

Biotechnology and Plant-Incorporated Protectants: Saga of Starlink Corn

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1

Overview

- ◆ Biopesticides
 - History, regulations, modern applications
- ◆ Biotechnology and biopesticides
 - Plant-incorporated protectants (PIP's) for crop protection
 - ◆ *Bacillus thuringiensis* (Bt)
 - From biopesticide to biotechnology (plant incorporated protectant)
- ◆ Risk assessment from dietary exposure
 - Starlink corn as example

2

What is a Pesticide? What is a Biopesticide?

- ◆ Pesticide: a chemical intended to kill, injure, or repel a pest
 - Defined under FIFRA (Federal Insecticide, Fungicide, and Rodenticide Act)
 - ◆ Includes fungicides, insecticides, herbicides, rodenticides, antimicrobials, repellents
- ◆ Biopesticide: pesticides derived from natural materials
 - Regulated under FIFRA, FQPA (Food Quality Protection Act, 1996)
 - ◆ Plants
 - ◆ Microorganisms
 - ◆ Minerals
 - ◆ Animals



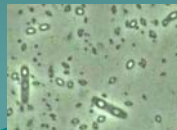
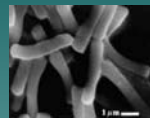
Classifications of Biopesticides

- ◆ Microbial biopesticides
 - *Bacillus thuringiensis*
 - *Aspergillus flavus*, strain AF 36
- ◆ Plant-incorporated protectants (PIP's)
 - Applications of biotechnology
 - ◆ Genetic incorporation of chemical resistance factors or microbial pest control factors into plant tissues
- ◆ Biochemical biopesticides
 - Naturally occurring chemicals
 - Mechanisms other than direct toxicity
 - ◆ Sex pheromones (interfere with mating)
 - ◆ Repellent chemicals



Microbial Biopesticides: *Bacillus thuringiensis* as Example

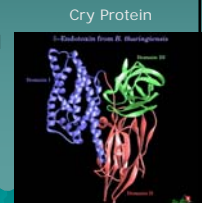
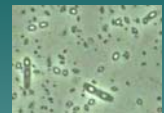
- ◆ Most widely utilized and studied microbial pesticide
- ◆ Gram-positive, motile, rod-shaped bacterium
 - Ubiquitous (naturally occurring)
 - In environment, exists as a dormant (spore) form
 - ◆ Forms protein crystals (Cry) in spore form
 - Registered for use by U.S. EPA as biopesticide in U.S. since 1961
 - ◆ Crop protection, forestry, greenhouses, vector control, outdoor residential uses



3

Bacillus thuringiensis (Bt) and Crystal Proteins (Insecticides)

- ◆ Cry proteins (produced by Bt)
 - a.k.a. *delta*-endotoxin
- ◆ Insecticidal properties are selective for the insect
 - Contrast with other insecticides
 - After ingestion, Cry proteins are cleaved to form active insecticidal protein
 - ◆ Binds to receptors in insect midgut
 - ◆ Leads to cessation of insect feeding
 - Reasons for selectivity
 - ◆ Receptor selectivity (insects)
 - ◆ Alkaline environment required for cleavage to active protein



Bt and Human Health Risks

- The toxicology pathway
 - Hazard** is ubiquitous
 - Exposure** (contact) is not unusual
 - Doses** are low (below threshold for response)
 - Results of mammalian, human studies
 - No effects at doses > 5,000 mg/kg Cry proteins
 - Contrast with other insecticides
 - Response** follows the dose
- Other (non-toxicological) risks from environmental, dietary exposure to Bt?

From Bt to Plant-Incorporated Protectant

- Applications of biotechnology
- Several important Bt genes encoding Cry proteins have been characterized
 - Amino acid sequences, protein structure
 - Modification of gene sequences can yield Cry proteins with enhanced insecticidal selectivity
 - Relevance of limiting target range
 - Can Cry genes be introduced into plants to confer protection against specific target pests?

