Petition 10-336-01p

RECEIVED
By APHIS BRS Document Control Officer at 9:39 am, Nov 08, 2011

ENVIRONMENTAL REPORT Petition for Determination of Nonregulated Status of Event 5307 Corn

OECD Unique Identifier: SYN-Ø53Ø7-1

Prepared for **Syngenta Biotechnology, Inc.**
Research Triangle Park, North Carolina

Prepared by ***VHB*/Vanasse Hangen Brustlin, Inc.**
Watertown, Massachusetts

Syngenta Reference No. 5307-USDA-3a

October 31, 2011

Table of Contents

APPENDICES

List of Tables

ACRONYMS AND ABBREVIATIONS

µg/g	Micrograms per gram
ACHP	Advisory Council on Historic Properties
AIA	Advanced Informed Agreement
AMS	Agricultural Marketing Service (also USDA-AMS)
AOSCA	Association of Official Seed Certifying Agencies
APHIS	Animal and Plant Health Inspection Service (also USDA-APHIS)
ARMS	Agricultural Resource Management Survey
ARS	Agricultural Research Services (also USDA-ARS)
ATTRA	Appropriate Technology Transfer for Rural Areas
BRS	Biotechnology Regulatory Service
Bt	*Bacillus thuringiensis*
C	Centigrade
C3	Three-carbon molecule photosynthetic pathway
C4	Four-carbon molecule photosynthetic pathway
CBD	*Convention on Biological Diversity*
CEQ	Council on Environmental Quality
CFR	Code of Federal Regulations
CO_2	Carbon dioxide
CRP	Conservation Reserve Program
Cry	Crystal proteins
EA	Environmental Assessment
EIS	Environmental Impact Statement
EO	Executive Order
EPA	Environmental Protection Agency (also USEPA)
ER	Environmental Report
ERS	Economic Research Service (also USDA-ERS)
ESA	*Endangered Species Act*
FATUS	Foreign Agricultural Trade of the United States
FAS	Foreign Agricultural Service (also USDA-FAS)
FB_1	Fumonisin B_1
FDA	Food and Drug Administration (also USDHHS-FDA)
FFP	Food, feed, or processing
FIFRA	*Federal Insecticide, Fungicide, and Rodenticide Act*
FESTF	FIFRA - Endangered Species Task Force
FONSI	Finding of No Significant Impact
FR	Federal Register
FWS	Fish and Wildlife Service (also USFWS)
GHG	Greenhouse gases
IMS	Information Management System
IP	Identity Preservation
IPM	Integrated Pest Management
IPCC	Intergovernmental Panel on Climate Change
IPPC	International Plant Protection Convention
IRM	Insect Resistance Management
LMO	Living modified organisms
LOQ	Limit of quantification
MJD	Multi-Jurisdictional Database
N_2O	Nitrous oxide
NAPPO	North American Plant Protection Organization

NASS	National Agricultural Statistics Service (also USDA-NASS)
NCGA	National Corn Growers Association
NEPA	*National Environmental Policy Act*
NHPA	*National Historic Preservation Act*
NOP	National Organic Program
NPS	Nonpoint source
NPTN	National Pesticide Telecommunications Network
NRC	National Research Council
NRCA	Natural Resources Conservation Service (also USDA-NRCS)
OECD	Organization for Economic Cooperation and Development
OTA	Organic Trade Association
PIPs	Plant-incorporated protectants
pmi	Phosphomannose isomerase gene (also known as *manA*)
PMI	Phosphomannose isomerase protein
PPA	*Plant Protection Act*
PPRA	Plant Pest Risk Assessment
PRA	Pest Risk Analysis
RR2	Roundup-Ready© Corn 2
RSPM	Regional Standards for Phytosanitary Measures
SCGO	Seed Corn Growers of Ontario
TSCA	*Toxic Substances Control Act*
US	United States
USACOE	United States Army Corps of Engineers
USDA	United States Department of Agriculture
USDHHS	United States Department of Health and Human Services
USEPA	United States Environmental Protection Agency (also EPA)
USFWS	United States Fish and Wildlife Service (also FWS)
USGC	U.S. Grains Council
Vip	Vegetative insecticidal protein

1

Purpose and Need

This Chapter describes the purpose of and need for determining a nonregulated status for Event 5307 corn, and includes an explanation of the regulatory context of the decision.

1.1 Regulatory Authority

"Protecting American agriculture" is the basic charge of the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS). APHIS provides leadership in ensuring the health and care of plants and animals. The agency improves agricultural productivity and competitiveness, and contributes to the national economy and public health. USDA policy states that all methods of agricultural production (resulting in conventional, genetically engineered [transgenic], and organic varieties) can provide benefits to the environment, consumers, and producers.

Since 1986, the United States government has regulated genetically engineered organisms pursuant to a regulatory framework known as the *Coordinated Framework for the Regulation of Biotechnology*[1] (Coordinated Framework). The Coordinated Framework describes the comprehensive federal regulatory policy for ensuring the safety of biotechnology research and products and explains how federal agencies will use existing federal statutes in a manner that ensures public health and environmental safety while maintaining regulatory flexibility to avoid impeding the growth of the biotechnology industry.

The Coordinated Framework is based on several important guiding principles:

(1) Agencies should define those transgenic organisms subject to review to the extent permitted by their respective statutory authorities;

1 Executive Office of the President, Office of Science and Technology Policy. 1986.

(2) Agencies are required to focus on the characteristics and risks of the biotechnology product, not the process by which it is created; and

(3) Agencies are mandated to exercise oversight of genetically engineered organisms only when there is evidence of "unreasonable" risk.

The Coordinated Framework explains the regulatory roles and authorities for the three major federal agencies involved in regulating genetically engineered organisms: APHIS, the US Food and Drug Administration (FDA), and the US Environmental Protection Agency (EPA):

- APHIS is responsible for regulating genetically engineered organisms pursuant to the plant pest authorities in the *Plant Protection Act*[2] (PPA) to ensure that genetically engineered organisms do not pose a plant pest risk to the environment.

- The FDA regulates genetically engineered organisms under the authority of the *Federal Food, Drug, and Cosmetic Act*.[3] The FDA is responsible for ensuring the safety and proper labeling of all plant-derived foods and feeds, including those that are genetically engineered. To help developers of food and feed derived from genetically engineered crops comply with their obligations under federal food safety laws, FDA encourages them to participate in a voluntary consultation process. In the FDA policy statement[4] concerning regulation of products derived from new plant varieties, including those genetically engineered, FDA uses this consultation process to ensure that human food and animal feed safety issues or other regulatory issues (e.g., labeling) are resolved prior to commercial distribution of bioengineered food. All food and feed derived from genetically engineered crops currently on the market in the United States have successfully completed this consultation process.

- The EPA regulates plant-incorporated protectants (PIPs) under the *Federal Insecticide, Fungicide, and Rodenticide Act*[5] (FIFRA) and certain biological control organisms under the *Toxic Substances Control Act*[6] (TSCA). The EPA is responsible for regulating the sale, distribution and use of pesticides, including pesticides that are produced by an organism through biotechnology.

1.2 Regulated Organisms

The mission of APHIS Biotechnology Regulatory Service (BRS) is to protect America's agriculture and environment using a dynamic and science-based regulatory

▼

2 *Plant Protection Act*. 2000.
3 *Federal Food, Drug, and Cosmetic Act*. 1938.
4 USDHHS-FDA. 1992.
5 *Federal Insecticide, Fungicide, and Rodenticide Act.* 1947.
6 *Toxic Substances Control Act*. 1976.

framework that allows for the safe development and use of genetically engineered organisms. APHIS regulations[7] promulgated pursuant to authority granted by the PPA govern the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms. A genetically engineered organism is no longer subject to the plant pest provisions of the PPA or the applicable regulatory requirements after APHIS determines that it is unlikely to pose a plant pest risk. A genetically engineered organism is considered a regulated article if the donor organism, recipient organism, vector, or vector agent used in engineering the organism belongs to one of the taxa listed in the regulation[8] and is also considered a plant pest. A genetically engineered organism is also regulated when APHIS has reason to believe that the genetically engineered organism may be a plant pest or APHIS does not have information to determine if the genetically engineered organism is unlikely to pose a plant pest risk.

A person may petition the agency for a determination that a particular regulated article is unlikely to pose a plant pest risk, and, therefore, is no longer regulated under the plant pest provisions of the PPA or the applicable regulations. The petitioner is required[9] to provide information related to plant pest risk that the agency may use to determine whether the regulated article is unlikely to present a greater plant pest risk than the unmodified organism. A genetically engineered organism is no longer subject to the regulatory requirements or the plant pest provisions of the PPA when APHIS determines that it is unlikely to pose a plant pest risk.

1.3 Petition for Determination of Nonregulated Status: Syngenta Event 5307 Corn

Syngenta Biotechnology, Inc. submitted an initial petition (10-336-01p) to APHIS in 2010, which was revised in 2011, [10] for determination of nonregulated status for Event 5307 rootworm-resistant corn (hereafter referred to as Event 5307 or 5307 corn). Nonregulated status would include 5307 corn, progeny from crosses between 5307 corn and conventional corn, and progeny from crosses of 5307 corn with other transgenic corn that has been deregulated. Event 5307 corn is currently regulated under the PPA. Interstate movements and field trials of 5307 corn have been conducted under permits issued or notifications acknowledged by APHIS since 2005. Data resulting from these field trials are described in the Syngenta petition.

▼

7 USDA-APHIS. 1987.
8 USDA-APHIS. 1995. 7 CFR Section 340.2.
9 USDA-APHIS. 1995. 7 CFR Section 340.6(c)(4).
10 Vlachos and Huber. 2011.

1.4 Purpose of and Need for Product

Corn rootworm (*Diabrotica*) larvae feed on the roots of growing corn plants and are widespread and major pests of US corn. Corn (*Zea mays* L., maize) derived from Syngenta's transformation Event 5307 contains a unique engineered insecticidal protein, eCry3.1Ab, that is active against three economically important corn rootworm species that cause significant damage to the US corn crop annually. This engineered pesticide provides corn plants with high resistance to larval feeding damage by western corn rootworm (*Diabrotica virgifera virgifera*), northern corn rootworm (*D. longicornis barberi*) and Mexican corn rootworm (*D. virgifera zeae*). Event 5307 corn demonstrates excellent efficacy in controlling these damaging pests.

Corn varieties containing the transgene *ecry3.1Ab* have the potential to displace applications of conventional rootworm insecticides that are of concern due to human and environmental risk factors. The new protein, eCry3.1Ab, acts on target pests via a unique mode of action, reducing the selection pressure on pest populations to evolve resistance to other Cry proteins and control methods. Growers are expected to derive benefits from the convenience and ease-of-use of 5307 corn as an alternative to the application of conventional insecticides, as well as realize economic benefits through increased crop yield under conditions of pest pressure, and reduced costs of insecticide applications. The availability of 5307 corn is expected to contribute to the significant trend in reduced use of hazardous insecticides and extends the useful life of other commercially available corn rootworm-protected *Bt* cultivars (Cry3Bb1, Cry34Ab1/Cry35Ab1, and mCry3A corn).

The benefits of 5307 corn include reduced insecticide use, improved worker safety, reductions in the use of fossil fuels to apply chemical insecticides, economic benefits for growers, improved insect resistance management, and increased competition in the marketplace for insect-protected seed products.

1.5 APHIS Response to Petition for Nonregulated Status

Under the authority of the plant pest provisions of the PPA, APHIS has issued regulations for the safe development and use of genetically engineered organisms. APHIS must respond to petitioners that request a determination of the regulated status of genetically engineered organisms,[11] including transgenic plants such as Syngenta's 5307 corn. When a petition for nonregulated status is submitted, APHIS must make a determination if the genetically engineered organism is unlikely to pose a plant pest risk.

▼

11 USDA- APHIS 1987. 7 CFR Section 340.6.

APHIS will prepare an environmental assessment (EA) that will consider the potential environmental effects as part of an agency determination of nonregulated status consistent with the *National Environmental Policy Act*[12] (NEPA) and implementing regulations from the Council on Environmental Quality (CEQ)[13] and APHIS,[14] as well as APHIS BRS procedures. This Environmental Report (ER) has been prepared in order to specifically evaluate the effects that may result from deregulation of Syngenta's 5307 corn on the physical, biological, and human environment.

1.6 Coordinated Framework Review

Syngenta has obtained an Experimental Use Permit (67979-EUP-8) from EPA that allows for broad-scale field testing of 5307 corn and various breeding stack combinations that include 5307 corn. The Experimental Use Permit[15] was initially granted on June 1, 2010 with effect through February 28, 2012 and was extended[16] on March 3, 2011 with effect through December 31, 2013. In connection with this Experimental Use Permit, EPA established[17] and extended[18] a temporary exemption from the requirement of a tolerance for eCry3.1Ab residues in corn commodities, pursuant to §408(d) of the *Federal Food, Drug, and Cosmetic Act*. Phosphomannose isomerase (PMI), the selectable marker protein produced by 5307 corn plants, is exempt from food and feed tolerances.[19]

In April 2011 Syngenta submitted applications to the EPA for registration of the eCry3.1Ab PIP in 5307 corn (Appendix A) and in two breeding stacks including 5307 corn, specifically Bt11 × MIR604 × TC1507 × 5307 × GA21 and Bt11 × MIR162 × MIR604 × TC1507 × 5307 × GA21 corn. In addition to the corn rootworm control provided by 5307 corn, the controls provided by other constituents of these combinations are:

- A Cry1Ab protein for lepidopteran control (Bt11);
- A modified Cry3A protein for corn rootworm control (MIR604);
- A Cry1F protein for lepidopteran control (TC1507);
- A double mutated 5-enolpyruvylshikimate-3-phosphate synthase enzyme for glyphosate tolerance (GA21);
- A Vip3Aa20 protein for lepidopteran control (MIR162); and
- A phosphinothricin acetyl transferase enzyme for glufosinate tolerance (Bt11 and TC1507).

▼

12 *National Environmental Policy Act*. 1970.
13 CEQ. 1978.
14 USDA-APHIS. 1995.
15 USEPA. 2010c.
16 USEPA. 2011c.
17 USEPA. 2010a.
18 USEPA. 2011a.
19 USEPA. 2007b.

The registration sought for the PIP in 5307 corn as a stand-alone cultivar (i.e., not part of a breeding stack) will be for a manufacturing-use product; Syngenta will not seek an end-use product registration from EPA for 5307 corn. Rather, commercial registrations will be sought from EPA for the two breeding stack products that include 5307 corn. Concurrently, Syngenta also submitted a petition (Petition No. 1F7857) to the EPA to establish a nonexpiring exemption from the requirement of a tolerance for eCry3.1Ab residues in food and feed commodities.

Event 5307 corn falls within the scope of the FDA policy statement concerning regulation of food products derived from new plant varieties, including those developed by genetic engineering.[20] Syngenta initiated a voluntary pre-market consultation process with FDA and submitted a Safety and Nutritional Assessment for 5307 corn in January 2011 (Appendix B).[21]

1.7 Public Involvement

APHIS routinely seeks public comment on draft environmental assessments prepared in response to petitions to deregulate genetically engineered organisms. APHIS does this through a notice published in the Federal Register. This ER, the EA, the petition submitted by Syngenta, and APHIS' Plant Pest Risk Assessment of 5307 corn will be available for public comment for a period to be specified by APHIS. Comments received by the end of the period will be analyzed and used to inform APHIS' determination decision of the regulated status of 5307 corn and to assist APHIS in determining whether an Environmental Impact Statement (EIS) is required prior to the determination decision of the regulated status of this corn line.

1.8 Issues Considered

The list of issues considered in this ER were developed in accordance with the requirements of NEPA, and through coordination with APHIS. The list was developed through experience in considering potential environmental impacts relevant to transgenic field crops like 5307 corn, as well as concerns and issues raised in public comments submitted for other EAs of transgenic organisms. The issues considered also address concerns raised in previous and unrelated court decisions, as well as questions that have been raised by various stakeholders in the past. The issues considered in this ER are listed below.

- Corn Production
  - Acreage and areas of production
  - Agronomic practices

▼

20 USDHHS-FDA. 1992.
21 Vlachos and Ward. 2011. (Appendix B)

  - Specialty systems
  - Raw and processed agricultural commodities
  - Persistence in the environment and weediness potential

- Physical and Natural Environment
  - Water quality and use
  - Soil
  - Air quality
  - Climate
  - Animals
  - Plants
  - Soil microorganisms
  - Biodiversity
  - Gene movement in the natural environment
  - Threatened and endangered species

- Public Health
  - Human health
  - Animal (livestock) health
  - Worker safety

- Socioeconomic Factors
  - Domestic economic environment
  - Trade economic environment
  - Social environment

- Cumulative Effects

This page intentionally left blank.

2

Affected Environment

This Chapter describes corn biology, corn production, and the environmental and human resources that could potentially be affected by deregulation of Event 5307 corn.

2.1 Corn Biology

Maize, or corn, is a member of the *Maydeae* tribe of the grass family *Poaceae*.[22] The western hemisphere genera *Zea* and *Tripsacum* are included in the tribe *Maydeae*. The Asian genera of *Maydeae* are *Coix*, *Polytoca*, *Chionachne*, *Schlerachne* and *Trilobachne*.[23]

Corn is a robust monoecious (i.e., having separate male and female flowers for each plant) annual plant, which was developed through artificial selection and does not occur in natural ecosystems outside of cultivation. The corn plant is adapted for high productivity because it has a large leaf area and a modified photosynthetic pathway that allows it to survive extended periods of drought.[24] The corn plant, along with a limited number of other plants like sugarcane, millet, and sorghum, uses the C4 photosynthetic pathway. At a biochemical level, these plants convert carbon dioxide (CO_2) into a four-carbon molecule in contrast to the more common photosynthetic pathway in which CO_2 is converted into a three-carbon molecule.[25] C4 photosynthesis allows the corn plant to produce more dry matter (i.e., in various combinations of carbohydrates, proteins, oils, and mineral nutrients) per unit of water transported than the C3 photosynthetic pathway. Because of their high photosynthetic efficiency, the C4 crops like corn and sugarcane are favored for ethanol production as well.

Zea mays is an allogamous plant (i.e., cross-fertilizes by pollen from one flower to stigmas on another) that propagates through seed produced predominantly by cross pollination. Pollen is transferred by wind and, in controlled breeding situations, by

▼

22 Organization for Economic Co-operation and Development. 2003. Pg. 11.
23 Ibid. Pg. 14.
24 Frost and Gilman. 2011. Pg. 1.
25 Ibid. Pg. 1.

humans. Corn can only be crossed experimentally with the genus *Tripsacum*; however, wild member species of its own genus (teosinte: *Z. diploperennis, Z. perennis, Z. luxurians, Z. nicaraguanensis*) may hybridize with corn under natural conditions (see Section 2.4.4, *Gene Movement in the Natural Environment*). Corn is not weedy, and does not persist outside of cultivation. There are no reports in which corn propagated vegetatively under field conditions, and the only known propagation method for corn is through seed germination. Corn is primarily grown in warm temperate climates. Corn seed is sensitive to cold and typically does not survive freezing winter conditions.[26] Consequently, corn has no innate dormancy.[27]

Humans have been selectively breeding corn for thousands of years to emphasize desired characteristics such as increased yield. Beginning in 1996, transgenic corn products were introduced in the US market by seed companies, thereby adding important new genetic traits to modern corn varieties. Corn cultivars representing 30 different transformation events have been deregulated by APHIS[28] in connection with 26 petitions for deregulation; these cultivars were genetically engineered to offer insect resistance, herbicide tolerance, and other traits. Sixteen of these cultivars, as listed in Table 2-1, contain proteins derived from *Bacillus thuringiensis* (*Bt*) and are known as *Bt* corn cultivars. *Bt* corn cultivars assist growers in preventing insect damage that would otherwise cause yield loss. The *Bt* "crystal" proteins (referred to as Cry proteins) in these cultivars are insecticidal against certain Coleoptera or Lepidoptera and exert their insecticidal activity when they:

- Are ingested by the insect and solubilized in the insect gut;
- Are activated by specific proteolytic cleavage by midgut enzymes;
- Bind to specific receptors on the surface of the insect midgut; and
- Form ion channels in the gut membrane.

▼

26 Organization for Economic Co-operation and Development. 2003. Pg. 23.
27 Ibid. Pg. 25.
28 USDA-APHIS. 2011b.

Table 2-1 Deregulated Transgenic Corn Cultivars Containing *Bt*-derived Proteins

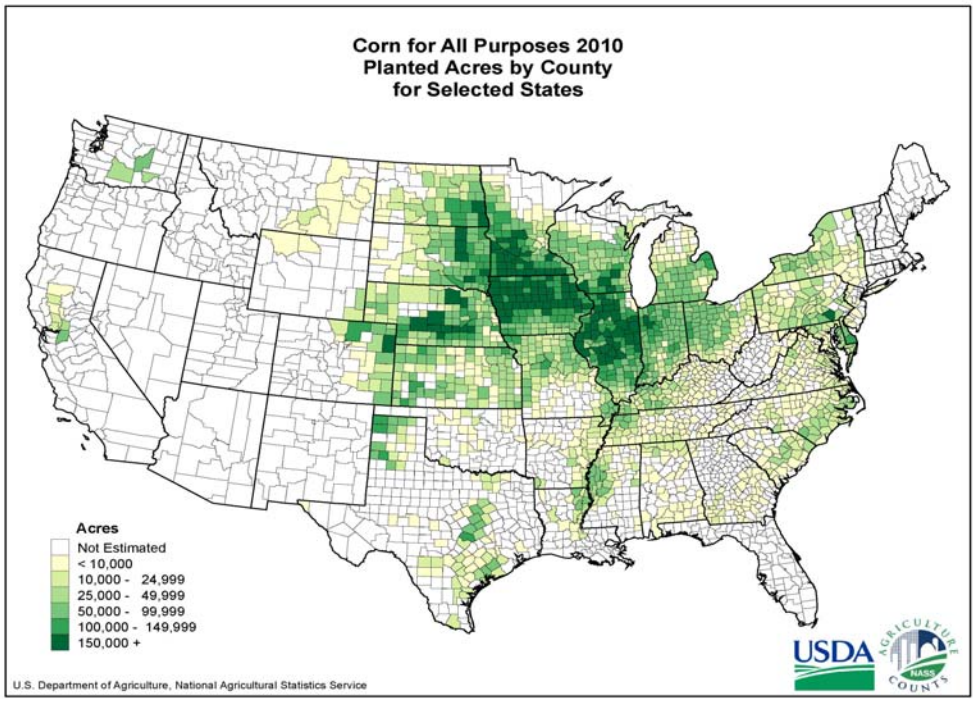
Cultivar	Filed By/Petition Number	Transgenic feature	NEPA Status (by APHIS)
Event 176	Ciba Seeds (petition 94-319-01p)	Lepidopteran (European corn borer) resistant, expressing Cry1Ab	FONSI and EA issued June 13, 1995
MON 80100	Monsanto (petition 95-093-01p)	Lepidopteran resistant, expressing Cry1Ab	FONSI and EA issued August 28, 1995
Bt11	Northrup King Company (petition 95-195-01p)	Lepidopteran resistant, expressing Cry1Ab, and glufosinate tolerant	FONSI and EA issued January 22, 1996
DBT418	DeKalb (petition 96-291-01p)	Lepidopteran resistant, expressing Cry1Ac, and glufosinate tolerant	FONSI and EA issued April 9, 1997
MON 809 and MON 810	Monsanto (petition 96-017-01p)	Lepidopteran resistant, expressing Cry1Ab	FR notice published March 15, 1996
MON 802	Monsanto (petition 95-317-01p)	Lepidopteran resistant, expressing Cry1Ab, and glyphosate tolerant	FONSI and EA issued May 27, 1997
CBH-351	AgrEvo (petition 97-265-01p)	Lepidopteran resistant, expressing Cry9C, and glufosinate tolerant	FONSI and EA issued May 11, 1998
Line 1507	Mycogen (petition 00-136-01p)	Lepidopteran resistant, expressing Cry1F, and glufosinate tolerant	FONSI and EA issued August 2, 2001
MON 863	Monsanto (petition 01-137-01p)	Corn rootworm resistant, expressing Cry3Bb1	FONSI and EA issued October 17, 2002
DAS 6275	Dow (petition 03-181-01p)	Lepidopteran resistant, expressing Cry1F, and glufosinate tolerant	FONSI and EA issued October 20, 2004
DAS 59122	Dow (petition 03-353-01p)	Corn rootworm resistant, expressing Cry34/35Ab1, and glufosinate tolerant	FONSI and EA issued September 23, 2005
MON 88017	Monsanto (petition 04-125-01p)	Corn rootworm resistant, expressing Cry3Bb1, and glyphosate tolerant	FONSI and EA issued December 14, 2005
MIR604	Syngenta (petition 04-362-01p)	Corn rootworm resistant, expressing modified Cry3A	FONSI and EA issued March 16, 2007
MON 89034	Monsanto (petition 06-298-01p)	Lepidopteran resistant, expressing Cry1A.105 and Cry2Ab2	FONSI and EA issued July 15, 2008
MIR162	Syngenta (petition 07-253-01p)	Lepidopteran resistant, expressing Vip3Aa20	FONSI and EA issued April 9, 2010

2.2 Corn Production

This section describes corn production and yield in the US, current agronomic practices, specialty corn production systems, raw and processed corn commodities, and corn's persistence in the environment and weediness potential. Corn is grown for animal feed, human food, vegetable oil, high fructose corn syrup, starch, fermentation into ethanol, and many other uses.

2.2.1 Production and Yield

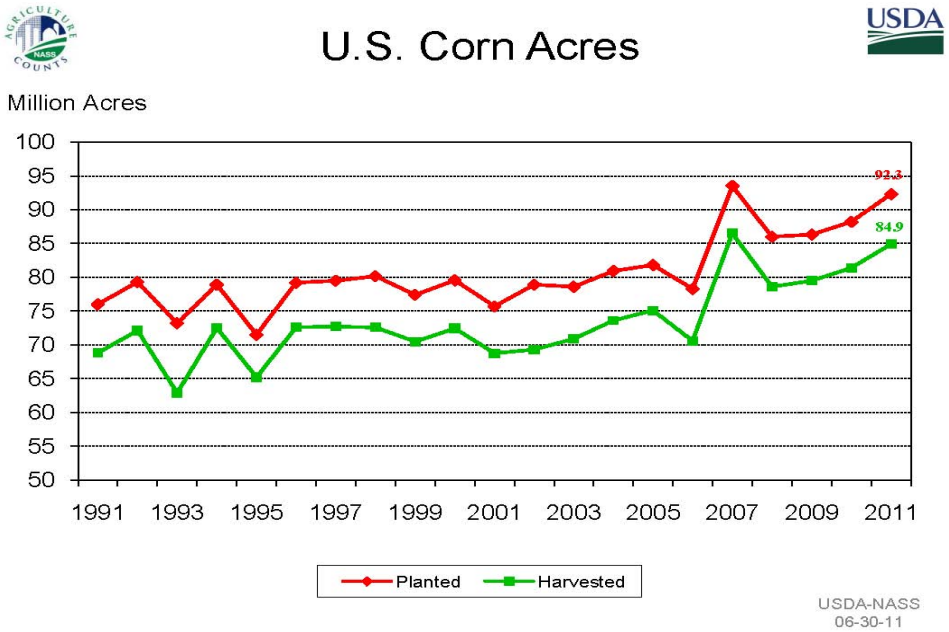
Corn is usually cultivated in temperate regions that provide sufficient moisture and an adequate number of frost-free days to reach maturity. US corn production is primarily focused in the Corn Belt, an area that includes Iowa, Illinois, Nebraska, and Minnesota, and parts of Indiana, South Dakota, Kansas, Ohio, Wisconsin, and Missouri. The Corn Belt has a combination of seasonal warm weather, rainfall, and favorable soil conditions for corn growth. Approximately 68.7 million acres of corn, representing approximately 78 percent of the US total of 88.2 million acres, was planted in these ten states in 2010.[29] The planted acres of corn for each county in selected states in 2010 are depicted below.



Source: USDA-NASS. 2011c.

29 USDA-NASS. 2011d. Pg. 8.

Nationally, planted corn acreage has increased from approximately 75 million acres in 1990 to a peak of nearly 95 million acres in 2008, as shown in the graph below. Corn growers were projected to plant more than 92 million acres of corn in 2011. Harvested acreage lags planted acreage by 5 to 7 million acres each year due to losses from adverse weather and insect damage.



Source: USDA-NASS. 2011a.

The majority of domestic corn is produced for livestock feed (grain and silage) and fuel (ethanol), comprising 39 and 36 percent, respectively of the market. Corn for grain production in 2010 was estimated at 12.4 billion bushels, 5 percent below the record high production of 13.1 billion bushels set in 2009.[30] Grain yield was approximately 152.8 bushels per acre, 11.9 bushels below the record high yield of 164.7 bushels per acre set in 2009. Since 1990, corn yields have increased by about 2.0 bushels per acre per year.[31]

The USDA-Economic Research Service (ERS) provides 10-year projections of supply and utilization for major field crops, including corn, grown in the US. ERS projects that an average of 89.5 million acres of corn will be planted each year through 2019.[32] Output will increase during this period, according to ERS:

> Expanding output is attributable to yield growth. The projected 2010/11 corn yield is based on the simple linear trend since 1990. The longer term trend for 2011/12 and later years reflects an annual yield increase of 2.0 bushels per acre per year, resulting in record corn production in 2011/12 and beyond.

30 USDA-NASS. 2011d.
31 Savage. 2011. Pg. 1.
32 USDA-ERS. 2011b. Pg. 6.

> Increases in corn yields have been driven by improvements in plant genetics, machinery, and cultivation practices that have allowed for faster, more precise planting and earlier harvesting. The latest round of advances in genetics and planting technology is expected to be fully adopted by the early years of the projections. Thus, longer term yield gains are expected to be somewhat slower than during the late 1990s and early 2000s. Gains continue to be supported by improved genetics, including advances in plant utilization of water and fertilizer.

In 2011, approximately 88 percent of the US corn fields were planted with transgenic crops for pest management.[33] As described in Section 2.2.2.3, *Insect Management*, and Section 2.2.2.5, *Weed Management*, the transgenic crops provide insect resistance and herbicide tolerance, as well as production-related traits. Insect-resistant varieties containing *Bt*-derived transgenes comprise about 16 percent of the US corn acreage, and herbicide-tolerant varieties some 23 percent; products combining both traits were planted on 49 percent of the corn acreage.[34]

2.2.2 Agronomic Practices

Corn growers choose agronomic practices that maximize grain yield. Grain yield can be affected by cropping practices such as crop rotation and tillage techniques, supplemental irrigation based on soil and climate conditions, and methods to manage insects, disease, and weeds. This section describes common agronomic practices for corn.

2.2.2.1 Cropping Practices

Crops grown in rotation typically have higher yields, lower weed biomass, and lower insect populations than those grown continuously.[35] Corn is typically rotated with legumes such as soybeans or alfalfa. Corn/soybean rotations have been found to have 9 percent higher yields than corn/corn rotations.[36] Weed population density and biomass may be reduced using crop rotation (temporal diversification) and intercropping (spatial diversification) strategies.[37] The risk of yield losses due to damage from some insects, particularly corn rootworm, decreases when corn is rotated with other crops.[38]

Legumes provide the greatest rotation benefits for corn; the benefit is principally attributed to the nitrogen-fixing property of legumes but other factors (such as increased organic matter and reduced weed seed banks[39]) may also contribute to

▼

33 USDA-NASS. 2011a. Pg. 22.
34 Ibid. Pg. 22.
35 Hicks and Thomison. 2004. Pgs. 504-505.
36 Hicks and Hoverstad. 2007. Pg. 1.
37 Liebman and Dyck. 1993. Pg. 92.
38 Bessin. 2004. Pg. 1.
39 Eisenthal. 2011. Pg. 1.

higher yield. Corn is rotated with soybeans annually in much of the Corn Belt, but oats and alfalfa may also be rotated with corn and soybeans or during fallow years in semi-annual to five-year cycles.[40] In 2010, 71 percent of the acres planted in corn were in a rotation program in the last three years.[41]

Tilling the soil reduces weeds that would otherwise crowd or compete with the corn crop.[42] Until recently, conventional tillage was commonplace, using moldboard plows prior to and after planting. Reduced or conservation tillage methods have largely replaced conventional methods. Reduced tillage uses chisel plows and results in less soil disturbance. Conservation tillage also uses chisel plows and includes no-till and focused methods such as strip, ridge, or mulch tillage. The intensive plowing of conventional tillage results in less than 15 percent crop residue (unharvested plant material); reduced tillage is associated with 15 to 30 percent crop residue; and conservation tillage is associated with at least 30 percent crop residue and substantially less soil erosion than other tillage practices.[43] Because of its low cost and positive impact on soil quality, conservation tillage is currently and widely practiced in the Midwestern US. In 2010, 62 percent of the acres planted in corn used a no-till or minimum tillage method.[44]

Herbicides are used to kill weeds, as described in Section 2.2.2.5, *Weed Management*, in all of these tillage methods. In no-till systems, the herbicide is applied directly to the last season's crop residue. In the other methods, soil is tilled before the herbicide is applied. The amount of herbicide used is somewhat independent of tillage method,[45] although transgenic crops may facilitate no-till methods by reducing the need for soil application of herbicides.

2.2.2.2 Irrigation

Soil and water (rainfall or irrigation) are the key resources in all crop production. Soil supports the basic physical, chemical, and biological processes for corn to grow, and specific soil characteristics regulate water flow between infiltration, root-zone storage, deep percolation, and storm water runoff.[46] Corn Belt soils are deep, fertile, and rich in organic material and nitrogen, and the land is relatively level. The warm nights, hot days, and well-distributed rainfall of the region during the growing season are ideal conditions for raising corn. Irrigation early in the season helps establish a uniform stand and water availability, whether by rainfall or irrigation, during flowering is critical to achieve good seed set.[47]

▼

40 Hicks and Thomison. 2004. Table 3.2.4.4.
41 USDA-NASS. 2011b. Pg. 2.
42 USEPA. 2009. Pg. 1.
43 Ibid. Pg. 1.
44 USDA-NASS. 2011b. Pg. 2.
45 USEPA. 2009. Pg. 1.
46 Christensen. 2002. Pg. 9.
47 Beck. 2004. Pg. 577.

Corn grown for grain has substantially more irrigated area than any other single crop in the US, about 10.6 million acres in 1997, but only about 15 percent of the total corn acreage is irrigated.[48] Irrigated corn yields are about 29 percent higher than unirrigated corn yields. Irrigated corn is geographically distributed in the Corn Belt: eastern states irrigate only about 3 percent of the total corn area, while western states irrigate about 50 percent.

2.2.2.3 Insect Management

Corn is susceptible to attack by a variety of insects throughout its life cycle. Insects can be categorized as major and consistent pests, major and sporadic pests, and moderate to minor pests, based on annual destructiveness and their geographic distribution. The most economically significant corn pests include *Diabrotica* species (the corn rootworm complex) and *Ostrinia nubilalis* (European corn borer). The damage inflicted by rootworm larvae can significantly reduce grain yield by interfering with photosynthetic rates, limiting the uptake of water and nutrients, and by increasing the plant's susceptibility to lodging.[49] Lodging further reduces the effective grain yield by making the plants more susceptible to breaking, reducing their access to sunlight, and increasing the difficulty with which the grain can be harvested efficiently. Table 2-2 lists corn insect pests found in the US in several corn growth stages.

Insect pest management consists of several practices:

- **Crop Rotation:** Crop rotation is the most utilized method for insect management and is often the lowest cost tactic. Rotation with a non-grass crop reduces the levels of many pests through starvation or eliminating insect reproduction. Section 2.2.2.1, *Cropping Practices*, describes crop rotation practices.
- **Selection of Planting and Harvest:** Plant phenology can be manipulated to disrupt synchronization with the phenology of the pest insects. This can be achieved by either delaying or advancing planting dates. Early-planted corn has shown lower susceptibility to corn earworm (*Helicoverpa zea*) and southwestern corn borer (*Diatraea grandiosella*) damage than late-planted crops.[50] The female *D. grandiosella* tends to lay fewer eggs on more mature plants and the plants have already passed their critical developmental stage. Early harvest often produces phenological asynchronies with a pest's life cycle, allowing harvest before the damaging phase occurs. Early-planted corn can be harvested before many fully grown pre-diapause larvae have girdled the mature plants and caused yield losses through lodging of the plants.[51]

48 Christensen. 2002. Pg. 31.
49 Olesen *et al.* 2005. Pg. 1.
50 Koul *et al.* 2004. Pg. 29.
51 Ibid. Pg. 29.

Table 2-2 Corn Insect Pests

Growth Stage	Description of Damage	Pests
From planting to full emergence	Seedlings are pulled up and eaten	Seedcorn maggots *(Delia platura)*
	Seeds are bored or completely hollowed out	Seedcorn beetle *(Stenolophus lecontei)*
		Wireworms *(Melanotus* spp.*)*
Emergence to knee-high corn	Stunting and wilting	White grubs (*Cyclocarpa* spp., *Papillia japonica*, *Phyllophaga* spp.)
	Unnatural growth (stem twisting or excessive tillering)	Grape colaspis *(Colaspis brunnea)*
	Sandblasted leaves (leaves with speckled appearance)	Chinch bug *(Blissus leucopterus)*
	Removal of plant tissue (chunks of leaves eaten, plants cut off near base, etc.)	Black cutworm *(Agrotis ipsilon)*
		Stink bugs, several species
		Stalk Borer *(Papaipema nebris)*
		Thrips, several species
		Corn flea beetle *(Chaetocnema ectypa)*
		Sod webworm, several species
		Southern corn leaf beetle *(Myochorus denticollis)*
		Billbugs, several species
		Armyworm *(Pseudaletia unipunctata)*
Knee-high to tasseling corn	Leaf tissues are removed (margin feeding or ragged holes in leaves, elongated lesions)	Corn rootworms *(Diabrotica* spp.*)*
	Stalks malformed (gooseneck growth)	Fall armyworm *(Spodoptera frugiperda)*
	Holes bored in the stalk	Grasshopper, several species
		European corn borer *(Ostrinia nubilalis)*
		Southwestern corn borer *(Diatraea grandiosella)*
		Stalk borer (*Papaipema nebris*)
Tasseling to corn maturity	Leaf tissue removed (chunks of leaf removed)	Fall armyworm
	Stalks malformed or broken	Grasshoppers
	Tassel damaged (tassel broken, eaten in whorl or discolored)	European corn borer
		Southwestern corn borer
	Silks clipped	Corn rootworm (especially western corn rootworm)
	Ear damaged (chunks of kernel removed, chewing damage, ear drop)	Corn leaf aphid *(Rhopalosiphum maidis)*
		Corn earworm *(Helicoverpa zea)*
		Japanese beetle *(Popillia japonica)*

Source: Summarized from O'Day *et al*. 1998.

- **Hybrid selection:** Hybrids vary in their ability to withstand and resist insect pests such as European corn borer. Rapid germination, early vigor, strong ear shanks, tight husks, resistance to stalk rots and other pests, strong stalks, and uniform performance over a wide population range are all factors influenced by hybrid genetics that may influence losses to insects.[52] Seedling insects, stalk borers, and ear feeding insects are most influenced by hybrid traits. Some hybrids have European corn borer resistance traits that reduce susceptibility to this pest.[53]

- **Chemical Management:** Insecticides can be used selectively through spot treatments by placing the chemical in the area occupied only by the pest to be controlled. This also helps protect non-target organisms and natural enemies. Recently developed insecticides are more selective in their toxicity spectrum. Naturally derived products like *Bacillus thuringiensis* and spinosad affect only certain orders of insect pests and work only if they are ingested. Other products are selective because of the way they are presented to the pest. Imidacloprid, for example, works because it is absorbed and incorporated into the corn vascular system and selectively kills insects such as aphids and stink bugs that suck plant juices. Chemical management of corn rootworm includes preventive seed treatments and in-furrow soil applications. Foliar applications are made if they are needed for adult corn rootworm beetles later in the season. In areas where corn rootworm is common, growers basically treat routinely and prophylactically, not selectively.

- **Insecticides Used in Organic Production:** There are a number of insecticides approved for use in certified organic production systems, mainly non-synthetic compounds or biocontrols. Conditions for use of an insecticide must be documented under the National Organic Standard. The *Organic Crops Workbook*[54] lists the approved classes of insecticides used for organic production (Table 2-3) based on the USDA's regulatory lists of approved and prohibited synthetic and non-synthetic substances.[55]

- **Transgenic Products:** Corn seed companies have developed a number of transgenic products that incorporate insect resistance traits, as described in Section 2.1, *Corn Biology*. These products, alone or in combinations with other traits (such as herbicide tolerance), provide corn growers with an additional tool to combat insect pests.

52 VanDuyn *et al.* 2005. Pg. 2.
53 Ibid. Pg. 2.
54 ATTRA. 2003. Pgs. 28-29.
55 USDA-AMS. 2007.

Table 2-3 National List of Approved Insecticides for Organic Production Systems

Class	General Description	Examples	Notes
Botanicals	Derived from plants	pyrethrum, rotenone, sabadilla, neem	Strychnine and nicotine are also botanicals, but are expressly prohibited under the National List; Rotenone, an insecticide approved for use in organic production, is highly toxic to fish.
Biologicals	Insecticides containing disease organisms or toxins derived from disease organisms	*Bacillus thuringiensis*, *Beauveria bassiana*, *Trichoderma harzianum*, *Spinosad*	Much like synthetic insecticides, insect pests have been observed to develop resistance to biological insecticides.
Spray Oils	Vegetable or animal derived oils Petroleum derived oils (narrow-range oils)	-	Commonly used to control scale and mite pests
Insecticidal soaps	Fatty acid insecticidal soaps	-	Although synthetic insecticides, they are allowed in organic production; Used on predatory mites
Minerals	Mineral-based insecticides	Sulfur, copper products, diatomaceous earth and kaolin clay	Natural minerals like arsenic, lead, and sodium fluoaluminate are prohibited; Sulfur can reduce populations of beneficial insects; Diatomaceous earth can cause respiratory problems in animals and humans
Pheromones	Hormones used in products called *mating disrupters*	-	-

Source: ATTRA. 2003.

Farmers have increasingly turned to integrated pest management practices (IPM), which allow them to reduce energy use, environmental risk, and production costs while maintaining the quality of agricultural products and helping improve water, air, and soil quality. IPM was introduced in the late 1960s and shifted the emphasis in pest control from a single-tactic, chemically based approach to a multi-tactic, economically based system.[56] IPM is site-specific in nature, and includes prevention, avoidance, monitoring and suppression of weeds, insects, diseases, and other pests. IPM includes insect scouting or monitoring to determine pest populations; applying compatible alternative biological, cultural, mechanical and chemical controls; and establishing action thresholds for agricultural inputs. Timely and targeted delivery of pest management interventions is key to successful IPM.

56 Koul *et al*. 2004. Pg. 29.

Corn Rootworm - Biology, Feeding Behavior and Economic Loss

Corn rootworms are important insect pests in the Corn Belt. There are at least four different corn rootworm taxa in the US: western corn rootworm *(D. virgifera virgifera*), the northern corn rootworm *(D. longicornis barberi*), the Mexican corn rootworm *(D. virgifera zeae*), and the southern corn rootworm *(D. undecimpunctata howardi*; also known as the spotted cucumber beetle).

Western and northern corn rootworms have similar life cycles. Both have a single generation each year, and corn is the only economic host. In general, corn rootworms cannot complete their life cycle without the food supplied by corn plants. Beginning in July, females lay eggs in the soil at a depth of two to four inches near the base of the corn plant.[57] The eggs overwinter, and the onset of hatch ranges from late May to mid June. Rootworm larvae feed on corn roots for three to four weeks, passing through three growth stages (instars). The second instar larvae are often the first detected because first instars are very small (only 1/16th of an inch long).[58]

Western and northern corn rootworm larvae feed first on roots near the soil surface; when these are consumed, the next lower node is attacked. First and second instars leave brown feeding scars (lesions) as they tunnel from root tips to the plant base, destroying root hairs and small roots. Third instars cause the majority of root damage and they generally feed on the large primary roots near the stalk. Larval corn rootworm injury results in yield losses in three ways:

- Root pruning and tunneling disrupt nutrients and water transport from the root system;
- Lack of root support causes goosenecking and lodging, which may limit sunlight capture by the plants and complicate harvesting; and
- Root feeding promotes invasions by secondary pathogens such as bacteria and fungi which increase the incidence of corn rots.[59]

Additionally, adult corn rootworm beetles can cause silk clipping injury to corn plants, resulting in poor pollination and incomplete kernel set.

The Mexican corn rootworm has similar biology to the western corn rootworm (both are *D. virgifera* subspecies), but is primarily an economic pest in Texas.[60]

The corn rootworm genus is one of the most damaging corn pests. In the US, approximately 20 to 25 million acres of corn are treated annually with soil insecticides to protect crops from feeding damage caused by corn rootworm larvae.[61]

▼

57 O'Day *et al.*1998. Pg. 39.
58 Ibid. Pg. 39.
59 Ibid. Pg. 40.
60 Rice. 2004. Pg. 1.
61 Roehrdanz *et al*. 2003. Pg. 901.

Corn rootworm larval feeding can disrupt the movement of water and nutrients, which can slow development and stunt plants, ultimately leading to yield loss. During dry periods, when conditions suppress root generation, rootworm damage is amplified. In 2006 (prior to widespread adoption of transgenic crops for rootworm control), the USDA estimated that corn rootworms caused $1 billion in lost revenue each year,[62] which includes $800 million in yield loss and $200 million in treatment costs for corn growers.[63]

Corn Rootworm Management

Corn rootworm can be managed by crop rotation, insecticide application, and transgenic corn products. Aspects of each method unique to corn rootworm are described in the following paragraphs.

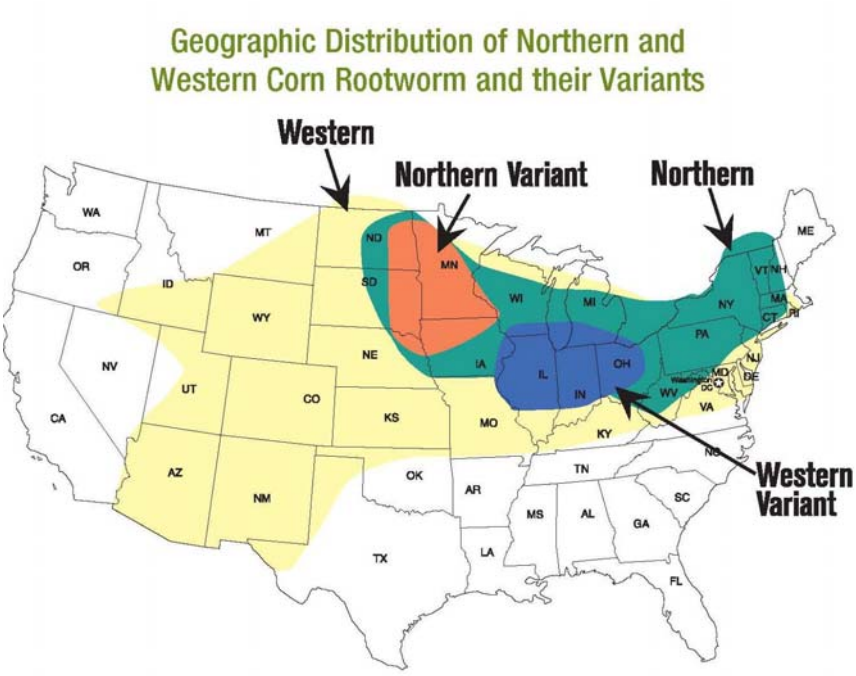
About 29 percent of US corn acreage is not rotated but rather is planted to continuous corn.[64] Insecticide application rates often increase when crop rotation is decreased.[65] Until recently corn rootworm caused damage almost exclusively in fields where corn was grown at least two years in a row. In addition, new strains of corn rootworm have evolved that survive intervening years. Corn producers have used crop rotation (usually with soybeans) to control corn rootworm, but this method has some economic and biological limitations. Rotations have become less effective for the following reasons:

- A new biotype of western corn rootworm (the western variant) has appeared in central Illinois, northern Indiana, and parts of Michigan that can lay eggs in soybean fields. The eggs hatch in the following season, coinciding with the corn rotation.[66] This strain has spread rapidly since it was first observed in 1992.
- A new northern corn rootworm biotype (the northern variant) has exhibited extended diapause in which some eggs can survive through a non-corn rotation to attack corn in a subsequent season. In South Dakota, Minnesota, Iowa, and Nebraska, the new northern corn rootworm biotype can diapause for two winters, which allows the eggs to bypass the rotated crop and hatch in time to feed on the next corn crop.[67]
- Southern corn rootworm overwinters as an adult and has a varied diet, reducing the effectiveness of crop rotation in controlling this pest.[68] Larvae of southern corn rootworm can attack more than 200 plant species, including soybeans.

▼

62 USDA-ARS. 2006.
63 Fyksen. 2003. Pg. 1.
64 Gianessi *et al*. 2002. Pg. 4.
65 USDA-ERS. nd. Pg. 146.
66 Gray *et al*. 2009. Pg. 303.
67 Gianessi *et al*. 2002. Pgs. 9-10.
68 Kuepper. 2002. Pg. 8.

The geographic distribution of the northern and western corn rootworms, and their variant populations, over the US is shown in the following map.



Source: Syngenta. 2010.

Much like resistance to crop rotation techniques, corn rootworm has developed resistance to some chemical insecticides.[69] The EPA believes that resistance to corn rootworm insecticides may result in increased chemical use and a greater dependence on insecticides.[70]

Before corn rootworm-protected *Bt* corn varieties were introduced in 2003, an estimated 14 million acres were treated annually with conventional insecticides to control corn rootworms.[71] This translated to the annual application of more than 7.7 million pounds of insecticide active ingredient to corn fields for corn rootworm control.[72] Controlling *Diabrotica* rootworms accounted for the largest single use of conventional insecticides in the US at that time.

The EPA has registered numerous insecticide products for the control of corn rootworm (a list of representative products is provided in Table C-1, Appendix C). The insecticides used to control corn rootworm in conventionally grown corn consist mainly of organophosphates, carbamates, synthetic pyrethroids and phenyl benzyl classes of chemistry. The majority of these products are classified as "Restricted Use." The EPA requires application control measures for such insecticides to limit human and environmental exposure.

▼

69 Meinke *et al.* 1998. Pg. 598.
70 Vlachos and Ward. 2011. Pg. 14.
71 Ibid. Pg. 14.
72 Ibid. Pg. 14.

In organic production systems, *Bt* foliar sprays may be used as a natural insecticide. However, the use of foliar sprays of *Bt* has been limited because of lack of persistence, limited coverage, relatively high cost, and the difficulty in determining the proper dose.[73]

Several varieties of transgenic rootworm-resistant corn have been developed by seed producers; these products provide several benefits:

- **Increased Root Protection**: Field studies indicate that transgenic rootworm-protected corn provides as good as or better efficacy than soil insecticides in protecting corn roots from significant corn rootworm larval injury.[74] In an Iowa study, transgenic corn hybrids were 100 percent effective in protecting roots from economic damage (i.e., damage that would result in yield loss), whereas the insecticide was only 63 percent effective and the untreated nontransgenic hybrid offered no protection from insect damage.[75] An added benefit of transgenic rootworm-protected corn is that root protection does not depend upon planting time, weather influences, calibration of application equipment, or soil conditions for optimum performance.
- **Reduced insecticide use.** Replacing insecticides with transgenic plants reduces insecticide use against the target pest. In the period from 1996 to 2008, the volume of active ingredients in insecticides applied to corn fields was reduced by 35 percent as a result of insect resistant transgenic corn use[76] for control of European corn borer and other lepidopteran pests (beginning in 1996) and corn rootworm (beginning in 2003). In 2008 alone, the annual savings in the volume of insectide active ingredient use was 4 million kilograms.[77]
- **Increased farm worker safety.** The majority of corn rootworm insecticides (both granular and liquid formulations) are labeled as Restricted Use Pesticides by the EPA. These insecticides are products that, without additional regulatory restrictions, could cause unreasonable adverse effects on wildlife and/or injury to the applicator. Transgenic insect-control technologies pose no such safety risks to applicators or the environment. Use of transgenic rootworm-protected corn decreases exposure of farm workers to chemical insecticides. Planting transgenic rootworm-protected corn on 10 million acres would avoid the application of 5.3 million pounds of insecticide.[78]

Transgenic corn varieties may be limited in controlling corn rootworm if the insects develop resistance to these products. To delay evolution of resistance to transgenic crops producing *Bt* toxins, nearby refuges of corn not producing *Bt* toxins for control of the same pests are required by the EPA. The refuges promote survival of

▼

73 Rhoush. 1994. Pg. 504.
74 Rice *et al*. 2004. Pg. 2.
75 Ibid. Pg. 2.
76 Brookes and Barfoot. 2010. Pg. 98.
77 Ibid. Pg. 98.
78 Rice. 2004. Pg. 5.

susceptible pests, which mate with resistant insects and slow the evolution of resistance.[79] Such refuges are expected to be most effective in slowing resistance when the toxin concentration in the adjacent *Bt* crops is high enough to kill all or nearly all target insects.[80]

The widespread use of transgenic *Bt* corn could generate selection pressures for insect resistance.[81] Although one recent study indicates that western corn rootworm in a localized area may have developed resistance to one Cry protein under intense cultivation,[82] combining *Bt* proteins in the same plant that bind to different binding sites in the target pest limits the potential for resistance to develop to any one product.[83] Other insect pest resistance that has been found may be related to insufficiently high Cry protein concentrations in the transgenic crop.[84] Nonetheless, insect resistance to *Bt* crops has not caused widespread failure of control measures, in part due to insect resistance management (IRM) strategies, including supplemental pesticide use and refuges.[85] In the case of *Bt* corn grown in the Corn Belt, refuge acres are typically 5 to 20 percent of the grower's corn area, depending on the product's requirements. Greenhouse and laboratory tests suggest that insects under intense selection pressure by Cry proteins over multiple generations may develop resistance rapidly in the absence of a refuge to sustain susceptible populations. These data in combination with the report of field resistance to a *Bt* product further emphasize the importance of effective refuges for resistance management.[86] Resistance management strategies, which are mandated by EPA's terms of *Bt* corn product registrations, have been developed for all *Bt* corn products to mitigate the risk of pest resistance and to implement additional measures if resistance occurs.

2.2.2.4 Disease and Other Pest Management

In addition to direct damage caused by feeding on plant tissue, insects play an important role in the transmission and dissemination of pathogenic organisms during corn development. Soil contains microorganisms, particularly fungi, that may infect plant parts injured by soil-dwelling insects. Primary roots of the seedling and the radical and seminal roots are commonly infected with *Fusarium* species after the roots have served their function and become senescent. Feeding by corn rootworms has been associated with increased frequencies of *Fusarium* infection;[87] rootworm feeding may also lead to increased incidences of stalk rots. These pathogen infections can reduce crop quality, harvestability, and yield.

▼

79 Tabashnik *et al*. 2008. Pg. 199.
80 Meihls *et al* 2008. Pg. 19177.
81 Tabashnik *et al*. 2008. Pg. 199.
82 Gassmann *et. al*. 2011. Pg. 22629.
83 Tabashnik *et al*. 2008. Pg.201.
84 Ibid. Pg. 201.
85 Tabashnik *et al*. 2008. Pg. 201.
86 Meihls *et al*. 2008. Pg. 19177.
87 Dicke and Guthrie. 1988. Pg. 813.

Crop disease reduces both the quantity and quality of grain harvested. Disease loss estimates for corn production in the US range between 2 and 15 percent each year and reached 20 percent in 1970 with the southern corn leaf blight epidemic.[88] Losses estimated for diseases are difficult to determine due to the following factors:

- Variation in yield potential between different years;
- Differences in genetic background of germplasm being grown; and
- Potential for unfavorable environmental conditions.

Table C-2 (Appendix C) lists corn diseases, their pathogens, the conditions that favor the spread of disease, and current management practices. In addition, mycotoxins such as aflatoxins and fumonisins may accumulate in corn as it matures in the field, and inappropriate storage conditions may facilitate additional fungal deterioration and contribute to the accumulation of aflatoxins.[89] A transgenic corn product has not yet been developed specifically to combat mycotoxins, although *Bt* corn used for control of ear-feeding lepidopteran pests has been shown to reduce the incidence of some mycotoxins in grain.[90] Minimizing insect damage through pest control measures such as *Bt* corn can reduce the incidence of fungal infection and accumulation of the associated mycotoxins.

2.2.2.5 Weed Management

Weeds compete with corn for light, nutrients, and water, especially during the first 3 to 5 weeks following emergence of the crop.[91] Late-season weed infestations do not reduce corn yield nearly as much as early weed competition, but late-season weeds can harbor destructive insect pests. Competitive weeds include nightshade, smartweed, nutsedge, foxtail, velvetleaf, lambs-quarters, pigweed, and waterhemp.[92]

Weeds can survive in crop production systems because of natural herbicide tolerance and because of growth types or life cycles that help them avoid being treated, such as some winter annual weed species. Industry practice includes weed resistance management training to reduce the potential for weeds to develop tolerance to herbicides.[93]

Weed control methods differ depending on a number of factors. No single weed control regime is effective for all growing conditions. The management practices utilized by a grower will depend on the types of pests in their field, level of infestation, cropping system, type of soil, cost, weather, time, and labor. An integrated weed management program utilizes a combination of cultural (planting),

▼

88 Jeffers. 2004. Pg. 670.
89 Rooney *et al*. 2004. Pgs. 290-291.
90 Council for Biotechnology Information. 2001. Pgs. 1 and 2.
91 Wright *et al*. 2009. Pg. 1.
92 Hager *et al*. 1998. Pg. 3.
93 NCGA. 2011. Slide 2.

mechanical (tillage), and chemical (herbicide) methods for consistent, effective weed control.[94] Integrated weed management is a USDA policy.[95] An integrated weed management program can help prevent the development of weed resistance to herbicides and the emergence of dominant weeds.

In 2010, herbicide active ingredients were applied to 98 percent of acres planted to corn.[96] The most widely used herbicide was glyphosate, applied to 66 percent of the planted acreage. Atrazine was applied to 61 percent of the planted acres and acetochlor was applied to 25 percent of the planted acres. Between 1996 and 2009, the rate of glyphosate application per crop year rose 39 percent for corn[97] while broad-based herbicide usage decreased.[98]

Herbicide-tolerant corn products have been genetically engineered to allow use of herbicides without harming the crop. Herbicide-tolerant corn has been widely adopted by growers in North America and offers enhanced weed control. In 2010, approximately 70 percent of the US corn crop was herbicide-tolerant.[99] Currently available transgenic herbicide-tolerant corn cultivars include three glyphosate-tolerant cultivars and four glufosinate- (phosphinothricin) tolerant cultivars.[100] However, over-reliance on herbicide-tolerant crops may under certain conditions contribute to the development of herbicide-tolerant weeds. Before glyphosate-tolerant crops were introduced, only three weed species in the world were known to have developed resistance to glyphosate. Glyphosate- or glufosinate-tolerant weeds now found in the US include common waterhemp (*Amaranthus rudis*), horseweed (*Conzya* spp.), giant ragweed (*Ambrosia trifida*), common ragweed (*Ambrosia artemisiifolia*), palmer amaranth (*Amaranthus palmeri*), hairy fleabane (*Conzya conariensis*), Italian ryegrass (*Lolium multiflorum*), rigid ryegrass (*Lolium perenne*), and Johnsongrass (*Sorghum halpense*).[101]

2.2.3 Specialty Systems

Specialty systems include organic corn and specialty products such as white and waxy corn, sweet corn and popcorn. These corn products comprise about 8 percent of the US corn market.[102]

▼

94 Wright *et al*. 2009. Pg. 1.
95 USDA. 1990. Pg. 1.
96 USDA-NASS. 2011b. Pg. 2.
97 Benbrook. 2009. Pg. 5.
98 USEPA. 2011d. Pg. 28.
99 USDA-ERS. 2011a.
100 USDA-APHIS. 2011b.
101 Knezevic. 2010. Pg. 3.
102 USGC. 2006. Pg. 12.

2.2.3.1 Organic Crop Production

Organic farming as defined in this report includes any production system that falls within the USDA Agricultural Marketing Service (AMS) National Organic Program (NOP) as a certified organic production system. The NOP was established by the *Organic Foods Production Act of 1990*[103] and implementing USDA regulations.[104]

Organic production operators must develop and maintain an organic system plan approved by an accredited certifying agent.[105] The plan describes how the operation will achieve and document compliance with the NOP's National Organic Standards. The NOP requires organic farming operations to have distinct, defined boundaries and buffer zones to prevent unintended contact with excluded methods from adjoining land that is not under organic management.[106] Use of synthetic insecticides, fertilizers, and transgenic crops is excluded. Natural products, including some *Bt* foliar spray insecticides, are allowed. Since organic certification is process-based, the presence of genetically modified organism residues (e.g., transgenic corn) does not by itself constitute a violation of the NOP regulations.[107] Growers use a variety of methods, including temporal and physical isolation, to prevent the commingling of organic crops with non-organic crops. For example organic corn may be planted at a different time than neighboring non-organic corn to avoid cross-pollination due to overlapping flowering periods, or a buffer zone may place the organic corn farther away from non-organic corn than wind-borne non-organic corn pollen will typically travel.

An integrated strategy[108] for controlling selected insect pests in corn using organic practices might be comprised of:

- Monitoring to determine pest pressure and need for treatment and, if necessary;
- Direct treatment of each ear with a microbial or botanical insecticide carried in vegetable oil to control corn earworm;
- *Trichogramma* releases and/or foliar applications of *Bt* or spinosad to control European corn borer; and/or
- Foliar applications of *Bt* or spinosad for fall armyworm control.

In 2008 (the most recent data available[109]), approximately 168,000 acres of certified organic corn were planted (for seed, grain, or silage),[110] representing 0.20 percent of

▼

103 *Organic Foods Production Act of 1990*, as amended.
104 USDA. 2011a. 7 CFR Part 205.
105 USDA. 2011a. 7 CFR Part 205, Section 201.
106 USDA. 2011a. 7 CFR Part 205, Paragraph 205.202(c).
107 USDA-AMS. 2011. Pg. 1.
108 Hazzard and Westgate. 2004. Pg. 1.

the 93.6 million acres of corn planted in the US that year.[111] The top three states planted with organic corn for grain or seed were Wisconsin (33,619 acres), Minnesota (27,565 acres) and Iowa (25,419 acres). Organic corn grown for grain or seed produced 15,749,401 bushels from 143,432 harvested acres,[112] or an average of 109.8 bushels per acre. Comparatively, non-organic corn yielded an average of 153.9 bushels per acre in 2008.[113] On a bushels-per-acre basis, organic corn yield is 72 percent of non-organic corn.[114] To produce the volume of organic corn that was generated in 2008 via non-organic methods would have required an additional 32,000,000 acres of planted organic corn, a 40 percent increase. Although both organic and non-organic yields have been increasing an average of 2.0 bushels per acre per year in recent decades, organic corn yields currently lag non-organic corn yields by about 21.5 years. Organic corn yields in 2011 are similar to the non-organic corn yields of 1980.

2.2.3.2 Seed Production

Seed corn production differs from commercial grain production because seed companies impose strict requirements to maintain seed identity and high levels of genetic purity of the final product. As described in Section 2.2.3.3, *Other Specialty Production Systems*, seed purity is accomplished using contracts, tracking and traceability systems, quality assurance processes, record maintenance, auditing, proper labeling, appropriate sampling and testing and identity preservation systems.

Seed increase for a new corn variety encompasses larger and larger acreage for each successive generation. The initial generation of the new inbred line consists of one or only a few plants. These plants will produce a few ears. The seeds from these few ears are used to plant a small plot, approximately 0.1 acres. Early generation plants are self pollinated and pollen control is generally achieved by using bags over the tassels and ears. Using this system of pollen control allows many lines to be grown in a small area with an extremely low possibility of cross pollination with other lines grown in the immediate area. The seeds harvested are used to plant several acres for the next increase. Plants at this stage are also self pollinated to increase the seed; however, the area is likely too large for bagging as a means of pollen control. Pollen control is achieved by means of temporal or physical isolation from other lines. This increase produces inbred line seed. In the next stage, which produces single cross hybrid seed to be used for planting commercial corn grain fields, two different inbred lines (both are from the previous stage increase) are planted in the same field. One inbred line is considered the male line providing the pollen and the second line

109 The USDA-NASS conducted a detailed survey of organic farming in 2008. USDA-ERS and USDA-NASS both prepare annual reports of overall crop production that do not provide as detailed information about organic farms. This summary therefore compares organic and non-organic farming in 2008, using USDA-NASS and USDA-ERS data as cited.
110 USDA-NASS. 2008a. Pgs. 51-52.
111 USDA-ERS. 2010. Pg. 1.
112 USDA-NASS. 2008a. Pg. 2.
113 USDA-NASS. 2009b. Pg. 1.
114 Savage. 2011. Pg. 1.

is the female line, from which the hybrid seed will be harvested. Procedures must be used to prevent these plants of the female line from producing viable fertile pollen.

The field sizes used for any one corn variety at each step of increase can vary considerably depending on the present or expected market share of each variety, the level of testing to be conducted during the increase phase, and the expected yield of the inbred lines. In the case of corn, based on current production data, the amount of seed of all varieties combined must be sufficient to plant over 90 million acres each year.

2.2.3.3 Other Specialty Production Systems

Approximately 8 percent of corn grown in the US is specialty corn, which includes sweet corn, popcorn, white, waxy, hard endosperm, high oil, non-transgenic, and organic corn.[115] These corn varieties are specified by buyers and end-users of corn for production, and premiums are paid for delivering a product that meets purity and quality standards for the corn variety. Product differentiation and market segmentation in the specialty corn industry includes mechanisms to keep track of the grain (traceability) for Identity Preservation (IP) and quality assurance processes (e.g., ISO9001-2000 certification), as well as contracts between growers and buyers that specify delivery agreements.[116] Systems used by specialty corn growers and end-users to maintain identity of the production include:

- Contracts – written agreements detailing responsibilities and duties of both parties including premiums for reaching goals and penalties for failing to attain specifications.
- Tracking and Traceability Systems – correct labeling of all products (planting seeds and harvested material) and testing procedures for identifying and detecting acceptability of materials.
- Quality Assurance Processes – oversight on handling procedures, testing planting seeds, and testing harvested materials to determine acceptability of use and product requirements, and assuring testing procedures are appropriate.
- Closed-Loop Systems – the end-user supplies the planting seeds and guarantees to purchase final products. This may also require that the end-user conduct intermediate procedures such as planting, providing oversight during the growing season, harvesting, and transportation to processing plant.
- Identity Preservation Systems – using systems of identity preservation that have been shown to be successful in the past such as the seed certification systems conducted by members of the Association of Official Seed Certifying Agencies

▼

115 USGC. 2006. Pg.12.
116 Sundstrom *et al.* 2002. Pg. 14.

(AOSCA).[117] To maintain the purity of the corn product, this production system is based on controlling, tracking and documenting each step from seed production to end use (processing plants).

2.2.4 Raw and Processed Corn Commodities

Corn is processed to make it more palatable or isolate functional ingredients suitable for specific purposes.[118] Dry or wet milling is used to separate the bran and germ from the endosperm; wet milling further separates the endosperm into its chemical components, principally starch and protein. Breeding and genetic engineering for certain traits have improved the concentrations of desired ingredients, but there are no differences in handling requirements for processing transgenic and non-transgenic corn except for certain products like Syngenta's alpha-amylase ("Enogen") corn, which requires special handling procedures to ensure that this corn is not used for unintended purposes.

2.2.5 Persistence in the Environment/Weediness Potential

Corn, a highly domesticated plant,[119] is dependent upon humans for survival.[120] It does not persist in the environment outside of cultivated areas and does not have a potential to develop as a weed. Corn is not listed on the Federal noxious weed list.[121] It is grown throughout the world without any report that it is a weed or that it forms persistent feral populations, although corn seed from a previous year's crop can overwinter in fields and germinate the following year in warmer areas. Manual or chemical measures are often applied to remove these volunteers, but the plants that are not removed do not result in feral populations in following years.

Transgenic insect-resistant corn plants are no better at establishing feral populations than non-transgenic corn.[122] A field study of transgenic insect-resistant hybrids, non-transgenic hybrids, and native Mexican landraces planted and allowed to naturally propagate for two years resulted in no viable plants, indicating that the populations had died out.[123] There were no differences in the replacement capacity of transgenic corn hybrids and the nontransgenic control hybrids.

▼

117 AOSCA. 2011. Pg. 2.
118 Orthoefer and Eastman. 2004. Pg. 868.
119 Troyer. 2004. Pg. 134.
120 Hallauer. 2004. Pgs 899-900.
121 USDA-APHIS. 2010a.
122 Raybould *et al*, 2011. Pg 8.
123 Raybould *et al*, 2011. Pg 7.

2.3 Physical Environment

Water, soil, and air affect, and are affected by, corn agriculture; the methods described in Section 2.2.2, *Agronomic Practices*, may reduce some adverse impacts. This section describes water quality and use, soil characteristics, and air quality impacted by corn agriculture. Climatic conditions, in the context of potential climate change, are also discussed.

2.3.1 Water Quality and Use

Agriculture can affect water quality and use in irrigation. The following subsections describe corn agriculture's impact to water quality and use.

2.3.1.1 Water Quality

Agricultural nonpoint source (NPS) pollution is the leading source of water quality impacts to rivers and lakes, the second largest source of impairments to wetlands, and a major contributor to contamination of estuaries and groundwater.[124] The primary cause of NPS pollution is increased sedimentation from soil erosion. Soil erosion can introduce sediments, fertilizers, and insecticides to nearby lakes and streams when they are carried from corn fields by rain or irrigation water.[125] Insecticides or their degradates have been detected in many of the nation's streams,[126] and agricultural stream sites in the Corn Belt have been documented with chemical herbicide concentrations that exceed the human-health benchmark established by the EPA.[127]

Certain agronomic practices, including conservation tillage methods and reduced fertilizer or insecticide application rates, may reduce adverse impacts. The EPA recommends[128] several Best Management Practices for protecting water quality:

- **Conservation Tillage** - leaving crop residue (plant materials from past harvests) on the soil surface reduces runoff and soil erosion, conserves soil moisture, helps keep nutrients and insecticides on the field, and improves soil, water, and air quality;
- **Crop Nutrient Management** - fully managing and accounting for all nutrient inputs helps ensure nutrients are available to meet crop needs while reducing nutrient movements off fields. It also helps prevent excessive buildup in soils and helps protect air quality;

▼

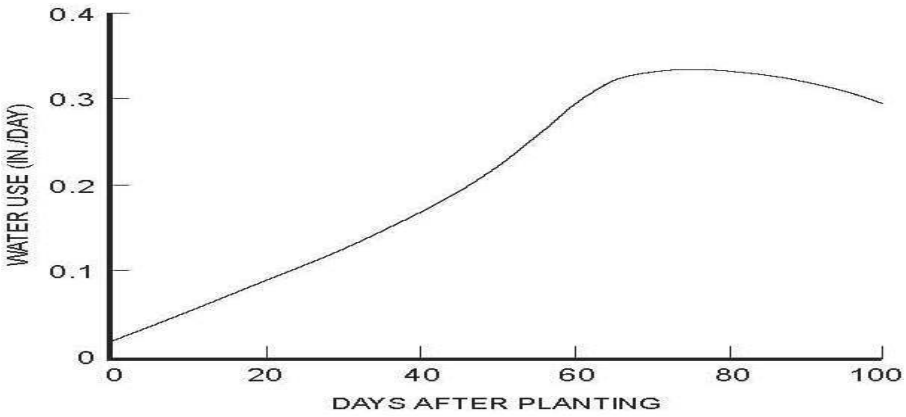
124 USEPA. 2005. Pg.1.
125 Ibid. Pg. 1.
126 Gilliom *et al*. 2007. Pg. 4.
127 Ibid. Pg. 6.
128 USEPA. 2008. Pg. 1.

- **Pest Management** - varied methods for keeping insects, weeds, disease, and other pests below economically harmful levels while protecting soil, water, and air quality; and

- **Conservation Buffers** - from simple grassed waterways to riparian areas, buffers provide an additional barrier of protection by capturing potential pollutants that might otherwise move into surface waters.

2.3.1.2 Water Use

Corn requires a steady supply of moisture, totaling approximately 4,000 gallons through the growing season, to produce one bushel of grain.[129] Rainfall, stored soil moisture from precipitation before the growing season, and supplemental irrigation during the growing season provide water for corn, as described in Section 2.2.2.2, *Irrigation*. Supplemental irrigation was used on 12 million acres of corn fields in the US in 2008, reflecting 15 percent of all corn acres harvested for grain.[130] Groundwater is the major source for irrigation, used on almost 90 percent of irrigated corn acreage in the US.



Water Use by Corn Plant in the First 100 Days After Planting

Source: Rhoades and Yonts. 2011.

The total amount of water used by corn varies from season to season and location to location, and is dependent on temperature, humidity, soil fertility, wind, solar radiation, total leaf area of the crop and the interaction of these factors.[131] The typical water use by corn in the first 100 days after planting, as calculated from rainfall and irrigation, is illustrated in the graph above.

▼

129 South Dakota Corn Growers Association. 2010. Pg. 1.
130 Christensen. 2002. Pg. 31.
131 Krantz *et al*. 2008. Pg. 3.

Corn does not extract water uniformly throughout its rooting depth. Generally, more water is extracted from shallow depths and less from deeper depths. If water is applied to the soil surface, the typical extraction pattern follows the 4-3-2-1 rule: 40 percent of the water comes from the top quarter of the root zone, 30 percent comes from the second quarter, and so on.[132]

2.3.2 Soil

The interaction between the below-ground community of microorganisms and arthropods, plant-root structure, and organic residues in the soil is central to soil ecological processes including decomposing organic material, subsequent nutrient cycling and release, and maintaining soil structure and composition. Cultivating corn directly impacts these biological attributes. Agronomic practices such as crop type, tillage, and pest management regime have greater effects on the biology of the soil than the type of corn that is cultivated.[133] For example, conventional tillage and mechanized harvesting machinery may disturb and expose the top soil surface layer, leaving the land prone to degradation. Soil degradation can lead to a decline in water quality and contribute to the greenhouse effect.[134] A decline in soil quality and soil resilience enhances the greenhouse effect through emissions of radiatively active gases (CO_2, nitrous oxide [NO_X]).[135] Land that is prone to degradation is also more likely to adversely impact water resource quality and communities of organisms dependent on those water resources.

Conservation tillage methods, including no-till, ridge-till, low-till and minimum-till, leave a crop mulch on the ground to provide a protective cover to the soil between seasons and improve soil fertility by maintaining nutrient-rich organic matter on the field.[136] Organic matter builds up in the soil, absorbing CO_2 and helping to reduce a significant amount of greenhouse gas.

2.3.3 Air Quality

Air quality may potentially be directly affected by agricultural activities such as burning, tilling, harvesting, spraying, and fertilizing. Smoke from burning agricultural waste releases particulates. Tilling and harvesting with motorized equipment release emissions that include carbon monoxide, nitrogen oxides, reactive organic gases, particulate matter, sulfur oxides, and greenhouse gases (GHG).[137] Tillage also releases GHG because of the loss of CO_2 to the atmosphere, and the exposure and oxidation of soil organic matter.[138] Aerial application of insecticides may cause impacts from drift and diffusion. Insecticides may volatilize after

▼

132 Krantz *et al.* 2008. Pg. 4.
133 Griffiths *et al.* 2007. Pg. 196.
134 Lal and Bruce. 1999. Pg. 177.
135 Ibid. Pg. 178.
136 NCGA. 2007. Pg. 1.
137 USEPA. 2011b. Pgs. 6-17.
138 Baker *et al.* 2005. Pg. 11260.

application to soil or plant surfaces and move following wind erosion.[139] NO_X may also be released following nitrogen fertilizer application.[140] Agriculture, including land-use changes for farming, is responsible for an estimated 6 percent of all human-induced GHG emissions in the US.[141] NO_X emissions from agricultural soil management comprise 68 percent of all US N_2O emissions.[142]

2.3.4 Climate

Corn agriculture may potentially affect or be affected by climate change.[143, 144] Agriculture-related activities are recognized as both direct (e.g., exhaust from motorized equipment) and indirect (e.g., agricultural-related soil disturbance) sources of GHGs.[145] Greenhouse gases collectively function as retainers of solar radiation and contribute to climate change. The agricultural sector is identified as the second largest contributor to GHG emissions in the US, ranking only behind the energy sector.[146]

Climate change may also potentially affect agricultural crop production. These potential impacts on the agro-environment and individual crops may be direct, including changing patterns in precipitation, temperature, and duration of growing season, or may cause indirect impacts extending the ranges of weeds and other pests.[147] One recent study[148] of aggregate North American impacts on agriculture from climate change projects yield increases of 5 to 20 percent for this century, while other data suggest a reduction in yields.[149] Certain regions of the US are likely to be more heavily impacted than others because water resources may be substantially reduced. North American production is expected to adapt with improved cultivars and responsive farm management[150]

2.4 Biological Environment

The biological environment described in this section includes animals, plants, biodiversity, and corn gene movement. Threatened and endangered species are discussed in Section 2.5.

▼

139 Vogel *et al*. 2008. Pg. 1101.
140 Aneja *et al*. 2009. Pg. 4236.
141 USEPA. 2011b. Pg. 6-1.
142 Ibid. Pg. 6-1.
143 Iserman. 1994. Pg. 106.
144 Aneja *et al*. 2009. Pg. 4238.
145 USEPA. 2011b. Pg. 6-17.
146 USEPA. 2011b. Pg. 2-23.
147 IPCC. 2007. Pg. 48.
148 Ibid. Pg. 48.
149 Gillis. 2011. Pg. 7.
150 IPCC. 2007. Pg. 52.

2.4.1 Animals

Animals that may be found in corn fields include birds, large and small mammals, and invertebrates. Reptiles and amphibians, although potentially present, have little reported effect on corn.

Birds can be beneficial or detrimental to the agro-environment. Although many birds visit row crop fields such as corn, resident numbers are low and few nest there, likely due to overlap between nesting phenology and mechanized harvest.[151] Bird species that have been observed in row crop fields during the growing season include red-winged blackbirds (*Agelaius phoeniceus*), brown-headed cowbirds (*Molothrus ater*), and vesper sparrows (*Pooecetes gramineus*).[152] Red-winged blackbirds are often initially attracted to corn fields to feed on insect pests, but then also feed on corn seeds. Red-winged blackbirds can destroy more than 360,000 tons of field corn and substantial amounts of sweet corn annually.[153] Other species, such as horned larks (*Eremophila alpestris*), may use fallow fields as foraging habitat.[154]

Throughout the latter half of the growing season, field corn functions as food and cover for white-tailed deer (*Odocoileus virginianus*).[155] Deer can significantly damage or completely destroy small corn fields that are surrounded by woody or brushy areas. Deer damage to large corn fields is often limited to a few rows closest to the wooded areas.

Corn fields are utilized for feeding and cover by small mammals such as raccoons (*Procyon lotor*), woodchuck (*Marmota monax*), deer mice (*Peromyscus maniculatus*), meadow voles (*Microtus pennsylvanicus*), and thirteen-lined ground squirrels (*Spermophilus tridecemlineatus*). Raccoons can damage field corn. The deer mouse is the most common small mammal in some corn production regions. Deer mice feed on a wide variety of plant and animal matter, but feed primarily on seeds and insects. They are considered beneficial in agroecosystems because they consume both weed and pest insect species. The meadow vole feeds primarily on fresh grass, sedges, and herbs, but also on seeds and grains. Meadow voles also can be considered beneficial for their role in the consumption of weeds, but can be an agricultural pest where abundant. The thirteen-lined ground squirrel feeds primarily on seeds of weeds and crops such as corn and wheat. Thirteen-lined ground squirrels have the potential to damage agricultural crops, although they can also be beneficial when eating pest insects such as grasshoppers and cutworms.

Many of the invertebrate organisms found in corn-producing areas are considered pests, such as corn earworm, European corn borer, fall armyworm, and corn rootworm. Many other invertebrates are considered beneficial. Numerous

▼

151 Patterson and Best. 1996. Pg. 153.
152 Best *et al.* 1990. Pg. 89.
153 Dolbeer. 1990. Pg. 309.
154 Best *et al.* 1990. Pg. 89.
155 Vercauteren and Hygnstrom. 1993. Pg. 218.

invertebrates perform valuable functions such as pollinating plants (e.g., bees), contributing to the decay of organic matter and cycling soil nutrients (e.g., earthworms), and attacking other pest insects and mites (e.g., ladybird beetles). Other beneficial insects include lacewing, minute pirate bug, syrphid fly, damselbug, ground beetle, praying mantis, spined soldier bug, tachinid fly, and parasitic wasps.[156]

2.4.2 Plants

Corn fields can be bordered by other agricultural fields (including other corn varieties), woodlands, or pasture and grasslands. From an agronomic perspective, the most relevant members of a surrounding plant community are those that can behave as weeds. Corn agronomic performance can be reduced by weed competition for water, nutrients, and light. US corn yields are threatened by more than 200 weed species annually.[157]

Common corn field weeds include giant foxtail (*Setaria faberi*), giant ragweed, velvetleaf (*Abutilon theophrasti*), common cocklebur (*Xanthium strumarium*), Canada thistle (*Cirsium arvense*), common lambsquarters (*Chenopodium album*), Johnsongrass, fall panicum (*Panicum dichotomiflorum*), and hairy fleabane.[158] Weeds such as giant foxtail and barnyard grass (*Echinochloa crusgalli*) have been shown to reduce corn yields by up to 14[159] and 35[160] percent, respectively. Weed management strategies that corn growers use, including strategies to address weed resistance to herbicides, are discussed in Section 2.2.2.5, *Weed Management*.

2.4.3 Biodiversity

Biodiversity within agricultural ecosystems is strongly impacted by agricultural practices, including the type of cultivated plant and crop-specific management practices. Species diversity and abundance in corn agroecosystems may differ between transgenic, non-transgenic, and organic corn. Relative to any natural ecosystem, species abundance and richness will generally be less in intensively managed agroecosystems.

2.4.4 Gene Movement in the Natural Environment

Corn is self-compatible and wind-pollinated. In the US, there are no native plant species that can be pollinated by corn pollen without human intervention. However, teosinte (the wild progenitor of corn) can sometimes be found as introduced populations in botanical gardens in the US and as feral populations of *Zea mexicana* in Florida, Alabama,

▼

156 SCGO. 2007. Pgs. 1-4.
157 Heap. 2008. Pg.1.
158 Childs. 1996. Pg. 1.
159 Fausey *et al*. 1997. Pg.256.
160 Bosnic and Swanton. 1997. Pg. 281.

and Maryland,[161] and *Zea perennis* in South Carolina.[162] Evidence of introgression of genes from corn into US teosinte populations has not been sought but complex mechanisms of incompatibility have been described that are barriers to this potential.[163]

2.5 Threatened and Endangered Species

This section identifies the coleopteran species protected under the federal *Endangered Species Act* (ESA)[164] that occur within the region where corn is grown. The consideration of protected species is limited to Coleoptera, as this is the insect group to which corn rootworms belong, and the insecticidal protein produced in 5307 corn (eCry3.1Ab) has been shown to be active only on certain Coleoptera and not on other invertebrate or vertebrate species. The proposed action's lack of potential impacts to these species and their critical habitat is discussed in Section 4.5, *Threatened and Endangered Species*.

Syngenta obtained data on listed coleopteran species and critical habitat from the US Fish and Wildlife Service[165] (FWS). County-level species location information was obtained from a work product of the FIFRA Endangered Species Task Force (FESTF) including the Information Management System (IMS) and the NatureServe Multi-Jurisdictional Database (MJD) licensed by FESTF.[166] This database contains county-level species data provided from the FWS and crop data from the USDA Census of Agriculture. This provides the best available aggregated data on federally listed species habitat and occurrence. Syngenta Crop Protection LLC is a full member of FESTF and has access to this licensed dataset. This database provides information only for extant species locations.

Syngenta's analysis identified a total of 17 coleopteran species listed by the FWS as Endangered or Threatened under the ESA, two of which do not occur in corn-growing regions. One coleopteran species was subsequently listed as endangered (Casey's June beetle, *Dinacoma caseyi*) but does not occur in corn-growing regions.[167] Table 2-4 lists the 15 threatened or endangered species of Coleoptera found within corn-growing regions. Critical habitat, where established by the FWS, is also indicated.

▼

161 USDA-NRCS. 2011a. Pg. 1.
162 USDA-NRCS. 2011b. Pg. 1.
163 Kermicle and Evans. 2010. Pg. 737.
164 *Endangered Species Act of 1973*, as amended.
165 USFWS. 2011a. Pgs. 1-3.
166 See the FESTF website at http://www.festf.org/visitors/default.asp.
167 USFWS. 2011b. Pg. 58954.

Table 2-4 Threatened or Endangered Coleopteran Species Occurring in Corn-Growing Regions

Scientific Name	Family	Common Name	ESA Status	Critical Habitat
Batrisodes texansus	Staphylinidae	Coffin Cave mold beetle	Endangered	None
Batrisodes venyivi	Staphylinidae	Helotes mold beetle	Endangered	Bexar Co., TX
Brychius hungerfordii	Halipidae	Hungerford's crawling water beetle	Endangered	None
Cicindela dorsalis dorsalis	Carabidae	Northeastern beach tiger beetle	Threatened	None
Cicindela nevadica lincolniana	Carabidae	Salt Creek tiger beetle	Endangered	Lancaster and Saunders Co., NE
Cicindela puritan	Carabidae	Puritan tiger beetle	Threatened	None
Desmocerus californicus dimorphus	Cerambycidae	Valley elderberry longhorn beetle	Threatened	Sacramento Co., CA
Elaphus viridus	Carabidae	Delta green ground beetle	Threatened	Solano Co., CA
Heterelmis comalensis	Byrrhoidae	Comal Springs riffle beetle	Endangered	Hays and Comal Co., TX
Nicrophorus americanus	Silphidae	American burying beetle	Endangered	None
Rhadine exilis	Carabidae	None	Endangered	Bexar Co., TX
Rhadine infernalis	Carabidae	None	Endangered	Bexar Co., TX
Rhadine Persephone	Carabidae	Tooth Cave ground beetle	Endangered	None
Stygoparnus comalensis	Dryopidae	Comal Springs dryopid beetle	Endangered	Hays and Comal Co., TX
Texamaurops reddelli	Staphylinidae	Kretschmarr Cave mold beetle	Endangered	none

Source: FESTF

2.6 Public Health

Public health concerns related to corn stem from human consumption of corn and corn products, animal (livestock) consumption of feed corn and corn products, and the indirect effect on human health and worker safety from laborers' exposure to agricultural chemicals.

2.6.1 Human Health

Corn has no known human health risks except allergenicity. The *Food Allergen Labelling and Consumer Protection Act*[168] requires disclosure of the presence of eight specific food groups which are designated as "major food allergens" on the package labels.[169] These eight food groups (milk, eggs, fish, crustacean shellfish, tree nuts, peanuts, wheat and soybean) account for 90 percent of food allergic reactions in the US.[170] Corn is not designated as a "major food allergen" and the FDA guidance on compliance with the Act does not include reference to corn or refined corn products.[171] There are no known human health effects from the novel proteins in *Bt* corn.

168 *Food Allergen Labelling and Consumer Protection Act of 2004.*
169 USDHHS-FDA. 1992. Pg. 1.
170 Ibid. Pg. 1.
171 Corn Refiners Association. 2006. Pg. 1.

At present, the prevalence of corn food allergy in the US is exceedingly low, estimated to affect no more than 0.016 percent of the general population.[172] Exposure to corn allergens can occur externally (direct skin contact) or internally (ingestion). Some attributed symptoms of corn allergy include dermatitis, asthma, urticaria, migraine headache, ulcerative colitis, irritable bowel disease, celiac sprue, and anaphylaxis under severe exposure.[173] Currently there is no cure for corn allergy. The only mechanism for managing the hypersensitivity is to avoid consuming foods that contain the allergen.

