics — length, length uniformity, strength, micronaire[2], color, and trash — vary with environment and variety. Fiber length is the average length of the upper half mean length measured in 100ths and 32nds of an inch. Length uniformity is the ratio between the mean length and the upper half mean length of the fibers expressed in percentages. Fiber strength is measured in grams per tex. A tex is a unit equal to the weight in grams of 1000 meters of fiber. Strength is the force in grams necessary to break a bundle of fibers of one tex unit size. This measurement is accomplished by determining the air permeability of a constant mass of compressed cotton fibers in fixed volume. Fiber color is determined by quantifying the degree of reflectance and yellowness. Reflectance indicates the brightness or dullness of a sample. Yellowishness indicates the degree of pigmentation. The color measurements are done using a Nickerson-Hunter cotton colorimeter diagram for Upland cotton. Trash measurements quantify the amount of non-lint material, such as leaf and bark from the cotton plant. Trash particles are calculated by using a video camera, which scans the surface of a cotton sample and calculates, in percentage, the amount of surface area covered with trash (Cotton Incorporated 2001). The classification of Pima cotton is similar to upland cotton except that Pima cotton classification uses a different color standard. Pima cotton fiber naturally has a deeper yellow color than upland cotton fiber.

ENVIRONMENTAL IMPACTS OF BIOTECHNOLOGY-DERIVED AND TRADITIONAL COTTON

Changes in Pesticide Use

Insect Pests and Pest Control Methods

In the late 1800s, bollworm (*Helicoverpa zea*) was the chief insect pest of U.S. cotton. As cotton production moved into Texas in the 1890s, the boll weevil became an important pest of cotton. More recently, the tobacco budworm (*Heliothis virescens*) has became a major pest. Historically, high budworm pressure areas have been Alabama, Mississippi, Arkansas, Louisiana, South Georgia, and the Florida panhandle. The first reports of pink bollworm in North America were in Mexico. This pest was believed to have arrived by cottonseed shipments from Egypt. In 1917, the pink bollworm was identified in Texas and was probably imported from Mexico in cottonseed shipments. Within ten years, pink bollworm had spread throughout Texas, New Mexico, and eastern Arizona cotton-producing areas. From 1934 to 1946, Arizona declared bollworm eradicated; however, by 1965 it had moved through Arizona into Southern California. Pink bollworm has not established economically damaging populations in the midsouth and southeastern regions of the United States, even though there have been outbreaks in Florida, Georgia, Louisiana, Mississippi, Arkansas and Missouri.

The major insect pests of cotton in China are bollworm and the cotton aphid. In Pakistan the major pests are whiteflies and leafhoppers (*Amrasca tabaci*). In Brazil the boll weevil and the tobacco budworm are the most important pests (ICAC 1995).

Outbreaks of pests including insects, weeds, and plant pathogens occurred during the Greek and Roman classical era (NRC 2000). These pests were controlled in various ways. Ancient Romans killed insect pests by burning sulfur and controlled weeds with salt. In the 1600s, ants were controlled with mixtures of honey and arsenic (Delaplane 1996). Control methods changed dramatically by the nineteenth century when farmers began using copper acetoarsenite, calcium arsenate, nicotine sulfate, and sulfur to control insets pests in field crops. However, the results were often ineffective and unsatisfactory because of the primitive chemistry and application methods.

In the early 1900s, cotton insect pests were controlled primarily through cultural and physical methods. After 1890, the boll weevil became the primary pest of concern, and the insecticides available at the time—Paris green and lead arsenate—did not provide effective control. Early maturing or shorter season cotton varieties were grown instead of the longer season cottons to limit late-season boll weevil attacks. Combining the early fall destruction of the harvested cotton plants and the sowing of shorter season, early maturing cottons provided an economic production system with minimal insecticide use. The tradeoff with this system was an inferior cotton fiber length as compared with the longer season varieties. In 1918, calcium arsenate was found to be effective against the boll weevil, thus enhancing insect control. This method eventually was developed for aerial application and continued to be the method of choice through World War II. Some farmers using calcium arsenate, however, reported poor results, so they began scouting for insects in order to properly time treatments in their efforts to improve treatment results.

This method of control — scouting combined with calcium arsenate treatments —continued until organochlorine insecticides were introduced after World War II. During the 1940s and 1950s, effective organochlorine insecticides that controlled the serious cotton insect pests became commercially available. The organic insecticides (organochlorines) proved more effective than the inorganic insecticides (calcium arsenate) and higher yields resulted from their use. Improved yields fostered an increase in acreage treated with organochlorine compounds. These compounds were known as DDT, benzene hexachloride, toxaphene, chlordane, and methoxychlor. Average yields were approximately 251 pounds per acre before the widespread use of organochlorine insecticides. However, after extensive use of organochlorine compounds, including DDT and other organic insecticides, average yields increased to approximately 300 pounds per acre. These new insecticides allowed for longer growing seasons with longer season cotton varieties having higher quality, longer staple lint, and greater yield. (Gianessi and Carpenter 1999).

Insect resistance developed to organochlorine insecticides over time. This led to the need and greater demand for organophosphate and carbamate insecticides. These were effective against the boll weevil, but relatively ineffective against the budworm/bollworm. Organophosphate and carbamate insecticides were used to overcome the insect resistance (boll weevil resistance) to organochlorine insecticides. The problems associated with organophosphate and carbamate insecticide use were destruction of beneficial insects, natural parasites, and predators of bollworm/budworm. As organophosphate use increased, the budworm/bollworm complex became more economically important, leading to the development of more insecticides to control budworm/bollworm outbreaks. The insecticides developed at this stage were synthetic pyrethroids. They were commercially introduced in the 1970s. Pyrethroid insecticides are modeled after pyrethrins (natural, plant-derived insecticides), which have been used for hundreds of years (Delaplane 1996). At the time of their development, synthetic pyrethroids were considered the best method for economic control of the budworm/bollworm complex (Gianessi and Carpenter 1999). However, as with previous insecticides, insect resistance developed. Nevertheless, improved control of budworm/bollworm by pyrethroids permitted cotton production to expand.

Most of the bollworm/tobacco budworm insecticides are targeted at controlling larvae (larvicides). Amitraz, methomyl, and thiodicarb target the egg stage (ovicides) of the pest. Several commonly used insecticides for bollworm/budworm are pyrethroids; for example, bifenthrin, cyfluthrin, cypermethrin, esfenvalerate, lambda-cyhalothrin, and tralomethrin. These are preferred because they are cheaper and require less active ingredient per acre than other insecticides and are safer than the organophosphates. Their use has diminished over time due to pyrethroid resistance in budworm populations. This resistance has been documented in Louisiana, Arkansas, Alabama, Texas, and Mississippi. Also, resistance has been reported in North Carolina (Bacheler 1999). Insecticide resistance in bollworm populations has shown up as well, although not to the same extent as in budworm. As resistance to available insecticides develops, new alternative insect control methods are sought. Traditional insecticides will likely continue to be used in integrated pest management (IPM) systems, but with the development of insect resistance to the older insecticides, new and novel approaches to control cotton pests are of interest (Gianessi and Carpenter 1999).

The pink bollworm established its presence in Texas in 1922, but did not become a serious cotton pest until the 1950s. Texas farmers used harvest-aid chemicals and mechanical tilling to effectively control the pink bollworm. However, in California and Arizona large quantities of organophosphate insecticides were used to control the pink bollworm thus causing these areas to have the highest per acre cost for cotton insect control by the 1960s (Carpenter and Gianessi 1999).

One new class of insecticides is the spinosyns derived by the fermentation of the metabolites of a species of Actinomycetes. Spinosad (tradename: Tracer®) is a recently commercialized insecticide in this group. It is effective against tobacco budworm and bollworm while being relatively safe to nontarget and beneficial insects. Chlorfenapyr (tradename: Pirate®) is developed from another class of insecticides, the pyrroles, which are developed from a strain of Streptomyces. To date, chlorfenapyr has not been registered for use in the United States due to questions regarding potential negative impacts on birds (USEPA 2000). Three other classes of insecticides have been identified for potential use in cotton production. Indoxacarb (tradename: Steward®) is an oxadiazine with good larvicidal activity and limited impact on beneficial insects. Methoxyfenozide (tradename: Intrepid®) is a diacylhydrazine – an insect growth regulator. Two other important insect control methods, biological controls, also provide potential benefits. These are sterile insect release programs and the use of natural insect pathogens (Freeman 1999).

The major chemical classes presently in use (organophosphates, carbamates, synthetic pyrethroids) are inexpensive and broad-spectrum. They are, however, significantly disruptive to most beneficial insects and they have significant environmental residue problems. New guidelines outlined in the Food Quality Protection Act of 1996 will decrease the availability of broad-spectrum pesticides, such as pyrethroids and organophosphates, (Alabama Cooperative Extension System 2001). The new generation of pesticides, which are more selective (i.e., targeted to specific pests), less disruptive to predators and the environment, and have modes of action capable of overcoming resistance problems, promotes IPM strategies with more positive social benefits (Fitt 2000). Increasingly, cotton farmers rely not only on newer classes of insecticides but also on new technologies such as plant-incorporated protectants (biotechnology-derived insect-resistant cotton) that provide alternatives to chemical pesticides.

Twenty-five percent of all pesticides used globally are applied to cotton crops. In 1999 cotton was the second most heavily pesticide treated crop in the United States, with approximately 81 million pounds of pesticides being applied on upland cotton (USDA-NASS 2001d). In 1994 more than 90% of total world cotton area was treated with one or more insecticide applications per season. (International Cotton Advisory Council 1995). The major insecticide groups used were organochlorines, organophosphates, pyrethroids, and carbamates.

Improper use of chemical pesticides is hazardous to human health and may affect biological diversity, as well as surface and groundwater quality. The full impact on human health is difficult to quantify, but symptoms of pesticide poisoning are widespread, especially in developing countries.

China is rapidly developing a biotechnology industry, currently second in size only to the United States and cotton is the primary biotechnology-derived crop plant in China. Evidence from China shows that while 22% of farmers of non-*Bt* cotton reported symptoms of pesticide poisoning, only 4.7% of *Bt* cotton farmers reported similar symptoms (Huang et al. 2002). China has produced at least 20 new biotechnology-derived cotton varieties containing the *Bt* gene. The Biotechnology Research Center of the China Academy of Agricultural Sciences (CAAS) has developed a new variety SGK 321 using modern biotechnology methods. The SGK321 contains two pesticidal genes – one that produces the familiar *Bt* toxin and another that produces a cowpea trypsin inhibitor (Pray et al. 2001). CAAS developed this two-gene variety because it is commonly believed that bollworm resistance to cotton varieties will develop slower with the two genes than with the one gene for *Bt* toxin.

Changes in Pesticide Use Patterns Related to Biotechnology-derived Cotton

As a result of the adoption of insect-resistant cotton, the number of insecticide applications and pounds of insecticide used per acres of cotton have decreased dramatically compared to the use of insecticides in cotton production prior to the introduction biotechnology-derived insect-protected varieties. Insecticides are used on 75% of total U.S. cotton acreage (Carpenter and Gianessi 2001; Peferoen 1997). Cotton herbicide application rates between 1994 and 1999 averaged 1.74lbs/a. with an average of 2.8 applications each year (Carpenter 2000; Carpenter and Gianessi 2001). Reductions in bollworm, tobacco budworm, and pink bollworm insecticide use in the United States after the introduction of *Bt* cotton were evaluated for the periods 1995 to 1998 and 1995 to 1999. The insecticides evaluated were amitraz, cyfluthrin, cypermethrin, deltamethrin, esfenvalerate, lambdacyhalothrin, methomyl, profenofos, spinosad, thiodicarb, tralomethrin, and zeta-cupermethrin. From 1995 to 1998 total use of these insecticides was estimated to be decreased by 2,008,000 pounds. The number of applications was estimated to be decreased by 8,738,000. For the study period of 1995 to 1999 insecticide use decreased by 2,715,000 pounds and the number of applications decreased by 15,142,000 (Carpenter 2000; Carpenter and Gianessi 2001). Hagerty et al. (2000) suggest that decreasing applications of broad-spectrum insecticides are due to early season applications being significantly decreased, thus protecting beneficial insect populations from disruption. The United States, China, Australia, Mexico, and Spain have achieved an overall reduction in broad-spectrum insecticide sprays (Bacheler et al. 1997; Benedict and Altman 2001; Edge et al. 2001; ICAC 2000a; Mullins and Mills 1999; Rejesus et al. 1997; Roof et al. 1997; Stark 1997; Yousefi 1999). The number of spray reductions ranges from 1 to 7.7 sprays per crop season. In China, *Bt* cotton decreased total insecticide use by 60 to 80%. Traditional cotton in China can require 15 to 20 sprays per growing season to control lepidopteran pests. The introduction of *Bt* cotton decreased the applications to control these pests by 90 to 100% (Edge et al. 2001). In Mexico, studies have indicated a reduction of insecticide use as well (Obando-Rodriguez et al. 1999). After the introduction of *Bt* cotton in Spain, the number of insecticidal sprays was decreased by five per season compared with traditional cotton (Edge et al. 2001; Novillo et al. 1999). A study conducted in Australia found that the average number of sprays for all pests was decreased by 40%, from 10.3 in traditional cotton to 6.2 in Ingard® cotton. For *Heliothis* the reduction in sprays was 47%, from 9.7 to 5.1 in Ingard® cotton (Pyke 2000). In some areas of Africa, farmers do not use fertilizer or herbicides due to the expense (Ismael et al. 2001). However, since insect pests are a major cause of cotton yield loss there was no alternative, before *Bt* cotton, but to apply insecticides. The common insecticides used are Monostem (monocrotophos), Cypermethrin (pyrethroid), Decis (pyrethroid), and Cruiser (thiamethoxam). Five to eight insecticide applications were made each season. Herbicide and insecticide use patterns by state are given in Gianessi and Silvers (2000) and a comparative study of 2000 insecticide sprays in *Bt* and traditional cotton in selected states is given in Oppenhuizen et al. (2001). A similar study for Louisiana is discussed by Leonard et al. (2001). For pesticide use patterns in other countries up to 1995 see International Cotton Advisory Council (1995).