From Bt to Plant-Incorporated Protectant: Biotechnology

- Methods of incorporation of Cry genes into plant genomes
 - Microprojectile bombardment
 - Gene directly delivered to host cells at high speed (penetrating nucleus)
 - Agrobacterium-mediated transformation
 - A. tumefaciens* (naturally occurring)
 - Plant pathogen
 - Plasmid gene of *A. tumefaciens* can incorporate into host genome, altering gene expression of the host
 - Cry genes can be introduced to this plasmid, and incorporated into host genome

Examples of Plant-Incorporated Protectants (Cry Proteins)

- Maize, potato, cotton
- Applications to corn
 - European corn borer as major pathogen of corn
- Modified Cry genes have been introduced into certain strains of corn (Bt corn)
 - Cry gene modifications are selective for the European corn borer
 - Incorporated gene remains stable in subsequent generations
 - Gene expression of Cry proteins (in target plant) is consistent when grown in different geographies
 - Highest expression in leaf of plant

Bt Corn in the United States

- Increasing utilization of Bt corn in U.S. agriculture since 1996
 - 1% in 1996 (.4 million acres)
 - 6% in 1997
 - 18% in 1998
 - 26% in 1999 (19.5 million acres)
- First Cry protein (as PIP) approved for use in corn (for human consumption) in 1995

Tissue	Leaf	Stem	Whole Plant ^{3,4}		Chlorophyll ⁵	
			100 mg DW	100 mg DW	100 mg DW	100 mg DW
Protein	1.10	0.10	1.10	0.00	1.70	0.10
DM of PIP	NA ⁶	NA ⁶	NA ⁶	NA ⁶	NA ⁶	NA ⁶
DM	NA ⁶	NA ⁶	NA ⁶	NA ⁶	NA ⁶	NA ⁶

¹ The tissues were analyzed from the samples of 10 plants sampled from each of the field sites, unless otherwise indicated.
² All values are expressed on a dry weight basis (by the weight of the sample fresh weight).
³ The DM values were calculated from analysis of one sample from one site.
⁴ The DM values were calculated from analysis of one sample from one site.
⁵ The DM values were calculated from analysis of one sample from one site.
⁶ Not available.

Bt corn: Risks and Benefits

- Effective pest control
- Reduced use of conventional pesticides
- Reduced risk of mycotoxin contamination?
 - Studies in Europe, United States
- Improved crop yields
- Pest resistance to Cry protein
- Effects on non-target organisms
- Transfer of gene to other plants
- Risk perception
 - Biotechnology and "Frankenfoods"
- Dietary risks from Cry proteins?

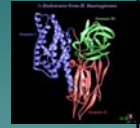
Risk/Benefits: Bt Corn and Mycotoxins

RISK ASSESSMENT		
Table 1. Studies demonstrating current events of Bt corn's control, or lack thereof, of fumonisin, aflatoxin, DON, and zearalenone in field trials.		
Bt corn lower mycotoxins than non-Bt isolines?	Yes	No
Fumonisin	<ul style="list-style-type: none"> U.S. Midwest Throughout U.S. U.S. Midwest when European corn borer favored France, Italy, Turkey, Argentina Germany Philippines 	<ul style="list-style-type: none"> U.S. Midwest when corn earworm favored
Aflatoxin	<ul style="list-style-type: none"> U.S. South when aflatoxin levels high, or fungus applied through non-wounding technique U.S. South, some years U.S. South, new varieties controlling corn earworm and fall armyworm 	<ul style="list-style-type: none"> U.S. South when aflatoxin levels low, or fungus applied by wounding technique U.S. South U.S. South, some years Italy
Deoxynivalenol	<ul style="list-style-type: none"> Canada Germany (animal feed) Eastern Europe, some years 	<ul style="list-style-type: none"> Eastern Europe, some years Germany
Zearalenone	<ul style="list-style-type: none"> France and Spain Germany (animal feed) 	

13

The Saga of Starlink Corn

- Genetically modified corn (PIP)
 - Carrying Cry9c gene and protein
 - Gene inserted to protect against European corn borer, cornstalk borer, corn earworm
- Manufacturer applied to U.S. EPA for use in animal feed and foods
 - 1998: EPA granted limited registration for animal feed (not for human consumption)
 - EPA planned for additional risk assessment
 - Rationale: distinctions of Cry9C from other insecticidal Cry proteins
 - Chemical stability (heat and acid)
 - structural characteristics (allergenicity?)



14

The Saga of Starlink Corn

- 9/2000: Environmental group reported detection of Cry9c DNA (not protein product) in commercial foods
 - Taco shells
- Received significant media coverage
- Led to food recalls, and reassessment of risks by regulatory agencies
 - > 2.5 million boxes of taco shells recalled
 - High cost to manufacturer (millions\$)
- Followed by reports of adverse food reactions associated with taco shells, other corn products to FDA
 - Reports suggestive of possible food allergy reactions



15

The Saga of Starlink Corn

- What was unique about Starlink corn?
 - Starlink was not first instance of human exposure to genetically modified plants
 - Another PIP (Cry1ab) had received regulatory approval for human consumption uses prior to Starlink (1995)
 - US EPA approved its use as PIP
 - No reports of adverse food reactions had been reported in association with dietary exposure
 - Starlink was an example of PIP genes being discovered in food where it was not expected
 - And had not received regulatory approval



16

Adverse Reactions to Foods Derived from Starlink Corn?