2.6.2 Animal (Livestock) Health

Corn has no known animal (livestock) health risks except from the presence of mycotoxins, which are secondary metabolites produced by certain fungi. Mycotoxins are considered unavoidable contaminants in food as there is no known technology that can completely eliminate their presence in crops. Insect damage is one factor that predisposes corn to mycotoxin contamination, as insect herbivory creates stalk or kernel wounds that encourage fungal colonization.[174] Mycotoxins are of concern worldwide because of their toxic and carcinogenic effects in humans and animals feeding on infected corn. Table 2-5 provides a list of corn mycotoxins; the two categories of most concern are fumonisins and aflatoxins.

Fumonisins are found exclusively in corn, while aflatoxins are found in a variety of crops including corn, cotton, peanuts and some nuts.[175] Fumonisins are produced by the fungi *Fusarium verticillioides* and *F. proliferatum*. Since their first discovery in 1988, over 28 types of fumonisins have been isolated, of which fumonisin B_1 (FB_1) is the most common in corn.[176] Fumonisins can be highly toxic to animals, causing diseases such as leukoencephalomalacia in horses and pulmonary edema in swine.[177] Lepidopteran-protected *Bt* corn hybrids have reduced fumonisin levels.[178]

Aflatoxins are produced by the fungi *Aspergillus flavus* and *A. parasiticus*, and are the most potent chemical liver carcinogens.[179] In poultry, aflatoxin consumption results in liver damage, impaired productivity and reproductive efficiency, decreased egg production in hens, inferior eggshell quality, inferior carcass quality, and increased susceptibility to disease.[180] In cattle, the primary symptoms are reduced weight gain, liver and kidney damage, and reduced milk production.[181] Extensive testing and use have identified no animal (livestock) health risks for transgenic corn products.

▼

172 Ibid. Pg. 1.
173 USDHHS-FDA. 1992. Pg. 2.
174 Wu. 2006a. Pg. 121.
175 Wu. 2008. Pg. 1.
176 Wu. 2006b. Pg. 278.
177 Ibid. Pg. 278.
178 Munkvold *et al*. 1999. Pg. 133.
179 Wu. 2006b. Pg. 278.
180 Ibid. Pg. 278.
181 Ibid. Pg. 278.

Table 2-5 List of Corn Mycotoxins Toxic to Animals Consuming Contaminated Feed

Mycotoxin	Fungi Associated	Symptoms/Toxicology
Aflatoxin	*Aspergillus flavus, A. parasiticus*	liver necrosis, liver tumors, reduced growth, depressed immune response, carcinogen
Fumonisin	*Fusarium moniliforme, F. proliferatum*	equine leukoencephalomalacia, porcine pulmonary edema
Deoxynivalenol	*F. graminearum*	feed refusal, reduced weight gain, diarrhea, vomiting
Trichothecenes	*F. graminearum, F. culmorum, F. poae*	alimentary toxic aleukia, necrosis, hemorrhage, oral lesions in broiler chickens
Ochratoxins	*Penicillium verrucosum, Aspergillus ochraceus*	porcine nephropathy; various symptoms in poultry
Citrinin	*Penicillium* spp., *Aspergillus* spp.	kidney damage
Cyclopiazonic acid	*Penicillium* spp., *Aspergillus* spp.	neurotoxin
Sterigmatocystin	*Aspergillus* spp., and others	carcinogen, mutagen

Source: Koennig and Payne. 1999. Pg. 4.

2.6.3 Worker Safety

Workers engaged in corn production may encounter insecticides, herbicides, fungicides or fertilizers that may pose a worker health or safety risk unless used in accordance with the EPA-established agriculture-specific requirements in the Worker Protection Standard[182] that protect field workers from the hazards of chemical exposure. The Occupational Safety and Health Administration requires all employers to protect their employees from hazards associated with agricultural chemicals.

Pesticides are used on most corn acreage in the US, and changes in acreage, crops, or farming practices can affect the amounts and types of pesticides used and thus the risks to workers. Registered pesticides, including the representative products listed in Table C-1 (Appendix C), must have use restrictions that, if followed, have been determined to be protective of worker health.

2.7 Socioeconomics

Corn agriculture can affect socioeconomic resources such as the domestic economy, international trade economy, and the social environment. This section describes key current issues within each of these topics.

182 USEPA. 1992.

2.7.1 Domestic Economy

Domestic demand for corn in the US comes from its use for feed, ethanol production, food, and seed, and totaled 11.1 billion bushels in the 2009/2010 marketing year.[183] Exports (as described in Section 2.6.2, *Trade Economy*) added another 2 billion bushels to total US corn demand. Demand is satisfied almost entirely by domestic supply with few imports, the US being largely a net exporter of corn. In the 2009/2010 marketing year, feed was approximately 40 percent of US corn production; food, seed, and industrial uses were approximately 45 percent (including ethanol production, at about 36 percent); and exports the remaining 15 percent.[184]

US corn farms have increased cash receipts from $34.1 billion in 2007 to a projected $62.0 billion in 2011, representing an increase from 22.7 percent of the US crop market to 30.0 percent.[185] Annual cash receipts for corn as compared to all crops are shown in Table 2-6.

Table 2-6 Corn and Crop Cash Receipts

	Cash Receipts ($ billions)	
Year	Corn	All Crops
2007	34.1	150.1
2008	48.4	175.0
2009	42.5	168.3
2010	44.8	172.9
2011	62.0	206.5

Source: USDA-ERS. 2011c. Pg. 1.

Net returns for corn farmers fluctuated between $125 and $200 per acre from the mid-1990s until the mid-2000s, but more than doubled in price to nearly $400 per acre in the late 2000s.[186] Net returns are expected to remain near $350 per acre for the next decade. USDA-ERS attributes the recent increase and projected future high prices to ethanol demand, in part due to the renewable fuel standard component of the *Energy Policy Act*.[187] The recent average annual market price of corn[188] in the US has been relatively high, due in part to the ethanol demand:

- 2008-- $4.06/bushel
- 2009-- $3.55/bushel
- 2010-- $5.40/bushel

183 USDA. 2011b. Pg. 70.
184 Ibid. Pgs. 61 and 70.
185 USDA-ERS. 2011c. Pg. 1.
186 USDA-ERS. 2011b. Pg. 3.
187 *Energy Policy Act of 2005*.
188 USDA-NASS. 2011d. Pg. 17.

There is a niche market for non-transgenic food and feed in the US, as is evident from private labeling initiatives such as the Non-GMO Project. This initiative offers third-party product verification and labeling for non-transgenic products.[189] There also is a niche market for organic products in the US. Sales of organic products have been growing quickly, from $1 billion in 1990 to $26.7 billion in 2010, with a 7.7 percent increase between 2009 and 2010 alone.[190] To satisfy the demand for organic corn, producers have had to adopt specific production practices to maintain and prevent the use of excluded methods as dictated by the NOP. To offset the increase in investment related to these more extensive practices, premiums are often paid for non-transgenic or organic corn. For example, August 2011 non-organic corn in selected markets averaged $7.65 per bushel, whereas organic corn averaged $13.00 per bushel.[191]

2.7.2 Trade Economy

Agribusiness is one of the world's largest industries, employing 1.3 billion people and producing $1.3 trillion worth of goods each year.[192] The US is the largest world exporter of corn, averaging nearly 50 million metric tons per year between 1990 and 2010.[193] US corn exports peaked at 61 million metric tons in 2007/08.[194] In 2010, the total value of US corn exports was nearly $8 billion.[195] During the last half decade, the US share of world corn exports averaged 60 percent; the second largest exporter is Argentina. Japan is the world's largest corn importer, typically followed by South Korea, Mexico, Egypt, and Taiwan.[196]

The primary US corn export destinations are also the largest world importers of corn and do not have major barriers for importing transgenic products. Data on international trade in organic corn are not readily available but trade in organic corn is likely to be a very small share of the total corn trade.

The USDA Interagency Agricultural Projections Committee forecasts near-term production increases for grain products in general, in response to recent global supply-and-demand conditions.[197] Prices are projected to decline globally in the short term as production expands. Steady growth in production and historically high prices are projected long term, through 2020. Assuming that current subsidies and tariffs remain in effect, corn is expected to remain the primary feedstock for US ethanol production, comprising about 36 percent of the total corn use.

▼

189 See Non-GMO-Project website: http://www.nongmoproject.org/.
190 OTA. 2011. Pg. 1.
191 Rodale Institute. 2011. Pg. 1.
192 Shelton *et al*. 2002. Pg. 870.
193 USDA. 2011b. Pg. 26.
194 USDA-ERS. 2009. Pg. 2.
195 USDA-ERS. 2011d. Pg. 1.
196 USDA-FAS. 2011. Pg. 1.
197 USDA. 2011b. Pg. 26.

2.7.3 Social Environment

Social issues related to corn include farmer and consumer choice, as well as the structure of US corn farms. Farmers have a range of options in agronomic practices, seed products (non-transgenic, transgenic, and organic), and markets for their products. Consumers have a range of corn products to choose from in a free market system such as the US.

Genetic engineering has exerted a downward pressure on food prices by increasing agricultural productivity. Relatively lower food prices presumably allow consumers to choose between a greater variety of products that are now more affordable. Transgenic crops can positively contribute to sustainability for farmers. Traits such as herbicide tolerance and insect resistance, as described in Section 2.2, *Corn Production*, offer opportunities for making agriculture more sustainable.[198] The advent of the NOP in the US has also increased consumer choice, as is evident by the rapid growth of the organic market segment.

US corn farms and their operators are similar in many respects to those of other feed grain crops. According to data from the 2003 Agricultural Resource Management Survey (ARMS), the majority of feed grain farms (84 percent) raised corn;[199] overall feed grain data are therefore generally applicable to corn farms when grain-specific data are not available. Feed grain farms operate more acres per farm, have higher gross and net incomes per farm, and have higher values of farm equity per farm than nonfeed grain farms. Feed grain operators were much more likely to list farming as their occupation and were more likely than operators of nonfeed grain farms to operate a farm organized as a partnership or family corporation.[200] Only 29 percent of all feed grain farms specialized in feed grains, but those farms accounted for 51 percent of all feed grain production, and most of the specialized farms were corn farms.[201]

In 2003, feed grain farms' average annual net cash income was $45,916, compared to $8,875 for nonfeed grain crops.[202] The ratio of cash expense to gross cash income was 75 percent for feed grain farms, compared to 85 percent for nonfeed grainfarms.[203] The total household income (from all sources) for corn farm operators averaged about $70,000 in 2003, nearly $10,000 above the US average household income that year but nearly equal to the average income from nonfeed grain farm families.[204] Off-farm income sources include off-farm employment, investment income, pensions, Social Security payments, and gifts.[205] Additionally, there are four types of government payments to feed grain operators: direct; counter-cyclical and loan

▼

198 Franke *et al*. 2011. Pg. 88.
199 Hoffman *et al*. 2007. Pg. 22.
200 Hoffman *et al*. 2007. Pg. 23.
201 Hoffman *et al*. 2007. Pg. 24
202 Hoffman *et al*. 2007. Pg. 22.
203 Hoffman *et al*. 2007. Pg. 22.
204 Hoffman *et al*. 2007. Pg. 24.
205 Hoffman *et al.* 2007. Pg. 24.

deficiency; conservation reserve, wetland reserve, and environmental quality incentives program payments; and other.[206] For corn operators, these programs contribute some 15 percent of their cash income. Direct government payments accounted for about 8 percent of average gross cash income for corn farms in 2003[207] while the other three categories comprised another 7 percent of cash income for corn farmers.[208]

▼

206 Hoffman *et al*. 2007. Pg. 22.
207 Hoffman *et al*. 2007. Pg. 22.
208 Hoffman *et al*. 2007. Pg. 23.

3 Alternatives

This chapter describes the alternatives considered regarding deregulation of Event 5307 corn. To approve Syngenta's petition for nonregulated status, APHIS must first determine that 5307 corn is unlikely to pose a plant pest risk. If the Plant Pest Risk Assessment (PPRA) determines that 5307 corn is unlikely to pose a plant pest risk, APHIS must conclude that 5307 corn is no longer subject to Chapter 7, Part 340 of the Code of Federal Regulations (7 CFR Part 340), or the plant pest provisions of the PPA.

3.1 No Action: Continuation as a Regulated Article

Under the No Action alternative, APHIS would deny the petition and the status quo would be maintained. Event 5307 corn and progeny derived from 5307 corn would continue to be regulated articles. Event 5307 corn would be grown under USDA notification or permit and confined release conditions. It would have restricted availability to growers. Measures to ensure physical and reproductive confinement would continue to be implemented. Growers would continue to use deregulated transgenic corn, currently comprising some 88 percent of the US corn market, or non-transgenic corn (including organic corn) that is available without restrictions. APHIS could choose this alternative if there were insufficient evidence to demonstrate the lack of plant pest risk from the unconfined cultivation of 5307 corn.

Event 5307 corn is unlikely to pose a plant pest risk. Choosing this alternative would not satisfy the purpose and need of making a determination of plant pest risk status and responding to the petition for nonregulated status. Additionally, this alternative would not satisfy the product's purpose of providing growers with an additional product to control corn rootworm. This product is expected to contribute to the trend in reduced use of hazardous insecticides and extend the useful life of other commercially available corn rootworm-protected *Bt* products (Cry3Bb1, Cry34Ab1/Cry35Ab1, and modified Cry3A corn), improve worker safety, reduce the use of fossil fuels to apply chemical insecticides, provide economic benefits for growers, and increase competition in the marketplace for insect-protected seed products.

3.2 Proposed Action: Determination that Event 5307 Corn is No Longer a Regulated Article

This alternative would deregulate 5307 corn, offering growers a new cultivar that is highly resistant to larval feeding damage by three coleopteran pests:

- Western corn rootworm (*Diabrotica virgifera virgifera*);
- Northern corn rootworm (*D. longicornis barberi*); and
- Mexican corn rootworm (*D. virgifera zeae*).

Corn plants derived from transformation Event 5307 will contain the gene *ecry3.1Ab* encoding an eCry3.1Ab protein and the gene *pmi* (also known as *manA*) encoding the enzyme phosphomannose isomerase (PMI). The eCry3.1Ab protein is an engineered chimera of modified Cry3A (mCry3A) and Cry1Ab proteins, each of which is derived from a native gene of *Bacillus thuringiensis* (*Bt*), a soil bacterium. The mCry3A protein is active among certain Coleoptera, whereas Cry1Ab is active on certain Lepidoptera. The gene *pmi* was obtained from *Escherichia coli* strain K-12 and the PMI protein it encodes was utilized as a plant selectable marker during development of 5307 corn.

As an engineered protein, eCry3.1Ab has similarities to other well-characterized Cry (crystal) proteins, namely mCry3A and Cry1Ab. Cry proteins exert their insecticidal activity when they:

- Are ingested by the insect and solubilized in the insect gut;
- Are activated by specific proteolytic cleavage by midgut enzymes;
- Bind to specific receptors on the surface of the insect midgut; and
- Form ion channels in the gut membrane.

Collectively, these four processes result in disruption of the normal function of the midgut leading to the death of the insect. The eCry3.1Ab protein exhibits the same behavior as other coleopteran-active *Bt* Cry proteins, including alkaline solubility, cleavage by chymotrypsin, specificity of brush border membrane binding, and ion channel formation. Although mCry3A and eCry3.1Ab act on corn rootworms by the same general mechanism, eCry3.1Ab has a unique binding site in the target pest (different from mCry3A).[209] Combining eCry3.1Ab and mCry3A in a single product will not result in these proteins competing for the same binding site in the pest gut membrane.

The mode of action of eCry3.1Ab, like that of most other Cry proteins, is highly specific to insects and is ineffectual in mammalian or other vertebrate species. Studies conducted by Syngenta demonstrate that eCry3.1Ab is not active on species

▼

209 Walters *et al*. 2010. Pg. 3086.

outside the Chrysomelidae family of Coleoptera. It demonstrates no lepidopteran activity, despite containing sequences from a lepidopteran-active protein (Cry1Ab).

Event 5307 corn will not be sold as a stand-alone product. Rather, Syngenta will "stack" the 5307 corn transgenes with other transgenes by conventional plant breeding to deliver two modes of action against corn rootworm and (at least) two modes of action against lepidopteran pests as well as herbicide tolerance. The two breeding stacks planned that include 5307 corn are Bt11 × MIR604 × TC1507 × 5307 × GA21 and Bt11 × MIR162 × MIR604 × TC1507 × 5307 × GA21 corn. In addition to the corn rootworm control provided by 5307 corn, the controls provided by other constituents of these combinations are:

- A Cry1Ab protein for lepidopteran control (Bt11);
- A modified Cry3A protein for corn rootworm control (MIR604);
- A Cry1F protein for lepidopteran control (TC1507);
- A double mutated 5-enolpyruvylshikimate-3-phosphate synthase enzyme for glyphosate tolerance (GA21);
- A Vip3Aa20 protein for lepidopteran control (MIR162); and
- A phosphinothricin acetyltransferase enzyme for glufosinate tolerance (Bt11 and TC1507).

Under the Proposed Action alternative, 5307 corn and its progeny would no longer be regulated articles. Permits issued or notifications acknowledged by APHIS would no longer be required for introductions of 5307 corn and its progeny.

3.3 Alternatives Considered But Rejected from Further Evaluation

Other alternatives were considered but rejected from further evaluation. These alternatives were considered in light of APHIS's authority under the plant pest provisions of the PPA and the regulations at 7 CFR Part 340, with respect to the purpose and need for 5307 corn. APHIS has determined that it has no regulatory authority to impose restrictions on a product once it concludes that the product does not pose a plant pest risk. These alternatives are discussed briefly below along with the specific reasons for rejecting each.

3.3.1 Prohibit Any Event 5307 Corn from Being Released

Under this alternative, APHIS would decline to deregulate 5307 corn and prohibit the release of 5307 corn by denying any permits associated with its field testing. No such restriction has been imposed on other *Bt* corn products.

In enacting the *Plant Protection Act*, Congress found that

> [D]ecisions affecting imports, exports, and interstate movement of products regulated under [the *Plant Protection Act*] shall be based on sound science….[210]

The White House Emerging Technologies Interagency Policy Coordination Committee has developed broad principles,[211] consistent with Executive Order 13563,[212] to guide the development and implementation of policies for oversight of emerging technologies (such as genetic engineering) at the agency level. Agencies should adhere to Executive Order 13563 and the following principle to the extent permitted by law, when regulating emerging technologies:

> [D]ecisions should be based on the best reasonably obtainable scientific, technical, economic, and other information, within the boundaries of the authorities and mandates of each agency.

Based on the PPRA and the scientific data evaluated therein, 5307 corn is unlikely to pose a plant pest risk. Accordingly, there is no basis in science for prohibiting the release of Event 5307 corn, and this alternative is not appropriate for further consideration.

3.3.2 Establish an Isolation Distance Between Event 5307 Corn and Non-Transgenic Corn

Under this alternative, APHIS would establish an isolation distance separating 5307 corn from non-transgenic corn. Because 5307 corn is unlikely to pose a plant pest risk, any alternative requiring isolation distances would be inconsistent with the APHIS's statutory authority under the plant pest provisions of the PPA and regulations in 7 CFR Part 340.

The imposition of isolation distances also would not meet APHIS's purpose and need to respond appropriately to a petition for nonregulated status based on the requirements in 7 CFR Part 340 and the agency's authority under the plant pest provisions of the PPA. Additionally, this alternative would not satisfy the product's purpose of providing growers with an additional product to control corn rootworm. Individuals may choose on their own to use isolation distances and other management practices to minimize possible gene movement between corn fields.

▼

210 *Plant Protection Act* § 402(4).
211 White House Emerging Technologies Interagency Policy Coordination Committee. 2011.
212 Executive Order 13563. 2011.

3.3.3 Establish Geographic Restrictions for Event 5307 Corn

Under this alternative, APHIS would restrict the production of 5307 corn to certain geographic areas based on the location of organic production systems or production systems for markets sensitive to transgenic products. This approach might or might not address concerns over possible gene movement between transgenic and non-transgenic plants. Because 5307 corn does not pose a plant pest risk, however, imposing geographic restrictions where there is no greater plant pest risk would not be consistent with APHIS's statutory authority under the plant pest provisions of the PPA and regulations in 7 CFR Part 340.

The imposition of geographic restrictions also would not meet APHIS's purpose and need to respond appropriately to a petition for nonregulated status based on the requirements in 7 CFR Part 340 and the agency's authority under the plant pest provisions of the PPA. Additionally, this alternative would not satisfy the product's purpose of providing growers with an additional product to control corn rootworm. Individuals may choose on their own to geographically isolate their non-transgenic corn production systems from 5307 corn or to use other management practices to minimize possible gene movement between corn fields.

3.3.4 Require Testing for Event 5307 Corn

Under this alternative, APHIS would require and provide testing to identify the presence of transgenic products in systems that produce non-transgenic products. There are no nationally established regulations involving testing, criteria, or limits of transgenic material in systems that produce non-transgenic products. Such a requirement would be extremely difficult to implement and maintain. Additionally, because 5307 corn does not pose a plant pest risk, the imposition of any type of testing requirement is inconsistent with the plant pest provisions of the PPA and the regulations at 7 CFR Part 340. Therefore, imposing such a requirement for 5307 corn would not meet APHIS's purpose and need to respond appropriately to the petition in accordance with its regulatory authorities. Additionally, this alternative would not satisfy the product's purpose of providing growers with an additional product to control corn rootworm.

3.4 Comparison of Alternatives

Table 3-1 summarizes the potential environmental consequences, including cumulative effects, associated with selection of either the No Action or the Proposed Action alternatives. Based on the resources described in Chapter 2, *Affected Environment*, an objective evaluation of the No Action and Proposed Action alternatives' potential impacts is provided in Chapter 4, *Environmental Consequences*.

Table 3-1 Summary of Environmental Consequences

Resource	Alternative	
	No Action	Proposed Action
Corn Production		
Acreage and areas of corn production	Unchanged	Unchanged
Cropping practices	Unchanged	Unchanged
Pesticide use	Unchanged	Reduced
Organic farming	Unchanged	Unchanged
Specialty corn production	Unchanged	Unchanged
Physical Environment		
Water quality and use	Unchanged	Unchanged
Soil	Unchanged	Unchanged
Air quality	Unchanged	Unchanged
Climate	Unchanged	Unchanged
Biological Environment		
Animals	Unchanged	Unchanged
Plants	Unchanged	Unchanged
Biodiversity	Unchanged	Improved
Gene movement	Unchanged	Unchanged
Threatened and Endangered Species	Unchanged	Unchanged
Public Health		
Human health	Unchanged	Unchanged
Animal (livestock) health	Unchanged	Unchanged
Worker safety	Unchanged	Improved
Socioeconomics		
Domestic economy	Unchanged	Improved
Trade economy	Unchanged	Unchanged
Social environment	Unchanged	Unchanged
Cumulative Effects		
Agronomic practices	Unchanged	Reduced pesticide use
Physical environment	Unchanged	Unchanged
Biological environment	Unchanged	Improved biodiversity
Threatened and endangered species	Unchanged	Unchanged
Public health	Unchanged	Improved worker safety
Socioeconomics	Unchanged	Improved domestic economy

4

Environmental Consequences

This Chapter describes the potential environmental consequences of the two alternatives under consideration: No Action and the Proposed Action. Under the No Action alternative, Event 5307 corn would continue to be a regulated article as defined by the PPA.[213] Environmental releases of 5307 corn would be under APHIS regulation, as they have been since 2005.[214] Limited field tests would be allowed, but the product would not be marketed. Under the Proposed Action alternative, APHIS would determine that 5307 corn does not pose a plant pest risk. Event 5307 corn would no longer be regulated under the PPA and Syngenta would market this product in stacks with other transgenic corn traits; there are no plans to market 5307 corn as a stand-alone product.

4.1 Introduction

This Section 4.1 describes the scope and methodology of the analysis, and assumptions adopted in Chapter 4. Sections 4.2 through 4.7 describe the potential direct and indirect impacts to environmental and human resources from the No Action and the Proposed Action alternatives for 5307 corn. A cumulative effects analysis for Event 5307 corn, stacked with other transgenic traits, is provided in Section 4.8.

4.1.1 Scope of the Environmental Analysis

The environmental consequences of selecting either alternative may directly, indirectly, or cumulatively affect certain resources in some areas. As required by regulations implementing the *National Environmental Policy Act* (NEPA) promulgated by the Council on Environmental Quality[215] (CEQ), an analysis of the environmental

▼

213 *Plant Protection Act*. 2000. Section 403, *Definitions*.
214 Appendix A of the Petition lists permits/authorizations in chronological order.
215 CEQ. 1978. 40 CFR Part 1500; Section 1502.16, *Environmental Consequences*.

consequences discusses the direct and indirect effects of a proposed action, and their significance. APHIS's agency-specific NEPA regulations refer to CEQ guidance in defining environmental consequences.[216] Direct effects are defined by the CEQ as those "which are caused by the action and occur at the same time and place."[217] Indirect effects are defined as those "which are caused by the action and are later in time or farther removed in distance, but are still reasonably foreseeable. Indirect effects may include growth inducing effects and other effects related to induced changes in the pattern of land use, population density or growth rate, and related effects on air and water and other natural systems, including ecosystems."[218] The CEQ's definition of cumulative effects is provided in Section 4.8, *Cumulative Effects*.

The potential impacts to the resources described in Chapter 2 were analyzed, including corn production practices as well as physical, biological, public health, and socioeconomic resources. The resources analyzed are of public interest or are potentially affected by the Proposed Action and are commonly addressed in APHIS's NEPA evaluations of their plant pest risk decisions. Resources that have no potential to be affected by the Proposed Action, such as coastal zones or noise levels, are not addressed in this report. The Proposed Action's potential direct or indirect effects from 5307 corn as a stand-alone product on the analyzed resources were compared to how those resources may be affected if the No Action alternative is selected. The scope of the cumulative effects analysis is provided in Section 4.8, *Cumulative Effects*.

The geographic scope of this analysis included any US land currently producing corn or producing crops that could incorporate a corn rotation, land that could be converted from inactive cropland to active cropland, and land currently in the Conservation Reserve Program (CRP) that could be removed from the program and farmed for corn. Geographic areas outside of the US were not considered in this report, except to address international trade impacts.

4.1.2 Methodology

The potential direct and indirect effects of the No Action alternative were evaluated by a review and analysis of readily available published literature (peer-reviewed technical articles, industry information, and agency data), as well as Syngenta's Petition[219] and Public Interest Assessment[220] for Event 5307 corn. The potential direct and indirect effects of the Proposed Action alternative to physical and biological resources were determined by laboratory analyses and field studies conducted by Syngenta, as described in the Petition and Public Interest Assessment, supported by literature review and analysis. Potential direct and indirect effects of the Proposed Action to public health and socioeconomic issues were determined by Syngenta's studies described in the Public Interest Assessment, supported by

▼

216 USDA-APHIS. 1995. 7 CFR Part 372, Section 372.4, *Definitions*.
217 CEQ. 1978. 40 CFR Part 1508, Section 1508.8, *Effects*, Paragraph (a).
218 CEQ. 1978. 40 CFR Part 1508, Section 1508.8, *Effects*, Paragraph (b).
219 Vlachos and Huber. 2011.
220 Vlachos and Ward. 2011. (Appendix A.)

literature review and analysis. The methodology used for the cumulative effects analysis is provided in Section 4.8, *Cumulative Effects*. All information sources are cited and full references are provided in Chapter 7.

4.1.3 Assumptions

The analysis of the potential environmental consequences of the No Action alternative was based on the assumption that regulated field trials of 5307 corn on small plots of land, as described in the Petition, would be continued. As noted above, the analysis of direct or indirect effects of the Proposed Action are based on 5307 corn in the absence of stacking. The cumulative effects analysis was based on an assumption of unlimited marketing of 5307 corn as a product stacked with other transgenic traits. The consequences of both alternatives were evaluated using the 5307 corn phenotype described in the Petition. Laboratory, greenhouse, growth chamber, and field investigations with 5307 corn confirmed that there were no changes in seed, pollen, plant phenotype, or composition parameters suggestive of increased plant pest risk. Assessments of the grain and forage from multiple US field sites demonstrate that 5307 corn is nutritionally and compositionally equivalent to, and as safe and nutritious as, its non-transgenic counterpart.

The environmental consequences of the two alternatives were analyzed under the assumption that the majority of farmers who produce transgenic, non-transgenic, or organic corn are using reasonable, commonly accepted best management practices for their chosen system and varieties during corn production. These management practices include standard planting dates, seeding rates, harvest times, and pest management methods as well as preservation and coexistence measures. The analyses also allow for effects that could result if some farmers do not follow best management practices.

The environmental consequences of both the No Action and the Proposed Action alternatives were evaluated in the context of existing transgenic seed products. Sixteen other transgenic corn cultivars containing *Bt*-derived proteins (Cry or Vip) that have a similar mode of action as the eCry3.1Ab protein of 5307 corn have been deregulated by APHIS[221] (see Table 2-1). Some of these are marketed as single trait products while others are marketed as stacked products; some of the cultivars are currently on the market and some have been discontinued or were never commercialized. Four include corn rootworm resistance traits (MON 863, DAS 59122-7, MON 88017 and MIR604 corn); these four cultivars are currently on the market.

As of October 12, 2011, one additional petition for deregulating a corn rootworm-resistant corn cultivar (Petition 11-244-01p for Event DP-004114-3 corn) is currently pending an APHIS decision; this cultivar is also resistant to Lepidoptera

▼

221 USDA-APHIS. 2011b.

and tolerant of glufosinate herbicide. Additionally, petitions for deregulation of three herbicide-tolerant and one drought-tolerant corn cultivar (MON 87427-7, DAS 40278-9, HCEM 485, and MON 87460) are pending APHIS's determination of plant pest risk.[222]

4.2 Corn Production

This section describes the potential impacts to agronomic practices, specialty corn systems, and raw and processed corn commodities that may result from the No Action and Proposed Action alternatives.

4.2.1 Agronomic Practices

Potential changes in agronomic practices, including acreage and areas of corn production, cropping practices, irrigation, and insect management, are described for the No Action and Proposed Action alternatives in the following paragraphs.

4.2.1.1 Acreage and Areas of Corn Production

The amount of transgenic corn planted in the US is increasing. Of the total corn acres planted in 2011, 88 percent were transgenic varieties,[223] up from 61 percent in 2006.[224] As described in Chapter 2, Section 2.2.1, *Production and Yield*, most increases in corn production are expected from yield growth rather than increases in planted areas. Substantive changes in corn production acreage and areas are more likely to result from market conditions and changes in federal policies than any other source.

Arable land for increased corn planting acreage could result from the return of CRP lands to agricultural production. The 2008 US Farm Bill[225] reduced CRP lands from 39.2 million acres to 32 million acres.[226] Available acreage for corn production could increase by this federal policy change. The conversion of CRP acres, if it were to occur, would be independent of the adoption of transgenic corn products.

No Action Alternative

The acreage and areas of corn production are not likely to change as a direct or indirect result of the No Action alternative. Conventional production practices that use transgenic varieties would continue to increase without granting nonregulated status to 5307 corn under the No Action alternative, based on current acreage trends.

▼

222 USDA-APHIS. 2011b.
223 USDA-NASS, 2011d. Pg.25.
224 USDA-NASS. 2006. Pg. 24.
225 *Food, Conservation, and Energy Act of 2008* (FCEA).
226 Ibid. Section 2103, Paragraph (3).

It is anticipated that seed with transgenic traits and non-transgenic hybrids would continue to be available under the No Action alternative. Corn is currently produced in 49 states (all states but Alaska, according to the 2002 Census of Agriculture) and under the No Action alternative the range of production would be unchanged. CRP acreage converted to corn production would not be affected by this alternative. Current trends in the acreage and areas of production are likely to continue to be driven by market conditions and federal policy even if 5307 corn continues to remain a regulated article. Regulated field trials of 5307 corn would only impact small plots of land with no effect on the acreage and areas of corn production.

Proposed Action Alternative

The acreage and areas of corn production are not likely to change as a direct or indirect result of the Proposed Action alternative. In 2011, transgenic corn accounted for 88 percent of all corn acres in production in the US and, as noted above, the use of transgenic corn seed has been increasing. Most corn is planted in fields that have been in crop production for years, rather than converted CRP lands. Granting nonregulated status to 5307 corn is not expected to alter the range of corn cultivation as the new transgenic trait (rootworm resistance) does not otherwise change the plant's agronomic performance compared to non-transgenic varieties.[227]

Event 5307 corn has normal agronomic characteristics: there were no statistically significant differences in most agronomic traits between 5307 corn and a non-transgenic, near-isogenic control hybrid.[228] Syngenta studies identified small but statistically significant differences in grain moisture, plant height, the number of heat units to 50 percent pollen shed, and grain yield.[229] However, there were no observed deficits in agronomic performance of 5307 corn.

Growers are not expected to change the acreage or areas of corn production as a result of deregulating 5307 corn. CRP acreage converted to corn production would not be affected by this alternative. Current trends in the acreage and areas of corn production are likely to continue to be driven by market conditions and federal policy if 5307 corn is deregulated.

4.2.1.2 Cropping Practices

Corn growers choose from many corn hybrids (including transgenic varieties) marketed by seed companies. Hybrids generally differ in agronomic characteristics, including disease and pest resistance and length of growing period.[230] The optimum planting dates for corn in the Corn Belt usually are in April or May but are influenced by factors such as growing locality, environmental conditions, seed

▼

227 Vlachos and Huber. 2011. Pg. 99.
228 Vlachos and Huber. 2011. Pg. 98.
229 Vlachos and Huber. 2011. Pg. 99.
230 Olson and Sander. 1988. Pg. 648.

growing period, and seed variety. Harvesting corn in the Corn Belt generally occurs from mid-to-late September through November.

Crop rotations are used to optimize soil nutrition and fertility, and reduce pathogen loads. As described in Chapter 2, Section 2.2.2.1, *Cropping Practices*, crop rotation is an effective measure for controlling corn rootworm, although in some areas variant rootworm populations display behavioral changes that circumvent rotation strategies. Some northern corn rootworm populations have an extended diapause that allows eggs to hatch when the crop rotation returns to corn rather than in the non-corn rotation crop in the growing season that follows corn. A variant western corn rootworm population now lays its eggs in soybean fields rather than corn fields, allowing eggs to hatch in fields rotating to corn.

Corn rootworm-resistant products may reduce the need to rotate crops for this purpose, but other benefits of crop rotation (e.g., increased yield) would remain. Corn-on-corn ("continuous corn") is used by some growers in response to market demands and expectations of higher economic returns.[231, 232]

Changes in rotation practices are more likely to result from market conditions than other factors. A recent increase in corn-to-corn rotations has been attributed to the increase in corn prices due to higher demand, driven primarily by ethanol production.[233, 234] In fact, corn-on-corn rotation was used prior to the increase in corn for ethanol.[235] Corn-on-corn rotations may provide a continual host environment for some insects and diseases. However, in a farm on a corn-soybean rotation, continuously growing corn for multiple seasons can decrease populations of soybean pests, such as soybean cyst nematode. In some areas, corn-on-corn rotation has increased levels of fertilizer inputs.[236]

Tillage practices are unlikely to change, although conservation methods have been increasingly adopted for other reasons. The use of tilling for weed control and impacts to soil moisture and carbon content, air quality, and erosion are unaffected by transgenic varieties for insect management.

No Action Alternative

Cropping practices are not likely to change as a direct or indirect result of the No Action alternative. Cropping practices are likely to continue to be driven by market conditions even if 5307 corn continues to remain a regulated article. The current economics of corn production are driving the change or perceived change in crop rotation practices. Growers make choices to plant certain corn varieties and use

▼

231 Erickson and Lowenberg-DeBoer. 2005. Pg. 1.
232 Malcolm *et al*. 2009. Pg. 24.
233 Hart. 2006. Pg. 5.
234 Vyn. 2006. Pg. 1.
235 Erickson and Lowenberg-DeBoer. 2005. Pgs. 1-2.
236 Sawyer. 2007. Pg. 27.

certain crop rotation practices based on factors such as yield, weed and disease pressures, cost of seed and other inputs, technology fees, worker safety, potential for crop injury, and ease and flexibility of the production system.[237, 238] Under the No Action alternative, the demands and price of corn would continue to depend on the market for field corn, and corn-to-corn rotations would continue to be used by farmers if this cropping practice meets the economic and marketing strategy for the particular farmer. Regulated field trials of 5307 corn would only impact small plots of land with no effect on cropping practices.

Proposed Action Alternative

Cropping practices such as rotation are not likely to change as a direct or indirect result of the Proposed Action alternative. Introduction of Event 5307 corn for corn rootworm control would not result in additional corn-on-corn rotation, but could allow farmers to implement corn-on-corn rotation more easily. The current economics of corn production are driving changes in crop rotation practices as farmers seek the most profitable production method. Granting nonregulated status to 5307 corn is unlikely to change the price of corn commodities in the US. Prices would continue to be set by market demand, without regard to the number or type of corn varieties available on the market. Deregulating 5307 corn is unlikely to affect a farmer's decision to either stop using a corn-on-corn rotation, or to increase the overall use of corn-on-corn rotation as a cropping strategy with the US farming community. Deregulating 5307 corn would not directly or indirectly impact tillage or weed control practices, and therefore soil moisture, carbon content, air quality, and soil erosion would not be affected either

4.2.1.3 Irrigation

As described in Chapter 2, Section 2.2.2.2, *Irrigation*, supplemental irrigation is used in some corn production areas, such as the western portion of the Corn Belt during the growing season and during flowering. Irrigation rates are affected by local climate conditions (seasonal rainfall), and climate change could increase or decrease irrigation rates in some areas.

No Action Alternative

Irrigation practices are not likely to change as a direct or indirect result of the No Action alternative. Even if 5307 corn continues to remain a regulated article, irrigation rates are likely to continue to be driven by climate conditions. Regulated field trials of 5307 corn would only impact small plots of land with no effect on irrigation rates.

▼

237 Olson and Sander. 1988. Pgs. 646-647.
238 Gianessi. 2005. Pg. 1.

Proposed Action Alternative

Irrigation practices are not likely to change as a direct or indirect result of the Proposed Action alternative. The damage inflicted by corn rootworm larvae can significantly reduce grain yield by interfering with photosynthetic rates and by limiting the uptake of water and nutrients.[239] Reducing rootworm-caused damage could decrease, and would likely not increase, irrigation rates. If 5307 corn is deregulated, irrigation rates are likely to continue to be affected by climate conditions. Because the eCry3.1Ab protein prevents the negative physiological impact of root pruning by rootworms, 5307 corn may allow more efficient use of water and fertilizer.[240]

4.2.1.4 Pesticide Use

As described in Chapter 2, Section 2.2.2.3, *Insect Management*, corn growers use a variety of methods, including applying chemical pesticides (specifically, insecticides), to control insect pests. Broad-spectrum insecticides may impact agriculturally important non-target organisms, including beneficial insects such as honeybees or insects that prey on other insects. *Bt*-derived products have a limited activity spectrum and affect only certain orders of insect pests. *Bt*-derived products are active only if they are ingested and activated by certain organisms, and they are selective because only organisms with gut receptors that bind specific *Bt* proteins are affected. Use of broad-spectrum or *Bt*-derived insecticides, however, can result in insects developing resistance to the insecticide.

Yield losses in all crops due to weeds, diseases, and insects were substantial and widespread until the introduction and adoption of crop protection chemicals in the 1960s. Weeds compete with crops for light, nutrients, water, and other growth factors. If weeds are left uncontrolled, corn cannot be grown economically. Estimates of corn yield loss caused by pathogens have ranged from 2 to 17 percent.[241] A corn crop is susceptible to attack by a variety of insects from the time it is planted until it is consumed as food or feed.

Before the introduction of *Bt*-derived corn varieties, the tools available to growers for insect control consisted of insecticide applications, agronomic practices, and, to a limited extent, the use of corn varieties with a degree of native pest resistance. The introduction of the first *Bt* corn varieties in 1996 provided growers with an effective means of limiting damage caused by the European corn borer. Prior to the introduction of corn rootworm-protected *Bt* corn varieties in 2003, an estimated 14 million acres were treated annually with conventional insecticides to control corn rootworms. This equated to applications of more than 7.7 million pounds of

▼

239 Vlachos and Huber. 2011. Pg. 20.
240 Vlachos and Ward. 2011. Pg. 37.
241 Smith and White. 1988. Pg. 687.

insecticide active ingredients annually in corn fields for the control of *Diabrotica* species.[242] Control of *Diabrotica* accounted for the largest single use of conventional insecticides in the US at that time.

Increased adoption of corn rootworm-protected corn products is of environmental importance because many of the chemical insecticides registered for corn rootworm control present potential risks to applicators, other agricultural workers, and wildlife. The soil-applied and foliar chemical insecticides for larval and adult corn rootworm control subject to the greatest use reductions following the adoption of transgenic rootworm-protected corn varieties are organophosphates, carbamates, pyrethroids, and pyrazoles. Adoption of additional rootworm-protected corn varieties is not expected to markedly reduce the use of insecticidal seed treatments also used to control other soil pests such as wireworms and grubs; however, these seed treatment products are applied at a lower rate per acre than the soil-applied or foliar insecticides used for corn rootworm control.

Corn rootworm populations have developed resistance to some insecticides and to non-chemical control methods.[243] Resistance to some corn rootworm insecticides may result in increased chemical use.[244] As an alternative, crops engineered to produce *Bt* toxins that target specific pest taxa have had favorable environmental effects, particularly when replacing broad-spectrum insecticides.[245, 246] Insect-resistant transgenic corn, including stacked-trait varieties with herbicide tolerance, accounted for 65 percent of corn acres planted in 2011.[247]

Rootworm-protected *Bt* corn hybrids have been available to US growers since 2003. The current PIP proteins registered for corn rootworm control in field corn are Cry3Bb1 (in MON 863 and MON 88017), Cry34Ab1/Cry35Ab1 (in DAS 59122-7), and mCry3A (in MIR604). As the industry trend is towards combined-trait hybrid offerings, these Cry proteins are available in multiple combinations. From the perspective of preventing or mitigating resistance in target pest populations, the deployment of multiple corn rootworm traits in a single corn hybrid is essential to the durability of the registered PIPs in corn.

Transgenic varieties for corn rootworm control are as effective, or more effective, than chemical insecticides used for rootworm control.[248] Available data indicate that broad-spectrum insecticide use in corn agriculture has declined since the introduction of insect-resistant corn varieties. Some estimates indicate that reductions in chemical insecticide use on the order of 70[249] to 75[250] percent could result from widespread adoption of corn rootworm-protected varieties. For example, insecticide

▼

242 Ward *et al*. 2005. Pgs. 242-243.
243 Arthropod populations having pesticide resistance are listed on Arthropod Pesticide Resistance Database.
244 USEPA. 2010b. Pg. 122.
245 NRC. 2010.
246 Carpenter. 2011. Pg. 12.
247 USDA-ERS. 2011a. Pg. 2.
248 Gianessi. 2005. Pg. 245.
249 Oehme and Pickrell. 2003. Pg. 20.
250 Rice. 2004. Pg. 4.

use for corn rootworm control could be reduced by over 5 million pounds per year if 10 million acres of transgenic corn were planted[251] (compared to approximately 92 million acres of corn planted in the US in 2011).

The widespread use of transgenic *Bt* corn could generate selection pressures for insect resistance.[252] One recent study indicates that western corn rootworm in a localized area may have developed resistance to one Cry protein under intense cultivation.[253] Nonetheless, insect resistance to *Bt* crops has not caused widespread failure of control measures, in part due to insect resistance management (IRM) strategies, including supplemental pesticide use and requirements for growers to plant refuges of crop varieties lacking similar *Bt* traits.[254]

The EPA requires refuges to minimize the potential for corn rootworms to develop resistance. The refuge requirements are product-specific and range from 5 to 20 percent of each corn field, and can be spatial (percentage of the field area) or as a portion of the seed mix ("refuge in a bag"), depending upon the specific product.[255] Varieties with multiple transgenic traits acting against a particular pest typically have smaller percentage requirements for refuges than single-trait products. Additionally, the National Corn Growers Association promotes EPA-mandated IRM strategies for all *Bt* corn varieties, including corn rootworm-protected cultivars, to minimize the potential for the insects to develop such resistance.[256]

No Action Alternative

Trends in insecticide use are not likely to change as a direct or indirect result of the No Action alternative. Corn production, and pesticide use in corn, would remain as it is practiced today by the farming community. Insecticide use rates are likely to continue to decline even if 5307 corn continues to remain a regulated article, as growers will continue to have access to existing deregulated insect-resistant corn varieties. Growers make choices to use certain pesticides based on weed, insect and disease pressures, cost of seed and other inputs, technology fees, fuel costs, worker safety, potential for crop injury, and ease and flexibility of the system.[257, 258]

Any environmental effects due to pesticide use in the agricultural production of corn would remain the same under the No Action alternative. The availability of other corn rootworm-resistant varieties would continue if 5307 corn continues to remain a regulated article. Corn growers are likely to continue to use the wide spectrum of available conventional pesticides or any of the four other corn rootworm-resistant cultivars that are currently available, as listed in Table 2-1, *Deregulated Transgenic*

▼

251 Rice. 2004. Pg. 4.
252 Tabashnik *et al*. 2008. Pg. 199.
253 Gassmann *et. al*. 2011. Pg. 5.
254 Tabashnik *et al*. 2008. Pg. 201.
255 USEPA. 2007a. Pg. 140.
256 NCGA. nd.
257 Olson and Sander. 1988. Pgs. 646-647.
258 Gianessi. 2005. Pg. 241.

Corn Cultivars Containing Bt-derived Proteins, or any that may be developed and marketed in the future. Regulated field trials of 5307 corn would only impact small plots of land with little effect on insecticide use.

Proposed Action Alternative

If 5307 corn is deregulated, trends in reducing insecticide use are likely to continue. Corn growers would have a new corn rootworm-control option to use in addition to the four other corn rootworm-resistant products that are currently available, as listed in Table 2-1, *Deregulated Transgenic Corn Containing Bt-derived Proteins*, or any that may be developed and marketed in the future. There is concern about the ability of corn rootworms to evolve resistance to control mechanisms that include crop rotation, chemical insecticides, and rootworm-protected *Bt* corn products. As 5307 corn has demonstrated efficacy against corn rootworm and the eCry3.1Ab protein operates via a novel mode of action, 5307 corn is expected to extend the useful life of other commercially available corn rootworm-protected *Bt* corn cultivars (i.e., containing Cry3Bb1, Cry34Ab1/Cry35Ab1 or mCry3A). The availability of eCry3.1Ab as an additional tool for rootworm control would also reduce the selection pressure on rootworm populations to develop resistance to other methods of control. The potential environmental consequences of reduced pesticide use from 5307 corn stacked with other transgenic corn cultivars are described in Section 4.8, *Cumulative Effects*.

Granting nonregulated status to 5307 corn would provide growers with an alternative to other transgenic corn rootworm-protected varieties as well as use of conventional insecticides. The availability of 5307 corn is expected to contribute to the trend in reduced use of chemical insecticides.

4.2.2 Specialty Corn Systems

Potential changes in specialty corn systems, such as organic farming, seed production, and other specialty production, are described for the No Action and Proposed Action alternatives in the following sections.

4.2.2.1 Organic Farming

Organic farming operations coexist with farming operations using non-transgenic and transgenic varieties for insect resistance. As described in Chapter 2, Section 2.2.3.1, *Organic Crop Production*, the National Organic Program (NOP) requires that organic farms have distinct, defined boundaries and buffer zones separating adjoining land not under organic management to prevent unintended contact with prohibited substances. Organic production operations must also develop and maintain an organic production system plan. Excluded production methods include methods used to genetically modify organisms or otherwise

influence their growth and development by means not possible under natural conditions or processes.

In organic systems, the use of synthetic pesticides and fertilizers is strictly limited, and transgenic crops and inputs are prohibited. Event 5307 corn would not be approved for use in organic systems because it is transgenic. Practices growers may use to exclude transgenic products include planting only organic seed, planting earlier or later than neighboring farmers who may be using transgenic crops so that the crops will flower at different times (temporal isolation), and employing adequate isolation distances between the organic field and the fields of neighbors to minimize the chance that pollen will be carried between the fields (physical isolation). Organic growers must also maintain records to show that production and handling procedures comply with USDA organic standards.

Certified organic corn acreage is a small but increasing percentage of overall corn production. The most recent data indicates that the certified organic corn acreage in 2008 was approximately 195,000 acres, representing 0.21 percent of the total field corn acreage[259] in the US. This was an increase of nearly 50 percent from the 131,000 acres in organic corn production in 2005, which represented 0.16 percent of the total field corn acreage that year. Comparatively, transgenic corn accounted for 80 percent of the 87.3 million acres of field corn planted in 2008,[260] about 69.8 million acres. That number represents an increase in plantings for transgenic corn since 2005, when transgenic corn accounted for 52 percent of the 81.6 million acres of field corn planted that year,[261] about 42.4 million acres.

Organic corn growers may benefit from transgenic crops grown nearby. Reducing populations of insect pests in transgenic crop fields can help lower population pressure in adjacent fields, organic or not, as is evident from economic analyses of avoided costs. For example, one study found that cumulative yield losses from *Bt* corn-suppressed European corn borer that would have occurred if the populations had remained at historic levels have been valued at over $4.3 billion in a 5-state region over 14 years.[262] Such area-wide pest suppression could conceivably occur following sustained use of rootworm-resistant corn varieties, thus resulting in benefits to organic corn growers.

Growers may choose to grow transgenic or non-transgenic corn, and obtain price premiums for growing varieties of corn for particular markets (e.g., using organic methods for corn production or producing a specialty corn variety for particular processing needs). For example, in August, 2011, non-organic corn averaged $7.65 per bushel,[263] whereas organic corn averaged $13.00 per bushel.[264] Transgenic,

▼

259 USDA-ERS. 2010.
260 USDA-NASS. 2008b. Pg. 32.
261 USDA-NASS. 2005. Pg. 24.
262 Hutchison *et al.* 2011. Pg. 224.
263 USDA-NASS. 2009b. Pg.15.
264 Rodale Institute. 2011. Pg. 1.

non-transgenic, and organic production systems can all provide benefits to the environment, consumers, and/or farm income.

Gene movement into and out of organic production systems has been managed using various types of isolation practices, such as differences in planting time (which result in differences in flowering time) or making sure that fields are distant from other compatible crops (by using isolation buffers) to minimize gene movement by pollen transmission.

The commonly used corn production practices and the practical methods typically used by organic corn farmers reduce the likelihood of incidental gene movement between fields of transgenic and non-transgenic corn. These practices protect organic crops and thus maximize profits and price premiums accorded to corn under organic production. It is assumed that farmers are already using, or have the ability to use, these common practices.

No Action Alternative

Organic farming practices, including field management to avoid excluded methods and gene movement through pollen drift or other means, would not change as a direct or indirect result of the No Action alternative. Organic corn seed availability would be unaffected by the No Action alternative. Organic production systems and non-organic production systems would continue to coexist in accordance with NOP requirements. Organic corn production would continue to be driven by market conditions if 5307 corn continues to remain a regulated article.

The amount of transgenic corn and organic corn in the US is increasing, and current trends suggest that both production practices will likely continue to increase. The use of coexistence measures (e.g., isolation of the farm, physical barriers or buffer zones between organic production and non-organic production, as well as formal communications between neighboring farms) are expected to continue. Organic farms would continue to benefit from the insect suppression provided by proximate farms with transgenic crops. The availability of seed for corn varieties that are transgenic, non-transgenic, or used for organic production would remain the same. Regulated field trials of 5307 corn would only impact small plots of land with no effect on organic farming.

Proposed Action Alternative

Organic farming practices, including field management to avoid excluded methods and gene movement through pollen drift or other means, are not likely to change as a direct or indirect result of the Proposed Action alternative. Organic corn seed availability would be unaffected by deregulation of 5307 corn. Consistent with NOP standards and practices, organic and non-organic corn production systems would continue to coexist. Organic farms would continue to benefit from the insect suppression provided by proximate farms with transgenic crops. Organic corn

production would continue to be regulated by the NOP and driven by market conditions if 5307 corn is deregulated. Nonregulated status for 5307 corn would not change current practices of organic corn producers or the growth of organic corn production.

Currently, the use of transgenic corn varieties and the use of organic corn production systems are both increasing due to market demands, and these markets would likely continue to increase under the Proposed Action alternative. Event 5307 corn incorporating the eCry3.1Ab protein would not present new and different issues than existing insect-resistant *Bt* corn cultivars (see Table 2-1) with respect to impacts on organic farmers. APHIS has granted nonregulated status to the products listed in Table 2-1 based on a finding that these other products do not pose a plant pest risk to organic or non-transgenic corn.

4.2.2.2 Seed Production

As described in Chapter 2, Section 2.2.3.3, *Other Specialty Production Systems*, seed purity is accomplished using contracts, tracking and traceability systems, quality assurance processes, closed loop systems, and identity preservation systems. Seed corn production differs from commercial grain production because seed companies impose strict requirements to maintain seed identity and high levels of genetic purity of the final product. The practices used to maintain seed purity do not vary substantively between transgenic and non-transgenic varieties.

No Action Alternative

The availability of methods used to produce seed corn under the No Action Alternative would be the same as in current seed corn production systems.

Proposed Action Alternative

Seed corn production methods are not likely to change as a direct or indirect result of the Proposed Action alternative. If 5307 corn is deregulated, specialty products would continue to be protected by the identity systems established in the industry. Event 5307 corn would be produced in a manner similar to other seed corn inbred lines and resulting hybrids. The inbreds and resulting hybrids are typically produced under identity preservation systems that include contracts with growers, traceability, product tracking, and process verification. Syngenta and other seed corn companies take precautions to ensure that inbred parent lines are not misappropriated by third parties. These procedures greatly minimize any chances of commingling of 5307 corn seed with other seed and, ultimately, commercial grain.

4.2.2.3 Other Specialty Corn Production Systems

As with the purity processes described above for seed production, specialty corn growers and end users maintain the identity of their products by contracts, tracking and traceability systems, quality assurance programs, closed loop systems, and identity preservation systems.

No Action Alternative

Other specialty corn production systems are not likely to change as a direct or indirect result of the No Action alternative. Specialty products are likely to continue to be protected by the identity-preservation systems established in the industry even if 5307 corn continues to remain a regulated article. Regulated field trials of 5307 corn would only impact small plots of land with no effect on other specialty corn production systems.

Proposed Action Alternative

Other specialty corn production systems are not likely to change as a direct or indirect result of the Proposed Action alternative. If 5307 corn is deregulated, specialty products would continue to be protected by the identity-preservation systems established in the industry.

4.2.2.4 Raw and Processed Commodities

As described in Chapter 2, Section 2.2.4, *Raw and Processed Corn Commodities*, there are no differences in handling requirements for processing transgenic and non-transgenic corn.

No Action Alternative

Raw and processed corn commodities are not likely to change as a direct or indirect result of the No Action alternative. Raw and processed corn commodities would continue to be handled in accordance with regulatory standards and industry practices even if 5307 corn continues to remain a regulated article. Regulated field trials of 5307 corn would only impact small plots of land with no effect on raw or processed commodities.

Proposed Action Alternative

Raw and processed corn commodities are not likely to change as a direct or indirect result of the Proposed Action alternative. Neither a direct nor indirect plant pest

effect is foreseen on any raw or processed 5307 corn commodity. Based on Syngenta's field trials, compositional profiles of grain and forage from 5307 corn hybrids are equivalent to those of the corresponding non-transgenic hybrids and commercial hybrids.[265, 266] Apart from the expected insect control benefits and associated benefits in crop yield and crop health, 5307 corn hybrids are agronomically equivalent to their non-transgenic counterparts.[267] Channeling/stewardship plans documenting product purity would remain unchanged under the Proposed Action. If 5307 corn is deregulated, raw and processed corn commodities would continue to be handled in accordance with regulatory standards and industry practices.

4.3 Physical Environment

This section describes the potential impacts to water quality and use, soil, air quality, and climate that may result from the No Action and Proposed Action alternatives.

4.3.1 Water Quality and Use

Potential changes to water quality and use are described for the No Action and Proposed Action alternatives in the following sections.

4.3.1.1 Water Quality

As described in Chapter 2, Section 2.3.1.1, *Water Quality*, the primary cause of agricultural NPS pollution is increased sedimentation from soil erosion, which can introduce sediments, fertilizers, and pesticides to nearby lakes and streams (Transgenic corn is neither sediment nor fertilizer.) Agronomic practices such as conservation tillage, crop nutrient management, pest management, and conservation buffers help protect water quality from agricultural runoff.

Corn pollen may be deposited into water bodies adjacent to corn fields, contributing to turbidity or suspended solids. However, corn pollen is heavy and wind-borne pollen densities decrease rapidly from the source.[268] Corn anthesis lasts for up to 14 days, and any pollen deposited into water bodies from adjacent fields during this period is unlikely to remain in suspension.[269] Corn plant matter could be transferred to water bodies after field harvest, potentially accumulating in stream or lake sediments.[270] There are no federal water quality standards for the insecticidal proteins present in transgenic insect-resistant plant varieties.

▼

265 Vlachos and Huber. 2011. Pg. 99.
266 Vlachos and Huber. 2011. Pg. 102.
267 Vlachos and Huber. 2011. Pg. 127.
268 Luna *et al*. 2001. Pg. 1556.
269 Webster *et al*. 1999. Pg. 698.
270 Rosi-Marshall *et al*. 2007. Figure 1, *Potential Fate of Corn Byproducts in Streams Adjacent to Corn Fields.*

No Action Alternative

Water quality is not likely to change as a direct or indirect result of the No Action alternative. Water quality would continue to be regulated by federal programs (some of which have been delegated to certain states) and agronomic practices to protect water quality would continue to be implemented even if 5307 corn continues to remain a regulated article. Chemical insecticide use would continue to be reduced as transgenic corn products with insect resistance traits are developed, marketed, and adopted. Regulated field trials of 5307 corn would only impact small plots of land with no effect on water quality.

Proposed Action Alternative

Water quality is not likely to change as a direct or indirect result of the Proposed Action alternative. Water quality would continue to be regulated by federal programs (some of which have been delegated to certain states) and agronomic practices to protect water quality would continue to be implemented if 5307 corn is deregulated. Event 5307 corn does not contain any substances which have the potential to affect water quality. The eCry3.1Ab protein concentrations in 5307 corn are lowest at senescence, when grain is harvested, and any residual eCry3.1Ab protein is likely to degrade significantly during the period between harvest and transfer of any plant matter to the waterways.[271] Chemical insecticide use would continue to be reduced as transgenic corn products with insect-resistance traits are developed, marketed, and adopted, potentially improving water quality. Chemical herbicide use is not likely to change as a result of deregulating 5307 corn; water quality would not be affected by the Proposed Action.

4.3.1.2 Water Use

As described in Chapter 2, Section 2.3.1.2, *Water Use*, the production of a bushel of corn requires some 4,000 gallons of water, by either rainfall or supplemental irrigation. Section 4.2.1.3, *Irrigation*, explains that transgenic corn products do not affect irrigation rates.

No Action Alternative

Water use is not likely to change as a direct or indirect result of the No Action alternative. Even if 5307 corn continues to remain a regulated article, irrigation rates would continue to driven by local conditions (e.g., climate, water availability, water pumping capacity, and fuel and electrical costs). Regulated field trials of 5307 corn would only impact small plots of land with no effect on water use.

▼

271 Vlachos and Huber. 2011. Pg. 139.

Proposed Action Alternative

Water use is not likely to change as a direct or indirect result of the Proposed Action alternative. As described in Section 4.2.1.3, *Irrigation*, 5307 corn does not change corn's water requirements. If 5307 corn is deregulated, irrigation rates would continue to driven by local conditions.

4.3.2 Soil

As described in Chapter 2, Section 2.3.2, *Soil*, agronomic practices such as crop type, tillage, and pest management regimes have greater effects on the biology of the soil than the type of corn cultivated. Degraded soil structure and composition may lead to decreased water retention, a decrease in soil carbon aggregation and net positive carbon sequestration, and increased greenhouse gas emissions. Conservation tillage methods can reduce these adverse effects and have been increasingly adopted by corn growers.

Most proteins do not persist or accumulate in soil because they are inherently degradable in soils that have normal microbial populations.[272, 273] Cry proteins from *B. thuringiensis* are rapidly degraded in a variety of soil types and these proteins do not accumulate.[274, 275, 276]

No Action Alternative

Soil characteristics are not likely to change as a direct or indirect result of the No Action alternative. The adoption of insect-resistant corn varieties would be expected to continue. Corn growers would continue current agronomic practices and further adoption of conservation tillage methods is expected even if 5307 corn continues to remain a regulated article. Regulated field trials of 5307 corn would only impact small plots of land with no effect on soil characteristics.

Proposed Action Alternative

Soil characteristics are not likely to change as a direct or indirect result of the Proposed Action alternative. Event 5307 corn production would not change land acreage or cultivation practices for transgenic or non-transgenic corn. Corn growers would continue current agronomic practices and further adoption of conservation tillage methods is expected regardless of whether 5307 corn is deregulated or not. Deregulating 5307 corn would not adversely impact soil.

▼

272 Burns. 1982. Pg. 425.
273 Marx *et al*. 2005. Pg. 35.
274 Mendelsohn *et al*. 2003. Pg.1007.
275 Dubelman *et al*. 2005. Pg. 915.
276 Head *et al*. 2002. Pg. 34.

4.3.3 Air Quality

As described in Chapter 2, Section 2.3.3, *Air Quality*, agricultural activities such as burning, tilling, harvesting, spraying pesticides, and fertilizing, including the emissions from farm equipment, can directly affect air quality. With regard to transgenic corn with insect resistance traits, tilling, harvesting, and fertilizing practices do not vary between transgenic and non-transgenic agricultural varieties. Air quality impacts from aerial application of insecticides may vary between transgenic corn varieties with insect resistance traits and non-transgenic varieties because less insecticide application would be needed for transgenic varieties. Aerial application of insecticides may impact air quality from drift, diffusion, and volatilization of the chemicals, as well as motor vehicle emissions from airplanes or helicopters. Section 4.2.1.4, *Pesticide Use*, explains that insecticide use can be reduced by transgenic insect-resistant products, with resultant air quality benefits.

No Action Alternative

Air quality is not likely to change as a direct or indirect result of the No Action alternative. Corn growers would continue current trends in agricultural activities even if 5307 corn continues to remain a regulated article; application of insecticides would continue to be reduced as additional insect-resistant products are adopted by growers. Regulated field trials of 5307 corn would only impact small plots of land with no effect on air quality.

Proposed Action Alternative

Air quality is not likely to change as a direct result of the Proposed Action alternative. However, air quality would be indirectly improved if aerial application of corn rootworm pesticides is reduced. Corn growers would continue current trends in agricultural activities if 5307 corn is deregulated. Spray application of insecticides could continue to be reduced as additional insect-resistant varieties are adopted by growers because 5307 corn would give growers another option to combat corn rootworm. Event 5307 corn production would not change land acreage or cultivation practices for transgenic or non-transgenic corn.

4.3.4 Climate

As described in Chapter 2, Section 2.3.4, *Climate*, agriculture-related activities are recognized as both direct (e.g., exhaust from motorized equipment) and indirect (e.g., agriculture-related soil disturbance, fertilizer production) sources of greenhouse gases (GHGs). Transgenic crops in general have reduced the release of GHGs from agriculture, equivalent to removing 5 million cars from the roads each

year,[277] although the portion attributable directly to insect-resistant corn products is not known.

The climate can also affect agricultural crop production, and climate change could affect corn yields either positively or negatively. Transgenic corn products with insect resistance traits are not expected to differ from non-transgenic corn in response to climate change.

No Action Alternative

The climate is not likely to change as a direct or indirect result of the No Action alternative. Corn growers would continue current trends in agricultural activities even if 5307 corn continues to remain a regulated article. GHG emission reductions would continue as other transgenic varieties are adopted by growers and they seek more energy-efficient methods of agriculture. Regulated field trials of 5307 corn would only impact small plots of land with no effect on the climate. Increases or decreases in corn yields resulting from climate change would not be affected by the No Action alternative.

Proposed Action Alternative

The climate is not likely to change as a direct or indirect result of the Proposed Action alternative. Event 5307 corn production would not change land acreage or cultivation practices for transgenic or non-transgenic corn. Corn growers would continue current trends in agricultural activities if 5307 corn is deregulated. GHG emission reductions would continue as other transgenic varieties are adopted by growers and they seek more energy-efficient methods of agriculture. Increases or decreases in corn yields resulting from climate change would not be affected by the Proposed Action alternative.

4.4 Biological Environment

This section describes the potential impacts to animals, plants, biodiversity, gene movement in the natural environment, and threatened and endangered species that may result from the No Action and Proposed Action alternatives.

4.4.1 Animals

As described in Chapter 2, Section 2.4.1, *Animals*, corn fields may be temporarily or permanently occupied by invertebrates, birds, reptiles and amphibians, and mammals. Aquatic organisms may reside in water bodies adjacent to corn fields.

▼

277 Brookes and Barfoot. 2005. Pg.195.

Animals can have positive, negative, or no effect on corn production; insect-resistant corn varieties are targeted to certain invertebrate species that negatively impact corn. Table 2-1, *Deregulated Transgenic Corn Cultivars Containing Bt-derived Proteins*, lists the transgenic corn cultivars containing Cry or Vip proteins for insect resistance that have been deregulated by APHIS. These cultivars are lepidopteran (European corn borer and other species) or coleopteran (corn rootworm) resistant.

As described in Chapter 2, Section 2.2.2.3, *Insect Management*, some species of corn rootworm have developed resistance to some chemical pesticides. Similarly, insects may develop resistance to the insecticidal proteins in transgenic *Bt* crops. However, refuges of non-transgenic corn, and stacking traits to provide multiple modes of action against the target insect, minimize the potential for insects to develop resistance.

The following paragraphs summarize potential exposure pathways and available information on the effects of Cry proteins on non-target invertebrates, aquatic organisms, birds, and mammals.[278] Conventional broad-spectrum insecticides are potentially toxic to invertebrates and vertebrates (including humans) as discussed in Section 2.2.2.3, *Insect Management*. The EPA requires application control measures for certain Restricted Use insecticides, including several used for corn rootworm control, to limit human and environmental exposure. *Bt* foliar sprays contain Cry proteins and many are approved insecticides for use in organic production systems to control moths, beetles, mosquitoes, and flies (Lepidoptera, Coleoptera, and Diptera), and exhibit low toxicity to non-target organisms.[279]

Non-Target Invertebrates

Transgenic *Bt* crops, including corn, containing Cry proteins could potentially affect non-target invertebrates that directly consume *Bt* plant material or are exposed via Cry protein residues in soil, water, or prey species. However, for a Cry protein to exert toxicity, the appropriate activating enzyme(s) and receptor binding sites would need to exist in the midgut of the non-target species, and sufficiently high concentrations of active Cry protein would have to reach these binding sites.

Non-target arthropods typically do not feed on corn plants: most species that feed on corn plants are, by definition, pests. The more likely route of exposure to transgenic proteins is consumption of prey that have fed on transgenic corn plants or consumption of transgenic pollen if prey is scarce.[280] The concentration of Cry proteins in the prey of non-target arthropods will vary depending on the prey species, its developmental stage, and the concentration of Cry protein in plant parts on which they are feeding. Several studies have examined the concentration of a representative Cry protein (Cry1Ab) in herbivores relative to the concentration in plants on which they are

▼

278 Potential impacts to threatened and endangered species are separately discussed in Section 4.4.5.
279 NPTN. 2000. Pgs 2-3.
280 Harwood *et al*. 2005. Pg. 2820.

feeding. In general, the results show that herbivorous arthropods contain lower concentrations of *Bt* toxin than the plants on which they are feeding:

- Sucking insects, such as aphids, contain only trace amounts of Cry1Ab when feeding on *Bt* corn;[281, 282, 283, 284]
- Lepidopteran larvae contain between 0.1 and 0.25 times the concentration of Cry1Ab in *Bt* corn on which they are feeding;[285, 286]
- Thrips (*Frankliniella tenuicornis*) contain up to 0.35 times the concentration of Cry1Ab in *Bt* corn, although adults contain about half this amount and pupae less than 1/40th the concentration in larvae;[287] and
- Spider mites (*Tetranychus urticae*) have been found to contain between 0.7 and approximately 4.6 times the concentration of Cry1Ab in *Bt* corn.[288, 289, 290, 291]

Multiple indicator species have been exposed to purified *Bt* proteins in direct feeding studies; these studies have typically not shown any hazard to the tested species, despite exposure to very high test concentrations under "no choice" conditions where the species was continually exposed through its diet. Some laboratory studies have found an effect, but concluded through refinement of exposure models and estimates, or via field studies, that the effect was not adverse or not representative of field conditions.[292]

Corn rootworm predators could ingest Cry proteins when consuming corn rootworms that have consumed *Bt* corn plant material. A study of a transgenic corn product expressing the Cry3Bb1 protein found no significant effects on predators for any measured parameter (including larval development, physical characteristics, and mortality).[293]

Honey bees could potentially forage for corn pollen and therefore could be exposed to Cry proteins.[294] Honey bees can successfully rear young on a diet of 100 percent corn pollen; however, it is unlikely that corn pollen regularly comprises more than 50 percent of their diet.[295] Laboratory studies indicate that Cry proteins have no adverse effects on honey bees.[296]

▼

281 Head *et al*. 2001. Pg. 38.
282 Raps *et al*. 2001. Pg. 531.
283 Dutton *et al*. 2002. Pg. 444.
284 Obrist *et al*. 2006a. Pg. 42.
285 Dutton *et al*. 2002. Pg. 445.
286 Obrist *et al* 2006b. Pgs. 148-149.
287 Obrist *et al*. 2005. Pg. 413.
288 Dutton *et al*. 2002. Pg. 444.
289 Obrist *et al*. 2006a. Pg. 44.
290 Obrist *et al*, 2006b. Pg. 146.
291 Alvarez-Alfageme *et al*. 2008. Pg. 951.
292 Duan *et al*. 2010. Pg. 76.
293 Ahmad *et al*. 2006. Pg. 10693.
294 Severson and Parry. 1981. Pg. 99.
295 Babendreier *et al*. 2004. Pg. 294.
296 Duan *et al*. 2008. Pg. 1415.

A meta-analysis of 74 laboratory studies of Cry protein toxicity and 52 field studies of *Bt* crops containing the same Cry proteins revealed that laboratory studies correctly predicted the reduced field abundance of certain non-target Lepidoptera exposed to lepidopteran-active proteins.[297] In the case of predators consuming prey that had fed on lepidopteran-active *Bt* plants, some laboratory tri-trophic studies identified reduced abundances that were, nevertheless, not observed under field conditions; thus, the laboratory studies overestimated risk. However, laboratory studies incorporating tri-trophic interactions of lepidopteran-active *Bt* plants, herbivores, and parasitoids were better correlated with the decreased field abundance of parasitoids than were direct-exposure assays. This result is not surprising because many parasitoids are associated specifically with target pests of *Bt* crops. Control of pest species by *Bt* crops can be expected to indirectly affect the abundance of their specialist parasitoids under field conditions. In similar studies with coleopteran-active Cry proteins and *Bt* plants, the meta-analysis revealed no adverse effects on survival in laboratory studies or field abundance for any functional group of non-target arthropods examined, including coleopteran and non-coleopteran species.

Transgenic insect-resistant products may reduce broad-spectrum insecticide use, as described in Section 4.2.1.4, *Pesticide Use*. Since the commercialization of *Bt* crops, there have been a substantial number of field studies that have demonstrated that non-target invertebrates are generally more abundant in *Bt* cotton and *Bt* corn fields than in non-transgenic fields managed with chemical insecticides.[298] These studies demonstrate that, not only are the *Bt* crops not causing any unreasonable adverse effects in the environment, but arthropod prevalence and diversity is greater in *Bt* crop fields.

Aquatic Organisms

A potential route of exposure of aquatic organisms to insecticidal proteins from *Bt* corn is through pollen deposited into water bodies adjacent to corn fields. As explained in Section 4.3.1.1, *Water Quality*, corn pollen is heavy and wind-borne pollen densities decrease rapidly from the source. Any pollen deposited into water bodies from adjacent fields during the corn anthesis period (up to 14 days) is unlikely to remain in suspension. In addition, the bioactivity of transgenic proteins is likely to degrade in pollen grains deposited in water.

Unharvested corn material deposited in water bodies as litter could also potentially introduce Cry proteins to aquatic organisms. However, significant degradation of protein is likely to occur during the period between harvest and transfer to the waterways[299] and between when the material is deposited in waterways and when it becomes palatable to aquatic organisms.

▼

297 Duan *et al.* 2010. Pg. 76.
298 USEPA. 2007a. Pg. 6.
299 Vlachos and Huber. 2011. Pg. 139.

Commercially raised fish may also be exposed to corn grain containing Cry proteins. About 30 percent corn grain by weight is typical in commercial fish feeds used in aquaculture.[300] Fish feed is heat-treated during preparation and it is likely that Cry proteins in feed prepared from insect-resistant corn would be at least partially denatured by heat and lose activity. Corn in fish feed is unlikely to comprise 100 percent insect-resistant corn grain.[301] The mode of action of most Cry proteins is highly specific to insects and is not biologically active in vertebrate species, including fish.[302]

Birds

The principal potential route of exposure of birds to Cry proteins is through ingestion of kernels. Some birds such as crows (*Corvus brachyrhynchos*), grackles (*Quiscalus quiscula*), and sandhill cranes (*Grus canadensis*) uproot sprouting corn to feed on the germinating kernels.[303, 304, 305] Red-winged blackbirds typically slit open husks with their bills and puncture kernels in the milk stage.[306] Blackbirds also forage for spilled kernels and weed seeds in corn stubble.[307] Wild birds are unlikely to consume a diet of 100 percent corn kernels. The diets of red-winged blackbirds and common grackles, for example, are comprised of up to 50 percent corn kernels.[308, 309] The mode of action of most Cry proteins is highly specific to insects and these proteins are not biologically active in vertebrate species, including birds.[310] Cry proteins are therefore are not expected to adversely affect non-target vertebrates.[311]

Mammals

The principal potential route of exposure of wild mammals to Cry proteins from *Bt* corn is through ingestion of corn kernels. Rodents such as thirteen-lined ground squirrels, deer mice, house mice, prairie and meadow voles (*Microtus* spp.), and woodchucks feed on germinating corn seeds. Larger mammals such as white-tailed deer typically nip off ear tips and raccoons chew through husks. Wild mammals are unlikely to consume a diet of 100 percent corn kernels. For example, the proportion of corn kernels in wild rodent diets varies greatly according to species, but can be up to 73 percent.[312] The mode of action of most Cry proteins is highly specific to insects and these proteins are not biologically active in vertebrate species, including

▼

300 NRC. 1983. Pg. 48.
301 USDA-NASS. 2011a. Pg. 25.
302 Vlachos and Huber. 2011. Pg. 79.
303 Steffey *et al*. 1999. Pg. 21.
304 Blackwell *et al*. 2001. Pg. 65.
305 Sterner *et al*. 2003.
306 Steffey *et al*. 1999. Pg. 21.
307 Linz *et al*. 2003. Pg. 263.
308 McNichol *et al*. 1979. Pg. 276.
309 Homan *et al*. 1994. Pg. 381.
310 Vlachos and Huber. 2011. Pg. 79.
311 Shimada *et al*. 2006. Pg. 1115.
312 Vlachos and Huber. 2011. Pg. 137.

mammals.[313] Cry proteins are therefore are not expected to adversely affect non-target vertebrates.[314]

No Action Alternative

Non-target species of animals would not be affected as a direct or indirect result of the No Action alternative. Animal exposure to genetically modified crops containing Cry proteins would be nearly identical to current conditions even if 5307 corn continues to remain a regulated article. Approved genetically modified crops containing Cry proteins would continue to be used by growers and there would be no change in animal exposure rates outside of controlled environments. Pesticide usage would continue to have the same effects on animal species. Chemical insecticides would continue to be applied, following current trends in application rates, and organic corn growers would continue to apply microbial *Bt* foliar sprays. Regulated field trials of 5307 corn would only impact small plots of land with controlled animal incursions.

Proposed Action Alternative

Non-target species of animals are not likely to be affected as a direct or indirect result of the Proposed Action alternative. If 5307 corn is deregulated, animal exposure to genetically modified crops containing Cry proteins would be similar to current conditions. Event 5307 corn has been demonstrated to not adversely impact non-target species.

Widespread cultivation of 5307 corn would not substantively change the overall usage of transgenic corn products with insect resistance traits. Other approved genetically modified crops containing Cry proteins have been demonstrated to not have adverse effects on animals and would continue to be used by growers. Pesticide usage would continue to have the same effects on animal species. Chemical insecticides would continue to be applied, following current trends in application rates, and organic corn growers would continue to apply microbial *Bt* foliar sprays. Adoption of 5307 corn may reduce the use of broad-spectrum insecticides, with a consequent reduction of potential impacts on the diversity of non-target insects.[315, 316] This potential impact is therefore addressed as a cumulative effect in Section 4.8.3.2, *Biodiversity*. Additionally, because it represents a new tool for corn rootworm control, the use of 5307 corn could help reduce the likelihood that rootworm resistance to chemical pesticides would increase.[317] This could help avoid future increases in the use rates of chemical insecticides that might otherwise result if current use rates became less effective in controlling rootworms.

▼

313 Vlachos and Huber. 2011. Pg. 79.
314 Shimada *et al*. 2006. Pg. 1115.
315 Dively. 2005. Pg. 1267.
316 Marvier *et al*. 2007. Pg. 1476.
317 Vlachos and Huber. 2011. Pg. 154.

Event 5307 corn is not substantively different in its effects on non-target species from other approved insect-resistant corn products with Cry proteins. The eCry3.1Ab protein present in 5307 corn has a unique binding site in the target pest but acts by the same general mechanism (i.e., pore formation in the target pest gut) as the approved Cry protein products. Syngenta's plant tissue studies determined that, on a fresh-weight basis, the concentrations of eCry3.1Ab in individual samples across all locations and plant stages ranged from less than the lower limit of quantification (< LOQ[318]) to 71.21 micrograms per gram (µg/g) in leaves, 0.40 µg/g to 9.29 µg/g in roots, 1.60 µg/g to 7.29 µg/g in kernels, < LOQ[319] to 0.09 µg/g in pollen and 1.70 µg/g to 28.64 µg/g in whole plants.[320] Activity spectrum data indicate that the insecticidal effects of eCry3.1Ab are limited to certain species of the Chrysomelidae family of Coleoptera. The eCry3.1Ab protein demonstrates no lepidopteran activity, despite containing sequences from a lepidopteran-active protein,[321] because of the specificity of the eCry3.1Ab protein. There would be no additional risk to animals from widespread cultivation of 5307 corn.

Well-characterized modes of action, physicochemical properties, and results of safety studies (as summarized below) demonstrate that the eCry3.1Ab protein present in 5307 corn presents no evidence of risk of harm for avian, fish, or mammalian species. Laboratory testing has similarly shown no adverse effects on survival associated with exposures of eCry3.1Ab in a range of non-target invertebrate species.[322]

Non-Target Invertebrates

Syngenta has conducted laboratory tests on six species representing non-target invertebrates potentially exposed to eCry3.1Ab via cultivation of 5307 corn. In summary, there were no statistically significant differences in survival between treatment and control groups of:

- Honey bees (*Apis mellifera*),[323]
- Spotted ladybird beetle larvae (*Coleomegilla maculata*),[324]
- Second-instar flower bugs (*Orius laevigatus*),[325]
- Larvae of *Poecilus cupreus*, a carabid beetle,[326]
- Adult rove beetles (*Aleochara bilineata*),[327] or
- Adult earthworms (*Eisenia fetida*).[328]

Aquatic Organisms

▼

318 LOQ for leaves was 0.02 µg/g; Vlachos and Huber. 2011. Pg. 88.
319 LOQ for pollen was 0.08 µg/g; Vlachos and Huber. 2011. Pg. 88.
320 Vlachos and Huber, 2011. Pg. 87.
321 Vlachos and Huber. 2011. Pgs. 79 and 129.
322 Vlachos and Huber. 2011. Pg. 151.
323 Vlachos and Huber. 2011. Pg. 145.
324 Vlachos and Huber. 2011. Pg. 145.
325 Vlachos and Huber. 2011. Pg. 146.
326 Vlachos and Huber. 2011. Pg. 146.
327 Vlachos and Huber. 2011. Pg. 147.
328 Vlachos and Huber. 2011. Pg. 147.

Syngenta has conducted laboratory tests on two species representing aquatic organisms potentially exposed to eCry3.1Ab via cultivation of 5307 corn. Aquatic concentrations of eCry3.1Ab are expected to be far below those at which biological activity is observed among known eCry3.1Ab-sensitive species. In summary, there were no statistically significant differences between treatment and control groups of adult gammarid freshwater shrimp (*Gammarus fasciatus*)[329] or juvenile channel catfish (*Ictalurus punctatus*).[330]

Birds

Syngenta has conducted laboratory tests on two species representing birds potentially exposed to eCry3.1Ab via cultivation of 5307 corn. In summary, there were no statistically significant differences in survival or overall performance between treatment and control groups of juvenile bobwhite quail (*Colinus virginianus*)[331] or broiler chickens (*Gallus gallus domesticus*).[332]

Mammals

Syngenta has conducted laboratory tests on one species representing mammals potentially exposed to eCry3.1Ab via cultivation of 5307 corn. There were no statistically significant differences between treatment and control groups of mice (*Mus musculus*).[333]

Syngenta initiated a voluntary pre-market consultation process with FDA and submitted a Safety and NutritionalAassessment for 5307 corn in January 2011.[334] The assessment demonstrated that the introduced proteins, eCry3.1Ab[335] and phosphomannose isomerase (PMI),[336] in 5307 corn are not toxic to human and animal consumers and have minimal allergenic potential. Additionally, Syngenta's assessment showed that 5307 corn is nutritionally equivalent to non-transgenic corn and other corn in commerce.

4.4.2 Plants

As described in Chapter 2, Section 2.4.2, *Plants*, corn fields can be bordered by other agricultural fields (including other corn varieties), woodlands, or pasture and grasslands. The most agronomically important members of a surrounding plant community are those that behave as weeds. Chapter 2, Section 2.2.2.5, *Weed Management*, explains the range of methods that growers use for weed control, including integrated weed management. These methods may be cultural (planting),

▼

329 Vlachos and Huber. 2011. Pg. 148.
330 Vlachos and Huber. 2011. Pg. 149.
331 Vlachos and Huber. 2011. Pg. 142.
332 Vlachos and Huber. 2011. Pg. 143.
333 Vlachos and Huber. 2011. Pg. 143.
334 Zeph and Vlachos. 2011. (Appendix B.)
335 EPA has issued a temporary tolerance exemption for eCry3.1Ab in corn (Federal Register 76:180; Pgs. 57653-57657; USEPA. 2011a) and Syngenta has petitioned for a non-expiring tolerance exemption.
336 EPA has granted a permanent tolerance exemption for PMI in all crops (40 CFR Part 180.1252; USEPA. 2007b).

mechanical (tillage), and chemical (herbicide), or some combination thereof. As described in Section 2.2.5, *Persistence in the Environment/Weediness Potential*, cultivated corn is not a weed and transgenic cultivars currently on the market have not changed corn's weediness potential.

No Action Alternative

Plants would not be affected as a direct or indirect result of the No Action alternative. Weed management methods would continue following current trends even if 5307 corn continues to remain a regulated article. Regulated field trials of 5307 corn would only impact small plots of land with controlled plant exposures. No impacts to plant species compared to any effects from current agronomic practices are anticipated.

Proposed Action Alternative

Plants would not be affected as a direct or indirect result of the Proposed Action alternative. Event 5307 corn production would not change land acreage or cultivation practices for transgenic or non-transgenic corn. Event 5307 corn does not have increased persistence in the environment or weediness potential compared with non-transgenic corn.[337] No changes in impacts to non-weed plant species compared to any effects from current agronomic practices are anticipated. A description of the potential impacts to plants from a biodiversity perspective from 5307 corn in stacked combinations is provided in Section 4.8, *Cumulative Effects*.

4.4.3 Soil Microorganisms

Plant type and root exudates influence the microorganisms that colonize the rhizosphere.[338] While *B. thuringiensis* occurs naturally in soil, growing transgenic *Bt* corn increases the amount of Cry endotoxins present in agroecosystems.[339] Cry proteins are typically expressed in all or most tissues throughout the plant's life cycle, potentially creating routes of exposure for soil organisms through root exudation, trophic level interactions, plant decomposition after harvest, and toxin persistence in the soil.[340] However, different effects of *Bt* plants on microbial communities in soil, ranging from no effects to minor and statistically significant effects, have been reported.[341]

Soil bacterial communities are influenced by plant species and cultivars as well as other environmental factors, such as soil type and agricultural practices.[342] One study concluded that plant variety (but not the presence of Cry proteins) had a

▼

337 Vlachos and Huber. 2011. Pg. 152.
338 Icoz *et al*. 2008. Pg. 658.
339 Blackwood and Buyer. 2004. Pg. 832.
340 Devare *et al*. 2004. Pg. 837.
341 Icoz *et al*. 2008. Pg. 657.
342 Icoz *et al*. 2008. Pg. 659.

significant but transient effect on the numbers of microorganisms and the activities of enzymes involved in the degradation of plant biomass,[343] while another study found no deleterious effect of growing corn-rootworm-resistant *Bt* corn on microbial biomass, activity, or bacterial community structure.[344]

As noted in Section 4.3.2, *Soil*, most proteins do not persist or accumulate in soil because they are inherently degradable in soils that have normal microbial populations.[345, 346] Cry proteins derived from *Bt* are rapidly degraded in a variety of soil types and the proteins typically do not accumulate in soil.[347, 348, 349] Soil type has a large effect on the microbial community and availability of Cry proteins.[350] Certain Cry proteins may adsorb rapidly to clay minerals, on the clay-sized fraction of soil, on humic soils, and on complexes of montmorillonite-humic acids-aluminum hydroxypolymers.[351] Some field studies on the persistence of Cry proteins released by transgenic plants showed that Cry proteins do not persist and degrade rapidly in soil, although a small fraction may be protected from biodegradation in the plant matrix or bound on surface-active particles.[352]

The numbers of microorganisms and the activity of some enzymes involved in the degradation of plant biomass differ significantly by season, probably as a result of differences in the water content of soils, ambient temperatures, and plant stage growth at the time of sampling.[353] Cry protein concentrations in the rhizosphere vary during the growth of the plant and can be affected by microbial activity, which depends in part on soil temperature and humidity.[354] A comparison of a corn-rootworm-resistant *Bt* corn variety and a non-transgenic control corn treated with a conventional insecticide concluded that the addition of the conventional insecticide had greater effects on the microbial function in soil and decaying roots than *Bt* corn.[355]

The EPA has concluded that *Bt* crops have a positive effect on soil flora compared to non-selective synthetic chemical pesticides.[356] Even though *Bt* is naturally ubiquitous in soil, the presence and release of *Bt* toxins from the aboveground and belowground parts of *Bt* plants may influence microbial diversity. *Bt* toxins have been found to be present in most tissues of *Bt* plants.[357] However, studies have found no differences in

▼

343 Icoz *et al*. 2008. Pg. 658.
344 Devare *et al.* 2004. Pg. 842.
345 Burns. 1982. Pg. 425.
346 Marx *et al*. 2005. Pg. 35.
347 Mendelsohn *et al*. 2003. Pg. 1007.
348 Head *et al*. 2002. Pg. 34.
349 Dubelman *et al*. 2005. Pg. 915.
350 Blackwood and Buyer. 2004. Pg. 835.
351 Saxena and Stotzky. 2001. Pg. 1225.
352 Icoz *et al.* 2008. Pg. 660.
353 Icoz *et al*. 2008. Pg. 650.
354 Baumgarte and Tebbe. 2005. Pg. 2547.
355 Lawhorn *et al*. 2009. Pg. 367.
356 USEPA. 2001. Pg. IIC53.
357 Sivasupramaniam *et al.* 2008. Pg.547.

microbial biodiversity or activity between fields cultivated with *Bt* corn or the corresponding non-*Bt* corn.[358]

The Cry proteins released in root exudates of *Bt* corn or from the degradation of biomass of *Bt* corn are not toxic to earthworms, nematodes, protozoa, bacteria, or fungi.[359] The EPA has determined that Cry proteins do not have any measurable adverse effect on microbial populations in the soil and that horizontal transfer of genes from transgenic plants to soil bacteria has not been demonstrated.[360]

No Action Alternative

Soil microorganisms would not be affected as a direct or indirect result of the No Action alternative. The use of transgenic corn producing Cry proteins and the use of other *Bt*-derived insecticides would continue following current trends even if 5307 corn continues to remain a regulated article. Regulated field trials of 5307 corn would only impact small plots of land with controlled plant exposures. No impacts to soil microorganisms compared to any effects from current agronomic practices are anticipated.