Aggregate pesticide and biotechnology-derived crop sales for the United States are estimated in National Research Council (2000) for 1995 to 1997. In 1995 sales of pesticides in the United States were estimated at $30.2 billion and sales of biotechnology-derived crops estimated at $75 million. In 1996 total pesticides sales were $31.0 billion and total biotechnology-derived crop sales were $235 million. In 1997 total sales of pesticides were $30.9 billion and sales of biotechnology-derived crops were estimated at $650 million. Total pesticide use declined by 0.37% between 1996 and 1997 and total biotechnology-derived crop sales increased by 176.6%.

Weed pests in Cotton Farming

The International Cotton Advisory Council (1995) indicates that herbicides are used extensively only in Colombia, Greece, Israel, Spain, Syria, and the United States. Herbicides are least used in Togo and Uganda where less than one percent of cotton area is treated. Inexpensive labor and mechanical eradication are alternate methods of weed control used in these cotton-producing countries. However, the general trend in is away from mechanical eradication and toward the targeted use of herbicides for more economically efficient control of weeds and minimum damage to the cotton plant.

Cotton producers have continuously searched for innovative and more efficient weed control methods. Approaches to weed control include hand pulling; hoeing; cultivation with many types, shapes, and designs of plows; grazing with geese; burning; and application of herbicides. As innovative control methods evolve, populations of weed species shift and weed concern shifts as well. Johnsongrass was considered extremely problematic in the 1970s. Currently, Johnsongrass is managed well where it still exists. Nevertheless, weeds remain a concern to cotton producers. In Arkansas, Johnsongrass as a major pest has been replaced by morning glory and pigweed. Efforts to combat the constantly evolving weed problems have led to the development of biotechnology-derived cotton varieties tolerant to glyphosate and bromoxynil (Smith 1999).

Changes in Tillage Practices and Soil Impacts Related to the Adoption of Biotechnology-Derived Cotton

The advent of biotechnology-derived crops, in particular cotton, provides farmers with the ability to implement more flexible farming practices. Options for time management, weed management, and insect pest management are enhanced. For example, the use of biotechnology-derived crops is consistent with conservation tillage – the practice of planting directly over the previous season's crop residue instead of plowing and disking the field. This allows soil moisture conservation leading to decreased irrigation needs in some regions. Also, conservation tillage decreases soil erosion by 90 % (CTIC 1998), which decreases the movement of silt and sediment into rivers, lakes, coastal waters, and wetlands. Prevention of siltation and sedimentation enhances fish respiration, plant productivity, and water depth, thus preserving habitat of aquatic organisms. Using less pesticide also decreases the amount of pesticide residue in the soil, surface, groundwater, and in the air.

With biotechnology-derived crops as with traditional crops, crop residues are plowed back into the soil. Questions have been raised relative to the persistence of delta-endotoxins from crop residues and whether they may cause impacts on soil invertebrates and microbial communities. However, according to Cohen (1999), delta endotoxins from *Bt* bind to clay, resist microbial degradation, and retain their insecticidal properties. Other studies have been conducted indicating that delta-endotoxins from biotechnology-derived crop residues have no short-term acute effects on soil health, but monitoring is necessary to ascertain the long-term effects. These studies have been conducted on temperate aerated soil and not tropical soils or submerged anaerobic soils such as rice paddies (Cohen 1999). However, Koskella and Stotzky (1997) suggest that *Bt* toxins from plants may accumulate, increasing the levels of active toxins in the soil relative to sprayed *Bt*. Donegan et al. (1995) conducted a study to determine the potential for detrimental effects on the soil ecosystem from residual plant material following harvesting and tillage from biotechnology-derived plants. In a review of several microcosm and field studies evaluating the persistence of biotechnology-derived plant products (endotoxins) and their effects in the soil and on plant microorganisms, Donegan and Seidler (1999) hypothesize that repeated planting of biotechnology-derived crops in an area may result in the accumulation of antimicrobial compounds in the ecosystem. When evaluated, in-field measurements showed a lack of accumulation (Head et al. 2002). With respect to biotechnology-derived herbicide-tolerant crops, the use of broad-spectrum herbicides on herbicide-tolerant crops is less environmentally damaging because these herbicides are less persistent in the soil relative to the alternatives.

Organochlorines, such as DDT, endrin, and dieldrin persist in soils for years. Although no longer used for crop protection, their residues persist in wildlife (Edwards 1993; Palmer and Bromley 2001). Carbamates are more persistent in the soil than organophosphates and thus have the potential for causing significant environmental damage in soils. Nematicides are transient in the soil, but can cause severe persistent, localized ecological effects. Although not causing direct environmental problems except non-breakdown of residues from previous treatments, their indirect effects include leaving bare soil susceptible to erosion. Fungicides are not specific in their target impacts. While they are applied to control pathogens, they may also reduce populations of beneficial and nontarget soil microbes (Anderson 1978; Horton, Carner, and Turnipseed 1980). Organic farming practices produce positive effects on natural soil fertility including higher organic content, lower soil acidity, better soil structure and activity, and more soil fauna (Bodenmüller 2001).

One of the promising environmental impacts of biotechnology-derived cropping systems is the opportunity to practice conservation tillage. Herbicide-resistant cultivars enhance the use of soil-protecting farming systems that promise positive impacts directly on water and soil resources, and indirectly on air quality and other environmental indicators.

Gene Flow and Outcrossing

Ellstrand et al. (2000) indicate that of the world's 13 most important food crops, 12 crops hybridize with wild relatives in some part of their agricultural distribution. Ellstrand and colleagues used population genetic theory to predict the evolutionary consequences of crop to wild gene flow. In a case study of cottonseed allozyme, DNA analyses demonstrated that limited interspecific introgression has occurred from cultivated cotton species to certain wild relatives. In the subtropics, *G. barbadense*-specific alleles were found in wild or feral populations of *G. hirsutum* that are sympatric with the crop. Alleles from *G. hirsutum* occur in wild *G. barbadense* populations that are sympatric with cultivated *G. hirsutum*. No other incidences of gene flow in cotton were reported.

Cook (2000) hypothesizes that although transfer from plants to microorganisms is possible based on laboratory studies, and has happened from an evolutionary perspective, the probability of a functional and medically important antibiotic gene movement from plant to a human pathogen is negligible. The trait may provide no competitive or reproductive advantage to fertile offspring. Although there is the possibility of high exposure, there is low risk or no risk because there is no identifiable hazard. Input traits meant to improve crop production, and probably most output trait characteristics, fall into this category (Cook 2000). However, the concern with respect to biotechnology-derived crops stems from the promiscuous sexual habits of plants or the movement of transgenes into wild relatives via pollen flow (Glausiusz 1998; Hancock, Grumet, and Hokanson 1996). Not only can pollen from one plant variety fertilize another, but also different species can sometimes mate and produce hybrids that are fertile. Genes from crops can thus move into populations of weeds (Glausiusz 1998).

ICAC (2000a) indicates that for gene flow to occur via normal sexual transmission, certain conditions must exist. The two parents must be sexually compatible, their periods of fecundity must coincide, a suitable pollen vector must be present and capable of transferring pollen between two parents, and the resulting progeny must be fertile and ecologically fit for the environment in which they would exist. Pollination among different plant species is discussed in Hancock, Grumet, and Hokanson 1996. The specific environmental risk must be assessed to determine if the gene flow is advantageous or not. Adverse effects of gene outcrossing are outlined in Raybould and Gray (1993) and Smith (2000b).

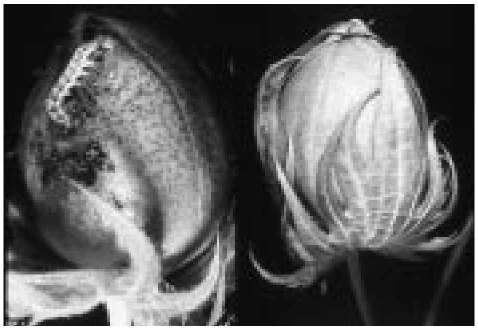
Malik (2000) discusses the horizontal gene transfer of selectable marker genes. Many cultivated plants that are being developed using biotechnology methods are not considered weeds and are unlikely to become weeds due to the introduction of a selectable marker. However, Hails (2000) suggests that the outcrossing of transgenes into wild relatives is a two-step process involving hybridization and establishment. Biotechnology-derived crops most likely hybridize with wild relatives, but the frequency with which it occurs depends on the crop and on the number and closeness of wild relatives to the relevant crop. Glausiusz (1998) suggests that the problem of gene flow and herbicide tolerance among wild plants is not a serious problem because herbicide use is mainly found only on farms. More important is the possibility that genes for the resistance to insects, viruses, and fungi could be spread to the wild. Research has shown that virus-resistant genes can escape from some crops into wild relatives. Whether or not the newly resistant wild relative can out-compete other native wild plants remains unanswered. Nevertheless, transgenes will likely move quickly from crop species enhanced using modern biotechnology methods to natural ecosystems whenever compatible relatives are in close proximity (Hancock, Grumet, and Hokanson 1996; Royal Society 1998).

In summary, findings indicate that the risks of new plant varieties developed through biotechnology becoming weedy or outcrossed are similar to varieties developed using traditional breeding methods. Thill (1996) suggests that there is no perceived risk of lateral gene transfer from cotton. However, the EPA has determined that the potential of gene capture and expression of the *Cry*1Ac endotoxin in cotton in wild or weedy relatives is possible in Hawaii and Florida where wild or feral cotton relatives exist (USEPA 2000). An evaluation by the Genetic Manipulation Advisory Committee (GMAC) in Australia concluded that there is no additional risk to the environment from Roundup Ready® or Roundup Ready®/INGARD® cotton relative to traditional cotton varieties. The risk of them spreading as a weed is low; the likelihood of gene transfer is low; and any transfer is unlikely to pose any hazard to human health or the environment.

Several technologies have been suggested to contain gene flow and to decrease the risk of escape of herbicide-resistance genes to weeds (Riches and Valverde 2002). One seed protection technology patented by the U.S. Department of Agriculture is a Technology Protection Systems (TPS) (euphemistically referred to as “terminator” technology) in which the viability of the progeny seed is genetically controlled. Seed protection technology was proposed for cotton and other crops, but due to public objection to this type of gene flow control technology, the further development of these products has not progressed (ICAC 2000a). Other approaches proposed to abate gene flow still in experimentation include chloroplast transformation of herbicide-resistance traits and use of tandem constructs. In a tandem construct, the herbicide-resistance gene is tightly linked to another gene that codes for traits harmful to weeds, but not to crop. The future availability and use of these approaches will further reduce the potential risks associated with gene flow between crop and its wild relatives and may lead to increased adoption of herbicide-tolerant crops.

Resistance in Cotton Pests

The development of resistance to chemical insecticides was a serious problem in U.S. cotton production in the mid-1990s. Had it not been for the introduction of biotechnology-derived *Bt* cotton, many cotton farmers in Alabama and other U.S. Cotton Belt locations would not have been able to grow their crop successfully. But the development of resistance to insecticides is not unique either to cotton pests or to the United States. Resistance is the result of selection, a process whereby a few insects in the population that have genes of specific resistance mechanisms survive the insecticide sprays and multiply, thereby increasing the proportion of resistant insects in the population. Resistance development in insects threatens both the high benefits and the low risks of using *Bt* toxins in transgenic crops and in microbial spray formulations.



Insect damaged cotton boll (left) and healthy cotton boll (right).

Pyrethroid use has decreased in Colombia and China due to resistance developed by the bollworm (*Helicoverpa armigera)*, and in Africa due to increases in mite populations. Organophosphate use in South Africa has decreased due to increases in aphid populations. Cote d’Ivoire, Egypt, Iran, and Togo have stopped using organochlorines because of high toxicity and residual effects (International Cotton Advisory Council 1995). The problem is severe in China and

Colombia where *Helicoverpa armigera* has developed a high degree of resistance to pyrethroids. Also, insecticide tank mixtures containing organophosphates were used to control bollworm without success. And according to Zhang and Tang (2000), changing climatic and ecological conditions hasten the development of insect resistance to pesticides. *H. armigera* resistance to chemical pesticides in China has increased significantly since 1980. Despite increased dosages of pesticides, *H. armigera* infested the cotton crops causing substantial losses economically and environmentally through increased pollution and disruption of the ecological balance.