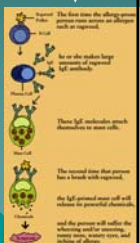
- Reports of adverse food reactions followed quickly after media reports of Cry9c gene in taco shells
- CDC conducted a study of suspected cases
 - Case definition: suspected anaphylactic (allergic) reaction within 1 hour of consumption of corn-derived products
 - Hives, rash, swelling of mouth or throat
 - Vomiting, diarrhea, cramping involving only one individual
 - Symptoms not obviously explained by a pre-existing medical condition
 - Of 51 adverse reports, 28 met case definition
 - 24 participated in subsequent investigation





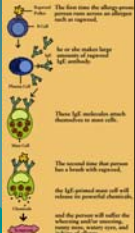
17

Allergic Reactions to Foods Derived from Starlink Corn?

- Investigation consisted of assessment of plausibility of allergic reaction to Cry9c protein
 - Protein as hazard (in contrast to the gene encoding the protein)
 - Distinctions between allergic responses and toxic responses
 - Hazard → Exposure → Dose → Response
 - Production of antibodies (immunoglobulin E – aka IgE) mediate the allergic response
 - In an allergic individual, the allergic response can occur from very low doses





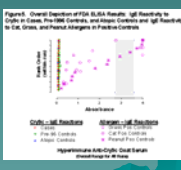
CDC **Allergic Reactions to Foods Derived from Starlink Corn?**

- Individuals with suspected allergic food reactions were tested for a specific IgE for the Cry9c protein
 - Positive antibody test would suggest Cry9c allergy as possible cause of adverse reaction
- Two other groups of individuals were tested for same antibody
 - Pooled blood samples from prior to 1996 (when Starlink was introduced)
 - Control group
 - Individuals with known history of food allergies



CDC **Allergic Reactions to Foods Derived from Starlink Corn?**

<http://www.cdc.gov/nceh/ohha/Cry9cReport/default.htm>

- Investigation results
 - Cry9c IgE antibodies were not detectable in any of the blood samples
 - Other IgE antibodies were detectable, but they were to common environmental allergens
 - Animal dander, peanuts, etc.
 - Results did not confirm an allergic reaction to Cry9c protein as cause of reported adverse food reactions

Starlink Corn: The Aftermath

- November, 2000
- EPA re-assessed potential allergenicity of Starlink corn
 - Accepted findings of CDC, but could not rule out the possibility of allergic reactions to Cry9c
 - Manufacturer of Starlink subsequently withdrew its petition for food registration
- EPA decided to no longer allow split-use registration for PIP's
 - Animal feed and food intended for human consumption
- EPA subsequently developed additional criteria for risk assessment of possible allergic responses to PIP's
 - Resistance to acid treatment and protein digestion
 - Molecular weight range
 - Immunologic (IgE) responses in rat models
 - Detection of the protein product in the bloodstream

Federal Register

Friday, April 25, 2003

Part V

**Environmental Protection Agency
Department of Health and Human Services**

Food and Drug Administration

EPA White Paper Regarding Starlink® Corn Safety, Exposure and Risk Assessment: Next Guidance for Industry on FDA Recommendations for Sampling and Testing Yellow Corn and Dry Milled Yellow Corn Aligned with the Human Use for Cry9c Protein Studies, Withdrawal of Guidance Notice


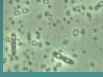

SUMMARY: EPA is making available a final White Paper that reviews data on the level in the human food supply of Cry9c protein from StarLink® corn grain. It concludes that the protein has been sufficiently removed from the human food supply to render the level of risk low enough that continued testing for the protein in yellow corn at dry mills and mass production facilities provides no added public health protection. The White Paper therefore recommends that the Food and Drug Administration (FDA) withdraw its guidance recommending testing yellow corn grain for Cry9c at dry mills and mass production facilities. Concurrent with this notice, FDA is publishing a notice in the Federal Register withdrawing their guidance.

Bt corn: Risks and Benefits

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Bt Corn: Conclusions

- PIP's (and Starlink) as example of emerging challenges in biotechnology
 - New paradigms in health risk assessment
 - Toxicity vs. other health endpoints
 - Public perceptions relating to the technology used to create food products
 - Risk assessment methods for food biotechnology will continue to evolve in the future

Questions for Discussion

- ◆ How do the risks of exposure to *Bacillus thuringiensis* differ from exposure to Bt corn?
- ◆ Are plant-incorporated protectants containing Bt (Cry) proteins adequately regulated?

25