Proposed Action Alternative

Soil microorganisms would not be affected as a direct or indirect result of the Proposed Action alternative. Syngenta conducted a laboratory study to examine the fate of eCry3.1Ab in live soil. A rapid decline in eCry3.1Ab bioactivity was observed: no biological activity above background control levels was detected after 14 days of eCry3.1Ab incubation in live soil.[361] The Cry protein present in 5307 corn does not bioaccumulate and a laboratory study demonstrated that eCry3.1Ab biological activity is rapidly degraded in healthy soils. No changes in impacts to soil microorganisms compared to any effects from current agronomic practices are anticipated.

4.4.4 Biodiversity

Biodiversity in an agroecosystem depends on the diversity of vegetation within and around the agroecosystem; permanence of crops within the agroecosystem; intensity of management; and the extent of isolation from natural vegetation.[362] Determining the level of biological diversity associated within any crop agroecosystem is complex because biological diversity can be defined and measured in many ways. Another difficulty with biodiversity studies is separating expected impacts from indirect impacts. For example, reductions of biological control organisms are seen in some

▼

358 Icoz *et al*. 2008. Pg. 660.
359 Saxena and Stotzky. 2001. Pg. 1228.
360 USEPA. 2001. Pg. IIC15.
361 Vlachos and Huber. 2011. Pg. 135.
362 Altieri. 1999. Pg. 21.

Bt-expressing transgenic crops but are caused by reduction of the pest host population rather than as a direct effect.[363]

Many studies over the last 10 years have investigated the differences in biological diversity and abundance between transgenic and non-transgenic crop fields, particularly those transgenic crops that are resistant to insects (e.g., *Bt* crops) or herbicides (e.g., glyphosate- or glufosinate-tolerant). Conflicting results are often reported. Some studies have found negligible to modest decreases in biological diversity or abundance due to crops genetically engineered to produce insecticidal proteins or tolerate herbicide application for weed management.[364, 365, 366] Other studies compared *Bt* crops to non-transgenic crops that were unsprayed or sprayed with insecticides and found that *Bt* crops do not cause any overall changes in arthropod abundance or diversity.[367, 368, 369, 370] A review of over 360 research papers concluded that there is no evidence of landscape-level effects from *Bt* crops.[371] Compared to the use of broad-spectrum insecticides in agriculture, *Bt* crops may increase biological diversity in agroecosystems by reducing broad-spectrum insecticide use, thus allowing more non-target species to survive.[372, 373] Most transgenic crops increase the productivity of cultivated lands, so biodiversity is protected because additional land is not needed for the same volume of crop production.[374]

No Action Alternative

Biodiversity would not be affected as a direct or indirect result of the No Action alternative. Biodiversity within agroecosystems would continue to be affected by agricultural practices following current trends. Growers would continue to have access to existing deregulated insect-resistant corn varieties, and adoption of new transgenic corn varieties would be expected to continue even if 5307 corn continues to remain a regulated article. Regulated field trials of 5307 corn would only impact small plots of land with controlled animal and plant incursions. No changes in biodiversity compared to effects from current agronomic practices are anticipated.

Proposed Action Alternative

Biodiversity would be improved as a direct result of the Proposed Action alternative, as chemical pesticide use rates would decrease because of the introduction of this insect-resitant cultivar. More non-target species would survive. Event 5307 corn

▼

363 USDA-APHIS. 2011a. Pg. 16.
364 Marshall *et al.* 2003. Pg.85.
365 Pilcher *et al.* 2005. Pg. 1312.
366 Ponsard *et al*. 2002. Pg. 1204.
367 Chen *et al.* 2008. Pg. 4.
368 Romeis *et al*. 2006. Pgs. 67-68.
369 Torres and Ruberson. 2005. Pg. 1254.
370 Wolfenbarger *et al*. 2008. Pg. 8.
371 Carpenter. 2011. Pg. 6.
372 Marvier *et al.* 2007. Pg. 1476.
373 Romeis *et al.* 2006. Pg. 67.
374 Raven. 2010. Pg. 528.

production would not change land acreage or cultivation practices for transgenic or non-transgenic corn. Event 5307 corn incorporates a Cry protein with a similar receptor-specific mode of action as other Cry proteins present in approved insect-resistant corn cultivars and does not have increased persistence in the environment or weediness potential over non-transgenic corn.[375] As described in Section 4.2, *Corn Production*, 5307 corn does not exhibit any different agronomic traits, apart from resistance to corn rootworms, or require different agronomic practices. As described in Section 4.4.1, *Animals*, and Section 4.4.2, *Plants*, animal and plant species that typically inhabit corn production areas would be managed the same as other for other insect-resistant corn products on the market. The potential biodiversity effects of 5307 corn when stacked with other transgenic traits are described in Section 4.8, *Cumulative Effects*.

4.4.5 Gene Movement in the Natural Environment

Corn is a highly domesticated plant with limited gene movement in the natural environment, and gene movement from transgenic corn is not any different than that of other cultivated corn varieties.[376] As described in Chapter 2, Section 2.2.5, *Persistence in the Environment/Weediness Potential*, corn is dependent upon humans for survival. Cultivated corn has limited sexual compatibility with its closest relative, teosinte, but no wild populations of teosinte exist in the US outside of some feral populations in Alabama, Florida, and Maryland.[377] Corn can only be crossed experimentally with the genus *Tripsacum*, and does not persist outside of cultivation. The only known propagation method for corn is through seed germination. Like many domesticated crops, corn seed from a previous year's crop can overwinter and germinate the following year. Corn seedlings may appear in soybean fields following a corn crop in a corn-on-soybean rotation. These plants do not result in sustained populations in subsequent years. Corn does not possess the suite of traits that are characteristic of successful weeds.

An extensive review of information relevant to the potential risks of horizontal gene transfer for *Bt* crops to soil microbes was conducted by the EPA. No evidence of horizontal gene transfer under field conditions was found, and there was only equivocal evidence for horizontal gene transfer under laboratory conditions designed to maximize the recovery of transformants.[378]

No Action Alternative

Corn's characteristic of limited gene movement in the natural environment would not be affected as a direct or indirect result of the No Action alternative. Corn breeding by traditional or transgenic means would continue even if 5307 corn

▼

375 Vlachos and Huber. 2011. Pg. 152.
376 USDA-APHIS. 2011a. Pg. 53.
377 USDA-NRCS. 2011a.
378 USEPA. 2001. Pg. IIC16.

continues to remain a regulated article. Regulated field trials of 5307 corn would only impact small plots of land with minimal potential for exposure to sexually compatible plants (i.e., other corn plants). No changes in gene movement characteristics of current corn varieties are anticipated.

Proposed Action Alternative

Corn's characteristic of limited gene movement in the natural environment would not be affected as a direct or indirect result of the Proposed Action alternative. Event 5307 corn has no more potential for gene introgression to sexually compatible wild relatives than other transgenic or non-transgenic corn varieties.[379] The codons in the *ecry3.1Ab* gene are optimized for expression in plants, and hence the gene is likely to have low sequence homology with genes of soil microbes.[380] Horizontal gene transfer of *ecry3.1Ab* from 5307 corn to soil microbes is highly unlikely. The probability of spread of *ecry3.1Ab* outside corn cultivation by horizontal gene transfer is negligible. No changes in gene movement characteristics compared to current corn varieties are anticipated.

4.5 Threatened and Endangered Species

The *Endangered Species Act*[381] (ESA) establishes the federal program to protect threatened and endangered species. Once an animal or a plant is added to the list of threatened or endangered species, protective measures apply to the species and its habitat. These measures include protection from adverse effects of federal activities, such as APHIS's determination of nonregulated status for transgenic crops. Section 7(a)(2) of the ESA requires that federal agencies, in consultation with the US Fish and Wildlife Service (USFWS), ensure that any action they authorize, fund, or carry out is not likely to jeopardize the continued existence of a listed species or result in the destruction or adverse modification of designated critical habitat. It is the responsibility of the federal agency taking the action to assess the effects of their action and to consult with the USFWS if it is determined that the action "may affect" listed species or critical habitat.

This section evaluates the potential for effects from cultivation of 5307 corn and its progeny on federally listed threatened and endangered species and species proposed for listing, as well as designated critical habitat and habitat proposed for designation, as required under Section 7 of the ESA. Direct effects are analyzed by considering the response that these species could have if exposed to 5307 corn. Indirect effects are those that could result from the use of 5307 corn which would occur later in time but are still reasonably foreseeable.

▼

379 Vlachos and Huber. 2011. Pg. 152.
380 Vlachos and Huber. 2011. Pg. 153.
381 *Endangered Species Act of 1973, as amended.*

4.5.1 APHIS Section 7 Process

Working with the USFWS, APHIS has developed a process for conducting an effects determination for petitions for nonregulated status. This process[382] is used by APHIS to fulfill their obligations and responsibilities under Section 7 of the ESA for biotechnology regulatory actions. APHIS considers the following information in its analysis of transgenic plants:

- The biology, taxonomy, and weediness potential of the crop plant and its sexually compatible relatives;
- Characteristics of each transgene with respect to its structure and function and the nature of the organism from which it was obtained;
- Where the new transgene and its products are produced in the plant and their quality;
- The agronomic performance of the plant, including disease and pest susceptibilities, weediness potential, and agronomic and environmental impact;
- Determination of the concentration of known plant toxicants; and
- Determination whether the transgenic plant is sexually compatible with any threatened or endangered plant species.

4.5.2 Potential Effects

Based on the process described above, the following paragraphs describe the potential effects to threatened or endangered species that may result from the No Action or Proposed Action alternatives.

No Action Alternative

The No Action alternative would not affect any listed threatened or endangered species. Under this alternative, 5307 corn could continue to be grown as a regulated article in limited field trials. Corn that would be widely cultivated consists of non-transgenic hybrids and a range of deregulated transgenic corn varieties that are herbicide-tolerant or which are insecticidal to certain Lepidoptera (e.g., European corn borer and corn earworm) or Coleoptera (corn rootworm) (see Table 2-1, *Deregulated Transgenic Corn Cultivars Containing Bt-derived Proteins*). APHIS has determined, as documented in prior Environmental Assessments, that none of the deregulated transgenic corn cultivars have an adverse effect on species protected under the ESA.[383, 384, 385]

382 USDA-APHIS. nd.
383 USDA-APHIS. 2007. Pg. 14.
384 USDA-APHIS. 2009. Pg. 15.
385 USDA-APHIS. 2010b. Pg. 18.

Proposed Action Alternative

Wide cultivation of 5307 corn is not expected to differ from practices normally used for commercial corn production. Event 5307 corn production would not change land acreage or cultivation practices for transgenic or non-transgenic corn. The potential environmental impacts on listed threatened or endangered species from 5307 corn are those associated with typical commercial corn production, in areas where commercial corn is typically produced, and therefore would not affect the natural habitat of any listed species. The analysis of potential effects considered the potential for 5307 corn to extend the range of corn production and the potential to expand agricultural production into new areas.

Event 5307 corn does not express any phenotypic traits that would allow it to be commercially viable outside of the existing range of corn cultivation, and is targeted specifically for use in the regions where corn rootworm is a recognized agricultural pest (see *Geographic Distribution of Northern and Western Corn Rootworm and their Variants*, in *Corn Rootworm Biology, Feeding Behavior and Economic Loss*; Chapter 2, Section 2.2.2.3, *Insect Management*). As described in Section 4.2.1, *Agronomic Practices*, considering that 88 percent of the corn currently grown in the US is genetically modified, it is expected that 5307 corn, if deregulated, would be planted in areas where corn is currently grown. The agronomic analyses show that 5307 corn would use the same agronomic practices – including the same fertilizers, herbicides, fungicides, irrigation, crop rotations, and tillage – as non-transgenic corn hybrids. There is no hazard associated with the cultivation of 5307 corn that would be different from production of non-transgenic corn. There is no evidence that deregulating 5307 corn would affect any listed species or critical habitat protected under the ESA.

Due to the selectivity of eCry3.1Ab for certain coleopteran species, potential exposure to threatened or endangered species is restricted to the order Coleoptera. Chapter 2, Section 2.5, *Threatened and Endangered Species*, describes the coleopteran species listed by the USFWS as endangered or threatened under the ESA. At present, there are no additional coleopteran species proposed for listing. A geospatial analysis was conducted for the listed coleopteran species; 15 were found within corn production counties. Refined geospatial analysis showed that only three of these co-occurred with potential corn production locations:

- Kretschmarr Cave mold beetle (*Texamaurops reddelli*),
- American burying beetle (*Nicrophorus americanus*), and
- Salt Creek tiger beetle (*Cicindela nevadica lincolniana*).

The analysis showed that there would be no exposure of two of these species to 5307 corn. The Kretschmarr Cave mold beetle inhabits small isolated caves within the Edwards Limestone formation in Texas,[386] making contact with corn unlikely. The

386 USFWS. 1988. Pg. 36030.

American burying beetle relies on carrion (mammals, birds, reptiles) as a food source[387] and would not be expected to ingest 5307 corn or its insecticidal protein because it does not eat corn or insects. The Salt Creek tiger beetle is a predatory beetle that feeds on other insects, and due to its observed proximity to corn production areas it may conceivably be exposed to very low eCry3.1Ab concentrations (compared to levels measured in 5307 corn) via predation. While predation upon arthropods containing trace amounts of eCry3.1Ab is possible, the beetle's predation habits would greatly limit the opportunity for such exposure. Salt Creek tiger beetle larvae are known to prey on insects from the entrance of permanent burrows built in saline stream banks and barren salt flats of saline wetlands, habitats which do not occur in cornfields.[388] The Salt Creek tiger beetle is a member of the Carabidae family. Biosafety analyses documented in the Petition[389] showed no effects of eCry3.1Ab on the survival of carabid beetles when tested at levels 62 times greater than the worst-case estimated environmental concentration.[390] Therefore, any exposure of Salt Creek tiger beetles to trace amounts of eCry3.1Ab via their prey would be unlikely. Further, if such exposure were to occur, no measurable hazard is predicted.

A safety assessment[391] conducted on 5307 corn showed that the insecticidal activity of eCry3.1Ab is limited to certain Chrysomelidae species within the order Coleoptera. The chrysomelids shown to be sensitive to the insecticidal effects of eCry3.1Ab are the larvae of the western corn rootworm (*Diabrotica virgifera virgifera*), northern corn rootworm *(D. longicornis barberi)*, Mexican corn rootworm (*D. virgifera zeae),* and the Colorado potato beetle *(Leptinotarsa decemlineata)*.[392]

Apart from the transgene that encodes eCry3.1Ab in 5307 corn, 5307 corn also contains the gene *pmi* (also known as *manA*) that encodes the selectable marker PMI enzyme, which is not a toxin. Event 5307 corn does not express additional proteins, natural toxicants, allelochemicals, pheromones, or hormones, etc. that could directly or indirectly affect a listed species or species proposed for listing. The high specificity of the eCry3.1Ab protein makes it unlikely that non-target or threatened or endangered insects would be affected.

As indicated by the studies conducted by Syngenta, the eCry3.1Ab protein is not insecticidal to species outside the Chrysomelidae family of Coleoptera. The eCry3.1Ab protein demonstrates no lepidopteran activity, despite containing sequences from a lepidopteran-active protein (Cry1Ab). The absence of lepidopteran activity is consistent with the fact that, although eCry3.1Ab contains a portion of Cry1Ab, that portion does not include the region responsible for lepidopteran activity.

▼

387 USFWS. 2011a. Pg. 2.
388 USFWS. 2005. Pg. 2.
389 Vlachos and Huber. 2011. Pg. 150.
390 The worst-case estimate assumes that the organisms' diet is comprised of 100 percent 5307 corn material.
391 Vlachos and Huber. 2011. Pg. 150.
392 The Colorado potato beetle is not a pest of corn, but it is sensitive to eCry3.1Ab in direct laboratory feeding studies.

Corn, including 5307 corn, has no sexually compatible relatives found in natural areas, and is only able to reproduce with other corn plants in the US. Corn cannot naturalize (as discussed in Chapter 2, Section 2.1, *Corn Biology*) and has no potential to become weedy. The biosafety studies conducted by Syngenta[393] show no toxic effects to birds, earthworms, fish, freshwater shrimp, non-target insects, or mammals, and show that the insecticidal effects of 5307 corn are limited to corn rootworm larvae, which are not pollinators. As described in the Petition, Syngenta evaluated the composition and nutritional quality of 5307 corn and compared its composition to the composition of a corresponding non-transgenic corn variety and other corn varieties for which composition data were available. The data suggest that there is no difference in composition and nutritional quality between 5307 corn and commercial corn varieties. It is therefore not anticipated that 5307 corn would have an adverse effect on non-target animals that consume it, or on listed animal species, including animals such as insects, bats or birds that may be pollinators of plants, or on listed plant species.

4.6 Public Health

This section describes the potential impacts to human health, animal (livestock) health, and worker safety that may result from the No Action and Proposed Action alternatives.

4.6.1 Human Health

The general public is often concerned about the potential impacts that transgenic products could have on human health because of possible toxic or nutritional effects of the products, or how the product might change the allergenicity of food products. Insecticidal Cry proteins from *B. thuringiensis* have a long history of safe use in food crops. Their modes of action are highly specific within narrow ranges of related insect species, and are not relevant to mammals or other vertebrates. PMI, the selectable marker protein produced by 5307 corn plants, is exempt from food and feed tolerances.[394]

The EPA requires seed registrants to submit tests of potential toxicity and allergenicity of the transgenic proteins in *Bt* corn cultivars before they can be approved for human consumption. All tests that have been performed for adverse mammalian impact from ingesting Cry proteins have been negative, even at extremely high doses.[395] The toxicity of insecticidal *Bt* proteins depends on binding to specific receptors present in the insect midgut. With regard to the specific Cry

393 Vlachos and Huber. 2011. Pg. 151.
394 USEPA. 2007b.
395 Wu. 2006. Pg. 121.

proteins produced in *Bt* crops, research demonstrates that this specificity limits each protein's toxic effect to certain insect species.

As described in Chapter 1, Section 1.1, *Regulatory Authority*, it is the responsibility of food and feed manufacturers to ensure that the products they market are safe and properly labeled. Food and feed derived from transgenic products must be in compliance with all applicable legal and regulatory requirements. Transgenic products for food and feed may undergo a voluntary consultation process with the FDA prior to release onto the market. To date, all transgenic crops marketed in the US have been the subject of pre-marketing consultations with the FDA.

Certain *Bt* corn cultivars can reduce mold infestation on corn grain.[396] One study found that transgenic hybrids with *cry* genes for lepidopteran resistance had fumonisin concentrations as low as 10 percent of those found in non-transgenic counterparts.[397] Any reduction in mold toxins resulting from use of *Bt* corn can provide direct benefits to people and corn-fed livestock by reducing exposure to mycotoxins. In a variety of field studies, lepidopteran-protected corn varieties expressing *Bt* proteins have been shown to have significantly lower levels of the common mycotoxins that are produced by fungal pathogens.[398]

No Action Alternative

Human health is not likely to be affected as a direct or indirect result of the No Action alternative. Previously deregulated transgenic corn would continue to be used as food for human consumption. However, the Cry proteins of *Bt* corn products are not toxic to humans and do not have any known allergenic properties for humans. Human exposure to 5307 corn would be limited to those individuals involved in cultivation under regulated conditions even if 5307 corn continues to remain a regulated article. Regulated field trials of 5307 corn would only impact small plots of land with no potential for exposure to the general public. Exposure to existing transgenic and non-transgenic corn would not change under this alternative.

Proposed Action Alternative

Human health is not likely to be affected as a direct or indirect result of the Proposed Action alternative. If 5307 corn is deregulated, the general public would primarily come in contact with the introduced transgenic proteins (i.e., eCry3.1Ab and PMI) through dietary exposure to food and products derived from 5307 corn, although most processed products would contain no detectable eCry3.1Ab or PMI residues. However, the Cry proteins of *Bt* corn products, including 5307 corn, are not toxic to humans and do not have any known allergenic properties for humans. The donor organism for the source genes used to create eCry3.1Ab is *B. thuringiensis*, a

▼

396 Carpenter *et al*. 2002. Section 1, Pg. 3.
397 Munkvold *et al*. 1999. Pg. 133.
398 Wu. 2006. Pgs. 404-410.

ubiquitous soil bacterium. The eCry3.1Ab protein is derived from a family of *Bt* proteins that has a long history of safe use in food crops. Similarly, the PMI marker protein present in 5307 corn raises no human health concerns with respect to toxicity or allergenicity. Exposure to existing transgenic and non-transgenic corn would not change under this alternative.

A discussion on the mechanism of action for eCry3.1Ab, its spectrum of activity, and its lack of toxicity to non-coleopteran species is presented in the Petition[399] and the Safety and Nutritional Assessment.[400] A comprehensive assessment of the safety of eCry3.1Ab demonstrated that the protein is nontoxic to mammals and unlikely to be a food allergen. The eCry3.1Ab protein is considered nontoxic because it does not share significant amino acid similarity with known protein toxins, is nontoxic to mice at a very high dose, is rapidly degraded in simulated mammalian gastric fluid, and the insecticidal mode of action is not relevant to mammals. The eCry3.1Ab protein is not likely to become a food allergen because it is not derived from a known source of allergenic proteins, it does not have significant amino acid sequence identity to known allergenic proteins, it is rapidly degraded in simulated mammalian gastric fluid, it is not glycosylated, and it is labile upon heating at temperatures of 37°C and above. The PMI protein is also considered nontoxic because it does not share significant amino acid homology with known protein toxins, it is nontoxic to mice at a very high dose, and it is rapidly degraded in simulated mammalian gastric fluid. PMI is not likely to become a food allergen because it is not derived from a known source of allergenic proteins, it does not have any significant amino acid sequence identity to known allergenic proteins with implications for its allergenic potential, it is rapidly degraded in simulated mammalian gastric fluid, it is not glycosylated, and it is labile upon heating at temperatures of 37°C and above.

Syngenta initiated a voluntary pre-market consultation process with FDA and submitted a Safety and Nutritional Assessment for 5307 corn in January 2011.[401] The assessment demonstrated a lack of toxicity and allergenicity of 5307 corn for human and animal consumption. With the exception of the worker safety benefits described in Section 4.6.3, *Worker Safety,* no impacts to humans, either directly or indirectly, are expected from deregulating 5307 corn; effects would be similar to the No Action alternative.

4.6.2 Animal (Livestock) Health

As described in Chapter 2, Section 2.5.2, *Animal (Livestock) Health,* livestock ingestion of feed from transgenic crops, with subsequent human ingestion of livestock food products, is a potential concern about transgenic crops.

▼

399 Vlachos and Huber. 2011. Pgs. 134-139.
400 Zepha and Vlachos, 2011. (Appendix B)
401 Zeph and Vlachos. 2011. (Appendix B)

Chapter 2, Section 2.2.1, *Production and Yield*, explains that approximately 39 percent of the corn produced in the US is used for livestock feed and most of the corn used currently for livestock feed is transgenic. As discussed in Section 4.6.1, *Human Health*, Cry proteins are not expected to be allergenic, toxic, or pathogenic in mammals or poultry. Additionally, no gene transfer to gastrointestinal flora is expected. Insecticidal Cry proteins from *B. thuringiensis* have a long history of safe use in feed crops.[402] Their modes of action are highly specific within narrow ranges of related insect species, and are not relevant to mammals, including domestic livestock, or other vertebrates. Cry proteins also have a history of safe consumption in the context of other food and feeds.[403] Additionally, the selectable marker PMI protein produced by 5307 corn plants is exempt from food and feed tolerances.[404]

No Action Alternative

Animal (livestock) health would not be affected as a direct or indirect result of the No Action alternative. Livestock would not be exposed to 5307 corn if it continues to remain a regulated article. Previously deregulated transgenic corn will continue to be used as feed for animal consumption, and adoption of transgenic corn varieties would be expected to continue. Exposure to existing transgenic and non-transgenic corn would not change under this alternative. Regulated field trials of 5307 corn would only impact small plots of land with no potential for exposure to livestock.

Proposed Action Alternative

Animal (livestock) health is not likely to be affected as a direct or indirect result of the Proposed Action alternative. If 5307 corn is deregulated, livestock would primarily come in contact with the introduced eCry3.1Ab and PMI proteins in 5307 corn through feed products derived from 5307 corn. The eCry3.1Ab protein is derived from a family of *Bt* proteins that has a long history of safe use in food crops. There are no animal health or environmental concerns with respect to toxicity or allergenicity of eCry3.1Ab or PMI. Exposure to existing transgenic and non-transgenic corn would not change under this alternative. A compositional analysis concluded that forage and grain from 5307 corn hybrids are considered similar in composition to forage and grain from both the non-transgenic comparator and conventional corn hybrids.[405] Event 5307 corn will be as safe and nutritious as conventional corn for livestock.

Syngenta initiated a voluntary pre-market consultation process with FDA and submitted a Safety and Nutritional Assessment for 5307 corn in January 2011.[406] The assessment demonstrated a lack of toxicity and allergenicity of the eCry3.1Ab and

▼

402 USEPA. 2001. Pg. 23.
403 USDHHS-FDA. 2010.
404 USEPA. 2007b.
405 Vlachos and Huber. 2011. Pg. 108.
406 Zeph and Vlachos. 2011. (Appendix B)

PMI proteins in 5307 corn for human and animal consumption. No adverse impacts to livestock, either directly or indirectly, are expected from deregulating 5307 corn.

4.6.3 Worker Safety

As described in Chapter 2, Section 2.6.3, *Worker Safety,* pesticides, including insecticides and herbicides, are used on most corn acreage in the US. The EPA's Worker Protection Standard[407] requires employers to take actions to reduce the risk of pesticide poisonings and injuries among agricultural workers and pesticide handlers. The restrictions and precautions for worker safety associated with several conventional insecticides for rootworm control include:

- Protective clothing (chemical-resistant gloves and other skin protection, eye protection, respirators, etc.) or other measures (closed-system applications) to minimize applicator exposure;
- Minimum worker reentry intervals post application; and
- Minimum preharvest intervals post application.

Large-scale cultivation of transgenic *Bt* corn has increased since its introduction in 1996. A number of studies indicate that the application of pesticides has decreased since the introduction of transgenic crops,[408] reducing exposure of agricultural workers to the hazards of pesticide mixing, loading and application.

EPA requires several types of data for *Bt* PIPs to provide a reasonable certainty that no harm to workers will result from the aggregate exposure of these proteins. None of the *Bt* proteins registered as plant pesticides in the US are toxic or have been shown to have any significant effect on humans.[409]

No Action Alternative

Agricultural workers and pesticide applicators would continue to be exposed to a variety of EPA-registered pesticides such as those approved for control of corn rootworm (Table C-1, Appendix C) under the No Action alternative. Chemical insecticide use rates would likely continue to decrease as insect-resistant transgenic corn continues to be adopted. Approved transgenic *Bt* corn cultivars would continue to be used even if 5307 corn continues to remain a regulated article. Regulated field trials of 5307 corn would only impact small plots of land with limited potential for exposure to agricultural workers. Under the No Action alternative, agricultural workers and pesticide applicators would continue to be exposed to a variety of chemicals.

▼

407 USEPA. 1992. 40 CFR Part 170.1, *Scope and Purpose*.
408 Kleter *et al*. 2007. Pg. 111.
409 USEPA. 2010b. Pg. 23.

Proposed Action Alternative

Similar to the No Action alternative, EPA-registered pesticides that are currently used for corn production would continue to be used by growers under the Proposed Action alternative. As described in Section 4.2.1.4, *Pesticide Use*, trends in reduced insecticide use are likely to continue as a direct result of the Proposed Action alternative. The safety and convenience of planting insect-protected corn compared to the application of conventional insecticides are consistently cited by growers as benefits of transgenic crops. The further adoption of transgenic varieties, including 5307 corn, would continue to extend these benefits to workers. Worker exposure to insecticides would continue to decline. If 5307 corn is deregulated, agricultural workers and pesticide applicators would likely benefit from the use of 5307 corn due to a reduction in the use of corn rootworm insecticide applications and the number of acre-treatments per year.

4.7 Socioeconomics

This section describes the potential impacts to the domestic economic environment, trade economic environment, and social environment that may result from the No Action and Proposed Action alternatives.

4.7.1 Domestic Economic Environment

As described in Chapter 2, Section 2.7.1, *Domestic Economy,* domestic demand for corn in the US comes primarily from its use for feed, ethanol production, food, and seed. In 2011, transgenic products comprised 88 percent of the planted corn to satisfy this demand.[410] The transgenic traits include herbicide tolerance, insect resistance, and other traits. Approximately 65 percent of the US corn crop in 2011 is comprised of *Bt* corn.[411] Overall, harvest security and quality is better with *Bt* corn. Farm income is positively impacted by *Bt* corn by reducing production costs or increasing revenues. Pest-resistant corn generally has a positive impact on farm income due to cost savings from reduced pesticide use.

Section 2.2.2.3, *Insect Management*, explains that the corn rootworm family is one of the most damaging corn pests, and was often referred to as the "billion dollar bug" due to annual yield losses and control costs prior to the introduction of transgenic cultivars for rootworm control, beginning in 2003.[412] Seed companies have developed transgenic crops with corn rootworm resistance specifically to address this economic loss. Four corn rootworm-resistant cultivars (MON 863, DAS 59122-7, MON 88017 and MIR604 corn) are currently available to growers to control this economically important pest.

▼

410 USDA-ERS. 2011b. Pg. 2.
411 Ibid. Pg. 2.
412 Metcalf. 1986. Pg. vii.

No Action Alternative

The domestic economic environment would not be affected as a direct or indirect result of the No Action alternative. Growers would continue to make choices to plant certain corn varieties and use certain crop rotation practices based on factors such as yield, weed and disease pressures, cost of seed and other inputs, technology fees, worker safety, potential for crop injury, and ease and flexibility of the production system.[413, 414] The No Action alternative would not affect available options for growers and therefore not affect the domestic economy. Event 5307 corn would remain a regulated article and would require an APHIS permit or notification for release into the environment. Regulated field trials of 5307 corn would only impact small plots of land with no impact on the domestic economy.

Proposed Action Alternative

The domestic economic environment would be positively affected as a direct and indirect result of the Proposed Action alternative. Growers would have an additional tool to use against corn rootworms if 5307 corn is deregulated, directly reducing economic loss from this pest. The Proposed Action could indirectly result in economic benefit from increased competition in the seed market. The availability of multiple corn rootworm-resistant products would increase grower choice and price competition, potentially resulting in lower seed prices for growers, and assist in managing insect resistance to existing products for corn rootworm control. Specific economic projections are not available.

To the extent that the planting of 5307 corn results in a decrease of insecticide applications for corn rootworm, or an increase in yields, farms adopting 5307 corn might experience an increase in net income. Event 5307 corn hybrids demonstrated an average grain yield advantage of 63 bushels per acre over control hybrids in the presence of intense larval rootworm pressure during field tests.[415] Growers are expected to realize a real-world increase in yield and would therefore likely realize direct economic gain from this product.[416] A description of the potential impacts to the domestic economic environment from 5307 corn in stacked combinations is provided in Section 4.8, *Cumulative Effects*.

4.7.2 Trade Economic Environment

As described in Chapter 2, Section 2.7.2, *Trade Economy,* the primary US corn export destinations are also the largest world importers of corn and do not have major barriers for importing food or feed commodities produced from transgenic crops, including those with insect resistance traits. Developing countries' demand for corn

▼

413 Olson and Sander. 1988. Pgs. 646-647.
414 Gianessi. 2005. Pg. 241.
415 Vlachos and Ward. 2011. Pg. 37.
416 Ibid. Pg. 37.

has increased steadily for the past three decades, propelling the global trade market above 70 million metric tons every year since 1999/2000.[417] Corn imports to European Union countries have declined steadily since the Common Agricultural Policy limited grain imports and EU membership has expanded.[418]

No Action Alternative

The trade economic environment would not be affected as a direct or indirect result of the No Action alternative. Additional genetically modified corn varieties would continue to be developed and available in the trade market. Event 5307 corn would remain a regulated article and would require an APHIS permit or notification for release into the environment. Regulated field trials of 5307 corn would only impact small plots of land with no impact on the worldwide corn trade.

Proposed Action Alternative

The trade economic environment would not be affected as a direct or indirect result of the Proposed Action alternative. Worldwide market conditions and destination country approval of transgenic crop commodities would continue to be factors for international corn prices, without regard to the presence or absence of 5307 corn on the market. Deregulating 5307 corn would not adversely impact the trade economy and may potentially enhance it through more efficient production of corn supplies worldwide.

Syngenta has applied to Canadian agencies for approval of the unconfined environmental release and food and feed use of corn commodities and processed goods containing 5307 corn. To avoid adversely affecting international trade in corn commodities exported from the US (and Canada), regulatory filings for 5307 corn import approvals have been made in Japan, South Korea, Taiwan, Australia/New Zealand, South Africa, Colombia, and the European Union. Applications are planned for additional countries including Mexico, China, the Philippines, Indonesia, and Russia. The trade economic impacts associated with the deregulation of 5307 corn are anticipated to be very similar to the No Action alternative.

4.7.3 Social Environment

As described in Chapter 2, Section 2.7.3, *Social Environment,* farmers have a range of options in agronomic practices, seed products to choose from, and opportunities for sale to customers. Consumers have a range of corn products to choose from. Section 4.2.2.1, *Organic Farming*, explains that growers may choose to grow transgenic, non-transgenic, or organic corn, and obtain price premiums for growing varieties of corn for particular markets. Regulatory agency requirements (under the

▼

417 USDA-ERS. 2009. Pg. 3.
418 Ibid. Pg. 3.

Federal Food, Drug, and Cosmetic Act and the NOP, for example) and consumer advocacy groups promote food product safety and consumer choice.

No Action Alternative

The social environment would not be affected as a direct or indirect result of the No Action alternative. Event 5307 corn would remain a regulated article and would require an APHIS permit or notification for release into the environment. Regulated field trials of 5307 corn would only impact small plots of land with no impact on the social environment.

Proposed Action Alternative

The social environment would not be affected as a direct or indirect result of the Proposed Action alternative. Regulatory programs and consumer choicewould be unchanged by granting nonregulated status to 5307 corn.

4.8 Cumulative Effects

This section describes the potential cumulative effects that may result from the Proposed Action alternative. In accordance with CEQ regulations, this evaluation considers the potential effects that, although individually minor, could have a collectively significant impact on the environment when added to other past, present, and reasonably foreseeable future actions.

4.8.1 Methodology and Assumptions

The CEQ regulations define a cumulative effect as "the impact on the environment which results from the incremental impact of the action when added to other past, present, and reasonably foreseeable future actions regardless of what agency (Federal or non-Federal) or person undertakes such other actions. Cumulative impacts can result from individually minor but collectively significant actions taking place over a period of time."[419]

Based on the information provided in Sections 4.2 through 4.7, which is summarized in Table 3-1, the Proposed Action would not adversely impact the physical, biological, or socioeconomic environment. Furthermore, as discussed in this section, there are no past, present or reasonably foreseeable actions that, in aggregate with the Proposed Action alternative, would adversely affect these resources. Based on the evaluation provided in previous sections of this Chapter, if 5307 corn is deregulated corn production practices would continue unchanged and most environmental resources would be unaffected. There would be no cumulative adverse effects to corn

▼

419 CEQ. 1978. 40 CFR Part 1508, Section 1508.7, *Cumulative impact*.

production, the physical environment, the biological environment, public health, or the socioeconomic environment. Event 5307 corn, in stacked combinations, is likely to have beneficial cumulative effects on pesticide usage, biodiversity, and the domestic economy.

4.8.2 Reasonably Foreseeable Future Actions

Transgenic corn varieties marketed in the US today typically contain multiple transgenic traits, some of which have been combined by traditional breeding between different deregulated cultivars. In the event that APHIS reaches a determination of nonregulated status, 5307 corn would potentially be combined with non-transgenic and transgenic corn cultivars by traditional breeding techniques, resulting in a plant variety that, for example, may be resistant to one or more herbicides and contain other insect-resistance traits. APHIS's current regulations at 7 CFR Part 340 do not provide for agency oversight of transgenic corn varieties previously deregulated pursuant to Part 340 and the PPA, nor over stacked varieties combining deregulated transgenic cultivars, unless it can be positively shown that such stacked varieties were likely to pose a plant pest risk.

There is no certainty that 5307 corn will be stacked with any particular deregulated transgenic variety, as company plans and market demands play a significant role in those business decisions. Predicting all potential combinations of stacked varieties that could be created using both deregulated transgenic corn cultivars and non-transgenic corn cultivars is hypothetical and purely speculative. However, Syngenta intends to offer 5307 corn for sale to US growers in two combinations comprised of the insect protection and herbicide tolerance traits; these combinations are listed as A and B in Table 4-1. Syngenta has requested authorization of these stacked products from the EPA, with regard to the specific combinations of insecticidal traits present in each product.[420]

Table 4-1 Planned Stacking Combinations

Cultivar	Traits	Combination
5307	Corn rootworm resistant	A, B
Bt11	Lepidopteran resistant and glufosinate tolerant	A, B
MIR604	Corn rootworm resistant	A, B
TC1507	Lepidopteran resistant and glufosinate tolerant	A, B
GA21	Glyphosate tolerant	A, B
MIR162	Lepidopteran resistant	B

420 Vlachos and Ward. 2011.

The combination hybrids A and B listed in Table 4-1 will combine two corn rootworm-active proteins (eCry3.1Ab from 5307 corn and mCry3A from MIR604 corn) that each provide control of western, northern, and Mexican corn rootworms. The hybrids will also combine other *Bt*-derived proteins that will deliver broad-spectrum control of economically important lepidopteran pests and traits that confer tolerance to glyphosate and glufosinate herbicide applications as additional weed-control options. Apart from 5307 corn, all of the cultivars derived from other transgenic events in these breeding stacks have already been deregulated by APHIS. Breeding stacks for insect control currently approved for sale or use in the US combine various insect resistance and herbicide tolerance traits, as listed in Table 4-2.

Table 4-2 Commercially Available Corn Breeding Stack Combinations with Insect Control Traits

Product	Event Names	Resistance/Tolerance Traits
Agrisure® GT/CB/LL	Bt11/GA21	Lepidoptera, glyphosate, glufosinate
Agrisure Viptera™ 3110	Bt11/MIR162/GA21	Lepidoptera, glyphosate, glufosinate
Agrisure CB/LL/RW	Bt11/MIR604	Lepidoptera, rootworm, glufosinate
Agrisure 3000GT	Bt11/MIR604/GA21	Lepidoptera, rootworm, glyphosate, glufosinate
Agrisure Viptera 3111	Bt11/MIR604/MIR162/GA21	Lepidoptera, rootworm, glyphosate, glufosinate
Syngenta GT/RW	MIR604/GA21	Rootworm, glyphosate
YieldGard® Corn Borer with Roundup Ready® Corn 2 (RR2)	MON 810/NK603	Lepidoptera, glyphosate
YieldGard Rootworm/RR2	MON 863/NK603	Rootworm, glyphosate
Genuity™ VT Double PRO™	MON 89034/NK603	Lepidoptera, glyphosate
YieldGard Plus	MON 810/MON 863	Lepidoptera, rootworm
YieldGard Plus/RR2	MON 810/MON 863/NK603	Lepidoptera, rootworm, glyphosate
YieldGard VT Triple® and Genuity™ VT Triple	MON 810/MON 88017	Lepidoptera, rootworm, glyphosate
(Genuity) Smart Stax™	MON 89034 /MON 88017/ TC1507/DAS-59122-7	Lepidoptera, rootworm, glyphosate, glufosinate
Genuity VT Triple PRO	MON 89034/MON 88017	Lepidoptera, rootworm, glyphosate
Herculex® I/RR2	TC1507/NK603	Lepidoptera, glyphosate, glufosinate
Herculex RW/RR2	DAS 59122-7/NK603	Rootworm, glyphosate, glufosinate
Herculex XTRA	TC1507/DAS 59122-7	Lepidoptera, rootworm, glufosinate
Herculex XTRA/RR2	TC1507/DAS 59122-7/NK603	Lepidoptera, rootworm, glyphosate, glufosinate
Optimum® AcreMax® 1	Seed blend of 90% TC1507/DAS-59122-7/NK603 and 10% TC1507/NK603 refuge seed	90% Lepidoptera, rootworm, glyphosate; 10% Lepidoptera, glyphosate
Optimum AcreMax RW	Seed blend of 90% DAS-59122-7/NK603 and 10% NK603 refuge seed	90% rootworm, glyphosate; 10% glyphosate

Sources: NCGA website http://ncga.com/know-before-you-grow/ and Pioneer website http://www.pioneer.com/home/site/about/products/product-traits-technology/optimum-acre-max/

For purposes of cumulative impact analysis, reasonably foreseeable future actions include the potential for stacking certain already approved transgenic corn cultivars with 5307 corn, or for creating new stacks with similar combinations of traits.

4.8.3 Cumulative Effects Evaluation

This section considers the potential cumulative effects resulting from including 5307 corn in multi-trait (stacked) combinations. Syngenta intends to commercialize the 5307 corn rootworm-resistance trait in combination with other insect-resistance traits (effective in controlling corn rootworm and Lepidoptera) and herbicide-tolerance traits, as listed in Table 4-1. Although APHIS has found that none of the 30 currently approved transgenic corn cultivars (as of October 12, 2011) presents a significant environmental impact alone or cumulatively, APHIS has not specifically addressed the cumulative impact of stacking these products with 5307 corn. The first step in evaluating the cumulative impacts of deregulating 5307 corn is to identify the resources that might be affected by that action. Areas of concern with regard to these stacked combinations include agronomic practices (insecticide use and potential development of resistant insect populations; herbicide use and potential development of herbicide-tolerant weeds), biodiversity, and domestic economic issues (cost to farmers).

Potential cumulative effects of the Proposed Action on these resources were evaluated by comparing the results of the direct and indirect impacts analyses with data from a literature review. The analysis provided below discusses the potential cumulative effects of the Proposed Action alternative in reference to these potentially affected resources. The potential cumulative effects of the No Action alternative would be a combination of the impacts of the existing approved transgenic corn cultivars and the impacts from limited field trials of 5307 corn as a regulated article. The potential cumulative effects of the Proposed Action alternative would be a combination of the impacts from existing approved transgenic corn products and the impacts of the incorporation of 5307 corn in new stacking combinations.

4.8.3.1 Pesticide Use

As noted in Table 3-1 and discussed in Section 4.2.1, *Agronomic Practices*, the Proposed Action is expected to have the effect of reducing insecticide use. The Proposed Action is not expected to change overall herbicide use. The past and current actions potentially contributing to cumulative effects on this resource are pest management strategies including conventional insecticide and herbicide use, crop rotation practices, and the introduction of transgenic corn varieties and their increasing use. The array of transgenic corn cultivars currently available for insect pest management is listed in Table 2-1. The future actions potentially contributing to this cumulative effect are the combination of 5307 corn stacked with transgenic corn cultivars exhibiting insect resistance and/or herbicide tolerance, as listed in Table 4-1.

Insecticide Use

Corn rootworm has evolved resistance to chemical insecticides and crop rotation practices that have historically been used to control these economically destructive pests. As described in Chapter 2, Section 2.2.2.3, *Insect Management*, use of conventional (chemical) pesticides to control corn rootworm has been reduced since the introduction of transgenic *Bt* corn for insect control.

A key factor in the continued use of *Bt* corn for insect control is product durability (i.e., delaying or avoiding the development of resistance to *Bt* proteins in insect pests). The widespread use of transgenic *Bt* corn could generate selection pressures for insect resistance.[421] Nonetheless, insect resistance to *Bt* crops has not caused widespread failure of control measures, in part due to IRM strategies, including rescue applications of insecticides and nearby refuges of crop varieties lacking the insecticidal trait. Although one recent study indicates that, in one localized area, western corn rootworm may have developed resistance to a Cry protein under intense cultivation,[422] stacking two transgenic corn traits with different modes of action against the target pest limits the potential for resistance to develop to any one product.[423]

Refuge strategies include planting a designated percentage of corn refuge acres (in the Corn Belt, typically 5 to 20 percent of a grower's corn field area[424]), either in a designated area that is a percent of the total area (a block) or a percent of the total seed planted ("refuge-in-a-bag"). Refuge sizes are determined for each specific insect-resistant corn or stacked product, and are mandated by the EPA as part of product registration. IRM practices are required by seed companies as a condition of seed purchase, and implemented through agreements signed by growers.

Corn rootworm-resistant cultivars with a different mode of action that may be stacked with 5307 corn in the future include Syngenta's previously approved rootworm-resistant *Bt* corn variety, MIR604 corn. APHIS granted nonregulated status for this product in March 2007.[425] APHIS determined that cultivating MIR604 corn would not necessarily result in the development of resistant rootworm populations. EPA has mandated in the terms of registration that growers use IRM strategies for MIR604 and other *Bt* corn varieties expressing PIPs for the control of insect pests.

No Action Alternative

As described in Section 4.2.1.4, *Pesticide Use*, insecticide use would not be affected as a direct or indirect result of the No Action alternative. For the cumulative effects

▼

421 Tabashnik *et al*. 2008. Pg. 199.
422 Gassmann *et al*. 2011. Pg. 4.
423 Tabashnik *et al*. 2008. Pg. 201.
425 USDA-APHIS. 2007.
425 USDA-APHIS. 2007.

analysis of the No Action alternative, the reasonably foreseeable future would not include stacking 5307 corn with other transgenic corn cultivars. Event 5307 corn would remain a regulated article and would require an APHIS permit or notification for release into the environment. Corn rootworms are not likely to develop resistance to *Bt* corn as a result of the continued regulated status of 5307 corn. Regulated field trials of 5307 corn would only impact small plots of land with no impact on insecticide use or corn rootworm resistance to *Bt* corn.

Insect-resistant transgenic corn varieties would continue to be available, and insects could potentially develop resistance to these varieties. When combined with the past, present, and reasonably foreseeable future actions, there would be no cumulative effect from the No Action alternative on insecticide use or corn rootworm resistance to *Bt* corn. However, without the additional rootworm control option offered by 5307 corn, fewer PIP traits would be available to growers to combat the evolution of insect resistance.

Proposed Action Alternative

As described in Section 4.2.1.4, *Pesticide Use*, if 5307 corn is deregulated, trends of reduced insecticide use are likely to continue. Corn growers would have a new corn rootworm-control cultivar in addition to the four other corn rootworm-control cultivars that are currently available. This would allow for more effective IRM strategies. Because 5307 corn has excellent efficacy against corn rootworm and because the eCry3.1Ab protein operates via a unique mode of action, introduction of the 5307 corn breeding stacks is expected to further extend the useful life of other commercially available corn rootworm-protected products (i.e., the PIPs Cry3Bb1, Cry34Ab1/Cry35Ab1, and mCry3A).[426]

The combination of eCry3.1Ab from 5307 corn and mCry3A via Syngenta's deregulated MIR604 corn in the same corn hybrids may also justify a reduction in the size of the required on-farm refuge from a minimum of 20 percent of a grower's corn acres to 5 percent.[427] Appropriate refuge requirements would be implemented in the context of a comprehensive IRM program mandated by the EPA. IRM would include insect scouting or monitoring to determine pest populations, considering and applying compatible alternative biological, cultural, mechanical and chemical controls, and establishing action thresholds for agricultural inputs. The timely and targeted delivery of pest management interventions is key to successful IRM. Planting 5307 corn stacks would eliminate the need to target and time applications of rootworm insecticides, due to the sustained production of the insect control proteins (eCry3.1Ab and mCry3A) in vulnerable root tissues.[428] The 5307 corn breeding stacks are consistent with the concept of IRM for corn.

▼

426 Vlachos and Ward. 2011. Pg. 30.
427 Vlachos and Huber. 2011. Pg. 154.
428 Vlachos and Ward. 2011. Pgs. 31-32.

With a reduced minimum refuge requirement (i.e., from 20 to 5 percent of a grower's corn acres), the use of transgenic *Bt* corn may be expected to increase. Increased use of transgenic *Bt* corn is correlated with reduced reliance on, and use of, insecticides to control corn rootworm. It is also correlated with higher corn yields than can typically be achieved with insecticides alone. Thus, when combined with past, present, and reasonably foreseeable future actions, the cumulative effects of the Proposed Action alternative would likely result in increased corn yields (on what previously would have been refuge acres), further reduction of insecticide use, and a reduced likelihood that corn rootworm populations will develop resistance to Cry proteins, extending the effectiveness of these tools.

Herbicide Use

Herbicide-tolerant transgenic corn cultivars have been developed to allow use of herbicides without harming the crop. As described in Chapter 2, Section 2.2.2.5, *Weed Management*, herbicide-tolerant corn has been widely adopted by growers in North America and offers enhanced weed control. Currently available transgenic herbicide-tolerant corn cultivars include multiple glyphosate- or glufosinate-(phosphinothricin) tolerant cultivars.[429]

In 2011, approximately 72 percent of the US corn crop was planted to transgenic varieties that were herbicide tolerant.[430] However, over-reliance on herbicide-tolerant crops may under certain conditions promote the development of herbicide-tolerant weeds. Weeds can potentially survive in crop production systems because of natural tolerance to the chemical(s) and because of growth types or life cycles that help them avoid being treated, such as some winter annual weed species. As described in Chapter 2, Section 2.2.2.5, *Weed Management*, some weeds have developed herbicide tolerance and the increased use of herbicide-tolerant corn may increase the prevalence of herbicide-tolerant weeds. Industry practice includes weed resistance management training to reduce the potential for weeds to develop tolerance to herbicides.[431] Because there are several herbicides available, and transgenic corn varieties tolerant to different herbicides available, growers have the ability to rotate herbicide use, rather than relying on a single herbicide.

The adoption of herbicide-tolerant crops also has been associated with the increased adoption of conservation tillage, which decreases runoff, increases water infiltration, and reduces soil erosion.[432]

▼

429 USDA-APHIS. 2011b.
430 USDA-ERS. 2011a. Pg. 2.
431 NCGA. 2011. Slide 2.
432 Carpenter. 2011. Pg. 8.

No Action Alternative

Herbicide use would not be affected as a direct or indirect result of the No Action alternative. The reasonably foreseeable future for the No Action Alternative would not include stacking 5307 corn with other transgenic corn cultivars. Event 5307 corn would remain a regulated article and would require an APHIS permit or notification for release into the environment. Transgenic corn varieties with herbicide tolerance would continue to be available, and weeds could continue to develop resistance to herbicides. Thus, when combined with past, present, and reasonably foreseeable future actions, there would be no cumulative effect from the No Action alternative on herbicide use or on the evolution of weed tolerance to herbicides.

Proposed Action Alternative

Although 5307 corn does not have an herbicide tolerance trait, under the Proposed Action alternative it will be stacked with other transgenic corn cultivars that do have herbicide tolerance traits. Syngenta would market 5307 corn stacked with its own herbicide-tolerant cultivars. If 5307 corn is deregulated and stacked with herbicide-tolerant cultivars, weed management methods may be altered to a minor degree but would generally continue following current trends.

In APHIS's recent analysis of the DP-098140-6 herbicide-tolerant corn, it was noted that the acres of transgenic corn planted with herbicide-tolerant varieties declined in 2008 but that corn varieties with insect resistance, whether alone or stacked with an herbicide tolerance trait, are planted more readily than those varieties conferring tolerance to herbicides alone.[433] Stacked products combining insect resistance and herbicide tolerance (and potentially other traits) are likely to become more common regardless of the regulatory status of 5307 corn. The Proposed Action would likely continue this trend.

As described in Chapter 2, Section 2.2.2.5, *Weed Management*, glyphosate application rates have increased recently and it is reasonable to conclude that they will continue to do so. Weeds may continue to develop tolerance to herbicides. Given the widespread adoption of herbicide-tolerant corn that has already occurred, stacking 5307 corn with herbicide tolerance traits is unlikely to significantly change current trends in herbicide use and increasing weed tolerance to herbicides. When combined with the past, present, and reasonably foreseeable future actions, the Proposed Action would have a negligible cumulative effect on herbicide use or weed tolerance to herbicides.

▼

433 USDA-APHIS. 2009. Pg. 5.

4.8.3.2 Biodiversity

The past and current actions potentially contributing to a cumulative effect on biodiversity are the introduction of transgenic corn varieties and their increasing use. The array of transgenic corn varieties currently available for pest management is outlined above. The future actions potentially contributing to cumulative effects on biodiversity are any combination of 5307 corn stacked with transgenic corn cultivars exhibiting insecticidal properties or herbicide tolerance.

This cumulative effects analysis focuses on potential changes in biodiversity that may result from impacts to non-target plants and animals within the agroecosystem. Other aspects of biodiversity would not be affected by the Proposed Action, as described in previous sections of this Chapter. Insect biodiversity within agricultural production areas could increase with the use of transgenic crops that reduce the use of broad-spectrum insecticides.

Several different Cry proteins are incorporated in various *Bt* corn varieties to provide insect resistance. EPA conducted a comprehensive environmental assessment of the registered *Bt* PIPs in 2001.[434] Although other *Bt* corn cultivars incorporating Cry proteins have been introduced in the intervening 10 years, and many of the PIPs in earlier cultivars were re-registered in 2010, the 2001 EPA review provides a general assessment of the risks to biodiversity associated with *Bt* corn varieties. Summarizing then-existing published studies and EPA's reviews of submitted studies on potential Cry protein impacts to non-target species (vertebrates and invertebrates), EPA concluded that "the weight of evidence from the reviewed data indicate that there is no hazard to non-target wildlife from the continued registration of *Bt* crops."[435] Minimal to undetectable adverse impacts, and in some cases beneficial impacts, to non-target insect populations were shown.[436] EPA also noted that *Bt* crops have a positive effect on soil flora compared to non-selective synthetic chemical pesticides.[437] More recent studies have also concluded that the use of *Bt* crops, rather than broad-spectrum insecticides, could allow larger populations of beneficial insects and non-pest herbivores to persist in crop fields.[438]

Cultivars expressing other *Bt*-derived proteins may be stacked with 5307 corn in the future, including Syngenta's most recently approved lepidopteran-resistant cultivar, MIR162 corn. APHIS granted nonregulated status for this cultivar in April 2010.[439] APHIS determined that there were no past, present, or reasonably foreseeable actions that would aggregate with effects of granting nonregulated status to MIR162 corn to create cumulative impacts or reduce the long-term productivity or sustainability of any of the resources associated with the ecosystem in which the MIR162 corn is

▼

434 USEPA. 2001. Pg. IIC1.
435 USEPA. 2001. Pg. IIC81.
436 USEPA. 2001. Pg. IIC84.
437 USEPA. 2001. Pg. IIC53.
438 Pilson and Prendeville. 2004. Pg. 164.
439 USDA-APHIS. 2010b.

planted.[440] APHIS also noted that transgenic corn varieties with *Bt* traits have been available on the market and the body of evidence in peer-reviewed literature does not suggest any negative effect on biodiversity.

In general, applying less toxic herbicides (e.g., glyphosate) may be more environmentally beneficial than traditional herbicides.[441] However, animals that consume the weeds targeted by the herbicides could potentially be impacted if the weeds are eradicated. These relationships and the actual impact on biodiversity are difficult to determine. For example, one study suggested that reductions in monarch butterfly (*Danaus plexippus*) populations overwintering in Mexico might be attributed in part to loss of host milkweed (*Asclepias syriaca*) plants in the Corn Belt from the extensive use of glyphosate.[442] However, other studies[443] suggest that monarch populations are very dynamic because of the high reproductive potential of this species, and no similar reduction was observed in populations studied in the US.

No Action Alternative

As described in Section 4.4.4, *Biodiversity*, the No Action alternative would not directly or indirectly affect biodiversity. For the cumulative effects analysis of the No Action alternative, stacking 5307 corn with other transgenic corn cultivars would not occur under the No Action alternative. Event 5307 corn would remain a regulated article and would require an APHIS permit or notification for release into the environment. Non-target plants and animals would not be affected as a result of the continued regulated status of 5307 corn. Regulated field trials of 5307 corn would only impact small plots of land with no impact on biodiversity. When combined with the past, present, and reasonably foreseeable future actions, there would be no cumulative effect from the No Action alternative on biodiversity.

Proposed Action Alternative

As described in Section 4.4.4, *Biodiversity*, the Proposed Action alternative would not adversely affect biodiversity. Event 5307 corn would only be stacked with approved transgenic cultivars that have been demonstrated by APHIS review to have insignificant impacts to animals. The hybrids under consideration by Syngenta would combine two rootworm-active proteins that each provide control of western, northern, and Mexican corn rootworms but do not compete for the same binding site in western corn rootworm gut membranes.[444] If 5307 corn is deregulated and stacked with herbicide-tolerant products, weed management methods may be altered to a minor degree, but would generally continue following current trends. Event 5307 corn would only be stacked with approved transgenic cultivars that have been demonstrated to have insignificant impacts to non-weed plants.

▼

440 USDA-APHIS. 2010b. Pg. 28.
441 Pilson and Prendeville. 2004. Pg. 155.
442 Brower *et al.* 2011. Pg. 2.
443 Davis. 2011. Pg. 3.
444 Vlachos and Huber. 2011. Pg. 154.

Corn growers would have a new corn rootworm-control product, stacked with other control products, to add to the tools available for pest management. Representative non-target animal species were not harmed when exposed to high concentrations of eCry3.1Ab, the protein expressed in 5307 corn.[445] APHIS has determined that the other cultivars would not have a significant adverse impact on biodiversity and, in some cases could have a beneficial impact. Stacking 5307 corn with the existing transgenic corn cultivars would only add incrementally to these beneficial impacts. Section 4.8.3.1, *Pesticide Use*, explains that stacking 5307 corn with herbicide-tolerance traits may incrementally increase the use rates or displacement of certain herbicides, potentially contributing to a negligible degree to increased weed tolerance to herbicides. Stacking with other insect-resistance traits would potentially decrease broad-spectrum insecticide use, cumulatively reducing the selection pressure for insect resistance and adverse impacts to non-target insects. On balance, these effects are expected to improve biodiversity. When combined with the past, present, and reasonably foreseeable future actions, the cumulative effects of the Proposed Action alternative would likely improve biodiversity within agroecosystems.

4.8.3.3 Domestic Economy

The past and current actions potentially contributing to cumulative effects on the domestic economy are the introduction of transgenic corn varieties and their increasing use. The array of transgenic corn varieties currently available for pest management is outlined above. The future actions potentially contributing to cumulative effects to the domestic economy are any combination of 5307 corn stacked with transgenic corn with PIPs exhibiting herbicide tolerance, or other desirable traits.

Farmers are expected to economically benefit from corn rootworm-resistant transgenic corn products. Chapter 2, Section 2.2.2.3, *Insect Management*, explains that the corn rootworm family is one of the most damaging corn pests, affecting as much as 32 million acres each year. Prior to the introduction of rootworm-protected transgenic corn varieties to the market, corn rootworms cost US farmers nearly $1 billion annually in crop losses and control costs.[446] Using corn rootworm-protected varieties offsets these costs by increasing yield and reducing the risk, cost, and time associated with insecticide use.[447] Seed companies and growers could also see an economic benefit, from increased profits.

Similar benefits are expected from insect-resistant crops targeted at other economically significant pests. For example, lepidopteran-active *Bt* corn has remained effective against the European corn borer for more than a decade, yielding

▼

445 Vlachos and Huber. 2011. Pg. 149.
446 Rice. 2004. Pg. 1.
447 Rice. 2004. Pg. 3.

billions of dollars of estimated benefits to farmers in the midwestern US. The cumulative economic benefit to corn growers in a five-state region over a 14-year period ending in 2009 has been estimated at $6.9 billion.[448] These growers include many who did not plant *Bt* corn varieties, but nevertheless benefited from area-wide suppression of European corn borer populations as a result of sustained *Bt* corn use in the region.

Herbicide-tolerant transgenic corn also has benefits for the domestic economy. For farmers, the general increase in yield, reduction in some input costs, improvement in pest control, increase in worker safety, and time-management benefits have generally outweighed the additional costs of transgenic seed.[449] Herbicide-tolerant crops have not greatly increased yields but have generally improved weed control and improved farmers' incomes by saving time.

Combining insect resistance traits and herbicide tolerance traits in a single stacked product would likely convey the individual economic benefits of each trait to farmers and seed companies. Available information does not allow specific quantification of the economic benefit that any given product would provide because of the complexity and variability of the market. Corn farmers' profitability is subject to resource costs (seeds, fuel), environmental factors (weather, pests), and labor costs (planting, harvesting, and processing). Corn commodity prices fluctuate in accordance with supply and demand, the latter of which can be driven by food, feed, or fuel needs as well as federal government policy.

No Action Alternative

As described in Section 4.7.1, *Domestic Economic Environment*, the No Action alternative would not directly or indirectly affect the domestic environment, and existing trends would continue. For the cumulative effects analysis of the No Action alternative, stacking 5307 corn with other transgenic corn cultivars, would not occur. Event 5307 corn would remain a regulated article and would require an APHIS permit or notification for release into the environment. The commercial market would not be affected as a result of the continued regulated status of 5307 corn. Regulated field trials of 5307 corn would only impact small plots of land with no impact on the domestic economy. However, this would not increase the number of different stacked corn varieties available, and growers would not have an increased choice about what to plant. When combined with the past, present, and reasonably foreseeable future actions, there would be no cumulative effect from the No Action alternative on the domestic economy.

▼

448 Hutchison *et al*. 2011. Pg. 224.
449 NRC. 2010. Pg. 174.

Proposed Action Alternative

As described in Section 4.7.1, *Domestic Economic Environment*, the Proposed Action alternative would positively affect, both directly and indirectly, the domestic economy. Growers would have a broader range of tools to use to combat insects and weeds if 5307 corn is deregulated, directly reducing economic loss from economically important pests. For stacked varieties in particular, the combination of eCry3.1Ab and modified Cry3A (via Syngenta's deregulated MIR604 corn) in the same corn hybrids could also justify a reduction in the size of the required on-farm refuge from a minimum of 20 percent of a grower's corn acres to 5 percent.[450] This would have economic benefits for the grower from increased yield, and would further reduce insecticide use for rootworm control. Although the specific domestic economy benefits cannot be calculated because of the variability and complexity of the market, it is assumed that growers will make rational decisions to maximize economic gain from their operations, and 5307 corn stacked with other transgenic corn traits will increase the varieties available to growers.

▼

450 Vlachos and Huber. 2011. Pg. 154.

5

Consideration of Executive Orders and Other Federal Laws Relating to Environmental Impacts

As required by the CEQ, this Chapter considers the Executive Orders (EOs) and other federal laws related to the potential environmental impacts of the Proposed Action.

5.1 Executive Orders with Domestic Implications

Four EOs have domestic implications that are relevant to the environmental assessment of the petition to deregulate 5307 corn.

5.1.1 Executive Order 12898: Environmental Justice

EO 12898, *Federal Actions to Address Environmental Justice in Minority Populations and Low-Income Populations*,[451] requires federal agencies to conduct their programs, policies, and activities that substantially affect human health or the environment in a manner so as not to exclude persons and populations from participation in or benefiting from such programs. It also enforces existing statutes to prevent minority or low-income communities from being subjected to disproportionately high and adverse human health or environmental effects.

Event 5307 corn is not significantly different from other transgenic or non-transgenic corn. As described in Chapter 4, Section 4.4.1, *Animals*, the eCry3.1Ab and PMI proteins do not pose a hazard to humans. A voluntary FDA consultation for food

▼

451 Executive Order 12898 of February 11, 1994.

and feed use of 5307 corn was initiated in January 2011 (Appendix B). Data and information provided by Syngenta support the safe use of 5307 corn and indicate that it will be as nutritious and wholesome as other corn currently used as food and feed. Based on these analyses, 5307 corn is not expected to have a disproportionate adverse effect on minorities or low-income populations.

As described in Chapter 4, Section 4.2.1, *Agronomic Practices*, the cultivation of previously deregulated corn varieties with similar insect resistance traits has been associated with a decrease in insecticide applications. If insecticide applications are reduced, there may be a beneficial effect on minority populations. These populations might include farm workers and their families, and other rural dwelling individuals who are potentially exposed to insecticides through aerial application, groundwater, or other routes of exposure.

5.1.2 Executive Order 13045: Protection of Children

EO 13045, *Protection of Children from Environmental Health Risks and Safety Risks*,[452] acknowledges that children may suffer disproportionately from environmental health and safety risks because of their developmental stage, greater metabolic activity levels, and behavior patterns, as compared to adults. This EO requires each federal agency (to the extent permitted by law and consistent with the agency's mission) to identify, assess, and address environmental health risks and safety risks that may disproportionately affect children.

Event 5307 corn is not significantly different from other transgenic or non-transgenic corn. As described in Chapter 4, Section 4.4.1, *Animals*, the eCry3.1Ab and PMI proteins do not pose a hazard to humans. A voluntary FDA consultation for food and feed use of 5307 corn was initiated in January, 2011 (Appendix B). Data and information provided by Syngenta support the safe use of 5307 corn and indicate that it will be as nutritious and wholesome as other corn current used as food and feed. Based on these analyses, 5307 corn is not expected to have a disproportionate adverse effect on children.

As described in Chapter 4, Section 4.2.1, *Agronomic Practices*, the cultivation of previously deregulated corn varieties with similar insect resistance traits has been associated with a decrease in insecticide applications. If insecticide applications are reduced, there may be a beneficial effect on children that might be exposed to the chemicals. Similar to minority populations, these children might include families of farm workers and other rural dwelling individuals who are exposed to insecticides through aerial application, groundwater contamination, or other routes.

▼

452 Executive Order 13045 of April 21, 1997.

5.1.3 Executive Order 13112: Invasive Species

EO 13112, *Invasive Species,*[453] requires federal agencies to take action to prevent the introduction of invasive species, to provide for their control, and to minimize the economic, ecological, and human health impacts that invasive species cause.

Corn is a highly domesticated plant and is not an invasive species. Both non-transgenic and transgenic corn varieties that have been granted nonregulated status are widely grown in the US and have not developed weedy or invasive characteristics. As described in Section 4.4.5, *Gene Movement in the Natural Environment*, 5307 corn plants are very similar in agronomic characteristics to other corn varieties that are currently grown and are not expected to become weedy or invasive. Accordingly, the Proposed Action would not raise concerns addressed by EO 13112, *Invasive Species*.

5.1.4 Executive Order 13186: Migratory Birds

EO 13186, *Responsibilities of Federal Agencies to Protect Migratory Birds,*[454] requires each federal agency to avoid and minimize, to the extent practicable, adverse impacts on migratory bird populations when conducting agency actions.

Although migratory birds forage in corn fields, as described in Section 4.4.1, *Animals*, 5307 corn is not expected to have any adverse impacts on migratory birds because the eCry3.1Ab protein is not biologically active in avian species. To confirm the absence of any impact on avian species, Syngenta has conducted an eCry3.1Ab toxicity study on juvenile bobwhite quail and a 5307 corn feeding study on broiler chickens, in which no harmful effects to quail or chickens were observed. Granting nonregulated status to this corn cultivar therefore is not expected to have a negative effect on migratory bird populations. The Proposed Action accordingly would be in compliance with EO 13186, *Responsibilities of Federal Agencies to Protect Migratory Birds*.

5.2 Executive Order with International Implications

EO 12114, *Environmental Effects Abroad of Major Federal Actions,*[455]requires federal officials to take into consideration any potential environmental effects outside the US, its territories, and possessions that may result from actions being taken.

All of the existing national and international regulatory authorities and phytosanitary regimes that currently apply to introduction of new corn cultivars internationally apply equally to those covered by an APHIS determination of

▼

453 Exectuive Order 13112 of February 3, 1999.
454 Executive Order 13186 of January 10, 2001.
455 Executive Order 12114 of January 4, 1979.

nonregulated status under 7 CFR Part 340. International trade of 5307 corn subsequent to a determination of nonregulated status for the product would be fully subject to national phytosanitary requirements and be in accordance with phytosanitary standards developed under the International Plant Protection Convention (IPPC).[456]

The purpose of the IPPC "is to secure a common and effective action to prevent the spread and introduction of pests of plants and plant products and to promote appropriate measures for their control."[457] The protection it affords extends to natural flora and plant products and includes both direct and indirect damage by pests, including weeds. The IPPC set a standard for the reciprocal acceptance of phytosanitary certification among the 177 nations that have signed or acceded to the convention.[458] In April 2004, a standard for pest risk analysis (PRA) of living modified organisms (LMOs) was adopted at a meeting of the governing body of the IPPC as a supplement to the existing *International Standard for Phytosanitary Measure No. 11*.[459] The standard acknowledges that LMOs will not present a pest risk and that a determination needs to be made early in the PRA for importation as to whether the LMO poses a potential pest risk resulting from the genetic modification. APHIS pest risk assessment procedures for genetically engineered organisms are consistent with the guidance developed under the IPPC. In addition, issues that may relate to commercialization and transboundary movement of particular agricultural commodities produced through biotechnology are being addressed in other international forums and through national regulations.

The *Cartagena Protocol on Biosafety* (the Protocol) is a treaty under the United Nations *Convention on Biological Diversity* (CBD) that established a framework for the safe transboundary movement, with respect to the environment and biodiversity, of LMOs, which includes those modified through biotechnology. The Protocol came into force on September 11, 2003, and 161 countries are currently parties to it.[460] Although the US is not a party to the CBD and thus not a party to the Protocol, US exporters will still need to comply with domestic regulations that importing countries that are parties to the Protocol have put in place to comply with their obligations.

The first intentional transboundary movement of LMOs intended for environmental release (field trials or commercial planting) will require consent from the importing country under an advanced informed agreement (AIA) provision, which includes a requirement for a risk assessment consistent with Annex III of the Protocol, and the required documentation. LMOs imported for food, feed, or processing (FFP) are exempt from the AIA procedure, and are covered under Article 11 and Annex II of the Protocol. Under Article 11, parties must post decisions to the Biosafety

▼

456 IPPC. 1997.
457 IPPC. 1997. Pg.1.
458 IPPC. 2011.
459 IPPC. 2004.
460 CBD. 2011.

Clearinghouse database on domestic use of LMOs for FFP that may be subject to transboundary movement. To facilitate compliance with obligations to this protocol, the US government has developed a website that provides the status of all regulatory reviews completed for different uses of bioengineered products.[461] These data will be available to the Biosafety Clearinghouse. International trade of 5307 corn would be conducted in compliance with the Protocol.

Biosafety and biotechnology consensus documents, guidelines, and regulations, are managed by APHIS in accordance with the requirements of the North American Plant Protection Organization (NAPPO) and the Organization for Economic Cooperation and Development (OECD). NAPPO has completed three modules of the Regional Standards for Phytosanitary Measures (RSPM) No. 14, *Importation and Release (into the environment) of Transgenic Plants in NAPPO Member Countries*.[462] The Proposed Action is not expected to affect APHIS's participation in NAPPO or the OECD.

North American Biotechnology Initiative is a forum for information exchange and cooperation on agricultural biotechnology issues for the US, Mexico, and Canada. Bilateral discussions on biotechnology regulatory issues are also held regularly with other countries including Argentina, Brazil, Japan, China, and South Korea. Several countries including Argentina, Brazil, Australia, Canada, China, Colombia, Japan, Mexico, New Zealand, South Korea, the Philippines, South Africa, and the European Union have already approved *Bt* corn varieties to be grown or imported for food or feed.

As described in Section 4.7.2, *Trade Economic Environment*, the Proposed Action is not expected to affect the US corn export market since Syngenta is actively pursuing regulatory approvals for 5307 corn in countries with functioning regulatory systems for genetically modified organisms and that import corn from the US or Canada. Regulatory filings for 5307 corn import approvals have been made in Australia, Japan, New Zealand, South Africa, South Korea, Taiwan, and the European Union. Applications are planned for additional countries including Mexico, China, the Philippines, Indonesia, and Russia.

5.3 Other Federal Laws

In addition to the *National Environmental Policy Act* and the *Plant Protection Act*, three other federal environmental laws are potentially relevant to the environmental assessment of the petition to deregulate 5307 corn. Other federal land management laws and regulations address the unique characteristics of certain geographic areas, and are also potentially relevant to deregulation of 5307 corn.

▼

461 See http://usbiotechreg.nbii.gov
462 NAPPO. 2003.

5.3.1 Clean Water Act

The *Federal Water Pollution Control Act*[463] (commonly referred to as the Clean Water Act) and implementing regulations[464, 465] require entities that discharge regulated materials to certain surface water bodies (including wetlands) obtain authorization to do so from federal or state agencies under various permit programs.

As described in Section 4.3.1, *Water Quality and Use*, water quality is not likely to change as a direct or indirect result of the Proposed Action. Deregulating 5307 corn would not result in a change of agricultural practices or any new discharge of pollutants to surface water bodies. Water quality would continue to be regulated and agronomic practices to protect water quality would continue to be implemented if 5307 corn is deregulated. Chemical insecticide use would continue to be reduced as transgenic corn products with insect-resistant traits are developed, marketed, and adopted. Cultivating 5307 corn would not require Clean Water Act permits different than those already required for agricultural activities.

5.3.2 Clean Air Act

The *Clean Air Act Amendments of 1990*[466] (commonly abbreviated as the Clean Air Act) and implementing regulations[467] require entities that discharge regulated materials into the atmosphere obtain authorization to do so from federal or state agencies under various permit programs.

As described in Section 4.3.3, *Air Quality*, the Proposed Action is not likely to directly change air quality. Event 5307 corn production would not change land acreage or cultivation practices for transgenic or non-transgenic corn. Air quality would be indirectly improved if aerial application of corn rootworm pesticides is reduced. Corn growers would continue current trends in agricultural activities if 5307 corn is deregulated. Spray application of insecticides could continue to be reduced as additional insect-resistant products are adopted by growers because 5307 corn would give growers another option to combat corn rootworm. Cultivating 5307 corn would not require Clean Air Act permits different than those already required for agricultural activities.

5.3.3 National Historic Preservation Act

The *National Historic Preservation Act*[468](NHPA) and its implementing regulations[469] require federal agencies to:

▼

463 *Federal Water Pollution Control Act,* as amended. 33 USC 1251-1376.
464 USACOE. Various dates. 33 CFR Parts 320 through 332.
465 USEPA. Various dates. 40 CFR Parts 230 through 233.
466 *Clean Air Act Amendments of 1990*, as amended. 40 USC 7401-7671.
467 USEPA. Various dates. 40 CFR Parts 50 through 99.
468 *National Historic Preservation Act of 1966*, as amended. 16 USC 470 *et seq.*
469 ACHP. Various dates. 36 CFR Parts 800 through 801.

1) Determine whether activities they propose constitute "undertakings" that have the potential to cause effects on historic properties, and

2) If so, to evaluate the effects of such undertakings on such historic resources and consult with the Advisory Council on Historic Preservation (i.e., State Historic Preservation Offices, Tribal Historic Preservation Officers), as appropriate.

The Proposed Action is not expected to adversely impact cultural resources on tribal properties. Any farming activity that may be taken by farmers on tribal lands would be undertaken by the tribe or at the tribe's request. The tribes would have control over any potential conflict with cultural resources on tribal properties. The Proposed Action would also have no impact on districts, sites, highways, structures, or objects listed in or eligible for listing in the National Register of Historic Places, nor would it cause any loss or destruction of significant scientific, cultural, or historical resources.

The Proposed Action is not an undertaking that may directly or indirectly cause alteration in the character or use of historic properties protected under the NHPA. In general, common agricultural activities conducted under this action do not have the potential to introduce new visual, atmospheric, or noise elements to areas in which they are used that are different from current agricultural practices and could result in effects on the character or use of historic properties. The cultivation of 5307 corn alone or in approved stacks is not expected to change any of these agronomic practices that would result in an adverse impact under the NHPA.

5.3.4 Federal Laws Regarding Unique Characteristics of Geographic Areas

Other federal land management laws and regulations protect park lands, prime farm lands, wetlands, wild and scenic areas, or ecologically critical areas. A determination of nonregulated status for 5307 corn is not expected to affect unique characteristics of these geographic areas, and the common agricultural practices that would be carried out in the cultivation of 5307 corn are not expected to deviate from current practices. As described in Section 4.2.1, *Agronomic Practices*, 5307 corn, when stacked with other deregulated corn traits, is expected to be deployed on agricultural land currently suitable for corn production and replace existing varieties, and is not expected to increase the acreage of corn production.

The Proposed Action would not include any major new ground disturbances; new physical destruction or damage to property; alterations of property, wildlife habitat, or landscapes; or prescribed sale, lease, or transfer of ownership of any property. The Proposed Action is limited to a determination of nonregulated status of Event 5307 corn. This action would not convert agricultural land use to nonagricultural use and therefore would have no adverse impact on prime farm land. Standard agricultural practices for land preparation, planting, pest control, irrigation, and harvesting of plants would be used on agricultural lands planted to 5307 corn, alone or when stacked with other deregulated corn traits.

This page intentionally left blank.

6

List of Preparers

Vanasse Hangen Brustlin, Inc.	
Jonathan Feinstein Senior Vice President Senior Technical Advisor	MCP Community Planning and Area Development, University of Rhode Island B.S., Natural Resources Development, University of Rhode Island Over 30 years of experience in environmental consulting and preparing NEPA documents
Lisa A. Standley, Ph.D. Chief Environmental Scientist Technical Document Lead	Ph.D., Botany, University of Washington M.S., Botany, Cornell University B.S., Ecology and Systematics, Cornell University 25 years of experience in environmental consulting and 20 years of experience in preparing NEPA documents
Christopher Rife Senior Project Manager Lead Document Author	Masters of Environmental Policy and Management, University of Denver B.A., Humboldt State University 20 years of experience in environmental consulting and 15 years of experience in preparing NEPA documents
Devleena Ghosh Environmental Scientist Researcher	B.S., Biology and Environment, Pine Manor College 5 years of experience in environmental consulting and 2 years of experience in preparing NEPA documents

Rhodes Life Sciences, LLC	
Dr. Richard C. Rhodes III Associate Director, Rhode Island Agricultural Experiment Station and Rhode Island Cooperative Extension Associate Dean, College of the Environment and Live Sciences	Ph.D., Texas A&M University M.S., Texas A&M University B.S., University of Delaware 32 years of research experience in endocrinology, physiology and agriculture

Syngenta Biotechnology	
Demetra Vlachos Global Crop Lead, Seeds Regulatory Affairs	M.S., Genetics, Rutgers University B.A., Biological Sciences, Douglass College 19 years of experience in agricultural biotechnology regulatory affairs and environmental safety assessment of transgenic crops; 30 years experience in mammalian toxicology and risk assessment of pesticides
Dr. Dennis Ward US Crop Lead, Seeds Regulatory Affairs	Ph.D., Pharmacology and Technology, University of California B.S., Biochemistry, University of California 25 years of experience in toxicology, risk assessment and regulatory affairs within the agricultural technology industry
Dr. Lawrence Zeph Senior Regulatory Manager	Ph.D., Molecular and Cell Biology, Pennsylvania State University B.A., Biology, Indiana University 22 years of experience in regulatory policy for transgenic crops

7 References

Advisory Council on Historic Preservation (ACHP). Various dates. 36 CFR Parts 800 through 801.

Ahmad, A., G.E. Wilde, J.R. Whitworth, and G. Zolnerowich. 2006. Effect of corn hybrids expressing the coleopteran-specific Cry3Bb1 protein for corn rootworm control on aboveground insect predators. *Journal of Economic Entomology* 99(4).

Altieri, M.A. 1999. The ecological role of biodiversity in agroecosystems. *Agriculture, Ecosystems and Environment* 74:19-31.

Alvarez-Alfageme, F., N. Ferry, P. Castanera, F. Ortego, and A.M.R. Gatehouse. 2008. Prey mediated effects of Bt maize on fitness and digestive physiology of the red spider mite predator *Stethorus punctillum* Wise (*Coleoptera: Coccinellidae*). *Transgenic Research* 17:943-954.

Aneja, V.P., *et al*. 2009. Effects of agriculture upon the air quality and climate: research, policy, and regulations. *Environmental Science and Technology* 43:4234–4240.

Association of Official Seed Certifying Agencies. (AOSCA). 2011. *Programs and Services*. AOSCA Programs and Services website: http://www.aosca.org/programs%20services.htm. Accessed June 23, 2011.

ATTRA. 2003. *NCAT's Organic Crops Workbook.* National Sustainable Agricultural Information Service website: https://attra.ncat.org/attra%e2%80%90pub/PDF/cropsworkbook.pdf. Accessed June 23, 2011.

Babendreier, D., N. Kalberer, J. Romeis, P. Fluri, and F. Bigler. 2004. Pollen consumption in honey bee larvae: a step forward in the risk assessment of transgenic plants. *Apidologie* 35:293-300.

Baker, J., *et al*. 2005. Agriculture dust production in standard and conservation tillage system in the San Joaquin Valley. *Journal of Environmental Quality* 34:1260-1269.

Baumgarte, S., and C.C. Tebbe. 2005. Field studies on the environmental fate of the Cry1Ab Bt-toxin produced by transgenic maize (MON810) and its effect on bacterial communities in the maize rhizosphere. *Molecular Ecology* 14:2539-2551.

Beck, D. 2004. Hybrid Corn Seed Production. Chapter 3.4 in *Corn: Origin, History, Technology and Production*. C.W. Smith, J. Betran, and E.C.A. Runge (eds.). Hoboken, NJ: John Wiley & Sons, Inc.

Benbrook, C. 2009. *Impacts of Genetically Engineered Crops on Pesticide Use in the United States: The First Thirteen Years*. The Organic Center website: www.organic-center.org.

Bessin, E. 2004. *Insect Management with Continuous Corn*. University of Kentucky, College of Agriculture website: http://www.ca.uky.edu/entomology/entfacts/ef141.asp. Accessed June 22, 2011.

Best, L.B., R.C. Whitmore, and G.M. Booth. 1990. Use of cornfields by birds during the breeding season: the importance of edge habitat. *American Midland Naturalist* 123(1):84-99.

Blackwell, B.F., D.A. Helon, and R.A. Dolbeer. 2001. Repelling sandhill cranes from corn: whole-kernel experiments with captive birds. *Crop Protection* 20:65-68.

Blackwood, C.B., and J.S. Buyer. 2004. Soil microbial communities associated with Bt and non-Bt Corn in three soils. *Journal for Environmental Quality*. 33:832-836.

Bosnic, A.C., and C.J. Swanton. 1997. Influence of barnyardgrass (*Echinochloa crusgalli*) time of emergence and density on corn (*Zea Mays*). *Weed Science*. 45(2):276-282.

Brookes, G., and P. Barfoot. 2005. GM crops: the global economic and environmental impact – the first nine years 1996 - 2004. *AgBioForum* 8(2&3):187-196.

Brookes, G., and P. Barfoot. 2010. *GM Grops: Global Socio-Economic and Environmental Impacts 1996 - 2008*. PG Economics Ltd: Dorchester, UK website: http://www.pgeconomics.co.uk/pdf/2010-global-gm-crop-impact-study-final-April-2010.pdf. Accessed July 29, 2011.

Brower, L.P., O.R. Taylor, E.H. Williams, D.A. Slayback, R.R. Zubieta, and M.I. Ramirez. 2011. Decline of monarch butterflies overwintering in Mexico: is the migratory phenomenon at risk? *The Royal Entomological Society, Insect Conservation and Diversity*: 1-6.

Burns, R.G. 1982. Enzyme activity in soil: location and a possible role in microbial ecology. *Soil Biology and Biochemistry* 14:423-427.

Carpenter, J., A. Felsot, T. Goode, M. Hammig, D. Onstad, and S. Sankula. 2002. *Comparative Environmental Impacts of Biotechnology-derived and Traditional Soybean, Corn, and Cotton Crops.* Ames, Iowa: Council for Agricultural Science and Technology.

Carpenter, J.E. 2011. Impacts of GM crops on biodiversity. *GM Crops* 2(1):1-17.

Chen, M., *et al*. 2008. A critical assessment of the effects of Bt transgenic plants on parasitoids. PLoS ONE 3(5):e2284.

Childs, D. 1996. *Top Ten Weeds.* Plant and Pest Diagnostic Laboratory. Purdue University website: http://www.ppdl.purdue.edu/ppdl/expert/Top_Ten_Weeds.html, Accessed August 31, 2011.

Christensen, L.A. 2002. *Soil, Nutrient, and Water Management Systems Used in U.S. Corn Production.* USDA Agricultural Information Bulletin No. 774. USDA-ERS website: http://www.ers.usda.gov/publications/aib774/aib774.pdf. Accessed June 22, 2011.

Clean Air Act Amendments of 1990, as amended. Codified at 40 USC § 7401-7671.

Convention on Biological Diversity (CBD). 2011. *Parties to the Protocol and Signatories to the Supplementary Protocol*. CBD website: http://bch.cbd.int/protocol/parties/ Accessed August 25, 2011.

Corn Refiners Association. 2006. *Allergens.* Food Safety Information Papers.

Council for Biotechnology Information. 2001. *Bt Corn and Mycotoxins*. Kansas State University Food Safety Network website: http://www.foodsafety.ksu.edu/articles/103/Bt_Corn_and_Mycotoxins.pdf. Accessed June 23, 2011.

Council on Environmental Quality (CEQ). 1978. Code of Federal Regulations (CFR), Title 40: *Protection of the Environment*, Parts 1500-1508 *Regulations for Implementing NEPA*. (40 CFR Part 1500 *et seq*.). Published at 43 FR 55990 on November 28, 1978, as amended.

Davis, A.K. 2011. Are migratory monarchs really declining in eastern North America? Examining evidence from two fall census programs. *The Royal Entomological Society, Insect Conservation and Diversity:* 1-5.

Devare, M.H., C.M. Jones, and J.E. Thies. 2004. Effect of Cry3Bb transgenic corn and tefluthrin on the soil microbial community: biomass, activity and diversity. *Journal for Environmental Quality* 33:837-843.

Dicke, F.F., and W.D. Guthrie. 1988. The Most Important Corn Insects.In *Corn and Corn Improvement*. 3rd Edition. Dudley, J. W and G. F. Sprague (eds.). Madison, WI: American Society of Agronomy, Crop Science Society of America and Soil Science Society of America.

Dively, G.P. 2005. Impact of transgenic VIP3A X Cry1Ab lepidopteran-resistant field corn on the nontarget arthropod community. *Environmental Entomology* 35(5):1267-1291.

Dolbeer, R.A. 1990. Ornithology and integrated pest management: red winged blackbirds *Agleaius phoeniceus* and corn. *The International Journal of Avian Science* 132:309-322.

Duan, J.J., M. Marvier, J. Huesing, G. Dively, and Z.Y. Huang. 2008. A meta-analysis of effects of Bt crops on honey bees (*Hymenoptera: Apidae*). PLoS ONE 3(1):e1415.