Other countries where insect resistance has developed include Thailand, Pakistan, Israel, Greece, South Africa, Spain, India and the United States. In Thailand, resistance to pyrethroids in bollworm and aphids, and resistance to organophosphates in jassids has developed. Pakistan and India may have developed bollworm resistance to pyrethroids (International Cotton Advisory Council 1995). Israel has seen the bollworm, *Spodoptera*, whitefly, and aphids develop resistance to chemical insecticides such as organochlorines, organophosphates, carbamates and pyrethroids (International Cotton Advisory Council 1995; Horowitz, Weintraub, and Ishaaya 1997). In Greece aphids have demonstrated resistance to carbamates and organophosphates (International Cotton Advisory Council 1995). South Africa has seen resistance in mites to organophosphates seven times the lethal dose. Also in South Africa, aphids appear to have developed resistance to organophosphates, and *H. armigera* appear to have developed resistance to pyrethroids. In Spain problems appear to be emerging with aphids (*Aphis gossypii* and *Myzus persicae*) being resistant to organophosphates and pyrethroids, and *H. armigera* is developing resistance to pyrethroids (International Cotton Advisory Council 1995; Vinuela 2001). The United States has seen tobacco budworm and aphids develop resistance to pyrethroids (International Cotton Advisory Council 1995; Smith 2000a). This is especially a concern with *H. armigera*, which has developed resistance to synthetic pesticides (Fernandez-Cornejo, Caswell and Klotz-Ingram 1999; Fitt 2000; Royal Society 1998; Smith 2000b).

Sharma and Ortiz (2000) suggest that it was initially thought that development of resistance to *Bt* crops was not an issue because *Bt* and pests have evolved together for millions of years. Due to the limited exposure and several toxins produced by *Bt* it is believed that development of resistance under natural conditions may not be high. However, most of the biotechnology-derived crops express only one toxin gene and lack the complexity of *Bt* formulations. This, combined with the plants continuously producing the *Bt* toxin, thus exposing the insects throughout the feeding cycle and season, puts the insect population under selection pressure and may cause resistance to the *Bt* toxins found in biotechnology-derived crops, as suggested by Sharma and Ortiz (2000). The intense selection pressure imposed over 3-4 generations per year by continuous expression of *Bt* toxins in cotton plants makes resistance a serious concern, thus resistance management plans are a precondition of commercialization of new biotechnology-derived cotton varieties in the United States.

After collecting corn earworm larvae from the ears of *Bt* sweet corn in North Carolina, Burd et al. (2000) found that, after only 6 generations of laboratory selection on artificial diet containing *Cry*1Ac toxin, the selected colony had developed about 50-fold resistance to *Cry*1Ac. Nearly 100-fold resistance was seen after 10 generations. They believe that resistance to *Bt* can be inherited as a dominant or incompletely dominant trait**.** Burd et al. (2001) estimated the frequency of non-recessive *Bt* genes in CEW. The estimated gene frequency for resistance to the *Cry*1Ac toxin was 0.00043 (Burd et al. 2001).

Several studies concerning the resistance management of *Bt* cotton have been conducted. Olsen and Daly (2000) conducted a study in Australia to determine the toxicity of *Bt* cotton and its effects on mortality of *H. armigera* at different plant growth stages. They suggest that plant-toxin interactions in fruiting cotton reduce the toxicity of the *Cry*1Ac protein. Another Australian study evaluated potential ecological impacts of biotechnology-derived cotton, determining effective resistance management strategies including effective refugia (i.e., areas in which a species can survive during difficult periods of time.) (Fitt, Mares, and Llewellyn 1994). Refugia provide a habitat for target pests to live without the constant impact of the insecticidal activity of the biotechnology-derived varieties, thus reducing the likelihood of resistance development.

Zhang and Tang (2000) discuss strategies to delay resistance in China, including developing varieties with more complex toxicity, timing of insecticide applications at early stages of plant growth when resistance is highest, and refuge management. China currently has three varieties of biotechnology-derived *Bt* insect-resistant cotton germplasm lines, which may be used in rotation or multiple *Bt* may be stacked into the same plant to delay the development of resistance. Sumerford et al. (1999) report on a three-year study of tolerance to *Cry*1Ac in populations of *Heliocoverpa zea* and *Heliothis virescens* in the eastern U.S. Cotton Belt. Results indicate that while there was some sign of tolerance in *H. zea* in some locations, the current tolerance level is transient and not high enough to cause control failures in the field.

A promising resistance management strategy exemplified by Bollgard II cotton is the development of multiple toxin traits with different modes of action in a single cotton plant (Mahaffey et al. 2001). Penn et al. (2001) conducted a study to test the lepidopteran activity level of Bollgard II cotton throughout the growing season. Results showed that Bollgard II provides significantly greater likelihood of protection from lepidopteran pests than Bollgard. Using broccoli as a model system, Shelton et al. (2000) conducted field tests on managing resistance to *Bt* cotton plants validating the need for refuges. Armstrong, Leser, and Kraemer (2000) sampled and compared major predators in *Bt* and non-*Bt* cotton in the Texas High Plains before the initiation of boll weevil eradication. This study helps to explain predator densities in *Bt* and non-*Bt* cotton and investigates the use of *Bt* cotton to act as a refuge for predaceous insects and spiders that are negatively impacted in non-*Bt* cotton fields. Carriere et al. (2001a) conducted a study to predict pink bollworm emergence and the importance of planting date as a component of resistance management strategies. Carriere (2001b) noted the importance of farmer participation in development of large-scale resistance management plans.

Resistance management for biotechnology-derived crops differs from most other crops in that strategies are in place to accompany introduction of the new biotechnology-derived varieties that will mitigate the development of resistance. The predominant approach to resistance management is the planting of refuge areas consisting of traditional crop varieties (Gould 1998; Royal Society 1998). The EPA requires that farmers planting a *Bt* crop must also plant traditional refuges (EPA 2001; Tabashnik 2001). As part of the registration of *Bt* crops, companies must provide a resistance management strategy outlining mitigation procedures and recommendations to farmers regarding monitoring protocols to analyze resistance development in the fields (Peferoen 1997).

The new refuge requirements for insect resistance management in Bollgard I cotton went into effect for the 2001growing season. These requirements and recommendations set forth by the EPA include the refuge size, structure, and deployment necessary to make the refuge strategy successful. The three alternative requirements are 95% *Bt* cotton to5% (95:5) external unsprayed refuge at least 150 feet wide and planted within _ mile; 95:5 embedded refuge at least 150 feet wide; and 80:20 external sprayed refuge planted within 1 mile, but preferably within _ mile (Matten 2001). The 95:5 external unsprayed refuge ensures that at least 5 a. of non-*Bt* cotton (refuge cotton) must be planted for every 95 a. of *Bt* cotton. This refuge may not be treated with any insecticide labeled for control of tobacco budworm, bollworm, or pink bollworm. The 80:20 external sprayed refuge ensures that at least 20 a. of non-*Bt* cotton must be planted for every 80 a. of *Bt* cotton. All the cotton may be treated with insecticides labeled for control of tobacco budworm, bollworm, or pink bollworm. For the option of embedded refuge, the refuge must be embedded as a contiguous block within the *Bt* cotton field (Matten 2001; Mullins 2001). A pilot program of community refuge requirements initiated in 2001 will continue in 2002. The community refuge option does not permit imbedded refuges (Matten 2002).

In summary, the introduction of biotechnology-derived cotton represents a major tool farmers have in addressing cotton pests that have already developed resistance to commercially available chemical pesticides. Because the potential to develop resistance to plant-incorporated protectants such as *Bt* cotton, a number of resistance management strategies have been implemented and this continues to be an active area for research and regulation.

Nontarget Species

In the past, the impacts of insecticides on both nontarget vertebrates (high life forms such as fish, birds, and mammals that have a spinal column) and nontarget invertebrates (lower life forms such as bees, butterflies, and earthworms that lack a spinal column) have led to research on alternative pest control methods . Such methods include more-targeted insecticide application strategies and more-targeted insecticides. More recently, biotechnology-derived plant-incorporated protectants have been designed to control specific pests by their mode of action and mode of delivery to the pest. Researchers have demonstrated that all commercially available plant-incorporated protectants, especially *Bt* cotton, have a positive impact on nontarget invertebrates and vertebrates when compared with traditional chemical insecticides.

For example, organophosphates and pyrethroids have been associated with major fish kills after aerial spraying (NRC 2000). Organophosphates are less persistent than organochlorines, but have higher mammalian toxicities also with potential to kill birds and other wildlife (Edwards 1993). Organophosphates and carbamates when used other than as instructed have caused nontarget effects on songbirds, waterfowl, and gamebirds (NRC 2000). Palmer and Bromley (2001) describe the effects of pesticides on nontarget species in noncrop areas, such as quail in habitats adjacent to cotton fields. Nontarget species have been found to have the insecticide methyl parathion in their bodies at levels high enough to cause sickness and/or death (methyl parathion is a chemical that affects the central nervous system). Organochlorines do not have high acute mammalian toxicities but are persistent and have the tendency to bioconcentrate into living tissue moving through the food chain. Thus, they have been phased out in most areas, except for some developing countries (Edwards 1993).

Synthetic pyrethroids are modeled after pyrethrins, which are natural, plant-derived pesticides used as insecticides for hundreds of years. They have low mammalian toxicity and persistence, but are very toxic to insects, enabling them to be used at low dosages (Delaplane 1996). These broad-spectrum insecticides are highly toxic to fish and other aquatic organisms and their use affects beneficial aquatic species. Pyrethroids decrease populations of natural control agents, thereby increasing the need for chemical control (Edwards 1993).

Carbamates are insecticides, acaricides, fungicides, and nematicides. They are more persistent than organophosphates with less mammalian toxicity. Because they are broad-spectrum, they have the potential to affect many organisms causing extreme environmental damage and soil problems (Edwards 1993). Some soil nematicides have high mammalian toxicity with broad-spectrum toxicity to a wide range of organisms (Edwards 1993). Some systemic herbicides such as 2,4-D, MCPA (2-methyl-4-chlorophenoxyacetic acid), and Mecoprop (CMPP) are moderately toxic to fish, although they have relatively low mammalian toxicity and do not cause serious direct environmental problems.

Herbicides can also cause problems to nontarget species and cause cotton seedling injury and stunting (Marquardt 2001). Unfavorable weather after planting with wet, cool conditions combined with soil-applied herbicides, nematicides, and insecticides increases the potential for injury. Fungicides have low mammalian toxicity (with the exception of carbamates), but may have severe environmental impacts on some soil organisms and beneficial fungi that are toxic to insect pests (Anderson 1978; Horton, Carner, and Turnipseed 1980).

Due to the impact on rural ecosystems by traditional cotton farming practices in India, Paarlberg (2001) favors the introduction of biotechnology-derived crops to produce increased yields, thus reducing the need to clear new land for farming in the rural areas and destroying habitats. Furthermore, Paarlberg infers that use of biotechnology-derived *Bt* crops would mitigate human health problems, as well as decrease environmental pollution and eliminate the killing of many nontarget species as is the case with current conventional chemical spray practices.

The cultivation (planting) of *Bt* crops leads to a reduction in application of synthetic insecticides directed against species of Lepidoptera and Coleoptera. This should decrease pesticide runoff and soil residue problems, and enhance the naturally occurring biological control of pests not controlled by *Bt* such as mites, aphids, and thrips (Cohen 1999). Cook (2000) suggests that agriculture by its very nature is disruptive to nontarget organisms in the environment. Tillage destroys populations of earthworms that have been shown to return in significant levels after only a few years of no-till practices. Other practices have similar impacts.

Stewart, Richards, and Halfhill (2000) indicate that the decreased use of broad-spectrum insecticides benefits both humans and nontarget insect populations. Whereas *Bt* cotton may require 3 or 4 sprays of insecticide treatments per year, traditional cotton required approximately 5-12 spray treatments. Riddick and Barbosa (1998) and Pilcher et al. (1997) indicate that the consumption of pests that have fed on *Bt* crops does not harm predators. Studies conducted on nontarget organisms such as honeybees, green lacewing, ladybird beetles, parasitic Hymenoptera, common soil organisms, earthworms, and springtails showed that they suffered no toxic effects from *Cry*1Ac proteins above maximum predicted environmental exposure (ICAC 2000a). Additionally, field observation studies of impacts of *Bt* cotton on nontarget organisms have shown increases in populations due to the reduction in non-specific pesticide use (ICAC 2000a). Head et al. (2001) studied the effects of Bollgard cotton on natural enemy populations with results indicating that natural enemy populations were in abundance. Van Tol and Lentz (1998) conducted a study to determine the influence of *Bt* cotton on beneficial arthropod populations, finding little difference between populations in traditional and *Bt* fields. Sims (1995) studied the effects of *Bt* cotton on beneficial and other nontarget insects. Results indicate that the protein expressed in biotechnology-derived cotton has biological activity specific for Lepidoptera and that risks to beneficial non-Lepidoptera insect species are negligible. Schuler et al. (1999) discuss the direct and indirect effects of *Bt* plants on arthropod natural enemies.