Duan, J.J., J.G. Lundgren, S. Naranjo, and M. Marvier. 2010. Extrapolating non-target risk of Bt crops from laboratory to field. *Biology Letters* 6:74-77.

Dubelman, S., B.R. Ayden, B.M. Bader, C.R. Brown, C. Jiang, and D. Vlachos. 2005. Cry1Ab protein does not persist in soil after 3 years of sustained Bt corn use. *Environmental Entomology* 34:915-921.

Dutton, A., H. Klein, J. Romeis, and F. Bigler. 2002. Uptake of Bt-toxin by herbivores feeding on transgenic maize and consequences for the predator *Chrysoperla carnea*. *Ecological Entomology* 27:441-447.

Eisenthal, J. 2011. *On-farm Demonstrations Show Real World Benefits of Alfalfa-Corn Rotation*. Minnesota Corn Growers Association website: http://www.mncorn.org/index.php?option=com_content&view=article&id=317:on-farm-demonstrations-show-real-world-benefits-of-alfalfa-corn-rotation&catid=1:daily-stories&Itemid=85. Accessed June 22, 2011.

Energy Policy Act of 2005. Public Law 109-58. August 8, 2005. Codified at 42 USC § 15801 *et seq.*

Endangered Species Act of 1973, as amended. Public Law 93-205. Codified at 7 USC § 126 and 16 USC § 1531 *et seq*.

Erickson, B., and J. Lowenberg-DeBoer. 2005. *Weighing the Returns of Rotated vs. Continuous Corn*. Purdue Top Farmer Crop Workshop. Monthly Update, February 2005 website: http://www.agecon.purdue.edu/topfarmer/update.asp. Accessed July 29, 2011.

Executive Office of the President, Office of Science and Technology Policy. 1986. *Coordinated Framework for Regulation of Biotechnology*. Published in the Federal Register (FR) at 51 FR 23302 on June 26, 1986.

Executive Order 12114 of January 4, 1979. *Environmental Effects Abroad of Major Federal Actions*. Published at 44 FR 1957 on January 4, 1979.

Executive Order 12898 of February 11, 1994. *Federal Actions to Address Environmental Justice in Minority Populations and Low-Income Populations*. Published at 59 FR 7629 on February 11, 1994.

Executive Order 13045 of April 21, 1987. *Protection of Children from Environmental Health Risks and Safety Risks*. Published at 62 FR 19883 on April 21, 1987.

Executive Order 13112 of February 3, 1999. *Invasive Species*. Published at 64 FR 6183 on February 3, 1999.

Executive Order 13186 of January 10, 2001. *Responsibilities of Federal Agencies to Protect Migratory Birds*. Published at 66 FR 3853 on January 10, 2001.

Executive Order 13563 of January 18, 2011. *Improving Regulation and Regulatory Review*. Published at 76 FR 14 on January 21, 2011.

Federal Food, Drug, and Cosmetic Act, as amended. 1938. Public Law 75-717. Codified at 21 USC § 301 *et seq*.

Federal Insecticide, Fungicide, and Rodenticide Act, as amended. 1940. Public Law 80-104. Codified at 7 USC § 136 *et seq*.

Federal Water Pollution Control Act (Clean Water Act). 1948. Public Law. 845. Codified at 33 USC § 1251 *et seq*.

Fausey, J.C., J.J. Kells, S.M. Swinton, and K.A. Renner. 1997. Giant foxtail (*Setaria faberi*) interference in nonirrigated corn (*Zea Mays*). *Weed Science* 45(2):256-260.

FIFRA Endangered Species Task Force (FESTF). 2005. *FESTF Visitors Website*. FESTF website: http://www.festf.org/visitors/default.asp. Accessed June 30, 2011.

Food Allergen Labeling and Consumer Protection Act of 2004. Public Law 108-282, Title II. Codified 21 USC § 343 *et seq.*

Food, Conservation, and Energy Act of 2008. Public Law 110-234. May 22, 2008. Codified at 7 USC § 8711 *et seq*., 8751 *et seq*.

Franke, A.C., M.L.H. Breukers, W. Broer, F. Bunte, O. Dolstra, F.M. d'Engelbronner-Kolff, L.A.P. Lotz, J. van Montfort, J. Nikoloyuk, M.M. Rutten, M.J.M. Smulders, C.C.M. van de Wiel, and M. van Zijl. 2011. *Sustainability of Current GM Crop Cultivation*. Wageningen, The Netherlands: Wageningen UR, Plant Research International, Report 386.

Frost, R., and L. Gilman. 2011. *Corn (Maize).* International Maize and Wheat Improvement Center website: http://science.jrank.org/pages/1806/Corn-Maize.html. Accessed August 15, 2011.

Fyksen, J. 2003. *EPA Registers Biotech Corn that Resists Borers and Rootworms.* Minnesota Farm Guide website: http://www.minnesotafarmguide.com/news/regional/epa-registers-biotech-corn-that-resists-borers-and-rootworms/article_a33a6f89-9241-5250-a8c3-cb4f88dcc71a.html. Accessed August 30, 2011.

Gassmann, A.J., J.L. Petzold-Maxwell, R.S. Keweshan, and M.W. Dunbar. 2011. Field-evolved resistance to Bt maize by western corn rootworm. PLoS ONE 6(7):e22629.

Gianessi, L.P, C.S. Silvers, S. Sankula, and J.E. Carpenter. 2002. *Plant Biotechnology: Current and Potential Impact for Improving Pest Management in U.S. Agriculture – An Analysis of 40 Case Studies*. National Center for Food and Agricultural Policy website: www.ncfap.org. Accessed June 22, 2011.

Gianessi, L.P. 2005. Economic and herbicide use impacts of glyphosate-resistant crops. *Pest Management Science* 61:241-245.

Gilliom, R.J., J.E. Barbash, C.G. Crawford, P.A. Hamilton, J.D. Martin, N. Nakagaki, L.H. Nowell, J.C. Scott, P.E. Stackelberg, G.P. Thelin, and D.M. Wolock. 2007. *The Quality of Our Nation's Waters: Pesticides in the Nation's Streams and Ground Water, 1992-2001*. US Department of the Interior, US Geological Survey Circular 1291. Washington DC: USGS.

Gillis, J.. 2011. A warming planet struggles to feed itself. *New York Times.* June 4, 2011.

Gray, M.E., T.W. Sappington, N. Miller, J. Moeser, and M.O. Bohn. 2009. *Adaptation and Invasiveness of Western Corn Rootworm: Intensifying Research on a Worsening Pest.* Department of Entomology Faculty Publications. University of Nebraska-Lincoln, pp 303-321.

Griffiths, B.S., S. Caul, J. Thompson, A.N.E. Birch, J. Cortet, M.N. Andersen, and P.H. Krogh. 2007. Microbial and microfaunal community structure in cropping systems with genetically modified plants. *Pedobiologia* 51(3):195-206.

Hager, A., L. Wax, and M. McGlannery. 1998. *Weed/Crop Competition: Factors to Consider*. Bulletin No. 2, University of Illinois at Urbana-Champaign, Cooperative Extension Service website: http://bulletin.ipm.illinois.edu/pastpest/articles/v982h.html. Accessed June 22, 2011.

Hallauer, A.R. 2004. Specialty Corn. Chapter 4.4 in *Corn: Origin, History, Technology and Production*. C.W. Smith, J. Betran, and E.C.A. Runge (eds.). Hoboken, NJ: John Wiley & Sons, Inc.

Hart, C.E. 2006. Feeding the ethanol boom: where will the corn come from? *Iowa Agriculture Review* 12(4):4-5.

Harwood, J.D., W.G. Wallin, and J.J. Obrycki. 2005. Uptake of Bt endotoxins by nontarget herbivores and higher order arthropod predators: molecular evidence from a transgenic corn agroecosystem. *Molecular Ecology* 14:2815-2823.

Hazzard, R., and P. Westgate. 2004. *Organic Insect Control in Sweet Corn*. University of Massachusetts Extension website: www.umassextension.org/Merchant2/merchant.mv. Accessed June 29, 2011.

Head, G., J.B. Surber, J.A. Watson, J.W. Martin, and J.J. Duan. 2002. No detection of Cry1Ac protein in soil after multiple years of Bt cotton (Bollgard) use. *Environmental Entomology* 31:30-36.

Heap, I.M. 2008. *International Survey of Herbicide Resistant Weeds.* Weed Science website: http://www.weedscience.org/In.asp. Accessed August 31, 2011.

Hicks, D.R., and T.R. Hoverstad. 2007. *The Rotation Effect for Corn Yields*. The University of Minnesota, Department of Agronomy website: http://www.extension.umn.edu/cropenews/2007/07MNCN09.htm. Accessed June 22, 2011.

Hicks, D.R., and P.R. Thomison. 2004. Corn Management. Chapter 3.2 in *Corn: Origin, History, Technology and Production*. C.W. Smith, J. Betran, and E.C.A. Runge (eds.). Hoboken, NJ: John Wiley & Sons, Inc.

Hoffman, L., A. Baker, L. Foreman, and E. Young. 2007. *Feed Grains Backgrounder*. Outlook Report from the USDA-ERS. FDS-07c-01: 1-58.

Homan, H.J., G.M. Linz, and W.J. Bleier. 1994. Effect of crop phenology and habitat on the diet of common grackles *(Quiscalus quiscula)*. *American Midland Naturalist* 131:381-385.

Hutchison, W.D., *et al.* 2011. Areawide suppression of European corn borer with Bt maize reaps savings to non-Bt maize growers. *Science* 330:222-225.

Icoz, I., D. Saxena, D.A. Andow, C. Zwahlen, and G. Stotzky. 2008. Microbial populations and enzyme activities in soil in situ under transgenic corn expressing Cry proteins from *Bacillus thuringiensis*. *Journal of Environmental Quality* 37:647-662.

Intergovernmental Panel on Climate Change (IPCC). 2007. *14.4.4.4. Agriculture, forestry and fisheries IPPC Fourth Assessment Report Climate Change 2007: Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, 2007*. M.L. Parry, O.F. Canziani, J.P. Palutikof, P.J. van der Linden, and C.E. Hanson. Cambridge, United Kingdom and New York, USA: IPCC.

International Plant Protection Convention (IPPC). 1997. *International Plant Protection Convention*. (New Revised Text approved by the FAO Conference at its 29th Session – November 1997).

_____. 2004. *International Standards for Phytosanitary Measures, ISPM No. 11, Pest Risk Analysis for Quarantive Pests Including Analysis of Environmental Risks and Living Modified Organisms*. Produced by the Secretariat of the IPPC. IPPC website: https://www.ippc.int/file_uploaded/1146658377367_ISPM11.pdf. Accessed August 25, 2011.

_____. 2011. *List of Countries*. IPPC website: https://www.ippc.int/index.php?id=1110618&L=0. Accessed August 25, 2011.

Iserman, K. 1993. Agriculture's share in the emission of trace gases affecting the climate and some cause-oriented proposals for sufficiently reducing this share. *Environmental Pollution* 83:95-111.

Jeffers, D.P. 2004. Disease Control. Chapter 3.6 in *Corn: Origin, History, Technology and Production*. C.W. Smith, J. Betran, and E.C.A. Runge (eds.). Hoboken, NJ: John Wiley & Sons, Inc.

Kermicle, J.L., and M.M.S. Evans. 2010. The *Zea mays* sexual compatibility gene ga2: naturally occurring alleles, their distribution, and role in reproductive isolation. *Journal of Heredity* 101(6):737-749.

Kleter, G.A., R. Bhula, K. Bodnaruk, E. Carazo, A.S. Felsot, C.A. Harris, A. Katayama, H.A. Kuiper, K.D. Racke, B. Rubin, Y. Shevah, G.R. Stephenson, K. Tanaka, J. Unsworth, R.D. Wauchope, and S. Wong. 2007. Altered pesticide use on transgenic crops and the associated general impact from an environmental perspective. *Pest Management Science* 63:1107–1115.

Knezevic, S.Z. 2010. *Use of Herbicide-Tolerant Crops as Part of an Integrated Weed Management Program*. The Board of Regents of the University of Nebraska, on behalf of the University of Nebraska-Lincoln Extension, Institute of Agriculture and Natural Resources website:

http://www.ianrpubs.unl.edu/epublic/pages/publicationD.jsp?publicationId=108. Accessed June 22, 2011.

Koul, O., G.S. Shaliwal, and G.W. Cuperus. 2004. *Integrated Pest Management-Potential, Constraints and Challenges*. CAB International Publishing ISBN 0-85199-686-8. Available at Google Books: http://books.google.com/books?id=LSJfge1E1xkC&printsec=frontcover&dq=Koul+integrated+pest+management&hl=en&ei=UI1fTunwHOLD0AGI6vTqAg&sa=X&oi=book_result&ct=result&resnum=1&ved=0CDsQ6AEwAA#v=onepage&q&f=false, pg 29.

Krantz, W.L., *et al*. 2008. *Irrigation Management System for Corn*. University of Nebraska, Lincoln Institute of Agriculture and Natural Resources website: http://elkhorn.unl.edu/epublic/live/g1850/build/#target2. Accessed June 23, 2011.

Kuepper, G. 2002. *Organic Field Corn Production*. Appropriate Technology Transfer for Rural Areas (ATTRA) website: http://www.attra.nact.org.

Lal, R., and J. Bruce. 1999. The potential of world cropland soils to sequester C and mitigate the greenhouse effect. *Environmental Science & Policy* 2(2):177-185.

Lawhorn, C.N., D.A. Neher, and G.P. Dively. 2009. Impact of coleopteran targeting toxin (Cry3Bb1) of Bt corn on microbially mediated decomposition. *Applied Soil Ecology* 41:364-368.

Liebman, M., and E. Dyck. 1993. Crop rotation and intercropping strategies for weed management. *Ecological Applications* 3(1).

Linz, G.M., G.A. Knutsen, H.J. Homan, and W.J. Bleier. 2003. Baiting blackbirds (*Icteridae*) in stubble grain fields during spring migration in South Dakota. *Crop Protection* 22:261-264.

Luna, S., J. Figuerroa, B. Balthazar, R. Gomez, R. Townsend, and J.B. Schoper. 2001. Maize pollen longevity and distance isolation requirements for effective pollen control. *Crop Science* 41:1551-1557.

Malcolm, S.A., M. Aillery and M. Weinberg. 2009. *Ethanol and a changing agricultural landscape*. USDA-ERS Economic Research Report Number 86.

Marshall, E.J.P., *et al*. 2003. The role of weeds in supporting biological diversity within crop fields. *European Weed Research Society* 43:77-89.

Marvier, M., *et al*. 2007. A meta-analysis of effects of Bt cotton and maize on nontarget invertebrates. *Science* 316:1475-1477.

Marx, M-C., E. Kandeler, M. Wood, N. Wermbter, and S.C. Jarvis. 2005. Exploring the enzymatic landscape: distribution and kinetics of hydrolytic enzymes in soil particle-size fractions. *Soil Biology and Biochemistry* 37:35-48.

McNichol, D.K., R.J. Robertson, and P.J. Weatherhead. 1979. Seasonal, habitat and sex-specific patterns of food utilization by red-winged blackbirds (*Agelaius phoeniceous*) in eastern Ontario and their economic importance. *Wildlife Damage Management, Internet Center for Bird Control Seminars Proceedings:* 273-290.

Meihls, L.N., M.L. Higdon, B.D. Siegfried, N.J. Miller, T.W. Sappington, M.R. Ellersieck, T.A. Spencer, and B.E. Hibbard. 2008. Increased survival of western corn rootworm on transgenic corn within three generations of on-plant greenhouse selection. *Proceedings of the National Academy of Sciences* 105(49):19177-19782.

Meinke, L.J., B.D. Siegfried, R.J. Wright, and L.D. Chandler. 1998. Adult susceptibility of Nebraska western corn rootworm (*Coleoptera: Chrysomelidae*) populations to selected insecticides. *Journal of Economic Entomology* 91:594-600.

Mendelsohn, M., J. Kough, Z. Vaituzis, and K. Matthews. 2003. Are Bt crops safe? *Nature Biotechnology* 21:1003-1009.

Metcalf, R.L. 1986. The Ecology of Insecticides and the Chemical Control of Insects. Foreword in *Ecological Theory and Integrated Pest Management Practice*. M. Kogan (ed.). New York: John Wiley & Sons.

Munkvold, G.P., R.L. Hellmich, and L.G. Rice. 1999. Comparison of fumonisin concentrations in kernels of transgenic maize hybrids and nontransgenic hybrids. *Plant Disease* 83(2):130-138.

National Corn Growers Association (NCGA). nd. *Insect Resistance Management Fact Sheet for Bt Corn*. NCGA website: http://www.ncga.com/managing-bt-technology/. Accessed July 15, 2011.

_____. 2007. *Sustainability. Conserving and Preserving: Soil Management and Tillage.* NCGA website: http://ncga.com/files/pdf/ConservingPreservingSoilManagement.pdf. Accessed June 23, 2011.

_____. 2011. *Weed Resistance Management*. National Corn Growers Association website: http://ncga.adayana.com/. Accessed June 22, 2011.

National Environmental Policy Act, as amended. 1970. Public Law 91-190. Codified at 42 USC § 4321 *et seq*.

National Historic Preservation Act of 1966, as amended. Public Law 89-665. October 15, 1966. Codified at 16 USC § 470 *et seq.*

National Pesticide Telecommunications Network (NPTN). 2000. *Bacillus thuringiensis Technical Fact Sheet*. Oregon State University and US EPA NPTN website: http://npic.orst.edu/factsheets/BTtech.pdf. Accessed July 25, 2011.

National Research Council (NRC). 1983. *Diet formulation and processing.* In *Nutrient Requirements of Warmwater Fishes and Shellfishes*. Washington, DC: National Academies Press.

_____. 2010. *Impact of Genetically Engineered Crops on Farm Sustainability in the United States.* Committee on the Impact of Biotechnology on Farm-Level Economics and Sustainability. Washington DC: National Academies Press. NAP website http://www.nap.edu/catalog.php?record_id=12804 Accessed July 20, 2011.

Non-GMO Project. 2011. *Welcome to the Non-GMO Project*. Website: http://www.nongmoproject.org/. Accessed June 30, 2011.

North American Plant Protection Organization (NAPPO). 2003. *NAPPO Regional Standards for Phytosanitary Measures (RSPM), RSPM No. 14, Importation and Release (into the environment) of Transgenic Plants, in NAPPO Member Countries.* Ottawa, Ontario: The Secretariat of the NAPPO.

Obrist, L.B., H. Klein, A. Dutton, and F. Bigler. 2005. Effects of Bt maize on *Franliniella tenuicornis* and exposure of thrips predators to prey-mediated Bt toxin. *Entolomologia Experimentalis et Applicata* 115:409-416.

Obrist, L.B., A. Dutton, J. Romeis, and F. Bigler. 2006a. Biological activity of Cry1Ab toxin expressed by Bt maize following ingestion by herbivorous arthropods and exposure of the predator *Chrysoperla carnea*. *BioControl* 51:31-48.

Obrist, L.B., A. Dutton, R. Albajes, and F. Bigler. 2006b. Exposure of arthropod predators to Cry1Ab toxin in Bt maize fields. *Ecological Entomology* 31:143-154.

O'Day, M., A. Becker, A. Keaster, L. Kabrick, and K. Steffy. 1998. *Corn Insect Pests: A Diagnostic Guide*. Columbia, MO: University of Missouri—Columbia.

Oehme, F.W., and J.A. Pickrell. 2003. Genetically engineered corn rootworm resistance: Potential for reduction of human health effects from pesticides. *Biomedical Environmental Science* 16:17–28.

Oleson, J.D., Y. Park, T.M. Nowatzki and J.J. Tollefson. 2005. Node-injury scale to evaluate root injury by corn rootworms. *Journal of Economic Entomology* 98:1-8.

Olson, R.A., and D.H. Sander. 1988. Corn Production. Chapter 11 in *Corn and Corn Improvement (3rd Edition).* G.F. Sprague and J.W. Dudley (eds). Madison, WI: American Society of Agronomy, Crop Science Society of America and Soil Science Society of America.

Organic Foods Production Act of 1990. Title XXI of the *Food, Agriculture, Conservation, and Trade Act of 1990.* Public Law 101-624, as amended through Public Law 109-97. November 10, 2005.

Organic Trade Association (OTA). 2011. *Industry Statistics and Projected Growth*. OTA website: http://www.ota.com/index.html. Accessed June 30, 2011.

Organization for Economic Co-operation and Development (OECD). 2003. *Consensus Document on the Biology of Zea mays subsp. mays (Maize).* Environmental Directorate No. 27, July 23, 2003.

Orthoefer, F.T., and J. Eastman. 2004. *Corn Processing and Products*. Chapter 4.3 in *Corn: Origin, History, Technology and Production*. C.W. Smith, J. Betran, and E.C.A. Runge (eds.). Hoboken, NJ: John Wiley & Sons, Inc.

Patterson, M.P., and L.B. Best. 1996. Abundance and besting success in Iowa CRP fields: the importance of vegetation, structure and composition. *American Midland Naturalist* 135(1):153-167.

Pilcher, C.D., M.E. Rice, and J.J. Obrycki. 2005. Impact of transgenic *Bacillus thuringiensis* corn and crop phenology on five nontarget arthropods. *Environmental Entomology* 34(55):13.2-1315.

Pilson, D., and H.R. Prendeville. 2004. Ecological effects of transgenic crops and the escape of transgenes into wild populations. *Annual Review of Ecology, Evolution and Systematics* 35:149-174.

Plant Protection Act. 2000. Title IV of the *Agriculture Risk Protection Act of 2000.* Public Law 106-224. Codified at 7 USC § 7701 *et seq*.

Ponsard, S., A.P. Gutierrez, and N.J. Mills. 2002. Effect of Bt-toxin (Cry1Ac) in transgenic cotton on adult longevity of four heteropteran predators. *Environmental Entomology* 31(6):1197-1205.

Raps, A., J. Kehr, P. Gugerli, W.J. Moar, F. Bigler, and A. Hilbeck. 2001. Immunological analysis of phloem sap of *Bacillus thuringiensis* corn and of the nontarget herbivore *Rhopalosiphum padi* (*Homoptera: Aphididae*) for the presence of Cry1Ab. *Molecular Ecology* 10:525-533.

Raven, P.H. 2010. Does the use of transgenic plants diminish or promote biodiversity? *New Biotechnology* 27(5):528-533.

Rhoades, F.M., and C.D. Yonts. 2011. Irrigation Schedule for Corn - Why and How. Chapter 20 in *National Corn Handbook*. Wisconsin Corn Agronomy website: http://corn.agronomy.wisc.edu/Management/NCH.aspx. Accessed June 23, 2011.

Rice, M.E. 2004. Transgenic rootworm corn: Assessing potential agronomic, economic, and environmental benefits. Online. *Plant Health Progress.* doi:10.1094/PHP-2004-0301-01-RV.

The Rodale Institute. 2011. *Organic Price Report.* Website: http://newfarm.rodaleinstitute.org/embedopx/product.php?prid=42. Accessed September 2, 2011.

Roehrdanz, R.L., A.L. Szalanski, and E. Levine. 2003. Mitochondrial DNA and IRS1 differentiation in geographical populations of northern corn rootworm, *Diabrotica barberi* (*Coleoptera: Chrysomelidae*): identification of distinct genetic populations. *Annals of Entomological Society of America* 96(6):901-913.

Romeis, J., M. Meissle, and F. Bigler. 2006. Transgenic crops expressing *Bacillus thuringiensis* toxins and biological control. *Nature Biotechnology* 24(1):63-71.

Rooney, L.W., C.M. McDonough, and R.D. Waniska. 2004. The Corn Kernel. Chapter 2.2 in *Corn: Origin, History, Technology and Production*. C.W. Smith, J. Betran, and E.C.A. Runge (eds.). Hoboken, NJ: John Wiley & Sons, Inc.

Rosi-Marshall, E.J., J.L. Tank, T.V. Royer, M.R. Whiles, M. Evans-White, C. Chambers, N.A. Griffiths, J. Pokelsek, and M.L. Stephen. 2007. Toxins in transgenic crop by-products may affect headwater stream ecosystems. *Proceedings of the National Academy of Science USA* 104:16204-16208.

Roush, R.T. 1994. Managing pests and their resistance to *Bacillus thuringiensis:* can transgenic crops be better than sprays? *Biocontrol Science and Technology* 4:501-516

Savage, S. 2011. *Today's Organic, Yesterday's Yields*. Biofortified website: http://www.biofortified.org/2011/02/todays-organic-yesterdays-yields/. Accessed June 22, 2011.

Sawyer, J. 2007. *Nitrogen Fertilization for Corn Following Corn*. Iowa State University. IC-498 (1): 27-28.

Saxena, D., and G. Stotsky. *Bacillus thuringiensis* (Bt) toxin released from root exudates and biomass of Bt Corn has no apparent effect on earthworms, nematodes, protozoa, bacteria and fungi in soil. *Soil Biology & Biochemistry* 33:1225-1230.

Seed Corn Growers of Ontario (SCGO). 2007. *Seed Corn Insect Images 3 – Beneficial Insects.* SCGO Seed Corn Best Management Practices for Ontario website:

http://www.seedcorngrowers.on.ca/SCManual/Seed%20Corn%20Insect%20Images%203.htm. Accessed June 27, 2011.

Severson, D.W., and J.E. Parry. 1981. A chronology of pollen collection by honeybees. *Journal of Apicultural Research* 20:97-103.

Shelton, A.M., J.Z. Zhao, and R.T. Roush. 2002. Economic, ecological, food safety, and social consequences of the development of Bt transgenic plants. *Annual Review of Entomology* 47:845-881.

Shimada, N., H. Murata, O. Mikami, M. Yoshioka, K.S. Guruge, N. Yamanaka, Y. Nakajima, and S. Miyazaki. 2006. Effects of feeding calves genetically modified corn Bt11: a clinico-biochemical study. *Journal of Veterinary Medical Science* 68(10):1113-1115.

Sivasupramaniam, S., W.J. Moar, L.G. Ruschke, J.A. Osborn, C. Jiang, J.L. Sebaugh, G.R. Brown, Z.W. Shappley, M.E. Oppenhuizen, J.W. Mullins, and J.T. Greenplate. 2008. Toxicity and characterization of cotton expressing *Bacillus thuringiensis* Cry1Ac and Cry2Ab2 proteins for contol of lepidopteran pests. *Journal of Economic Entomology* 101(2):546-554.

Smith, D.R., and D.G. White. 1988. Diseases of Corn. Chapter 12 in *Corn and Corn Improvement*. Dudley, J.W. and G.F. Sprague (eds). 3rd Edition. Madison, WI: American Society of Agronomy, Inc., Crop Science Society of America, Inc., Soil Science Society of America, Inc.

South Dakota Corn Growers Association. 2010. *SDCGA: GAO Report doesn't Tread Water.* SDCGA website: http://www.sdcorn.org/page/News/sub/News_Releases/shownews/story/id/261. Accessed August 17, 2011.

Steffey, K.L., M.E. Rice, J. All, D.A. Andow, M.E. Gray, and J.W. van Duyn. 1999. Corn Pests. In *Handbook of Corn Insects*. Lanham, MD: Entomological Society of America.

Sterner, R.T., B.E. Petersen, S.E. Gaddis, K.L. Tope, and D.J. Poss. 2003. *Impacts of Small Mammals and Birds on Low-Tillage, Dryland Crops*. Lincoln, NE: Internet Center for Wildlife Damage Management, USDA National Wildlife Research Center- Staff Publications; University of Nebraska- Lincoln.

Sundstrom, F., J. Williams, A. Van Deynze, and K. Bradford. 2002. *Identity Preservation of Agricultural Commodities.* Davis, CA: University of California Division of Agriculture and Natural Resources Publications and University of California—Davis Seed Biotechnology Center.

Syngenta. 2010. *Corn Rootworm Management Guide*. Bulletin by Syngenta Agronomy Research. C804-10, pp 1-2.

Tabashnik, B.E., A.J. Gassmann, D.W. Crowder, and Y. Carrière. 2008. Insect resistance to Bt crops: evidence versus theory. *Nature Biotechnology* 26:199-202.

Torres, J.B., and J.R. Ruberson. 2005. Canopy-and ground dwelling predatory arthropods in commercial Bt and non-Bt cotton fields: patterns and mechanisms. *Environmental Entomology* 34(5):1242-1256.

Toxic Substances Control Act, as amended. 1976. Public Law 94-469. Codified at 15 USC § 2601 *et seq*.

Troyer, A.F. 2004. Persistent and Popular Germplasm in Seventy Centuries of Corn Evolution. Chapter 1.4 in *Corn: Origin, History, Technology and Production*. C.W. Smith, J. Betran, and E.C.A. Runge (eds.). Hoboken, NJ: John Wiley & Sons, Inc.

United States Army Corps of Engineers. Various dates. 33 CFR Parts 320 through 332.

United States Department of Agriculture (USDA). 1990. *Policy of Noxious Weed Management*. Departmental Regulation Number 9500-10. Washington, DC: USDA.

_____. 2011a. *National Organic Program*. 7 CFR Part 205.

_____. 2011b. *USDA Agricultural Projections to 2020*. In *Long-term Projections Report* OCE-2011-1. Washington DC: USDA Office of the Chief Economist, World Agricultural Outlook Board; prepared by the Interagency Agricultural Projections Committee.

USDA-Agricultural Research Services (USDA-ARS). 2006. *Biorational Technologies for Management of Chrysomelid Beetle Pests of Agricultural Crops.* Invasive Insect and Biocontrol Laboratory, 2006 Annual Report. http://www.ars.usda.gov/research/projects/projects.htm?ACCN_NO=409669&fy=2006. Accessed August 30, 2011.

USDA-Agricultural Marketing Service (USDA-AMS). 2007. *The National List of Allowed and Prohibited Substances*. 7 CFR Part 205.600 *et seq*.

_____ National Organic Program. 2011. *Policy Memorandum: Genetically Modified Organisms*. Washington DC: USDA Agricultural Marketing Service, National Organic Program.

USDA-Animal and Plant Health Inspection Service (USDA-APHIS). nd. *Threatened and Endangered Species Decision Tree for FWS Consultations*.

_____. 1987. *Introduction of Organisms and Products Altered or Produced Through Genetic Engineering Which are Plant Pests or Which There is Reason to Believe are Plant Pests*. 7 CFR Part 340.

_____. 1995. *National Environmental Policy Act Implementing Procedures*. 7 CFR Part 372.

_____. 2007. *Finding of No Significant Impact, Animal and Plant Health Inspection Service, Petition for Non-regulated Status for Corn Line MIR604 (APHIS 04-362-01p)*. Riverdale, MD: USDA-APHIS Biotechnology Regulatory Services.

_____. 2009. *Finding of No Significant Impact, Animal and Plant Health Inspection Service, Petition for Nonregulated Status for Pioneer Corn DP-098140-6 (APHIS 07-152-01p)*. Riverdale, MD: USDA-APHIS Biotechnology Regulatory Services.

_____. 2010a. *Federal Noxious Weed List (as of Dec. 10, 2010)*. APHIS Plant Health website: http://www.aphis.usda.gov/plant_health/plant_pest_info/weeds/downloads/weedlist.pdf Accessed June 23, 2011.

_____. 2010b. *National Environmental Policy Act Decision and Finding of No Significant Impact, Syngenta Biotechnology Incorporated MIR162 Maize SYN-IR162-4*. Riverdale, MD: APHIS Biotechnology Regulatory Services.

_____. 2011a. *Monsanto Company Petition (070CR-191U) for Determination of Non-regulated Status of Event 87460, Draft Environmental Assessment*. Riverdale, MD: APHIS Biotechnology Regulatory Services.

_____. 2011b. *Petitions for Nonregulated Status Granted or Pending by APHIS as of September 29, 2011.* USDA-APHIS website: http://www.aphis.usda.gov/biotechnology/not_reg.html. Accessed October 6, 2011.

USDA-Economic Research Services (USDA-ERS). nd. *Crop Rotations.* USDA-ERS Publication website: http://www.ers.usda.gov/publications/arei/ah705/AREI4-2.PDF. Accessed August 31, 2011.

_____. 2009. *Corn: Trade*. USDA-ERS Briefing Room website: http://www.ers.usda.gov/Briefing/corn/trade.htm. Accessed June 30, 2011.

_____. 2010. *Organic Production; Table 3. Certified organic and total U.S. acreage, selected crops and livestock 1995-2008*. USDA-ERS website: http://www.ers.usda.gov/Data/Organic/. Accessed June 22, 2011.

_____. 2011a. *Adoption of Genetically Engineered Crops in the U.S*. USDA-ERS Data Sets website: http://www.ers.usda.gov/Data/BiotechCrops/. Accessed June 29, 2011.

_____. 2011b. *Corn: Market Outlook*. USDA-ERS Briefing Room website: http://www.ers.usda.gov/Briefing/Corn/2010baseline.htm. Accessed June 16, 2011.

_____. 2011c. *Farm Income and Costs: Farm Sector Income Forecast*. USDA-ERS Briefing Room website: http://www.ers.usda.gov/Briefing/FarmIncome/nationalestimates.htm. Accessed October 6, 2011.

_____. 2011d. *US Agricultural Exports, year-to-date and current months*. USDA-ERS Data Sets, Foreign Agricultural Trade of the United States (FATUS) website: http://www.ers.usda.gov/Data/FATUS/. Accessed June 30, 2011.

USDA-Foreign Agricultural Service (USDA-FAS). 2011. *World Corn Trade, October/September Year, Thousand Metric Tons*. USDA-FAS Grain: World Markets and Trade Monthly Circular website: http://www.fas.usda.gov/psdonline/psdreport.aspx?hidReportRetrievalName=BVS&hidReportRetrievalID=455&hidReportRetrievalTemplateID=7. Accessed June 30, 2011.

USDA-National Agricultural Statistics Service (USDA-NASS). 2005. *Acreage.* USDA-NASS Agricultural Statistics Board website: http://usda.mannlib.cornell.edu/usda/nass/Acre//2000s/2005/Acre-06-30-2005.pdf. Accessed July 26, 2011.

_____. 2006. *Acreage*. USDA-NASS website: http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1000. Accessed July 29, 2011.

_____. 2008a. *2008 Organic Survey*. USDA-NASS Census of Agriculture website: http://www.agcensus.usda.gov/Publications/2007/Online_Highlights/Organics/index.asp. Accessed July 26, 2011.

_____. 2008b. *Acreage.* USDA-NASS Agricultural Statistics Board website: http://usda.mannlib.cornell.edu/usda/nass/Acre//2000s/2008/Acre-06-30-2008.pdf. Accessed July 26, 2011.

_____. 2009a. *Acreage.* USDA-NASS website: http://usda.mannlib.cornell.edu/usda/current/Acre/Acre-06-30-2009.pdf. Accessed July 27, 2010.

_____. 2009b. *Crop Values 2009 Summary*. USDA-NASS Economics, Statistics, and Market Information Systems website: http://usda.mannlib.cornell.edu/usda/current/CropValuSu/CropValuSu-02-16-2009.pdf. Accessed June 30, 2011.

_____. 2011a. *Acreage.* USDA-NASS website: http://usda.mannlib.cornell.edu/usda/current/Acre/Acre-06-30-2011.pdf. Accessed July 27, 2010.

_____. 2011b. *Agricultural Chemical Use: Corn, Upland Cotton and Fall Potatoes 2010*. USDA-NASS website: http://www.nass.usda.gov/Surveys/Guide_to_NASS_Surveys/Chemical_Use/FieldCropChemicalUseFactSheet06.09.11.pdf. Accessed June 22, 2011.

_____. 2011c. *Corn for All Purposes 2010 Planted Acres by County for Selected States*. USDA-NASS website: http://www.nass.usda.gov/Charts_and_Maps/Crops_County/pdf/CR-PL10-RGBChor.pdf. Accessed June 16, 2011.

_____. 2011d. *Crop Values 2010 Summary*. USDA-NASS Economics, Statistics, and Market Information Systems website: http://usda.mannlib.cornell.edu/usda/current/CropValuSu/CropValuSu-02-16-2011.pdf. Accessed June 30, 2011.

USDA-Natural Resources Conservation Service (USDA-NRCS). 2011a. *Zea mexicana*. USDA-NRCS PLANTS Profile website: http://plants.usda.gov/java/nameSearch?keywordquery=Zea+mexicana&mode=sciname. Accessed June 30, 2011.

_____. 2011b. *Zea perennis*. USDA-NRCS PLANTS Profile website: http://plants.usda.gov/java/nameSearch?keywordquery=Zea+perennis&mode=sciname&submit.x=13&submit.y=9. Accessed June 30, 2011.

United States Department of Health & Human Services- U.S. Food and Drug Administration (USDHHS-USFDA). 1992. *Statement of Policy: Foods Derived From New Plant Varieties*. 57 FR 22984, May 29, 1992.

United States Environmental Protection Agency (USEPA). Various dates. 40 CFR Parts 50 through 59 and 40 CFR Parts 230 through 233.

_____. 1992. *Worker Protection Standard*. 40 CFR Part 170. USEPA website: http://www.epa.gov/pesticides/safety/workers/PART170.htm. Accessed August 31, 2011.

_____. 2001. *Biopesticides Registration Action Document - Bacillus thuringiensis Plant-Incorporated Protectants*. Washington DC: USEPA Office of Pesticide Programs, Biopesticides and Pollution Prevention Division.

_____. 2005. *Protecting Water Quality from Agricultural Runoff*. Fact Sheet 841-F-05-001. Washington, DC: USEPA.

_____. 2007a. *Biopesticides Registration Action Document: Modified Cry3A Protein and the Genetic Material Necessary for its Production (Via Elements of pZM26) in Event MIR604 Corn SYN-IR604-8*. Washington, DC: USEPA Office of Pesticide Programs, Biopesticides and Pollution Prevention Division.

_____. 2007b. *Phosphomannose isomerase in all plants; exemption from the requirement of a tolerance*. 40 CFR § 174.527.

_____. 2008. *Agriculture Management Practices for Water Quality Protection.* USEPA website: http://www.epa.gov/owow/watershed/wacademy/acad2000/agmodule/. Accessed June 23, 2011.

_____. 2009. *Soil Preparation*. USEPA Ag 101 website: http://www.epa.gov/agriculture/ag101/cropsoil.html. Accessed June 22, 2011.

_____. 2010a. *Bacillus thuringiensis eCry3.1Ab protein in corn; temporary exemption from the requirement of a tolerance*. 40 CFR § 174.532.

_____. 2010b. *Biopesticides Registration Action Document: Cry1Ab and Cry1F Bt Plant-Incorporated Protectants.* Washington, DC: EPA Office of Pesticide Programs, Biopesticides and Pollution Prevention Division.

_____. 2010c. *Issuance of an Experimental Use Permit*. Published in 75 FR 57778 on September 22, 2010.

_____. 2011a. *Bacillus thuringiensis eCry3.1Ab protein in corn; temporary exemption from the requirement of a tolerance*. 40 CFR § 174.532.

_____. 2011b. *Inventory of U.S. Greenhouse Gas Emissions and Sinks: 1990-2009*. USEPA Report 430-R-11-05. Washington, DC: USEPA.

_____. 2011c. *Pesticide Experimental Use Permit, receipt of extension application; comment request*. Published in 76 FR 3135 on January 19, 2011.

_____. 2011d. *Pesticides Industry Sales and Usage: 2006 and 2007 Market Estimates*. Washington, DC: USEPA Office of Chemical Safety and Pollution Prevention, Office of Pesticide Programs, Biological and Economic Analysis Division.

United States Fish and Wildlife Service (USFWS). 1988. *Endangered and Threatened Wildlife Plants; Final Rule to Determine Five Texas Cave Invertebrates to be Endangered Species.* Department of the Interior, 50 CFR Part 17, Vol. 53, No. 180. Published at 53 FR 36029 on September 16. 1988.

_____. 2005. *Service Lists Salt Creek Tiger Beetle as Endangered.* Department of the Interior News Release published on October 6, 2005.

_____. 2011a. *American Burying Beetle (Nicrophorous americanus).* Species summary.

_____. 2011b. *Endangered and Threatened Wildlife and Plants; Determination of Endangered Status for Casey's June Beetle and Designation of Critical Habitat*. Department of the Interior, 50 CFR Part 17. Published at 76 FR 58954 on September 22, 2011.

U.S. Grains Council (USGC). *Value Enhanced Grain Report 2006*. USGC website: http://www.grains.org/images/stories/technical_publications/USGC_Report__Final__04_28_06.pdf. Accessed June 30, 2011.

Van Duyn, J., *et al*. 2005. *Organic Insect Pest Management: Field Corn*. North Carolina State University.

Vercauteren, K.C., and S.E. Hygnstrom. 1993. White-Tailed Deer Home Range Characteristics and Impacts Relative to Field Corn Damage. *Wildlife Damage Management. Great Plains Wildlife Damage Control Workshop Proceedings.* University of Nebraska, Lincoln.

Vlachos, D., and D. Ward. 2011. *Public Interest Assessment Supporting US EPA Registration of 5307 Corn and the Breeding Stacks Bt11 × MIR604 × TC1507 × 5307 and Bt11 × MIR162 × MIR604 × TC1507 × 5307 Corn.* Research Triangle Park, NC: Syngenta Seeds, Inc. – Field Crops—NAFTA.

Vlachos, D., and S.A. Huber. 2011. *Petition for Determination of Nonregulated Status for Rootworm-Resistant Event 5307 Corn*. Research Triangle Park, NC: Syngenta Biotechnology, Inc.

Vogel, J.R., *et al*. 2008. Pesticides in rain in four agricultural watersheds in the United States. *Journal of Environmental Quality* 37:1101-1115.

Vyn, T.J. 2006. *Meeting the Ethanol Demand: Consequences and Compromises Associated with More Corn on Corn in Indiana.* Purdue University. ID-336: 1-4.

Walters, F.S., C.M. deFontes, H. Hart, G.W. Warren, and J.S. Chen. 2010. Lepidopteran-active variable-region sequence imparts coleopteran activity in eCry3.1Ab, an engineered *Bacillus thuringiensis* hybrid insecticidal protein. *Applied and Environmental Microbiology* 76:3082-3088.

Ward, D., T. DeGooyer, T. Vaughn, G. Head, M. McKee, J. Astwood, and J. Pershing. 2005. Genetically enhanced maize as a potential management option for corn rootworm: YieldGard® rootworm maize case study. In *Western Corn Rootworm: Ecology and Management.* S. Vidal, U. Kuhlmann, and C. Edwards (eds.). CAB International, Wallingford, Oxfordshire.

Webster, J.R., E.F. Benfield, T.P. Ehrman, M.A. Schaeffer, J.L. Tank, J.J. Hutchens, and D.J. D'Angelo. 1999. What happens to allochthonous material that falls into streams? A synthesis of new and published information from Coweeta. *Freshwater Biology* 41:687-705.

White House Emerging Technologies Interagency Policy Coordination Committee. 2011. *Principles for Regulation and Oversight of Emerging Technologies.* Washington DC: Executive Office of the President, Office of Science and

Technology Policy; United States Trade Representative; and Administrator, Office of Information and Regulatory Affairs.

Wolfenbarger, L.L., S.E. Naranjo, J.G. Lundgren, R.J. Bitzer, and L.S. Watrud. 2008. Bt crop effects on functional guilds of non-target arthropods: a meta-analysis. PLoS One 3(5):e2118.

Wright, S.D., W.M. Canevari, and D.J. Munier. 2009. *How to Manage Pests, UC Pest Management Guidelines: Corn, Integrated Weed Management*. The Regents of the University of California. University of California Integrated Pest Management, UC ANR Publication 3443. UC Davis website: http://www.ipm.ucdavis.edu/PMG/r113700111.html. Accessed June 22, 2011.

Wu, F. 2006a. An analysis of Bt corn benefits and risks for national and regional policymakers considering Bt corn adoption. *International Journal of Technology and Globalization* 2(1 and 2):115-136.

Wu, F. 2006b. Mycotoxin reduction in Bt corn: potential economic, health, and regulatory impacts. *Transgenic Research* 15:277-289.

Wu, F. 2008. *Field Evidence: Bt Corn and Mycotoxin Reduction.* ISB News Report.

Zeph, L. and D. Vlachos. 2011. *Biotechnology Consultation Document for the United States Food and Drug Administration, Safety and Nutritional Assessment of Event 5307 Corn. OECD Unique Identifier: SYN-Ø53Ø7-1.*

Appendix A
Public Interest Assessment

Title

Public Interest Assessment
Supporting US EPA Registration of 5307 Corn and the Breeding Stacks Bt11 × MIR604 × TC1507 × 5307 and Bt11 × MIR162 × MIR604 × TC1507 × 5307 Corn

Data Requirement

Federal Register **51**:7626 (1986)

Authors

Demetra Vlachos, M.S.
Dennis Ward, Ph.D.

Date

March 31, 2011

Submitter

Syngenta Seeds, Inc. – Field Crops - NAFTA
P.O. Box 12257
3054 East Cornwallis Road
Research Triangle Park, NC 27709

Report ID

SSB-201-10

Statement of No Data Confidentiality Claim

No claim of confidentiality is being made for information in this report on the basis of its falling within the scope of FIFRA §10(d)(1)(A), (B), or (C).

Syngenta submits this material to the United States Environmental Protection Agency specifically under provisions contained in FIFRA, as amended, and consents to use and disclosure of this material by EPA according to FIFRA. In submitting this material to EPA according to method and format requirements contained in PR Notice 86-5 and 40 CFR §158.33, Syngenta does not waive any protection of rights involving this material that would have been claimed by the company if this material had not been submitted to the EPA.

Company: Syngenta Seeds, Inc. – Field Crops - NAFTA

Submitter:

Demetra Vlachos, M.S.
Regulatory Affairs Manager

Date: March 31, 2011

Statement Concerning Good Laboratory Practices

This assessment is not a study as defined by the Good Laboratory Practice (GLP) Standards as set forth in 40 CFR §160. Thus, the GLP Standards do not apply.

Study Director: There was no GLP Study Director for this assessment.

Submitter:

Demetra Vlachos, M.S.
Regulatory Affairs Manager
Syngenta Seeds, Inc. – Field Crops - NAFTA
P.O. Box 12257
3054 East Cornwallis Road
Research Triangle Park, NC 27709

Date: March 31, 2011

Table of Contents

Page

List of Tables

Page

List of Figures

Page

Summary

Syngenta is seeking a manufacturing-use registration under FIFRA Section 3 for a new plant-incorporated protectant (PIP), the eCry3.1Ab protein, as produced in corn derived from transformation event 5307 ("5307 corn" or "Event 5307 corn"). Additionally, Syngenta seeks commercial registrations for two new products that include the 5307 corn PIP in breeding combinations with other registered PIPs: Bt11 × MIR604 × TC1507 × 5307 corn and Bt11 × MIR162 × MIR604 × TC1507 × 5307 corn. Data and information are provided herein to support a finding that issuance of these registrations will be in the public interest.

Corn is the most widely cultivated U.S. crop, in terms of acreage planted and net value. Transgenic corn hybrids that produce *Bacillus thuringiensis*-derived Cry proteins for targeted rootworm control have been available in the U.S. since 2003. Prior to their introduction, control of *Diabrotica* rootworms accounted for the largest single use of conventional insecticides in the U.S. Transgenic corn hybrids currently available to U.S. growers for rootworm control have significantly improved growers' ability to control these pests effectively and easily, while dramatically reducing growers' use of broad-spectrum soil-applied insecticides. The attendant benefits of the current PIP rootworm-control traits on the market include higher and more consistent grain yield, economic benefits to growers, healthier plants, improved worker safety, and reduced use of fossil fuels to apply insecticide treatments. The deployment of 5307 corn, in Bt11 × MIR604 × TC1507 × 5307 corn and Bt11 × MIR162 × MIR604 × TC1507 × 5307 corn, will also offer these qualitative benefits.

Reduction of broad-spectrum insecticide use by growers is of special importance because several conventional pesticide products registered for corn rootworm control have use restrictions or label warnings related to their potential to be highly toxic to humans and/or wildlife. Registration of the PIP in 5307 corn and the breeding combinations thereof is expected to further reduce chemical insecticide use by corn growers. Based on an extensive battery of safety studies provided for review by the U.S. Environmental Protection Agency, the eCry3.1Ab protein poses no significant human health or environmental risks, either in 5307 corn alone or in the PIP breeding combinations described above.

Field studies demonstrate that the eCry3.1Ab protein in 5307 corn is highly effective in controlling the larvae of three important U.S. rootworm pests: *Diabrotica virgifera virgifera* LeConte (western corn rootworm), *D. longicornis barberi* Smith and Lawrence (northern corn rootworm), and *D. virgifera zeae* Krysan and Smith (Mexican corn rootworm). In the presence of high rootworm infestations, 5307 corn hybrids have also demonstrated a significant yield advantage in side-by-side comparisons with nontransgenic, near-isogenic control hybrids.

Event 5307 corn, offered in the breeding combinations Bt11 × MIR604 × TC1507 × 5307 corn and Bt11 × MIR162 × MIR604 × TC1507 × 5307 corn, will provide important new choices in pest control, thus promoting marketplace competition and

higher adoption rates of biotechnology-derived corn varieties. Moreover, studies demonstrate that the eCry3.1Ab protein in 5307 corn has unique properties and targets a different gut binding site in corn rootworm larvae than does mCry3A (in Syngenta's registered MIR604 corn, a component of both breeding stacks above). The concurrent deployment of both eCry3.1Ab and mCry3A in the same hybrid offerings to growers is expected to help preserve target pest susceptibility to both Cry proteins. Additionally, by reducing the selection pressure on rootworm populations to evolve resistance to any single method of control, this strategy is predicted to help prolong pest susceptibility to other *B. thuringiensis* (Bt)-derived rootworm-control proteins in transgenic corn cultivars, as well as to other established control methods, including conventional insecticides and crop rotation strategies.

Current insect resistance management strategies for Bt corn products are centered around the planting of a structured refuge that can provide a source of susceptible adult insects with which rare resistant insects can mate, thereby preventing or delaying the establishment of resistance genes in a population. The size and configuration of the structured refuge are determined by toxin dose and insect biology. A second important aspect of combining eCry3.1Ab and mCry3A in a single product is that the minimum size of the on-farm refuge required to delay resistance can be reduced from 20% of a grower's total corn acreage (e.g., for MIR604 corn or other corn products with single rootworm-control traits) to 5% of a grower's acreage. This will offer additional pest control benefits on what would previously have been refuge acres, will reduce the environmental impact of insecticide use on refuge acres, and will facilitate grower compliance with refuge requirements. Corn hybrids producing both eCry3.1Ab and mCry3A will represent an important and effective new tool in the corn grower's arsenal of rootworm control mechanisms.

I. Introduction

At the time of application for a pesticide registration under Section 3 of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) it is often not possible to determine if the registration will be granted under special circumstances. Since their introduction, plant-incorporated protectant (PIP) registrations have only been granted under special circumstances. FIFRA authorizes the Administrator of the Environmental Protection Agency (EPA) to conditionally register a pesticide containing a new active ingredient for a period sufficient to generate and submit additional data. Conditional registrations are granted if the Administrator determines that use of the pesticide during such period will not cause any unreasonable adverse effect on human health or the environment and that use of the pesticide is in the public interest.

The introduction of crops improved through modern biotechnology has been the single most important technological innovation in United States (U.S.) agriculture in recent years. As a result of the benefits growers derive from genetically modified crops, adoption of crops with insect and herbicide tolerance traits has increased dramatically since their commercial introduction. Between 1996 and 2008, the use of transgenic crops boosted global farm incomes by $52 billion, of which $7.1 billion was directly attributable the use of insect-resistant corn hybrids in the U.S. alone (Brookes and Barfoot, 2010). Improved insect protection and weed control provided by transgenic crops have led to increased crop yields and reductions in conventional pesticide applications (Marra et al., 2002). The continued development and introduction of such products are expected to benefit growers, agricultural workers, consumers, and the environment.

Event 5307 corn was developed by Syngenta Biotechnology, Inc. to provide U.S. growers with corn hybrids that are resistant to feeding damage caused by coleopteran insect pests, specifically corn rootworms. There is no herbicide tolerance trait in 5307 corn; the selectable marker used during transformation was phosphomannose isomerase (PMI), which has no agronomic utility. Event 5307 corn will not be offered as a stand-alone product, but will be offered to U.S. growers in breeding combinations with other approved PIPs to provide control of multiple lepidopteran and coleopteran pests as well as herbicide tolerance. The specific products will represent PIP combinations[1] contained in breeding crosses of transgenic Bt11 × MIR604 × TC1507 × 5307 corn and Bt11 × MIR162 × MIR604 × TC1507 × 5307 corn.

This assessment characterizes several anticipated benefits of 5307 corn and the two 5307 stacked-trait products to support a determination that the associated PIP registrations will be in the public interest.

[1] Bt11, TC1507 and MIR162 corn produce the lepidopteran-active PIPs Cry1Ab, Cry1F and Vip3Aa20, respectively. MIR604 and 5307 corn produce the coleopteran-active PIPs mCry3A and eCry3.1Ab.

II. Overview of Corn

Zea mays Linnaeus, known as maize throughout the world and as corn in the United States, is one of the few major crop species indigenous to the western hemisphere. It has been cultivated in the Americas since early historic times. Corn is the leading production crop globally; the 2008/2009 growing season yielded approximately 792 million metric tons of grain (USDA, 2010a).

A. Importance of Corn to the U.S. Economy

Corn is the largest crop grown in the U.S. in terms of both volume and value. The U.S. accounted for nearly 39% of global corn production in 2008/2009 (USDA, 2010a), while utilizing only 20% of the global corn area harvested (NCGA, 2010b). In 2009, nearly 80 million acres of corn were harvested for grain in the U.S., yielding 13.2 billion bushels (335 metric tons) (USDA, 2010b).

Corn is grown for animal feed, human food, vegetable oil, high fructose corn syrups (HFCS), starch, fermentation into ethanol, and a multitude of industrial and consumer uses. U.S. corn usage by market segment is shown in Figure 1. Corn grain used as a feedstock for fuel ethanol production has increased dramatically in recent years; this trend is expected to continue as the U.S. seeks renewable sources of energy. Domestic corn production contributes significantly to the positive U.S. trade balance in agricultural products.

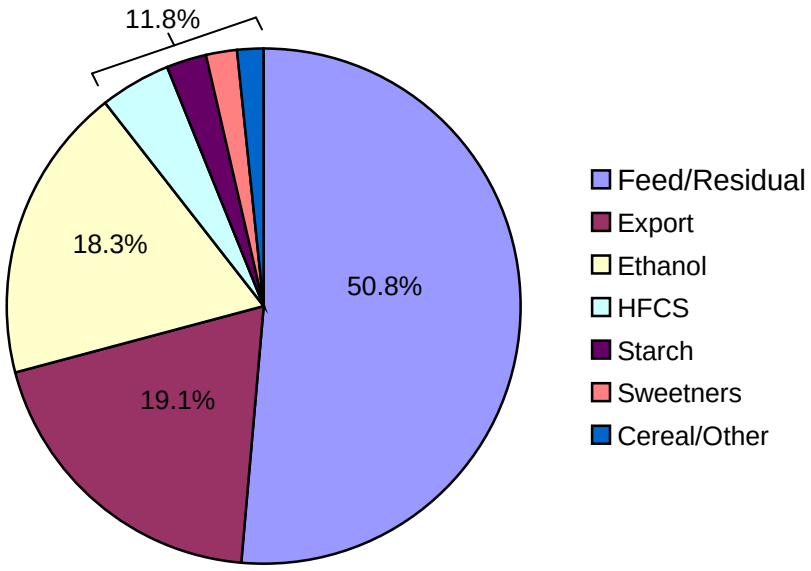


Figure 1. U.S. corn usage by segment, 2009 (Baker et al., 2010).

Total U.S. agricultural exports in 2010 were estimated to be valued at about $100 billion, and over 10% were attributable to corn. Agricultural exports generate employment, income, and purchasing power in both farm and nonfarm sectors of the economy. The production equivalent of over one-fourth of U.S. cropland moved into export channels in 2008 (USDA, 2010c). On a raw crop basis, the U.S. exported 40% of food-grain production, 15% of feed grains, and more than 43% of oilseeds. The value of all agricultural exports increased more than imports; net agricultural exports in 2008 contributed $35.0 billion to the overall U.S. economy, an increase of $16.4 billion over 2007. Technology advances increase agricultural productivity and promote the competitiveness of U.S. growers in the global market. Moreover, such advances are becoming increasingly important to global food security (Flavell, 2010).

B. Corn Agronomics

Z. mays is a large, annual monoecious grass; the duration of its life cycle depends on the cultivar and the environment in which the cultivar is grown. The bulk of corn is produced between latitudes 30° and 47°. Practically no corn is grown where the mean midsummer temperature is less than 19°C or where the average nighttime temperature during the summer months falls much below 13°C. The greatest production occurs where the warmest month isotherms range between 21° and 27°C and the freeze-free season lasts
120 to 180 days. Corn is grown in areas where annual precipitation ranges from 25 to over 500 cm. Summer rainfall of 15 cm is approximately the lower limit for corn production without irrigation.

The upper midwest region of the U.S. provides an ideal combination of temperature, rainfall, and soil type for the cultivation of corn. Iowa, Illinois, Nebraska, Minnesota, Indiana, Ohio, Wisconsin, Missouri, Kansas, and South Dakota are major corn-growing states. Production in these ten states accounted for over 80% of total production in 2009 (USDA, 2010b). Figure 2 displays the geographic distribution of acres planted in 2008.

Growers have hundreds of corn hybrids from which to choose. Available varieties differ widely in agronomic characteristics, including length of growing period and yield. Technology providers continue to develop varieties with desirable traits and increasing yield. Corn yields have increased an average of 3.5 bushels per year over the past decade. The average yield reported for the 2008 U.S. growing season was 153.9 bushels/acre (NCGAa, 2010).

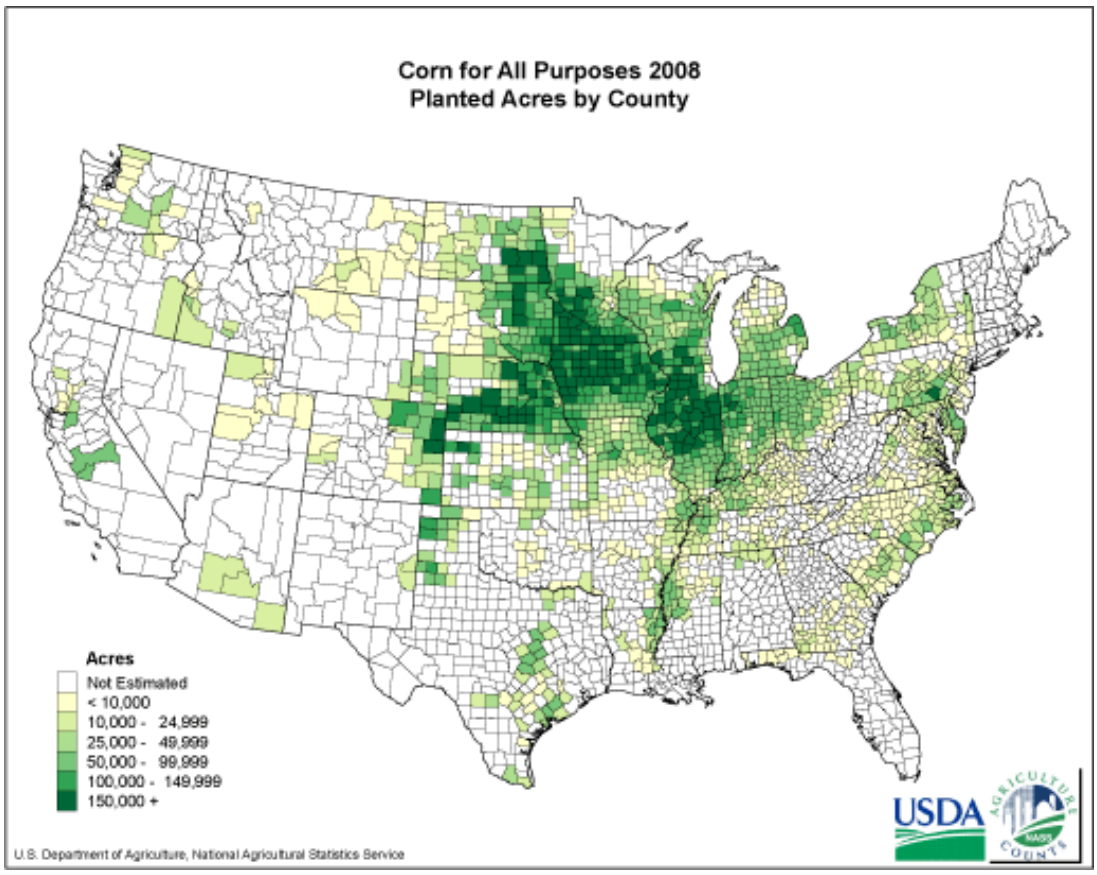


Figure 2. Geographic distribution by county of acres harvested for corn grain in 2008 (source USDA/NASS Charts & Maps).

C. Pests of Corn

Yield losses in all crops due to weeds, diseases, and insects were substantial and widespread until the introduction and adoption of crop protection chemicals in the 1960s. Weeds compete with crops for light, nutrients, water, and other growth factors. If weeds are left uncontrolled, corn simply cannot be grown successfully. Estimates of corn yield loss caused by pathogens have ranged from 2 to 17% (Smith and White, 1988). In addition, a corn crop is susceptible to attack by a variety of insects from the time it is planted until it is consumed as food or feed.

Insect pests can be categorized as major and consistent pests, major and sporadic, and moderate to minor based on annual destructiveness and their geographic distribution. Table 1 categorizes most of the insect pests of corn found in the U.S. The most economically significant of these corn are *Diabrotica* spp. (the corn rootworm complex) and *Ostrinia nubilalis* (European corn borer).

Table 1. Categorization of corn insect pests based on their potential for causing economic losses (modified from Gray and Luckmann, 1994).

Major & Consistent Insect Pests	**Moderate to Minor Insect Pests (cont.)**
Western corn rootworm (*Diabrotica virgifera virgifera*)	Cinch bug (*Blissus leucopterus*)
Northern corn rootworm (*Diabrotica longicornis barberi*)	Southern corn rootworm (*Diabrotica undecimpunctata*)
European corn borer (*Ostrinia nubilalis*)	Other cutworms, many species
Corn earworm (*Helicoverpa zea*)	Seedcorn beetle (*Stenolophus lecontei*)
Fall armyworm (*Spodoptera frugiperda*)	Seedcorn maggot (*Delia platura*)
	Banks grass mite (*Oligonychus pratensis*)
Major Sporadic Insect Pests	Two-spotted spider mite (*Tetranychus urticae*)
Black cutworm (*Agrotis ipsilon*)	Billbugs, many species
Corn leaf aphid (*Rhopalosiphum maidis*)	White grubs, many species
Southwest corn borer (*Diatraea grandiosella*)	Stalk borer (*Papaipema nebris*)
Sugarcane borer (*Diatraea saccharalis*)	Garden symphylan (*Scutigerella immaculata*)
Lesser cornstalk borer (*Elasmopalpus lignosellus*)	Japanese beetle (*Popillia japonica*)
Western bean cutworm (*Striacosta albicosta*)	Sod webworms, several species
	Grape colaspis (*Colaspis brunnea*)
Moderate to Minor Insect Pests	Thrips, several species
Wireworms, many species	Dusky sap beetle (*Carpophilus lugubris*)
Armyworm (*Pseudaletia unipunctata*)	Stink bugs, several species
White-fringed beetles (*Graphognathus spp.*)	Southern cornstalk borer (*Diatraea crambidoides*)
Grasshoppers, many species	Corn root aphid (*Anuraphis maidiradicis*)
Corn flea beetle (*Chaetocnema pulicaria*)	

The damage inflicted by rootworm larvae can significantly reduce grain yield by interfering with photosynthetic rates, limiting the uptake of water and nutrients, and by increasing the plant's susceptibility to lodging (Oleson et al., 2005). Lodging (leaning) further reduces the effective grain yield by making the plants more susceptible to breaking, reducing their access to sunlight, and increasing the difficulty with which the grain can be harvested efficiently.

In addition to direct damage caused by feeding on plant tissue, insects play an important role in the transmission and dissemination of pathogenic organisms during

corn development. Soil contains microorganisms, particularly fungi, that may infect plant parts injured by soil-dwelling insects. Primary roots of the seedling and the radical and seminal roots are commonly infected with *Fusarium* spp. after the roots have served their function and become senescent. Feeding by corn rootworms has been associated with increased frequencies of *Fusarium* infection (Dicke and Guthrie, 1988); rootworm feeding may also lead to increased incidences of stalk rots. These pathogen infections can reduce crop quality, harvestability, and yield.

D. Current Insect Control Practices

The most widespread and damaging insects of corn in the U.S. Corn Belt have been *Diabrotica* species and *O. nubilalis*. Before the introduction of *Bacillus thuringiensis* (Bt)-derived corn varieties, the tools available to growers for pest control consisted of insecticide applications, agronomic practices, and to a limited extent, the use of varieties with a degree of native pest resistance. The introduction of the first *Bt* corn varieties in 1996 provided growers with an effective means of limiting damage caused by *O. nubilalis*. Prior to the introduction of corn rootworm-protected *Bt* corn varieties in 2003, an estimated 14 million acres were treated annually with conventional insecticides to control corn rootworms. This equated to applications of more than 7.7 million pounds of insecticide active ingredient annually in corn fields for the control of *Diabrotica* species (Ward et al., 2005). Control of *Diabrotica* rootworms accounted for the largest single use of conventional insecticides in the U.S. at that time.

Insect-resistant transgenic corn, including stacked-trait varieties with herbicide tolerance, accounted for 63% of corn acres planted in 2009 (USDA, 2009a). Crops engineered to produce Bt toxins that target specific pest taxa have had favorable environmental effects, particularly when replacing the use of broad-spectrum insecticides that may also impact agriculturally important nontarget organisms, including beneficial insects such as honeybees or natural enemies that prey on other insects (NRC, 2010; Carpenter, 2011).

Prior to the advent of insect protected field corn, corn rootworm was controlled through the use of crop rotation (e.g., corn/soybean rotation) and insecticides applied to the soil, plant or seed. It is significant that corn rootworm populations have developed resistance to some insecticides and to non-chemical control methods. Resistance to some corn rootworm insecticides may result in increased chemical use (EPA, 2010). Although crop rotation has historically been an effective control tool, in some areas, variant rootworm populations display behavioral changes that circumvent rotation strategies. Some northern corn rootworm populations have an extended diapause that allows eggs to hatch when the crop rotation returns to corn rather than in the non-corn rotation crop in the growing season that follows corn. A variant western corn rootworm population now lays its eggs in soybean fields rather than corn fields, allowing eggs to hatch in fields rotating to corn.

Rootworm-protected Bt corn hybrids have been available to U.S. growers since 2003. The current PIP proteins registered for corn rootworm control in field corn are Cry3Bb1 (in events MON 863 and MON 88017 corn), Cry34Ab1/Cry35Ab1 (in event DAS-59122-7 corn) and mCry3A (in Syngenta's event MIR604 corn). As the industry trend is towards combined-trait hybrid offerings, it is important to note that the aforementioned Cry proteins are available in multiple combinations. From the perspective of preventing or mitigating resistance in target pest populations, the deployment of multiple corn rootworm traits in a single corn hybrid is key to the durability of the registered PIPs in corn.

III. Characteristics of 5307 Corn

A. Transgenes and Novel Proteins in 5307 Corn

Using the techniques of modern molecular biology, Syngenta has transformed corn (*Zea mays* L.) to produce 5307 corn, a new cultivar that produces an insecticidal protein with activity against certain corn rootworm (*Diabrotica*) species. Event 5307 corn contains the transgene *ecry3.1Ab* encoding an eCry3.1Ab protein and the transgene *pmi* (also known as *manA*) encoding the enzyme phosphomannose isomerase (PMI). The eCry3.1Ab protein is an engineered chimera of modified Cry3A (mCry3A) and Cry1Ab proteins, the native forms of which occur in *B. thuringiensis*. The gene *pmi* was obtained from *Escherichia coli* strain K-12 and the PMI protein it encodes was utilized as a plant selectable marker during development of 5307 corn. PMI allows cultured plant cells to utilize mannose as the primary carbon source. It does not confer herbicide tolerance or other agronomic characteristics.

Syngenta conducted mortality bioassays with the eCry3.1Ab protein in a range of insect species to identify those that are susceptible. The results of these bioassays demonstrate that the activity of eCry3.1Ab protein is limited to species within the Order Coleoptera, yet not all coleopteran species are sensitive to the protein. Although part of the amino acid sequence of the eCry3.1Ab protein is derived from Cry1Ab, a lepidopteran-active protein, the portion of Cry1Ab represented in eCry3.1Ab does not include the N-terminal lepidopteran-active region. This likely accounts for why eCry3.1Ab demonstrates no activity against several lepidopteran species tested (Nelson, 2010).

The native Cry3A protein from *B. thuringiensis* is highly toxic to Colorado potato beetle larvae (*Leptinotarsa decemlineata*), but has very little or no toxicity to related beetles in the genus *Diabrotica*. By modifying the native gene *cry3A*, Syngenta introduced a protease recognition site into the native Cry3A protein, thereby creating a modified Cry3A (mCry3A) that was active on the larvae of three *Diabrotica* spp.: western, northern and Mexican corn rootworms. Modified Cry3A is the registered PIP in Syngenta's MIR604 corn (Agrisure® RW corn). *L. decemlineata* and the same *Diabrotica* spp. that are sensitive to mCry3A are also sensitive to eCry3.1Ab. Although the eCry3.1Ab protein contains a portion of mCry3A in its amino acid

sequence, it is significant that eCry3.1Ab and mCry3A have unique biochemical properties and do not compete for the same binding site in the guts of corn rootworm larvae.

The species specificity of each Bt δ-endotoxin is the result of the efficiency of the various steps involved in producing an active protein toxin and its subsequent interaction with the epithelial cells in the insect midgut. To be insecticidal, most known Bt δ–endotoxins must: (1) be ingested by the insect and solubilized in the gut, (2) be activated by specific proteolytic cleavages, (3) bind to specific receptors on the surface of the insect midgut, and (4) form ion channels. The completion of all these four processes results in disruption of the normal function of the midgut leading to the death of the insect.

The eCry3.1Ab protein induces toxicity via the four steps noted above (Jones, 2010). Data support the conclusion that eCry3.1Ab recognizes different insect midgut receptor binding sites than the activated form of mCry3A in the brush border membrane of western corn rootworm larvae, the primary target pest of 5307 corn. This differential binding, in conjunction with ion channel profiles, is indicative of a unique mode of action for eCry3.1Ab relative to the related mCry3A protein (Jones, 2010; Walters et al., 2010).

Syngenta conducted or sponsored an extensive battery of studies to support an environmental safety assessment for the eCry3.1Ab protein and 5307 corn. No harmful effects were observed in studies with a range of nontarget organisms, which were exposed to levels of eCry3.1Ab protein at or above expected environmental concentrations. A comprehensive environmental risk assessment describing the results of these studies has been submitted for EPA review (Nelson, 2010).

The absence of adverse effects in nontarget species is consistent with the known mechanism by which Cry proteins exert their selective toxicity and the relatively narrow insecticidal spectrum of a given Cry protein. Individual Cry proteins are usually active against only a few related species within any phylogenetic Order. Most of the insect-specific toxins identified to date have shown activity among the Orders of Lepidoptera and/or Diptera, or Coleoptera. A few Bt crystal toxins have shown activity against nematodes (Marroquin et al., 2000; Wei et al., 2003).

Accompanying the registration applications for the 5307 corn breeding stacks (Bt11 × MIR604 × TC1507 × 5307 corn and Bt11 × MIR162 × MIR604 × TC1507 × 5307 corn), Syngenta is providing studies that demonstrate the absence of synergistic effects among the combined PIP proteins in these products (Seastrum et al., 2010; Seastrum, 2010). These studies support a conclusion that there will not be adverse effects on nontarget species as a result of exposure to the combined PIPs in the stacked-trait corn hybrids (Raybould, 2011a, 2011b).

Additionally, a standard battery of studies to identify hazards for mammalian species potentially exposed to plant-incorporated eCry3.1Ab has been conducted. These

eCry3.1Ab studies include an acute oral toxicity study with mice (Korgaonkar, 2009), *in vitro* digestive fate assays (Song, 2010; Seastrum, 2009), a comparison of amino acid sequence to that of known toxins and allergens (Harper, 2011; McClain, 2011), and a characterization of the biochemical properties of the eCry3.1Ab protein (Nelson, 2008; Nelson, 2009). The results of the safety studies demonstrated that no adverse effects were observed in mice exposed to a maximum attainable oral dose, that the protein is rapidly degraded in a simulated gastric environment, that the protein does not share sequence homology with known mammalian toxins or allergens, and that it does not possess properties suggestive of food allergen potential. A temporary exemption from the requirement of food and feed tolerances has been established for eCry3.1Ab (40 CFR §174.532) in connection with the current Experimental Use Permit for 5307 corn and associated stacked-trait corn (67979-EUP-8). Concurrently with its application for registration of the eCry3.1Ab PIP in 5307, Syngenta has submitted a petition for a permanent tolerance exemption.

The PMI marker protein in 5307 corn is considered an "inert ingredient" with respect to pesticidal activity. PMI is exempt from tolerances in all plants (40 CFR §174.527); a battery of safety studies previously submitted by Syngenta support this exemption.

B. Field Performance of 5307 Corn

Other than its resistance to corn rootworm larval damage, 5307 corn is phenotypically equivalent to conventional corn. In the presence of populations of western, northern or Mexican corn rootworm larvae, production of eCry3.1Ab in the roots of 5307 corn plants will protect the plants from significant damage by rootworm larvae and will preserve the inherent yield potential of the corn varieties that contain the trait.

1. Efficacy studies of 5307 corn and stacked MIR604 × 5307 corn

a) Western corn rootworm efficacy as assessed by root damage ratings

Syngenta conducted field trials at two Illinois locations in 2006 (Haney et al., 2008) and four locations in Illinois and Minnesota in 2008 (Haney et al., 2009) to assess the efficacy of 5307 corn hybrids in controlling western corn rootworm larvae. Cultural practices used in the trials (tillage, fertilization, herbicide application, etc.) were typical of the recommended agricultural procedures for each area, except that all insecticides (including seed treatments) were avoided.

The trial entries were planted in a randomized complete block design in fields that had been planted to a trap crop of cucurbits the previous season to attract corn rootworm beetles for increased egg accumulation (Branson and Sutter, 1989). In some locations, additional western corn rootworm eggs were also applied to artificially infest corn seedlings. These methods were used to assess 5307 corn efficacy at high levels of pest pressure.

At both locations in the 2006 trials, four replicate plots of each trial entry were planted in four-row plots, with 25 plants per row. For each plot, root masses were evaluated from four random plants in the center two rows, avoiding the row ends. At each

location in the 2008 trials, three replicate plots of each trial entry were planted in three-row plots, with 15 plants per row. For each plot, root masses were evaluated from six random plants in the center row, avoiding the row ends.

Damage ratings were assessed on roots collected and washed at the R1 stage of plant development (silking stage, beginning when silks are visible outside husks). Ratings were made on a scale of 0.01 (no damage) to 3.0 (heavy damage) using a modification of the Iowa State University node-injury linear (0 – 3) scale for rating root damage by corn rootworm larvae (Oleson et al., 2005). The root damage rating scale used is described in Table 2. For each set of trials (2006 and 2008), mean damage ratings were compared across entries by analysis of variance, and statistical significance was assigned at the customary 5% level.

Table 2. Damage rating scale used to assess root damage by corn rootworm larvae.
Rating scale was modified from the 0 – 3 scale of Oleson et al. (2005).

Rating	Description of Rootworm Damage
0.01	No damage to 1 – 2 light surface scars on roots
0.02	3+ light surface scars ≤ 4 moderate scars (combined across all roots on a plant)
0.05	5+ heavy scars (long, deep scars), but no root pruning (pruning ≤ 1.5 inches from crown)
0.10	1 root pruned to ≤ 1.5 inches accompanied by heavy scars
0.25	2+ roots pruned to ≤ 1.5 inches (up to ¼ nodes, equivalent, pruned)
0.50	Equivalent of 0.50 node of roots pruned
0.75	Equivalent of 0.75 node of roots pruned
1.00	Equivalent of 1.00 node of roots pruned
1.25	Equivalent of 1.25 nodes of roots pruned
1.50	Equivalent of 1.50 nodes of roots pruned
1.75	Equivalent of 1.75 nodes of roots pruned
2.00	Equivalent of 2.00 nodes of roots pruned
2.25	Equivalent of 2.25 nodes of roots pruned
2.50	Equivalent of 2.50 nodes of roots pruned
2.75	Equivalent of 2.75 nodes of roots pruned
3.00	Equivalent of 3.00 nodes of roots pruned

Western corn rootworm larvae were very destructive to the nontransgenic, near-isogenic control hybrids at all trial locations in both years, but caused significantly less injury to the 5307 corn hybrids. Mean root damage ratings in the 2006 trials (Figure 3) ranged from 0.12 to 0.20 for the 5307 hybrids across both locations and were 1.53 and 1.94 for the controls. These ratings demonstrated that the 5307 hybrids sustained only 6.3% to 12.3% of the damage observed in the control hybrids in both locations. In 2008 (Figure 4), mean root damage ratings ranged from 0.04 to 0.06 for the 5307 hybrid entries across the four test locations, while the control ratings ranged

from 1.42 to 2.81. The 5307 hybrids sustained only 1.5% to 4.2% of the damage observed in the control hybrids across the four test locations. In all cases, the differences between the 5307 and control hybrids were statistically significant.

The photograph in Figure 5 illustrates how destructive western corn rootworm larvae were to the control hybrid under high pest pressure and how highly effective the 5307 hybrid was in preventing damage under the same pest pressure.

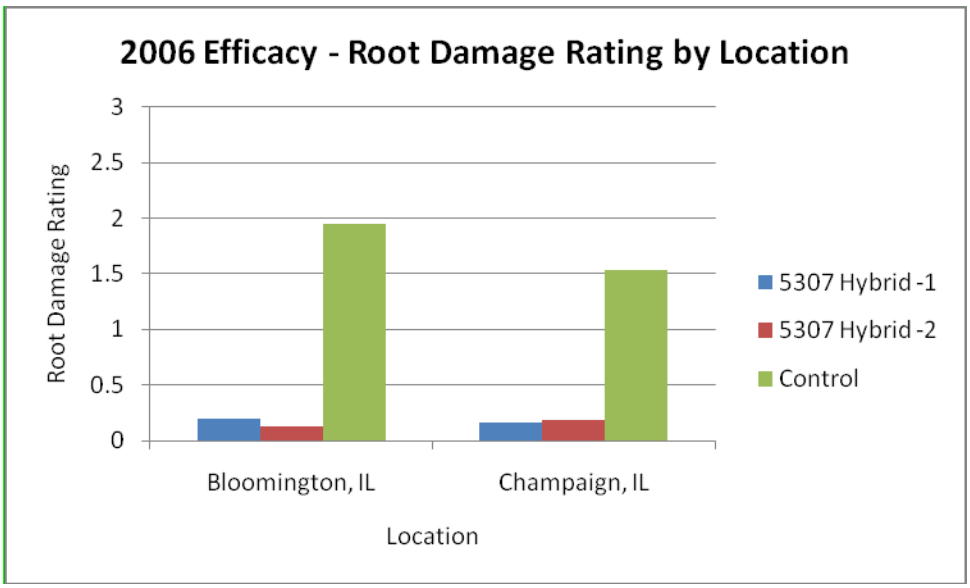


Figure 3. Root damage ratings from western corn rootworm field efficacy trials conducted in 2006 with 5307 corn (Haney et al., 2008)

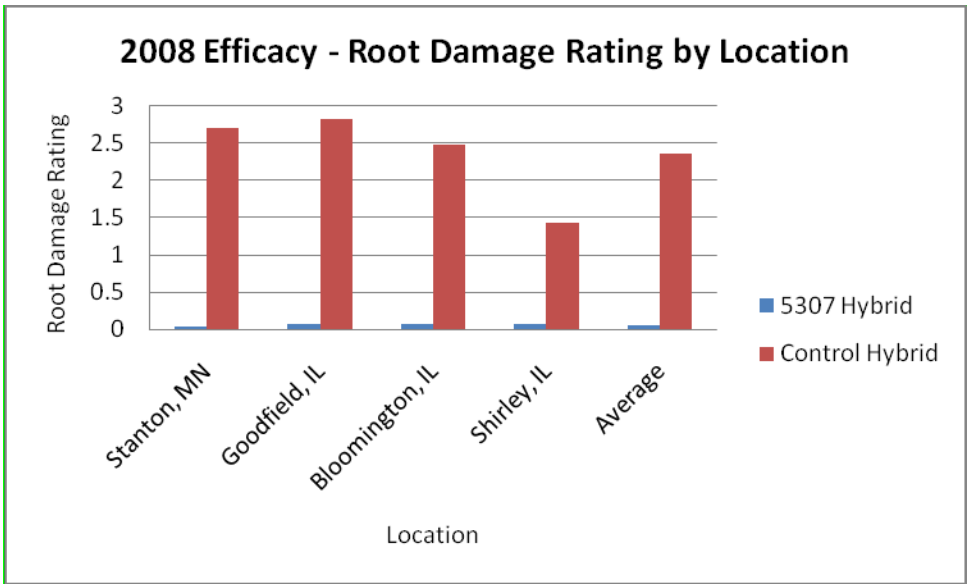


Figure 4. Root damage ratings from western corn rootworm field efficacy trials conducted in 2008 with 5307 corn (Haney et al., 2009)

Figure 5. Root mass of a 5307 hybrid compared to a control hybrid under high western corn rootworm pressure.
A typical root mass of a 5307 corn hybrid is shown on the left (Shirley, IL, 2008). Note the loss of entire nodes of roots from the control hybrid on the right due to extensive feeding by corn rootworm larvae.

The efficacy of 5307 corn in preventing root feeding damage was also examined in a broader set of field trials conducted in 2005 through 2009, at a total of 21 trial locations in Illinois and Minnesota. In addition to comparing the performance of a 5307 corn hybrid to a nontransgenic control hybrid, comparisons were also made to a MIR604 × 5307 hybrid and a MIR604 hybrid. All hybrids tested were in the same background genotype. The trials were conducted under natural rootworm infestation, which was facilitated by planting a cucurbit trap crop the previous season. The design and methods used in these trials were otherwise similar to those employed in the western corn rootworm trials described above, and root damage ratings were assigned using the same 0.01 – 3.0 rating scale (Table 2). At the Illinois trial locations, the predominant rootworm species was western corn rootworm, whereas the trials in Minnesota had mixed infestations of western and northern corn rootworms.

The results of these five years of trials are summarized in Figure 6 and again demonstrate the high rootworm control efficacy of the eCry3.1Ab protein in 5307 hybrids. This was evident whether the trait was present alone via 5307 corn or in combination with the mCry3A trait in MIR604 corn. The combined statistical analysis across this set of trials indicated that the root damage ratings for all the transgenic hybrids were significantly lower than the control hybrid. Although both

the 5307 hybrid and the MIR604 × 5307 hybrid had the lowest mean root damage ratings across all locations (0.05 on a scale of 0.01 – 3.0), these ratings were not statistically lower than the damage ratings for the MIR604 hybrid (0.17). By comparison, the control hybrid had mean damage ratings of 1.75.

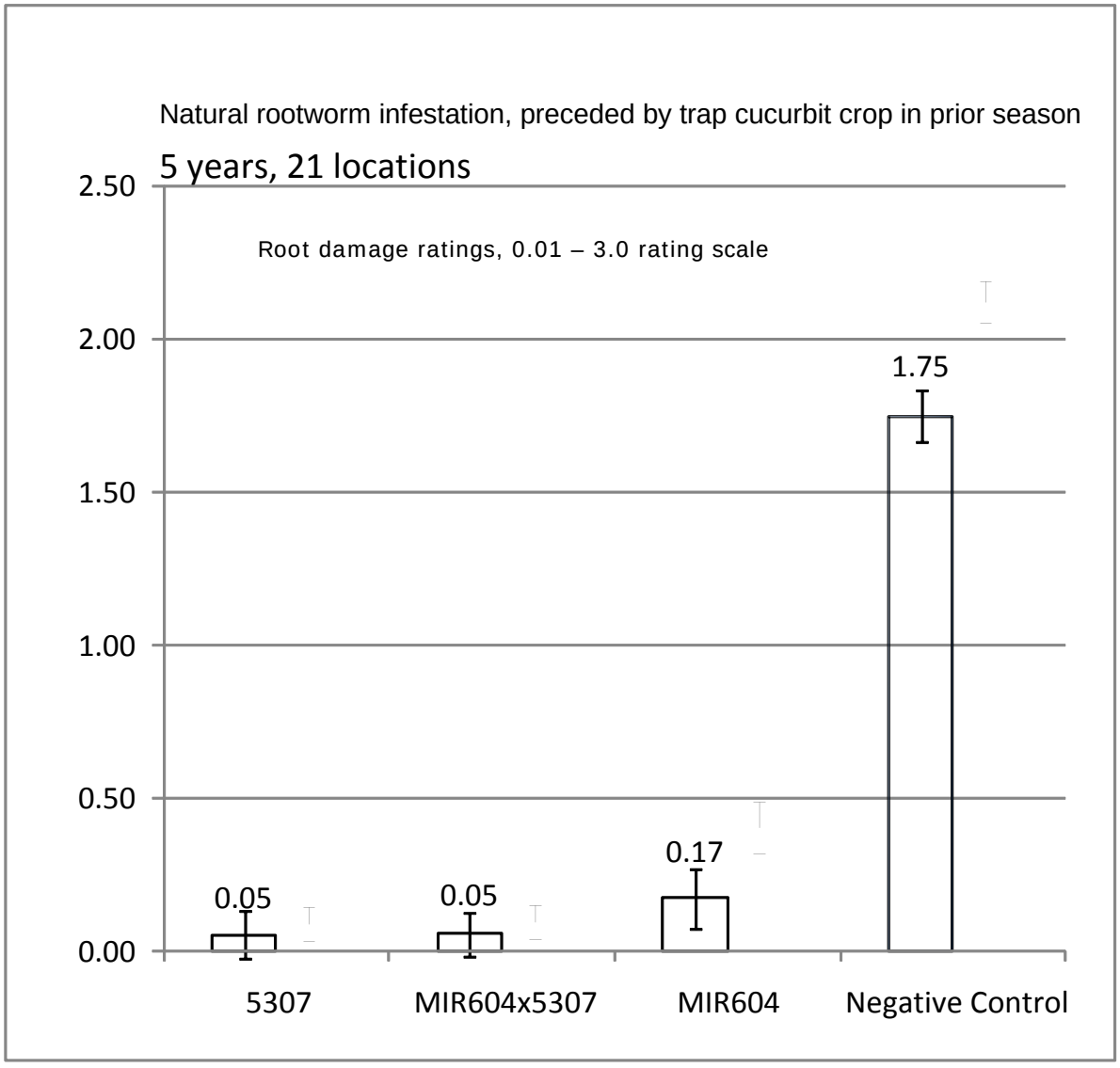


Figure 6. Rootworm efficacy of 5307, MIR604 × 5307, and MIR604 corn hybrids; Illinois and Minnesota trials, 2005-2009.

Western corn rootworm was the predominant rootworm species in the Illinois trials, while mixed infestations of western and northern corn rootworms were present in the Minnesota trials.

b) Western corn rootworm efficacy as assessed by adult emergence counts

Root damage ratings are commonly used to measure the efficacy of insect control tactics against corn rootworms, however, several other methods are also employed (Oleson et al., 2005). Collecting and counting rootworm adults as they emerge from the soil represent an alternative method (Hein et al., 1985). Studies of adult rootworm emergence were conducted in controlled field experiments using 5307 corn, MIR604 corn, MIR604 × 5307 corn, and nontransgenic control corn. These field assessments were conducted in multiple locations in Missouri in 2007 to 2009 by Bruce Hibbard, Ph.D. (Plant Genetics Research Unit; USDA Agricultural Research Service; Columbia, Missouri), as part of an insect resistance management research effort to characterize 5307 corn performance (Hibbard, 2009).