Greenplate (1999) reports a study to develop a sensitive quantitative bioassay for the measurement of *Bacillus thuringiensis* Berliner endotoxin, *Cry*1Ab, in Bollgard cotton tissue. Analyses were conducted in this study to effectively quantify insect-active *Cry*1Ab in collected plant tissue. The samples were tested for the effects of environment, plant age, and tissue type on *Cry*1Ab concentrations. Pilcher et al. (1997b) and Yu, Berry, and Croft (1997) describe tests on secondary lepidopteran pests and nontarget soil arthropods and orbatid mites.

In summary, the impact on nontarget species by broad-spectrum cotton insecticides is greater than that of targeted plant-incorporated protectants such as *Bt* cotton.

Weed Management in Cotton Farming

Using chemicals to regulate plant growth has enabled agriculturists to provide food to the expanding world population and prevent crop losses while improving crop quality. This is accomplished by limiting the competition for soil nutrients, water, and exposure to light between weeds and the desired crop. Weeds, plant pathogens, and insects decrease crop yields (Kuiper et al. 2000; Songstad 2000). However, cotton is more susceptible to yield reductions from weed competition than corn or soybean (Zimdahl 1980).

Overuse of herbicides increases the probability of herbicide resistance in weeds. Weed control in cotton is more difficult in the mid-south and southeastern United States due to warm and humid growing conditions (Snipes and Mueller 1992a , 1992b; Wilcut et al. 1996). These weather conditions break down herbicides, reducing their efficacy allowing for weed germination and growth (Wilcut et al. 1996). Numerous studies have been conducted documenting yield loss from weeds, and competitive indices have been developed for the common annual broadleaf weeds in cotton (Coble and Byrd 1992). Controlling weeds in cotton requires use of herbicides to prevent economic damage.

The number of herbicide-resistant weeds increased from 12 at the end of the 1980s to over 250 by early 2000. These include 53 resistant to acetolactate (ALS) inhibitors, 26 to sulfonylurea and imidazolinone classes, and 19 to acetic coenzyme A carboxylase (ACC) inhibitors, with most resistance found in high-input agricultural regions such as the United States, Europe, Canada, and Australia (Rubin 1996; Schutte 2000).

Biotechnology-derived herbicide-tolerant cotton is more widely adopted in the United States than *Bt* cotton (ICAC 2000a). Songstad (2000) demonstrates that the advent of herbicide-tolerant crops through modern biotechnology has provided farmers with a new management tool to control weeds. Herbicide-tolerant crops are the most field-tested and planted biotechnology-derived trait worldwide. Three different herbicide-tolerant cotton varieties have been developed for the U.S. market: these are glyphosate, sulfonylurea, and bromoxynil (Schutte 2000). However, only glyphosate-tolerant and bromoxynil-tolerant cotton were commercially available by late 1999 (Liebman and Brummer 2000). Tolerance to more than one herbicide would allow for the use of additional herbicides, increasing the flexibility of weed control measures and mitigating the potential for development of herbicide resistance (Kuiper et al. 2000).

Resistance in Traditionally Bred Cotton

Glyphosate Resistance

Glyphosate is the active ingredient in a popular herbicide used worldwide, Roundup®. It is a desirable broad-spectrum weed control agent in many settings, including ecologically sensitive areas, due to its rapid degradation (Songstad 2000). Glyphosate resistance, previously thought unlikely to occur in weeds, has evolved in five species in seven locations worldwide.

- In 1997, in Malaysia, goosegrass (*Eleusine indica*) evolved resistance to glyphosate.

- In 2000, in Delaware, horseweed (*Conyza Canadensis*), a dicot weed in the Asteraceae family, first reported resistance to glyphosate.

- In Chile in 2001, Italian ryegrass (*Lolium multiforum*), a monocot weed in the Poaceae family, reported resistance. It is estimated that 2-5 sites totaling 101-500 a. are infested with Italian ryegrass resistant to glyphosate.

- In Victoria and New South Wales, Australia; California, the United States; and South Africa, rigid ryegrass (*Lolium rigidum*), a monocot weed also in the *Poacease* family, reported resistance to glyphosate. (Neve 2001; Weed Science.Com 2001).

- The detection of glyphosate resistance in a few populations of annual rye grass has increased concerns that glyphosate-tolerant canola will increase selection for glyphosate resistance in annual ryegrass and other weeds (Roush 2001).

Bromoxynil Resistance

The herbicide Bromoxynil (3,5-dibromo-4-hydroxybenzonitrile) falls under the class of nitriles and others (Weed Science.Com 2001). This class of herbicides is known for the inhibition of photosynthesis and photosystem II. In Oregon in 1995, bromoxynil resistance was reported in the common groundsel (*Senecio vulgaris*), another dicot weed in the Asteraceae family. It is estimated that one site totaling approximately 6-10 a. planted in traditional cotton was infested with common groundsel resistant to bromoxynil (Weed Science.Com 2001).

Sulfonylurea Resistance

The sulfonylureas are a family of compounds that kill broadleaf plants. This is done by blocking the plant enzyme acetolactate synthase (ALS), an enzyme important to the plant for the synthesis of some amino acids (leucine, isoleucine, and valine). Without the synthesis of these amino acids, the plant is not able to synthesize proteins, which results in plant death. Sulfonylureas were introduced in the early 1980s and can be preemergence and/or postemergence applied on annual and perennial weeds (Defelice 1999). These herbicides have medium to long soil persistence lasting from several weeks to several years depending on the particular herbicide type and application rate.

Widespread use of these herbicides has led to the appearance of numerous resistant weed populations around the world (weedscience.org 2001). Weed resistance to ALS herbicides was first reported in the western United States after five to six years of continuous chlorsulfuron (Glean®) use in wheat. ALS herbicide-resistant weed populations have increased at a greater annual rate than any other herbicide mode of action over the last ten years. There are currently 19 different weed species that have evolved resistance to ALS inhibitor herbicides in the United States and 53 ALS-resistant weed species worldwide in 14 countries. ALS inhibitor-resistant weeds have appeared in small grains, corn, soybean, rice, highway rights-of-way, and forests (Defelice 1999; weedscience.org 2001). Herbicide resistance management practices are now encouraged whenever ALS herbicides are used in weed management programs. For a comprehensive review of the weeds and locations worldwide where sulfonylurea-resistant weeds are found, see weedscience.org.

Herbicide Tolerance Traits in Biotechnology-derived Cotton

Glyphosate herbicide is used to control most annual broadleaf and grass species. In a study conducted in 1992 and 1993, Roundup® at 0.5lb/a. provided 90% control of seedling Johnsongrass and Texas panicum. Formulations of 0.75lb/a. provided 80% control of large crabgrass and crowfoot grass. At 0.25lb/a. Roundup® provided 95% control of smallflower morning glory and cocklebur, and 100% Ipomoea morning glory control was achieved with 0.5lb/a. Roundup® at 0.75lb/a. provided 85% control of Florida beggarweed and sicklepod (Richburg et al. 1994). These application rates and their corresponding levels of control may vary significantly depending on time of application and environmental conditions such as weather. Glyphosate also would be effective to control dinitroaniline herbicide-resistant goosegrass (*Eleusine indica* [L.]Gaertn) (Wilcut et al. 1996). Glyphosate and bromoxynil herbicides would be viable options to control arsenical herbicide-resistant and ALS herbicide-resistant common cockelbur, and dinitroaniline herbicide-resistant Palmer amaranth (*Amaranthus palmeri* S. Wats) (Wilcut et al. 1996).

Glyphosate Tolerance

Monsanto developed the glyphosate-tolerant cotton through modern biotechnology; it became commercially available in 1997 (Carpenter and Gianessi 2001). Glyphosate, also known as N-[phosphonomethyl]glycine is the active ingredient in Roundup® herbicide. Plants manufacture essential aromatic amino acids such as phenylalanine through the shikimic acid pathway, housed in their chloroplasts. When Glyphosate is applied to plants it, blocks a key enzyme in the pathway, 5-enolpyruvylshikimate-3-phosphate synthase, or EPSPS. Monsanto discovered a gene for glyphosate-resistant EPSPS in Agrobacterium species CP4 and through modern biotechnology methods is capable of producing crop plants resistant to the effects of glyphosate (Palevitz 2000). The inserted gene in cotton should make the plants able to withstand two applications of Roundup® herbicide per season.

Viator et al. (2000) conducted a study to determine a critical rate of Roundup® applied over the top of herbicide-tolerant, Roundup Ready® cotton. He determined that application rate influences fruit abscission and whether the application method and timing for midseason Roundup® application affects cotton yield, fiber quality, percentage gin out, boll distribution, and abnormality of bolls. They found that Roundup® should be applied to the herbicide-tolerant cotton plants before the appearance of buds, flowers and bolls. Farmers may need to prevent glyphosate contact with both cotton stems and leaves when applying glyphosate after the fourth leaf stage to prevent possible yield losses. This means that glyphosate can be applied only over the top for the first four leaves of growth without a delay of boll production, which has not stopped adoption of Roundup Ready® varieties (ICAC 2000a). In another field study conducted to determine the effect of glyphosate on pollen viability and pollination in Roundup Ready® cotton, Pline et al. (2001) determined which floral organs were affected by glyphosate applications, the male portions (anthers, pollen) or the female portions (stigma, ovary). As observed in previous studies, the male portions of the cotton flower develop earlier and therefore the potential exists for the damage to male organs with early glyphosate applications.

Adoption of herbicide-tolerant crops leads to substitution of glyphosate herbicides for the previously used herbicides (Heimlich et al. 2000). A study evaluating the cost of Roundup® treatments compared with traditional herbicide treatments in cotton suggests that Roundup® treatments may be the most economical (Sciumbato and Hurst 2001). Mackey and Santerre (2000) report that planting of glyphosate-tolerant crops leads to less total herbicide use in cotton production. Kalaitzandonakes and Suntornpithug (2001) observe that the use of a nonselective herbicide, such as Roundup® may require less management and the effectiveness of weed control programs may improve. McCarty et al. (2001) found that Roundup Ready® cotton treated with Roundup Ultra® herbicide provided weed control as effectively as traditional herbicide control in traditional cotton. Yields of the Roundup Ready® cotton from large scale, commercial-size plots were comparable to those of traditional varieties.

Bromoxynil Tolerance

In 1995 Bromoxynil-tolerant (BXN) cotton was introduced. It was produced by Calgene and marketed as Stoneville BXN varieties. Bromoxynil herbicide (3,5-dibromo-4-hydroxybenzonitrile) is known as Buctril® and is a postemergence broadleaf herbicide used with cotton (Panter et al. 1997). BXN cotton is highly tolerant of Buctril®. In field tests, BXN cotton was able to tolerate Buctril® levels ten times the labeled rates without damaging the plants. Buctril® provided superior control of morning glory species and available weed efficacy data suggest that it will be useful for the cotton weed complex commonly found in the mid-South (ICAC 2000a). BXN cotton was made tolerant to the herbicide bromoxynil through the transfer of the oxy gene from the soil bacterium *Klebsiella pneumoniae* subspecies ozaenae. This gene codes for a degradation enzyme, nitrilase, which breaks down bromoxynil into nonphytotoxic compounds (ANZFA 2001).

Bromoxynil does not control grasses, which makes continued use of soil-applied herbicides more probable when planting BXN cotton (Carpenter and Gianessi 2001). BXN cotton is adopted at greater rates in areas where bromoxynil effectively controls the predominant problem weeds, such as morning glory and cocklebur. However, where sicklepod is predominant, adoption rates tend to be low. Everitt et al. (1999, 2001) conducted research pertaining to bromoxynil-tolerant cotton and glyphosate-tolerant cotton and perennial weed management. They found that these biotechnology-derived alternatives provide increased yields and net returns over weed control costs compared to cultivation alone for all weed species. Vargas et al. (2000) conducted field trials of BXN cotton in California to determine weed control efficacy and the tolerance of BXN cotton to bromoxynil applied over the top. In their study, bromoxynil provided 95 to 100% control of most annual broadleaf weeds tested. Several other studies have been conducted to determine the efficacy of weed control using BXN cotton (Baumann and Morgan 1997; Burris et al. 1997; Collins et al. 1998; Guy 1998; Hurst 1998; Murdock et al. 1997; Paulsgrove et al. 1997, 1998). In general, the results show that BXN cotton with appropriate herbicide applications provides at least as high lint yield with reduced herbicide applications in comparison with traditional systems. When some weed species are a problem, using PRE and/or POST herbicides, in addition to bromoxynil, improves cotton production. Askew et al. (1999b) conducted studies to overcome weaknesses in BXN cotton weed management using preemergence and preplant herbicides. Wilcut et al. (1999) conducted a two-year study to evaluate weed control, cotton tolerance, and cotton yield response to postemergence applications of bromoxynil, Staple®[3], and tank mixtures of Buctril® plus Staple®. Cotton yields with bromoxynil plus Staple® were at least as high as traditional control methods. Askew et al. (1999a) studied weed management in traditional and no-tillage cotton using BXN cotton and Roundup Ready® cotton, reporting no statistical difference in yield among the biotechnology-derived varieties. York and Culpepper (1999) conducted an economic study comparing the weed management systems in BXN, Roundup Ready®, and traditional cotton. Lint yields were statistically equivalent for the three varieties studied under standard herbicide programs.