The trials were planted in a randomized complete block design in one location with three replications in 2007, in one location with five replications in 2008, and in two locations with five replications and one location with four replications in 2009. Due to prior cropping practices, the plots were not expected to already contain western corn rootworms at the time of planting. Cultural practices used in the trials (tillage, fertilization, herbicide application, etc.) were typical of the recommended agricultural procedures for each area, except that all insecticides (including seed treatments) were avoided. Following artificial infestation of the test plots with western corn rootworm eggs, individual tents were erected over each replicate plot to collect and count the adult rootworm beetles as they emerged. The Missouri locations where the trials were conducted do not have the rotation-resistant (extended diapause) variant of western corn rootworm that lays eggs outside of corn (in soybeans and other crops). Thus, it was assumed that all western corn rootworm adults recovered in these trials survived from eggs infested in these trials.

As shown in Table 3, the mean number of western corn rootworm adults that survived to emerge from the transgenic corn plots was markedly and statistically significantly lower than in the control plots in all individual trials. Reflecting the increased statistical power of the combined data across all trials in 2007 - 2009, both the 5307 corn hybrid and the MIR604 × 5307 stacked hybrid were statistically significantly more efficacious than the MIR604 hybrid alone. The mean number of beetles recovered per tent for these hybrids were 1.9, 0.8, and 19, respectively, compared with 879 beetles per tent in the control plots. Although only half the number of beetles emerged from the MIR604 × 5307 stacked hybrid as emerged from the 5307 hybrid, the difference between them was not statistically significant; both hybrids provided high levels of control. Because so few western corn rootworm adults emerged from the 5307 corn plots as well as the MIR604 × 5307 corn plots, this likely indicates that the eCry3.1Ab trait alone in 5307 corn is highly effective in controlling the larvae. Using 100% control survival as a comparator, the mean percent survival ranged from only 0.03% – 1.92% across all five trials for hybrids containing the eCry3.1Ab trait, whether as a single 5307 hybrid or in a breeding stack combination with the mCry3A trait in MIR604 corn. By comparison, the percent survival in the single-event

MIR604 hybrid plots ranged from 1.15% - 3.12%; this demonstrated slightly lower but, nevertheless, very good efficacy of the mCry3A trait against the target pest.

Table 3. Surviving adult western corn rootworm beetles recovered in five artificially infested field trials in Missouri, 2007 - 2009

Location - Year	Corn Hybrid	Adult WCRW Beetles Recovered	
		Mean No. per Tent ± SEM	% Relative Survival
Site 1 - 2007	5307	2.7 ± 0.7 b	1.92
	MIR604 x 5307	0.3 ± 0.3 b	0.24
	MIR604	4.3 ± 3.4 b	3.12
	Nontransgenic control	139 ± 44 a	100
Site 2 - 2008	5307	0.6 ± 0.4 b	0.06
	MIR604 x 5307	0.6 ± 0.2 b	0.06
	MIR604	14.0 ± 3.4 b	1.45
	Nontransgenic control	964 ± 147 a	100
Site 2 - 2009	5307	5.2 ± 1.6 b	0.48
	MIR604 x 5307	2.2 ± 0.9 b	0.20
	MIR604	22.4 ± 6.2 b	2.07
	Nontransgenic control	1081 ± 116 a	100
Site 3 - 2009	5307	0.4 ± 0.2 b	0.19
	MIR604 x 5307	0.2 ± 0.2 b	0.10
	MIR604	2.4 ± 1.4 b	1.15
	Nontransgenic control	208 ± 45 a	100
Site 4 - 2009	5307	0.5 ± 0.5 b	0.03
	MIR604 x 5307	0.5 ± 0.5 b	0.03
	MIR604	52.8 ± 29.4 b	2.75
	Nontransgenic control	1916 ± 295 a	100
All	5307	1.9 ± 0.6 c	0.21
	MIR604 x 5307	0.8 ± 0.3 c	0.09
	MIR604	19.0 ± 6.3 b	2.16
	Nontransgenic control	879 ± 149 a	100

Data from Hibbard (2009)

WCRW = western corn rootworm

Means followed by the same letter are not statistically different (i.e., $p > 0.05$).

All = combined data from all trial sites and years.

c) Northern corn rootworm efficacy as assessed by adult emergence counts

When considered across the US Corn Belt, northern corn rootworms do not typically cause as much economic damage as do western corn rootworms, the primary target

pest of 5307 corn. Nevertheless, northern corn rootworms remain a significant pest in many corn-growing areas, particularly in cooler regions.

It is difficult to successfully mass rear northern corn rootworms in culture, hence it is not feasible to artificially infest test plots with eggs of this species to conduct controlled trials under high population pressure. However, natural infestations of northern corn rootworm larvae were evident in two of the five Missouri trials conducted by Bruce Hibbard (Hibbard, 2009). At one trial location in 2007 and another location in 2008, sufficient northern corn rootworm beetles were captured in the tents placed over the corn plots to observe 5307 corn efficacy against this pest.

As shown in Table 4, a mean of over 21 northern corn rootworm beetles per plot survived to emerge from the control corn plots, whereas a mean of less than one beetle per plot survived to emerge from the plots containing the 5307 and/or MIR604 traits; the differences between the control and the transgenic plots were significant for all comparisons ($p < 0.05$). Although not a single surviving adult northern corn rootworm beetle emerged in any of the MIR604 × 5307 corn plots, the differences between the 5307, MIR604 × 5307, and MIR604 hybrids were not statistically significant. This may reflect the limited statistical power afforded by the data for the two trial sites. Nevertheless, these trials demonstrated high efficacy of eCry3.1Ab and 5307 corn against northern corn rootworms, both alone and in combination with mCry3A in MIR604 corn.

Table 4. Surviving adult northern corn rootworm beetles recovered in two naturally infested trials in Missouri

Location - Year	Corn Hybrid	Adult NCRW Beetles Recovered	
		Mean No. per Tent ± SEM	% Relative Survival
Site 1 - 2007	5307	0.3 ± 0.3 b	1.49
	MIR604 x 5307	0.0 ± 0.0 b	0.00
	MIR604	0.3 ± 0.3 b	1.49
	Nontransgenic control	22.3 ± 5.5 a	100
Site 2 - 2008	5307	0.2 ± 0.2 b	0.93
	MIR604 x 5307	0.0 ± 0.0 b	0.00
	MIR604	0.8 ± 0.4 b	3.74
	Nontransgenic control	21.4 ± 2.5 a	100

Data from Hibbard (2009)

Means followed by the same letter are not statistically different (i.e., $p > 0.05$).

NCRW = northern corn rootworm

d) Mexican corn rootworm efficacy as assessed by root damage ratings

While western and northern corn rootworms are pests of corn throughout the Midwestern states, the Mexican corn rootworm occurs in Texas, Oklahoma and New Mexico (Krysan, 1986). The efficacy of 5307 corn hybrids in controlling Mexican corn rootworm was investigated in 2006 and 2007 under naturally infested conditions at one Texas location where corn had been grown for several consecutive seasons. The performance of 5307 corn was compared to that of MIR604 corn, MIR604 × 5307 corn, one or two corn rootworm insecticide treatments applied at the label rate to nontransgenic corn, and an untreated nontransgenic control corn hybrid. All hybrids were of near-isogenic genotypes and were planted in four replicates of two-row plots in a randomized complete block design. The trials were managed under typical local agricultural practices, however, insecticide applications (including seed treatments) were not applied, except where noted as the experimental variable. Six root masses per replicate plot were evaluated for feeding damage according to the 0.01 to 3.00 rating scale described in Table 2. The ratings were compared by an analysis of variance appropriate for a randomized complete block design, and statistical significance was assigned at the customary 5% level.

In the 2006 trial (Figure 7), all entries had significantly less root damage than the control hybrid, but none was statistically different from another. In the 2007 trial (Figure 8), all entries again had significantly less root damage than the control. The 5307 hybrid was significantly more efficacious than the nontransgenic hybrid treated with Force® 3G (tefluthrin granular) insecticide. However, there were no statistically significant differences in root ratings among the 5307 hybrid, the MIR604 × 5307 hybrid, the MIR604 hybrid, the MIR604 hybrid treated with Force 3G insecticide, or the MIR604 hybrid treated with Cruiser® 0.25 insecticide (a thiamethoxam seed treatment). Across both trials, mean root damage ratings in the 5307 and MIR604 × 5307 plots were very low, and ranged from 0.01 to 0.045. By contrast, the control plants had mean root damage ratings of 0.71 and 0.69.

Although the statistical power of this small dataset of Mexican corn rootworm trials to-date is limited, the trials clearly demonstrated that 5307 corn, either alone or in a breeding stack with MIR604 corn, was highly effective in controlling this damaging pest.

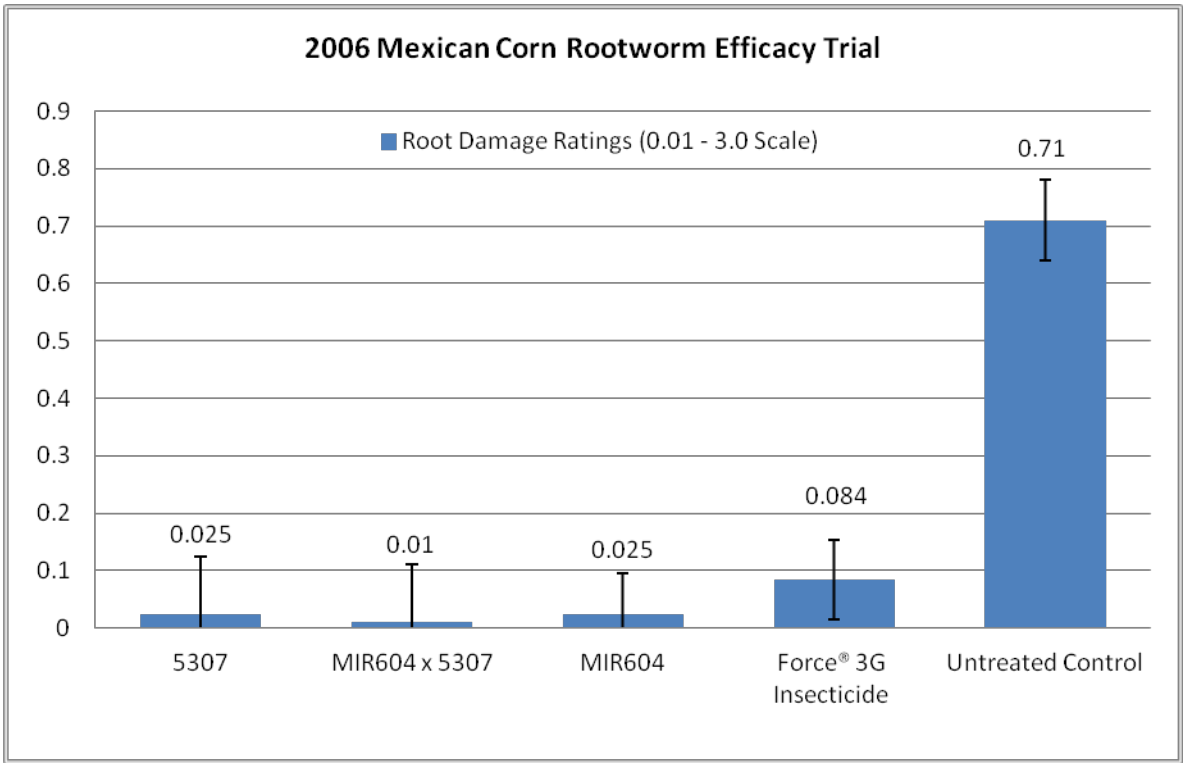


Figure 7. Efficacy of 5307 corn against Mexican corn rootworm in Texas, 2006

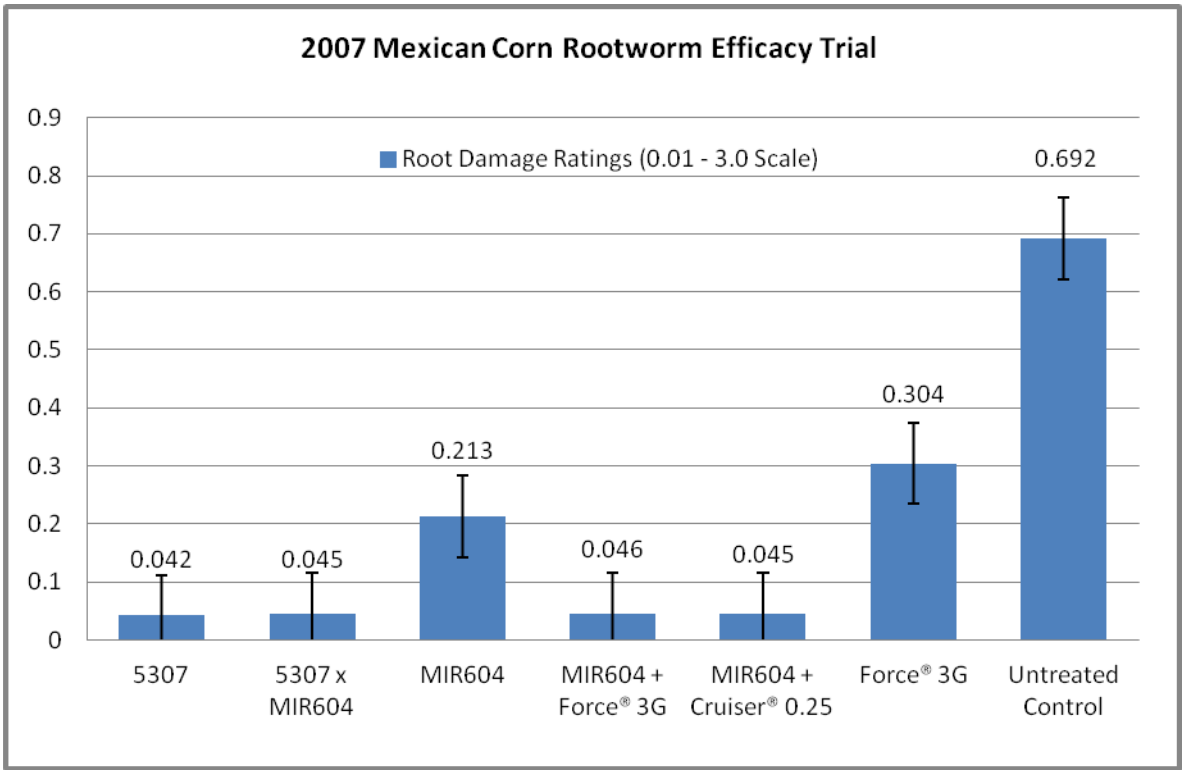


Figure 8. Efficacy of 5307 corn against Mexican corn rootworm in Texas, 2007

2. Summary of efficacy trials of 5307 corn against target pests

Multi-year and multi-location field assessments of the efficacy of 5307 corn against western corn rootworm larvae were conducted by measuring feeding damage to corn roots and by counting the numbers of adult beetles that survived to emerge from infested corn plots. Both methods independently demonstrated that 5307 corn provides excellent control of this primary target pest. Where 5307 corn was also tested in a breeding stack with MIR604 corn, as MIR604 × 5307 hybrids, efficacy was also excellent.

Although the efficacy of 5307 corn against northern and Mexican corn rootworm larvae has not been as extensively studied, the trials to-date also indicate that 5307 corn is highly efficacious in reducing root feeding damage by these significant pests. Similarly, the MIR604 × 5307 breeding stack also appears to be highly effective.

Data in some trials suggested a possible efficacy advantage of combining the eCry3.1Ab trait in 5307 corn with the mCry3A trait in MIR604 corn, however, the numerical differences in efficacy ratings were not statistically significant. This may reflect the fact that the eCry3.1Ab protein in 5307 corn alone is highly effective in controlling rootworm larvae; incremental efficacy afforded by stacking the eCry3.1Ab and mCry3A traits may, therefore, be difficult to discern. Expanded pre-commercial product testing will more precisely define the relative efficacy profiles of 5307 and MIR604 × 5307 hybrids under a variety of growing conditions and levels of pest pressure. Nevertheless, as detailed in the discussion of insect resistance management benefits of 5307 corn there are compelling reasons to combine the traits in 5307 and MIR604 corn in breeding stacks for commercial deployment.

3. Grain yield studies of 5307 corn and stacked MIR604 × 5307 corn

Syngenta conducted field studies of 5307 corn grain yield over three years in 13 U.S. locations across the Corn Belt. The trial entries were planted in a randomized complete block design in fields that had been planted to a trap crop of cucurbits the previous season to attract corn rootworm beetles for increased egg accumulation (Branson and Sutter, 1989). The test and control corn hybrids evaluated were near-isogenic (i.e., in the same germplasm background). In most locations, 5307 corn was also compared to MIR604 corn, a MIR604 x 5307 corn breeding stack, as well as nontransgenic corn treated with Force® 3G insecticide, a granular soil applied tefluthrin (pyrethroid) formulation. Cultural practices used in the trials (tillage, fertilization, herbicide application, etc.) were typical of the recommended agricultural procedures for each area, except that all other insecticides (including seed treatments) were avoided.

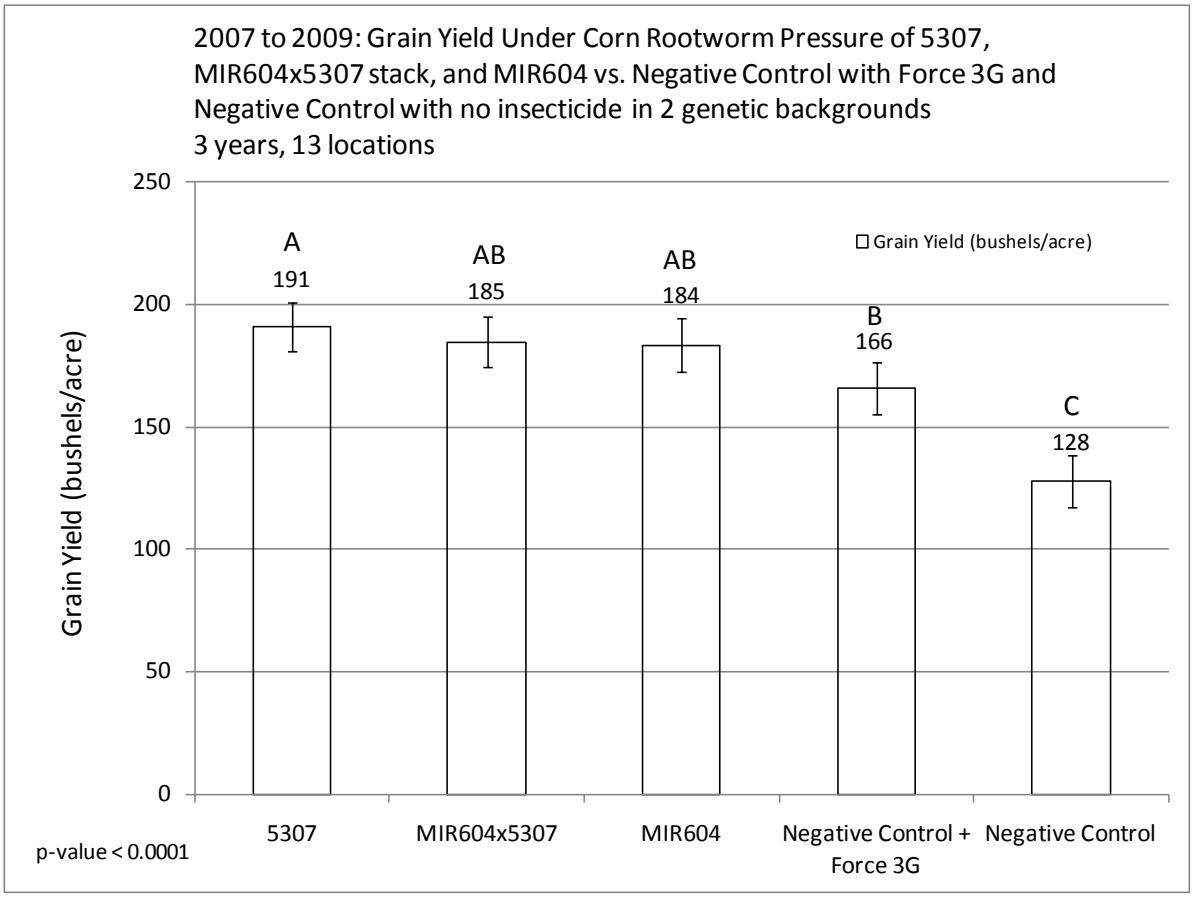
Under these conditions of rootworm pressure, the average yield advantage of 5307 corn was 63 bushels/acre compared to untreated control corn and 25 bushels/acre compared to control corn treated with Force 3G insecticide (Figure 9). Grain yield

was not statistically different between the 5307, MIR604 and MIR604 x 5307 corn test plots.

The 63 bushels/acre mean yield advantage for 5307 corn compares very favorably with a recently compiled, more extensive set of trial data for MIR604 hybrids (Agrisure® RW corn). These data demonstrate a 25 to 57 bushels/acre yield advantage of MIR604 corn over untreated control hybrids under conditions where rootworm populations reached economically damaging levels (Syngenta Agronomy Research, 2010).

Figure 9. Grain yield performance of 5307 corn and MIR604 × 5307 corn under corn rootworm pressure.

Means and standard errors are shown; means not labeled with the same letter differ statistically ($p < 0.05$).



IV. Public Interest Factors Applicable to 5307 Corn and the Breeding Stacks Thereof

The U.S. EPA's 1986 policy notice (EPA, 1996) regarding conditional registrations indicates that EPA will consider a variety of factors pertaining to the need for a new pesticide active ingredient, specifically its comparative benefits, risks, and costs. EPA policy (EPA, 1986) states: "The Agency must determine that (1) there is a need for the new chemical that is not being met with other currently registered pesticides or non-pesticide alternatives; (2) the new pesticide is comparatively less risky to health or the environment than currently registered pesticides; or (3) the benefits (including economic benefits) from the use of the new chemical exceed those of alternative registered pesticides and other available non-chemical techniques." A consideration of these factors, as they relate to 5307 corn and the breeding stacks thereof (Bt11 × MIR604 × TC1507 × 5307 and Bt11 × MIR162 × MIR604 × TC1507 × 5307 corn), clearly demonstrates that the associated PIP registrations will be in the public interest.

Registration of the Bt-derived eCry3.1Ab protein encoded by the pSYN12274 vector in 5307 corn can be presumed to be in the public interest because it meets the criteria for a conditional registration. Registration of a new pesticide is presumed to be in the public interest if it is a replacement for another pesticide that is of continuing concern to EPA. Corn varieties containing the 5307 transgenes have the potential to displace applications of conventional rootworm insecticides that are of concern to EPA, growers, and the public due to human and environmental risk factors. Additionally, the safety, convenience, and simplicity of the 5307 corn breeding stacks (Bt11 × MIR604 × TC1507 × 5307 and Bt11 × MIR162 × MIR604 × TC1507 × 5307 corn) compared to the application of conventional insecticides, along with the opportunity to extract an economic benefit through increased crop yield, are expected to be attractive to growers.

A. Need Factors

Prior to the introduction of rootworm-protected Bt corn varieties in 2003, the primary tactics for corn rootworm control were crop rotation (e.g., corn/soybean rotation), soil-applied insecticides, foliar insecticides and, to a lesser extent, insecticidal seed treatments. The current PIP proteins registered for corn rootworm control are Cry3Bb1 (in events MON 863 and MON 88017 corn), Cry34Ab1/Cry35Ab1 (in event DAS-59122-7 corn) and mCry3A (in Syngenta's event MIR604 corn).

Grower demand for corn rootworm control technologies is influenced by the level of corn rootworm infestation (acreage and degree of infestation), the comparative cost vs. benefit of competing corn rootworm control technologies, U.S. and global market acceptance and approval of a technology, and other regulatory constraints (e.g., refuge requirements). In the year 2000, prior to the introduction of corn rootworm-protected transgenic hybrids, almost 8 million pounds of corn rootworm insecticide, costing

$172 million ($12.29/acre), were applied to 14 million acres, or 17% of U.S. corn acreage. Between 2005 and 2013, corn-rootworm-infested acreage was projected to increase from approximately 31.8 million acres to 39 million acres (EPA, 2010). EPA estimated that the market for transgenic in-plant corn rootworm protection would increase by 2.6% per year, reaching 18 to 19 million acres by the year 2013. Product efficacy data provided herein indicate that hybrids containing the eCry3A.1Ab trait from 5307 corn will provide excellent protection against corn rootworm feeding damage, and that this protection will likely meet or exceed that of current control alternatives.

Increased adoption of corn rootworm-protected corn products is of special importance because many of the chemical insecticides registered for corn rootworm control present potential risks to applicators, other agricultural workers, and wildlife, and bear Restricted Use labels. The chemical insecticides for larval and adult corn rootworm control subject to the greatest use reductions following the adoption of transgenic rootworm-protected corn varieties are organophosphates, carbamates, pyrethroids, and pyrazoles. The availability of 5307 corn breeding stacks is expected to contribute to this significant trend in reduced use of hazardous insecticides. Adoption of rootworm-protected corn products is not expected to markedly reduce the use of insecticidal seed treatments; however, seed treatment products are applied at a lower rate per acre than soil-applied or foliar insecticides. (In addition to use of seed treatments for larval rootworm control, they are also used to control other soil pests such as wireworms and grubs.)

There is concern about the ability of corn rootworms to evolve resistance to control mechanisms, including crop rotation, chemical insecticides, and rootworm-protected Bt corn products. Because 5307 corn has excellent efficacy against corn rootworm and because the eCry3.1Ab protein operates via a unique mode of action, introduction of the 5307 corn breeding stacks is expected to extend the useful life of other commercially available corn rootworm-protected Bt corn products (i.e., the PIPs Cry3Bb1, Cry34Ab1/Cry35Ab1 and mCry3A). The availability of eCry3.1Ab as an additional tool for rootworm control will also reduce the selection pressure on rootworm populations to evolve resistance to other methods of control.

B. Composition Factors

The active insecticidal component of 5307 corn plants, eCry3.1Ab, is plant-incorporated, thus it fundamentally differs from the composition of conventional pest control products. The characteristics of PIPs virtually eliminate the occupational and environmental risks currently associated with the application of chemical controls for corn insect pests. These product characteristics support a conclusion that registration of 5307 corn is in the public interest.

C. Usage Factors

The safety, convenience, and simplicity of planting insect-protected corn compared to the application of conventional insecticides, along with the opportunity to extract an economic benefit through increased crop yield, are expected to make the 5307 breeding stack products highly attractive to growers. The restrictions and precautions associated with several conventional insecticides (Table 5) for rootworm control include:

- protective clothing (chemical-resistant gloves and other skin protection, eye protection, respirators, etc.) or other measures (closed-system applications) to minimize applicator exposure
- minimum worker reentry intervals post application
- minimum preharvest intervals post application
- restrictions against applying the product near bodies of water, when bees are present, or when spray drift is likely
- ensuring that granular insecticides are covered with soil post-application to protect wildlife
- recommendations regarding insect resistance management

By contrast, from the grower's perspective, the use of 5307 breeding stacks for insect control warrants only restrictions and obligations related to insect resistance management, including planting and managing refuge acres.

Registration of the PIPs in 5307 corn and the 5307 corn breeding stacks will help reduce the manufacture, transportation, storage, and disposal of millions of pounds of hazardous chemicals annually and reduce the fossil fuel consumption and greenhouse gas emissions associated with these activities.

D. Performance Factors

Multi-year and multi-location field efficacy trials demonstrate the substantial rootworm protection and yield advantages of 5307 corn, both alone and in combination with Syngenta's existing PIP rootworm control product, MIR604 corn (see Part III). The eCry3.1Ab protein is present in all tissues of 5307 corn plants, although the concentrations in pollen are extremely low (Nelson, 2010). Production of eCry3.1Ab in roots throughout the corn plant's development ensures protection where it is needed and eliminates the risk of insecticide failures associated with timing of soil-applied or foliar insecticide applications or unfavorable environmental conditions.

Integrated pest management (IPM) in agriculture includes insect scouting or monitoring to determine pest populations, consideration and application of compatible alternative biological, cultural, mechanical and chemical controls, and the establishment of action thresholds for agricultural inputs. The timely and targeted delivery of pest management interventions is key to successful IPM. The planting of

5307 corn stacks eliminates the need to target and time applications of rootworm insecticides, due to the sustained production of the insect control proteins (eCry3.1Ab and mCry3A) in vulnerable root tissues. The planting of 5307 corn stacks is compatible with current insect scouting and monitoring programs that provide data upon which to base crop management decisions. These seed products are also fully compatible with cultural control measures such as crop rotation. The 5307 corn breeding stacks will fit seamlessly into the concept of IPM for corn.

Table 5. Representative conventional insecticide products labeled for use in field corn to control corn rootworm larvae and/or adults.

Product	Active Ingredient(s); % of Formulation	Signal Word/ Classification	Environmental Hazards and/or Reasons for Restricted Use Classification
Ambush® Insecticide	permethrin; 25.6%	WARNING Restricted Use	Extremely toxic to fish and aquatic invertebrates, highly toxic to bees
Avicta® Duo Nematicide/Insecticide (seed treatment)	abamectin; 12.4% thiamethoxam; 28.1%	WARNING Restricted Use	Toxic to fish and wildlife, highly toxic to aquatic invertebrates, exposed treated seeds may be hazardous to wildlife
Aztec® 2.1% Granular Insecticide	tebupirimfos; 2.0% cyfluthrin; 0.1%	WARNING Restricted Use	Toxic to fish and wildlife
Baythroid® 2 Emulsifiable Pyrethroid Insecticide	cyfluthrin; 25%	DANGER Restricted Use	Extremely toxic to fish and aquatic invertebrates, highly toxic to bees
Capture® 2EC Insecticide/Miticide	bifenthrin; 25.1%	WARNING Restricted Use	Extremely toxic to fish and aquatic invertebrates, highly toxic to bees
Capture® LFR Insecticide	bifenthrin; 17.15%	WARNING Restricted Use	Extremely toxic to fish and aquatic invertebrates, highly toxic to bees
Counter® 15G Systemic Insecticide Nematicide	terbufos; 15%	DANGER Restricted Use	Highly toxic to fish and wildlife, known to cause fish kills, birds and mammals may be killed if granules not covered with soil. Fatal if swallowed, inhaled or absorbed through skin, corrosive, causes irreversible eye damage.
Counter 20G Systemic Insecticide - Nematicide	terbufos; 20%	DANGER Restricted Use	Highly toxic to fish and wildlife, known to cause fish kills, birds and mammals may be killed if granules not covered with soil. Acute dermal, oral and inhalation toxicity.
Cruiser® 5FS (seed treatment)	thiamethoxam; 47.6%	CAUTION	Toxic to wildlife, highly toxic to aquatic invertebrates, treated seeds exposed on soil surface may be hazardous to wildlife
DuPont Asana®XL Insecticide - 0.66 emulsible concentrate	esfenvalerate; 8.4%	WARNING Restricted Use	Extremely toxic to fish and aquatic invertebrates, highly toxic to bees
Declare® Emulsifiable Insecticide Concentrate	methyl parathion; 45.11%	DANGER. Restricted Use	Fatal if swallowed, inhaled or absorbed through skin, highly toxic to aquatic invertebrates and wildlife, highly toxic to bees
Dimethoate 4 EC Systemic Insecticide	dimethoate; 44.8%	WARNING	Toxic to wildlife and aquatic invertebrates, highly toxic to bees
Dimethoate 400 Systemic Insecticide - Miticide	dimethoate; 43.5%	WARNING	Toxic to wildlife and aquatic invertebrates, highly toxic to bees

Product	Active Ingredient(s); % of Formulation	Signal Word/ Classification	Environmental Hazards and/or Reasons for Restricted Use Classification
Force® 3G Insecticide	tefluthrin; 3%	CAUTION Restricted Use	very highly toxic to freshwater and estuarine fish and invertebrates
Force® ST Insecticide for corn seed treatment	tefluthrin; 26.8%	CAUTION	very highly toxic to freshwater and estuarine fish and invertebrates; exposed treated seeds may be hazardous to birds and other wildlife
Force® CS Insecticide	tefluthrin; 23.4%	WARNING Restricted Use	very highly toxic to freshwater and estuarine fish and invertebrates
Fortress® 2.5G granular insecticide	chlorethoxyfos; 2.5%	WARNING Restricted Use	toxic to wild mammals, birds, fish and aquatic invertebrates; harmful if absorbed through the skin
Fortress® 5G granular insecticide	chlorethoxyfos; 5%	DANGER Restricted Use	toxic to wild mammals, birds, fish and aquatic invertebrates; fatal if swallowed; may be fatal if inhaled; harmful if absorbed through skin
Furadan® 4F insecticide/ nematicide	carbofuran; 44%	DANGER Restricted Use	toxic to fish, birds and other wildlife; highly toxic to bees; can seep or leach through soil and can contaminate groundwater; poisonous if swallowed or inhaled; may be fatal or harmful as a result of skin or eye contact or breathing spray mist.
Furadan® LFR insecticide/ nematicide	carbofuran; 40.64%	DANGER Restricted Use	toxic to fish, birds and other wildlife; highly toxic to bees; can seep or leach through soil and can contaminate groundwater; poisonous if swallowed or inhaled; may be fatal or harmful as a result of skin or eye contact or breathing spray mist.
Gaucho® 600 Flowable (seed treatment)	imidacloprid; 48.7%	CAUTION	Highly toxic to birds and aquatic invertebrates
Gaucho® 75 ST Insecticide (seed treatment)	imidacloprid; 75%	CAUTION	Highly toxic to birds and aquatic invertebrates
Lambda 25 CS	lambda-cyhalothrin; 23.6%	WARNING Restricted Use	Extremely toxic to fish and aquatic invertebrates; toxic to wildlife; highly toxic to bees
Lannate® LV insecticide	methomyl; 29%	DANGER Restricted Use	Fatal if swallowed, toxic to fish, aquatic invertebrates and mammals, highly toxic to bees, known to leach through soil into groundwater
Lannate® SP insecticide	methomyl; 90%	DANGER Restricted Use	Fatal if swallowed, may cause blindness, toxic to fish, aquatic invertebrates and mammals, highly toxic to bees, known to leach through soil into groundwater
Lorsban® 15G Granular Insecticide	chlorpyrifos; 15%	CAUTION	Toxic to fish, aquatic invertebrates, birds and small mammals, highly toxic to bees
Lorsban® -4E Insecticide	chlorpyrifos; 44.9%	WARNING Restricted Use	Toxic to fish, aquatic invertebrates, birds and small mammals, highly toxic to bees

Product	Active Ingredient(s); % of Formulation	Signal Word/ Classification	Environmental Hazards and/or Reasons for Restricted Use Classification
Lorsban® 75WG Insecticide	chlorpyrifos; 75%	WARNING	Toxic to fish, aquatic invertebrates, birds and small mammals, highly toxic to bees
Mocap® 15% Granular Nematicide/ Insecticide	ethoprop; 15%	DANGER Restricted Use	Toxic to aquatic organisms and wildlife; extremely toxic to birds; toxic to bees; fatal if absorbed through skin; may be fatal if swallowed; causes irreversible eye damage; harmful if inhaled
Penncap-M® Microencapsulated Insecticide	methyl parathion; 22.0%	WARNING Restricted Use	Highly toxic to aquatic invertebrates and wildlife
Phorate 20 G Organophosphate Insecticide	phorate; 20.0%	DANGER Restricted Use	Very highly toxic to fish and wildlife. Birds and mammals may be killed if granules are not properly covered with soil. Fatal if swallowed, inhaled or absorbed through the skin; corrosive; causes irreversible eye damage
Poncho® 600 (seed treatment)	clothianidin; 48%	CAUTION	Toxic to aquatic invertebrates
Pounce® 3.2 EC Insecticide	permethrin; 38.4%	CAUTION. Restricted Use	Extremely toxic to fish and aquatic invertebrates, highly toxic to bees
Pounce® 25 WP Insecticide	permethrin; 25%	WARNING. Restricted Use	Extremely toxic to fish and aquatic invertebrates, highly toxic to bees
Sevin® 80 Solupak	carbaryl; 80%	WARNING	Extremely toxic to aquatic invertebrates, highly toxic to bees
Sevin® brand XLR PLUS Carbaryl Insecticide	carbaryl; 44.1%	CAUTION	Extremely toxic to aquatic invertebrates, highly toxic to bees
Tundra® Max Insecticide	chlorpyrifos; 28.6% bifenthrin; 9.0%	WARNING Restricted Use	Extremely toxic to fish, aquatic invertebrates, small mammals, and birds. Highly toxic to bees.
Warrior II with Zeon Technology® Insecticide	lambda-cyhalothrin; 22.8%	WARNING. Restricted Use	Extremely toxic to fish and aquatic organisms and toxic to wildlife, highly toxic to bees
Zeta-Cype 0.8EW Insecticide	zeta-cypermethrin; 9.2%	WARNING Restricted Use	Extremely toxic to fish, aquatic invertebrates, oysters and shrimp; highly toxic to bees

E. Risk Factors

1. Reduced-risk alternative to conventional insecticides

Representative conventional insecticide products registered for control of corn rootworm in field corn are listed in Table 5. Several are classified as Restricted Use Pesticides due to human and/or environmental risk concerns. The Restricted Use classification, imposed due to adverse environmental effects under normal use practices or applicator safety concerns [40 CFR 152.171(a)], limits the use of these chemicals to certified pesticide applicators who have received special training needed for safe handling and application of these products. Personal protective equipment to reduce occupational exposure, special labeling, and special record keeping are required for the sale and use of Restricted Use Pesticides. Without these restrictions, EPA has determined that use of these products may cause unreasonable adverse effects on humans and/or the environment. This classification is an indication of EPA concern about the safety of these products.

Event 5307 corn has the potential to displace the use of many of the Restricted Use Pesticides that are currently being used for control of corn rootworms. Based on this consideration alone, the plant-incorporated eCry3.1Ab pesticidal protein encoded in 5307 corn is entitled to a presumption of public interest.

2. Safety profile of 5307 corn and 5307 corn breeding stacks

A standard battery of mammalian toxicity studies were conducted for the eCry3.1Ab protein produced in5307 corn. These studies found no evidence of eCry3.1Ab protein-induced adverse effects. The protein is rapidly degraded in mammalian digestive systems and bears no amino acid sequence similarities to known toxins or allergens. Because the insecticidal protein is plant-incorporated, the opportunity for exposure when handling and planting seed is insignificant. Planting of 5307 corn breeding stacks (Bt11 × MIR604 × TC1507 × 5307 and Bt11 × MIR162 × MIR604 × TC1507 × 5307 corn) by growers will essentially eliminate the environmental and occupational health risks currently associated with chemical controls for corm rootworm pests (via the rootworm-control PIPs in 5307 and MIR604 corn) as well as for the lepidopteran pests that will otherwise be controlled by the PIP traits in one or both of the 5307 breeding stacks (i.e., the PIPs in Bt11, TC1507, and MIR162 corn).

The selectivity of eCry3.1Ab for certain coleopteran pests minimizes risk for nontarget organisms. A series of hazard identification studies has been conducted with nontarget indicator species, including many species that are part of the corn ecosystem. No adverse effects attributable to eCry3.1Ab proteins were observed in these studies, even at exposure levels exceeding expected environmental concentrations (Nelson, 2010). Additionally, no adverse effects are predicted from use of the 5307 corn breeding stacks (Raybould, 2011a, 2011b).

Primary roots of the seedling and the radical and seminal roots are commonly infected with *Fusarium* spp. after they have served their function and become senescent. Feeding

by corn rootworms has been associated with increased frequencies of *Fusarium* infection (Dicke and Guthrie, 1988). Rootworm feeding may also lead to increased incidences of stalk rots. These pathogen infections can lead to reductions in crop quality, harvestability, and yield. Due to the superior protection from damage that will be afforded by planting 5307 corn there is a potential health benefit for the livestock industry resulting from reduced *Fusarium* levels in livestock feed.

F. Economic Factors

The adoption of new varieties improved through biotechnology has added greatly to farm productivity and profits since their introduction (Brookes and Barfoot, 2010). Varieties containing herbicide tolerance and insect protection traits have been widely adopted by corn, soybean, and cotton growers because they protect the inherent yield potential of these crops and typically reduce grower input costs. Insect-protected varieties, including stacked-trait varieties that offer insect protection and herbicide tolerance, accounted for 63% of corn planted in 2009 (USDA, 2009a). The adoption of transgenic corn varieties is estimated to have reduced the application of conventional pesticides by more than 20 million pounds annually (NCGA, 2010b; Doane, 2010).

Event 5307 corn demonstrates excellent efficacy against western, northern and Mexican corn rootworms. Moreover, 5307 corn hybrids demonstrated an average grain yield advantage of 63 bushels/acre over the corresponding control hybrids in the presence of significant larval rootworm pressure. Growers will realize significant economic gain due to the insect-control efficacy and associated yield advantage of the 5307 corn breeding stacks under rootworm pressure.

In breeding stacks with MIR604 corn, a justified reduction in the size of the previously required 20% structured corn rootworm refuge to 5% of a grower's corn acres will have further economic benefits. The cost of chemical insecticide control on the previous refuge acres will be reduced, while grain yields for these acres will potentially increase.

Because the eCry3.1Ab protein in 5307 corn does not compete for the same larval gut binding site as mCry3A in MIR604 corn (Walters et al., 2010), it offers key advantages for insect resistance management when stacked with MIR604 corn. Moreover, it can be expected to extend the durability of other commercially available corn rootworm PIPs as well as other methods of corn rootworm control, thereby helping to maintain lower overall costs for pest control.

The availability of multiple rootworm-protected corn products will increase grower choice and price competition, potentially resulting in lower seed prices for growers and higher adoption rates. Additionally, the availability of a new and reliable pest-control option that promotes higher yield, more efficient use of water and fertilizer, healthier plants, and better grain quality, while reducing the environmental impacts of agriculture, will operate to reduce the costs of producing corn and contribute to global food security. These substantial economic benefits indicate that registration of 5307 corn is in the public interest.

V. References

Baker, A., Allen, E. and H. Lutman (2010). Feed outlook. A Report from the Economic Research Service, United States Department of Agriculture. FDS-10b, January 14, 2010. Accessed March 13, 2010: http://usda.mannlib.cornell.edu/usda/ers/FDS//2000s/2010/FDS-01-14-2010.pdf

Branson, T.F. and G.R. Sutter (1989). Evaluating and Breeding for Maize Resistance to the Rootworm Complex. *In*: Toward Insect Resistant Maize for the Third World: Proceedings of the International Symposium on Methodologies for Developing Host Plant Resistance to Maize Insects. Mexico, D., F.: CIMMYT.

Brookes, G. and P. Barfoot (2010). GM crops: global socio-economic and environmental impacts 1996- 2008. PG Economics Ltd: Dorchester, UK. Accessed March 29, 2011: http://www.pgeconomics.co.uk/pdf/2010-global-gm-crop-impact-study-final-April-2010.pdf

Carpenter, J. (2011). Impacts of GM crops on biodiversity. *GM Crops* 2:1, 1-17; January/February/March 2011. published online in advance of print edition. https://www.landesbioscience.com/journals/gmcrops/article/15086/

Dicke, F. F. and W. D. Guthrie (1988). The most important corn insects. Pp 767-867. *In* Corn and Corn Improvement. Third Edition. Sprague, G. F. and J. W. Dudley (eds.). American Society of Agronomy, Crop Science Society of America and Soil Science Society of America, Madison, Wisconsin.

Doane (2010). 2009 AgroTrak. The US agrochemical usage study. A Doane Marketing Research syndicated tracking study. St. Louis, Missouri.

EPA (1986). U.S. Environmental Protection Agency. Conditional registration of new pesticides. *Federal Register* **51**:7628.

EPA (2010). U.S. Environmental Protection Agency. Biopesticides Registration Action Document - Modified Cry3A Protein and the Genetic Material Necessary for its Production (via Elements of pZM26) in Event MIR604 Corn SYN-IR604-8. Office of Pesticide Programs; Biopesticides and Pollution Prevention Division. Accessed March 30, 2011: http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2010-0607-0027.

Flavell, R. (2010). Knowledge and technologies for sustainable intensification of food production. *New Biotechnology* 27: 505-516. Transgenic Plants for Food Security in the Context of Development – Proceedings of a Study Week of the Pontifical Academy of Sciences, 30 November 2010. http://www.sciencedirect.com/science/article/B8JG4-507CS31-1/2/2f9dde686fc1dcc2390c7e2106d21004

Gray, M. and W. Luckmann (1994). Integrating the cropping system for corn insect pest management. pp 507-541 *In* Introduction to Insect Pest Management. 3rd Ed. Metcalf, R. L. and W. H. Luckmann (eds.). John Wiley & Sons, Inc., New York,

New York.

Haney, L., J. White and M. Meghji (2008). Efficacy of 5307 Maize Hybrids Against Western Corn Rootworm in the Field. Unpublished Syngenta Report No. SSB-505-08. US EPA MRID 47734718.

Haney L., J. White and M. Meghji (2009). Efficacy of a 5307 Maize Hybrid Against Western Corn Rootworm in the Field. Unpublished Syngenta Report No. SSB-139-09. US EPA MRID 47734719.

Harper, B. (2011). eCry3.1Ab (Entrez® Database Accession Number ADC30135): Assessment of Amino Acid Sequence Similarity to Known or Putative Allergens. Unpublished Syngenta report, No. SSB-112-11. (Included as a data volume in submission for EPA registration.)

Hein, G.L., M.K. Bergman, R.G. Bruss, and J.J. Tollefson (1985). Absolute sampling technique for corn rootworm (Coleoptera: Chrysomelidae) adult emergence that adjusts to common-row spacing. *J. Econ. Entomol.* 78: 1503-1506.

Hibbard, B. (2009) Selection intensity of SYN101 and its stack with MIR604. Columbia, MO. Unpublished report to Syngenta Biotechnology, Inc.

Jones, W. (2009) Characterization and Safety of eCry3.1Ab Protein and Corn Plants Derived from Event 5307. Unpublished Syngenta Document No. 5307-EPA-EUP. US EPA MRID 47734701.

Korgaonkar, C. K. (2009) ECRY3.1AB-0208: Single-Dose Oral (Gavage) Toxicity Study in Mice with a 14-Day Observation Period. Unpublished Syngenta Study No. WIL-639031. US EPA MRID 47753901.

Krysan, J.L. (1986). Introduction: biology, distribution, and identification of pest *Diabrotica*, pp. 1-24, *In* J.L. Krysan and T.A. Miller (eds.), Methods for the Study of Pest *Diabrotica*. Springer-Verlag, New York.

Marra, M., P. Pardey, and J. Alston (2002). The payoffs to transgenic field crops: An assessment of the evidence. *AgBioForum* 5:43-50. http://www.agbioforum.org/v5n2/v5n2a02-marra.htm

Marroquin, L.D., D. Elyassnia, J.S. Griffitts, J.S. Feitelson, R.V. Aroian (2000) *Bacillus thuringiensis* susceptibility and isolation of resistance mutants in the nematode *Caenorhabditis elegans*. *Genetics* 155: 1693-1699.

McClain, S. (2011). eCry3.1Ab (Entrez® Database Accession Number ADC30135): Assessment of Amino Acid Sequence Similarity to Known or Putative Toxins. Unpublished Syngenta Report No. SSB-165-11. (Included as a data volume in submission for EPA registration.)

National Corn Growers Association (2010a). Corn Production Trends. Accessed February 28, 2010: http://www.ncga.com/corn-production-trends

National Corn Growers Association (2010b). 2010 World of corn. Innovation in Action. National Corn Grower's Association. www.worldofcorn.com. Accessed May 10, 2010: http://ncga.com/files/pdf/WOC2010.pdf

Nelson, A. (2008) Characterization of Test Substance ECRY3.1AB-0208 and Certificate of Analysis. Unpublished Syngenta Report No. SSB-010-08. MRID 47734704.

Nelson, A. (2009) Comparison of eCry3.1Ab Protein Produced in Event 5307-Derived Maize Plants and eCry3.1Ab Protein Produced in Recombinant *Escherichia coli*. Unpublished Syngenta Report No. SSB-002-09. MRID 47734703.

Nelson, A. (2010). The Environmental Fate and Effects of eCry3.1Ab in Event 5307 Maize: Estimated Environmental Concentrations, Margins of Exposure in Nontarget Organism Studies, and US Endangered Species Assessment. Unpublished Syngenta Report No. SSB-235-10. (Included as a data volume in submission for EPA registration.)

Oleson, J.D., Y. Park, T.M. Nowatzki and J.J. Tollefson. (2005). Node-injury scale to evaluate root injury by corn rootworms. *J. Econ. Entomol.* 98(1): 1-8.

Raybould, A. (2011a). Non-Target Organism Risk Assessment for Bt11 x MIR604 x TC1507 x 5307 x GA21 Stacked Maize (Field Corn) Hybrids. Unpublished Syngenta Report No. NRA-115-11. (Included as a data volume in submission for EPA registration.)

Raybould, A. (2011b). Non-Target Organism Risk Assessment for Bt11 x MIR162 x MIR604 x TC1507 x 5307 x GA21 Stacked Maize (Field Corn) Hybrids. Unpublished Syngenta Report No. NRA-114-11. (Included as a data volume in submission for EPA registration.)

Seastrum, L. (2009). *In vitro* Digestibility of eCry3.1Ab Protein as Contained in Test Substance ECRY3.1AB-0208 Under Simulated Mammalian Intestinal Conditions. Unpublished Syngenta Report No. SSB-015-09. (Included as a data volume in submission for EPA registration.)

Seastrum, L., K. Ward, and A. Raybould (2010). Investigation of the Potential Interaction between Lepidopteran-active (Cry1Ab + Vip3Aa20 + Cry1F) and Coleopteran-active (mCry3A + eCry3.1Ab) Insecticidal Bt Proteins Using Target Insects *Ostrinia nubilalis* and *Leptinotarsa decemlineata*. Unpublished Syngenta Report No. TK0026520. (Included as a data volume in submission for EPA registration.)

Seastrum, L. (2010). Investigation of the Potential Interaction between Insecticidal Proteins mCry3A + eCry3.1Ab Using Colorado Potato Beetle (*Leptinotarsa decemlineata*). Unpublished Syngenta Report No. TK0026862. (Included as a data volume in submission for EPA registration.)

Song, S. (2010). *In vitro* digestibility of eCry3.1Ab Protein under simulated mammalian gastric conditions. Unpublished Syngenta Report No. TK0028111. (Included as a data volume in submission for EPA registration.)

Syngenta Agronomy Research (2010). Corn rootworm management guide. Accessed March 20, 2011: http://www.syngenta.com/country/us/en/Seeds/PDF%20and%20Other%20document%20Storage%20Library/Ag_Reports/CornRootworm_Syngenta_FINAL.pdf.

USDA (2009a). Acreage. June 30. Cr Pr 2-5 (6-09). U.S. Department of Agriculture –

National Agricultural Statistics Service. Washington, DC. Accessed May 12, 2010: http://usda.mannlib.cornell.edu/usda/current/Acre/Acre-06-30-2009.pdf.

USDA (2009b). Data and statistics: Quick stats. Washington, DC: U.S. Department ofAgriculture - National Agricultural Statistics Service. Accessed May 12, 2010: http://www.nass.usda.gov/Data_and_Statistics/Quick_Stats/index.asp

USDA (2010a). World agricultural production. Foreign Agricultural Service, United States Department of Agriculture. February 2010. Accessed March 13, 2010: http://www.fas.usda.gov/wap/circular/2010/10-02/productionfull02-10.pdf

USDA (2010b). Crop production 2009 summary. National Agricultural Statistics Service, United States Department of Agriculture. Report Cr Pr 2-1(10), January 2010. Accessed March 13, 2010: http://usda.mannlib.cornell.edu/usda/nass/CropProdSu//2010s/2010/CropProdSu-01-12-2010.pdf

USDA (2010c). Agricultural Trade Multipliers: Effects of Trade on the U.S. Economy. Updated February 8, 2010. Accessed April 29, 2010: http://www.ers.usda.gov/Data/TradeMultiplier/econeffects/2008overview.aspx

Walters, F.S., C.M. deFontes, H. Hart, G.W. Warren and J.S. Chen (2010). Lepidopteran-active variable region sequence imparts coleopteran activity in eCry3.1Ab, an engineered *Bacillus thuringiensis* hybrid insecticidal protein. *Appl. Env. Microbio.* 76: 3082–3088. (Included as a data volume in submission for EPA registration.)

Ward, D., T. DeGooyer, T. Vaughn, G. Head, M. McKee, J. Astwood, and J. Pershing. (2005). Genetically enhanced maize as a potential management option for corn rootworm: YieldGard® rootworm maize case study. Pp 239-262. *In* Western Corn Rootworm: Ecology and Management. Vidal, S., U. Kuhlmann, and C. Edwards (eds.). CAB International, Wallingford, Oxfordshire.

Wei, J.-Z., K. Hale, L. Carta, E. Platzer, C. Wong, S-C Fang, and R.V. Aroian (2003). *Bacillus thuringiensis* crystal proteins that target nematodes. *Proc. Natl. Acad. Sci. USA* 100: 2760-2765.

■

Appendix B
Safety and Nutritional Assessment

Biotechnology Consultation Document for the United States Food and Drug Administration

Safety and Nutritional Assessment of Event 5307 Corn

OECD Unique Identifier: SYN-Ø53Ø7-1

Submitted By:

Demetra Vlachos
Syngenta Seeds, Inc.
P.O. Box 12257
3054 East Cornwallis Road
Research Triangle Park, NC 27709-2257

Date:

January 27, 2011

Authors:

Lawrence Zeph, Ph.D. and Demetra Vlachos, M.S.

Syngenta Seeds, Inc.

I. Administrative

I.A. Table of Contents

I.B. List of Figures

I.C. List of Tables

I.D. Release of Information

Syngenta is submitting the information in this consultation document for review by the United States Food and Drug Administration (FDA) as part of a federal regulatory process. By submitting this information to FDA, Syngenta does not authorize its release to any third party. In responding to a request made under the Freedom of Information Act (FOIA), 5 U.S.C. §552, covering all or some of the information in this consultation document, Syngenta expects that, in advance of the release of the information, FDA will provide Syngenta with a copy of the FOIA request and the material proposed to be released, and the opportunity to object to the release of information based on appropriate legal grounds (e.g., responsiveness, trade secret, and/or commercial concerns). Except in accordance with the foregoing, Syngenta does not authorize the release, publication, or other distribution of this information (including internet posting) without prior notice and Syngenta consent.

I.E. Abbreviations and Acronyms

5307	Event 5307; derived from corn transformation Event 5307
ADF	acid detergent fiber
AEBSF	4-(2-aminoethyl)-benzenesulfonylfluoride HCl
ANOVA	analysis of variance
AOAC	Association of Analytical Communities
APHIS	Animal and Plant Health Inspection Service
BC	backcross
BCA	bicinchoninic acid
BME	beta-mercaptoethanol
bp	base pairs
BSA	bovine serum albumin
B.t.	Bacillus thuringiensis
cm	centimeter(s)
CMP	cestrum yellow leaf curling virus promoter
CFR	U.S. Code of Federal Regulations
Cry1	crystal protein from Bacillus thuringiensis
CTAB	hexadecyltrimethylammonium bromide
Da	daltons
DIG	digoxigenin
DNA	deoxyribonucleic acid
dsDNA	double-stranded deoxyribonucleic acid
DTT	dithiothreitol
DW	dry weight
EDTA	ethylenediamine tetraacetic acid
ELISA	enzyme-linked immunosorbent assay
EPA	U.S. Environmental Protection Agency
F1	first generation of progeny from a breeding cross
FARRP	Food Allergy Research and Resource Program
FDA	Food and Drug Administration
FFDCA	Federal Food, Drug and Cosmetic Act
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
FOIA	Freedom of Information Act
FW	fresh weight
G6PDH	glucose 6-phosphate dehydrogenase
gdw	grams dry weight
IgG	immunoglobulin G
ILSI	International Life Sciences Institute
kb	kilobase(s)
kDa	kilodaltons
LB	left border
LC50	median lethal concentration; a statistically derived estimate of the concentration resulting in mortality of 50% of the test organisms
LOD	limit of detection
LOQ	limit of quantification
LTR	long terminal repeat

MES	2-(N-morpholino)ethanesulfonic acid
MW	molecular weight
N	number of samples
NADP	β-nicotinamide adenine dinucleotide phosphate
NADPH	β-nicotinamide adenine dinucleotide phosphate reduced
NCBI	National Center for Biotechnology Information
NDF	neutral detergent fiber
No.	number
NOS	nopaline synthase
OD	optical density
OECD	Organisation for Economic Co-operation and Development
ORF	open reading frame
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PMI	phosphomannose isomerase
PVDF	polyvinylidene difloride
PVP	polyvinylpyrrolidone
RB	right border
SD	standard deviation
SDS	sodium dodecylsulfate
SDS-PAGE	sodium dodecylsulfate polyacrylamide gel electrophoresis
SEM	standard error of the mean
spec	streptomycin adenylyltransferase gene from Escherichia coli
SSC	saline-sodium citrate buffer
T-DNA	transferred deoxyribonucleic acid
TMB	tetramethylbenzidine
US	United States
U.S.C.	United States Code
USDA	U.S. Department of Agriculture
vir	virulence regulon in Agrobacterium tumefaciens
v/v	volume per volume
w/v	weight per volume
w/w	weight per weight
ZmUbiInt	Zea mays ubiquitin promoter with intron

I.F. Summary of the Safety and Nutritional Assessment of Event 5307 Corn

The proteins encoded by the transgenes *ecry3.1Ab* and *pmi* in Syngenta's Event 5307 corn have been evaluated for their food and feed safety, and additional studies have demonstrated that grain and forage from Event 5307 corn are nutritionally equivalent to conventional corn. The gene *ecry3.1Ab* encodes a chimeric protein, eCry3.1Ab, that represents a fusion between two *Bacillus thuringiensis*-derived proteins: modified Cry3A (mCry3A) and Cry1Ab. The gene *pmi* encodes the enzyme phosphomannose isomerase (PMI), which was used as a selectable marker in the production of Event 5307 corn.

The species-specific insecticidal mode of action of eCry3.1Ab and its similarity to other Cry proteins for which human safety has previously been established (e.g., mCry3A, Cry3A, and Cry1Ab) support the prediction that no adverse health effects will result from exposure to the eCry3.1Ab protein present in 5307 corn. The safety of PMI has been established previously, and it is exempt from food and feed tolerances in all crops.

No toxicity to mammals was observed following oral exposure to high doses of eCry3.1Ab, and the properties of this protein do not indicate allergenic potential. The large body of data and information described herein support the conclusion that there is reasonable certainty that no harm will result from mammalian (including human) exposure to eCry3.1Ab and PMI produced in 5307 corn.

Analyses of key nutritional components of forage and grain from 5307 corn showed that no biologically significant changes in composition occurred as an unintended result of the transformation process or expression of the transgenes in 5307 corn. Forage and grain from 5307 corn are similar in composition to forage and grain from conventional corn. In addition, a 49-day feeding study demonstrated that there were no adverse dietary effects on broiler chickens consuming diets prepared with 5307 corn grain when compared with those consuming diets prepared with nontransgenic control corn grain, either as a direct effect of the transgenic proteins in the diet or as a result of any unintended compositional changes in the grain that may have altered its nutritional value. These results support the conclusion that 5307 corn is nutritionally comparable to and as safe as conventional corn.

II. Synopsis of the Safety and Nutritional Assessment of Event 5307 Corn

II.A. Name and Address of the Submitter

Submitting company:

Syngenta Seeds, Inc.
P.O. Box 12257
3054 East Cornwallis Road
Research Triangle Park, North Carolina
27709-2257

Company representative:

Demetra Vlachos
Senior Regulatory Affairs Manager
Tel. 919-226-7383; Mobile 919-433-7642; Fax 919-226-7462
demetra.vlachos@syngenta.com

II.B. Subject of This Consultation and the Plant Species from Which it is Derived

The subject of this consultation is coleopteran-resistant corn (maize; *Zea mays* L.), derived from transformation Event 5307 (hereafter "Event 5307 corn" or "5307 corn"). The variety that was transformed to produce Event 5307 corn was NP2222, an elite Syngenta inbred field corn variety.

II.C. Designation of Transformation Event

The designation of the transformation event is Event 5307 corn, which has been assigned the OECD Unique Identifier SYN-Ø53Ø7-1.

II.D. Description of the Genetic Materials Introduced Into Event 5307 Corn

See description in Part IV below.

II.E. The Intended Technical Effect of Event 5307 Corn

Event 5307 corn contains the transgenes *ecry3.1Ab* and *pmi* (also known as *manA*), which encode the proteins eCry3.1Ab and phosphomannose isomerase (PMI), respectively. The eCry3.1Ab protein is a chimeric protein comprised largely of portions of a modified Cry3A (mCry3A) protein and a Cry1Ab protein derived from *Bacillus thuringiensis*, a ubiquitous

bacterium. The eCry3.1Ab[1] protein is insecticidally active against certain Coleoptera, including western corn rootworm *(Diabrotica virgifera virgifera)*, northern corn rootworm *(D. longicornis barberi)* and Mexican corn rootworm *(D. virgifera zeae)*, which are significant pests of US corn. PMI was utilized as a selectable marker during the development of Event 5307 corn, and serves no agronomic or other purpose in the plant. In breeding combinations with other insect-resistant corn varieties, 5307 corn will allow growers to have optimal broad-spectrum control of several significant corn pests. No intended compositional changes are expected as a result of the genetic modification in Event 5307 corn. Event 5307 corn is as safe and nutritious as food and feed derived from conventional corn varieties.

II.F. Application and Uses of Event 5307 Corn

Event 5307 corn will be grown for the same uses as current commercially available corn in the US. In 2010, there were an estimated nearly 88 million acres planted to corn in the US, producing over 13 billion bushels of grain (USDA-NASS 2010). Corn grown in the US is predominantly of the yellow dent type, a commodity crop largely used to feed domestic animals, either as grain or silage. The remainder of the crop is exported or processed by wet or dry milling to yield products such as high fructose corn syrup and starch or oil, grits and flour. These processed products are used extensively in the food industry. For example, corn starch serves as a raw material for an array of processed foods, and in industrial manufacturing processes. Since the early 1980s, a significant amount of corn grain has also been used for fuel ethanol production. The by-products from these distilling processes are often used in animal feeds.

II.G. Applications for Which Event 5307 Corn is Not Suitable

Event 5307 corn is suitable for all uses applicable for conventional corn.

[1] The descriptor "eCry3.1Ab" was assigned by Syngenta; the "e" denotes that it was engineered, and the "Cry3" and "1Ab" descriptors relate to the respective source Cry (crystal) proteins, mCry3A and Cry1Ab. The eCry3.1Ab protein has not been assigned an official Cry protein designation under the formal nomenclature scheme for *B. thuringiensis* Cry proteins (Crickmore et al. 2010).

III. Status with Other Regulatory Agencies

III.A. Submissions to US Agencies

III.A.1. Environmental Protection Agency

Substances that are pesticides, as defined under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), are subject to regulation by the US Environmental Protection Agency (EPA). The eCry3.1Ab protein encoded by the genetic insert in Event 5307 corn has insecticidal activity against several significant coleopteran pests and is, therefore, regulated by the EPA as a plant-incorporated protectant. Pursuant to § 408(d) of the Federal Food, Drug and Cosmetic Act, a temporary exemption from the requirement of food and feed tolerances was established, effective June 10, 2010, under 40 CFR §174.532 for eCry3.1Ab in corn. This exemption is currently set to expire June 1, 2012, however, a petition to renew the exemption is currently pending at the EPA. A petition for a permanent tolerance exemption for eCry3.1Ab will be submitted by Syngenta to the EPA in early 2011, concurrently with a FIFRA §3 registration application for use of eCry3.1Ab in Event 5307 corn.

A permanent exemption from the requirement of a tolerance exists under 40 CFR §174.527 for PMI in all plants, in connection with its use as a selectable marker in pesticidal transgenic plants.

III.A.2. Department of Agriculture

A petition (number 10-336-01p) for the determination of nonregulated status under 7 CFR Part 340.6 for Event 5307 corn was submitted to the US Department of Agriculture (USDA) on December 1, 2010.

III.B. Submissions to Foreign Agencies

Syngenta also intends to commercialize Event 5307 corn for cultivation in Canada and will seek the necessary food, feed and environmental approvals to do so. Syngenta will also pursue regulatory approvals for importation of Event 5307 corn in key export markets for US and Canadian corn (including Japan, Taiwan, South Korea, the Philippines, and Mexico) where functioning systems exist for regulating imports of genetically modified crops.

Syngenta will additionally examine commercial opportunities in other countries where corn rootworm species are significant economic pests of corn. In countries where commercial opportunities exist, Syngenta may apply for cultivation approvals in the future.

IV. Transformation and Development of Event 5307 Corn

IV.A. Characterization of the Parent Plant – Corn

IV.A.1. Description of *Zea mays* L. (Corn; Maize)

Zea is a genus of the family Graminae (Poaceae), commonly known as the grass family. Corn (*Zea mays* L.; maize) is a tall, monecious annual grass with overlapping sheaths and broad conspicuously distichous blades. Plants have staminate spikelets in long spike-like racemes that form large spreading terminal panicles (tassels) and pistillate inflorescences in the leaf axils, in which the spikelets occur in 8 to 16 rows, approximately 30 cm long, on a thickened, almost woody axis (cob). The whole structure (ear) is enclosed in numerous large foliaceous bracts and long styles (silks) protrude from the tip of the ear as a mass of silky threads (Hitchcock and Chase 1971). Pollen is produced entirely in the staminate inflorescence, and eggs entirely in the pistillate inflorescence. Corn is wind-pollinated and both self- and cross-pollination are usually possible. Shed pollen usually remains viable for 10 to 30 minutes, but can remain viable for longer durations under favorable conditions (Coe et al. 1988). Cultivated corn is presumed to have been derived from teosinte (*Z. mexicana*) and is thought to have been introduced into the old world in the sixteenth century. Corn is cultivated worldwide and represents a staple food for a significant proportion of the world's population. No significant native toxins are reported to be associated with the genus *Zea* (International Food Biotechnology Council 1990).

IV.A.2. Origin of the Species *Zea mays* L.

It is generally agreed that teosinte (*Z. mexicana*) is an ancestor of corn, although opinions vary as to whether corn is a domesticated version of teosinte (OECD 2003). Teosinte is an ancient wild grass found in Mexico and Guatemala. Because it has differentiated into various races, species and plant habits, taxonomic classification is still a matter of controversy. Doebley and Iltis (1980) and Iltis and Doebley (1980) classified the annual teosintes into two subspecies of *Z. mays,* ssp. *mexicana* (including races Chalco, Central Plateau and Nobogame) and ssp. *parviglumis* var. *parviglumis* (race Balsas) and var. *huehuetenangensis* (race Huehuetenango), and the species *Z. luxurians* (race Guatemala). The perennial teosintes from Jalisco, Mexico are separated into two more species according to ploidy, *Z. perennis* and *Z. diploperennis*. The Meso-American region located within middle South Mexico and Central America is recognized as one of the main centers of origin and development of agriculture as well as center of origin and diversification of more than one hundred crops (OECD 2003). At the present time, there is no agreement about where exactly corn was domesticated and there are several proposals in this regard. Based on the findings of archaeological materials from the corn plant (pollen, cobs, husks, and other remnants) in the US and Mexico that are older than those found in South America, Randolph proposed that corn was domesticated, independently, in the southwestern US, Mexico, and

Central America (OECD 2003). Mangelsdorf proposed that "corn had not one origin but several in both Mexico and South America," because the archaeological evidence is found in Mexico and several morphological characteristics of extant populations are found in the corn races of South America (Andes region) in comparison to those races of Meso-America (OECD 2003).

IV.B. Description of the Genetic Materials Introduced into 5307 Corn

Event 5307 corn was produced by *Agrobacterium tumefaciens*-mediated transformation of immature corn embryos using the transformation plasmid vector pSYN12274. The DNA region between the left and right borders of the transformation plasmid included gene expression cassettes for *ecry3.1Ab* and *pmi* (also known as *manA*); this T-DNA was transferred into the corn genome during transformation. The *ecry3.1Ab* expression cassette consisted of the *ecry3.1Ab* coding region regulated by a cestrum yellow leaf curling virus (CMP) promoter and a nopaline synthase (NOS) polyadenylation (terminator) sequence. The *pmi* expression cassette consisted of the *pmi* coding region regulated by a *Zea mays* polyubiquitin (ZmUbiInt) promoter and the NOS terminator sequence. A schematic of the plasmid vector is shown in Figure IV-1. The size and description of each genetic element in the vector are shown in Table IV-1 below.

Figure IV-1. Plasmid map for vector pSYN12274.

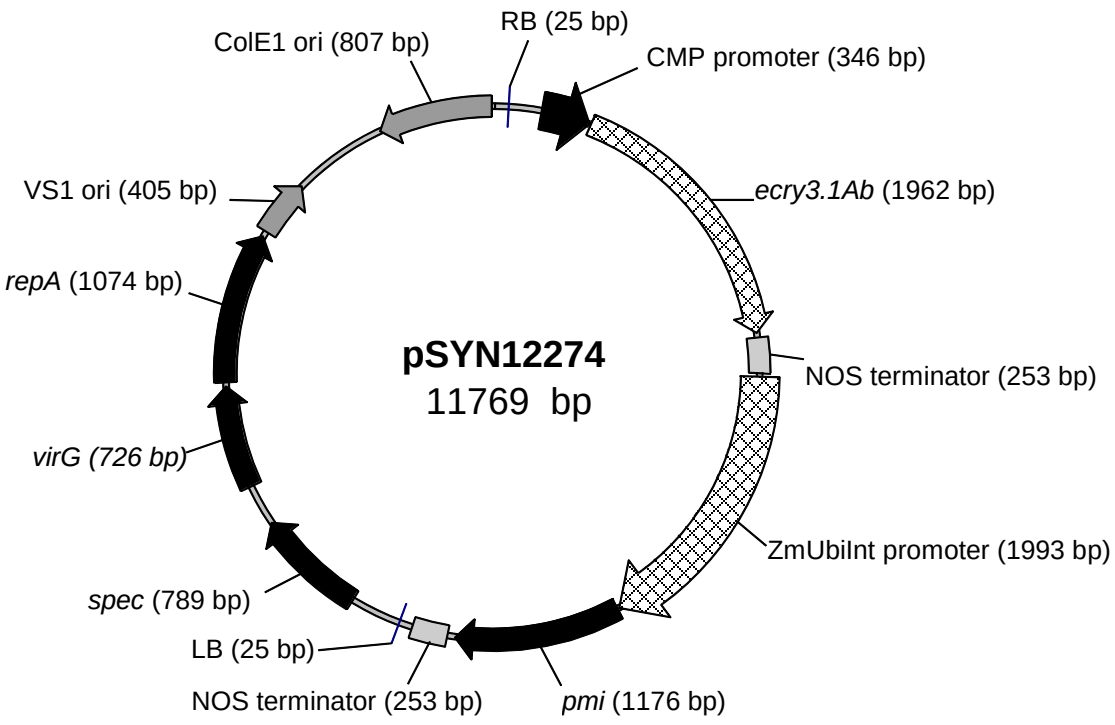


Table IV-1. Description of genetic elements in vector pSYN12274.

Genetic element	Size (bp)	Position	Description
Active ingredient cassette			
Intervening sequence	203	26 to 228	Intervening sequence with restriction sites used for cloning
CMP promoter	346	229 to 574	Cestrum Yellow Leaf Curling Virus promoter region (Hohn et al. 2007; Stavolone et al. 2003). Provides constitutive expression in corn.
Intervening sequence	9	575 to 583	Intervening sequence with restriction sites used for cloning
ecry3.1Ab	1962	584 to 2545	An engineered Cry gene active against certain corn rootworm (*Diabrotica*) species (Entrez® Accession No. GU327680 [NCBI 2010a]). The gene *ecry3.1Ab* (Walters et al. 2010) consists of a fusion between the 5′ end (Domain I, Domain II and 15 amino acids of Domain III) of a modified Cry3A gene (*mcry3A*) and the 3′ end (Domain III and Variable Region 6 [Höfte and Whiteley 1989]) of a synthetic Cry1Ab gene (see descriptions of *mcry3A* and *cry1Ab* and Figure IV-2 below). Upstream of the *mcry3A* domain, the gene *ecry3.1Ab* carries a 67-bp-long oligomer extension at its 5′ end, which was introduced during the engineering of the variable regions and is translated into the following 22 amino acid residues: MTSNGRQCAGIRPYDGRQQHRG. The next 459 amino acid residues are identical to a portion of mCry3A, followed by 172 residues that are identical to a portion of Cry1Ab. Figure IV-2 illustrates the origins of the corresponding amino acid sequences in eCry3.1Ab. Description of *mcry3A*: a corn-optimized *cry3A* was synthesized to accommodate the preferred codon usage for corn (Murray et al. 1989). The synthetic sequence was based on the native Cry3A protein sequence from *Bacillus thuringiensis* subsp. *tenebrionis* (Sekar et al. 1987). The corn-optimized gene was then modified to incorporate a consensus cathepsin-G protease recognition site within the expressed protein. The amino acid sequence of the encoded mCry3A corresponds to that of the native Cry3A, except that (1) its N-terminus corresponds to methionine 48 of the native protein and (2) a cathepsin G protease recognition site has been introduced, beginning at amino acid residue 155 of the native protein. This cathepsin-G recognition site has the sequence alanine-alanine-proline-phenylalanine, and has replaced the amino acids valine-155, serine-156, and serine-157 in the native protein (Chen and Stacy 2003). Description of *cry1Ab*: The gene *cry1Ab* was originally cloned from *Bacillus thuringiensis* subsp. *kurstaki* strain HD-1 (Geiser et al. 1986). Its sequence was codon-optimized (Koziel et al. 1997) to accommodate the preferred codon usage for corn (Murray et al. 1989).

Table IV-1 (*Continued*). Description of genetic elements in vector pSYN12274.

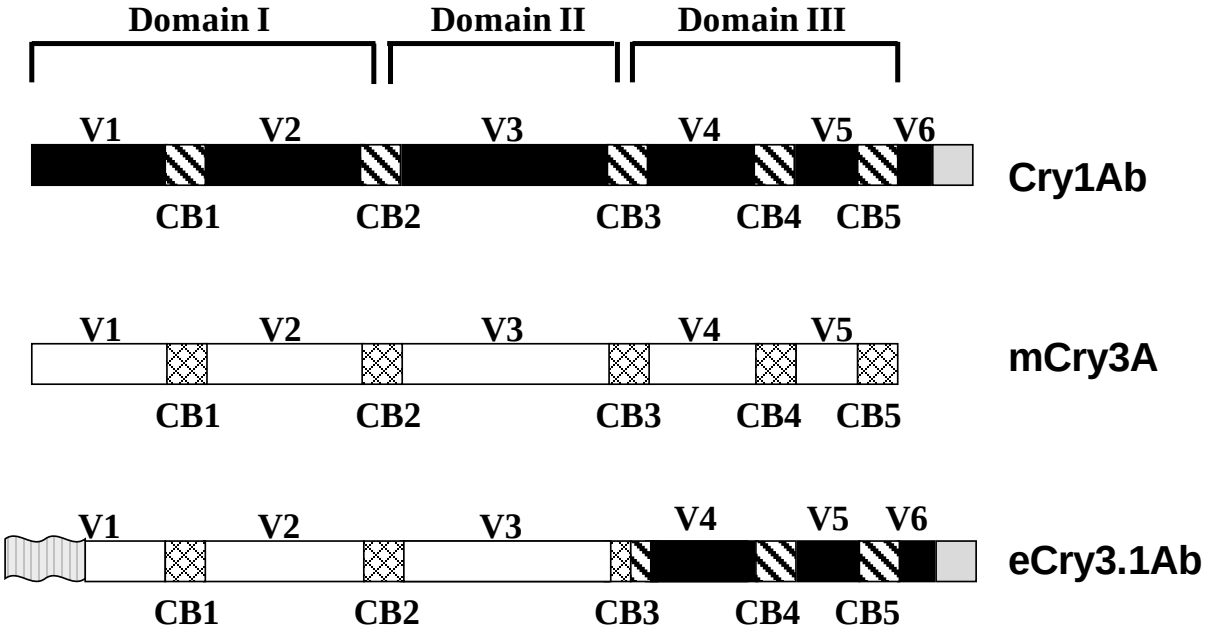
Genetic element	Size (bp)	Position	Description
Intervening sequence	30	2546 to 2575	Intervening sequence with restriction sites used for cloning
NOS	253	2576 to 2828	Terminator sequence from the nopaline synthase gene of *Agrobacterium tumefaciens* (Entrez Accession Number V00087 [NCBI 2010a]). This sequence provides a polyadenylation site (Depicker et al. 1982).
Selectable marker cassette			
Intervening sequence	25	2829 to 2853	Intervening sequence with restriction sites used for cloning
ZmUbiInt promoter	1993	2854 to 4846	Promoter region from the maize polyubiquitin gene which contains the first intron (Entrez® Accession Number S94464 [NCBI, 2010a]). Provides constitutive expression in monocots (Christensen et al. 1992)
Intervening sequence	12	4847 to 4858	Intervening sequence with restriction sites used for cloning
pmi	1176	4859 to 6034	*Escherichia coli* strain K-12 gene *pmi* encoding the enzyme phosphomannose isomerase (PMI) (Entrez Accession Number M15380 [NCBI, 2010a]); this gene is also known as *manA*. Catalyzes the isomerization of mannose-6-phosphate to fructose-6-phosphate (Negrotto et al., 2000).
Intervening sequence	60	6035 to 6094	Intervening sequence with restriction sites used for cloning
NOS	253	6095 to 6347	Terminator sequence from the nopaline synthase gene of *Agrobacterium tumefaciens* (Entrez Accession Number V00087 [NCBI, 2010a]). This sequence provides a polyadenylation site (Depicker et al. 1982).
Intervening sequence	88	6348 to 6435	Intervening sequence with restriction sites used for cloning
Plasmid backbone			
Left border (LB)	25	6436 to 6460	Left border region of T-DNA from *Agrobacterium tumefaciens* nopaline Ti-plasmid (Entrez® Accession Number J01825 [NCBI, 2010a]). Short direct repeat sequence that flanks the T-DNA and is required for the transfer of the T-DNA into the plant cell (Zambryski et al. 1982)
Intervening sequence	349	6461 to 6809	Intervening sequence with restriction sites used for cloning sequence

Table IV-1 (*Continued*). Description of genetic elements in vector pSYN12274.

Genetic element	Size (bp)	Position	Description
spec	789	6810 to 7598	Streptomycin adenylyltransferase gene, *aadA*, from *Escherichia coli* transposon Tn7 (similar to Entrez® Accession Number X03043 [NCBI 2010a]). Confers resistance to streptomycin and spectinomycin and is used as a bacterial selectable marker (Fling et al. 1985)
Intervening sequence	299	7599 to 7897	Intervening sequence with restriction sites used for cloning
virG	726	7898 to 8623	The VirGN54D gene (*virG*) from pAD1289 (similar to Entrez® Accession Number AF242881 [NCBI, 2010a). The N54D substitution results in a constitutive *virG* phenotype. VirG is part of the two-component regulatory system for the virulence (*vir*) regulon in *Agrobacterium tumefaciens* (Hansen et al. 1994).
Intervening sequence	29	8624 to 8652	Intervening sequence with restriction sites used for cloning
repA	1074	8653 to 9726	Gene encoding the pVS1 replication protein from *Pseudomonas aeruginosa* (similar to Entrez® Accession Number AF133831 [NCBI 2010a]), which is a part of the minimal pVS1 replicon that is functional in Gram-negative, plant-associated bacteria (Heeb et al. 2000)
Intervening sequence	42	9727 to 9768	Intervening sequence with restriction sites used for cloning
VS1 ori	405	9769 to 10173	Consensus sequence for the origin of replication and partitioning region from plasmid pVS1 of *Pseudomonas aeruginosa* (Entrez® Accession Number U10487 [NCBI 2010a]). Serves as origin of replication in *Agrobacterium tumefaciens* host (Itoh et al. 1984)
Intervening sequence	677	10174 to 10850	Intervening sequence with restriction sites used for cloning
ColE1 ori	807	10851 to 11657	Origin of replication (similar to Entrez® Accession Number V00268 [NCBI 2010a]) that permits replication of plasmids in *Escherichia coli* (Itoh and Tomizawa 1979)
Intervening sequence	112	11658 to 11769	Intervening sequence with restriction sites used for cloning
Right border (RB)	25	1 to 25	Right border region of T-DNA from *Agrobacterium tumefaciens* nopaline Ti-plasmid (Entrez® Accession Number J01826 [NCBI 2010a]). Short direct repeat that flanks the T-DNA and is required for the transfer of the T-DNA into the plant cell (Wang et al. 1984)

Figure IV-2. A. Schematic illustrating the origin of the amino acid residues present in eCry3.1Ab. B. Nucleotide alignment between *ecry3.1Ab* and *mcry3A* and corresponding amino acids present at the N-termini of eCry3.1Ab and mCry3A.

A.



B.

```
ecry3.1Ab   ATG ACT AGT AAC GGC CGC CAG TGT GCT GGT ATT CGC CCT TAT GAC GGC CGA
eCry3.1Ab   M   T   S   N   G   R   Q   C   A   G   I   R   P   Y   D   G   R

mcry3A      ....................................................ATG ACG GCC GAC
mCry3A                                                          M   T   A   D


ecry3.1Ab    CAA CAA CAC CGA GGC  C-TG GAC AGC AGC ACC ACC AAG GAC GTG
eCry3.1Ab    Q   Q   H   R   G    L   D   S   S   T   T   K   D   V

mcry3A       AAC AAC ACC GAG GCC  CTG GAC AGC AGC ACC ACC AAG GAC GTG
mCry3A       N   N   T   E   A    L   D   S   S   T   T   K   D   V
```

(A) The black rectangles represent the variable regions 1 through 6 (V1 through V6) of Cry1Ab; the rectangles with diagonal lines represent the conserved blocks 1 through 5 (CB1 through CB5) of Cry1Ab; the gray rectangles represent the Cry1Ab tail sequence. The white rectangles represent the variable regions 1 through 5 of mCry3A; the rectangles with crosshatch lines represent the conserved blocks 1 through 5 of mCry3A. The vertically striped portion of eCry3.1Ab represents the N-terminal amino acids unique to eCry3.1Ab (see Figure IV-2B).

(B) The dotted line symbolizes sequence absent from mCry3A; the dash indicates a one-base-pair deletion in *ecry3.1Ab*. The first 40 nucleotides of *ecry3.1Ab*, and the 13 amino acids they encode, are in bold text to highlight their addition during the engineering of the gene. The 9 amino acids LDSSTTKDV shown for both proteins in bold text indicate where the amino acid sequence of eCry3.1Ab is restored to that of mCry3A.

IV.C. Description of the Transformation System

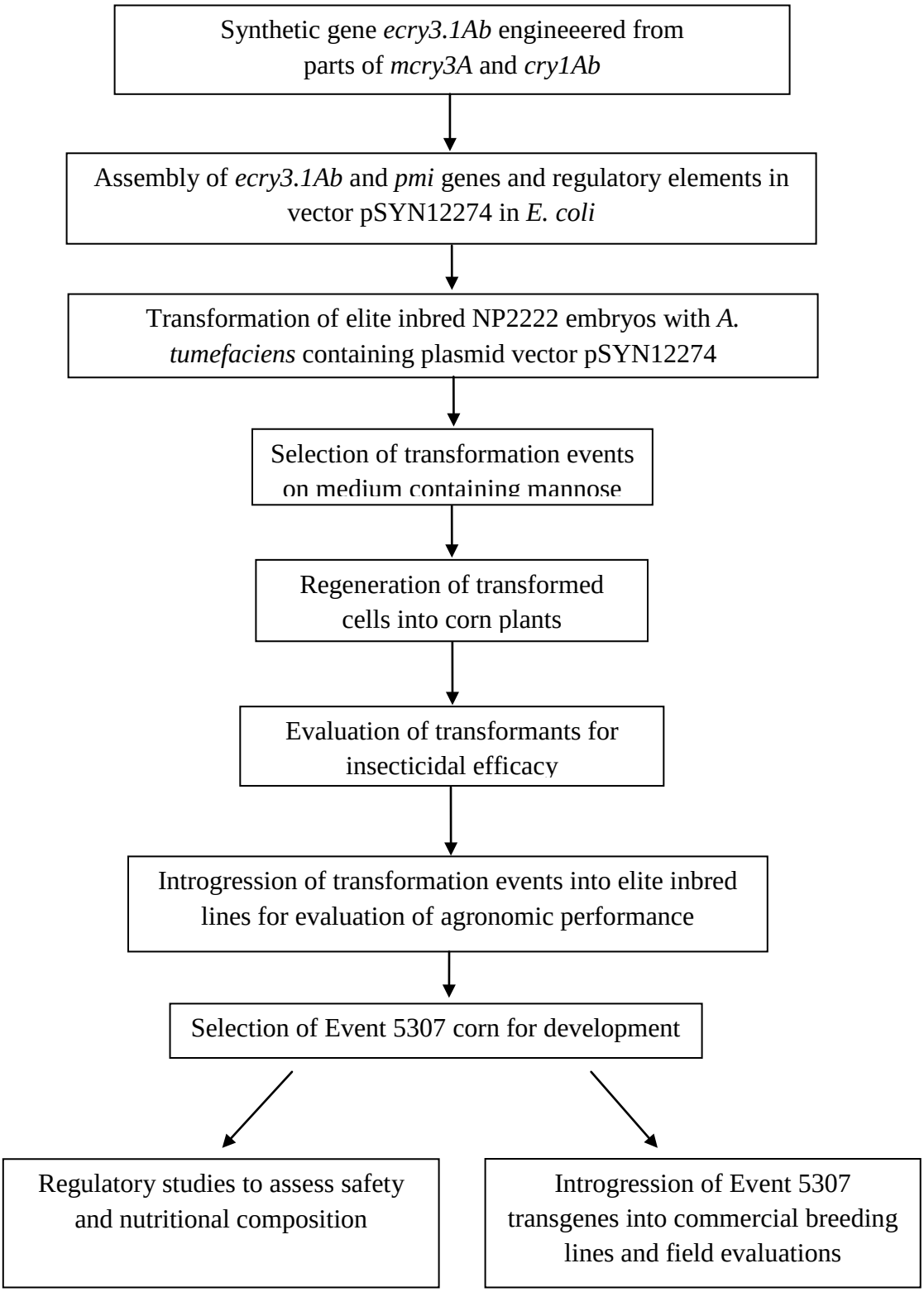
IV.C.1. Development of Event 5307 Corn

Transformation of *Z. mays* to produce Event 5307 corn was accomplished using immature embryos of a proprietary corn line via *Agrobacterium tumefaciens*-mediated transformation (Negrotto et al. 2000; Grimsley and Bisaro 1987; Ishida et al. 1996). Using this method, DNA within the left border (LB) and right border (RB) elements of a transformation plasmid, referred to as the transferred DNA (T-DNA), is integrated into the genome of infected cells, while genetic elements outside of the plasmid borders are generally not. Plants positive for both the *pmi* and the *ecry3.1Ab* genes and negative for the *spec* gene were transferred to the greenhouse for further propagation.

Event 5307 transformation employed a binary vector system (de Framond et al. 1983). Plasmid pSYN12274, which contained the *pmi* gene from *E. coli* and the coding sequence for *ecry3.1Ab* between the right and left borders of the T-DNA, was placed into *A. tumefaciens* strain LBA4404 (licensed from Japan Tobacco, Inc.); see Figure IV-1.