Sulfonylurea Tolerance

A sulfonylurea-tolerant cotton was developed by DuPont using biotechnology rDNA techniques. This biotechnology-derived cotton was developed in 1996 using an acetolactate synthase (ALS) gene from tobacco, *Nicotiana tabacum* cv. Xanthi. It was evaluated in field trials conducted under USDA Animal and Plant Health Inspection Service permits in 1991. Sulfonylurea herbicides are used at a small fraction of an ounce per acre (USDA-APHIS 2001a). Schutte (2000) suggests that only a few grams are needed on one hectare compared with 0.5 to 3 kilograms for other herbicides. However, the technology developer decided to discontinue further development of this product, so it was not commercialized and therefore provides no benefits or risks to the environment (Lemaux 1999).

Water Impacts from Cotton Farming

Use of water resources in cotton farming presents a significant environmental resource challenge. Irrigated cotton is frequently grown in regions where fresh water is in short supply, such as the Mediterranean and desert and/or near-desert areas in India, Pakistan, Uzbekistan, and Australia. Extensive irrigation of cotton impacts the regional water resources, possibly contributing to surface and groundwater depletion (WWF 1999). Furthermore, cotton production relies heavily on the use of agrochemicals (i.e., pesticides including organophosphates and pyrethroids, herbicides including 2,4-D, MCPA, and CMPP and nutrients including nitrogen fertilizers), which can runoff fields and pose risks to freshwater ecosystems (Yelverton 2001). In addition to runoff, extensive irrigation and rerouting of water can impact local ecosystems through drainage, dam construction, and land reclamation.

Problems in China, Egypt, and Uzbekistan due to inadequate or inappropriate drainage have contributed to the salinization of freshwater (Abbot and Leeds-Harrison 1998; WWF 1999). Other water impacts of cotton production include changes of the water table or depletion of groundwater (New South Wales, Australia), degradation of wetlands and lakes (Aral Sea, Yellow River Valley), and rising water tables and salinization of soil surface (Australia, Indus River Valley, Uzbekistan, Pakistan) (United Nations University 1999). While these are primarily problems in developing countries; they are not unique to these areas. In the Texas High Plains — a major cotton production area — agriculture may have been responsible for depleting one-quarter of the Texas portion of the Ogallala aquifer (Bonnis and Steenblik 1998). Currently, developed nations have widespread drainage infrastructure and are generally better equipped to address water management issues through regulatory institutions.

By contrast, planting biotechnology-derived cotton offers positive water management options. In China, millions of acres are now under *Bt* cotton production. The yields are increasing and pesticide applications are down from an average of 12 applications to 3 applications per season thus reducing the potential for runoff in local ground- and surface water. Use of *Bt* and herbicide tolerant cotton is also consistent with the adoption of conservation tillage programs, which result in increased soil moisture and decrease water demands. Becker (2001) conducted a study focusing on biotechnology-derived cotton in Mississippi. Runoff water sampled in the study was virtually free of insecticides during the four-year study. Samples were taken from both *Bt* and non-*Bt* cotton fields testing for pyrethroids and organophosphates. The conclusion was that there were no detrimental environmental effects from the runoff from any of the *Bt* test sites. Therefore, the use of biotechnology-derived cotton has positive environmental impacts on water quality and offers partial solutions to existing water management challenges in cotton growing regions.

Human Exposure

Planting of biotechnology-derived cotton varieties has resulted in the significant reduction of human pesticide exposures in China and other regions of the world where pesticide intensive cotton farming is practiced (Conway 2000; Pray et al. 2001).

According to National Academy of Sciences (2000), between 1951 and 1967, 151 deaths were attributed to agrochemicals in California with 34 of them being occupational exposures. Approximately 1500 poisonings were recorded between 1966 and 1970, with organophosphate insecticides the most common cause. The National Research Council (2000) suggests that to understand the impact of pesticides on humans it is necessary to understand the transport and fate of pesticides in the environment. Once a pesticide is applied to soil, it remains in the soil, transfer to air, transfer to surface runoff, or transfer to soil-pore water. Potential human exposure occurs through consumption of contaminated surface water. Pesticides posing the greatest risk are those that are very mobile, persistent, and highly toxic. Examples are discussed by Kurtz (1990), who observed that airborne pesticides can travel long distances to remote environments. Residues of organochlorines have been found in the polar regions in the body fat of Innuits, seals, and polar bears (Kurtz 1990, NRC 2000). Franzaring and Eerden (2000) provide a review of studies concerning the accumulation of airborne organic pollutants in plants in areas distant from the pesticide application. Airborne residues represent a direct human hazard, as well as hazards to vegetation and wildlife. Humans, such as farm workers, working directly with pesticides are the most susceptible. However, humans downwind from treated fields can become exposed as well (NRC 2000).

In Central Asia the drying of the Aral Sea has accelerated the desertification process. The chemicals used on irrigated fields drain into the Sea and sink to the seabed forming toxic salt pans as the Sea dries.

Kobori and Glantz (1998) hypothesize that these chemicals are then lifted by the winds and later fall with rain causing higher infant mortality rates, sickness, and death to the people of the region.

In Africa foliar application of pesticides followed by a heavy downpour of rain leads to pesticide runoff into nearby streams or water sources (Ismael, Bennett, and Morse 2001). Farmers wash pesticide containers in the same water sources as they use for household consumption without understanding the human and environmental health hazards of these actions. Similar conditions are found throughout the tropics where chemical-based farming systems pose significant threats to human health (Yousefi 1999).

In Pakistan, a number of studies have documented pesticide residues in water and soil samples, seed cake, and among people – cotton pickers – exposed to pesticide (Banuri 1998). Cottonseeds were also found to be contaminated with pesticide poisons, constituting a severe health hazard because 60% of edible oil in Pakistan is derived from cottonseed. Perhaps the most pressing concern is pesticide poisoning. From a study conducted by the Central Cotton Research Institute (CCRI), one out of 888 female cotton pickers had low-level pesticide poisoning, 74% had moderate pesticide poisoning, and approximately 25% had dangerous levels of pesticide poisoning (Banuri 1998).

Recent evidence from China demonstrates the direct human health advantage of biotechnology-derived cotton among farmers. Incidences of symptoms of pesticide poisonings were significantly reduced among those who planted *Bt* cotton compared to farmers of traditional varieties (Huang et al. 2002).

There have been no adverse effects of *Bt* proteins being observed in higher animals, including mammals (Sharma and Ortiz 2000). Mammals, including man, do not contain specific receptors for *Cry*1A(b) protein in their gastrointestinal tract. The concept of substantial equivalence has been accepted by the American Medical Association, the U.S. regulatory agencies and the international health community including the United Nations World Health Organization as the basis to determine the safety of biotechnology-derived food products. Plants are considered substantially equivalent or *as safe as* their conventional counter parts when testing results demonstrate that are no significant differences in the phenotypic characteristics and composition of the parent crop and the biotechnology-derived crop. The safety assessment of the selectable marker gene NPTII was studied by Fuchs et al. (1993) and the results were consistent with previous studies demonstrating that the NPTII is readily degraded like other dietary proteins and does not compromise the efficacy of aminoglycoside antibiotics, does not possess attributes of known protein food allergens, is not toxic to mammals, and therefore presents no risk to humans (Flavell et al. 1992; IFT 2000). Cotton fiber from biotechnology-derived cotton was evaluated and found to be *as safe as* traditional cotton (Gustafson et al. 2001).

The results from the Genetic Manipulation Advisory Committee in Australia indicate that biotechnology-derived Roundup Ready® and Round Ready®/INGARD® cotton are not potentially harmful nor do they pose any additional risks to public health and safety or to the environment relative to traditional cotton varieties. Herbicide-tolerant and the stacked herbicide-tolerant/insect-resistant cotton varieties are not likely to prove toxic or allergenic to humans, the likelihood of gene transfer is low, and any transfer is unlikely to pose a hazard to human health or the environment.

ECONOMICS

Adoption of Biotechnology-derived Cotton

Adoption of biotechnology-derived cotton in the United States has increased rapidly since the first plantings in 1995. *Bt* cotton accounted for 12% of U.S. acreage in 1996 and rose to 39% by 2000. Roundup Ready® cotton increased from 4% of acreage in 1997 to 54% in 2000. Bromoxynil (BXN) cotton was planted on 0.1% of U.S. acreage in 1995and 7.2 % in 2000 (Carpenter 2000; Carpenter and Gianessi 2001; James 2001). Total estimated cotton acreage planted in the United States, for both Pima and Upland cotton, remained relatively constant over the 1995-2000 time period, with an average of 15 million a. (6 million ha) (USDA-NASS 1995-2000). So in absolute numbers, acreage planted of traditional varieties decreased over this period. See Tables V-1 and V-2.

Global area planted to cotton is estimated at 34 million ha (84 million a.). Of the 34 million ha, 16% was planted in biotechnology-derived cotton in 2000 (James 2001). This represents an increase from 0.8 million ha in 1997 to 5.3 million ha in 2000 (James 2001). According to Frisvold and Tronstad (2001), the U.S. share of world *Bt* cotton acreage was 100% in 1996, but 75% in 2000.

Kalaitzandonakes and Suntornpithug (2001) address the question of why farmers adopt biotechnology-derived cotton. Primarily, adoption rates are driven by the effectiveness of biotechnology-derived crops to control target pests, decrease costs, and reduce production risks. Gianessi and Carpenter (1999) also examined factors driving adoption. They show that biotechnology adoption is based on economic benefits derived from decreased pesticide use and increased yields. Falck-Zepeda et al. (2000) and Frisvold et al. (2000) expand on the distribution of the economic benefits realized through biotechnology adoption. These are summarized by James (2001) and shown in Table V-14.

According to Kalaitzandonakes and Suntornpithug (2001), previous studies overlooked the dynamic effects of the adoption process including the possibility of substitution between technologies and the effects of adopted biotechnology on other pest control methods. Their study indicated that the adoption of Bollgard cotton may reduce production costs, improve pest control, decrease production risk, and allow adopters of the technology to capitalize on the sum of these individual effects on other technologies and inputs used in farming systems. For example, herbicide-resistant cotton facilitates adoption of alternative tillage practices. Although decreased production costs and risks, and the effectiveness of pest control, are primary reasons for adoption, the environmental benefits go further than simply reducing chemical pesticide use. These benefits extend to soil and water quality improvements, human health, biodiversity preservation, and others. Furthermore, the innovations achieved through modern biotechnology have been more rapidly adopted than traditionally bred varieties, which take much more time to develop (Fernandez-Cornejo et al. 1999).

The gains in food production obtainable through modern biotechnology and necessitated by increasing world population pressures contribute to the rapid adoption of biotechnology-derived crops in spite of the controversies surrounding modern biotechnology (Feldman et al. 2000; Mooney and Klein 2000; Reed et al. 1999). Pray and colleagues (2001) conducted a study, focusing on China, of the debate regarding biotechnology adoption in developing countries. The study was conducted to evaluate whether or not adoption of biotechnology-derived crops will help to alleviate world food problems, impact farmers' income, and decrease pollution. Because China's principal biotechnology-derived crops are tobacco and cotton, the direct impact on food production in China due to biotechnology adoption so far is nil. However, the study does show that small farmers, even some of the smallest, receive increased incomes from adopting *Bt* cotton. Similar results are observed among small farmers in South Africa (Bennett 2001).

Huang et al. (2002) provide a list of biotechnology-derived crop varieties that have been commercialized or are currently in trials in China. The list includes several food crops including staples such as rice, maize, wheat, and several fruits and vegetables. Developments are primarily focused on improving production efficiency and reducing chemical pesticide use. China, with the second largest biotechnology industry in the world, is likely to rapidly expand plantings of biotechnology-derived crops.

In 1998, Australia grew 80,000 ha of *Bt* cotton, an increase of approximately 20% from 1997. Mexico grew approximately 40,000 ha of *Bt* cotton, an increase from 15,000 ha the previous year. Also, 1998 was the first year Mexico grew the stacked *Bt*/herbicide-tolerant cotton on an estimated 1000 ha. Traxler et al. (2001) discuss adoption rates in Mexico specific to regions of interest. The Mexican government instituted a 40% limit on the amount of biotechnology-derived cotton that can be planted in the country (Gonzalez-Garcia et al. 2001). South Africa grew biotechnology-derived cotton for the first time in 1998 with an estimated 12,000 ha of *Bt* cotton being planted. Also in 1998, China and Argentina grew biotechnology-derived cotton for the first time. Estimated plantings for 1998 were 63,000 ha of *Bt* cotton in China and 8,000 ha of *Bt* cotton in Argentina (James 1998).

In 2000, Australia’s planting of biotechnology-derived cotton increased to an estimated 150,000 ha. China’s area increased to 0.5 million ha, a 250% increase from the previous year (ICAC Recorder 2000b; James 2001). China’s biotechnology-derived crop area increase was the largest relative change, increasing threefold from less than 0.1 million ha in 1998 to approximately 0.3 million ha in 1999 – equivalent to 1% of global share (James 2000). For a provincial breakdown of China’s *Bt* cotton area see Pray and colleagues (2001). The area estimated to be *Bt* cotton was between 300,000 ha and 1,000,000 ha out of an estimated 3,726,000 ha of total cotton area in 1999. Another study determining adoption determinants and economic impacts of *Bt* cotton was conducted by Ismael et al. (2001) in Africa. Their study indicates that only 12% of farmers in the region adopted the biotechnology-derived cotton. However, the major determinant to adoption of *Bt* cotton was farm size. Fifty-seven percent of the farmers having greater than 10 ha adopted *Bt* cotton compared with 7% of those with less than 2.5 ha. The authors indicate, however, that due to the short period of time addressed in the study no definitive conclusions of adoption dynamics can be drawn.