Progeny of the original transformant (T_0 plant) were field tested for resistance to insect feeding damage and for agronomic performance after introgression of the transgenes into multiple elite lines of corn. A schematic showing the steps in development of Event 5307 corn is shown in Figure IV-3.

Figure IV-3. Steps in the development of 5307 corn.



IV.C.2. Development of Test and Control Seed Materials

Table IV-2 lists the genotypes and descriptions of the various 5307 corn and nontransgenic control corn seed genotypes used in the studies described within this submission. The breeding pedigree diagram in Figure IV-4 indicates how each of the 5307 genotypes was derived via conventional breeding crosses from the original Event 5307 T_0 transformant. Seed genotypes used in studies described herein are identified in the pedigree diagram by a single letter code (A through F); these correspond to seed lot identifiers in Table IV-2. In Figure IV-4, genotypes enclosed in ovals represent transgenic 5307 corn genotypes that were used in studies described herein.

Several Syngenta studies described in this submission were conducted using 5307 hybrid corn of genotype NP2171 × NP2460(5307), which was hemizygous for the transgenes; a hemizygous genotype is representative of future hybrids containing the 5307 transgenes that would be grown commercially. This 5307 hybrid is indicated by the letter code "D" in the breeding diagram (Figure IV-4), and is alternatively referred to within this submission as "NP2171 × $BC5F_3$." As indicated in Figure IV-4, it was produced by crossing a nontransgenic inbred parent, NP2171, with the F_3 (third generation) transgenic progeny of self-pollinated plants following five successive generations of backcross (BC) breeding of the transgenes into to a recurrent NP2460 inbred. This backcross process fully introgressed the transgenes from the initial transformed germplasm (inbred line NP2222) into NP2460 germplasm. The corresponding nontransgenic control seed material (indicated by the letter code "E" in Figure IV-4) was produced by crossing nontransgenic inbreds NP2171 and NP2640. Except for the presence of the transgenes, control hybrid NP2171 × NP2460 is nearly genetically identical to the corresponding transgenic hybrid; thus, this control is considered near-isogenic to the NP2171 × NP2460(5307) hybrid.

Two genetic characterization studies described in this submission (genetic stability studies and Mendelian inheritance studies) required seed from multiple generations in the 5307 breeding pedigree, as indicated in Table IV-2.

Table IV-2. Event 5307 and control seed materials used in studies.

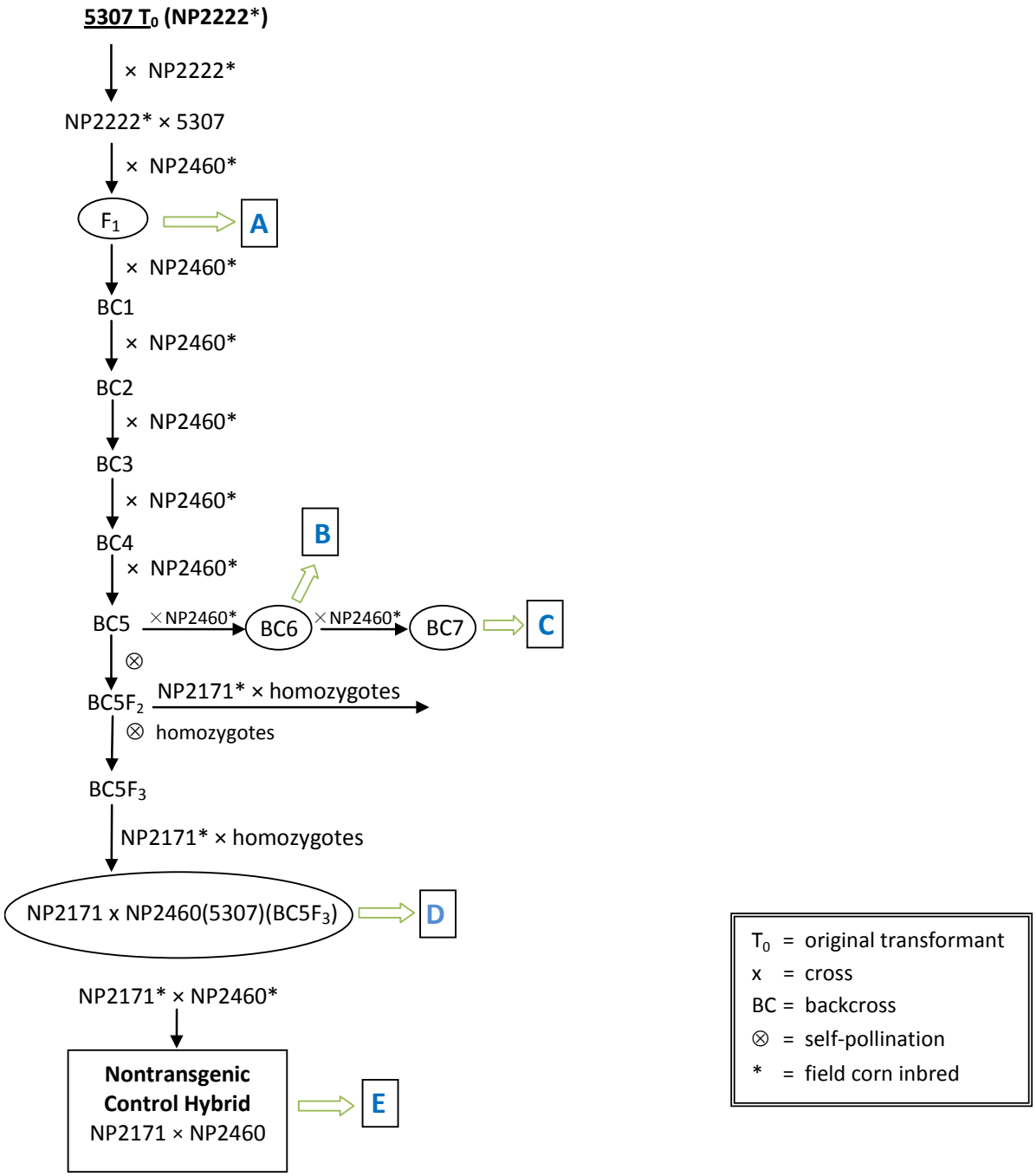
The female parent germplasm is shown first in each hybrid genotype description; the male parent germplasm is listed second. The pedigree diagram in Figure IV-4 indicates the origin of Event 5307 seed and control seed materials of these genotypes by seed lot identifier (Lot ID).

Study Description (Relevant Part of Submission)	Event 5307 Seed Material		Nontransgenic Control Seed Material	
	Lot ID	Genotype	Lot ID	Genotype
Nucleotide sequence inserted (Part IV.E.1)	D	NP2171 × NP2460(5307)($BC5F_3$)*		None
Southern blots for copy no. of functional elements (Part IV.E.2)	D	NP2171 × NP2460(5307)($BC5F_3$)*	E	NP2171 × NP2460 + parental inbred lines NP2222, NP2460, NP2171 (not shown in Fig. IV-4)
Southern blot analysis of genetic stability (Part IV.E.3)	A B C D	F_1 BC6 BC7 NP2171 × NP2460(5307)($BC5F_3$)*	 E	Parental inbred lines NP2222, NP2460, and NP2171 (not shown in Fig. IV-4) NP2171 × NP2460
Mendelian inheritance analysis (Part IV.E.4)	A B C	F_1 BC6 BC7		None
Determination of insert flanking sequences (Part IV.E.5)	D	NP2171 × NP2460(5307)($BC5F_3$)*		None
Equivalence of eCry3.1Ab produced in 5307 corn and recombinant *E. coli* (Part VI.C)	D	NP2171 × NP2460(5307)($BC5F_3$)*	E	NP2171 × NP2460
eCry3.1Ab and PMI concentrations in 5307 corn tissues (Part VI.N)	D	NP2171 × NP2460(5307)($BC5F_3$)*	E	NP2171 × NP2460
Compositional assessment of forage and grain (Part VII.C)	D	NP2171 × NP2460(5307)($BC5F_3$)*	E	NP2171 × NP2460
Broiler chicken feeding study with grain (Part VII.D)	D	NP2171 × NP2460(5307)($BC5F_3$)*	E	NP2171 × NP2460

* This 5307 hybrid is alternatively referred to as "NP2171 x $BC5F_3$" or "NP2171 x NP2640(5307)."

Figure IV-4. Pedigree diagram of Event 5307 and control seed materials.

Generations in ovals were Event 5307 materials used in studies described in this submission. Generations in rectangles were used as controls. Boxed letter codes correspond to specific seed lots used in studies listed in Table IV-2. A backcross (BC) is a cross of an individual with one of its parents. The initial Event 5307 F_1 generation was crossed with one of its inbred parents, NP2460, to create generation BC1. Likewise, BC2 originated from a cross of the BC1 with the parental line NP2460. Successive backcrosses with the recurrent parent followed similarly to introgress the transgenes into NP2460 germplasm.



IV.D. Quality Control Testing of Seed Materials

All test and control seed lots were analyzed for the presence of Event 5307 DNA and adventitious DNA from other transformation events using the real-time PCR method described in Appendix A. All Event 5307 seed lots were confirmed to contain the genes *ecry3.1Ab* and *pmi* based on nucleotide sequence. Additionally, all Event 5307 seed lots were confirmed to contain Event-5307-specific DNA based on nucleotide sequences at the junction of the T-DNA insert and the corn genome. The analyses did not detect these components in control seed lots. All test and control seed lots had no detectable sequences that would be indicative of DNA from other regulated events under development at Syngenta, or deregulated events for which testing methodology is available.

IV.E. Characterization of the Genetic Material in Event 5307 Corn

IV.E.1. DNA Insert Sequencing

Two overlapping DNA fragments that span the 5307 corn insert were amplified from genomic DNA extracted from 5307 corn using a polymerase chain reaction method. These fragments were cloned, and sequences of the clones were aligned to create a consensus of the T-DNA sequence. The consensus nucleotide sequence data for the 5307 corn insert were compared to the sequence of the transformation plasmid pSYN12274 (Figure IV-1). The data demonstrated that the insert was intact and that the organization of the functional elements within the insert, as present in plasmid pSYN12274, was maintained. The functional elements *ecry3.1Ab*, *pmi,* the CMP promoter, the ZmUbiInt promoter, and the NOS terminators in 5307 corn were identical to those in the transformation plasmid pSYN12274.

One nucleotide change was identified in the 5307 corn insert 48 bp upstream of the CMP promoter in a non-coding region of the T-DNA (Figure IV-1). This nucleotide change has no effect on the transgenes encoded by 5307 corn.

Sequence analysis revealed that some truncation occurred at the right border (RB) and left border (LB) ends of the T-DNA during the transformation process. The entire RB, three bp of non-coding sequence at the 5′ end of the insert, and eight bp of the LB were truncated. These deletions had no effect on the functionality of the insert; similar deletions have previously been observed in transformations with *Agrobacterium tumefaciens* (Tinland and Hohn 1995; Brunaud et al. 2002; Chilton and Que 2003).

IV.E.2. Analysis of DNA Insertion Site in Corn Genome

Sequence analysis of the 5307 maize genomic insertion site was conducted to determine whether any corn genomic DNA was deleted when the 5307 corn T-DNA insert integrated into the corn genome. This analysis was conducted by identifying the T-DNA insertion site

using PCR analysis of extracted genomic DNA, followed by cloning the PCR fragment into a vector and determining the nucleotide sequence of the PCR amplification product in the resulting plasmid preparations.

An alignment of the nucleotide sequence of the corn genomic sequence flanking the 5307 corn T-DNA insert with the sequence of the corn genomic insertion site, as determined from the nontransgenic control maize line used for transformation (Syngenta inbred NP2222), revealed that 33 base pairs of genomic corn DNA were deleted during integration of the 5307 T-DNA into the corn genome. This deletion has had no apparent deleterious effect on the phenotype or properties of Event 5307 corn.

IV.E.3. Copy Number of Functional Elements

Southern blot analyses demonstrated that the T-DNA insert in 5307 corn contains single copies of *ecry3.1Ab, pmi,* the CMP promoter sequence, and the ZmUbiInt promoter sequence and two copies of the NOS terminator sequence, as expected for a single insertion site. Results also indicated that there are no extraneous DNA fragments of the functional elements elsewhere in the 5307 corn genome, and that 5307 corn is free of backbone sequence from the transformation plasmid pSYN12274.

For each Southern blot, there is a map showing the location of the specific probe and the locations of the restriction enzyme sites used in that analysis. These can be found in Figure IV-5, Figure IV-7, Figure IV-9, Figure IV-11, and Figure IV-13. The results of these Southern blot analyses are shown in Figure IV-6, Figure IV-8, Figure IV-10, Figure IV-12, and Figure IV-14, respectively. In addition, comparisons of the expected and observed hybridization bands are displayed in table format (Table IV-3, Table IV-4, Table IV-5, Table IV-6, and Table IV-7, respectively) for each Southern blot.

IV.E.3.a. Copy Number of Functional Elements: **ecry3.1Ab-*Specific Probe***

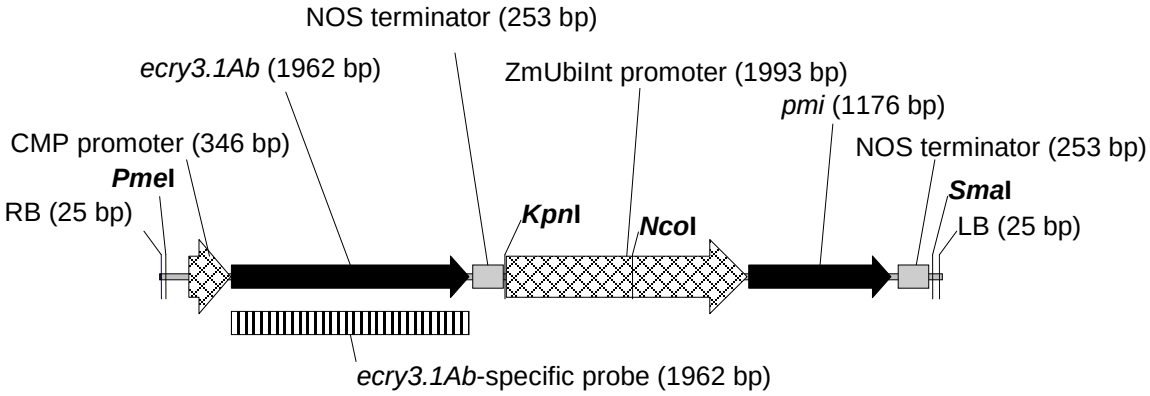
A map of the T-DNA of 5307 corn transformation plasmid pSYN12274, indicating the location of the *ecry3.1Ab*-specific probe and restriction sites for *Kpn*I, *Nco*I, *Sma*I, and *Pme*I is shown in Figure IV-5. The results of this analysis are shown in Table IV-3 and Figure IV-6.

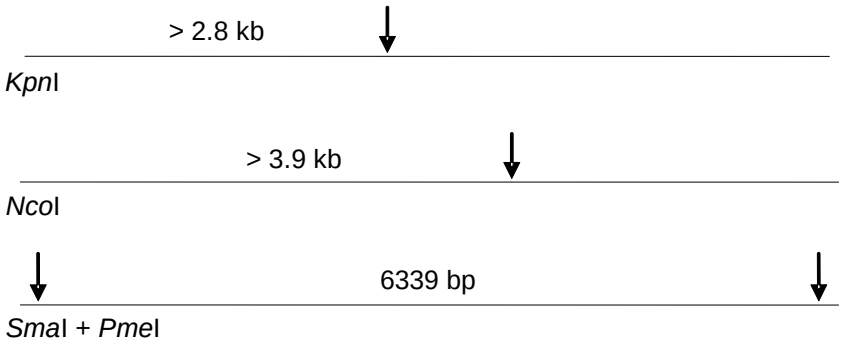
Genomic 5307 corn DNA digested with *Kpn*I (Figure IV-6A, Lane A3) produced a single hybridization band at approximately 8.5 kb, corresponding to a single copy of *ecry3.1Ab*. Genomic 5307 corn DNA digested with *Nco*I (Figure IV-6B, Lane B3) produced a single hybridization band at the expected size of approximately 19 kb, corresponding to a single copy of *ecry3.1Ab*. Genomic 5307 corn DNA digested with *Sma*I + *Pme*I (Figure IV-6C, Lane C3) produced a single hybridization band at approximately 6.3 kb, corresponding to a single copy of *ecry3.1Ab*.

The negative control corresponding to each digest showed no hybridization (*Kpn*I, Figure IV-6A, Lane A4; *Nco*I, Figure IV-6B, Lane B4; and *Sma*I + *Pme*I, Figure IV-6C, Lane C4). One hybridization band of the expected size was present in all lanes (Lanes A5, B5 and C5 of Figure IV-6A, B and C, respectively) containing positive control DNA from the plasmid pSYN12274.

For Southern blot analyses with the *ecry3.1Ab*-specfic probe, detection of only one hybridization band of the expected size for each restriction enzyme digestion strategy demonstrated that the T-DNA insert in 5307 corn contains a single copy of *ecry3.1Ab*. No unexpected bands were detected, indicating that there were no extraneous DNA fragments of *ecry3.1Ab* in the 5307 corn genome.

Figure IV-5. Location of the *Kpn*I, *Nco*I, *Sma*I, and *Pme*I restriction sites and position of the 1962 bp *ecry3.1Ab*-specific probe in the T-DNA region of the transformation plasmid pSYN12274.





The vertical arrows indicate the site of restriction digestion.
Sizes of the expected restriction fragments are indicated.

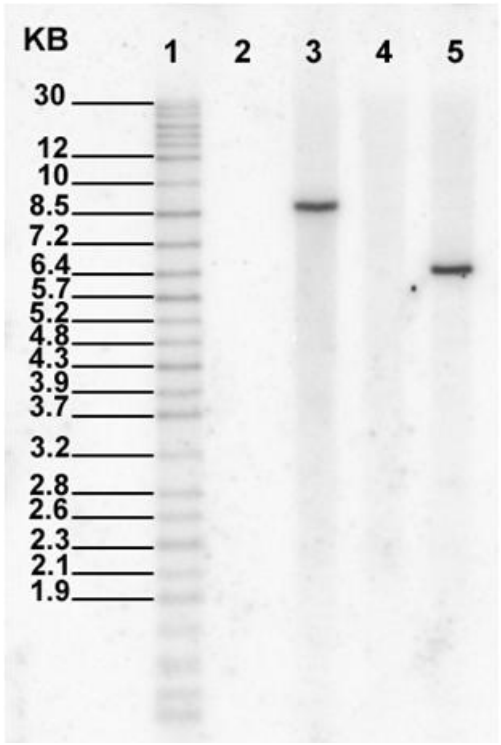
Table IV-3. Expected and observed hybridization band sizes in Southern blot analysis of 5307 corn, using an *ecry3.1Ab*-specific probe and restriction enzymes *Kpn*I, *Nco*I, and *Sma*I + *Pme*I.

Figure & Lane No.	Source of DNA	Restriction enzyme(s)	Expected number of bands	Expected band size (kb)	Observed band size (kb)
Figure IV-6A, Lane A3	5307 NP2171 × $BC5F_3$	*Kpn*I	1	>2.8	~8.5
Figure IV-6A, Lane A4	NP2171 × NP2460	*Kpn*I	none	none	none
Figure IV-6A, Lane A5	Positive control (16.53 pg of pSYN12274 mixed with NP2171 × NP2460 digested with *Kpn*I)	*Sma*I + *Pme*I	1	~6.3	~6.3
Figure IV-6B, Lane B3	5307 NP2171 × $BC5F_3$	*Nco*I	1	>3.9	~19
FigureIV-6B, Lane B4	NP2171 × NP2460	*Nco*I	none	none	none
Figure IV-6B, Lane B5	Positive control (16.53 pg of pSYN12274 mixed with NP2171 × NP2460 digested with *Nco*I)	*Sma*I + *Pme*I + *Nco*I[1]	1	~3.9	~3.9
Figure IV-6C, Lane C3	5307 NP2171 × $BC5F_3$	*Sma*I + *Pme*I	1	~6.3	~6.3
Figure IV-6C, Lane C4	NP2171 × NP2460	*Sma*I + *Pme*I	none	none	none
Figure IV-6C, Lane C5	Positive control (16.53 pg of pSYN12274 mixed with NP2171 × NP2460 digested with *Sma*I + *Pme*I)	SmaI + *Pme*I	1	~6.3	~6.3

[1] Digestion of pSYN12274 with *Nco*I was the result of addition to NP2171 × NP2460 digested with *Nco*I

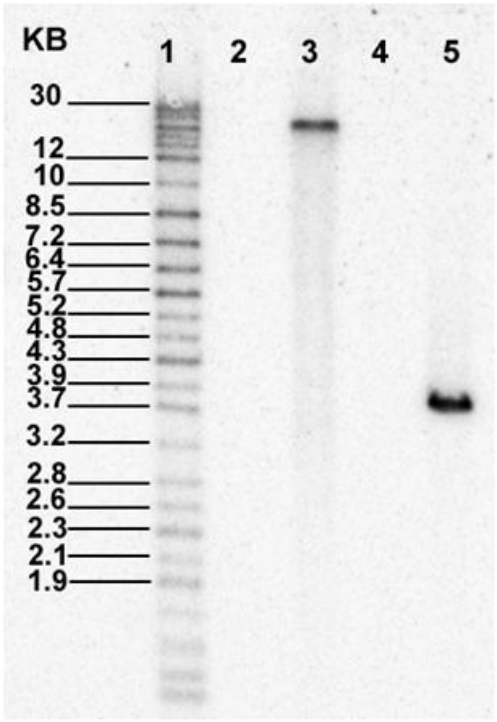
Figure IV-6. Southern blot analysis of 5307 corn for copy number of functional elements: 1962-bp *ecry3.1Ab*-specific probe, using restriction enzymes *Kpn*I, *Nco*I, *and Sma*I + *Pme*I

(A) *Kpn*I



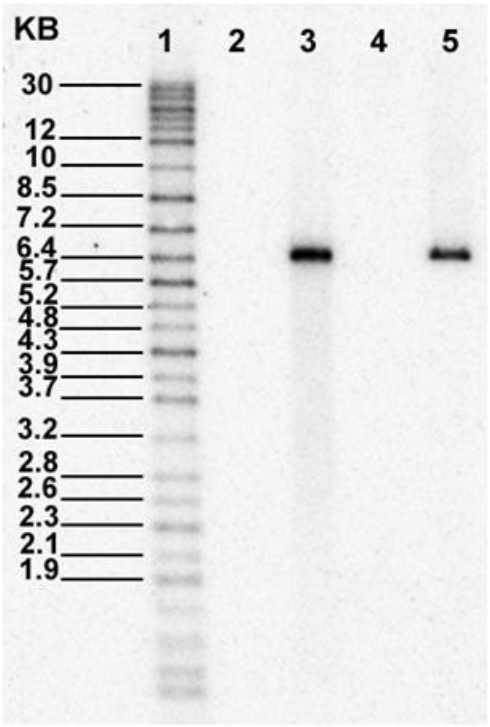
Lane A1 = molecular weight markers
Lane A2 = blank
Lane A3 = 5307 NP2171 × BC5F$_3$ digested with *Kpn*I
Lane A4 = NP2171 × NP2460 digested with *Kpn*I
Lane A5 = Positive control (NP2171 × NP2460 digested with *Kpn*I and 16.53 pg of pSYN12274 digested with *Sma*I + *Pme*I)

(B) *Nco*I



Lane B1 = molecular weight markers
Lane B2 = blank
Lane B3 = 5307 NP2171 × BC5F$_3$ digested with *Nco*I
Lane B4 = NP2171 × NP2460 digested with *Nco*I
Lane B5 = Positive control (NP2171 × NP2460 digested with *Nco*I and 16.53 pg of pSYN12274 digested with *Sma*I + *Pme*I + *Nco*I)

(C) *Sma*I + *Pme*I



Lane C1 = molecular weight markers
Lane C2 = blank
Lane C3 = 5307 NP2171 × BC5F$_3$ digested with *Sma*I + *Pme*I
Lane C4 = NP2171 × NP2460 digested with *Sma*I + *Pme*I
Lane C5 = Positive control (NP2171 × NP2460 digested with *Sma*I + *Pme*I and 16.53 pg of pSYN12274 digested with *Sma*I + *Pme*I)

IV.E.3.b. Copy Number of Functional Elements: **pmi-*Specific Probe***

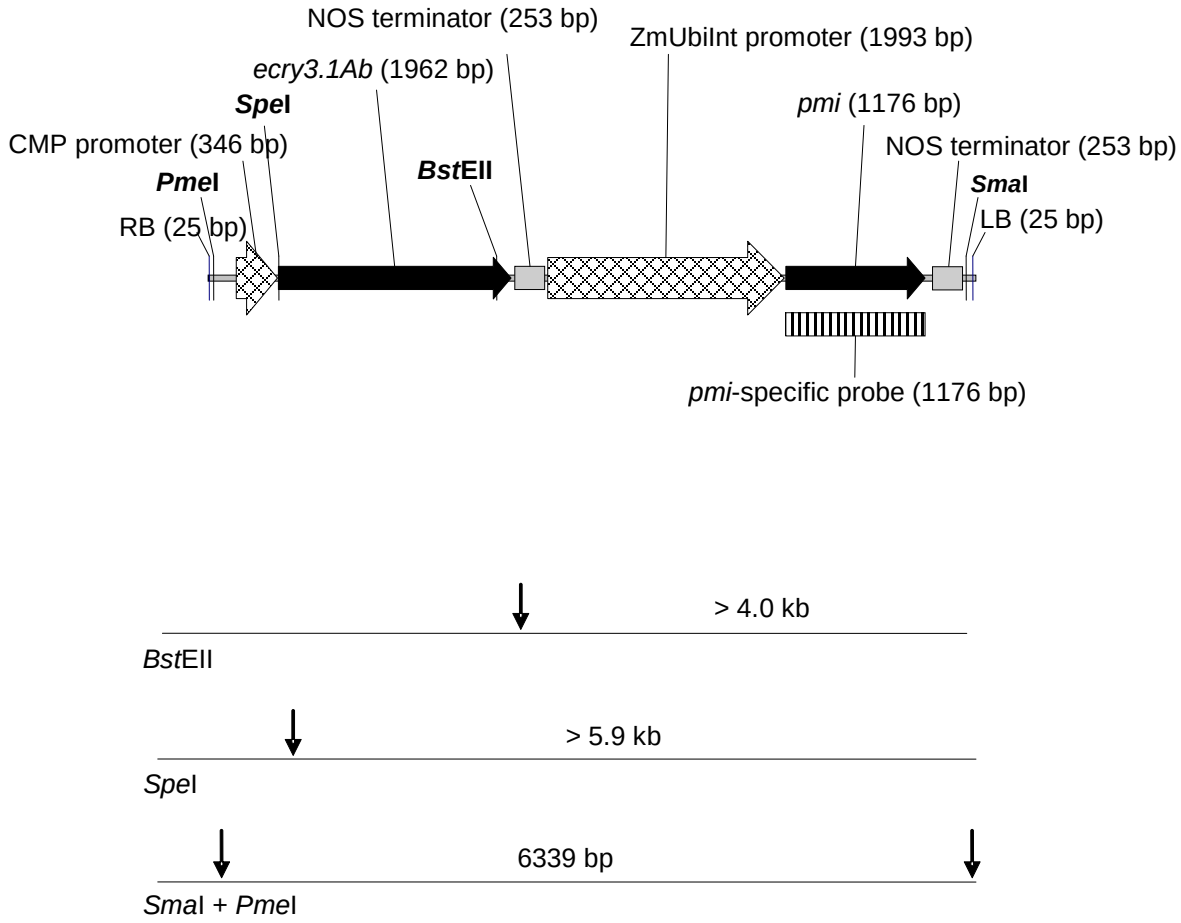
A map of the T-DNA region in the 5307 corn transformation vector pSYN12274 indicating the location of the *pmi*-specific probe, and the restriction enzymes used in this analysis, are shown in Figure IV-7. The results of this analysis are shown in Table IV-4 and Figure IV-8.

Genomic 5307 corn DNA digested with *Bst*EII (Figure IV-8, Lane 3) produced a single hybridization band of approximately 7.2 kb, corresponding to a single copy of *pmi*. Genomic 5307 corn DNA digested with *Spe*I (Lane 5) produced a single hybridization band at approximately 7.0 kb, corresponding to a single copy of *pmi*. Genomic 5307 corn DNA digested with *Sma*I + *Pme*I (Lane 7) produced a single hybridization band at the expected size of approximately 6.3 kb, corresponding to a single copy of *pmi* and confirming the intactness of the insert.

The negative control corresponding to each digest showed no hybridization (*Bst*EII Lane 4, *Spe*I Lane 6, and *Sma*I + *Pme*I Lane 8). One hybridization band of the expected size was present in the lane (Lane 9) containing positive control DNA from the plasmid pSYN12274.

For the *pmi*-specific probe, the restriction enzyme digests resulted in a single hybridization signal in each case, demonstrating that 5307 corn contains a single copy of *pmi*. No unexpected bands were detected, indicating that 5307 corn does not contain any additional *pmi* coding regions other than that associated with the DNA insert.

Figure IV-7. Location of the *Bst*EII, *Spe*I, *Sma*I, and *Pme*I restriction sites and position of the 1176 bp *pmi*-specific probe in the T-DNA region of the transformation plasmid pSYN12274

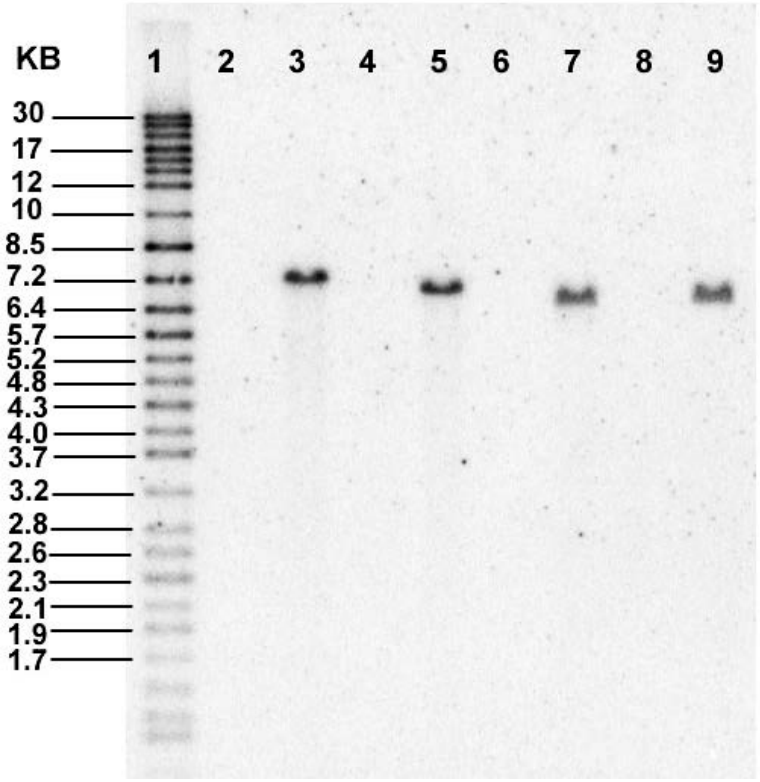


The vertical arrows indicate the site of restriction digestion.
Sizes of the expected restriction fragments are indicated.

Table IV-4. Expected and observed hybridization band sizes in Southern blot analysis of 5307 corn, using a *pmi*-specific probe and restriction enzymes *Bst*EII, *Spe*I, and *Sma*I + *Pme*I.

Figure & Lane No.	Source of DNA	Restriction enzyme(s)	Expected number of bands	Expected band size (kb)	Observed band size (kb)
Figure IV-8, Lane 3	5307 NP2171 × $BC5F_3$	*Bst*EII	1	> 4.0 kb	~ 7.2 kb
Figure IV-8, Lane 4	NP2171 × NP2460	*Bst*EII	none	none	none
Figure IV-8, Lane 5	5307 NP2171 × $BC5F_3$	*Spe*I	1	> 5.9 kb	~ 7.0 kb
Figure IV-8, Lane 6	NP2171 × NP2460	*Spe*I	none	none	none
Figure IV-8, Lane 7	5307 NP2171 × $BC5F_3$	*Sma*I + *Pme*I	1	~6.3 kb	~6.3 kb
Figure IV-8, Lane 8	NP2171 × NP2460	*Sma*I + *Pme*I	none	none	none
Figure IV-8, Lane 9	Positive control (16.53 pg of pSYN12274 mixed with NP2171 × NP2460 digested with *Sma*I + *Pme*I)	*Sma*I + *Pme*I	1	~6.3 kb	~6.3 kb

Figure IV-8. Southern blot analysis of 5307 corn for copy number of functional elements: 1176-bp *pmi*-specific probe, using restriction enzymes *Bst*EII, *Spe*I, and *Sma*I + *Pme*I.



Lane 1 = molecular weight markers
Lane 2 = blank
Lane 3 = 5307 NP2171 × $BC5F_3$ digested with *Bst*EII
Lane 4 = NP2171 × NP2460 digested with *Bst*EII
Lane 5 = 5307 NP2171 × $BC5F_3$ digested with *Spe*I
Lane 6 = NP2171 × NP2460 digested with *Spe*I
Lane 7 = 5307 NP2171 × $BC5F_3$ digested with *Sma*I + *Pme*I
Lane 8 = NP2171 × NP2460 digested with *Sma*I + *Pme*I
Lane 9 = Positive control (NP2171 × NP2460 digested with *Sma*I + *Pme*I and 16.53 pg of pSYN12274 digested with *Sma*I + *Pme*I)

IV.E.3.c. Copy Number of Functional Elements: CMP promoter-specific probe

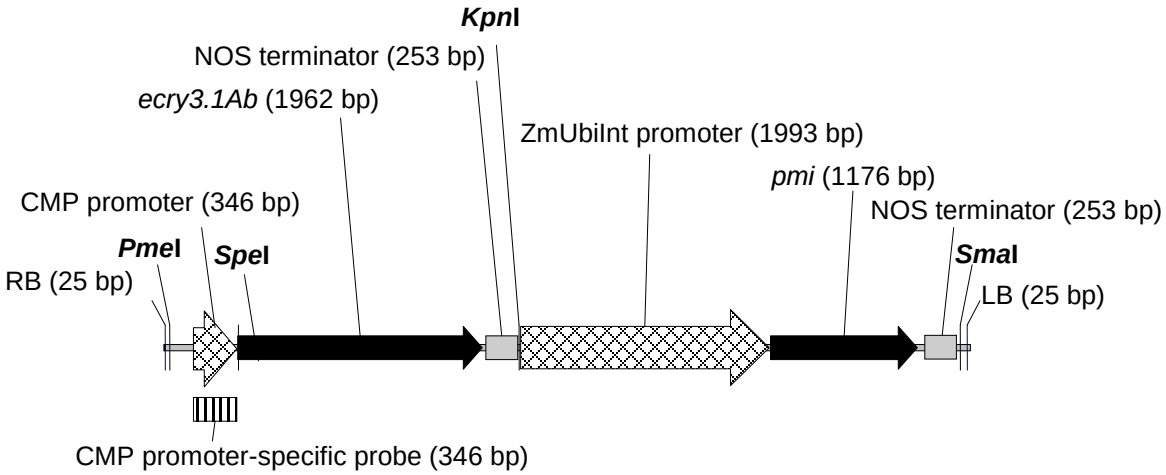
Figure IV-9 shows a map of the T-DNA of 5307 corn transformation plasmid pSYN12274, indicating the location of the CMP promoter-specific probe and restriction sites for *Kpn*I, *Spe*I, *SmaI*, and *Pme*I. Figure IV-10 depicts the results of the corresponding Southern blot analyses, and Table IV-5 outlines the expected and observed sizes of the hybridization bands.

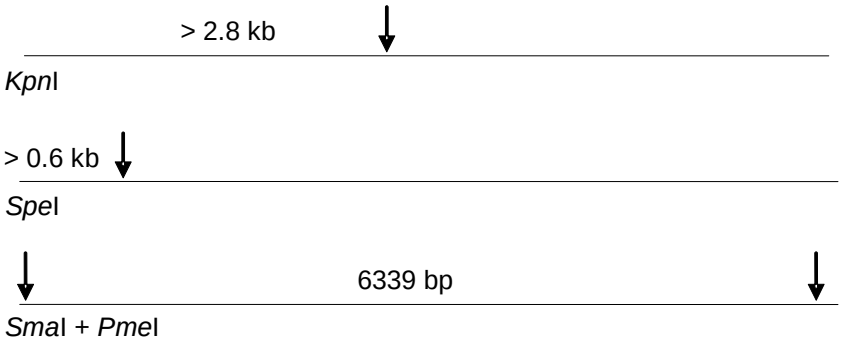
For Southern blot analysis with genomic DNA digested with *Kpn*I and probed with the CMP promoter-specific probe, one hybridization band of approximately 8.5 kb was observed as expected (Figure IV-10A, Lane A3) (Table IV-5). For Southern blot analysis with genomic DNA digested with *Spe*I and probed with the CMP promoter-specific probe, one hybridization band of approximately 2.6 kb was observed as expected (Figure IV-10B, Lane B3) (Table IV-5). For Southern blot analysis with genomic DNA digested with *Sma*I + *Pme*I and probed with the CMP promoter-specific probe, one hybridization band of approximately 6.3 kb was observed as expected (Figure IV-10C, Lane C3) (Table IV-5).

No CMP promoter-specific hybridization band was present in DNA extracted from the control substance plants (Figure IV-10A, B, and C, Lanes A4, B4 and C4, respectively) and therefore hybridization with this probe was specific to the 5307 corn insert. As expected, one hybridization band of approximately 6.3 kb was observed in the lane containing the positive control, plasmid pSYN12274 DNA, digested with *Sma*I + *Pme*I (Figure IV-10A, B, and C, Lanes A5, B5 and C5, respectively).

For Southern blot analyses with the CMP promoter-specfic probe, detection of only one hybridization band of the expected size for each restriction enzyme digestion strategy demonstrated that the 5307 corn T-DNA insert contains a single copy of the CMP promoter sequence. No unexpected bands were detected, indicating that there were no extraneous DNA fragments of the CMP promoter sequence in the 5307 corn genome.

Figure IV-9. Location of the *Kpn*I, *Spe*I, *Sma*I, and *Pme*I restriction sites and position of the 346 bp CMP promoter-specific probe in the T-DNA region of the transformation plasmid pSYN12274.





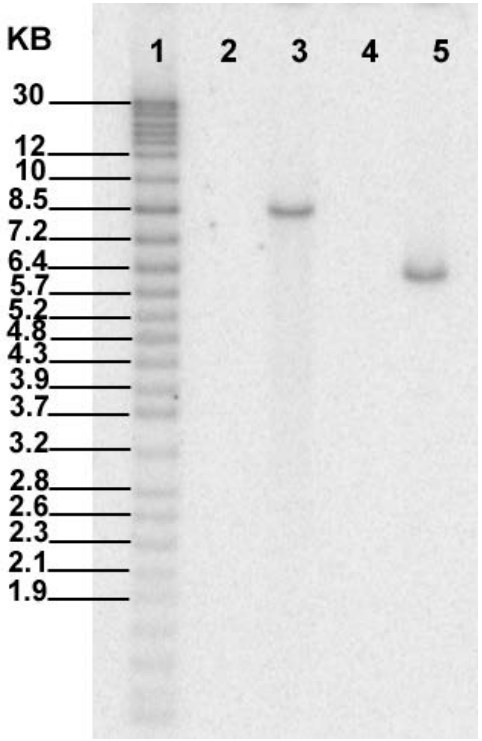
The vertical arrows indicate the site of restriction digestion.
Sizes of the expected restriction fragments are indicated.

Table IV-5. Expected and observed hybridization band sizes in Southern blot analysis of 5307 corn, using a CMP promoter-specific probe and restriction enzymes *Kpn*I, *Spe*I, and *Sma*I + *Pme*I.

Figure & Lane No.	Source of DNA	Restriction enzyme(s)	Expected number of bands	Expected band size (kb)	Observed band size (kb)
Figure IV-10A, Lane A3	5307 NP2171 × $BC5F_3$	*Kpn*I	1	>2.8	~8.5
Figure IV-10A, Lane A4	NP2171 × NP2460	*Kpn*I	none	none	None
Figure IV-10A, Lane A5	Positive control (16.53 pg of pSYN12274 mixed with NP2171 × NP2460 digested with *Kpn*I)	*Sma*I + *Pme*I	1	~6.3	~6.3
Figure IV-10B, Lane B3	5307 NP2171 × $BC5F_3$	*Spe*I	1	>0.6	~2.6
Figure IV-10B, Lane B4	NP2171 × NP2460	*Spe*I	none	none	none
Figure IV-10B, Lane B5	Positive control (16.53 pg of pSYN12274 mixed with NP2171 × NP2460 digested with *Spe*I)	*Sma*I + *Pme*I	1	~6.3	~6.3
Figure IV-10C, Lane C3	5307 NP2171 × $BC5F_3$	*Sma*I + *Pme*I	1	~6.3	~6.3
Figure IV-10C, Lane C4	NP2171 × NP2460	*Sma*I + *Pme*I	none	none	none
Figure IV-10C, Lane C5	Positive control (16.53 pg of pSYN12274 mixed with NP2171 × NP2460 digested with *Sma*I + *Pme*I)	*Sma*I + *Pme*I	1	~6.3	~6.3

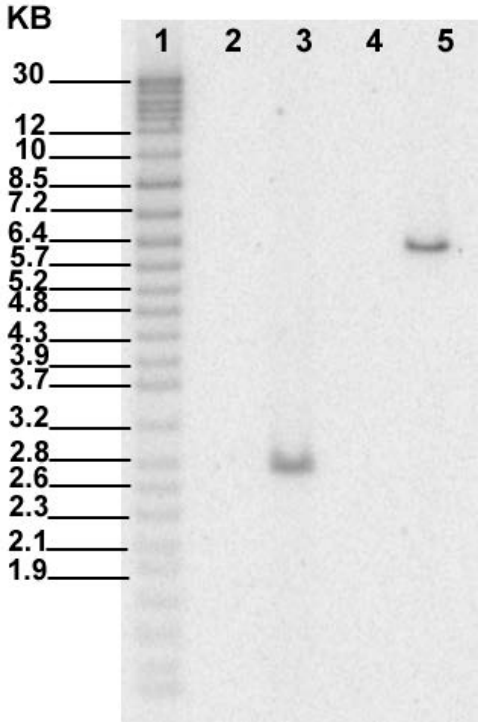
Figure IV-10. Southern blot analysis of 5307 corn for copy number of functional elements: 346-bp CMP promoter-specific probe, using restriction enzymes *Kpn*I, *Spe*I, and *Sma*I + *Pme*I

(A) *Kpn*I



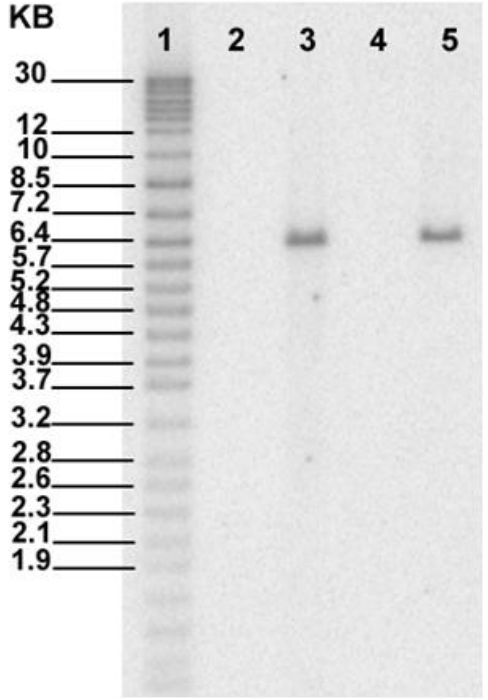
Lane A1 = molecular weight markers
Lane A2 = blank
Lane A3 = 5307 NP2171 × BC5F$_3$ digested with *Kpn*I
Lane A4 = NP2171 × NP2460 digested with *Kpn*I
Lane A5 = Positive control (NP2171 × NP2460 digested with *Kpn*I and 16.53 pg of pSYN12274 digested with *Sma*I + *Pme*I)

(B) *Spe*I



Lane B1 = molecular weight markers
Lane B2 = blank
Lane B3 = 5307 NP2171 × BC5F$_3$ digested with *Spe*I
Lane B4 = NP2171 × NP2460 digested with *Spe*I
Lane B5 = Positive control (NP2171 × NP2460 digested with *Spe*I and 16.53 pg of pSYN12274 digested with *Sma*I + *Pme*I)

(C) *Sma*I + *Pme*I



Lane C1 = molecular weight markers
Lane C2 = blank
Lane C3 = 5307 NP2171 × BC5F$_3$ digested with *Sma*I + *Pme*I
Lane C4 = NP2171 × NP2460 digested with *Sma*I + *Pme*I
Lane C5 = Positive control (NP2171 × NP2460 digested with *Sma*I + *Pme*I and 16.53 pg of pSYN12274 digested with *Sma*I + *Pme*I)

IV.E.3.d. Copy Number of Functional Elements: ZmUbiInt PromoterSpecific Probe

Figure IV-11 shows a map of the T-DNA of 5307 corn transformation plasmid pSYN12274, indicating the location of the ZmUbiInt promoter-specific probe and restriction sites for *Bst*EII, *Spe*I, *Sma*I, and *Pme*I. Figure IV-12 depicts the results of the corresponding Southern blot analyses, and Table IV-6 outlines the expected and observed sizes of the hybridization bands.

For Southern blot analysis with genomic DNA digested with *Bst*EII and probed with the ZmUbiInt promoter-specific probe, one hybridization band of approximately 7.2 kb was observed as expected (Figure IV-12A, Lane A3) (Table IV-6).

No hybridization band was present in DNA extracted from the control substance plants (Figure IV-12A, Lanes A4, A5, A6, and A7) and therefore hybridization was specific to the 5307 corn insert. As expected, three hybridization bands of approximately 3.9 kb, 8.4 kb, and 18 kb were observed in the lane containing the positive control DNA digested with *Sma*I + *Pme*I + *Bst*EII (Figure IV-12A, Lane A8).

Additional bands resulting from cross-hybridization of the ZmUbiInt promoter-specific probe with the endogenous maize polyubiquitin promoter sequence were also detected in all material analyzed. Two hybridization bands of approximately 12 kb and 18 kb corresponding to the hybridization bands observed in lanes containing DNA extracted from control plants (Figure IV-12A, Lanes A5 and A6), respectively, were also observed in the DNA extracted from 5307 corn plants (Figure IV-12A, Lane A3). Two hybridization bands of approximately 8.4 kb and 18 kb corresponding to the hybridization bands observed in lanes containing DNA extr acted from NP2460 plants (Figure IV-12A, Lane A7) and NP2171 plants (Figure IV-12A, Lane A5), respectively, were observed in the lane containing DNA extracted from NP2171 × NP2460 plants (Figure IV-12A, Lane A4) and the lane containing the positive control (Figure IV-12, Lane A8).

For Southern blot analysis with genomic DNA digested with *Spe*I and probed with the ZmUbiInt promoter-specific probe, one hybridization band of approximately 7.0 kb was observed in the lane containing DNA extracted from 5307 corn plants as expected (Figure IV-12B, Lane B3) (Table IV-6). No hybridization band was present in the DNA extracted from the control substance plants (Figure IV-12B, Lanes B4, B5, B6, and B7) and therefore hybridization was specific to the 5307 corn insert. As expected, three hybridization bands of approximately 6.3 kb, 14 kb, and 20 kb were observed in the lane containing the positive control DNA digested with *Sma*I + *Pme*I (Figure IV-12B, Lane B8).

Additional bands resulting from cross-hybridization of the ZmUbiInt promoter-specific probe with the endogenous maize polyubiquitin promoter sequence were also detected in all material analyzed. Two hybridization bands of approximately 25 kb and 14 kb corresponding to the hybridization bands observed in lanes containing DNA extracted from control substance plants (Figure IV-12B, Lanes B5 and B6) were also observed in the DNA extracted from 5307 corn plants (Figure IV-12B, Lane B3). Finally, two hybridization bands of approximately 20 kb and

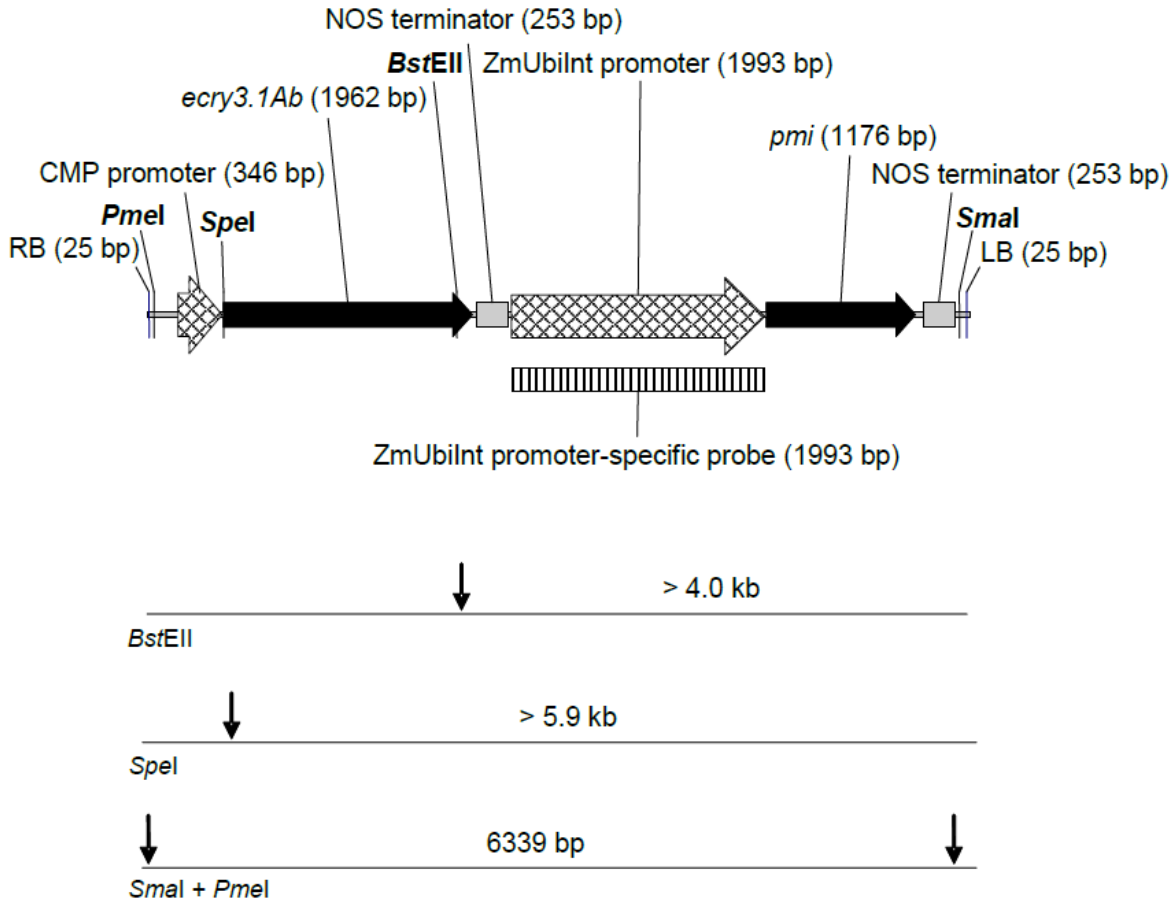
14 kb corresponding to the hybridization bands observed in lanes containing DNA extracted from NP2460 plants (Figure IV-12B, Lane B7) and NP2171 plants (Figure IV-12B, Lane B5), respectively, were observed in the lane containing DNA extracted from NP2171 × NP2460 plants (Figure IV-12B, Lane B4) and the lane containing the positive control (Figure IV-12B, Lane B8).

For Southern blot analysis with genomic DNA digested with *Sma*I + *Pme*I and probed with the ZmUbiInt promoter-specific probe, one hybridization band of approximately 6.3 kb was observed in the DNA extracted from 5307 corn plants as expected (Figure IV-12C, Lane C3) (Table IV-6). This hybridization band was absent in the lane containing DNA extracted from the control substance plants NP2171 × NP2460 (Figure IV-12C, Lanes C4, C5, C6, and C7) and therefore hybridization was specific to the 5307 corn insert. A hybridization band of approximately 6.3 kb and a high molecular weight band (greater than 30 kb) was observed in the lane containing the positive control DNA (Figure IV-12C, Lane C8).

At least one additional band resulting from cross-hybridization of the ZmUbiInt promoter-specific probe with the endogenous maize polyubiquitin promoter sequence was also detected in all material analyzed. One hybridization band of approximately 18 kb corresponding to the hybridization band observed in the lane containing DNA extracted from control substance plants (Figure IV-12C, Lane C6) was also observed in the lane containing DNA extracted from 5307 corn plants (Figure IV-12C, Lane C3). An additional high molecular weight band (greater than 30 kb) was observed in lanes containing DNA extracted from control substance plants (Figure IV-12C, Lanes C5 and C7). This faint high molecular weight band (greater than 30 kb) was also observed in the lane containing DNA extracted from 5307 corn plants (Figure IV-12C, Lane C3), control substance plants (Figure IV-12C, Lane C4), and the lane containing the positive control (Figure IV-12C, Lane C8).

For Southern blot analyses with the ZmUbiInt promoter-specfic probe, detection of only one hybridization band specific to 5307 corn for each restriction enzyme digestion demonstrated that 5307 corn contains a single copy of the ZmUbiInt promoter sequence. No unexpected bands were detected, indicating that there were no extraneous DNA fragments of the ZmUbiInt promoter sequence in the 5307 corn genome.

Figure IV-11. Location of the *Bst*EII, *Spe*I, *Sma*I, and *Pme*I restriction sites and position of the 1993-bp ZmUbiInt promoter-specific probe in the T-DNA region of the transformation plasmid pSYN12274.



The vertical arrows indicate the site of restriction digestion.
Sizes of the expected restriction fragments are indicated.

Table IV-6. Expected and observed hybridization band sizes in Southern blot analysis of 5307 corn, using a ZmUbiInt promoter-specific probe and restriction enzymes *Bst*EII, *Spe*I, and *Sma*I + *Pme*I.

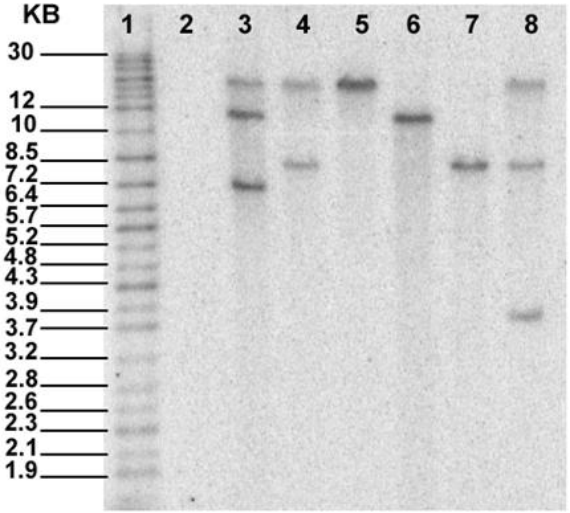
Figure & Lane No.	Source of DNA	Restriction enzyme(s)	Expected no. of bands	Expected band size (kb)	Observed band size (kb)
Figure IV-12A, Lane A3	5307 NP2171 × $BC5F_3$	*Bst*EII	1 5307 insert x endogenous	> 4.0 unknown	~ 7.2 ~ 12 (endogenous) ~ 18 (endogenous)
Figure IV-12A, Lane A4	NP2171 × NP2460	*Bst*EII	x endogenous	unknown	~ 8.4 (endogenous) ~ 18 (endogenous)
Figure IV-12A, Lane A5	NP2171	*Bst*EII	x endogenous	unknown	~ 18 (endogenous)
Figure IV-12A, Lane A6	NP2222	*Bst*EII	x endogenous	unknown	~ 12 (endogenous)
Figure IV-12A, Lane A7	NP2460	*Bst*EII	x endogenous	unknown	~ 8.4 (endogenous)
Figure IV-12A, Lane A8	Positive control (16.53 pg of pSYN12274 mixed with NP2171 × NP2460 digested with *Bst*EII)	*Sma*I + *Pme*I + *Bst*EII[1]	1 pSYN12274 x endogenous	~ 3.9 unknown	~ 3.9 ~ 8.4 (endogenous) ~ 18 (endogenous)
Figure IV-12B, Lane B3	5307 NP2171 × $BC5F_3$	*Spe*I	1 5307 insert x endogenous	> 5.9 unknown	~ 7.0 ~ 14 (endogenous) ~ 25 (endogenous)
Figure IV-12B, Lane B4	NP2171 × NP2460	*Spe*I	x endogenous	unknown	~ 14 (endogenous) ~ 20 (endogenous)
Figure IV-12B, Lane B5	NP2171	*Spe*I	x endogenous	unknown	~ 14 (endogenous)
Figure IV-12B, Lane B6	NP2222	*Spe*I	x endogenous	unknown	~ 25 (endogenous)
Figure IV-12B, Lane B7	NP2460	*Spe*I	x endogenous	unknown	~ 20 (endogenous)
Figure IV-12B, Lane B8	Positive control (16.53 pg of pSYN12274 mixed with NP2171 × NP2460 digested with *Spe*I)	*Sma*I + *Pme*I	1 pSYN12274 x endogenous	~ 6.3 unknown	~ 6.3 ~ 14 (endogenous) ~ 20 (endogenous)
Figure IV-12C, Lane B3	5307 NP2171 × $BC5F_3$	*Sma*I + *Pme*I	1 5307 insert x endogenous	~ 6.3 unknown	~ 6.3 ~ 18 (endogenous) > 30 (endogenous)
Figure IV-12C, Lane C4	NP2171 × NP2460	*Sma*I + *Pme*I	x endogenous	unknown	> 30 (endogenous)
Figure IV-12C, Lane C5	NP2171	*Sma*I + *Pme*I	x endogenous	unknown	> 30 (endogenous)
Figure IV-12C, Lane C6	NP2222	*Sma*I + *Pme*I	x endogenous	unknown	~ 18 (endogenous)
Figure IV-12C, Lane C7	NP2460	*Sma*I + *Pme*I	x endogenous	Unknown	> 30 (endogenous)
Figure IV-12C, Lane C8	Positive control (16.53 pg of pSYN12274 mixed with NP2171 × NP2460 digested with *Sma*I + *Pme*I)	*Sma*I + *Pme*I	1 pSYN12274 x endogenous	~ 6.3 unknown	~ 6.3 > 30 (endogenous)

x = unknown number

[1] Digestion of pSYN12274 with *Bst*EII was the result of addition to NP2171 × NP2460 digested with *Bst*EII

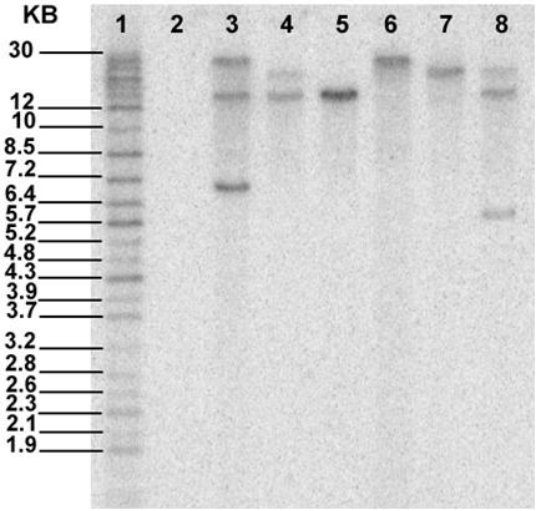
Figure IV-12. Southern blot analysis of 5307 corn for copy number of functional elements: 1993-bp ZmUbiInt promoter-specific probe, using restriction enzymes *Bst*EII, *Spe*I, and *Sma*I + *Pme*I.

(A) *Bst*EII



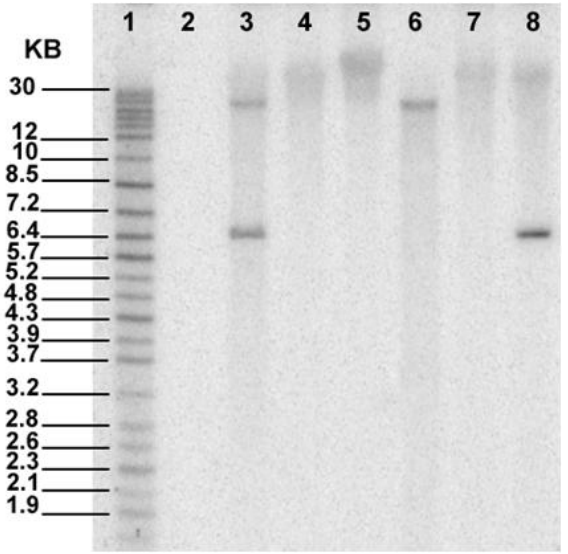
Lane A1 = molecular weight markers
Lane A2 = blank
Lane A3 = 5307 NP2171 × $BC5F_3$ digested with *Bst*EIII
Lane A4 = NP2171 × NP2460 digested with *Bst*EII
Lane A5 = NP2171 digested with *Bst*EII
Lane A6 = NP2222 digested with *Bst*EII
Lane A7 = NP2460 digested with *Bst*EII
Lane A8 = Positive control (NP2171 × NP2460 digested with *Bst*EII and 16.53 pg of pSYN12274 digested with *Sma*I + *Pme*I + *Bst*EII)

(B) *Spe*I



Lane B1 = molecular weight markers
Lane B2 = blank
Lane B3 = 5307 NP2171 × $BC5F_3$ digested with *Spe*I
Lane B4 = NP2171 × NP2460 digested with *Spe*I
Lane B5 = NP2171 digested with *Spe*I
Lane B6 = NP2222 digested with *Spe*I
Lane B7 = NP2460 digested with *Spe*I
Lane B8 = Positive control (NP2171 × NP2460 digested with *Spe*I and 16.53 pg of pSYN12274 digested with *Sma*I + *Pme*I)

(C) *SmaI + PmeI*



Lane C1 = molecular weight markers
Lane C2 = blank
Lane C3 = 5307 NP2171 × $BC5F_3$ digested with *Sma*I + *Pme*I
Lane C4 = NP2171 × NP2460 digested with *Sma*I + *Pme*I
Lane C5 = NP2171 digested with *Sma*I + *Pme*I
Lane C6 = NP2222 digested with *Sma*I + *Pme*I
Lane C7 = NP2460 digested with *Sma*I + *Pme*I
Lane C8 = Positive control (NP2171 × NP2460 digested with *Sma*I + *Pme*I and 16.53 pg of pSYN12274 digested with *Sma*I + *Pme*I)

IV.E.3.e. Copy Number of Functional Elements: NOS Terminator-Specific Probe

Figure IV-13 shows a map of the T-DNA of 5307 corn transformation plasmid pSYN12274, indicating the location of the NOS terminator-specific probe and restriction sites for *Kpn*I, *Nco*I, and *Sma*I + *Pme*I. Figure IV-14 depicts the results of the corresponding Southern blot analyses, and Table IV-7 outlines the expected and observed sizes of the hybridization bands.

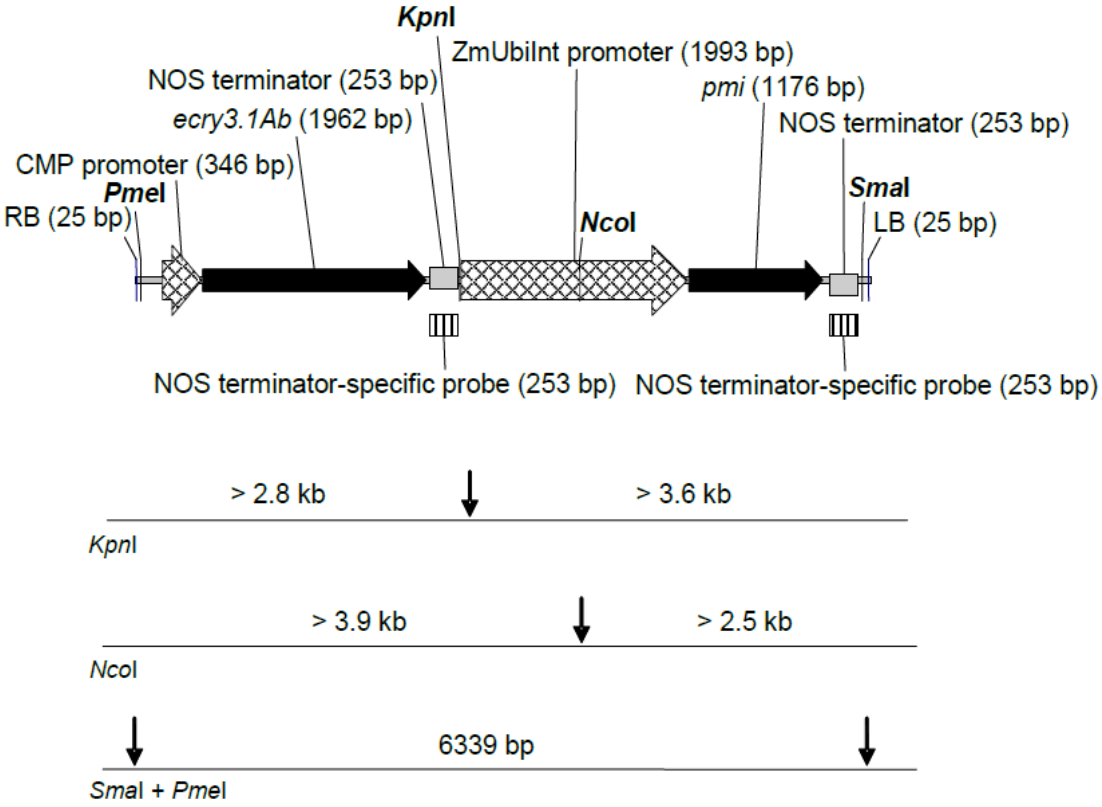
For Southern blot analysis with genomic DNA digested with *Kpn*I and probed with the NOS terminator-specific probe, two hybridization bands of approximately 6.4 kb and 8.5 kb were observed in the lane containing DNA extracted from 5307 corn plants as expected (Figure IV-14A, Lane A3) (Table IV-7). No hybridization bands were present in DNA extracted from the control substance plants (Figure IV-14A, Lane A4). Therefore, two copies of the NOS terminator sequence are present in the 5307 corn insert (one NOS terminator sequence regulating *ecry3.1Ab* and one NOS terminator sequence regulating *pmi*). One hybridization band of approximately 6.3 kb was observed in the lane containing the positive control, as expected (Figure IV-14A, Lane A5).

For Southern blot analysis with genomic DNA digested with *Nco*I and probed with the NOS terminator-specific probe, two hybridization bands of approximately 16 kb and 19 kb were observed in the lane containing DNA extracted from 5307 corn plants as expected (Figure IV-14B, Lane B3) (Table IV-7). These hybridization bands were absent in the lane containing DNA extracted from the control substance plants (Figure IV-14B, Lane B4) and were, therefore, specific to the two copies of the NOS terminator sequence in the 5307 corn insert. As expected, two hybridization bands of approximately 2.5 kb and 3.9 kb were observed in the lane containing the positive control digested with *Sma*I + *Pme*I + *Nco*I (Figure IV-14B, Lane B5).

For Southern blot analysis with genomic DNA digested with *Sma*I + *Pme*I and probed with the NOS terminator-specific probe, one hybridization band of approximately 6.3 kb was observed in the lane containing DNA extracted from 5307 corn plants as expected (Figure IV-14C, Lane C3) (Table IV-7). This hybridization band was absent in the lane containing DNA extracted from the control substance plants (Figure IV-14C, Lane C4) and was, therefore, specific to the 5307 corn insert. One hybridization band of approximately 6.3 kb was observed in the lane containing the positive control, as expected (Figure IV-14C, Lane C9).

For Southern blot analyses with the NOS terminator-specfic probe, detection of two hybridization bands of the expected size for each restriction enzyme digestion strategy demonstrated that 5307 corn contains two copies of the NOS terminator sequence (one NOS terminator sequence regulating *ecry3.1Ab* and one NOS terminator sequence regulating *pmi*). No unexpected bands were detected, indicating that there were no extraneous DNA fragments of the NOS terminator sequence in the 5307 corn genome.

Figure IV-13. Location of the *Kpn*I, *Nco*I, *Sma*I, and *Pme*I restriction sites and position of the 253-bp NOS terminator-specific probe in the T-DNA region of the transformation plasmid pSYN12274.



The vertical arrows indicate the site of restriction digestion.
Sizes of the expected restriction fragments are indicated.

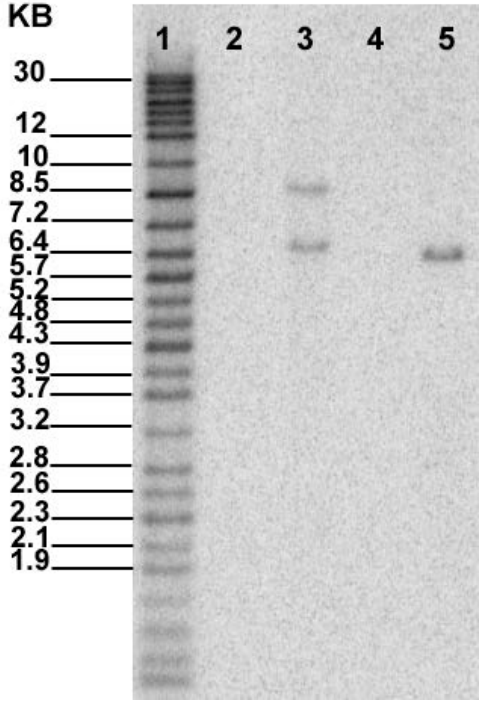
Table IV-7. Expected and observed hybridization band sizes in Southern blot analysis of 5307 corn, using a NOS terminator-specific probe and restriction enzymes *Kpn*I, *Nco*I, and *Sma*I + *Pme*I.

Figure & Lane No.	Source of DNA	Restriction enzyme(s)	Expected number of bands	Expected band size (kb)	Observed band size (kb)
Fig. IV-14A, Lane A3	5307 NP2171 × $BC5F_3$	*Kpn*I	2	> 2.8 > 3.6	~ 6.4 ~ 8.5
Fig. IV-14A, Lane A4	NP2171 × NP2460	*Kpn*I	none	none	none
Fig. IV-14A, Lane A5	Positive control (16.53 pg of pSYN12274 mixed with NP2171 × NP2460 digested with *Kpn*I)	*Sma*I + *Pme*I	1	~6.3	~ 6.3
Fig. IV-14B, Lane B3	5307 NP2171 × $BC5F_3$	*Nco*I	2	> 2.5 > 3.9	~ 16 ~ 19
Fig. IV-14B, Lane B4	NP2171 × NP2460	*Nco*I	none	none	None
Fig. IV-14B, Lane B5	Positive control (16.53 pg of pSYN12274 mixed with NP2171 × NP2460 digested with *Nco*I)	*Sma*I + *Pme*I + *Nco*I[1]	2	~ 2.5 ~ 3.9	~ 2.5 ~ 3.9
Fig. IV-14C, Lane C3	5307 NP2171 × $BC5F_3$	*Sma*I + *Pme*I	1	~ 6.3	~ 6.3
Fig. IV-14C, Lane C4	NP2171 ×NP2460	*Sma*I + *Pme*I	none	none	none
Fig. IV-14C, Lane C5	Positive control (16.53 pg of pSYN12274 mixed with NP2171 × NP2460 digested with *Sma*I + *Pme*I)	*Sma*I + *Pme*I	1	~ 6.3	~ 6.3

[1] Digestion of pSYN12274 with *Nco*I was the result of addition to NP2171 × NP2460 digested with *Nco*I

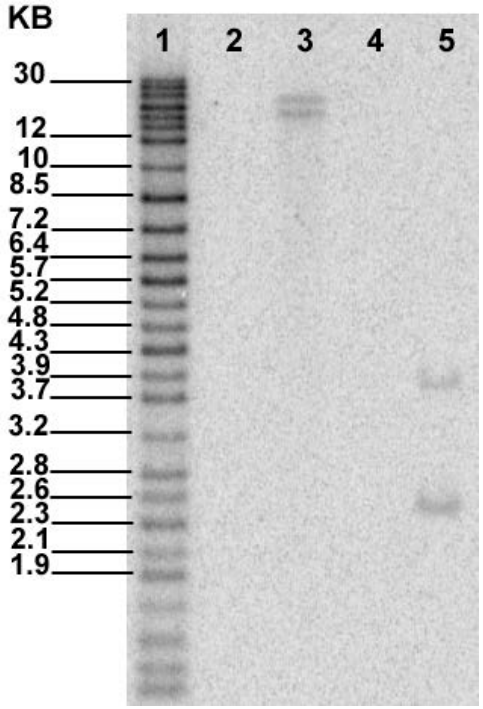
Figure IV-14. Southern blot analysis of 5307 corn for copy number of functional elements: 253-bp NOS terminator-specific probe, using restriction enzymes *Kpn*I, *Nco*I, and *Sma*I + *Pme*I.

A. *KpnI*



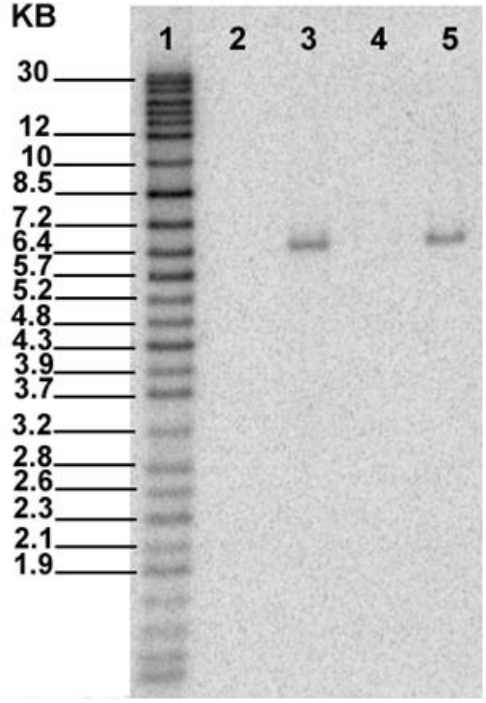
Lane A1 = molecular weight markers
Lane A2 = blank
Lane A3 = 5307 NP2171 × $BC5F_3$ digested with *Kpn*I
Lane A4 = NP2171 × NP2460 digested with *Kpn*I
Lane A5 = Positive control (NP2171 × NP2460 digested with *Kpn*I and 16.53 pg of pSYN12274 digested with *Sma*I + *Pme*I)

B. *NcoI*



Lane B1 = molecular weight markers
Lane B2 = blank
Lane B3 = 5307 NP2171 × $BC5F_3$ digested with *Nco*I
Lane B4 = NP2171 × NP2460 digested with *Nco*I
Lane B5 = Positive control (NP2171 × NP2460 digested with *Nco*I and 16.53 pg of pSYN12274 digested with *Sma*I + *Pme*I + *Nco*I)

C. *SmaI + PmeI*



Lane C1 = molecular weight markers
Lane C2 = blank
Lane C3 = 5307 NP2171 × $BC5F_3$ digested with *Sma*I + *Pme*I
Lane C4 = NP2171 × NP2460 digested with *Sma*I + *Pme*
Lane C5 = Positive control (NP2171 × NP2460 digested with *Sma*I + *Pme*I and 16.53 pg of pSYN12274 digested with *Sma*I + *Pme*I)

IV.E.4. Generational Stability

Molecular analyses were performed to demonstrate the genetic stability of the 5307 corn insert over four generations. Southern blot analyses were performed using standard molecular biology techniques. Each Southern blot contained a positive control and a negative control. The positive control, representing one copy of a fragment of known size in the corn genome, was included to demonstrate the sensitivity of each experiment; the negative control, DNA extracted from plants grown from nontransgenic corn seed, was included in order to identify possible endogenous DNA sequences that hybridize with the probe. Two probes were used: a full-length T-DNA-specific probe containing every base of the plasmid pSYN12274 T-DNA and a plasmid pSYN12274 backbone-specific probe containing every base of plasmid pSYN12274 present outside of the T-DNA region.

These Southern blot analyses demonstrated that 5307 corn contains a single, complete copy of the T-DNA insert and that there are no extraneous DNA fragments of plasmid pSYN12274 T-DNA inserted elsewhere in the 5307 corn genome. Identical hybridization patterns across all generations of 5307 corn analyzed in this study indicate that the insert is stably inherited from one generation to the next. Additionally, every generation of 5307 corn examined was free of backbone sequence from the transformation plasmid pSYN12274. Descriptions and results of these Southern blot analyses of 5307 corn are provided below. Details of the methods used are provided in Appendix A.

IV.E.4.a. Stability of the T-DNA Using a Full-Length T-DNA-specific Probe

Genetic stability of the insert during conventional breeding of 5307 corn was determined by Southern blot analyses using a full-length T-DNA-specific probe. The Southern blot analyses included genomic DNA extracted from 5307 plants of four generations (5307 F_1, 5307 BC6, 5307 BC7, and 5307 NP2171 × $BC5F_3$) and the corresponding nontransgenic, near-isogenic control genotypes (NP2171 × NP2460, NP2222, NP2460, and NP2171) (see Figure IV-4 breeding pedigree). The full-length T-DNA-specific probe, which contains sequence of the maize polyubiquitin promoter (ZmUbiInt), cross-hybridizes to genomic DNA fragments of different sizes in the different corn lines due to restriction fragment length polymorphism of the genomic DNA that carries the endogenous maize polyubiquitin promoter. Control genomic DNA from inbred lines NP2222, NP2460, and NP2171 was needed because the 5307 corn generations analyzed were created by crossing with these corn lines. For these experiments, genomic DNA was analyzed using two restriction enzyme digestion strategies.

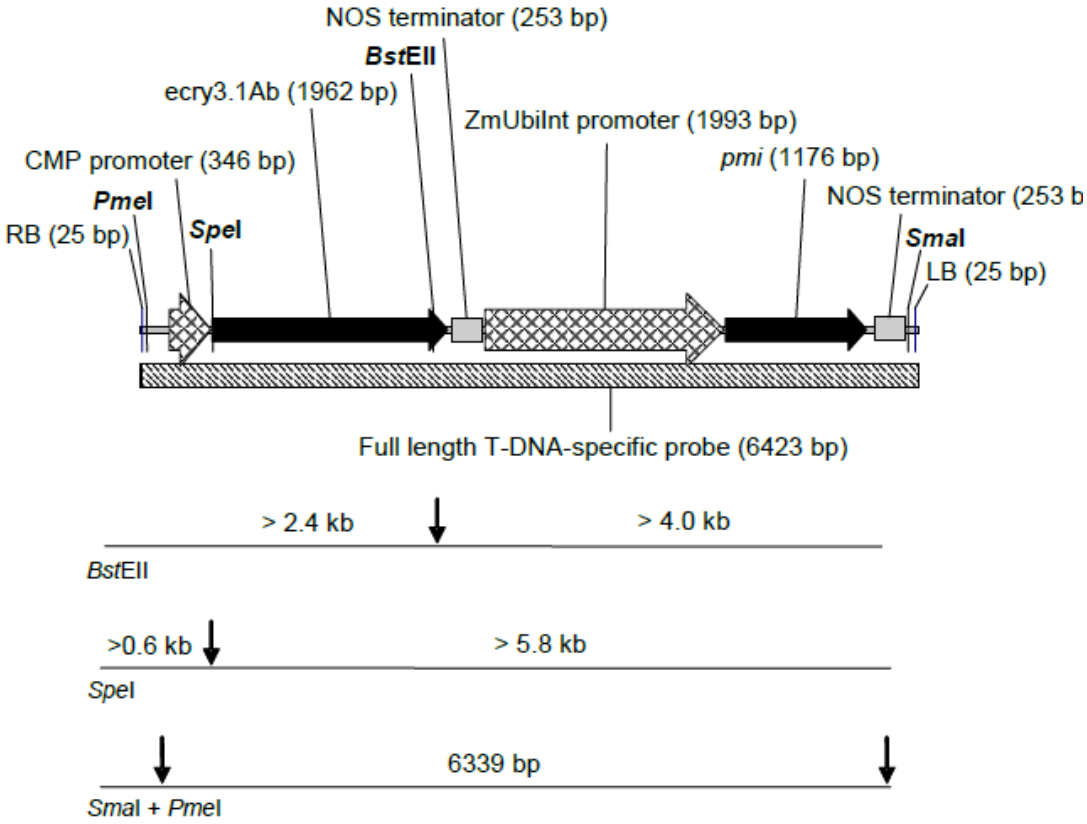
In the first strategy, the corn genomic DNA was digested with an enzyme that cut once within the 5307 corn insert. The other recognition sites for this enzyme were located in the corn genome flanking the 5307 corn insert. This first strategy was used twice with two different enzymes (*Bst*EII and *Spe*I) to determine the copy number of the 5307 corn insert and the presence or absence of extraneous DNA fragments of the plasmid pSYN12274 T-DNA in other

regions of the 5307 corn genome. These digests were expected to result in only two hybridization bands corresponding to the 5307 corn insert when a full length T-DNA-specific probe was used. More than two bands with either digest would have indicated that there were multiple copies of the insert in the plant genome.

In the second strategy, the corn genomic DNA was digested with *Sma*I + *Pme*I, which cut within the 5307 corn insert such that a DNA fragment of predictable size was released. This strategy was used to determine the presence of any closely linked extraneous DNA fragments of the plasmid pSYN12274 T-DNA.

Figure IV-15 shows a map of the T-DNA of the 5307 corn transformation plasmid pSYN12274, indicating the location of the full-length T-DNA-specific probe and restriction sites for *Bst*EII, *Spe*I, *Sma*I, and *Pme*I. Figure IV-16 depicts the results of the corresponding Southern blot analyses, and Table IV-8 provides the expected and observed sizes of the hybridization bands.

Figure IV-15. Location of the *Bst*EII, *Spe*I, *Sma*I, and *Pme*I restriction sites and position of the 6423-bp full-length T-DNA-specific probe in the T-DNA region of the transformation plasmid pSYN12274.



The vertical arrows indicate the site of restriction digestion
Sizes of the expected restriction fragments are indicated

Table IV-8. Expected and observed hybridization band sizes in Southern blot analysis of 5307 corn, using a full length T-DNA-specific probe and restriction enzymes *Bst*EII, *Spe*I, and *Sma*I + *Pme*I.

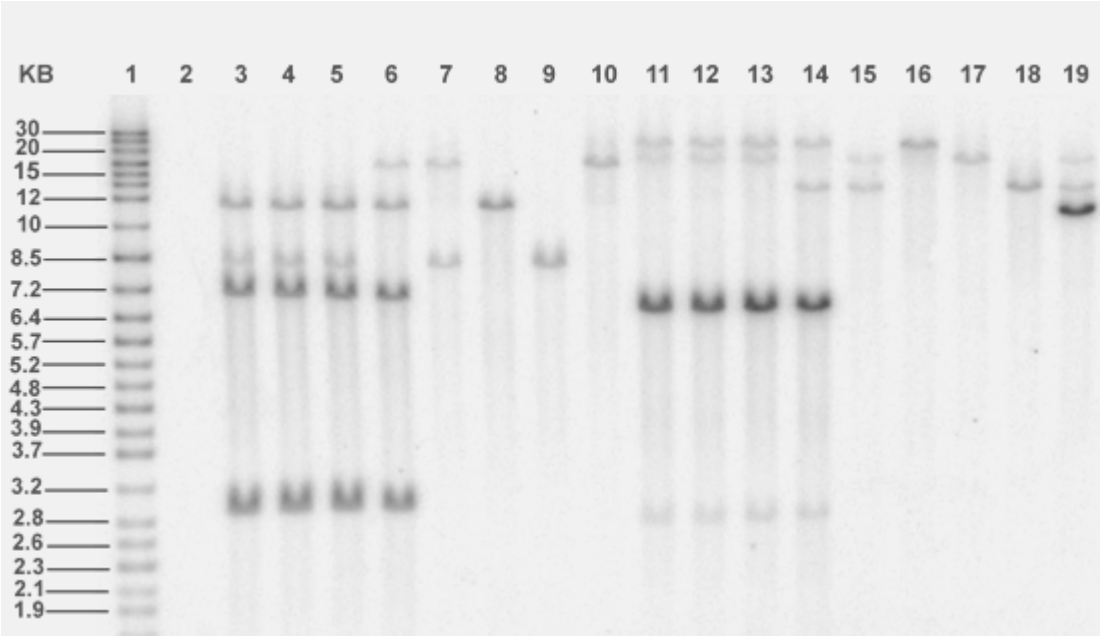
Figure & Lane No.	Source of DNA	Restriction enzyme(s)	Expected number of bands	Expected band size (kb)	Observed band size (kb)
Figure IV-16A, Lane A3	5307 F_1	*Bst*EII	2 5307 insert x endogenous	>2.4 >4 unknown	~2.9 ~7.2 ~8.4 (endogenous) ~12 (endogenous)
Figure IV-16A, Lane A4	5307 BC6	*Bst*EII	2 5307 insert x endogenous	>2.4 >4 unknown	~2.9 ~7.2 ~8.4 (endogenous) ~12 (endogenous)
Figure IV-16A, Lane A5	5307 BC7	*Bst*EII	2 5307 insert x endogenous	>2.4 >4 unknown	~2.9 ~7.2 ~8.4 (endogenous) ~12 (endogenous)
Figure IV-16A, Lane A6	5307 NP2171 × $BC5F_3$	*Bst*EII	2 5307 insert x endogenous	>2.4 >4 unknown	~2.9 ~7.2 ~12 (endogenous) ~18 (endogenous)
Figure IV-16A, Lane A7	NP2171 × NP2460	*Bst*EII	x endogenous	unknown	~8.4 (endogenous) ~18 (endogenous)
Figure IV-16A, Lane A8	NP2222	*Bst*EII	x endogenous	unknown	~12 (endogenous)
Figure IV-16A, Lane A9	NP2460	*Bst*EII	x endogenous	unknown	~8.4 (endogenous)
Figure IV-16A, Lane A10	NP2171	*Bst*EII	x endogenous	unknown	~18 (endogenous)
Figure IV-16A, Lane A11	5307 F_1	*Spe*I	2 5307 insert x endogenous	>0.6 >5.8 unknown	~2.6 ~7 ~20 (endogenous) ~25 (endogenous)
Figure IV-16A, Lane A12	5307 BC6	*Spe*I	2 5307 insert x endogenous	>0.6 >5.8 unknown	~2.6 ~7 ~20 (endogenous) ~25 (endogenous)
Figure IV-16A, Lane A13	5307 BC7	*Spe*I	2 5307 insert x endogenous	>0.6 >5.8 unknown	~2.6 ~7 ~20 (endogenous) ~25 (endogenous)
Figure IV-16A, Lane A14	5307 NP2171 × $BC5F_3$	*Spe*I	2 5307 insert x endogenous	>0.6 >5.8 unknown	~2.6 ~7 ~14 (endogenous) ~25 (endogenous)
Figure IV-16A, Lane A15	NP2171/NP2460	*Spe*I	x endogenous	unknown	~14 (endogenous) ~20 (endogenous)
Figure IV-16A, Lane A16	NP2222	*Spe*I	x endogenous	unknown	~25 (endogenous)
Figure IV-16A, Lane A17	NP2460	*Spe*I	x endogenous	unknown	~20 (endogenous)
Figure IV-16A, Lane A18	NP2171	*Spe*I	x endogenous	unknown	~14 (endogenous)
Figure IV-16A, Lane A19	Positive control (NP2171/NP2460 and 16.53 pg of	*Spe*I	1 pSYN12274 x endogenous	11.8 unknown	~11.8 ~14 (endogenous) ~20 (endogenous)

Table IV-8 (*continued*). Expected and observed hybridization band sizes in Southern blot analysis of 5307 corn, using a full length T-DNA-specific probe and restriction enzymes *Bst*EII, *Spe*I, and *Sma*I + *Pme*I

Figure & Lane No.	Source of DNA	Restriction enzyme(s)	Expected number of bands	Expected band size (kb)	Observed band size (kb)
Figure IV-16B, Lane B3	5307 F_1	*Sma*I + *Pme*I	1 5307 insert x endogenous	6.3 unknown	~6.3 ~18 (endogenous)
Figure IV-16B, Lane B4	5307 BC6	*Sma*I + *Pme*I	1 5307 insert x endogenous	6.3 unknown	~6.3 ~18 (endogenous)
Figure IV-16B, Lane B5	5307 BC7	*Sma*I + *Pme*I	1 5307 insert x endogenous	6.3 unknown	~6.3 ~18 (endogenous)
Figure IV-16B, Lane B6	5307 NP2171 × $BC5F_3$	*Sma*I + *Pme*I	1 5307 insert x endogenous	6.3 unknown	~6.3 ~18 (endogenous)
Figure IV-16B, Lane B7	NP2171 × NP2460	*Sma*I + *Pme*I	x endogenous	unknown	>30 (endogenous)
Figure IV-16B, Lane B8	NP2222	*Sma*I + *Pme*I	x endogenous	unknown	~18 (endogenous)
Figure IV-16B, Lane B9	NP2460	*Sma*I + *Pme*I	x endogenous	unknown	>30 (endogenous)
Figure IV-16B, Lane B10	NP2171	*Sma*I + *Pme*I	x endogenous	unknown	>30 (endogenous)
Figure IV-16B, Lane B11	Positive control (NP2171 × NP2460 and 16.53 pg of pSYN12274)	*Sma*I + *Pme*I	1 pSYN12274 x endogenous	6.3 unknown	~6.3 >30 (endogenous)

Figure IV-16. Genetic stability Southern blot analysis of 5307 corn with the 6423-bp full-length T-DNA-specific probe, using restriction enzymes *Bst*EII, *Spe*I, and *Sma*I + *Pme*I.