Biotechnology-derived cotton has been approved for commercial planting in the above-mentioned countries. Despite vocal opposition against biotechnology-derived cotton, adoption through unapproved means is becoming commonplace in some parts of the developing world. The primary motivations for adoption of biotechnology-derived cotton prior to local regulatory approval has been attributed to the farmers’ perceived and realized economic, environmental and worker safety benefits. In particular, biotechnology-derived cotton is rapidly entering into farm operations prior to receipt of local approval in several countries in Asia – India, Thailand, and Indonesia (Buffin and Jewel 2001). Thailand field tested *Bt* cotton in 1997 from seeds brought into the country in 1995. However, although *Bt* cotton has not been commercialized in Thailand, studies in 1999 demonstrated the presence of *Bt* cotton on farms outside the approved test sites. Opposition groups pressured the government to institute a ban on testing and commercial release of the *Bt* cotton in Thailand and Thailand has declared itself a “GMO-free” country. In spite of the ban, planting of *Bt* cotton continues in Thailand.

India is the third-largest cotton producer in the world. The approval of biotechnology-derived cotton cultivars in early 2002 by the Indian government demonstrates that farmer demand for the technology was outpacing the ability of regulatory institutions to manage the early adoption process. The original commercial release scheduled for June 2001 was stalled by pressure groups. However, it was discovered that *Bt* cotton had been sold as a hybrid in some regions of India for three years. It is estimated that *Bt* cotton is planted prior to regulatory approval in India on 10,000 ha. and similar pre-approval planting of *Bt* cotton has occurred in Indonesia (Buffin and Jewel 2001; Prakash 2001). Monsanto, a life science company developing biotechnology-derived crops, brought *Bt* cotton into India in 1996 to work with a local seed producer to develop cotton varieties optimized for India. The *Bt* varieties were backcrossed into elite Indian cotton varieties. By July 2000, the seed producer received approval to plant 150 ha to produce seed for 85 field testing, which continues today.

Global area of biotechnology-derived crops and the breakdown of biotechnology-derived cotton varieties are given in Table VI-2**.** In 1996, total global biotechnology-derived crop area was an estimated 800,000 ha, of which *Bt* comprised an estimated 27%. Herbicide-tolerant cotton comprised less than 1% of total biotechnology-derived crop area. Stacked *Bt*/herbicide-tolerant cotton was not planted in 1996. By 2000, the global area of biotechnology-derived cotton increased to an estimated 44.2 million ha comprised of 3% *Bt* cotton, 5% herbicide-tolerant cotton, and 4% stacked gene cotton.

Table VI-2: Global Area of Biotechnology-derived Crops and Cotton’s Share

	Percent of Global Biotechnology-derived Area			
	Global Biotechnology-derived Acreage-Millions Hectares	*Bt* Cotton	Herbicide-tolerant Cotton	Stacked *Bt*/Herbicide-tolerant Cotton
1996	0.8	27	<1.0	0
1997	11	2	3	<1.0
1998	27.8	1	[a]	1.5
1999	39.9	3	4	2
2000	44.2	3	5	4

Source: James, 2001, 1999, 1998, 1997.
Note a included in stacked variety.

United States production

The United States is the second largest producer of cotton, and the largest producer of biotechnology-derived cotton. Between the years 1996 and 2000, estimated biotechnology-derived cotton area in the United States increased from approximately 1.8 million a. (0.7 million ha) by approximately 5,600 farmers in 1996 to an estimated 3.8 million ha in 2000 (James 2001; USDA-NASS 1995-2001). Over the same period total estimated cotton acreage planted in the United States for both Pima and Upland cotton increased from 14,633,500 a. in 1996 to 15,536,500 a. in 2000 (USDA-NASS 1995-2001). Cotton-producing states, with acreage planted and yields for 1995-2001, are given in Table VI-3. Table VI-4 shows the distribution of biotechnology-derived cotton planted in the United States in 2000 and 2001.

Table VI-3: United States Cotton Production By State; Planted Acres and 480-Pound Bales

Upland	000 Acres 1995	000 Bales 1995	000 Acres 1996	000 Bales 1996	000 Acres 1997	000 Bales 1997	000 Acres 1998	000 Bales 1998	000 Acres 1999	000 Bales 1999	000 Acres 2000	000 Bales 2000	000 Acres 2001
Al	590	492	520	789	535	550	495	553	565	625	90	540	600
AZ	365	793	315	778	320	820	250	608	270	716	280	0	280
AR	1170	1468	1000	1636	950	1730	920	1209	970	1428	960	1450	1050
CA	1170	2312	1000	2390	880	2200	650	1146	610	1580	775	2200	660
FL	110	107	99	130.4	100	135	89	81.5	107	114	130	00	120
GA	1500	1941	1340	2079	1440	1900	1370	1542	1470	1567	1500	1640	1500
KS	3.8	1	4.5	4.1	15	16.9	17	13.9	33	21.9	40	23	44
LA	1085	1375	890	1286	630	985	535	641	615	901	710	910	800
MS	1460	1841	1120	1876	985	1810	950	1444	1200	1731	1300	1730	1500
MO	462	513	390	591	380	580	370	350	380	472	400	540	400
NM	61	71	59	84	70	88	66.3	80.4	84	109	90	130	75
NC	805	798	721	1002	670	930	710	1026	880	816	930	1440	1050
OK	380	123	290	134	200	200	160	140	240	144	280	155	300
SC	348	376	284	455	290	400	290	350	330	281	300	380	310
TN	700	724	540	675	500	656	450	546	570	595	570	715	600
TX	6400	4460	5700	4345	5500	5300	5650	3600	6150	5050	6400	3950	6000
VA	107	137	103	159	101	139	92	145.1	110	142.8	110	159	105
US	**16717**	**17532**	**14376**	**18414**	**13566**	**18440**	**13064**	**13476**	**14584**	**16294**	**15365**	**16822**	**15394**
American Pima													
AZ	48.6	72.2	42	74.4	22	45	15.9	26.8	9	16.3	6	10.3	7
CA	115	224.5	165	375	185	420	200	352.8	240	602.7	145	350	190
NM	5	18.9	14	19	13	17	7.3	10	7.5	10.7	4.5	7.2	7
TX	36	52	37	60.1	32	55	105	52.7	33	44.6	16	30	16
US	**214.6**	**367.6**	**258**	**528.5**	**252**	**537**	**328.2**	**442.3**	**289.5**	**674.3**	**171.5**	**397.5**	**220**
All Types													
Al	590	492	520	789	535	550	495	553	565	625	590	540	600
AZ	413.6	865.2	357	852.4	342	865	265.9	634.8	279	732.3	286	770.3	287
AR	1170	1468	1000	1636	950	1730	920	1209	970	1428	960	1450	1050
CA	1285	2536.5	1165	2765	1065	2620	850	1498.8	850	2182.7	920	2550	850
FL	110	107	99	130.4	100	135	89	81.5	107	114	130	100	120
GA	1500	1941	1340	2079	1440	1900	1370	1542	1470	1567	1500	1640	1500
KS	3.8	1	4.5	4.1	15	16.9	17	13.9	33	21.9	40	23	44
LA	1085	1375	890	1286	630	985	535	641	615	901	710	910	800
MS	1460	841	1120	1876	985	1810	950	1444	1200	1731	1300	1730	1500
MO	462	513	390	591	380	580	370	350	380	472	400	540	400
NM	76	89.9	73	103	83	105	73.6	90.4	91.5	119.7	94.5	137.2	82
NC	805	798	721	1002	670	930	710	1026	880	816	930	1440	1050
OK	380	123	290	134	200	200	160	140	240	144	280	155	300
SC	348	376	284	455	290	400	290	350	330	281	300	380	310
TN	700	724	540	675	500	656	450	546	570	595	570	715	600
TX	6436	4512	5737	4405.1	5532	5355	5755	3652.7	6183	5094.6	6416	3980	6016
VA	107	137	103	159	101	139	92	145.1	110	142.8	110	159	105
US	**16931**	**17900**	**14634**	**18942**	**13818**	**18977**	**13393**	**13918**	**14874**	**16968**	**15537**	**17220**	**15614**

Source: USDA-NASS 1995, 1996, 1997, 1998, 1999, 2000, 2001b

Table VI-4: Biotechnology-derived Upland Cotton Varieties In the United States By State 2000-2001 (percent of upland cotton acreage planted)

	Insect Resistance		Herbicide Tolerant		Stacked	
	2000	2001	2000	2001	2000	2001
AR	33	21	23	16	14	35
CA	3	5	17	17	4	2
GA	18	12	32	29	32	36
LA	37	39	13	10	30	37
MS	29	16	13	16	36	54
NC	11	6	29	22	36	43
TX	7	6	33	38	6	3
Other States	17	19	21	25	36	27
US	15	13	26	28	20	23

Source: USDA-NASS 2001b

Table VI-5: Summary of On-Farm Bollgard Comparisons in Mississippi

	1995			1996			1997		
	Bollgard		non-Bollgard	Bollgard		non-Bollgard	bollgard		non-Bollgard
Lint Yields (lbs/ac)	969		877	894		848	984		900
Insect Control Costs ($/ac)[1]	$71.31		$94.01	$63.35		$58.16	$84.42		$82.55
Return $/ac[2]									
Bollgard Advantage		$82.50			$24.71			$53.73	

Source: Reproduced from Wier, A.T., J. Walt Mullins and Jane M. Mills. 1998

1 Control costs include $32.ac technology fee for Bollgard

2 (yield x 0.65/lb lint)

Table VI-6: Summary of On-Farm Bollgard Comparisons Over 3 Cotton Production Regions, 1996-98

	Southeast			Delta			East[3] Texas		
	Bollgard		non-Bollgard	Bollgard		non-Bollgard	Bollgard		non-Bollgard
Lint Yields (lbs/ac)	933		819	964		919	543		489
Insect Control Costs ($/ac)[1]	$46.13		$26.56	$79.99		$86.27	$65.61		$41.53
Return $/ac[2]	$560.32		$505.79	$546.61		$511.08	$287.34		$276.32
Bollgard Advantage		$54.53			$35.53			$11.02	

Source: Reproduced from Wier, A. T, J. Mullins and Mills. 1998

1 Control costs include $32.ac technology fee for Bollgard

2 (yield x 0.65/lb lint)

3 1996 and 1997 only

Several studies of the economic impact of biotechnology-derived cotton have been conducted. Stark (1997) conducted an economic study of biotechnology-derived cotton in Georgia. The results indicated that *Bt* cotton outperformed the non-*Bt* variety with a high yield of 1,378 pounds of lint compared with a high of 1,239 pounds for the non-*Bt* cotton. The low yield of *Bt* cotton was 746 pounds of lint compared with 706 for the non-*Bt* cotton. The average yield across all field sites of *Bt* cotton was 1,027 pounds, and 923 pounds for the non-*Bt* cotton. The number of pesticide spray applications per acre for the *Bt* cotton was a high of 5, and for the non-*Bt* cotton, 10. The fewest number of sprays on the *Bt* cotton was zero compared with one for the non-*Bt* cotton. The average number of sprays across all sites on the *Bt* cotton was 1.1, and the average for non-*Bt* fields was 3.6. The *Bt* fields had a yield advantage of $72.80 per acre and a pesticide spray advantage of $27.50 per acre, for a total production cost advantage of $100.30.

Wier, Mullins, and Mills (1998) indicated that in a study in Mississippi over three years, Bollgard had an economic advantage over the non-Bollgard cotton. For the three-year study, Bollgard cotton had greater lint yields than the non-Bollgard cotton. Interestingly, the Bollgard varieties in year two and three actually had greater costs associated with insect control. Nevertheless, the economic advantage for the three years 1995-97 for Bollgard cotton was $82.50, $24.71, and $53.73, respectively. These results are shown in Table VI-5.

In the same study, Bollgard comparisons were done over three regions and averages for the three years were compared. The three regions were the Southeast, the Mississippi Delta, and East Texas. The Southeast includes Alabama, Florida, and Georgia; the Delta includes Mississippi, Arkansas, and Louisiana; and East Texas includes 5 picker regions –Rio Grande Valley, coastal Bend, Upper Gulf Coast, Brazos River Bottom, and the Blacklands. The results indicated that cotton lint yields (the average over the three years) were greater for Bollgard cotton in each of the three regions. The economic advantage again favored the Bollgard cotton by $54.33, $35.53, and $11.02 for the Southeast, Delta, and East Texas, respectively. Summary results of this study are given in Table VI-6.

Cooke et al. (2001) conducted a study for the years 1997 to 2000 that yielded a different story. The results represent costs associated with high tobacco budworm pressure comparing Bollgard to traditional cotton. Yields for the Bollgard cotton were less for the entire four years relative to the non-Bollgard cotton. Insect control costs were greater for the *Bt* cotton in years 1997, 1999, and 2000. And, yields were greater for traditional over *Bt* cotton for all four years. These results are summarized in Table VI-7and Table VI-8 showing the four-year averages for the traditional and *Bt* cotton. Developing sustainable, cost-efficient strategies for managing cotton insects is discussed in Reed et al. (1999). They compare lint yields, insect control costs, and economic benefits for biotechnology-derived and traditional cotton varieties in field-size plots in five Mississippi counties over two years. Results show that *Bt* cotton provides a greater return than traditional cotton in years of high tobacco budworm pressure, and lower returns in years of low tobacco budworm pressure. Gibson et al. (1997), Rejesus et al. (1997a, b), Spense et al. (1999), Magana et al. (1999), Stark (1997), and Cavin et al. (2001) all report on economic studies comparing biotechnology-derived and traditional cotton. Each study found that the biotechnology-derived cultivars provided economic benefits superior, at least on average, to the traditional varieties. Though there are conditions under which traditional cotton may be preferred, the consensus of these studies is that biotechnology-derived cultivars show significant promise for higher economic returns.

Table VI-7: Economic Data for Traditional & Bt Cotton 1997-2000

Item	Yield lb/Acre	Total Specified Insect Cost ($U.S./acre)
1997		
Traditional	981	$85.40
Bt	965	$91.34
Difference	16	($5.93)
1998		
Traditional	906	$126.99
Bt	902	$97.85
Difference	4	$29.14
1999		
Traditional	802	$70.09
Bt	799	$79.14
Difference	3	($9.05)
2000		
Traditional	829	$80.45
Bt	820	$83.97
Difference	9	($3.52)

Source: Reproduced from Cooke, F. T. et al. 2001

Table VI-8: Traditional & Bt Cotton Economic Data, 4-Year Averages

Item	Yield (lb/Acre)	Total Specified Insect Cost ($U.S./acre)
Traditional		
1997	981	$85.40
1998	906	$126.99
1999	802	$70.09
2000	829	$80.45
Average	879	$90.73
Bt		
1997	965	$91.34
1998	902	$97.85
1999	799	$79.14
2000	820	$83.97
Average	871	$88.07
Difference		
Traditional	879	$90.73
Bt	871	$88.07
Difference	8	$2.66

Source: Reproduced from Cooke, F. T. et al. 2001

Tables VI-9 and VI-10 give estimated world cotton yield comparisons for five geographic regions, as well as world totals and U.S. totals, for the years 1992 to 2000. The five regions are: North and Central America – the United States, El Salvador, Guatemala, Honduras, Mexico, Nicaragua, Costa Rica, Cuba, Dominican Republic, and Haiti; South America – Argentina, Bolivia, Brazil, Colombia, Ecuador, Paraguay, Peru, and Venezuela; Europe – Greece, Italy, Spain, Bulgaria, Yugoslavia, and Albania; Africa – Angola, Benin, Burkina, Cameroon, Central African Republic, Chad, Cote d'Ivoire, Egypt, Ethiopia, Ghana, Kenya, Madagascar, Mali, Morocco, Mozambique, Niger, Nigeria, Senegal, Somalia, South Africa, Sudan, Tanzania, Togo, Tunisia, Uganda, Zaire, Zambia, and Zimbabwe; and Asia/Mideast/Oceania – Afghanistan, Bangladesh, Burma, China, India, Indonesia, Korea (South and North), Malawi, Pakistan, Philippines, Sri Lanka, Thailand, Vietnam, Cyprus, Iran, Iraq, Israel, Syria, Turkey, Yemen, Australia, Republic of Azerbaijan, Republic of Kazakhstan, Republic of Kyrgyzstan, Republic of Tajikistan, Republic of Uzbekistan, and Turkmenistan.

Table VI-9: Estimated World Cotton Yield Comparisons By Region and Years 1992-2000 (Pounds/Acre)

Year	United States	World	North/ Central America	South America Europe	Europe	Africa	Asia/ Middle East Oceania
1992	700	601	652	356	866	302	542
1993	606	552	697	334	838	341	463
1994	708	542	605	365	842	362	449
1995	537	579	704	366	879	334	478
1996	705	563	539	422	895	341	515
1997	673	574	711	372	693	353	501
1998	625	591	682	377	859	347	532
1999	607	561	639	434	848	301	510
2000	631	591	613	594	912	290	543

Source: Compiled from USDA-NASS 2001, 2000, 1999, 1998, 1997, 1996, 1995, 1994

Table VI-10: Estimated World Cotton Yield Comparisons By Region and Years 1992-2000 (kg/Hectare)

Year	United States	World	North/ Central America	South America	Europe	Africa	Asia/ Middle East /Oceania
1992	785	601	731	399	971	339	607
1993	679	552	781	374	939	382	519
1994	794	542	678	409	944	406	503
1995	602	579	789	410	985	374	536
1996	790	563	604	473	1003	382	577
1997	754	574	797	417	777	396	561
1998	701	591	764	423	963	389	596
1999	680	561	716	487	950	337	572
2000	707	591	687	666	1022	325	609

Source: Compiled from USDA-NASS 2001, 2000, 1999, 1998, 1997, 1996, 1995, 1994

Estimated yield comparisons for the six biotechnology-derived cotton-producing countries – the United States, China, Australia, Argentina, Mexico, and South Africa – for the years 1992 to 2000 are given in Tables VI-11 and Table VI-12. Australia has consistently produced higher yields than the other biotechnology-derived cotton producing countries.

A common perception is that biotechnology-derived crops are not beneficial to consumers, particularly when evaluating biotechnology-derived cotton with input trait characteristics introduced. However, Falck-Zepeda et al. (1999, 2000) conducted an analysis of *Bt* cotton for the period 1996-1998 determining the rents created by *Bt* technology. A summary of the results of these studies is reproduced from James (2001) in Table VI-13. Of the total economic surplus generated, $240 million, from adoption of *Bt* cotton in the United States in 1996, an estimated $142 million (59.16%) went to farmers, $50 million (20.83%) to the developers of the technology, and $12 million (5%) to the seed suppliers. Consumers received an estimated $22 million (9.16%) and the rest of the world received $14 million (5.83%). The results for 1997 are depicted in the same table. Another study conducted by Pray and colleagues (2001) determines the economic advantage to small farmers in China. Eighty-three percent of the gains to biotechnology-derived cultivars accrued to cotton producers. The results of this study are also reproduced here from James (2001) in Table VI-14.

Table VI-11: Estimated Yield Comparisons of Countries Growing Biotechnology-derived Cotton, 1992-2000 (Pounds/Acre)

	United States	China	Australia	Argentina	Mexico	South Africa
1992	700	775	588	385	646	234
1993	606	588	1270	398	638	253
1994	708	668	1112	436	706	294
1995	537	699	1346	446	629	304
1996	705	784	1271	390	595	310
1997	673	794	1370	329	850	238
1998	625	913	1357	321	956	341
1999	607	902	1137	275	926	340
2000	631	917	1423	361	855	313

Source: Compiled from USDA-NASS 2001, 2000, 1999, 1998, 1997, 1996, 1995, 1994

Table VI-12: Estimated Yield Comparisons of Countries Growing Biotechnology-derived Cotton, 1992-2000 (kgs/Hectare)

	United States	China	Australia	Argentina	Mexico	South Africa
1992	785	869	1780	431	724	262
1993	679	659	1424	446	715	284
1994	794	749	1246	489	791	329
1995	602	784	1509	500	705	341
1996	790	879	1425	437	667	348
1997	754	890	1535	369	953	267
1998	701	1023	1521	360	1071	382
1999	680	1011	1274	308	1038	381
2000	707	1028	1595	405	958	351

Source: Compiled from USDA-NASS 2001, 2000, 1999, 1998, 1997, 1996, 1995, 1994

Table VI-13: Distribution of Economic Surplus Associated with Bt Cotton Planted in the United States in 1996 to 1997

	1996		1997	
Beneficiary	**$ million**	**% total**	**$ million**	**% total**
US Farmer Surplus	142	59	80	42
Technology Developer	50	21	67	35
Seed Supplier	12	5	18	9
US Consumer	22	9	14	7
Net Rest of World	14	6	11	7
Total Surplus	240	100	190	100

Source: Reproduced from James, C. 2001

Table VI-14: Distribution of Share (percent) of Economic Surplus from Biotechnology-derived Bt Cotton for Different Stakeholders

	***Bt* Cotton 1996 United States**	***Bt* Cotton 1997 United States**	***Bt* Cotton 1998 United States**	***Bt* Cotton 1997 Mexico**	***Bt* Cotton 1998 Mexico**	***Bt* Cotton Public 1999 China**	***Bt* Cotton Private 1999 China**
Farmer Surplus	59	42	46	61	90	83	83
Technology Developer	21	35	34	31	8	NA	12
Seed Supplier	5	9	9	8	2	17	NA
US Consumer	9	7	7	NA	NA	NA	NA
Net Rest of World	6	7	4	NA	NA	NA	NA

Source: reproduced from James, C. 2001

CONCLUSIONS

Several general conclusions can be drawn from the information gleaned from the available scientific literature on biotechnology-derived cotton.

- Herbicide-tolerant cotton permits the use of herbicides that are less persistent in the environment, substituting for others that are more persistent.
- Herbicide-tolerant cotton is a major factor in allowing greater implementation of conservation tillage practices, thus decreasing energy use and soil loss through erosion, improving water quality, and other positive environmental impacts.
- Herbicide-tolerant cotton increases the flexibility and reliability of cotton weed management.
- Biotechnology-derived insect-resistant cotton technology is highly transferable to developing nations because it does not require significant capital investment, changes in cultural practices, or training for adoption.
- Rapid adoption of *Bt* cotton in China serves as an example of how, in developing nations, a plant-incorporated protectant greatly reduces the volume of pesticides applied and the risks of pesticide runoff while increasing agricultural worker safety and health.
- Studies in the U.S. Cotton Belt and in Australia show that *Bt* cotton has a positive impact on beneficial insects in cotton fields, thus promoting biodiversity.
- The introduction of *Bt* cotton in Australia, China, and the United States demonstrates the ability of these varieties to alleviate problems with insect resistance to chemical pesticides. The future production of cotton in some regions of these countries was in jeopardy prior to the introduction of *Bt* cotton.
- The ability to add several different genes to control the same pest should delay the time it takes for pesticide resistance to develop.
- *Bt* and herbicide-tolerant cotton reduces production costs to farmers, increases efficiency, reduces risks, and increases the range of options available to whole-farm management systems.

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[1] Allotetraploids have four times the number of chromosomes normally occurring in the mature germ cell.

[2] Micronaire is a measurement of fiber fineness and maturity.

[3] Staple® (trademark DuPont) is an herbicide with active ingredient sodium 2-chloro-6-(4,6-dimethoxypyrimidin- 2-ylthio)benzoate used in conjunction with glyphosate on glyphosate-tolerant cotton for improved control of morningglory and broadleaf weeds.

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Appendix I: Glossary

Acre. 4840 square yards (220 by 22 yards). One acre equals 0.4047 hectares.

Amino acids. The fundamental building blocks of a protein molecule. A protein is a chain of hundreds or thousands of amino acids. Our bodies can make most of the amino acids from their component parts (carbon, nitrogen, oxygen, hydrogen, and sometimes sulfur.) However, eight amino acids (called essential amino acids) must be obtained from food.

Backcrossing. Mating of a hybrid to one of its parents.

Beneficial insect. Species providing control of pests such as insects and mites, of which the beneficial insect is a natural enemy.

Biotechnology. The tools and technology that are used to make products from biological systems (cheese making), to carry out processes using biological substances (enzyme-based processing such as wine making), or to modify biological systems in order to improve performance or produce bio-materials (breeding, tissue culture, cloning, transgenics).

Biotechnology-derived. Term for the use of molecular biology and/or recombinant DNA technology, or in vitro gene transfer, to develop products or to impart specific capabilities in plants or other living organisms.

***Bt*, *Bt* toxins, and *Bt* proteins.** Crystalline compounds produced by the bacteria *Bacillus thuringiensis* that are toxic to select insect orders: *lepidoptera*, *diptera*, and *coleoptera*. Genes encoding *Bt* toxins have been transferred to plants to confer protection from insects. *Bt* toxins (*Bt* proteins) are not harmful to humans.

Buffer strip. A strip of land often comprising windbreaks or hedgerows where disturbance is not allowed or is closely monitored to enhance and preserve environmental quality and to control non-point source pollution.

Conservation tillage. Method of preparing the soil; comprises tillage methods such as no-till, reduced till, and minimum till, which differ from each other in the degree to which soil disturbance occurs before planting. Conservation tillage leaves at least 30% of soil covered by crop residues; designed to minimize soil compaction and erosion by wind and water.

Cultivar. Term synonymous with variety; the international equivalent of variety.

DNA (deoxyribonucleic acid). The double-stranded molecule that encodes genetic information. It is made up of four different kinds of bases, which are abbreviated A, C, T, and G. A DNA fragment that is 10 bases long might have a sequence of, for example, ATCGTTCCTG. The particular sequence of bases encodes important information in an individual's genetic blueprint and is unique for each individual (except identical twins).

Endotoxin. A complex bacterial toxin composed of protein, lipid, and polysaccharide, which is released only when the cell opens to release the cell contents.

Gene. The fundamental unit of heredity. A gene is an ordered sequence located in a particular position on a particular chromosome that provides the code for a specific function or trait.

Gene expression. The process by which a gene's coded information is converted into the structures present and operating in the cell.