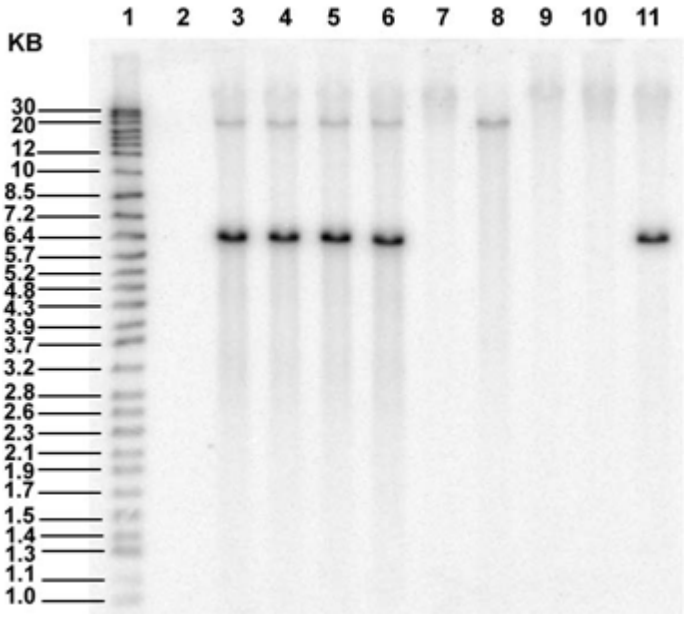
(A) *Bst*EII or *Spe*I



Lane A1 = molecular weight markers
Lane A2 = blank
Lane A3 = 5307 F_1 digested with *Bst*EII
Lane A4 = 5307 BC6 digested with *Bst*EII
Lane A5 = 5307 BC7 digested with *Bst*EII
Lane A6 = 5307 NP2171 × $BC5F_3$ digested with *Bst*EII
Lane A7 = NP2171 × NP2460 digested with *Bst*EII
Lane A8 = NP2222 digested with *Bst*EII
Lane A9 = NP2460 digested with *Bst*EII
Lane A10 = NP2171 digested with *Bst*EII
Lane A11 = 5307 F_1 digested with *Spe*I
Lane A12 = 5307 BC6 digested with *Spe*I
Lane A13 = 5307 BC7 digested with *Spe*I
Lane A14 = 5307 NP2171 × $BC5F_3$ digested with *Spe*I
Lane A15 = NP2171 × NP2460 digested with *Spe*I
Lane A16 = NP2222 digested with *Spe*I
Lane A17 = NP2460 digested with *Spe*I
Lane A18 = NP2171 digested with *Spe*I
Lane A19 = Positive control (NP2171 × NP2460 and 16.53 pg of pSYN12274 digested with *Spe*I)

Figure IV-16 *(continued)*. Genetic stability Southern blot analysis of 5307 corn with the 6423-bp full-length T-DNA-specific probe, using restriction enzymes *Bst*EII, *Spe*I, and *Sma*I + *Pme*I.

B. *Sma*I + *Pme*I



Lane B1 = molecular weight markers
Lane B2 = blank
Lane B3 = 5307 F_1 digested with *Sma*I + *Pme*I
Lane B4 = 5307 BC6 digested with *Sma*I + *Pme*I
Lane B5 = 5307 BC7 digested with *Sma*I + *Pme*I
Lane B6 = 5307 NP2171 × $BC5F_3$ digested with *Sma*I + *Pme*I
Lane B7 = NP2171 × NP2460 digested with *Sma*I + *Pme*I
Lane B8 = NP2222 digested with *Sma*I + *Pme*I
Lane B9 = NP2460 digested with *Sma*I + *Pme*I
Lane B10 = NP2171 digested with *Sma*I + *Pme*I
Lane B11 = Positive control (NP2171 × NP2460 and 16.53 pg of pSYN12274 digested with *Sma*I + *Pme*I)

For Southern blot analysis with genomic DNA digested with *Bst*EII and probed with the full-length T-DNA-specific probe, two hybridization bands of approximately 2.9 kb and 7.2 kb were observed in lanes containing DNA extracted from 5307 F_1, 5307 BC6, 5307 BC7, and 5307 NP2171 × $BC5F_3$ plants, as expected (Figure IV-16A, Lanes A3, A4, A5, and A6) (Table IV-8). These hybridization bands were absent in lanes containing DNA extracted from the control plants (Figure IV-16A, Lanes A7, A8, A9, and A10) and were, therefore, specific to the 5307 corn insert. Three hybridization bands of approximately 11.8 kb, 14 kb, and 20 kb were observed in the lane containing the positive control (16.53 pg of plasmid pSYN12274 DNA digested with *Spe*I and loaded with DNA extracted from NP2171 × NP2460 plants) (Figure IV-16A, Lane A19).

Additional bands resulting from cross-hybridization of the ZmUbiInt promoter sequence present on the full length T-DNA-specific probe with the endogenous maize polyubiquitin promoter sequence were also detected in all material analyzed. Two hybridization bands of approximately 8.4 kb and 12 kb, corresponding to the hybridization bands observed in lanes containing DNA extracted from NP2460 plants (Figure IV-16A, Lane A9) and NP2222 plants (Figure IV-16A, Lane A8), respectively, were observed in lanes containing DNA extracted from 5307 F_1, 5307 BC6, and 5307 BC7 plants (Figure IV-16A, Lanes A3, A4, and A5, respectively). Two hybridization bands of approximately 12 kb and 18 kb, corresponding to the hybridization bands observed in lanes containing DNA extracted from NP2222 plants (Figure IV-16A, Lane A8) and NP2171 plants (Figure IV-16A, Lane A10), respectively, were observed in the lane containing DNA extracted from 5307 NP2171 × $BC5F_3$ plants (Figure IV-16A, Lane A6). Finally, two hybridization bands of approximately 8.4 kb and 18 kb, corresponding to the hybridization bands observed in lanes containing DNA extracted from NP2460 plants (Figure IV-16A, Lane A9) and NP2171 plants (Figure IV-16A, Lane A10), respectively, were observed in lanes containing DNA extracted from NP2171 × NP2460 plants (Figure IV-16A, Lane A7).

For Southern blot analysis with genomic DNA digested with *Spe*I and probed with the full length T-DNA-specific probe, two hybridization bands of approximately 2.6 kb and 7.0 kb were observed in lanes containing DNA extracted from 5307 F_1, 5307 BC6, 5307 BC7, and 5307 NP2171 × $BC5F_3$ plants, as expected (Figure IV-16A, Lanes A11, A12, A13, and A14) (Table IV-8). These bands were absent in lanes containing DNA extracted from the control plants (Figure IV-16A, Lanes A15, A16, A17, and A18) and were, therefore, specific to the 5307 corn insert. Three hybridization bands of approximately 11.8 kb, 14 kb, and 20 kb were observed in the lane containing the positive control (16.53 pg of plasmid pSYN12274 DNA digested with *Spe*I and loaded with DNA extracted from NP2171 × NP2460 plants) (Figure IV-16A, Lane A19).

Additional bands resulting from cross-hybridization of the ZmUbiInt promoter sequence present in the full length T-DNA-specific probe with the endogenous maize polyubiquitin promoter sequence were also detected in all material analyzed. Two hybridization bands of approximately 20 kb and 25 kb, corresponding to the hybridization bands observed in lanes containing DNA

extracted from NP2460 plants (Figure IV-16A, Lane A17) and NP2222 plants (Figure IV-16A, Lane A16), respectively, were observed in lanes containing DNA extracted from 5307 F_1, 5307 BC6, and 5307 BC7 plants (Figure IV-16A, Lanes A11, A12, and A13). Two hybridization bands of approximately 14 kb and 25 kb, corresponding to the hybridization bands observed in lanes containing DNA extracted from NP2171 plants (Figure IV-16A, Lane A18) and NP2222 plants (Figure IV-16A, Lane A16), respectively, were observed in the lane containing DNA extracted from 5307 NP2171 × $BC5F_3$ plants (Figure IV-16A, Lane A14).

Finally, two hybridization bands of approximately 14 kb and 20 kb, corresponding to the hybridization bands observed in lanes containing DNA extracted from NP2171 plants (Figure IV-16A, Lane A18) and NP2460 plants (Figure IV-16A, Lane A17), respectively, were observed in lanes containing DNA extracted from NP2171 × NP2460 plants (Figure IV-16A, Lane A15).

For Southern blot analysis with genomic DNA digested with *Sma*I + *Pme*I and probed with the full length T-DNA-specific probe, one hybridization band of approximately 6.3 kb was observed in lanes containing DNA extracted from 5307 F_1, 5307 BC6, 5307 BC7, and 5307 NP2171 × $BC5F_3$ plants as expected (Figure IV-16B, Lanes B3, B4, B5, and B6) (Table IV-8). This hybridization band was absent in lanes containing DNA extracted from the control plants (Figure IV-16B, Lanes B7, B8, B9, and B10) and was, therefore, specific to the 5307 corn insert. One hybridization band of approximately 6.3 kb and a high molecular weight band (greater than 30 kb) was observed in the lane containing the positive control (16.53 pg of plasmid pSYN12274 DNA digested with *Sma*I + *Pme*I and loaded with DNA extracted from NP2171 × NP2460 plants) (Figure IV-16B, Lane B11). At least one additional band resulting from cross-hybridization of the ZmUbiInt promoter sequence present on the full length T-DNA-specific probe with the endogenous maize polyubiquitin promoter sequence was also detected in all material analyzed. One hybridization band of approximately 18 kb, corresponding to the hybridization band observed in the lane containing DNA extracted from NP2222 plants (Figure IV-16B, Lane B8), was observed in lanes containing DNA extracted from 5307 F_1, 5307 BC6, 5307 BC7 and 5307 NP2171 × $BC5F_3$ plants (Figure IV-16B, Lanes B3, B4, B5, and B6). An additional high molecular weight band (greater than 30 kb) was observed in lanes containing DNA extracted from NP2460 plants (Figure IV-16B, Lane B9) and NP2171 plants (Figure IV-16B, Lane B10). This faint high molecular weight band (greater than 30 kb) was observed in all lanes containing DNA extracted from plants carrying either the NP2460 polyubiquitin promoter allele (5307 F_1, 5307 BC6, 5307 BC7, and NP2171 × NP2460) (Figure IV-16B, Lanes B3, B4, B5, and B7) and/or the NP2171 polyubiquitin promoter allele (5307 NP2171 × $BC5F_3$ and NP2171 × NP2460) (Figure IV-16B, Lanes B6 and B7).

Data from these Southern blot analyses demonstrated that the 5307 corn insert integrated into a single locus of the corn genome as only two hybridization bands specific to the 5307 corn insert were observed when genomic DNA was digested with *Bst*EII and probed with a full length T-DNA-specific probe and only two hybridization bands specific to the 5307 corn insert were observed when the genomic DNA was digested with *Spe*I and probed with a full length T-DNA-specific probe. These hybridization bands were specific to the 5307 corn insert and corresponded to each side of the restriction site of the enzyme used for Southern blot analysis. Additional hybridization bands observed resulted from cross-hybridization between the ZmUbiInt promoter sequence present on the full length T-DNA-specific probe with the maize endogenous polyubiquitin promoter sequence; these bands were consistent with the genetic make-up of the various generations analyzed. As expected, the 5307 F_1, 5307 BC6, and 5307 BC7 generations carry the maize polyubiquitin promoter allelic forms present in NP2222 and NP2460, the 5307 NP2171 × $BC5F_3$ generation carries the maize polyubiquitin promoter allelic forms present in NP2171 and NP2222, and the control NP2171 × NP2460 material carries the maize polyubiquitin promoter allelic forms present in NP2171 and NP2460.

Data from these Southern blot analyses also demonstrated that a complete copy of the 5307 corn insert integrated into the corn genome as the hybridization band specific to the 5307 corn insert observed when the genomic DNA was digested with *Sma*I + *Pme*I was the predicted size. The approximately 18 kb band observed on this Southern blot was present in lanes containing DNA extracted from NP2222 plants and all generations carrying the NP2222 polyubiquitin promoter allelic form (5307 F_1, 5307 BC6, 5307 BC7, and 5307 NP2171 × $BC5F_3$). The faint and high molecular weight band (greater than 30 kb) observed on this Southern blot was present in lanes containing DNA extracted from NP2460 plants and NP2171 plants and all generations carrying either the NP2460 polyubiquitin promoter allelic form (5307 F_1, 5307 BC6, 5307 BC7, and NP2171 × NP2460) and/or the NP2171 polyubiquitin promoter allelic form (5307 NP2171 × $BC5F_3$ and NP2171 × NP2460). Because no additional bands were observed (other than those associated with the 5307 corn insert and the corn endogenous sequence), Southern blot analyses indicated that there were no extraneous DNA fragments of plasmid pSYN12274 T-DNA in other regions of the 5307 corn genome. The data depicted in the Southern blot analyses showed that the hybridization bands specific to the insert were identical in lanes containing DNA extracted from plants grown from all generations (5307 F_1, 5307 BC6, 5307 BC7, and 5307 NP2171 × $BC5F_3$); these results indicated that the 5307 corn insert is stably inherited from one generation to the next. Southern blot analyses demonstrated that 5307 corn contains a single, complete copy of the insert and that there are no extraneous DNA fragments of plasmid pSYN12274 T-DNA inserted elsewhere in the 5307 corn genome. Identical hybridization patterns across all generations of 5307 corn analyzed in this study indicates that the insert is stably inherited from one generation to the next.

IV.E.4.b. Stability of the T-DNA Using a Plasmid Backbone-Specific Probe

The absence of plasmid backbone sequence in 5307 corn was assessed by Southern blot analyses using plasmid pSYN12274 backbone sequence as a probe on Southern blots of DNA subjected to the two restriction enzyme digestion strategies described above. This plasmid backbone-specific probe contained every base of the plasmid pSYN12274 backbone present outside of the T-DNA region. With both restriction enzyme digestion strategies, no hybridization bands were expected.

The Southern blot analyses included genomic DNA from four generations of 5307 corn plants (5307 F_1, 5307 BC6, 5307 BC7, 5307 NP2171 × $BC5F_3$) and the corresponding nontransgenic control plants (NP2171 × NP2460, NP2222, NP2460, and NP2171) (see pedigree diagram,Figure IV-4). Figure IV-17 shows a map of the plasmid pSYN12274 indicating the location of the plasmid pSYN12274 backbone-specific probe and restriction sites for *Bst*EII, *Spe*I, *Sma*I, and *Pme*I. Figure IV-18 depicts the results of the corresponding Southern blot analyses, and Table IV-9 provides the expected and observed sizes of the hybridization bands.

For Southern blot analyses with genomic DNA digested with *Bst*EII and probed with the plasmid pSYN12274 backbone-specific probe, no hybridization bands were observed in the lanes containing DNA extracted from plants grown from the test and control substances (5307 F_1, 5307 BC6, 5307 BC7, 5307 NP2171 × $BC5F_3$, NP2171 × NP2460, NP2222, NP2460, and NP2171) (Figure IV-18A, Lanes 3, 4, 5, 6, 7, 8, 9, and 10) as expected. The positive control (16.53 pg of plasmid pSYN12274 DNA digested with *Spe*I and loaded with DNA extracted from NP2171 × NP2460 plants) produced the expected 11.8 kb band (Figure IV-18A, Lane 19). For Southern blot analyses with genomic DNA digested with *Spe*I and probed with the plasmid pSYN12274 backbone-specific probe, no hybridization bands were observed in the test and control substances (5307 F_1, 5307 BC6, 5307 BC7, 5307 NP2171 × $BC5F_3$, NP2171 × NP2460, NP2222, NP2460, and NP2171) (Figure IV-18A, Lanes A11, A12, A13, A14, A15, A16, A17, and A18) as expected. The positive control (16.53 pg of plasmid pSYN12274 DNA digested with *Spe*I and loaded with DNA extracted from NP2171 × NP2460 plants) produced the expected 11.8 kb band (Figure IV-18A, Lane A19). For Southern blot analyses with genomic DNA digested with *Sma*I + *Pme*I and probed with the backbone-specific probe, no hybridization bands were observed in the test and control substances (5307 F_1, 5307 BC6, 5307 BC7, 5307 NP2171 × $BC5F_3$, NP2171 × NP2460, NP2222, NP2460, and NP2171) (Figure IV-18B, Lanes B3, B4, B5, B6, B7, B8, B9, and B10). The positive control (16.53 pg of plasmid pSYN12274 DNA digested with *Sma*I + *Pme*I and loaded with DNA extracted from NP2171 × NP2460 plants) produced the expected 4.2 kb and 1.2 kb bands (Figure IV-18B, Lane B11).

The data from the three Southern blot analyses demonstrated that all the 5307 corn generations analyzed are free of plasmid pSYN12274 backbone sequence.

Figure IV-17. Location of the *Bst*EII, *Spe*I, *Sma*I, and *Pme*I restriction sites and position of the 5312-bp plasmid backbone-specific probe in the transformation plasmid pSYN12274.

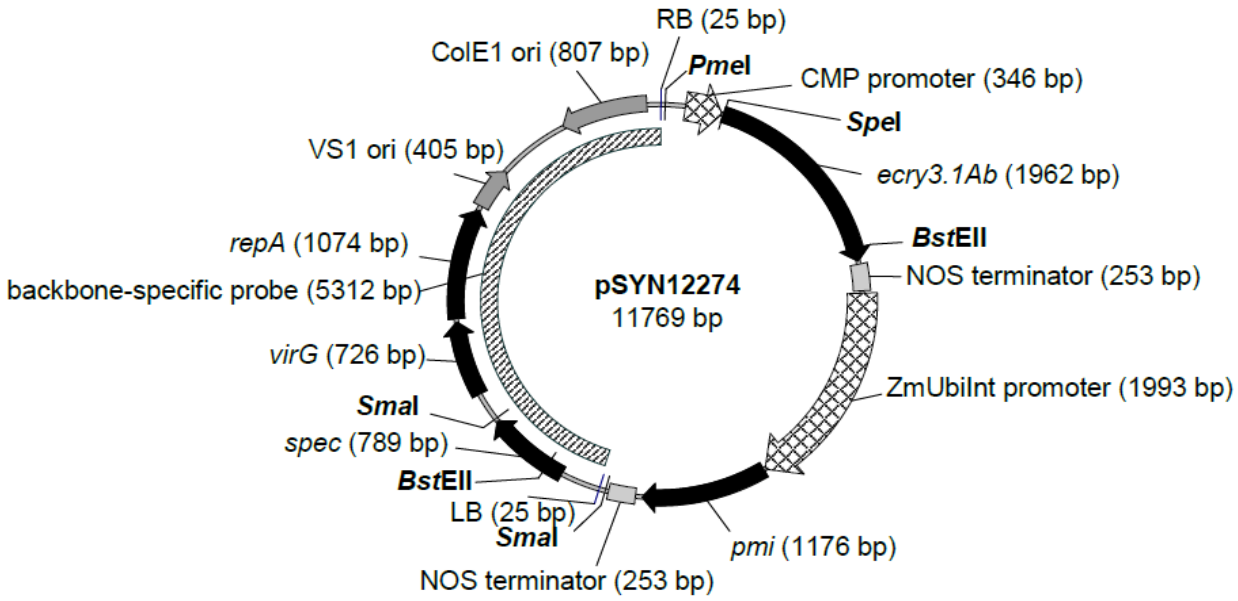
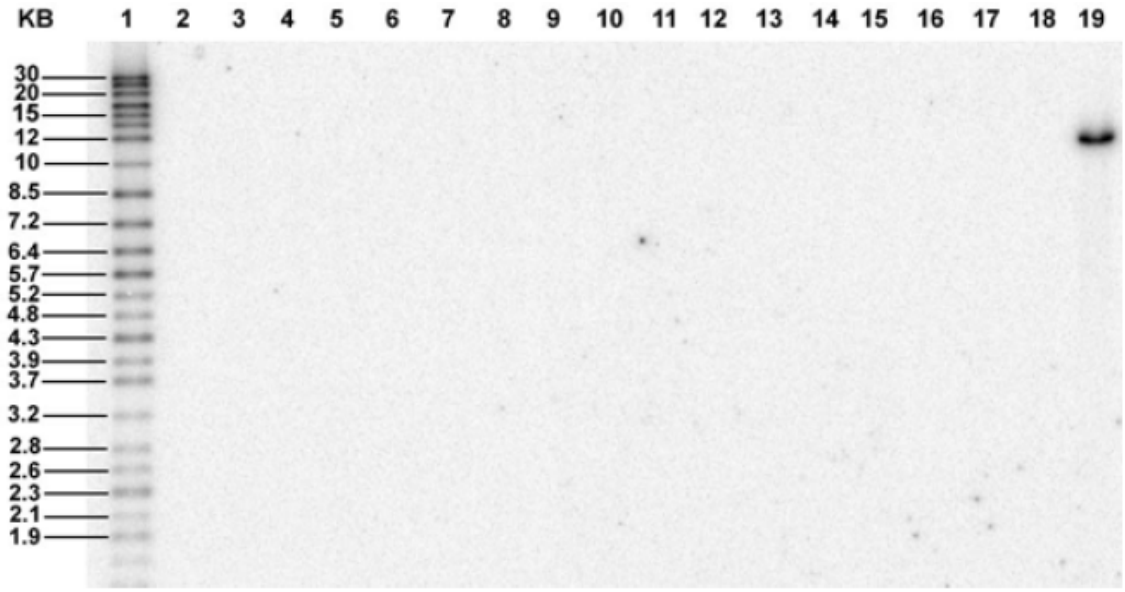


Table IV-9. Expected and observed hybridization band sizes in Southern blot analysis of 5307 corn, using a plasmid pSYN12274 backbone-specific probe and restriction enzymes *Bst*EII, *Spe*I, and *Sma*I + *Pme*I.

Figure & Lane Number	Source of DNA	Restriction enzyme(s)	Expected number of bands	Expected band size (kb)	Observed band size (kb)
Figure IV-18A, Lane A3	5307 F_1	*Bst*EII	none	none	none
Figure IV-18A, Lane A4	5307 BC6	*Bst*EII	none	none	none
Figure IV-18A, Lane A5	5307 BC7	*Bst*EII	none	none	none
Figure IV-18A, Lane A6	5307 NP2171 × $BC5F_3$	*Bst*EII	none	none	none
Figure IV-18A, Lane A7	NP2171 × NP2460	*Bst*EII	none	none	none
Figure IV-18A, Lane A8	NP2222	*Bst*EII	none	none	none
Figure IV-18A, Lane A9	NP2460	*Bst*EII	none	none	none
Figure IV-18A, Lane A10	NP2171	*Bst*EII	none	none	none
Figure IV-18A, Lane A11	5307 F_1	*Spe*I	none	none	none
Figure IV-18A, Lane A12	5307 BC6	*Spe*I	none	none	none
Figure IV-18A, Lane A13	5307 BC7	*Spe*I	none	none	none
Figure IV-18A, Lane A14	5307 NP2171 × $BC5F_3$	*Spe*I	none	none	none
Figure IV-18A, Lane A15	NP2171 × NP2460	*Spe*I	none	none	none
Figure IV-18A, Lane A16	NP2222	*Spe*I	none	none	none
Figure IV-18A, Lane A17	NP2460	*Spe*I	none	none	none
Figure IV-18A, Lane A18	NP2171	*Spe*I	none	none	none
Figure IV-18A, Lane A19	Positive control (NP2171×NP2460 and 16.53 pg of pSYN12274)	*Spe*I	1	11.8	~11.8
Figure IV-18B, Lane B3	5307 F_1	*Sma*I + *Pme*I	none	none	none
Figure IV-18B, Lane B4	5307 BC6	*Sma*I + *Pme*I	none	none	none
Figure IV-18B, Lane B5	5307 BC7	*Sma*I + *Pme*I	none	none	none
Figure IV-18B, Lane B6	5307 NP2171 × $BC5F_3$	*Sma*I + *Pme*I	none	none	none
Figure IV-18B, Lane B7	NP2171 × NP2460	*Sma*I + *Pme*I	none	none	none
Figure IV-18B, Lane B8	NP2222	*Sma*I + *Pme*I	none	none	none
Figure IV-18B, Lane B9	NP2460	*Sma*I + *Pme*I	none	none	none
Figure IV-18B,Lane B10	NP2171	*Sma*I + *Pme*I	none	none	none
Figure IV-18B,Lane B11	Positive control (NP2171×NP2460 and 16.53 pg of pSYN12274)	*Sma*I + *Pme*I	2	4.2 1.2	~4.2 ~1.2

Figure IV-18. Genetic stability Southern blot analysis of 5307 corn with the 5312-bp plasmid backbone-specific probe, using restriction enzymes *Bst*EII, *Spe*I, and *Sma*I + *Pme*I.

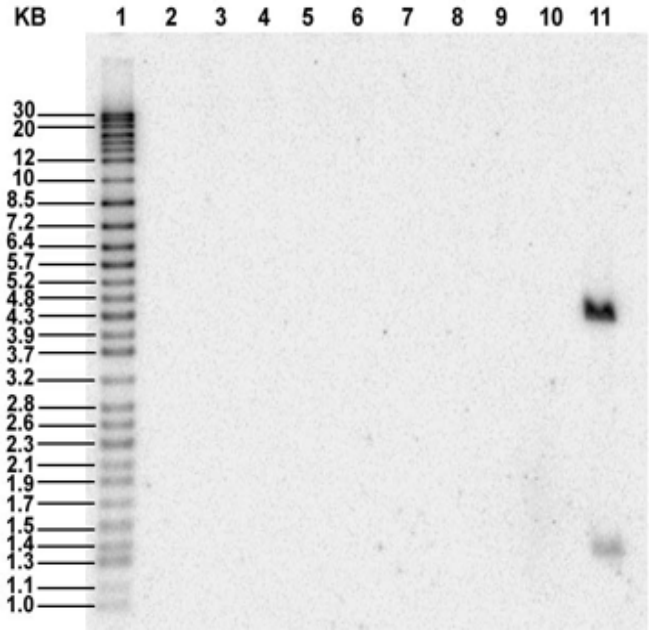
(A) *Bst*EII or *Spe*I



Lane A1 = molecular weight markers
Lane A2 = blank
Lane A3 = 5307 F_1 digested with *Bst*EII
Lane A4 = 5307 BC6 digested with *Bst*EII
Lane A5 = 5307 BC7 digested with *Bst*EII
Lane A6 = 5307 NP2171 × $BC5F_3$ digested with *Bst*EII
Lane A7 = NP2171 × NP2460 digested with *Bst*EII
Lane A8 = NP2222 digested with *Bst*EII
Lane A9 = NP2460 digested with *Bst*EII
Lane A10 = NP2171 digested with *Bst*EII
Lane A11 = 5307 F_1 digested with *Spe*I
Lane A12 = 5307 BC6 digested with *Spe*I
Lane A13 = 5307 BC7 digested with *Spe*I
Lane A14 = 5307 NP2171 × $BC5F_3$ digested with *Spe*I
Lane A15 = NP2171 × NP2460 digested with *Spe*I
Lane A16 = NP2222 digested with *Spe*I
Lane A17 = NP2460 digested with *Spe*I
Lane A18 = NP2171 digested with *Spe*I
Lane A19 = Positive control (NP2171 × NP2460 and 16.53 pg of pSYN12274 digested with *Spe*I)

Figure V-18. (*Continued*)

(B) Sma*I* + Pme*I*



Lane B1 = molecular weight markers
Lane B2 = blank
Lane B3 = 5307 F_1 digested with *Sma*I + *Pme*I
Lane B4 = 5307 BC6 digested with *Sma*I + *Pme*I
Lane B5 = 5307 BC7 digested with *Sma*I + *Pme*I
Lane B6 = 5307 NP2171 × $BC5F_3$ digested with *Sma*I + *Pme*I
Lane B7 = NP2171 × NP2460 digested with *Sma*I + *Pme*I
Lane B8 = NP2222 digested with *Sma*I + *Pme*I
Lane B9 = NP2460 digested with *Sma*I + *Pme*I
Lane B10 = NP2171 digested with *Sma*I + *Pme*I
Lane B11 = Positive control (NP2171 × NP2460 and 16.53 pg of pSYN12274 digested with *Sma*I + *Pme*I)

IV.E.5. Mendelian Inheritance of the DNA Insert

A Chi-square (X^2) analysis of *ecry3.1Ab* and *pmi* inheritance data over three generations of Event 5307 corn was performed to test the hypothesis that the transgenes are inherited in accordance with the laws of Mendelian genetics. The Chi-square analysis was based on a comparison of observed and expected gene segregation ratios from each generation. Real-time PCR analyses (Ingham et al. 2001) were conducted on DNA extracted from leaf tissue of three generations of 5307 corn plants to determine the number of plants that were positive or negative for both the transgenes *ecry3.1Ab* and *pmi*. The plants used for this analysis were grown from seeds of the 5307 F_1, 5307 BC6 and 5307 BC7 generations; these genotypes are also indicated in the list of seed materials used (Table IV-2) and the pedigree diagram (Figure IV-4). During breeding, only progeny that tested positive for the two transgenes were selected for further crossing; thus, the expected inheritance ratio for positive to negative plants was 1:1 in each generation.

Genotypic data generated for the three 5307 corn generations were used to assess the goodness-of-fit of the observed genotypic ratios to the expected genotypic ratios using Chi-square analysis with Yates' correction factor as in Armitage and Berry (1987).

$$X^2 = \sum [|(\text{observed} - \text{expected})| - 0.5]^2 / \text{expected}$$

All plants tested positive for the control assay targeting the endogenous corn gene *adh1*, confirming that DNA was present in all real-time PCR reactions. The expected and observed frequencies of *ecry3.1Ab* and *pmi* for each generation are presented in Table IV-10 and Table IV-11. The critical value for rejection of the null hypothesis at the 5% level was 3.84 (Strickberger 1976). The Chi-square values for each generation tested were found to be less than 3.84. This analysis demonstrates that both the *ecry3.1Ab* and *pmi* are inherited in a predictable manner according to Mendelian principles. These results are consistent with the genetic characterization data for 5307 corn, which indicate stable integration of the T-DNA at a single locus in the genome.

Table IV-10. Observed *versus* expected frequencies for *ecry3.1Ab* across generations.

Trait	F_1		BC6		BC7	
	Observed	Expected	Observed	Expected	Observed	Expected
Positive	44	46	40	45.5	43	45
Negative	48	46	51	45.5	47	45
Total	92	92	91	91	90	90
X^2 value	0.098		1.099		0.100	

Table IV-11. Observed *versus* expected frequencies for *pmi* across generations.

Trait	F_1		BC6		BC7	
	Observed	Expected	Observed	Expected	Observed	Expected
Positive	44	46	40	45.5	43	45
Negative	48	46	51	45.5	47	45
Total	92	92	91	91	90	90
X^2 value	0.098		1.099		0.100	

IV.E.6. Flanking Sequence and Analysis to Identify Putative Open Reading Frames

Nucleotide sequences flanking the 5′ and 3′ ends of the T-DNA in 5307 corn were screened for similarity with sequences found in public databases. This comparison provided an indication of whether the 5307 corn T-DNA inserted into a known plant gene. A sequence similarity analysis was performed using Basic Local Alignment Search Tool for Nucleotides (BLASTN) software, version 2.2.19 (Altschul et al. 1997) which compared the flanking sequences with nucleotide sequences in the National Center for Biotechnology Information Nucleotide Collection (nr/nt) database (NCBI 2010a). The nr/nt database contains all sequences from the National Institutes of Health genetic sequence database (GenBank®), RefSeq Nucleotides, the European Molecular Biology Laboratory, the DNA Database of Japan, and nucleotide sequences derived from the three-dimensional structures from the Brookhaven Protein Data Bank. At the time the BLASTN analysis was run (May 7, 2010), the nr/nt database contained over 11 million sequences. Details of the parameters used in the BLASTN analysis are provided in Appendix A.

BLASTN analysis was conducted using the 1000 bp of corn genomic sequence flanking the 5′ end of the 5307 T-DNA insert as the query sequence. The search identified all alignments to sequences in the nr/nt database with search results yielding an *E*-value of 10 or lower. This analysis was repeated using the 1000 bp of corn genomic sequence flanking the 3′ end of the 5307 T-DNA as the query sequence. The *E*-value, or "expectation value," is a measure of the probability that matches between sequences occurred by chance. Search results involving comparisons between nucleotide sequences with highly similar sequences yield *E*-values approaching zero. The probability that sequence similarities occurred by chance increases with higher *E*-values (Ponting 2001).

Using the results of each BLASTN query, the 20 alignments with the lowest *E*-values were examined for this analysis. The corn genomic sequences flanking the 5′ region and the 3′ region of the 5307 corn insert aligned to multiple bacterial artificial chromosome (BAC) corn sequences that were annotated as various corn chromosome numbers (i.e., corn chromosome numbers 4, 5, 6, 8, 9, and 10). This suggests that the 5307 corn insert is located in a repetitive region of the corn genome. Both flanking sequences aligned to NCBI nucleotide database accession numbers AY530950.1, AY530951.1 and AY530952.1, which were annotated as containing gene sequences. However, the regions of AY530950.1, AY530951.1, and AY530952.1 that were similar to the corn genomic sequences flanking the 5′ region and the 3′ region of the 5307 insert were not annotated as a gene. Both flanking sequences also aligned to accession numbers AY664416.1, AY664413.1, AY664419.1, and AY664415.1. (However, alignments of corn genomic sequence flanking the 3′ region of the 5307 insert to accession numbers AY664419.1 and AY664415.1 were not among those with the 20 highest similarity scores.) These accession numbers are all annotated as containing sequences of various genes and repeat regions. However, the regions of AY664416.1, AY664413.1, AY664419.1, and AY664415.1 that were similar to the corn genomic sequences flanking the 5′ region and the 3′ region of the 5307 insert were not annotated as a gene. The corn genomic sequences flanking the 5′ region and the 3′

region of the 5307 insert aligned to a region of AY664413.1 that was annotated as repeat region Giepumx19002_LTR retrotransposon. Additionally, short regions of both flanking sequences (i.e., ~20 to 40 base pairs in length) aligned to multiple regions of multiple sequences in the database. Some of these regions were also annotated as repeat regions in accession numbers AY664416.1, AY664413.1, AY664419.1, and AY664415.1. These repeat regions were also associated with retrotransposons. Approximately 49 to 78% of the corn genome is suggested to be comprised of retrotransposons (San Miguel et al. 1998). However, only approximately 5% of the retrotransposon sequences in the corn genome are predicted to produce proteins (Meyers et al. 2001). Because retrotranposons are repetitive, the repeat regions that align to corn genomic sequences flanking the 5′ region and the 3′ region of the 5307 insert are likely to be repeated elsewhere in the corn genome.

BLASTN analysis indicated that the corn genomic sequence flanking the 3′ region of the 5307 corn insert is also similar to accession number EU954153.1, which was annotated as a hypothetical protein mRNA sequence from *Zea mays*. The region of the flanking sequence that is similar to EU954153.1 was located 109 bp downstream of the genome-to-insert junction. BLASTN analysis also indicated that the corn genomic sequence flanking the 3′ region of the 5307 corn insert is similar to accession number EZ054274.1, annotated as mRNA sequence from *Zea mays*. The region of the flanking sequence that is similar to EZ054274.1 was located 376 bp downstream of the genome-to-insert junction. The corn genomic sequence flanking the 5′ region of the 5307 corn insert did not align to sequence EU954153.1 or EZ054274.1. None of the alignments retrieved indicates that a known endogenous corn gene was interrupted by the Event 5307 T-DNA insert.

The Vector NTI Advance™ program, version 10.3.0, was used to identify any putative open reading frames (ORFs) that span the junctions between the corn genomic sequence and the 5307 corn T-DNA insert. For this analysis, putative ORFs were defined as DNA sequences in any reading frame that are contained between a putative start codon (ATG) and a putative stop codon (TAG, TAA, or TGA), and have a minimum translation size of 30 amino acids. This analysis identified one putative ORF of 243 bp; it spanned the junction between the corn genomic sequence and the 3′ region of the 5307 corn insert.

Bioinformatic analyses demonstrated that the translated 81-amino-acid sequence of this putative ORF had no significant sequence similarity to known or putative toxins or allergens. The methods used for these toxin and allergen similarity searches are described in Appendix A.

IV.E.7. Summary of the Genetic Characterization of Event 5307 Corn

Southern blot analyses demonstrate that Event 5307 corn (1) contains, at a single locus within the corn genome, a single copy each of the gene *ecry3.1Ab*, its CMP promoter sequence, the marker gene *pmi*, its ZmUbiInt promoter sequence, and the two expected copies of the NOS terminator sequence, one NOS terminator sequence regulating *ecry3.1Ab* and one NOS terminator sequence regulating *pmi*; (2) does not contain any extraneous DNA fragments of these functional elements inserted elsewhere in the corn genome; (3) and does not contain plasmid backbone sequence from the transformation plasmid, pSYN12274.

Nucleotide sequence analysis of the entire Event 5307 insert confirms that the insert is intact and that the organization of the functional elements within the insert is identical to their organization within pSYN12274. One nucleotide change compared to the sequence of pSYN12274 was identified 48 base pairs (bp) upstream of the CMP promoter in a noncoding region of the insert in Event 5307 corn. However, this nucleotide change had no effect on the functionality of the insert. Additionally, the analysis indicates that some truncation of the nucleotide sequence occurred at the 5′ and 3′ ends of the T-DNA during the transformation process that resulted in Event 5307 corn; such truncation occurs commonly in transformation via *Agrobacterium*. The entire RB and three bp of noncoding sequence at the 5′ end of the insert, and eight bp of the LB were truncated; however, these deletions had no effect on the functionality of the insert.

Sequence analysis of the Event 5307 insertion site demonstrates that 33 bp of corn genomic sequence were deleted when the Event 5307 insert integrated into the corn genome. BLASTN analyses comparing the corn genomic sequence flanking the Event 5307 insert to sequences in public databases indicate that the insert does not disrupt any known endogenous corn gene.

A putative 243-bp novel open reading frame (ORF) spanning the junction between corn genomic sequence and the 3′ region of the 5307 corn insert was identified. The translated 81-amino-acid sequence encoded by the putative ORF was screened for amino acid sequence similarity to known or putative allergens or toxins. Comparisons to the FARRP AllergenOnline database indicate that the amino acid translation of the identified putative ORF shows no biologically relevant amino acid sequence similarity to any known or putative protein allergens. Additionally, the results of a comprehensive amino acid similarity search of the NCBI Entrez® Protein Database indicate that the amino acid translation of the identified putative ORF shows no biologically relevant amino acid sequence similarity to any known or putative toxins. These data collectively demonstrate that there are no deleterious changes in the 5307 corn genome as a result of the T-DNA insertion.

The *ecry3.1Ab* and *pmi* segregation ratios over several generations of Event 5307 corn plants are consistent with linkage of these transgenes at a single locus in the corn nuclear genome. These data and the results from Southern blot analyses of multiple generations of Event 5307 corn indicate that the transgenic locus is stably inherited during conventional breeding.

V. Absence of Genes That Encode Resistance to Antibiotics

As indicated in Figure IV-1 and described in Table IV-1, the intended T-DNA between the left and right borders of plasmid pSYN12257 does not contain any sequences that encode antibiotic resistance markers. Therefore, no genes encoding resistance to antibiotics are predicted to occur in Event 5307 corn. Although the backbone DNA of the pSYN12257 transformation plasmid (i.e., the region not included between the left and right borders) contains the antibiotic resistance gene *spec* (see Table IV-1), it is predicted that this gene would not have been transferred to the corn genome during transformation. Southern blot analyses (Figure IV-18) described in Part IV.E.3.b. demonstrated no hybridization of Event 5307 corn DNA with the pSYN12274 backbone-specific probe (Figure IV-17). This confirms that, as expected, no *spec* DNA sequences were incorporated into Event 5307 corn DNA.

VI. Safety Assessment of the Introduced eCry3.1Ab and PMI Proteins

This section provides a summary of the safety assessment for the eCry3.1Ab and PMI proteins produced in Event 5307 corn. This section includes (1) information about the identity and function of each protein, (2) information characterizing the plant-produced proteins and demonstrating equivalence to microbially produced test substances used in safety studies, (3) information about the toxicity of each protein, (4) information about the allergenic potential of each protein, (5) information about history of safe use, and (6) information about eCry3.1Ab and PMI protein levels in plant tissues and exposure potential.

VI.A. Identity and Function of the eCry3.1Ab Protein in Event 5307 Corn

The eCry3.1Ab protein produced in Event 5307 corn is a chimeric Cry protein comprised largely of portions of modified Cry3A (mCry3A) protein and Cry1Ab protein, which are derived from *Bacillus thuringiensis* (*B.t.*)(see Table IV-1). As illustrated in Figure IV-2, the coleopteran-active eCry3.1Ab protein was produced by exchange of the variable regions (V1 to V6) between mCry3A (a coleopteran-active protein) and Cry1Ab (a lepidopteran-active protein). At the N-terminus of eCry3.1Ab are 22 amino acid residues that are not derived from either mCry3A or Cry1Ab, per se, but were the result of a PCR-induced mutation that resulted in a reading-frame shift in the nucleotide sequence of a portion of the gene *mcry3A*. The next 459 residues are identical to those of mCry3A, followed by 172 residues of Cry1Ab at the C-terminus. The eCry3.1Ab protein is 653 amino acid residues in size and has a molecular weight of approximately 73.7 kDa.

The eCry3.1Ab protein is insecticidally active against the larvae of certain coleopteran pests in the family Chrysomelidae, including western corn rootworm (*Diabrotica virgifera virgifera*), northern corn rootworm (*D. longicornis barberi*), Mexican corn rootworm (*D. virgifera zeae*), and Colorado potato beetle (*Leptinotarsa decemlineata*). The rootworm species are all pests of corn, whereas Colorado potato beetle is a pest of other crops. The eCry3.1Ab protein has not demonstrated insecticidal activity among Coleoptera outside the family Chrysomelidae, and no biological activity has been observed in tests of multiple other organisms, including lepidopteran insects, other nontarget insects, and avian, mammalian, and aquatic species.

The spectrum of activity of any specific insecticidal Cry protein is quite narrow, and is usually restricted to a few related species. One given Cry protein is usually active against only a few species within any phylogenic Order. The specificity of each *B.t.* Cry protein (δ-endotoxin) is the result of the efficiency of the various steps involved in producing an active protein toxin and its subsequent interaction with the epithelial cells in the insect midgut. To be insecticidal, most known *B.t.* δ-endotoxins must: (1) be ingested by the insect and solubilized in the insect gut, (2) be activated by specific proteolytic cleavages, (3) bind to specific receptors on the surface of the insect midgut and (4) form ion channels. The completion of all these four processes results in disruption of the normal function of the midgut leading to the death of the insect. The

eCry3.1Ab protein exhibits the same behavior as other coleopteran active *B.t.* δ-endotoxins including alkaline solubility, cleavage by chymotrypsin, specificity of brush border membrane binding and ion channel formation. The mode of action of eCry3.1Ab, like that of other Cry proteins, is highly specific to insects and is not relevant to mammalian species.

VI.B. Production of eCry3.1Ab Test Substance for Safety Studies

It was not feasible to extract sufficient eCry3.1Ab protein from Event 5307 corn plants to conduct safety studies, because such studies require relatively large amounts of purified test protein. Syngenta undertook extensive efforts to produce the identical eCry3.1Ab protein present in 5307 plants via a microbial production system, for use as a test substance. However, these efforts did not yield sufficient quantities of bioactive protein, and it proved necessary to produce eCry3.1Ab with an N-terminal tag of histidine residues as a purification aid. This is a common procedure for protein synthesis and purification. Except for the N-terminal tag consisting of one methionine and six histidine residues, the eCry3.1Ab produced in recombinant *E. coli* has the identical amino acid sequence as eCry3.1Ab produced in 5307 plants. The resulting lyophilized test substance was designated ECRY3.1AB-0208; it was determined to be 89.6% pure eCry3.1Ab by weight and the intact mass of the eCry3.1Ab protein, as measured by mass spectrometry, was 74.8 kDa. The methods used to produce and characterize this test substance are described in Appendix B. Studies comparing this microbially produced eCry3.1Ab test substance to eCry3A.1Ab from 5307 plants are described below, and demonstrate that microbially produced eCry3.1Ab is a suitable surrogate for eCry3.1Ab in 5307 plants.

VI.C. Characterization of the Plant- and *Escherichia coli*-produced eCry3.1Ab Proteins

The amino acid sequence of the plant-produced eCry3.1Ab can be deduced from the observation that the nucleotide sequence of *ecry3.1Ab* recovered from 5307 plants is identical to the intended sequence (see Part IV.E.1. DNA Insert Sequencing). Nevertheless, a series of analytical methods were used to characterize the eCry3.1Ab protein produced in Event 5307 corn and to assess whether the microbially produced eCry3.1Ab test substance, ECRY3.1AB-0208, is a suitable surrogate for use in food and feed safety studies. A description of the results of the protein characterization is summarized below. A detailed description of the methods and results for the protein characterization can be found in Appendix B.

The identities of the plant-produced and microbially produced eCry3.1Ab proteins were confirmed by immunoreactivity, apparent molecular weight, glycosylation status, peptide mass mapping analysis, and N-terminal amino acid sequence. Both proteins were shown to have the predicted molecular weight and cross-reacted with the same antibodies, as shown by Western blot analysis. There was no evidence of post-translational glycosylation of eCry3.1Ab from the plant-produced protein or the microbially produced protein. Peptide mass mapping identified 76% and 87% of the predicted eCry3.1Ab amino acid sequence for the plant-produced protein

and microbially produced protein, respectively, confirming the identity of the insecticidal protein from both sources.

In addition to the biochemical analyses, the eCry3.1Ab proteins from both sources were evaluated for biological activity. In bioassays against first-instar Colorado potato beetle,[1] both the plant-produced protein and microbially produced protein were insecticidally active, with comparable LC_{50} values (Table VI-1).

Table VI-1. Comparison of Colorado potato beetle larvae LC_{50} values for eCry3.1Ab from 5307 plants and microbial eCry3.1Ab test substance.

First instars were exposed to diets containing eCry3.1Ab from 5307 plants or test substance ECRY3.1AB-0208. Leaf extract from control plants was included in the bioassays with ECRY3.1AB-0208 to control for leaf matrix effects.

Replicate	LP5307[a]		LP-NEG[b] + ECRY3.1AB-0208	
	µg eCry3.1Ab/ml insect diet			
	LC_{50}	95% CI	LC_{50}	95% CI
#1	1.316	0.631-3.187	1.780	0.924-3.190
#2	1.669	0.875-3.279	1.113	0.113-4.001
#3	2.888	1.765-4.655	3.226	1.955-5.134

[a] Leaf protein extract of 5307 plants containing eCry3.1Ab .

[b] Leaf protein extract of control plants, tested in combination with eCry3.1Ab from test substance ECRY3.1AB-0208 to control for effects of the leaf protein matrix.

Based on these results, the identities of the plant- and microbially produced eCry3.1Ab proteins have been verified and it can be concluded that eCry3.1Ab produced in recombinant *E. coli* and Event 5307 corn are biochemically and functionally equivalent. Therefore, the microbially-produced test substance is a suitable surrogate for eCry3.1Ab expressed in Event 5307 corn.

[1] Although Colorado potato beetle larvae are not corn pests, they are sensitive to eCry3.1Ab, and are members of the Chrysomelidae family of Coleoptera, the same family that includes corn rootworms. Colorado potato beetle larvae were used in these bioassays because they are more amenable than rootworm larvae to laboratory testing.

VI.D. Assessment of eCry3.1Ab Protein Toxicity

VI.D.1. Assessment of eCry3.1Ab Amino Acid Sequence Similarity to Known Toxins

An amino acid sequence comparison between a novel protein and known protein toxins can be a useful predictor of toxicity. To determine whether the eCry3.1Ab protein had any significant amino acid sequence similarity to proteins identified as toxins, the protein sequence (Entrez Accession No. ADC30135) was systematically compared to the latest posting of the National Center for Biotechnology Information (NCBI 2010b) Entrez Protein Database. The Basic Local Alignment Search Tool for Proteins program (BLASTP) was used to search the NCBI database. This process identified (1) whether any proteins in the database showed significant similarity to the eCry3.1Ab amino acid sequence (*i.e.*, alignments with BLASTP Expectation values [*E* - values] below an established threshold), indicating that the amino acid sequence might be closely related to the eCry3.1Ab amino acid sequence, and (2) whether any proteins showing sequence similarity to the eCry3.1Ab amino acid sequence were known or putative toxins.

Most proteins are modular in nature and contain repeating functional domains within the protein. Similarly, functional domains are conserved across different proteins from different species. The BLASTP algorithm is optimized to identify these domains or shorter sequence similarities present within the full-length query sequence; as a result, this approach detects more similarities than would a search started by aligning two sequences over their entire length. The search conservatively identified all sequences in the database with search results yielding an *E*-value of 10 or lower.

A threshold below which similarity to the query sequence is considered significant, and not the result of random similarity in amino acid composition, is required for meaningful analysis of database alignments. To assess the significance of sequence similarity to the eCry3.1Ab amino acid sequence, additional searches were conducted with shuffled versions of the eCry3.1Ab amino acid sequence. Five shuffled sequences were created through random shuffling of the eCry3.1Ab amino acid sequence. The resulting shuffled sequences all had the same amino acid *composition* as eCry3.1Ab (*i.e.,* the same number of residues of each specific amino acid), but were unlikely to have amino acid *sequences* similar to those of either eCry3.1Ab or other proteins found in the NCBI Entrez® Protein Database. Searches using these shuffled sequences provided an estimate of the background incidence of alignments that would be expected for any sequence with the same amino acid composition as eCry3.1Ab. Searches with the five shuffled versions of the eCry3.1Ab amino acid sequence identified alignments yielding *E*-values that ranged from 0.32 to 2.1; therefore, the threshold *E*-value for significant amino acid sequence similarity to eCry3.1Ab was considered to be 0.32. Proteins with significant amino acid sequence similarity to eCry3.1Ab (*i.e.,* with *E*-values less than 0.32) were evaluated for identity, source and biological function, if known. The NCBI Entrez® Protein Database records and supporting literature were accessed, if needed, using the Entrez® Accession Numbers.

The NCBI Entrez® Protein Database search identified 495 sequences with significant similarity to the eCry3.1Ab amino acid sequence (*i.e., E*-values less than 0.32). Of these, 449 were identified as known or putative delta-endotoxin proteins (also known as Cry proteins or insecticidal crystal proteins) from 14 species or synthetic gene constructs. The *E*-values for alignments between these sequences and the eCry3.1Ab amino acid sequence ranged from 0.0 to 0.24. An additional 31 sequences were identified as hypothetical proteins of unspecified function from six species. Two additional entries were similar to a twin arginine translocation pathway signal from *Vibrio angustum* and *Ralstonia eutropha* (Berks et al. 2000). These sequences were not known or putative toxins.

Additionally, 13 proteins or hypothetical proteins from *Bacillus thuringiensis* were described in the database supporting information as being part of the parasporin family of proteins. The term parasporin is defined as "*Bacillus thuringiensis* and related bacterial parasporal proteins that are non-hemolytic but capable of preferentially killing cancer cells" (Yamashita et al. 2005). A classification and nomenclature scheme similar to that of Cry proteins has now been developed for parasporins (Ohba et al. 2006); however, several proteins described in this search still retain their "Cry" nomenclature names. The parasporins have not been shown to have insecticidal activity to date. They have been shown to have strong *in vitro* cytocidal activity against sensitive cells derived from cancer cell lines, but low or no toxicity to other normal human or other mammalian cell lines (Mizuki et al. 2000; Katayama et al. 2005; Yamashita et al. 2005). There are no published reports of *in vivo* parasporin toxicity to humans or other mammals.

The results of a comprehensive amino acid similarity search of the NCBI Entrez® Protein Database support the conclusion that the eCry3.1Ab amino acid sequence shows no significant similarity with any known or putative toxins other than known or putative delta-endotoxin proteins (also described as Cry proteins or insecticidal crystal proteins).

VI.D.2. Acute Oral Toxicity Study of eCry3.1Ab

The potential toxicity of the eCry3.1Ab protein was evaluated in an acute oral toxicity study in the mouse conducted by WIL Research Laboratories, LLC (Ashland, OH, USA). The microbially produced, lyophilized test substance ECRY3.1AB-0208 containing eCry3.1Ab protein (89.6% purity, w/w; see Part VI.B) was administered as a single oral dose via gavage to groups of five male and five female Crl:CD-1 (ICR) mice at 0 or 2000 mg eCry3.1Ab/ kg body weight. The dosing vehicle, 0.5% (w/v) aqueous carboxymethylcellulose, was administered to the control group. The dosing formulations were administered at a dose volume of 10 mL/kg for all groups. All animals were euthanized after a 14-day observation period following dosing.

All animals were observed twice daily for mortality and moribundity. Clinical examinations were performed at the time of dosing, approximately 1-2 hours post-dosing and approximately 4-5 hours post-dosing on the day of dose administration (study day 0) and once daily on nondosing days (study days 1-13). Detailed physical examinations were performed weekly. Individual

body weights and food consumption were recorded daily during the study. Complete necropsies were conducted on all animals, and selected tissues were examined microscopically from all animals.

All animals survived the 14-day observation period to the scheduled necropsy. There were no observed clinical signs of distress or impairment. There were no test-substance-related clinical observations. All clinical findings in the test-substance-treated groups were limited to single animals and/or were common findings for laboratory mice of this age and strain. At the end of the 14-day observation period, a complete necropsy was conducted on all animals. The necropsies included, but were not limited to, examination of the external surface, all orifices, and the cranial, thoracic, abdominal and pelvic cavities, including viscera. Histopathology evaluations were conducted on all the gastrointestinal tissues of all the animals, as well as the spleen, thymus, mandibular and mesenteric lymph nodes, and any gross lesions.

Body weights and food consumption were unaffected by test substance administration. Statistically significantly higher mean body weight gain was noted for the 2000 mg/kg group males on study days 3-4 and 13-14 compared to the control group. Statistically significantly lower mean body weight gain was noted for the 2000 mg/kg females on study days 2-3 and 9-10. These differences in body weight gain were considered incidental and not related to test substance administration because the magnitude of the change was very small.

Statistically significantly higher mean food consumption was noted for the 2000 mg/kg group males on study days 8-9 and 12-13. This difference in food consumption was considered incidental and not related to test substance administration because the magnitude of the change was small and no other changes in food consumption were noted for the remaining study intervals.

Review of the gross necropsy observations revealed no observations that were considered to be associated with administration of the test substance. Occasional histologic changes were noted in both treated and control animals; these were all considered to be incidental findings or related to some aspect of experimental manipulation other than administration of the test substance. There was no test-substance-related alteration in the prevalence, severity, or histologic character of those incidental tissue alterations.

In summary, test substance ECRY3.1AB-0208, containing the active ingredient eCry3.1Ab protein (89.6% purity w/w), administered as a single oral dose at 2000 mg eCry3.1Ab/ kg body weight followed by a 14-day nondosing observation period was well tolerated in male and female CD-1 mice. No toxicity was observed in mice given an acute oral gavage dose of 2000 mg eCry3.1Ab/kg body weight. Based on the results of this study, the estimated no observable adverse effect level (NOAEL) for eCry3.1Ab protein in male and female mice was > 2000 mg/kg body weight.

VI.D.3. Conclusion of the eCry3.1Ab Toxicity Assessment

The eCry3.1Ab protein does not share significant amino acid sequence similarity to known protein toxins and no adverse test-substance-related effects were observed in mice administered a single high dose (2000 mg/kg body weight) of eCry3.1Ab protein. Therefore, eCry3.1Ab is considered nontoxic.

VI.E. Assessment of eCry3.1Ab Allergenic Potential

VI.E.1. Source of the eCry3.1Ab Protein

The eCry3.1Ab protein is a synthetic protein (chimera) containing regions from both mCry3A and Cry1Ab. The source of the native Cry3A and Cry1Ab proteins is *Bacillus thuringiensis* (see Table IV-1). Bacteria have no history of allergenicity (Taylor and Hefle 2001; FAO/WHO 2001). Additionally, during decades of widespread use of *Bacillus thuringiensis* formulations as insecticides, there have been no confirmed reports of immediate or delayed allergic reactions to the Cry protein components of these products, despite significant oral, dermal and inhalation exposure (EPA 2010). Therefore, eCry3.1Ab is not derived from a source known to produce allergenic proteins.

VI.E.2. Assessment of eCry3.1Ab Amino Acid Sequence Similarity to Known Allergens

Amino acid sequence comparisons between novel proteins and known allergens are part of the weight-of-evidence approach to assessing potential allergenicity. For example, in combination with other supporting data, the presence of significant sequence amino acid similarity to known allergens could indicate that the novel protein might elicit an allergic cross-reaction in sensitized individuals.

The eCry3.1Ab amino acid sequence was systematically compared to the protein sequences in the Food Allergy Research and Resource Program (FARRP) AllergenOnline database, version 10.0 (FARRP 2010), located online at www.allergenonline.org. The FARRP AllergenOnline database contains the amino acid sequences of known and putative protein allergens. It is a curated, peer-reviewed database containing proteins identified as food allergens, respiratory allergens, allergenic venom proteins, contact allergens, gliadins, and glutenins. Entries were compiled primarily from searches of publicly available protein databases using the National Center for Biotechnology Information (NCBI) Entrez® search and retrieval system. Proteins are classified as known or putative allergens according to predetermined criteria set by the FARRP expert review panel.

The FAARP AllergenOnline database (2010) contained 1,471 non-redundant entries at the time the searches were performed. Sequential 80-amino-acid peptides of the eCry3.1Ab sequence were compared to the protein sequences in the FAARP AllergenOnline database using the

FASTA search algorithm[1] (Pearson and Lipman 1988). Any 80-amino-acid peptide in the query sequence having greater than 35% amino acid identity to an allergen sequence would be considered to have significant identity to the allergen sequence, in accordance with the recommendations of the Codex Alimentarius Commission (Codex 2009). The results of this analysis revealed that there was no significant identity between any of the sequential 80-amino-acid peptides of eCry3.1Ab and any entry in the FAARP AllergenOnline database. Therefore, eCry3.1Ab does not share overall sequence similarity with any known allergenic protein.

The eCry3.1Ab protein sequence was also examined for matches of eight contiguous amino acids (Hileman et al. 2002) between the eCry3.1Ab sequence and the allergen sequences in the FAARP AllergenOnline database to screen for short, local regions of amino acid identity that might indicate the presence of common T-cell binding epitopes. There were no matches of eight contiguous amino acids between eCry3.1Ab and any proteins in the FAARP AllergenOnline database. These results support the conclusion that eCry3.1Ab shows no biologically relevant amino acid sequence similarity to any known or putative protein allergens.

VI.E.3. *In vitro* Pepsin Digestibility of eCry3.1Ab

A study was conducted to assess the *in vitro* digestibility of eCry3.1Ab in simulated mammalian gastric fluid (SGF). Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE), Western blot and densitometry analyses were used to evaluate the *in vitro* digestibility of eCry3.1Ab in SGF over a 15-minute time course at 37°C (Figure VI-1).

The eCry3.1Ab protein degraded rapidly upon exposure to the pepsin enzyme in SGF. Intact eCry3.1Ab (molecular weight 74.8 kDa) was readily digested in less than 30 seconds, as assessed by SDS-PAGE analysis. A very faint band corresponding to intact eCry3.1Ab (74.8 kDa) was visible following incubation in SGF for 15 seconds (Lane 8). However, this band was no longer visible in the digestibility assay sample taken following incubation in SGF for 30 seconds (Lane 9). Two very faint, diffuse bands with molecular weights of approximately 4 kDa and 5 kDa, respectively, were visible after incubation in SGF for 15 seconds (Lane 8). These two bands diminished in intensity over the time course and were no longer detectable after incubation in SGF for 10 minutes (Lane 18).

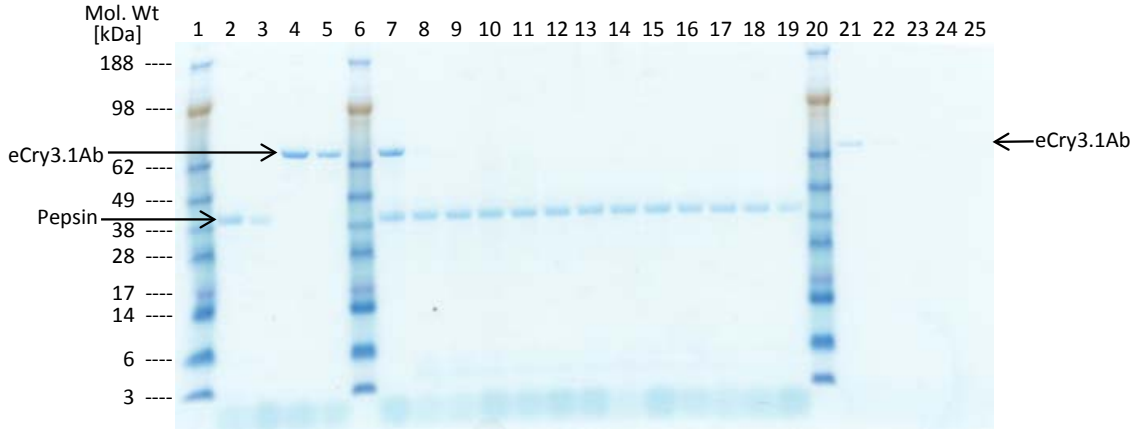
The eCry3.1Ab protein incubated in SGF without pepsin (eCry3.1Ab control) showed no significant degradation over 15 minutes (Lanes 4 and 5), which indicates that the hydrolysis of eCry3.1Ab, seen in the SGF samples (Lanes 7 through 19), can be attributed to pepsin. Similar band intensities were visualized for the time zero digestibility assay sample (Lane 7) and the eCry3.1Ab time zero control (Lane 4), confirming that equal amounts of eCry3.1Ab were

[1] FASTA version 3.45 was used with the following parameters: extension penalty of 2 and gap creation penalty of 12. The scoring matrix was the Blocks Substitution Matrix 50 (BLOSUM50).

applied to the SDS-PAGE. The lowest amount of eCry3.1Ab visible on the gel (Lanes 21 through 25) was 0.025 μg (Lane 22). Therefore, the limit of detection (LOD) of eCry3.1Ab for the SDS-PAGE used in this study was determined to be 0.025 µg.

Further analysis of the eCry3.1Ab protein bands in the SDS-PAGE gel by densitometry revealed that 3% and 0% of the eCry3.1Ab remained after incubation in SGF for 15 and 30 seconds, respectively (Table VI-2).

Figure VI-1. SDS-PAGE analysis of eCry3.1Ab following digestion in simulated gastric fluid.
The molecular weight of eCry3.1Ab is 74.8 kDa. The molecular weight of pepsin is 34.6 kDa[1].



Lane 1: molecular weight standard
Lane 2: SGF control – time zero
Lane 3: SGF control – 15 minutes
Lane 4: eCry3.1Ab control (eCry3.1Ab in SGF without pepsin) – time zero
Lane 5: eCry3.1Ab control (eCry3.1Ab in SGF without pepsin) – 15 minutes
Lane 6: molecular weight standard
Lane 7: *in vitro* digestibility assay - time zero[2]
Lane 8: *in vitro* digestibility assay - 15 seconds[2, 3, 4]
Lane 9: *in vitro* digestibility assay - 30 seconds[2]
Lane 10: *in vitro* digestibility assay - 45 seconds[2]
Lane 11: *in vitro* digestibility assay - 1 minute[2]
Lane 12: *in vitro* digestibility assay - 1 minute 15 seconds[2]
Lane 13: *in vitro* digestibility assay - 1 minute 30 seconds[2]
Lane 14: *in vitro* digestibility assay - 1 minute 45 seconds[2]
Lane 15: *in vitro* digestibility assay – 2 minutes[2]
Lane 16: *in vitro* digestibility assay – 3 minutes[2]
Lane 17: *in vitro* digestibility assay – 5 minutes[2]
Lane 18: *in vitro* digestibility assay – 10 minutes[2]
Lane 19: *in vitro* digestibility assay – 15 minutes[2]
Lane 20: molecular weight standard
Lane 21: 0.1 µg eCry3.1Ab for LOD determination
Lane 22: 0.025 µg eCry3.1Ab for LOD determination[4]
Lane 23: 0.0063 µg eCry3.1Ab for LOD determination
Lane 24: 0.0016 µg eCry3.1Ab for LOD determination
Lane 25: 0.0004 µg eCry3.1Ab for LOD determination

---

[1] The 34.6 kDa pepsin band showed slightly lower mobility and therefore appeared to have a higher apparent molecular weight (MW) when compared to the MW standards on the gel. The difference between the expected and observed MWs can be explained by the limitations of SDS-PAGE for accurate MW determination. Dube and Flynn (1988) reviewed the reliability of SDS-PAGE for MW determinations and concluded that the apparent MW of a protein by this method is typically within 10% of its true MW. This depends greatly on the similarity between the properties of the protein of interest and the proteins in the standard set (Sadeghi et al. 2003).
[2] The lanes selected for the eCry3.1Ab band detection and quantification by densitometry analysis (Table VI-2).
[3] Due to the resolution limits of the scanner and the printer, the very faint bands (approximately 4 kDa and 5 kDa) visible on the actual gel might not be visible on the printed image that appears here.
[4] Due to the resolution limits of the scanner and the printer, the very faint band (74.8 kDa) visible on the actual gel might not be visible on the printed image that appears here.

Table VI-2. The eCry3.1Ab remaining at each time point during exposure to simulated gastric fluid as determined by densitometry analysis.

Lane in Figure VI-1	Time point	Densitometric volume	Percent (%) of eCry3.1Ab remaining[1]	Amount (µg) of eCry3.1Ab remaining[2]
7	Time zero	373.9	100	0.50
8	15 seconds	12.3	3	0.02
9	30 seconds	0.7	0	0
10	45 seconds	1.0	0	0
11	1 minute	0.6	0	0
12	1 minute, 15 seconds	1.1	0	0
13	1 minute, 30 seconds	0.7	0	0
14	1 minute, 45 seconds	1.2	0	0
15	2 minutes	0.7	0	0
16	3 minutes	0.8	0	0
17	5 minutes	0.6	0	0
18	10 minutes	1.4	0	0
19	15 minutes	0.9	0	0

[1] Percent (%) of eCry3.1Ab remaining relative to the amount of eCry3.1Ab at time zero as determined by densitometry.

[2] The amount of eCry3.1Ab at time zero was equivalent to 0.5 µg. The amount of remaining eCry3.1Ab at each time point was calculated as follows:
amount of eCry3.1Ab remaining = amount of eCry3.1Ab at time zero (0.5 µg) × percent (%) of eCry3.1Ab remaining. This calculation was based on the fact that equivalent volumes of each SGF digestibility assay sample were subjected to SDS-PAGE.

The Western blot analysis results (Figure VI-2) confirm that eCry3.1Ab is readily digested in SGF in less than 30 seconds. After incubation of eCry3.1Ab in SGF for 30 seconds (Lane 9), no protein bands representing either intact eCry3.1Ab or eCry3.1Ab-derived fragments were visible.

A very faint band with an approximate molecular weight of 150 kDa was visible in the eCry3.1Ab control samples (Lanes 4 and 5) and the time-zero digestibility assay sample (Lane 7). This protein cross-reacted with the antibody capable of detecting eCry3.1Ab and displayed a mobility consistent with the molecular weight of two eCry3.1Ab molecules (150 kDa). Therefore, it most likely represents a dimer of eCry3.1Ab. An additional faint band with an approximate molecular weight of 47 kDa was also visible in the time zero digestibility assay sample (Lane 7). This band cross-reacted with the antibody capable of detecting eCry3.1Ab and was also present in the eCry3.1Ab control (Lanes 4 and 5). This suggests that the 47 kDa band most likely corresponds to a minor eCry3.1Ab hydrolysis product derived from the sample and is not related to pepsin digestion. Both bands (150 kDa and 47 kDa) were no longer detectable following exposure to SGF for 15 seconds (Lane 8).

The eCry3.1Ab protein incubated in SGF without pepsin (eCry3.1Ab control) showed no significant degradation over 15 minutes (Lanes 4 and 5), which indicates that the hydrolysis of eCry3.1Ab, seen in the SGF samples (Lanes 7 through 19), can be attributed to pepsin. Similar band intensities were visualized for the time-zero digestibility assay sample (Lane 7) and the eCry3.1Ab time-zero control (Lane 4), confirming that equal amounts of eCry3.1Ab were applied to the SDS-PAGE and electroblotted. The lowest amount of eCry3.1Ab visible on the blot (Lanes 21 through 25) was 0.4 ng (Lane 22). Therefore, the LOD of eCry3.1Ab for the Western blot used in this study was determined to be 0.4 ng.

Figure VI-2. Western blot analysis of eCry3.1Ab following digestion in simulated gastric fluid.

The molecular weight of eCry3.1Ab is 74.8 kDa.

Lane 1: molecular weight standard
Lane 2: SGF control – time zero
Lane 3: SGF control – 15 minutes
Lane 4: eCry3.1Ab control (eCry3.1Ab in SGF without pepsin) – time zero[1]
Lane 5: eCry3.1Ab control (eCry3.1Ab in SGF without pepsin) – 15 minutes[1]
Lane 6: molecular weight standard
Lane 7: *in vitro* digestibility assay - time zero[1]
Lane 8: *in vitro* digestibility assay - 15 seconds[2]
Lane 9: *in vitro* digestibility assay - 30 seconds
Lane 10: *in vitro* digestibility assay - 45 seconds
Lane 11: *in vitro* digestibility assay - 1 minute
Lane 12: *in vitro* digestibility assay - 1 minute 15 seconds
Lane 13: *in vitro* digestibility assay - 1 minute 30 seconds
Lane 14: *in vitro* digestibility assay - 1 minute 45 seconds
Lane 15: *in vitro* digestibility assay – 2 minutes
Lane 16: *in vitro* digestibility assay – 3 minutes
Lane 17: *in vitro* digestibility assay – 5 minutes
Lane 18: *in vitro* digestibility assay – 10 minutes
Lane 19: *in vitro* digestibility assay – 15 minutes
Lane 20: molecular weight standard
Lane 21: 1.6 ng eCry3.1Ab for LOD determination
Lane 22: 0.4 ng eCry3.1Ab for LOD determination[2]
Lane 23: 0.1 ng eCry3.1Ab for LOD determination
Lane 24: 0.024 ng eCry3.1Ab for LOD determination
Lane 25: 0.006 ng eCry3.1Ab for LOD determination

[1] Due to the resolution limits of the scanner and the printer, the very faint bands (47 kDa and 150 kDa) visible on the actual blot might not be visible on the printed image that appears here.
[2] Due to the resolution limits of the scanner and the printer, the very faint band (74.8 kDa) visible on the actual blot might not be visible on the printed image that appears here.

VI.E.4. Susceptibility of eCry3.1Ab to Inactivation by Heat

The temperature stability of eCry3.1Ab was evaluated by incubating the aliquots of an aqueous solution of the test substance at various temperatures [4ºC (control), 25ºC, 37ºC, 65ºC and 95ºC] for 30 minutes and determining the loss of insecticidal activity in an insect bioassay with Colorado potato beetle (*Leptinotarsa decemlineata*) larvae, a sensitive coleopteran species.

At 25ºC, 37ºC, and 65ºC, eCry3.1Ab retained bioactivity against Colorado potato beetle larvae when compared with that observed at 4ºC (Table VI-2). However, after treatment at 95ºC for 30 minutes, LC_{50} values and 95% confidence intervals could not be estimated due to the low mortality in these samples at all concentrations tested. These data demonstrate that eCry3.1Ab is inactivated, and therefore, denatured upon heating at temperatures of 95ºC and above.

Table VI-3. Effect of temperature on insecticidal activity of eCry3.1Ab in diet incorporation bioassay with Colorado potato beetle larvae: LC_{50} values and 95% confidence intervals at 144 hours.

Temperature [°C]	LC_{50} [µg eCry3.1Ab/ml]	95% Confidence Interval [µg eCry3.1Ab/ml]
4	0.918	0.419-1.617
25	1.802	1.066-2.714
37	1.814	0.411-4.714
65	4.682	1.321-10.092
95	>100	Not estimable

VI.E.5. Conclusion of the Assessment of eCry3.1Ab Allergenic Potential

The weight-of-evidence indicates that eCry3.1Ab is not likely to be a food allergen because:

- eCry3.1Ab is not derived from a known source of allergenic proteins,
- eCry3.1Ab does not have any significant amino acid sequence similarity to known or putative allergenic proteins,
- eCry3.1Ab is rapidly degraded in simulated mammalian gastric fluid, and
- eCry3.1Ab is inactivated, and thus denatured, at temperatures of 95ºC and above.

VI.F. eCry3.1Ab History of Safe Use

Prior dietary exposure to the eCry3.1Ab protein has not occurred because it is a novel protein engineered by Syngenta. Effective June 16, 2010, a temporary tolerance exemption was established for eCry3.1Ab (40 CFR 174.532) in connection with an Experimental Use Permit granted by the EPA.

Although there is no history of prior exposure to Cry3.1Ab, per se, the protein is similar to other well-characterized Cry proteins with a history of safe use. It is comprised largely (96.6%) of portions of mCry3A and Cry1Ab proteins (see Figure IV-2), which have been safely used in other genetically modified crops. The mCry3A protein is produced by Syngenta's MIR604 corn (the subject of BNF No. 99), a commercial product, and is a modified version of the native Cry3A protein from *B.t.* subsp. *tenebrionis*. Cry3A is produced in transgenic *B.t.* (NewLeaf) potatoes and is present in microbial *B.t.* formulations used for control of coleopteran pests in food crops. Pursuant to § 408(d) of the Federal Food, Drug and Cosmetic Act, food and feed tolerance exemptions have been established for mCry3A, Cry3A, and other Cry3 proteins used in transgenic crops (mCry3A, 40 CFR 174.505; Cry3A, 40 CFR 174.509; and Cry3Bb1, 40 CFR 174.518), reflecting their demonstrated mammalian safety.

The C-terminal portion of the native, full-length Cry1Ab *B.t.* protein contained in the eCry3.1Ab amino acid sequence produced 5307 corn is also present in Syngenta's transgenic COT67B cotton (the subject of BNF No. 112), which produces a full-length Cry1Ab. (Syngenta's Bt11 corn and Monsanto's MON810 corn produce truncated Cry1Ab proteins representing primarily the N-terminal lepidopteran-active region of the native Cry1Ab protein; this region is not present in eCry3.1Ab.) Native Cry1Ab is present in multiple microbial *B.t.* subsp. *kurstaki*-based insecticides used for lepidopteran control in food crops. Accordingly, multiple tolerance exemptions exist for Cry1Ab and other Cry1 proteins used in transgenic crops (including full-length Cry1Ab, 40 CFR 174.529; Cry1Ac, 40 CR 174.510; and Cry1F, 40 CFR 174.520). Spore preparations of multiple strains of *B.t.*, producing a variety of Cry proteins, are exempt from food and feed tolerances on food crops (40 CFR 180-1011). These multiple tolerance exemptions for Cry proteins are supported by extensive safety studies, and no documented food or feed safety issues have been identified during the use history of the associated products.

VI.G. Conclusion of the eCry3.1Ab Protein Safety Assessment

A substantial body of data exists to support the safety of eCry3.1Ab protein produced in 5307 corn.

The eCry3.1Ab protein is considered nontoxic:

- The mode of action for the insecticidal activity of eCry3.1Ab is not relevant to mammals,
- eCry3.1Ab does not have significant amino acid sequence similarity to known protein toxins,
- eCry3.1Ab was nontoxic to mice with no treatment-related effects at a single dose of 2000 mg eCry3.1Ab/kg body weight, and
- Cry1 and Cry3 proteins have a history of safe use in agriculture.

The weight-of-evidence supports the conclusion that eCry3.1Ab is not likely to be a food allergen:

- eCry3.1Ab is not derived from a known source of allergenic proteins,
- eCry3.1Ab does not have significant amino acid sequence similarity to known or putative allergenic proteins,
- eCry3.1Ab is rapidly degraded in simulated mammalian gastric fluid, and
- eCry3.1Ab is labile upon heating at temperatures of 95ºC and above.

VI.H. Identity and Function of the PMI Protein in Event 5307 Corn

Event 5307 corn contains the gene *pmi* (also known as *manA*[1]) from *Escherichia coli* strain K-12 (a nonpathogenic strain) (Miles and Guest 1984), as a selectable marker; it serves no agronomic or other purpose in 5307 plants. This gene encodes a phosphomannose isomerase (PMI) protein of 391 amino acids having a molecular weight of approximately 42.8 kDa. PMI enzymes serve an essential function in many organisms, including humans. PMI catalyzes the reversible interconversion of mannose 6-phosphate and fructose 6-phosphate and has utility as a selectable marker for transformation of many plant species (Negrotto et al. 2000). Plant cells that produce sufficient PMI are able to survive and grow on media containing mannose as the only or primary energy source (Miles and Guest 1984), whereas plant cells that do not produce PMI cannot.

VI.I. Regulatory Status and Established Safety of PMI

PMI proteins are present as selectable markers in three other Syngenta transgenic corn cultivars for which FDA consultations have been completed: MIR162 corn (BNF No. 113), MIR604 corn (BNF No. 99) and Event 3272 corn (BNF No. 95). The PMI protein encoded in 5307 corn is identical to that in MIR162 corn and 3272 corn. (The PMI variant in MIR604 corn differs by two amino acid substitutions from the PMI produced in MIR162 corn, 3272 corn, and 5307 corn.)

PMI has been granted an exemption from food and feed tolerances in all plants by the EPA (40 CFR 174.527). This tolerance exemption is supported by extensive data and information, submitted by Syngenta and previously reviewed by the EPA and FDA, demonstrating that PMI is nontoxic and is unlikely to become a food allergen. Therefore, detailed descriptions of these studies are not reiterated herein. However, the substantial body of data that exists to support the safety of PMI protein produced in 5307 maize may be summarized as follows:

PMI is considered nontoxic:

- PMI does not share significant amino acid similarity to known protein toxins. (See also the description of the updated search for amino acid sequence similarity to known toxins described in Part VI.K, below.)
- PMI was nontoxic to mice with no treatment-related effects at high acute doses.
- PMI proteins have a history of safe exposure due to their ubiquitous occurrence in nature and previous use in transgenic corn.

[1] Entrez Nucleotide Database Accession No. M15380 (NCBI 2010a).

A standard weight-of-evidence analysis for allergenic potential (Codex 2009) indicates that PMI is unlikely to be a food allergen and is unlikely to be cross-reactive to known allergens:

- PMI is not derived from a known source of allergenic proteins.
- PMI does not have any significant amino acid sequence similarity to known or putative allergenic proteins with implications for its allergenic potential. (See also the description of the updated search for amino acid sequence similarity to known allergens described in Part VI.L, below.)
- PMI is rapidly degraded in simulated mammalian gastric fluid containing pepsin.
- PMI is labile upon heating at temperatures of 65ºC and above.
- PMI is not glycosylated in corn.

To supplement the safety data summarized above, however, the following additional information is described herein:

- A characterization study (see Part VI.J, below) demonstrating that microbially produced PMI used as a test substance for safety studies is equivalent to, and a suitable surrogate for, PMI as produced in 5307 plants;
- Updated bioinformatic analyses, conducted in 2010, confirming that PMI does not share biologically significant amino acid sequence similarity to known toxins or allergens (see Part VI.K and Part VI.L, below).

VI.J. Characterization of the Plant- and *Escherichia coli*-produced PMI Proteins

Sequencing of the inserted T-DNA in 5307 plants confirmed the presence of the exact nucleotide sequence of *pmi*, as intended (see Part IV.E.1). This indicates that the intact PMI protein (391 amino acids), as intended, is encoded in 5307 plants.

Additionally, a characterization and comparison of PMI extracted from Event 5307 plants and PMI in a test substance produced in recombinant *E. coli* was conducted to assess whether the microbially produced PMI test substance (PMI-0105, containing 89.5% PMI by weight) used for safety studies is a suitable surrogate for PMI produced in 5307 corn plants. PMI proteins from both sources were demonstrated to have the predicted molecular weight of approximately 42.8 kDa, and both immunologically cross-reacted with the same anti-PMI antibodies, as determined by Western blot analysis, thus confirming their identity and integrity. It was also confirmed that

the PMI proteins from both sources catalyzed the same chemical reaction[1] and had similar specific activity (455.67 U/mg PMI for the plant-produced PMI and 526.26 U/mg PMI for the microbially produced PMI; see also Table B-1 in Appendix B).

Based on these results, the identities of the plant- and microbially produced PMI proteins have been verified and it can be concluded that the PMI produced in recombinant *E. coli* and Event 5307 corn are biochemically and functionally equivalent. Therefore, the microbially produced PMI is a suitable surrogate for PMI produced in Event 5307 corn.

VI.K. Updated Bioinformatic Assessment of PMI Amino Acid Sequence Similarity to Known Toxins

Bioinformatic analyses demonstrated that the PMI amino acid sequence (391 amino acids) has no significant sequence similarity to any toxins. This comparison was conducted using a 2010 posting of the National Center for Biotechnology Information (NCBI) Entrez® Protein Database (NCBI 2010b), and searching the database using the Basic Local Alignment Search Tool for Proteins (BLASTP) program[2] (Altschul et al. 1997). This procedure determined:

1. whether any proteins in the database shared significant similarity to the PMI amino acid sequence, indicating they may be closely related to PMI,

2. whether any proteins demonstrating significant sequence similarity to the PMI amino acid sequence were known or putative toxins, indicating possible implications for the toxic potential of PMI.

The NCBI Entrez® database search identified all amino acid sequences with Expectation values (*E*-values) of 10 or lower. The *E*-value is a measure of the probability that amino acid matches between sequences occurred by chance. Comparisons between highly similar sequences yield *E*-values approaching zero, whereas the probability that amino acid sequence similarities occurred only by chance increases with higher *E*-values (Ponting 2001). The PMI query sequence showed no significant sequence similarity to any known or putative toxins.

---

[1] One unit (U) of PMI activity is defined as the amount of enzyme required to catalyze the conversion of 1 µmol of mannose 6-phosphate to fructose 6-phosphate per minute (equivalent to 1 µmol NADP reduced per min). Reaction: Mannose 6-P (catalyzed by PMI) → Fructose 6-P (catalyzed by phosphoglucose isomerase) → Glucose 6-P +NADP (catalyzed by glucose 6-P dehydrogenase) → 6- Gluconolactone + NADPH

[2] BLASTP version 2.2.8 was used with the following parameters: no complexity filter; expectation score = 10; word size = 3; gap costs: existence = 11 and extension = 1. The similarity matrix was Blocks Substitution Matrix62 (BLOSUM62).

VI.L. Updated Bioinformatic Assessment of PMI Amino Acid Sequence Similarity to Known Allergens

Bioinformatic analyses showed that the PMI amino acid sequence has no biologically relevant similarity to the sequences of known or putative allergens in the current posting of FARRP AllergenOnline database, version 10.0 (FARRP 2010). Sequential 80-amino-acid peptides of the PMI sequence were compared to the protein sequences in the AllergenOnline database using the FASTA search algorithm[1] (Pearson and Lipman 1988). Additionally, the PMI sequence was also examined for matches of eight contiguous amino acids (Hileman et al. 2002) with any allergen sequences, to screen for short, local regions of amino acid identity that might indicate the presence of common T-cell binding epitopes. As previously identified, there was one sequence identity match of eight contiguous identical amino acids between PMI and a known allergen, α-parvalbumin from *Rana* species CH2001 (unidentified edible frog) (Hilger et al. 2002). Further investigation using IgE-specific serum screening methodology (Codex 2009) demonstrated no cross-reactivity between PMI and the allergen α-parvalbumin, using serum from the single individual known to have demonstrated IgE-mediated allergy to this specific α-parvalbumin. The allergic patient's serum IgE did not recognize any portion of PMI as an allergenic epitope. These results support the conclusion that the eight-amino-acid sequence identity between the PMI protein and α-parvalbumin from *Rana* species CH2001 is not biologically relevant and has no implications for the potential cross-reactivity between PMI and α-parvalbumin. Therefore, the short sequence identity match with α-parvalbumin from *Rana* species CH2001 has no implications for the potential allergenicity of PMI.

VI.M. PMI History of Safe Use

Data and information are available to support a history of safe use of PMI proteins. PMI proteins are present as the selectable marker in commercially available transgenic corn products (Syngenta's MIR162 corn and MIR604 corn). It is conceivable that small amounts of PMI proteins from various sources have always been present in the food and feed supply due to the ubiquitous occurrence of PMI proteins in nature, including food plants and animals. PMI proteins have been found in such diverse plant species as tobacco (Barb et al. 2002), walnut (Malvolti et al. 1993), and *Brassica* species (Chen et al. 1989), as well as in seeds of soybeans and other legumes (Lee and Matheson 1984). Genes encoding putative PMI proteins have been purified and characterized from many other organisms, including bacteria, yeast, rats, pigs, and humans (Proudfoot et al. 1994a, 1994b; Davis et al. 2002) and have been demonstrated to be essential for many organisms, including humans.

[1] FASTA version 3.45 was used with the following parameters: extension penalty of 2 and gap creation penalty of 12. The scoring matrix was the Blocks Substitution Matrix 50 (BLOSUM50).

VI.N. Conclusion of the PMI Safety Assessment

A substantial body of data exists to support the safety of PMI produced in Event 5307 corn. PMI has been granted an exemption from food and feed tolerances in all plants by the EPA (40 CFR 174.527) based on data demonstrating that PMI is nontoxic and that PMI is not likely to be a food allergen.