Gene flow. The exchange of genetic traits between populations by movement of individuals, gametes, or spores. It involves the spread of new gene variants among different populations through dispersal. Gene flow and mutation are, therefore, the only means by which new genetic factors may be introduced into a population.

Gene mapping. Determination of the relative positions of genes on a DNA molecule and in the genome.

Gene transfer, horizontal. Horizontal gene transfer consists of transfer of genetic material from one living thing to another, without the second living thing being the offspring of the first. By contrast, vertical gene transfer refers to transfer of genetic material from parent to offspring.

Genetic engineering. The technique of removing, modifying, or adding genes to a DNA molecule in order to change the information it contains. By changing this information, genetic engineering (also referred to as modern biotechnology) changes the type or amount of proteins an organism is capable of producing.

Genetically modified organism (GMO). Currently, the label GMO and the term "transgenic" are used to refer to organisms that have acquired novel genes from other organisms by laboratory "gene transfer" methods. The term GMO is a poor label for transgenic organisms because all organisms are genetically modified. When a plant or animal species breeds with another one, genetic material is exchanged and results in the offspring. This can happen in the natural reproduction process or in a lab process. More precisely referred to as biotechnology-derived.

Genetics. The study of the patterns of inheritance of specific traits.

Genome. All the genetic material in each organism.

Glufosinate. Common name for the herbicide Liberty™ ; 2-amino-4(hydroxymethylphosphonyl)butanoic acid. It provides both broadleaf and grass weed control and is non-selective in activity, killing the majority of plant species with which it comes in contact.

Glyphosate. N-(phosphonomethyl)glycine; herbicide marketed under trade names Roundup®, Rodeo®, and Accord®. It controls broadleaf and grass weeds and is non-selective in activity, i.e. , toxic to a majority of plant species with which it comes in contact.

Greenhouse gas. A gas that contributes to the natural "greenhouse effect (warming of the atmosphere due to the reduction in outgoing solar radiation resulting from concentrations of gases such as carbon dioxide)." The Kyoto Protocol covers six greenhouse gases (GHGs) produced by human activities: carbon dioxide, methane, nitrous oxide, hydrofluorocarbons, perfluorocarbons, and sulphur hexafluoride. An important natural GHG that is not covered by the Protocol is water vapor.

Hectare. 10,000 square meters. One hectare equals 2.47 acres.

Herbicide resistance (or herbicide tolerance). See herbicide tolerance.

Herbicide tolerance. The inherent or acquired ability of a plant to survive and reproduce following an exposure to a dose of herbicide that would normally be lethal to the targeted plants.

Herbicide-tolerant. Crop plants, cultivated by people, that have been enhanced to be able to survive application(s) of one or more commercially available herbicides by the incorporation of certain gene(s) via biotechnology methods (i.e., genetic engineering) or traditional breeding methods (i.e., natural, chemical, or radiation mutation).

Hybrid. Seed or plants produced as the result of controlled pollination as opposed to seed produced as the result of natural pollination.

Insect resistance. Resistance is the result of selection, a process whereby a few insects in the population with genes of specific resistance mechanisms survive the insecticide sprays and multiply, thereby increasing the proportion of resistant insects in the population. Resistance development in insects threatens the high benefits and low risks of using Bt toxins in transgenic crops and in microbial spray formulations.

Instar. Any of various life stages of an insect or other arthropod.

Isoflavone. Nutrients found in soybean.

Low-till or low-tillage farming. Method of preparing the soil for planting, which reduces intensive and repeated plowing of fields.

Mendelian inheritance. Inheritance of genes following the patterns of segregation and independent assortment as described by Gregor Mendel. Also referred to as classical inheritance.

Mutagenesis. The introduction into a gene of an alteration that results in a change in the structure or function of the gene product.

Mutation. A genetic change giving rise to heritable variations.

Nontarget organism. Any plant or animal other than the one a pesticide or plant-incorporated protectant is meant to control.

No-tillage crop production. A method of crop production in which the farmer uses virtually no mechanical cultivation: only one pass over the field with a planter instead of the conventional four passes per year with a mechanical cultivator plus one pass with a planter, as used for traditional crop production. This reduction in field soil disturbance leaves more carbon in the soil (thereby reducing greenhouse gasses in the atmosphere), leaves more earthworms (*Eisenia foetida*) per cubic foot living in the topsoil, and reduces soil compaction (thereby increasing the fertility of such no-till farm fields).

Outcrossing. The transfer of a given gene or genes from a domesticated organism to a wild type (plant relative).

Plant-incorporated protectants. Formerly referred to as plant-pesticides, plant-incorporated protectants (PIPs) are substances that act like pesticides, which are produced and used by a plant to protect it from pests such as insects, viruses, and fungi.

Plasmid. A small, self-replicating piece of DNA found outside the chromosome. Plasmids are the principal tools for inserting new genetics information into microorganisms or plants.

Postemergence herbicide (POST). Herbicides applied after the plant emerges from the soil, thus providing farmers the opportunity to determine which weed species are present before herbicide application.

Pre-emergence herbicide (PRE). Herbicides applied after planting, but before the crop appears above ground.

Protein. A complex biological molecule composed of a chain of units called amino acids. Proteins have many different functions: structure, movement, catalysis, transport, regulation of cellular processes, and response to stimuli. Protein function is dependent on the protein's three-dimensional structure, which is dependent upon the sequence of amino acids in the protein. The information for making proteins is stored in the sequence of nucleotides in the DNA molecule.

Proteinase. An enzyme that cleaves the peptide bonds of protein backbones.

Pyramiding or pyramided genes. Breeding or engineering two or more genes for the same trait into one cultivar or hybrid. The objective is usually to get better or more stable or durable resistance to a disease or pest. For example, Bollgard II cotton has two *Bt* genes pyramided into it.

Recombinant DNA technology. Procedure used to cut and join together DNA segments in a cell-free system (an environment outside a cell or organism). Under appropriate conditions, a recombinant DNA molecule can enter a cell and replicate there, either autonomously or after it has become integrated into a cellular chromosome.

Reference dose. Formerly known as the acceptable daily intake or ADI. The reference dose represents the maximum daily human exposure to a pesticide that results in no appreciable risk. The reference dose for each pesticide is determined from the no-observed-effect-level (NOEL) multiplied by a safety factor, where NOEL is the maximum dose level (amount of pesticide/amount of body weight/day) at which no effects attributable to the pesticide under examination can be found.

Refuge. An area in which a species can survive during difficult periods. In terms of insect resistance management for *Bt* crops, an area planted to non-*Bt* varieties.

Resistance management. Planning to ensure continued effectiveness of both the plant-incorporated protectant and the applied formulation of *Bt* proteins.

Roundup Ready. Species of plants that have been developed through modern biotechnology methods to withstand the applications of the herbicide Roundup or glyphosate are referred to as Roundup Ready. Because Roundup is non-selective (i.e., kills many plants), producers have to be careful not to get it on plants they want to grow.

Stacking or stacked genes. Breeding or engineering two or more genes for different traits into one cultivar or hybrid. For example, these days stacked cotton is both insect-resistant and herbicide-resistant.

Transgenic. Containing genes altered by insertion of DNA from an unrelated organism. Taking genes from one species and inserting them into another species in order to get that trait expressed in the offspring. The term “GMO” is often used mistakenly when “transgenic” or “biotechnology-derived” products are the intended reference.

Triazine. Triazine is an herbicide family that includes such herbicides as atrazine and simazine. Triazine herbicides control weeds by disrupting their photosynthesis ability. Atrazine is used for weed control in corn, sorghum, and sugarcane. Simazine is used in high-value crops, including citrus, nuts, vegetables, and ornamentals. Both herbicides control several broadleaf and certain grass weeds.

Variety. Subdivision of a species for taxonomic classification. Used interchangeably with the term cultivar to denote a group of individuals that is distinct genetically from other groups of individuals in the species. An agricultural variety is a group of similar plants that by structural features and performance can be identified from other varieties within the same species.

Weed. Simply any plant growing where it is not wanted. In agriculture, term used for a plant that has good colonizing capability in a disturbed environment and that usually can compete with a cultivated species therein. Weeds are typically considered as unwanted, economically useless, or pest species.

Weediness or crop weediness. The term weediness is used principally (1) to describe the degree to which a cultivated field is occupied by weeds; (2) to indicate the potential for a crop to transfer properties to a native plant and weed species that would make the native variety a pest problem or affect control of native weed species; and (3) to describe some plants and crops having properties that make them more likely to become weeds than other plants. Such properties include long-lived seeds that don't all germinate at the same time, rapid seedling growth, high tolerance to changes in environment, ability to grow in different environments, aggressive competition with other plants, continuous production of new seeds, production of a large number of seeds, and ability to disperse its seeds long distances. "There is an important distinction between increased *weediness* and increased *fitness*, however. Fitness is the ability of a plant to respond better to its environmental stresses and to be more successful at making viable seeds. Many of the traits presently genetically engineered into plants *do* increase the fitness of the plant— such as resistance to insects, viral disease, and herbicides— but *do not* affect the weediness of the plant. *Bt* corn, for example, is much more resistant to certain insects than non-*Bt* corn, but this improvement does not help corn overcome all of the other seed dispersal and growth habit traits that prevent corn plants from spreading out into the wild."
(http://www.comm.cornell.edu/gmo/issues/weeds.html)

Whole-farm systems. Systems that pull together decision making about environmental, economic, and production concerns on a farm-wide basis.

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Appendix II: Abbreviations, Acronyms, and Symbols

a.	acre
ACC	acetic coenzyme A carboxylase
ai	active ingredient
ALS	acetolactate synthase
AMPA	aminomethylphosphonic acid
APHIS	USDA Animal and Plant Health Inspection Service
ASA	American Soybean Association
BPMV	bean pod mottle virus
Bt	*Bacillus thuringiensis*
BXN	Bromoxynil-tolerant
CAAS	China Academy of Agricultural Sciences
CaMV	Cauliflower mosaic virus
CB	carbamate
CCRI	Central Cotton Research Institute
CDC	Centers for Disease Control and Prevention
CEW	corn earworm
CIMMYT	International Maize and Wheat Improvement Center
CLA	corn leaf aphid
CMPP	Mecoprop
CO_2	carbon dioxide
CP4 EPSPS	5-enol-pyruvylshikimate-3-phosphate synthase isolated from *Agrobacterium* sp. strain CP4
CRW	corn rootworm
Cry	crystalline
CSIRO	Commonwealth Science and Industrial Research Organization
CTP4	Chloroplast transit peptide 4
d	day
D&PL	Delta and Pineland
DCI	data call-in
DIMBOA	Hydroxamic acids, Hxs (4-hydroxy-1,4-benzoxazin-3-ones), including 2, 4-hydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one
DNA	deoxyribonucleic acid
DT_{50}	degradation time 50% is the length of time it takes for a pesticide active ingredient to transform (break down, degrade) to 50% of its initial concentration in soil
E35S	Cauliflower mosaic virus (CaMV) 35S promoter containing a duplication of the -90 to -300 bp region
EC	European Commission
ECB	European corn borer

EEC	expected environmental concentration
EIL	economic injury levels
ELISA	enzyme linked immunosorbent assay
ELS	Extra Long Staple
EPA	U.S. Environmental Protection Agency
EPSPS	5-enolpyruvylshikimate-3-phosphate synthetase
ERS	Economic Research Service
FAO	Food and Agriculture Organization of the United Nations
FDA	U.S. Food and Drug Administration
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
ft	feet
g	gram
g/cm^2	grams per centimeter squared
gal	gallon
GMAC	Genetic Manipulation Advisory Committee
GOX	glyphosate oxidoreductase
ha	hectare
hr	hour
HRAC	Herbicide Resistance Action Committee
HT	herbicide-tolerant
IPM	integrated pest management
IPSA	Independent Professional Seed Association
IRM	insect resistance management
kDa	kilo-Daltons
kg	kilogram
LC	lethal concentration
LD	lethal dose
L	liter
m	meter
MCL	maximum contamination level
MCPA	2-methyl-4-chlorophenoxyacetic acid
mg/kg	milligrams per kilogram
mL	milliliter
mmt	million metric tons
mo	month
mRNA	messenger ribonucleic acid

NASS	National Agricultural Statistic Service
NCR	northern corn rootworm
NOAEL	no observable adverse effect level
NOEC	no-observable-effect concentration
NOS	Nopaline synthase
NPTII	neomycin phosphotransferase II
NYU	New York University
OECD	Organisation for Economic Cooperation and Development
OP	organophosphate [also organophosphorus]
PAT	phosphinothricin acetyltransferase
PCR	polymerase chain reaction
PMRA	Pest Management Regulatory Agency of Canada
ppm	parts per million
PRA	probabilistic risk assessment
rDNA	recombinant DNA
RED	re-registration eligibility decision document
RR	Roundup Ready
SCN	soybean cyst nematode
SCR	southern corn rootworm
SMV	soybean mosaic virus
STS	sulfonylurea tolerant soybean
SU	sulfonylurea
SWCB	Southwestern corn borer
TPS	Technology Protection Systems
USDA	U.S. Department of Agriculture
USEPA	see EPA
WCR	western corn rootworm
WHO	World Health Organization of the United Nations
WSSA	Weed Science Society of American
yr	year

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