VI.O. eCry3.1Ab and PMI Protein Expression Levels in 5307 Corn and Exposure Potential

VI.O.1. Concentrations of eCry3.1Ab and PMI

The concentrations of eCry3.1Ab and PMI in various 5307 plant tissues were quantified using ELISA. The concentrations of these proteins were measured in leaves and kernels as well as whole plants at four growth stages (whorl, anthesis, maturity and senescence) from 5307 hybrid maize hybrid grown at four US locations in 2008. The means across all four locations for eCry3.1Ab (Table VI-3) and PMI (Table VI-4) concentrations were determined on a dry- and fresh-weight basis, and represent the levels of these proteins in 5307 corn in relevant tissue types across four different locations throughout the life of the plant. Details of the methods used to quantify eCry3.1Ab and PMI in 5307 plants are provided in Appendix C.

VI.O.2. Mammalian Exposure Potential

Kernels from 5307 maize are the most likely tissue to enter the food supply, either as grain or grain by-products. The average eCry3.1Ab concentration measured in kernels from 5307 maize (4.45 µg eCry3.1Ab/g dry weight [gdw] at senescence, Table VI-3) represents approximately 0.004% of the total protein in kernels. Humans would potentially consume corn grain at the senescence stage of plant development, whereas livestock would be more likely to consume the kernels at plant maturity. The average eCry3.1Ab concentration measured in kernels at maturity was 6.19 µg eCry3.1Ab/gdw (Table VI-3), representing approximately 0.006% of the total protein.

The average PMI concentration measured in kernels from 5307 corn (1.11 µg PMI/gdw at senescence, Table VI-4) represents approximately 0.001% of the total kernel protein. The average PMI concentration measured in kernels at maturity was 2.08 µg PMI/gdw (Table VI-4), representing approximately 0.002% of the total protein. (These calculations are based on corn grain/kernels containing 10% total protein by weight.)

Given the low levels of eCry3.1Ab and PMI in 5307 kernels, dietary exposure potential can be considered minimal. Because no health hazards have been identified for the eCry3.1Ab or PMI proteins, specific dietary exposure estimates for 5307 grain or grain by-products used in human food products or 5307 grain, grain by-products, forage or silage used as animal feed are not necessary to support a conclusion regarding the safety of 5307 corn.

Table VI-4. Concentrations of eCry3.1Ab in 5307 corn tissues at four stages on a dry-weight (DW) and a fresh-weight (FW) basis.

		µg/g DW		µg/g FW	
Stage	**Location**	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**
Whorl	Leaves	142.96 ± 53.44	88.65–279.79	23.75 ± 3.16	16.81–33.80
	Whole plants	111.08 ± 38.36	75.16–178.22	15.78 ± 2.47	11.41–28.64
Anthesis	Leaves	84.34 ± 9.85	61.37–112.62	20.23 ± 2.59	13.83–27.59
	Whole plants	38.14 ± 8.72	14.18–55.67	8.11 ± 2.11	3.10–13.12
Maturity	Leaves	49.04 ± 31.79	1.46–105.60	25.33 ± 17.99	0.89–71.21
	Whole plants	16.03 ± 5.45	6.37–38.94	8.86 ± 3.93	3.36–21.96
	Kernels	6.19 ±1.87	2.37–9.64	4.56 ± 1.40	1.60–7.29
Senescence	Leaves	–	< LOQ–26.50	–	< LOQ–20.29
	Whole plants	8.27 ± 2.90	3.41–25.46	3.60 ± 0.94	1.70–10.65
	Kernels	4.45 ± 0.82	2.92–6.76	3.24 ± 0.41	2.38–4.66

Means represent data from 4 locations. At each location, 5 plants were analyzed at each sampling stage. All data are corrected for extraction efficiency. Tissues of near-isogenic, nontransgenic control plants were assayed in parallel to assess any effects of the plant matrix on the ELISA.

– = Not Applicable. It was not possible to calculate the mean as some values were below the limit of quantification (LOQ). LOQ = 0.10 µg/g DW and 0.02 µg/g FW.

Table VI-5. Concentrations of PMI in 5307 corn tissues at four stages on a dry-weight (DW) and a fresh-weight (FW) basis.

		µg/g DW		µg/g FW	
Stage	**Location**	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**
Whorl	Leaves	4.83 ± 1.47	2.97–8.33	0.81 ± 0.10	0.59–1.13
	Whole plants	4.23 ± 1.38	2.59–7.69	0.62 ± 0.22	0.34–1.13
Anthesis	Leaves	2.91 ± 0.33	1.74–5.20	0.70 ± 0.08	0.43–1.28
	Whole plants	4.38 ± 2.43	2.00–8.83	0.93 ± 0.47	0.44–2.13
Maturity	Leaves	–	< LOQ–3.50	–	< LOQ–1.66
	Whole plants	1.83 ± 0.51	0.68–2.56	0.96 ± 0.31	0.41–1.57
	Kernels	2.08 ± 0.49	1.04–3.82	1.36 ± 0.29	0.74–2.38
Senescence	Leaves	–	< LOD–0.54	–	< LOD–0.42
	Whole plants	0.97 ± 0.37	0.39–2.02	0.43 ± 0.14	0.15–0.71
	Kernels	1.11 ± 0.05	0.70–1.62	0.82 ± 0.06	0.50–1.28

Means represent data from 4 locations. At each location, 5 plants were analyzed at each sampling stage. All data are corrected for extraction efficiency. Tissues of near-isogenic, nontransgenic control plants were assayed in parallel to assess any effects of the plant matrix on the ELISA.

– = Not Applicable. It was not possible to calculate the mean as some values were below the LOQ or Limit of Detection (LOD); LOQ = 0.06 µg/g DW and 0.03 µg/g FW for leaves at maturity; LOQ = 0.05 µg/g DW and 0.03 µg/g FW for leaves at senescence; LOD = 0.01 µg/g DW and FW for leaves at senescence.

VII. Food and Feed Nutritional Assessment of Event 5307 Corn

VII.A. Justification for Comparators

To confirm that Event 5307 corn and food and feed derived from Event 5307 corn are nutritionally comparable to and as safe as that derived from conventional corn, the composition of forage and grain from Event 5307 corn was compared to the composition of forage and grain from nontransgenic, near-isogenic corn. The Event 5307 corn and the corresponding conventional control corn were harvested from six locations in the USA during 2008. The locations were selected to be representative of the range of environmental conditions under which the hybrid varieties were expected to be grown. All plants were grown using local agronomic practices for the respective regions.

In addition, a 49-day broiler chicken feeding study was performed to evaluate whether standard poultry diets prepared with grain from Event 5307 corn supported broiler chicken survival, growth, and feed conversion to body weight that was not significantly different from survival, growth, and feed conversion of chickens consuming diets prepared with grain from nontransgenic, near-isogenic corn grown in the same location during the same growing season.

VII.B. Historic Use of Corn in the U.S.

Field corn or maize (*Zea mays* L.) is the most widely distributed cereal grain grown worldwide. Cultivated corn is presumed to have been derived from teosinte (*Z. mexicana*) and is thought to have been introduced into the old world in the sixteenth century. Corn represents a staple food for a significant proportion of the world's population. The modern era of corn hybrid production began in the US where research conducted in the early part of the 1900s proved that hybrid corn could produce a yield superior to open-pollinated varieties (Sprague and Eberhart 1977). Gradually, hybrid varieties replaced the open-pollinated types in the 1930s and 1940s. Almost all corn grown in the US now comes from hybrid seed that is obtained every planting season from private enterprises; the older open-pollinated varieties are virtually unknown in commerce (Hallauer et al. 1988). Corn grown in the US is predominantly of the yellow dent type, a commodity crop. Roughly 60% of the crop is fed to livestock either as grain or silage. Livestock that feed on corn include cattle, pigs, poultry, sheep and goats. The remainder of the crop is exported or processed by wet or dry milling to yield products such as high fructose corn syrup, starch, oil, grits and flour. These processed products are used extensively in the food industry. For example, corn starch serves as a raw material for an array of processed foods and in industrial manufacturing processes. Since the early 1980s a significant amount of grain has also been used for fuel ethanol production. The by-products from these processes are often used in animal feeds.

VII.C. Compositional Assessment of 5307 Forage and Grain

Compositional analyses of 5307 corn were performed to identify any changes in nutrient or anti-nutrient content of the new crop in the context of its use as food or feed and to assess its biochemical equivalence and familiarity to conventional corn. This assessment was undertaken by performing quantitative analyses of 59 biochemical components of 5307 hybrid corn forage and grain including key food and feed nutrients, antinutrients, and secondary plant metabolites. An identical set of analyses was performed on nontransgenic, near-isogenic control hybrid corn.

VII.C.1. Design and methods used in compositional analysis study

The 5307 hybrid plants were genotype NP2171 × NP2460(5307) and the control plants were genotype NP2171 × NP2460. The seed materials used to plant these composition trials are also identified in Table IV-2 and in the pedigree diagram in Figure IV-4. The trials were conducted under USDA APHIS notification 08-051-104n.

Forage and grain from the 5307 and control hybrids were harvested from six locations in the U.S. during 2008: Stanton, MN, Janesville, WI, New Haven, IN, Shirley, IL, Marshall, MO, and Bloomington, IL. These locations are representative of major corn growing regions of the U.S. At each location, the hybrids were planted in a randomized complete block design, with three replicates for each genotype. All plots were managed according to local agronomic practices for the respective regions. Plants were self-pollinated by hand and the developing ears were bagged to avoid cross-pollination.

The components measured in this study were selected based on recommendations of the Organisation for Economic Co-operation and Development (OECD 2002) for comparative assessment of composition of new varieties of corn. The components analyzed are listed in Table VII-1 below.

All analyses were conducted using methods published and approved by the Association of Analytical Communities (AOAC) International or other industry-standard analytical methods. Based on the moisture content of each sample, analyte levels were converted to equivalent units of dry weight. A detailed description of the methodology for the compositional analyses is provided in Appendix D.

VII.C.1.a. Statistical analysis for across-location comparisons

The data for each component were subjected to analysis of variance using the following mixed model:

$$Y_{ijk} = U + T_i + L_j + B(L)jk + LT_{ij} + e_{ijk}$$

In this model, Y_{ijk} is the observed response for genotype i at location j block k, U is the overall mean, T_i is the genotype effect, L_j is the location effect, $B(L)_{jk}$ is the effect of block within a

location, LT_{ij} is the location-by-genotype interaction effect, and e_{ijk} is the residual error. Genotype was regarded as a fixed effect, while the effects of location, block within location, and location-by-genotype were regarded as random.

For each quantifiable component, an F test was used to assess the statistical significance of the genotype effect with an alpha level of 0.05 and with the denominator degrees of freedom determined using the Kenward-Roger method (Kenward and Roger 1997). Moisture content of grain was not statistically analyzed because the samples had been mechanically dried.

VII.C.1.b. Statistical analysis for individual-location comparisons

The data for each component at each location were subjected to an analysis of variance with genotype and block included in the statistical model. Significance was based on an alpha level of 0.05.

Statistical analyses were performed using SAS v. 9.2 (SAS Institute, Inc.; Cary, NC).

VII.C.1.c. Comparison with ILSI Crop Composition Database

The mean levels of each component for each location and across locations were calculated and compared nonstatistically with means and ranges for forage and grain composition published in the ILSI Crop Composition Database (2008). The ILSI database is the most comprehensive and current source of crop composition data for most nutritional components.

Table VII-1. Forage and grain components measured in 5307 and conventional corn.

Forage	Grain			
Minerals	**Minerals**	**Amino acids**	**Secondary Metabolites**	**Fatty acids**
Calcium	Calcium	Alanine (Ala)	ρ-Coumaric acid	16:0 palmitic
Phosphorus	Copper	Arginine (Arg)	Ferulic acid	18:0 stearic
Proximates	Iron	Aspartic acid (Asp)	Furfural	18:1 oleic
Acid detergent fiber (ADF)	Magnesium	Cystine (Cys)	Inositol	18:2 linoleic
Ash	Manganese	Glutamic acid (Glu)	**Vitamins**	18:3 linolenic
Carbohydrates	Phosphorus	Glycine (Gly)	Vitamin A (β-carotene)	20:0 arachidic
Fat	Potassium	Histidine (His)	Vitamin B_1 (thiamine)	20:1 eicosenoic
Moisture	Selenium	Isoleucine (Ile)	Vitamin B_2 (riboflavin)	22:0 behenic
Neutral detergent fiber (NDF)	Sodium	Leucine (Leu)	Vitamin B_3 (niacin)	**Anti-nutrients**
Protein	Zinc	Lysine (Lys)	Vitamin B_6 (pyridoxine)	Phytic acid
	Proximates	Methionine (Met)	Vitamin B_9 (folic acid)	Raffinose
	ADF	Phenylalanine (Phe)	Vitamin E (α-tocopherol)	Trypsin inhibitor
	Ash	Proline (Pro)		
	Carbohydrates	Serine (Ser)		
	Fat	Threonine (Thr)		
	Moisture	Tryptophan (Trp)		
	NDF	Tyrosine (Tyr)		
	Protein	Valine (Val)		
	Starch			
	Total Dietary Fiber (TDF)			

Table VII-2 through Table VII-9 report the statistical comparisons of nutritional component levels in forage and grain between 5307 corn and nontransgenic corn, the mean levels for each genotype across locations and at each location, a range of individual replicate values, and the levels for conventional hybrid corn reported in the ILSI Crop Composition Database (2008).

All data were compared with the ranges reported in the ILSI database to establish whether the results were within the range of natural variation and to provide an indication of whether the results were likely to be of biological significance.

VII.C.2. Results of statistical analysis for forage

When analyzed across all six locations, there were no statistically significant differences in any of the measured forage components (proximates, calcium, and phosphorus) between genotypes (Table VII-2 and Table VII-3).

When analyzing the results at each individual location, statistically significant differences were observed in moisture, protein, calcium, and phosphorus values, but all of these components were only different at one location (out of six) and all means fell within the range of natural variation of corn reported in the ILSI database (ILSI 2008).

VII.C.3. Results of statistical analysis for grain

When analyzed across all six locations, there were no statistically significant differences in 52 of the 59 components including: proximates, starch, and fiber components (Table VII-4), minerals (Table VII-5), vitamins B_1, B_2, B_3 and E (Table VII-6), amino acids (Table VII-7), oleic, linoleic, arachidic, and behenic fatty acids (Table VII-8), antinutrients, or secondary metabolites (Table VII-9).

There were a few statistically significant differences observed in vitamins A, B_6, and B_9 (Table VII-6), as well as 16:0 palmitic, 18:0 stearic, 18:3 linolenic, and 20:1 eicosenoic acids (Table VII-8). However, the differences observed were small and the mean values observed for these vitamins and all but one fatty acid (18:3 linolenic) were all within the ranges of values observed for the nontransgenic grain, and all means fell within the natural variation of corn reported in the ILSI database (ILSI 2008).

Some statistically significant differences were observed in values for individual locations but all of these components were only different at one or two locations and all per-location means fell within the range of natural variation for corn reported in the ILSI database (ILSI 2008), with the exception of starch and Vitamin B_2 in the nontransgenic grain, for which the values exceeded the reported range.

VII.C.4. Conclusion of compositional analyses

For all 59 chemical components that were measured in 5307 forage and grain, including those for which statistically significant differences were observed, the average values (when quantifiable) were within the ranges of natural variation reported in the ILSI database (2008). No biologically significant changes in composition were found to have occurred as an unintended result of the transformation process or expression of the transgenes in 5307 corn. In conclusion, forage and grain from 5307 corn hybrids are considered similar in composition to forage and grain from both the nontransgenic comparator and conventional corn hybrids.

These data support the conclusion that 5307 corn will be as safe and nutritious as conventional corn.

Table VII-2. Proximate composition of forage from 5307 corn and nontransgenic corn.

Proximate levels are shown in % DW, except for moisture (% FW). Results significantly different at $P < 0.05$ are shown in bold italic type.
For across-location analyses, $N = 18$; the range of values among these replicates is provided.

Location	Data source	Statistic	Moisture	Protein	Fat	Ash	Carbo-hydrates	ADF	NDF
Across all	Event 5307	mean	73.0	7.72	1.90	4.12	86.3	29.1	44.9
		range	66.5–79.5	5.91–10.3	0.893–2.81	2.89–5.35	82.9–88.9	22.3–40.1	35.5–56.1
	Nontransgenic	mean	72.3	7.57	1.89	4.34	86.2	28.6	45.4
		range	66.7–78.0	6.27–10.0	0.843–2.63	3.43–6.18	82.3–89.0	19.0–41.5	32.3–57.4
	ANOVA (*F* test)								
	Genotype effect	*P*	0.126	0.525	0.893	0.076	0.895	0.696	0.785
		SEM	1.49	0.449	0.118	0.295	0.64	1.33	1.91
	ILSI (2008)	mean	70.2	7.78	2.039	4.628	85.6	27.00	41.51
		range	49.1–81.3	3.14–11.57	< LOQ–4.570	1.527–9.638	76.4–92.1	16.13–47.39	20.29–63.71
		N[a]	945	945	921	945	945	945	945

[a]*N* is the number of ILSI values used to calculate the mean and excludes values < LOQ

Table VII-2 (*Continued*). Proximate composition of forage from 5307 corn and nontransgenic corn.

Proximate levels are shown in % DW, except for moisture (% FW). Results significantly different at $P < 0.05$ are shown in bold italic type. For individual location means, $N = 3$.

Location	Data source	Statistic	Moisture	Protein	Fat	Ash	Carbo-hydrates	ADF	NDF
L1	Event 5307	mean	70.2	7.98	2.23	3.50	86.3	26.9	36.4
	Nontransgenic	mean	68.8	7.28	1.73	3.67	87.3	26.4	40.1
		P	0.208	0.286	0.415	0.162	0.162	0.887	0.480
		SEM	0.53	0.340	0.346	0.055	0.35	2.48	3.04
L2	Event 5307	mean	72.1	6.96	2.01	4.59	86.4	29.7	44.1
	Nontransgenic	mean	71.4	6.77	2.11	4.82	86.3	33.8	48.9
		P	0.630	0.731	0.772	0.363	0.961	0.433	0.563
		SEM	0.96	0.340	0.207	0.139	0.43	2.98	4.97
L4	Event 5307	mean	72.5	7.46	2.15	3.64	86.8	28.1	46.7
	Nontransgenic	mean	71.3	6.96	2.30	4.01	86.7	26.4	41.1
		P	0.105	***0.033***	0.795	0.417	0.771	0.738	0.295
		SEM	0.31	0.066	0.350	0.258	0.21	3.13	2.82
L6	Event 5307	mean	76.0	8.40	1.41	4.64	85.5	29.1	47.7
	Nontransgenic	mean	76.7	9.07	1.77	4.41	84.8	27.0	45.5
		P	0.654	0.521	0.192	0.646	0.349	0.092	0.077
		SEM	0.99	0.610	0.131	0.300	0.41	0.49	0.46
L7	Event 5307	mean	78.5	9.35	1.73	5.05	84.0	33.8	48.6
	Nontransgenic	mean	76.7	8.80	1.83	5.47	83.9	32.4	52.9
		P	***0.007***	0.141	0.695	0.377	0.804	0.672	0.541
		SEM	0.10	0.162	0.162	0.265	0.42	2.06	4.19
L8	Event 5307	mean	68.6	6.19	1.89	3.33	88.6	26.7	45.7
	Nontransgenic	mean	68.7	6.54	1.57	3.65	88.3	25.5	43.7
		P	0.225	0.057	0.323	0.162	0.625	0.688	0.594
		SEM	0.04	0.061	0.174	0.104	0.33	1.88	2.33

Table VII-3. Calcium and phosphorus composition of forage from 5307 corn and nontransgenic corn.

Calcium and phosphorus levels shown in mg/kg DW. Results significantly different at $P < 0.05$ are shown in bold italic type. For across-location analyses, $N = 18$; the range of values among these replicates is provided.

Location	Data source	Statistic	Ca	P
Across all	Event 5307	mean	2346	1906
		range	1450–3470	1420–2870
	Nontransgenic	mean	2354	1953
		range	1660–3350	1390–2890
	ANOVA (*F* test)			
	Genotype effect	*P*	0.886	0.491
		SEM	209.3	163.9
	ILSI (2008)	mean	2028.6	2066.1
		range	713.9–5767.9	936.2–3704.1
		N	481	481

Table VII-3 *(Continued)*. Calcium and phosphorus composition of forage from 5307 corn and nontransgenic corn.

Calcium and phosphorus levels shown in mg/kg DW. Results significantly different at *P* <0.05 are shown in bold italic type. For individual location means, *N* =3.

Location	Data source	Statistic	Ca	P
L1	Event 5307	mean	2370	1723
	Nontransgenic	mean	2210	1523
		P	0.208	***0.003***
		SEM	61.6	8.2
L2	Event 5307	mean	2130	1500
	Nontransgenic	mean	2223	1457
		P	0.711	0.694
		SEM	154.5	67.4
L4	Event 5307	mean	2217	1783
	Nontransgenic	mean	2303	1813
		P	0.087	0.869
		SEM	19.3	113.1
L6	Event 5307	mean	2497	2493
	Nontransgenic	mean	2407	2677
		P	0.771	0.662
		SEM	191.4	255.6
L7	Event 5307	mean	3287	1947
	Nontransgenic	mean	3190	2043
		P	***0.032***	0.585
		SEM	12.5	105.9
L8	Event 5307	mean	1573	1990
	Nontransgenic	mean	1793	2203
		P	0.138	0.076
		SEM	64.8	44.0

Table VII-4. Proximate and starch composition of grain from 5307 corn and nontransgenic corn.

Proximate and starch levels shown in % DW, except moisture (% FW). Results significantly different at $P < 0.05$ are shown in bold italic type.
For across-location analyses, $N = 18$; the range of values among these replicates is provided.

Location	Data source	Statistic	Moisturea	Protein	Fat	Ash	Carbo-hydrates	ADF	NDF	TDF	Starch
Across all	Event 5307	mean	10.13	10.86	4.54	1.46	83.1	2.74	8.85	11.8	69.4
		range	9.54–11.4	9.12–12.6	3.85–4.93	1.22–1.60	81.0–85.3	2.23–3.34	7.68–9.52	10.8–13.4	62.0–73.7
	Nontransgenic	mean	10.18	10.92	4.72	1.40	83.0	2.85	8.83	11.7	70.3
		range	9.21–12.2	9.20–13.0	4.43–5.09	1.09–1.67	80.7–84.7	2.47–3.48	7.79–10.2	10.6–13.5	63.1–77.3
	ANOVA (*F* test)										
	Genotype effect	*P*	–	0.737	0.053	0.138	0.515	0.281	0.930	0.700	0.589
		SEM	–	0.375	0.067	0.044	0.44	0.069	0.128	0.19	1.21
	ILSI (2008)	mean	11.3	10.30	3.555	1.439	84.6	4.05	11.23	16.43	57.7
		range	6.1–40.5	6.15–17.26	1.742–5.823	0.616–6.282	77.4–89.5	1.82–11.34	5.59–22.64	8.85–35.31	26.5–73.8
		N	1434	1434	1174	1410	1410	1350	1349	397	168

– = not applicable
[a] Grain was mechanically dried after harvest; moisture levels were not subject to ANOVA

Table VII-4 *(Continued)*. Proximate and starch composition of grain from 5307 corn and nontransgenic corn.

Proximate and starch levels shown in % DW, except moisture (% FW). Results significantly different at $P < 0.05$ are shown in bold italic type.
For individual location means, $N = 3$.

Location	Data source	Statistic	Moisturea	Protein	Fat	Ash	Carbo-hydrates	ADF	NDF	TDF	Starch
L1	Event 5307	mean	10.54	10.93	4.38	1.43	83.2	2.63	8.66	11.2	69.4
	Nontransgenic	mean	11.13	10.60	4.79	1.29	83.3	2.89	9.27	11.9	71.7
		P	–	0.405	0.068	***0.038***	0.578	0.390	0.623	0.456	0.401
		SEM	–	0.225	0.080	0.019	0.14	0.169	0.754	0.54	1.54
L2	Event 5307	mean	10.73	9.38	4.53	1.34	84.8	2.89	9.01	11.7	71.9
	Nontransgenic	mean	11.00	9.97	4.68	1.25	84.1	2.67	8.73	11.2	74.7
		P	–	***0.011***	0.358	0.594	0.070	0.152	0.407	0.431	0.090
		SEM	–	0.044	0.090	0.094	0.13	0.069	0.188	0.41	0.64
L4	Event 5307	mean	9.77	10.97	4.84	1.42	82.8	2.84	9.20	11.6	72.5
	Nontransgenic	mean	9.48	10.50	4.84	1.39	83.3	2.73	8.65	11.9	66.0
		P	–	0.630	0.974	0.628	0.621	0.391	0.406	0.710	***0.019***
		SEM	–	0.586	0.126	0.037	0.65	0.076	0.372	0.50	0.65
L6	Event 5307	mean	9.79	12.37	4.59	1.54	81.5	2.85	9.10	12.5	67.3
	Nontransgenic	mean	9.56	12.50	4.77	1.62	81.1	2.87	8.89	12.4	67.8
		P	–	0.801	0.257	0.159	0.593	0.962	0.442	0.978	0.940
		SEM	–	0.327	0.080	0.025	0.41	0.217	0.154	0.75	3.87
L7	Event 5307	mean	10.20	10.60	4.25	1.52	83.6	2.54	8.55	12.0	65.9
	Nontransgenic	mean	10.27	10.67	4.56	1.38	83.4	2.97	8.45	11.4	68.9
		P	–	0.868	0.384	0.112	0.805	0.188	0.842	0.135	0.222
		SEM	–	0.249	0.198	0.037	0.42	0.156	0.322	0.19	1.23
L8	Event 5307	mean	9.74	10.90	4.68	1.50	82.9	2.67	8.57	11.7	69.7
	Nontransgenic	mean	9.64	11.27	4.66	1.47	82.6	2.95	8.99	11.4	72.9
		P	–	0.053	0.792	0.159	0.057	0.479	0.519	0.189	0.283
		SEM	–	0.062	0.047	0.012	0.05	0.229	0.389	0.11	1.59

– = not applicable. [a] Grain was mechanically dried after harvest; moisture levels were not subject to ANOVA

Table VII-5. Mineral composition of grain from 5307 corn and nontransgenic corn.

Mineral levels shown in mg/kg DW. Results significant at $P < 0.05$ are shown in bold italic type.
For across-location analyses $N = 18$; the range of values among these replicates is provided.

Location	Data source	Statistic	Ca	Cu	Fe	Mg	Mn	P	K	Se[a,c]	Na[b,c]	Zn
Across	Event 5307	mean	43.9	1.52	23.7	1323	5.65	3228	3758	–	–	23.0
all		range	38.6–49.3	0.89–4.20	21.2–28.0	1150–1430	4.69–6.61	2620–3520	3400–4010	<LOQ–0.363	< LOQ	19.5–26.9
	Nontransgenic	mean	44.0	1.89	23.3	1336	5.43	3307	3776	–	–	23.4
		range	40.3–50.1	1.02–4.36	20.3–28.1	1220–1450	4.43–6.38	2650–3600	3240–4150	<LOQ–0.400	< LOQ	20.5–27.9
	ANOVA (*F* test)											
	Genotype effect	*P*	0.891	0.058	0.308	0.401	0.131	0.110	0.707	–	–	0.355
		SEM	1.28	0.253	0.85	21.2	0.249	94.7	81.0	–	–	0.78
	ILSI (2008)	mean	46.4	1.75	21.81	1193.8	6.18	3273.5	3842	0.20	31.75	21.6
		range	12.7–208.4	<LOQ–18.50	10.42–49.07	594.0–1940.0	1.69–14.30	1470.0–5330.0	1810.0–6030.0	<LOQ–0.75	<LOQ–731.54	6.5–37.2
		N[d]	1344	1249	1255	1257	1256	1349	1257	89	223	1257

– = not applicable
[a]The LOQ for selenium was 0.055–0.056 mg/kg DW
[b]The LOQ for sodium was 110–114 mg/kg DW
[c]Where some or all values were < LOQ, calculation of the mean and statistical comparison were not possible, thus only the range is shown
[d]*N* is the number of ILSI values used to calculate the mean and excludes values < LOQ

Table VII-5 *(Continued)*. Mineral composition of grain from 5307 corn and nontransgenic corn.

Mineral levels shown in mg/kg DW. Results significant at $P < 0.05$ are shown in bold italic type. For individual location means, $N = 3$.

Location	Data source	Statistic	Ca	Cu	Fe	Mg	Mn	P	K	Se[a]	Na[a]	Zn
L1	Event 5307	mean	47.6	2.66	23.6	1347	5.66	3163	3803	<LOQ–0.086	<LOQ	22.3
	Nontransgenic	mean	45.4	2.89	22.0	1330	5.03	3103	3680	0.079	<LOQ	22.1
		P	0.198	0.556	0.187	0.755	0.096	0.712	0.381	–	–	0.915
		SEM	0.81	0.232	0.55	33.0	0.149	99.7	78.1	–	–	0.78
L2	Event 5307	mean	41.6	1.35	22.4	1307	5.16	2857	3473	0.113	<LOQ	20.4
	Nontransgenic	mean	41.0	1.88	23.1	1337	5.31	2903	3403	0.125	<LOQ	22.1
		P	0.657	0.200	0.482	0.644	0.669	0.866	0.724	0.211	–	0.138
		SEM	0.87	0.198	0.63	39.4	0.219	172.4	121.7	0.0046	–	0.49
L4	Event 5307	mean	39.7	1.45	27.2	1297	5.09	3293	3657	0.348	<LOQ	25.7
	Nontransgenic	mean	41.2	1.28	27.5	1283	5.11	3413	3760	0.364	<LOQ	26.8
		P	0.200	0.453	0.701	0.732	0.910	0.230	0.446	0.569	–	***0.045***
		SEM	0.59	0.133	0.42	23.9	0.128	49.7	77.7	0.0160	–	0.16
L6	Event 5307	mean	46.7	0.95	22.5	1347	6.44	3383	3920	<LOQ–0.058	<LOQ	22.1
	Nontransgenic	mean	47.7	1.09	22.5	1353	6.25	3487	3930	<LOQ–0.063	<LOQ	21.4
		P	0.118	0.271	1.000	0.900	0.369	0.335	0.946	–	–	0.559
		SEM	0.27	0.066	0.27	33.2	0.117	58.1	92.7	–	–	0.78
L7	Event 5307	mean	46.8	1.36	24.3	1243	6.32	3227	3750	<LOQ–0.063	<LOQ	24.1
	Nontransgenic	mean	45.5	2.09	23.4	1297	6.14	3417	3883	<LOQ–0.107	<LOQ	23.9
		P	0.156	0.302	0.459	***0.047***	0.366	***0.033***	0.231	–	–	0.841
		SEM	0.40	0.372	0.73	8.5	0.108	24.8	55.4	–	–	0.83
L8	Event 5307	mean	40.8	1.34	22.1	1400	5.25	3443	3947	<LOQ–0.061	<LOQ	23.5
	Nontransgenic	mean	42.9	2.14	21.0	1417	4.73	3520	3997	<LOQ–0.062	<LOQ	24.2
		P	0.152	0.156	0.200	0.588	***0.018***	0.323	0.286	–	–	0.417
		SEM	0.66	0.252	0.39	18.4	0.049	41.7	24.5	–	–	0.51

– = not applicable

[a] Where some or all values were < LOQ, calculation of the mean and statistical comparison were not possible, thus only the range is shown

Table VII-6. Vitamin composition of grain from 5307 corn and nontransgenic corn.

Vitamin levels shown in mg/100 g DW except as indicated for vitamin E (mg/g). Results significant at $P < 0.05$ are shown in bold italic type.
For across-location analyses, $N = 18$; the range of values among these replicates is provided.

Location	Data source	Statistic	Vitamin A β-carotene	Vitamin B_1 Thiamine	Vitamin B_2 Riboflavin	Vitamin B_3 Niacin	Vitamin B_6 Pyridoxine	Vitamin B_9 Folic Acid	Vitamin E[a] α-tocopherol
Across all	Event 5307	mean	0.155	0.449	0.198	3.13	0.692	0.0397	0.0093
		range	0.133–0.185	0.399–0.511	0.156–0.264	2.53–4.11	0.587–0.769	0.0305–0.0460	0.00719–0.0111
	Nontransgenic	mean	0.176	0.458	0.198	3.18	0.737	0.0382	0.0090
		range	0.155–0.216	0.408–0.518	0.152–0.318	2.51–3.70	0.621–0.815	0.0289–0.0463	0.00607–0.0110
	ANOVA (*F* test)								
	Genotype effect	*P*	***<0.001***	0.146	0.941	0.674	***0.005***	***0.031***	0.074
		SEM	0.0049	0.0126	0.0096	0.104	0.0167	0.00199	0.00055
	ILSI (2008)	mean	0.684	0.530	0.125	2.376	0.644	0.0651	0.0103
		range	0.019–4.681	0.126–4.000	0.050–0.236	1.037–4.694	0.368–1.132	0.0147–0.1464	0.0015–0.0687
		N	276	894	704	415	415	895	863

[a] Original units of mg/100 g reported by the testing laboratory were converted to mg/g

Table VII-6 *(Continued)*. Vitamin composition of grain from 5307 corn and nontransgenic corn.

Vitamin levels shown in mg/100 g DW except as indicated for vitamin E (mg/g). Results significant at $P < 0.05$ are shown in bold italic type.
For individual location means, $N = 3$.

Location	Data source	Statistic	Vitamin A β-carotene	Vitamin B1 Thiamine	Vitamin B2 Riboflavin	Vitamin B3 Niacin	Vitamin B6 Pyridoxine	Vitamin B9 Folic Acid	Vitamin Ea α-tocopherol
L1	Event 5307	mean	0.152	0.473	0.219	3.27	0.611	0.0448	0.0075
	Nontransgenic	mean	0.171	0.476	0.205	3.29	0.716	0.0434	0.0069
		P	0.297	0.939	0.612	0.963	0.105	0.399	0.176
		SEM	0.0096	0.0220	0.0162	0.225	0.0261	0.00091	0.00022
L2	Event 5307	mean	0.151	0.456	0.175	3.53	0.660	0.0422	0.0082
	Nontransgenic	mean	0.167	0.476	0.202	3.33	0.684	0.0424	0.0078
		P	0.082	0.232	0.587	0.657	0.645	0.807	0.313
		SEM	0.0035	0.0083	0.0294	0.279	0.0321	0.00059	0.00018
L4	Event 5307	mean	0.141	0.488	0.197	3.16	0.739	0.0393	0.0093
	Nontransgenic	mean	0.159	0.504	0.169	3.20	0.748	0.0360	0.0090
		P	***0.016***	0.548	0.222	0.903	0.609	0.093	0.289
		SEM	0.0017	0.0164	0.0112	0.239	0.0098	0.00078	0.00011
L6	Event 5307	mean	0.172	0.432	0.213	2.89	0.699	0.0427	0.0095
	Nontransgenic	mean	0.188	0.424	0.246	2.85	0.749	0.0407	0.0094
		P	0.061	0.218	0.403	0.826	0.484	0.506	0.743
		SEM	0.0029	0.0033	0.0222	0.104	0.0421	0.00176	0.00028
L7	Event 5307	mean	0.157	0.434	0.183	3.16	0.721	0.0317	0.0107
	Nontransgenic	mean	0.176	0.435	0.172	3.25	0.759	0.0299	0.0109
		P	0.218	0.808	0.519	0.709	0.076	0.346	0.733
		SEM	0.0076	0.0009	0.0100	0.153	0.0079	0.00104	0.00030
L8	Event 5307	mean	0.160	0.410	0.203	2.77	0.723	0.0377	0.0105
	Nontransgenic	mean	0.195	0.432	0.191	3.17	0.767	0.0368	0.0098
		P	***0.025***	0.077	0.560	0.498	***0.003***	0.744	0.454
		SEM	0.0040	0.0045	0.0123	0.345	0.0018	0.00176	0.00056

[a] Original units of mg/100 g reported by testing laboratory were converted to mg/g

Table VII-7. Amino acid composition of grain from 5307 corn and nontransgenic corn.

Amino acid levels shown in mg/g DW. Results significant at $P < 0.05$ are shown in bold italic type.
For across-location analyses, $N = 18$; the range of values among these replicates is provided.

Location	Data source	Statistic	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Val
Across all	Event 5307	mean	6.93	3.80	5.14	20.4	9.23	3.95	8.21	2.33	5.12
		range	5.82–8.15	3.19–4.39	4.10–6.10	16.4–24.9	7.55–11.0	3.52–4.39	6.64–9.97	2.07–2.50	4.33–6.08
	Nontransgenic	mean	6.88	3.79	5.17	20.6	9.24	3.97	8.24	2.36	5.13
		range	6.00–8.20	3.36–4.47	4.44–6.28	17.4–25.3	7.84–10.9	3.61–4.35	7.06–10.0	2.14–2.59	4.28–6.01
	ANOVA (*F* test)										
	Genotype effect	*P*	0.625	0.908	0.736	0.715	0.973	0.761	0.846	0.284	0.877
		SEM	0.236	0.123	0.203	0.90	0.375	0.087	0.345	0.043	0.179
	ILSI (2008)	mean	6.88	3.75	5.12	20.09	9.51	3.85	7.90	2.21	4.90
		range	3.35–12.08	2.24–6.66	2.35–7.69	9.65–35.36	4.62–16.32	1.84–5.39	4.39–13.93	1.25–5.14	2.66–8.55
		N	1350	1350	1350	1350	1350	1350	1350	1350	1350

Table VII-7 *(Continued)*. Amino acid composition of grain from 5307 corn and nontransgenic corn.

Amino acid levels shown in mg/g DW. Results significant at $P < 0.05$ are shown in bold italic type. For individual location means, $N = 3$.

Location	Data source	Statistic	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Val
L1	Event 5307	mean	7.07	3.82	5.09	21.1	9.25	3.86	8.42	2.33	5.25
	Nontransgenic	mean	6.74	3.71	4.98	19.9	8.51	3.80	7.97	2.34	5.00
		P	0.133	0.197	0.431	0.239	0.408	0.059	0.212	0.707	0.179
		SEM	0.096	0.043	0.082	0.51	0.499	0.011	0.176	0.016	0.088
L2	Event 5307	mean	5.94	3.26	4.28	16.8	7.78	3.56	6.78	2.12	4.40
	Nontransgenic	mean	6.27	3.48	4.63	18.3	8.18	3.75	7.30	2.26	4.66
		P	***0.030***	***0.018***	0.118	***0.010***	0.380	***0.028***	***0.010***	0.148	***0.012***
		SEM	0.041	0.021	0.092	0.10	0.255	0.024	0.037	0.045	0.020
L4	Event 5307	mean	6.96	3.81	5.25	20.5	9.08	4.04	8.23	2.36	5.17
	Nontransgenic	mean	6.97	3.84	5.32	20.9	9.78	4.13	8.40	2.36	5.30
		P	0.961	0.833	0.761	0.591	0.247	0.485	0.591	0.940	0.367
		SEM	0.128	0.088	0.149	0.48	0.307	0.075	0.186	0.028	0.079
L6	Event 5307	mean	7.89	4.31	5.94	24.0	10.90	4.28	9.60	2.47	5.82
	Nontransgenic	mean	7.73	4.22	5.95	23.9	10.14	4.18	9.48	2.54	5.72
		P	0.747	0.668	0.976	0.967	0.301	0.606	0.849	0.277	0.820
		SEM	0.306	0.123	0.206	1.01	0.390	0.124	0.393	0.033	0.272
L7	Event 5307	mean	6.69	3.68	4.98	19.3	8.82	3.95	7.85	2.32	4.91
	Nontransgenic	mean	6.48	3.61	4.93	19.0	8.92	3.86	7.68	2.32	4.74
		P	0.514	0.680	0.885	0.772	0.763	0.554	0.725	0.840	0.533
		SEM	0.192	0.109	0.186	0.78	0.219	0.084	0.292	0.021	0.167
L8	Event 5307	mean	7.03	3.89	5.30	20.9	9.55	4.02	8.36	2.39	5.16
	Nontransgenic	mean	7.09	3.88	5.19	21.5	9.89	4.08	8.59	2.36	5.38
		P	0.835	0.933	0.519	0.661	0.584	0.594	0.648	0.311	0.508
		SEM	0.189	0.075	0.097	0.83	0.368	0.064	0.306	0.019	0.198

Table VII-7 (*Continued*). Amino acid composition of grain from 5307 corn and nontransgenic corn.

Amino acid levels shown in mg/g DW. Results significant at $P < 0.05$ are shown in bold italic type.
For across-location analyses, $N = 18$; the range of values among these replicates is provided.

Location	Data source	Statistic	Met	Ile	Leu	Tyr	Phe	Lys	His	Arg	Trp
Across all	Event 5307	mean	2.29	3.92	13.8	3.18	5.50	3.10	2.99	4.81	0.570
		range	1.97–2.51	3.19–4.77	10.8–17.1	1.57–4.18	4.34–6.68	2.76–3.36	2.57–3.44	3.72–5.56	0.381–0.704
	Nontransgenic	mean	2.36	3.91	13.8	3.26	5.52	3.09	3.01	4.82	0.557
		range	2.08–2.56	3.23–4.71	11.5–17.3	1.67–3.98	4.73–6.70	2.74–3.38	2.57–3.43	4.20–5.32	0.380–0.700
	ANOVA (*F* test)										
	Genotype effect	*P*	0.102	0.947	0.789	0.711	0.883	0.902	0.684	0.892	0.722
		SEM	0.049	0.163	0.66	0.153	0.239	0.059	0.088	0.144	0.0298
	ILSI (2008)	mean	2.09	3.68	13.41	3.36	5.25	3.15	2.96	4.33	0.627
		range	1.24–4.68	1.79–6.92	6.42–24.92	1.03–6.42	2.44–9.30	1.72–6.68	1.37–4.34	1.19–6.39	0.271–2.150
		N	1350	1350	1350	1350	1350	1350	1350	1350	1350

Table VII-7 (*Continued*). Amino acid composition of grain from 5307 corn and nontransgenic corn.

Amino acid levels shown in mg/g DW. Results significant at $P < 0.05$ are shown in bold italic type. For individual location means, $N = 3$.

Location	Data source	Statistic	Met	Ile	Leu	Tyr	Phe	Lys	His	Arg	Trp
L1	Event 5307	mean	2.32	4.03	14.3	2.88	5.63	3.08	3.01	4.43	0.497
	Nontransgenic	mean	2.32	3.74	13.3	3.47	5.26	3.06	2.92	4.61	0.601
		P	0.915	0.150	0.231	0.334	0.283	0.319	0.175	0.429	0.552
		SEM	0.039	0.092	0.39	0.330	0.177	0.014	0.031	0.132	0.1039
L2	Event 5307	mean	2.02	3.26	11.1	2.57	4.49	2.81	2.62	4.19	0.494
	Nontransgenic	mean	2.23	3.49	12.2	3.02	4.86	2.95	2.80	4.44	0.539
		P	0.144	0.066	***0.007***	0.228	0.079	0.072	***0.009***	0.161	0.656
		SEM	0.061	0.043	0.06	0.187	0.077	0.029	0.012	0.083	0.0605
L4	Event 5307	mean	2.28	3.95	13.8	3.16	5.50	3.19	3.03	5.02	0.680
	Nontransgenic	mean	2.36	4.01	14.1	3.52	5.63	3.22	3.11	5.29	0.588
		P	0.279	0.535	0.589	0.622	0.515	0.762	0.402	0.343	0.169
		SEM	0.037	0.057	0.30	0.441	0.114	0.068	0.058	0.157	0.0307
L6	Event 5307	mean	2.42	4.56	16.4	3.54	6.46	3.31	3.34	5.23	0.624
	Nontransgenic	mean	2.52	4.46	16.3	3.06	6.31	3.21	3.28	4.92	0.658
		P	0.115	0.793	0.931	0.524	0.724	0.578	0.721	0.523	0.427
		SEM	0.027	0.229	0.72	0.446	0.255	0.100	0.109	0.279	0.0238
L7	Event 5307	mean	2.37	3.74	13.0	3.43	5.27	3.08	2.91	5.02	0.554
	Nontransgenic	mean	2.39	3.58	12.7	3.39	5.18	2.97	2.82	4.84	0.502
		P	0.823	0.538	0.761	0.895	0.781	0.333	0.589	0.555	0.403
		SEM	0.037	0.154	0.61	0.189	0.193	0.063	0.100	0.178	0.0350
L8	Event 5307	mean	2.32	3.98	14.0	3.48	5.68	3.12	3.04	4.95	0.573
	Nontransgenic	mean	2.32	4.21	14.5	3.08	5.88	3.15	3.13	4.80	0.454
		P	0.919	0.477	0.644	0.665	0.592	0.607	0.509	0.703	0.134
		SEM	0.021	0.187	0.61	0.562	0.231	0.031	0.086	0.236	0.0342

Table VII-8. Fatty acid composition[a] of grain from 5307 corn and nontransgenic corn.

Fatty acids shown as % of total fatty acids. Results significant at $P < 0.05$ are shown in bold italic type.
For across-location analyses, $N = 18$; the range of values among these replicates is provided.

Location	Data source	Statistic	16:0 Palmitic	16:1 Palmitoleic[b]	18:0 Stearic	18:1 Oleic	18:2 Linoleic	18:3 Linolenic	20:0 Arachidic	20:1 Eicosenoic	22:0 Behenic
Across all	Event 5307	mean	15.7	–	1.74	24.5	55.6	1.60	0.392	0.250	0.220
		range	15.1–16.1	<LOQ–0.137	1.50–2.04	22.0–27.0	53.2–58.1	1.48–1.71	0.353–0.453	0.238–0.265	0.186–0.252
	Nontransgenic	mean	15.2	–	1.81	24.9	55.7	1.50	0.387	0.242	0.213
		range	14.6–15.9	<LOQ–0.450	1.54–2.17	22.6–26.4	53.8–58.4	1.40–1.57	0.361–0.437	0.232–0.261	0.194–0.247
	ANOVA (*F* test)										
	Genotype effect	*P*	***<0.001***	–	***0.038***	0.108	0.599	***<0.001***	0.186	***<0.001***	0.243
		SEM	0.07	–	0.059	0.54	0.60	0.017	0.0098	0.0029	0.0056
	ILSI (2008)	mean	11.50	0.154	1.82	25.8	57.6	1.20	0.412	0.297	0.176
		range	7.94–20.71	<LOQ–0.447	1.02–3.40	17.4–40.2	36.2–66.5	0.57–2.25	0.279–0.965	0.170–1.917	<LOQ–0.349
		N	1344	596	1344	1344	1344	1344	988	987	924

– = not applicable
[a] Where some or all values were <LOQ, % of total fatty acids could not be calculated and statistical analysis could not be performed. Levels <LOQ were observed for all replicates at all locations for 8:0 caprylic, 10:0 capric, 12:0 lauric, 14:0 myristic, 14:1 myristoleic, 15:0 pentadecanoic, 15:1 pentadecenoic, 17:0 heptadecanoic, 17:1 heptadecenoic, 20:2 eicosadienoic, 20:3 eicosatrienoic, and 20:4 arachidonic fatty acids
[b] Some values were <LOQ, therefore, only the range is shown

Table VII-8 (*Continued*). Fatty acid composition of grain from 5307 corn and nontransgenic corn.

Fatty acids shown as % of total fatty acids. Results significant at $P < 0.05$ are shown in bold italic type. For individual location means, $N = 3$.

Location	Data source	Statistic	16:0 Palmitic	16:1 Palmitoleic[a]	18:0 Stearic	18:1 Oleic	18:2 Linoleic	18:3 Linolenic	20:0 Arachidic	20:1 Eicosenoic	22:0 Behenic
L1	Event 5307	mean	15.5	<LOQ	1.53	22.4	58.1	1.63	0.374	0.247	0.210
	Nontransgenic	mean	15.1	<LOQ	1.58	22.7	58.2	1.51	0.371	0.237	0.204
		P	0.225	–	***0.013***	0.286	0.199	0.122	0.641	0.061	0.730
		SEM	0.16	–	0.004	0.15	0.06	0.033	0.0043	0.0019	0.0107
L2	Event 5307	mean	15.8	<LOQ	1.77	23.5	56.4	1.67	0.397	0.242	0.216
	Nontransgenic	mean	15.5	<LOQ–0.450	1.93	24.3	55.8	1.54	0.388	0.238	0.202
		P	0.149	–	0.202	0.120	0.406	***0.017***	0.467	0.476	0.100
		SEM	0.10	–	0.060	0.22	0.43	0.012	0.0077	0.0038	0.0035
L4	Event 5307	mean	15.7	0.134	1.76	25.3	54.8	1.57	0.391	0.245	0.200
	Nontransgenic	mean	15.2	0.132	1.86	25.5	55.0	1.47	0.391	0.240	0.212
		P	***0.013***	0.372	***0.023***	0.319	0.478	***0.019***	0.960	***0.047***	0.373
		SEM	0.04	0.0017	0.011	0.14	0.16	0.010	0.0042	0.0009	0.0075
L6	Event 5307	mean	15.8	<LOQ–0.132	1.68	24.5	55.5	1.60	0.368	0.246	0.228
	Nontransgenic	mean	15.1	<LOQ–0.134	1.72	24.7	56.1	1.54	0.370	0.239	0.206
		P	0.069	–	0.270	0.560	0.210	0.203	0.606	0.242	0.072
		SEM	0.14	–	0.019	0.17	0.25	0.023	0.0027	0.0029	0.0045
L7	Event 5307	mean	15.5	<LOQ	1.98	26.4	53.6	1.55	0.441	0.263	0.242
	Nontransgenic	mean	15.4	<LOQ–0.143	1.96	26.1	54.1	1.47	0.427	0.253	0.235
		P	***0.038***	–	0.701	0.493	0.291	0.086	0.336	0.132	0.678
		SEM	0.02	–	0.032	0.26	0.23	0.017	0.0077	0.0030	0.0098
L8	Event 5307	mean	15.7	<LOQ–0.137	1.71	24.9	55.1	1.59	0.380	0.254	0.221
	Nontransgenic	mean	15.0	<LOQ–0.124	1.80	25.9	54.9	1.45	0.378	0.247	0.220
		P	0.053	–	0.082	0.211	0.678	0.093	0.878	0.294	0.946
		SEM	0.13	–	0.020	0.39	0.29	0.032	0.0054	0.0037	0.0061

– = not applicable

[a] Where some or all values were < LOQ, % of total fatty acids could not be calculated, statistical analysis could not be performed, and only the range is shown

Table VII-9. Secondary metabolite and antinutrient composition of grain from 5307 corn and nontransgenic corn.

Analyte units as in column headings. Results significant at $P < 0.05$ are shown in bold italic type.
For across-location analyses, $N = 18$; the range of values among these replicates is provided.

Location	Data source	Statistic	Ferulic acid (mg/kg DW)	ρ-Coumaric acid (mg/kg DW)	Inositol (ppm DW)	Phytic acid (% DW)	Trypsin inhibitor (TIU/mg DW)	Furfural[a,b] (mg/kg DW)	Raffinose (% DW)
Across all	Event 5307	mean	1906	186	2510	0.910	3.34	–	0.156
		range	1670–2190	153–229	2120–3160	0.671–1.03	2.39–4.42	< LOQ	0.115–0.199
	Nontransgenic	mean	1889	186	2504	0.942	3.46	–	0.163
		range	1620–2090	148–226	1980–3060	0.729–1.06	2.22–3.94	< LOQ	0.119–0.188
	ANOVA (*F* test)								
	Genotype effect	*P*	0.691	0.926	0.951	0.216	0.393	–	0.066
		SEM	52.4	9.1	86.1	0.0261	0.118	–	0.0087
	ILSI (2008)	mean	2201.1	218.4	1331.5	0.745	2.73	3.697	0.312
		range	291.9–3885.8	53.4–576.2	89.0–3765.4	0.111–1.570	<LOQ–7.18	<LOQ–6.340	<LOQ–0.320
		N[c]	817	817	504	1196	696	14	701

– = not applicable
[a] The LOQ for furfural was 0.55–0.57 mg/kg DW
[b] All values were <LOQ and therefore statistical comparison was not possible
[c] *N* is the number of ILSI values used to calculate the mean and excludes values < LOQ

Table VII-9 (*Continued*). Secondary metabolite and antinutrient composition of grain from 5307 corn and nontransgenic corn.

Analyte units as in column headings. Results significant at $P < 0.05$ are shown in bold italic type. For individual location means, $N = 3$.

Location	Data source	Statistic	Ferulic acid (mg/kg DW)	ρ-Coumaric acid (mg/kg DW)	Inositol (ppm DW)	Phytic acid (% DW)	Trypsin inhibitor (TIU/mg DW)	Furfural[a] (mg/kg DW)	Raffinose (% DW)
L1	Event 5307	mean	1770	169	2323	0.903	3.31	<LOQ	0.179
	Nontransgenic	mean	1883	187	2160	0.878	3.58	<LOQ	0.174
		P	0.579	0.494	0.062	0.577	0.124	–	0.707
		SEM	122.2	15.9	30.1	0.0268	0.073	–	0.0082
L2	Event 5307	mean	1747	197	2517	0.840	2.90	<LOQ	0.166
	Nontransgenic	mean	1827	207	2230	0.848	3.48	<LOQ	0.178
		P	0.463	0.375	0.323	0.938	0.111	–	0.607
		SEM	62.8	6.5	155.8	0.0592	0.149	–	0.0133
L4	Event 5307	mean	2137	226	2790	1.000	3.58	<LOQ	0.164
	Nontransgenic	mean	1977	209	2760	1.006	3.73	<LOQ	0.175
		P	0.186	0.193	0.896	0.818	0.702	–	0.093
		SEM	57.2	6.3	143.5	0.0171	0.241	–	0.0026
L6	Event 5307	mean	2017	177	2527	0.842	3.44	<LOQ	0.167
	Nontransgenic	mean	1990	185	2523	0.965	3.64	<LOQ	0.173
		P	0.829	0.702	0.959	0.156	0.412	–	0.234
		SEM	76.6	12.8	40.9	0.0390	0.140	–	0.0027
L7	Event 5307	mean	1800	159	2470	0.912	3.40	<LOQ	0.139
	Nontransgenic	mean	1753	154	2627	0.984	3.28	<LOQ	0.156
		P	0.340	0.225	0.691	0.053	0.861	–	0.091
		SEM	26.6	2.0	241.1	0.0123	0.403	–	0.0039
L8	Event 5307	mean	1967	186	2433	0.964	3.41	<LOQ	0.122
	Nontransgenic	mean	1903	175	2727	0.971	3.02	<LOQ	0.123
		P	0.604	0.601	0.081	0.864	0.311	–	0.893
		SEM	73.5	13.0	62.8	0.0230	0.205	–	0.0031

– = not applicable

[a] All values were < LOQ and therefore statistical comparison was not possible

VII.D. Other Information Relevant to the Nutritional Assessment of Event 5307 Corn: Broiler Chicken Feeding Study

Chickens consume large quantities of corn grain in commercial feeds. Broiler chickens, in particular, have relatively high corn consumption because conventional feeding regimens have been designed to provide maximal body weight gain in the shortest amount of time. In addition, broiler chickens are highly sensitive to small nutrient changes within their diets because of their rapid growth rates. A 49-day feeding study was performed to evaluate whether standard poultry diets prepared with Event 5307 corn grain had any effect on male or female broiler chicken survival, growth, feed conversion (an indicator of how efficiently a bird converts feed to live body weight), or carcass yield when compared with control corn grain. Three lots of corn grain were used to prepare poultry diets as follows:

- diets prepared with grain from 5307 corn
- diets prepared with grain from nontransgenic, near-isogenic control corn
- diets prepared with a commercially available lot of North Carolina corn

The specific seed varieties planted to generate the 5307 and nontransgenic, near-isogenic control corn grain are specified in Table IV-2 and Figure IV-4. The poultry diets were formulated based on the individual nutrient analyses for each of the grain sources to meet standard nutritional recommendations for growing chickens (diets prepared with 55% to 63% maize) and were fed to groups of 90 male and 90 female birds for 49 consecutive days.

Parameters evaluated in the study included survival, body weight, feed conversion and carcass yield. Broiler chickens fed diets prepared with Event 5307 grain did not show any adverse effects compared to chickens fed diets prepared with either the nontransgenic, near-isogenic grain or the commercially available grain. There were no statistically significant differences between groups for body weight, feed consumption, survival, overall feed conversion, and carcass yield when expressed on an absolute weight basis. A few statistically significant differences were noted in carcass yield when expressed as a percentage of total body weight. Male broilers fed 5307 maize grain had decreased thigh weights compared with male broilers fed nontransgenic diets, but they were not different from the males consuming the commercially available control diets. Female broilers fed 5307 diets had decreased thigh and *pectoralis minor* weights compared with female broilers fed the nontransgenic and NCSU 2007 diets. There were no differences noted in the other carcass parts including fat pads, drums, wings, and *pectoralis major* muscles.

Overall, diets containing 5307 maize grain supported rapid broiler chicken growth at low mortality rates and excellent feed conversion ratios and there were no adverse effects on carcass yield. There were no adverse dietary effects on broiler chickens consuming diets prepared with Event 5307 corn grain when compared with those consuming diets prepared with nontransgenic corn grain, either as a direct effect of the transgenic proteins in the diet

or as a result of any unintended compositional changes in the grain that may have altered its nutritional value. The results of this study support the conclusion that Event 5307 corn is nutritionally comparable to and as safe as conventional corn for consumers.

VII.E. Summary of the Food and Feed Nutritional Assessment

Analysis of key nutritional components of forage and grain from 5307 corn showed that no biologically significant changes in composition occurred as an unintended result of the transformation process or expression of the transgenes in 5307 corn. Forage and grain from 5307 corn are considered similar in composition to forage and grain from conventional corn. In addition, a 49-day feeding study demonstrated that there were no adverse dietary effects on broiler chickens consuming diets prepared with 5307 corn grain when compared with those consuming diets prepared with nontransgenic, control corn grain, either as a direct effect of the transgenic proteins in the diet or as a result of any unintended compositional changes in the grain that may have altered its nutritional value. These results support the conclusion that 5307 corn is nutritionally comparable to and as safe as conventional corn.

VIII. References

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucl. Acids Res.* 25:3389-3402.

Armitage P, Berry G. 1987. Statistical Methods in Medical Research, 2nd ed. Oxford: Blackwell Scientific Publications. pp. 129-130.

Arumuganathan K, Earle ED. 1991. Nuclear DNA content of some important plant species. *Plant Mol. Biol. Rep.* 9:208–218.

Barb AW, Williamson JD, Pharr DM. 2002. Selection for mannose-6-phosphate isomerase activity in tobacco NT1 cultures: Partial physiological and molecular characterization. Direct unpublished submission to GenBank database; Accession no. AAN09933 (see NCBI 2010a).

Berks BC, Sargent F, Palmer T. 2000. The Tat protein export pathway. *Mol. Microbiol.* 35:260–274.

Brunaud V, Balzergue S, Dubreucq B, Aubourg S, Samson F, Chauvin S, Bechtold N, Cruaud C, DeRose R, Pelletier G, Lepiniec L, Caboche M, Lecharny A. 2002. T-DNA integration into the *Arabidopsis* genome depends on sequences of pre-insertion sites. *EMBO Reports* 3:1152-7.

Chen BY, Heneen WK, Simonsen V. 1989. Comparative and genetic studies of isozymes in resynthesized and cultivated *Brassica napus* L., *B. campestris* L. and *B. alboglabra* Bailey. *Theoret. Appl. Genet.* 77: 673-679.

Chen E, Stacy C. 2003 Modified Cry3A toxins and nucleic acid sequences coding therefor. Patent Application WO Patent No. 03/018810.

Chilton M, Que O. 2003. Targeted integration of T-DNA into the tobacco genome at double-stranded breaks: New insights on the mechanism of T-DNA integration. *Plant Physiol.* 133:956-65.

Christensen AH, Sharrock RA, Quail PH. 1992. Maize polyubiquitin genes: structure, thermal perturbation of expression and transcript splicing, and promoter activity following transfer to protoplasts by electroporation. *Plant Mol. Biol.* 18:675-689.

Chomczynski P. 1992. One-hour downward alkaline capillary transfer for blotting of DNA and RNA. *Anal. Biochem.* 201:134-139.

Codex. 2009. Codex Alimentarius Commission. Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants. pp. 7-23. Joint

FAO/WHO Food Standard Programme, Rome, Italy. ftp://ftp.fao.org/docrep/fao/011/a1554e/a1554e00.pdf.

Coe EH Jr., Nueffer MG, Hoisington DA. 1988. The genetics of maize. *In*: Corn and Corn Improvement, GF Sprague and JW Dudley (Eds.), (Agronomy Monographs No. 18; pp. 81-236). Madison, WI: American Society of Agronomy.

Crickmore N, Zeigler DR, Schnepf E, Van Rie J, Lereclus D, Baum J, Bravo A, Dean DH. 2010. *Bacillus thuringiensis* toxin nomenclature. http://www.lifesci.sussex.ac.uk/Home/Neil_Crickmore/Bt/

Davis JA, Wu XH, Wang L, DeRossi C, Westphal V, Wu R, Alton G, Srikrishna G, Freeze HH. 2002. Molecular cloning, gene organization, and expression of mouse Mpi encoding phosphomannose isomerase. *Glycobiology* 12:435-442.

deFramond A, Barton K, Chilton, M-D. 1983. Mini-Ti: A new vector strategy for plant genetic engineering. *Nat. Biotechnol.* 1:262-269.

Depicker A, Stachel S, Dhaese P, Zambryski P, Goodman HM. 1982. Nopaline synthase: transcript mapping and DNA sequence. *J. Molec. Appl. Genet.* 1:561-573.

Doebley JF, Iltis HH. 1980. Taxonomy of *Zea* (Graminae). I. Subspecific classification with key to taxa. *Am. J. Bot.* 67:986-983.

Dube S, Flynn E. 1988. Estimating protein molecular weights using SDS-PAGE. *Focus* 20: 24-25.

EPA. 2010. US Environmental Protection Agency. Biopesticides Registration Action Document. Cry1Ab and Cry1F *Bt* Plant-Incorporated Protectants.. Office of Pesticide Programs. Biopesticides and Pollution Prevention Division. http://www.regulations.gov/search/Regs/contentStreamer?objectId=0900006480b2916e&disposition=attachment&contentType=pdf.

Ewing B, Green P. 1998. Base-calling of automated sequencer traces using Phred II. Error probabilities. *Genome Res.* **8**: 186-194.

FAO/WHO 2001. Evaluation of allergenicity of genetically modified foods. Report of a Joint Food and Agriculture/World Health Organization Expert Consultation on Allergenicity of Foods Derived from Biotechnology. 22-25 January 2001. Rome, Italy.

Fling ME, Kopf J, Richards C. 1985. Nucleotide sequence of the transposon Tn7 gene encoding an aminoglycoside-modifying enzyme, 3"(9)-O-nucleotidyltransferase. *Nucleic Acids Res.* 13:7095-7106.

Geiser M, Schweizer S, Grimm C. 1986. The hypervariable region in the genes coding for entomopathogenic crystal proteins of *Bacillus thuringiensis*: nucleotide sequence of the *kurhd1* gene of subsp. *kurstaki* HD-1. *Gene* 48:109-118.

Gracy RW, Noltmann EA. 1968. Studies on phosphomannose isomerase. *J. Biol. Chem.* 243: 3161-3168.

Grimsley N, Bisaro D. 1987. Agroinfection. pp. 87-107 *In*: Plant DNA Infectious Agents..T. Hohn, J. Schell (Eds). New York: Springer-Verlag.

Hallauer AR, Russell WA, Lamkey KR. 1988. Corn Breeding. *In*: Corn and Corn Improvement. G.F. Sprague, J.W. Dudley (Eds.), Agronomy Monographs No 18; pp. 463-564. Madison, WI; American Society of Agronomy.

Hansen G, Das A, Chilton M-D. 1994. Constitutive expression of the virulence genes improves the efficiency of plant transformation by *Agrobacterium. Proc. Natl. Acad. Sci. USA* 91:7603-7607.

Heeb S, Itoh Y, Nishijyo T, Schnider U, Keel C, Wade J, Walsh U, O'Gara F, Haas D. 2000. Small, stable shuttle vectors based on the minimal pVS1 replicon for use in Gram-negative, plant-associated bacteria. *Mol. Plant Microbe In.* 13:232-237.

Hileman RE, Silvanovich A, Goodman RE, Rice EA, Holleschak G, Astwood JD, Hefle SL. 2002. Bioinformatic methods of allergenicity assessment using a comprehensive allergen database. *Int. Arch. Allergy Imm.* 128:280-291.

Hilger C, Grigioni F, Thill L, Mertens L, Hentges F. 2002. Severe IgE-mediated anaphylaxis following consumption of fried frog legs: definition of α-parvalbumin as the allergen in cause. *Allergy* 57:1053-1058.

Hill HD, Straka JG. 1988. Protein determination using bicinchoninic acid in the presence of sulfhydryl reagents. *Anal. Biochem.* 170:203-208.

Hitchcock AS, Chase A. 1971. Manual of the grasses of the United States (Vol. 2. pp. 790-796), New York: Dover Publications.

Höfte H, Whiteley H. 1989. Insecticidal crystal proteins of *Bacillus thuringiensis*. *Microbiol. Rev.* 53: 242-255.

International Food Biotechnology Council. 1990. Biotechnologies and food: assuring the safety of foods produced by genetic modification. *Reg. Tox. Pharma*. 12: S1-S196.

ILSI. 2008. *International Life Sciences Institute Crop Composition Database,* v. 3.0. http://www.cropcomposition.org/cgi-perl/search_ora.cgi; search parameters: crop type = "CORN_FIELD," tissue type = "FORAGE" or "GRAIN," crop year = "All",

country = “All,” and state = “All”; searching not restricted by analysis method or value range (accessed February 9, 2010).

Iltis HH, Doebley JF. 1980. Taxonomy of *Zea* (Graminae). II. Subspecific categories in the *Zea mays* complex and a generic synopsis. *Am. J. Bot.* 67:994-1004.

Ingham DJ, Beer S, Money S, Hansen G. 2001. Quantitative real-time PCR assay for determining transgene copy number in transformed plants. *BioTechniques* 31:136–140.

Ishida Y, Saito H, Ohta S, Hiei Y, Komari T, Kumashiro T. 1996. High efficiency transformation of maize (*Zea mays* L.) mediated by *Agrobacterium tumefaciens*. *Nat. Biotechnol.* 4:745-750.

Itoh T, Tomizawa J. 1979. Initiation of replication of plasmid ColE1 DNA by RNA polymerase, ribonuclease H and DNA polymerase I. *Cold Spring Harb. Sym. Quant. Biol.* 43:409-417.

Itoh Y, Watson J, Haas D, Leisinger T. 1984. Genetic and molecular characterization of the *Pseudomonas* plasmid pVS1. *Plasmid* 11:206-220.

Katayama H, Yokota H, Akao T, Nakamura O, Ohba M, Mekada E, Mizuki E. 2005. Parasporin-1, a novel cytotoxic protein to human cells from non-insecticidal parasporal inclusions of *Bacillus thuringiensis*. *J. Biochem.* 137:17–25.

Kenward MG, Roger JH. 1997. Small Sample Inference for Fixed effects from Restricted Maximum Likelihood. *Biometrics* 53:983–997.

Koziel MG, Desai NM, Lewis KS, Kramer VC, Warren GW, Evola SV, Crossland LD, Wright MS, Merlin EJ, Launis KL, Rothstein SJ, Bowman CG, Dawson JL, Dunder EM, Pace GM, Suttie JL. 1997. Synthetic DNA sequence having enhanced insecticidal activity in maize. Syngenta, assignee. U. S. Patent No. 5,625,136. Washington, DC: U.S. Patent Office.

Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227:680-685.

Lee BT, Matheson NK. 1984. Phosphomannoisomerase and phosphogluco-isomerase in seeds of *Cassia coluteoides* and some other legumes that synthesize galactomannan. *Phytochem*. 23:983-987.

Malvolti ME, Paciucci M, Cannata F, Fineschi S. 1993. Genetic variation in Italian populations of *Juglans regia* L. *Acta Hortic.* 311: 86-94.

Martinez A, Traverso JA, Valot B, Ferro M, Espagne C, Ephritikhine G, Zivy M, Giglione C, Meinnel T. 2008. Extent of N-terminal modifications in cytosolic proteins from eukaryotes. *Proteomics* 8:2809-2831.

Meyers BC, Tingey SV, Morgante M. 2001. Abundance, distribution, and transcriptional activity of repetitive elements in the maize genome. *Genome Res.* 11:1660–1676.

Miles JS, Guest JR. 1984. Nucleotide sequence and transcriptional start point of the phosphomannose isomerase gene (*manA*) of *Escherichia coli*. *Gene* 32:41-48.

Mizuki E, Park YS, Saitoh H, Yamashita S, Akao T, Higuchi K, Ohba M. 2000. Parasporin, a human leukemic cell-recognizing parasporal protein of *Bacillus thuringiensis. Clin. Diagn. Lab. Immunol.* 7:625–634.

Murray EE, Lotzer J, Eberle M. 1989. Codon usage in plant genes. *Nucleic Acids Res.* 17:477-498.

Nakamura K, Oshie K, Shimizu M, Takada Y, Oeda K, Ohkawa H. 1990. Construction of chimeric insecticidal proteins between the 130-kDa and 135-kDa proteins of *Bacillus thuringiensis* subsp. aizawai for analysis of structure-function relationship. *Agr. Biol. Chem. Tokyo* 54:715–724.

NCBI. 2009. Entrez® Protein database. Bethesda, MD: National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health. http://www.ncbi.nlm.nih.gov/sites/entrez?db=Protein. Accessed May 21, 2009.

NCBI. 2010a. Entrez® Nucleotide Database, including GenBank®. Bethesda, MD: National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health. http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucleotide.

NCBI. 2010b. Entrez® Protein Database, Bethesda, MD: National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Protein.

Negrotto D, Jolley M, Beer S, Wenck AR, Hansen G. 2000. The use of phosphomannose-isomerase as a selectable marker to recover transgenic maize plants (*Zea mays L.*) via *Agrobacterium* transformation. *Plant Cell Rep.* 19:798–803.

OECD. 2002. Consensus Document on Compositional Considerations for New Varieties of Maize (*Zea mays*): Key Food and Feed Nutrients, Anti-Nutrients and Secondary Plant Metabolites. Series on the Safety of Novel Foods and Feeds, No. 6. Paris, France: Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology, Environment Directorate, Organisation for Economic Co-operation and Development. 42 pp. http://www.olis.oecd.org/olis/2002doc.nsf/43bb6130e5e86e5fc12569fa005d004c/ef5

acb7d10c9cd66c1256c1b004323e8/$FILE/JT00130429.PDF (accessed January 28, 2010).

OECD. 2003. Consensus document on the biology of *Zea mays* subsp. *mays*. Series on Harmonisation of Regulatory Oversight in Biotechnology, No. 27. http://www.olis.oecd.org/olis/2003doc.nsf/43bb6130e5e86e5fc12569fa005d004c/f70b80eb7cd25728c1256d57003e5f0c/$FILE/JT00147699.PDF.

Ohba M, Mizuki E, Crickmore N, Cote JC, Nagamatsu Y, Kitada S, Sakai H, Harata K, Shin T. 2006. Parasporin nomenclature. http://parasporin.fitc.pref.fukuoka.jp/ (accessed March 16, 2009).

Pearson WR, Lipman DJ. 1988. Improved tools for biological sequence comparison. *Proc. Natl. Acad. Sci*. 85:2444-2448.

Ponting C. 2001. Issues in predicting protein function from sequence. *Brief. Bioinform.* 2:19-29.

Proudfoot AEI, Payton MA, Wells TNC. 1994a. Purification and characterization of fungal and mammalian phosphomannose isomerases. *J. Protein Chem.* 13:619-627.

Proudfoot AEI, Turcatti G, Wells TNC, Payton MA, Smith DJ. 1994b. Purification, cDNA cloning and heterologous expression of human phosphomannose isomerase. *Eur. J. Biochem*. 219:415-423.

Sadeghi M, Hajivandi M, Bogoev R, Amshey J. 2003. Molecular weight estimation of proteins by gel electrophoresis revisited. *Focus* 25:35-39.

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW. 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. *Proc. Natl. Acad. Sci. USA* 81:8014–8018.

San Miguel P, Bennetzen JL. 1998. Evidence that a recent increase in maize genome size was caused by the massive amplification of intergene retrotransposons. *Ann. Bot.* 82 (Supplement A):37-44.

Sekar V, Thompson D, Maroney MJ, Bookland RG, Adang MJ. 1987. Molecular cloning and characterization of the insecticidal crystal protein gene of *Bacillus thuringiensis* var. *tenebrionis*. *Proc. Natl. Acad. Sci. USA* 84:7036-7040.

Sprague GF, Eberhart SA. 1977. Maize breeding. *In:* Corn and Corn Improvement. (Agronomy Monographs No. 18, pp. 312-313), Madison, WI: American Society of Agronomy.

Stavolone L, Kononova M, Pauli S, Ragozzino A, de Hann P, Milligan S, Lawton K, Hohn T. 2003. Cestrum yellow leaf curling virus (CmYLCV) promoter: a new strong constitutive promoter for heterologous gene expression in a wide variety of crops. *Plant Mol. Biol.* 53:703-713.

Strickberger MW. 1976. Probability and statistical testing. pp 140-163 *In:* Genetics, 2nd ed. New York: Macmillan Publishing Company.

Taylor SL, Hefle SL. 2001. Will genetically modified foods be allergenic? *J. Allergy Clin. Immun.* 107:765-771.

Tijssen P. 1985. Processing of data and reporting of results of enzyme immunoassays. pp 385-421. *In* Practice and Theory of Enzyme Immunoassays. Laboratory Techniques in Biochemistry and Molecular Biology, Volume 15. Elsevier Science Publishers: Amsterdam.

Tinland B., Hohn B. 1995. Recombination between prokaryotic and eukaryotic DNA: Integration of *Agrobacterium tumefaciens* T-DNA into the plant genome. *Genet. Eng.* 17:209-229.

USDA-NASS. 2010. United States Department of Agriculture. National Agricultural Statistics Service. Acreage Report. Released June 30, 2010. http://usda.mannlib.cornell.edu/usda/current/Acre/Acre-06-30-2010.pdf.

Walling LL 2006. Recycling or regulation? The role of amino-terminal modifying enzymes. *Curr. Opin. Plant Biol.* 9:227-233.

Walters FS, deFontes CM, Hart H, Warren GW, Chen JS. 2010. Lepidopteran-active variable-region sequence imparts coleopteran activity in eCry3.1Ab, an engineered *Bacillus thuringiensis* hybrid insecticidal protein. *Appl. Environ. Microbiol.* 76:3082–3088.

Wang K, Herrera-Estrella L, Van Montagu M, Zambryski P. 1984. Right 25 bp terminus sequence of the nopaline T-DNA is essential for and determines direction of DNA transfer from *Agrobacterium* to the plant genome. *Cell* 38:455-462.

Yamashita S, Katayama H, Saitoh H, Akao T, Park YS, Mizuki E, Ohba M, Ito A. 2005. Typical three-domain Cry proteins of *Bacillus thuringiensis* strain A1462 exhibit cytocidal activity on limited human cancer cells. *J. Biochem.* 138:663–672.

Zambryski P, Depicker A, Kruger K., Goodman HM. 1982. Tumor induction by *Agrobacterium tumefaciens:* Analysis of the boundaries of T-DNA. *J. Mol. Appl. Genet.* 1: 361-370.

Appendix A: Materials and Methods Used in the Genetic Characterization of Event 5307 Corn

This appendix provides details of the materials and methods used in the various studies performed to genetically characterize Event 5307 corn. The design, results and conclusions of these experiments are described in Part IV. E. of this submission (**Characterization of the Genetic Material in Event 5307 Corn**).

A.1. Plant Material

Event 5307 corn and nontransgenic control corn seeds were planted and grown in a Syngenta Biotechnology, Inc. greenhouse in Research Triangle Park, North Carolina, USA under standard greenhouse conditions and then processed to extract genomic DNA. The specific generations of 5307 plants used and the corresponding control plants used are specified in the descriptions of the studies in Part IV. C.2. of this submission, **Development of Test and Control Seed Materials**. Appropriate quality control methods were used to verify the purity and identity of the plant material used in each study. The pedigree showing the genotypes of Event 5307 seed for the various studies is provided in Figure IV-4. Table IV-2 provides a list of the sources of plant material used for each study.

A.2. Real-Time PCR Analysis

All plants grown for genetic characterization studies were individually analyzed for the presence of *ecry3.1Ab* and *pmi* by real-time PCR analysis (Ingham et al. 2001). A control assay targeting the endogenous corn alcohol dehydrogenase gene 1 (*adh1*) was used to confirm the presence of DNA in each reaction. Leaf discs were sampled from each individual plant. Deoxyribonucleic acid (DNA) was isolated from leaf discs of each individual plant using a method adapted from the Wizard® Magnetic 96 DNA Plant System for real-time PCR analysis.

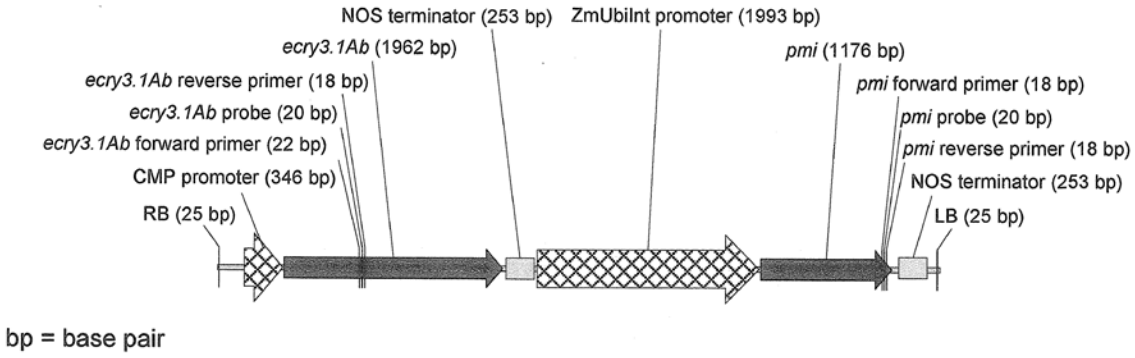
Table A-1 lists the primers and probes used to detect *ecry3.1Ab*, *pmi* and *adh1*. Figure A-1 shows the locations of the *ecry3.1Ab*-specific and *pmi*-specific primers and probes in the transferred DNA (T-DNA) of plasmid pSYN12274, the transformation plasmid used to generate 5307 corn.

The following cycling parameters were used for this reaction: 95°C for five minutes, followed by 40 cycles of 95°C for five seconds and 60°C for 30 seconds.

Table A-1. Real-time PCR primers and probes used for the detection of *ecry3.1Ab*, *pmi*, and *adh1*

Amplicon of interest	Forward primer sequence (5' to 3')	Reverse primer sequence (5' to 3')	Probe sequence (5' to 3')
ecry3.1Ab	TACGAGAGCTGGGTG AACTTCA	CGATCAGGTCCAGCA CGG	CCGCTACCGCCGCG AGATGA
pmi	CCGGGTGAATCAGCG TTT	GCCGTGGCCTTTGAC AGT	TGCCGCCAACGAATC ACCGG
adh1	GAACGGTGTTGGGTTT GCAT	TGCAGCCTAACCATG CGCAGGGTA	TCCAGCAATCCTTGC ACCTT

Figure A-1. Locations of real-time PCR primers and probes in the plasmid pSYN12274 T-DNA



A.3. Genomic DNA Extraction

Following verification of the plants' identity by real-time PCR analysis (see above), leaf tissue for 10 plants of each genotype was pooled into a sampling bag and stored at -80°C ± 10°C. Genomic DNA used for nucleotide sequencing and Southern blot analyses was isolated from the pooled leaf tissue from 10 plants per genotype using a modification of the method described in Saghai-Maroof et al. (1984). Pooled leaf tissue was ground into a fine powder using a pre-chilled mortar and pestle, with liquid nitrogen, and then placed into a bottle for storage. For each DNA extraction, approximately 40 g of this tissue and 200 ml of prewarmed CTAB buffer (100 mM Tris pH 8.0, 20 mM EDTA pH 8.0, 1.4 M NaCl, 2% CTAB [w/v], 0.2% [v/v] β-mercaptoethanol) were combined in a bottle; the sample was then mixed gently and incubated for 90 minutes at 65°C ± 5°C. An equal volume of chloroform: isoamyl alcohol (24:1) was then added, followed by gentle mixing and centrifugation for 10 minutes at 7277 × *g* at room temperature. The resulting aqueous phase was trans-ferred to a clean container, and 10 µg of ribonuclease per ml of aqueous phase was added. The sample was mixed and incubated for 30 minutes at 37°C ± 2°C. An equal volume of chloroform: isoamyl alcohol (24:1) was then added, followed by gentle mixing and centrifu-gation for 10 minutes at 7277 × *g* at room temperature. The aqueous phase was collected in a clean bottle,

and the DNA was precipitated with a 0.8 volume of isopropanol. The DNA was then pelleted by centrifugation at 291 × *g* and washed once with 70% ethanol. The DNA pellet was air dried and dissolved in 2.5 ml of prewarmed 0.1X Tris-EDTA.

A.4. DNA Quantitation

The concentration of DNA was measured using a Quant-iT™ PicoGreen® dsDNA kit. A two-point standard curve was generated using a Lambda DNA standard. Genomic DNA was quantified by interpolation from the two point standard curve using the TBS-380 Mini-Fluorometer.

A.5. Nucleotide Sequence of the T-DNA Insert

Two overlapping fragments that span the 5307 corn insert were amplified from genomic DNA using PCR analysis (Figure A-2). The 5307 plants used for this analysis were from the NP2171 × BC5F3 generation, as identified in the pedigree chart of plant materials (Figure IV-4). PCR amplification was carried out using the Expand™ Long Template PCR System. Table A-3 lists the primers used to amplify the insert fragments; Tables A-4 and A-5 contain the thermal cycling parameters.

Figure A-2. Map of the 5307 corn insert and location of PCR-amplified fragments from 5307 corn to determine insert sequence

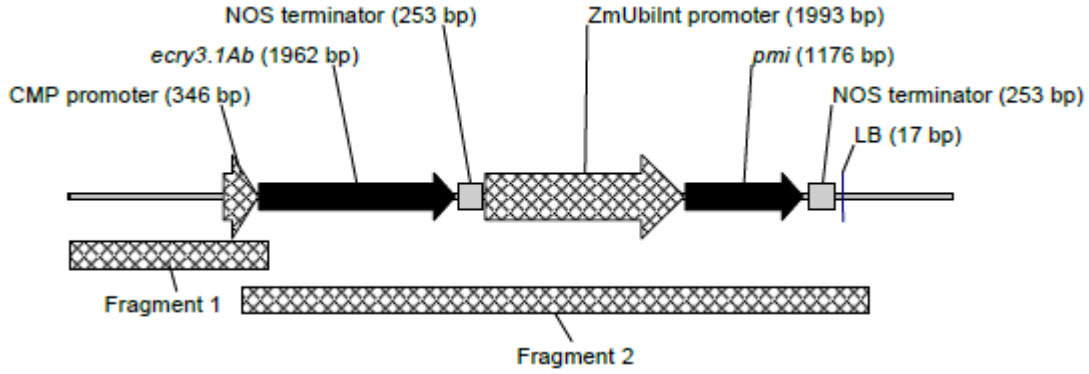


Table A-2. Primers used to amplify the insert of 5307 corn

Fragment	Forward primer sequence (5' to 3')	Reverse primer sequence (5' to 3')
1	GTGTAAGCCCCAAGCCATTTACTTCCTC	CGTCCTTGGTGGTGCTGCTGTCCAGGC
2	ATTCGTGGCCGACAGGTGG	AGCCGTACTATAAAGAGGGGTTGTCG

Table A-3. Cycling parameters for PCR amplification of insert Fragment 1

Cycle	Step	Temperature (°C)	Time	Number of cycles
A	1	94	2 min	1
B	1	94	15 sec	30
B	2	65	30 sec	30
B	3	72	90 sec	30
C	1	72	5 min	1
D	1	4	Hold	1

Table A-4. Cycling parameters for PCR amplification of insert Fragment 2

Cycle	Step	Temperature (°C)	Time	Number of cycles
A	1	94	2 min	1
B	1	94	10 sec	1
B	2	60	30 sec	1
B	3	68	5 min	1
C	1	94	15 sec	25
C	2	60	30 sec	25
C	3	68	5 min (+20 sec each cycle)	25
D	1	68	7 min	1
E	1	4	Hold	1

The PCR fragments were cloned into pCR®4-TOPO® vector, and three colonies for each PCR product were randomly selected and grown. The plasmid DNA was then independently extracted, and the resulting plasmid preparations, which contained the PCR amplification products, were subsequently sequenced.

Dye-terminator sequencing, a modification of the dideoxynucleotide chain-terminator sequencing method, was carried out using the ABI3730XL analyzer with ABI BigDye® 3.1 terminator chemistry. The sequence analysis was done using the Phred, Phrap, and Consed package (from the University of Washington), and was carried out to an error rate of less than 1 in 10,000 bases (Ewing and Green 1998).

Three individual clones for each PCR product were sequenced individually, and a consensus sequence was generated for each clone. These sequences were aligned using AlignX,™ a component of Vector NTI Advance,™ version 10.3.0, to obtain the final consensus sequence for each segment of the insert sequence.

A.6. Southern Blot Analyses

Southern blot analyses were performed using standard molecular biology techniques (Chomczynski 1992). Each lane contained 7.5 µg of genomic DNA that was digested with the appropriate restriction enzyme(s) for 8 to 16 hours.

A positive control, representing one copy of a fragment of known size in the corn genome, was included on each Southern blot. The positive control for these Southern blot analyses was digested DNA from plasmid pSYN12274. This positive control was loaded in a well together with 7.5 µg of digested DNA from NP2171 × NP2460 plants, so that the migration of this positive control DNA reflected, more accurately, the migration of the restriction fragment in the corn genome. The amount of positive control (picograms for one copy) was calculated by the following formula with a corn genome size of 2.67×10^9 bp (Arumuganathan and Earle 1991).

$$\left\{\left(\frac{Positive\,control\,size(bp)}{Genome\,size(bp) * Ploidy}\right) * \mu g\ loaded\right\} * 1 \times 10^6 = pg\ for\ 1\ copy$$

The following factors were used to calculate the amounts of positive control:

maize genome size (bp)	2.67×10^9
maize ploidy	2
DNA loaded in each lane (µg)	7.5
Positive control size (bp)	11,769

The following amount of positive control was calculated:

Plasmid pSYN12274 (pg)	16.53

The molecular weight marker (serving as the reference substance), the digested genomic DNA, and the positive control were loaded onto 1% SeaKem® Gold agarose gels, and the DNA fragments were then separated by electrophoresis in 1X Tris-acetate-EDTA buffer.

Following a 10 minute depurination in 0.25 N HCl, the DNA in the gel was denatured in 0.5 M NaOH and 1.5 M NaCl for 30 minutes. The DNA was then transferred to a Zeta-Probe GT membrane, by downward alkaline transfer, for 90 minutes using a Bio-Rad Appligene Vacuum Blotter. After rinsing the membrane briefly in 2X SSC, the DNA was cross-linked to the membrane using ultraviolet light.

All PCR-generated probes and the molecular weight marker-specific probe were labeled with phosphorus-32-deoxycytidine triphosphate ([α-^{32}P]-dCTP) by random priming using the Megaprime™ DNA labeling system. Unincorporated label ([α-^{32}P]-dCTP) was removed using the Micro Bio-Spin® Chromatography Columns.

Membranes were incubated in 30 ml of PerfectHyb™ Plus Hybridization Buffer (which contained 100 µg/ml denatured Calf Thymus DNA) for at least 30 minutes at 65°C ± 5°C. Both the molecular weight marker-specific probe and either the full length T-DNAspecific probe or backbone-specific probe were added to the hybridization solution, and the membranes were incubated for 16 hours at 65°C ± 5°C. Incubation was followed by a combination of washes at 65°C ± 5°C in 2X SSC with 0.1% SDS and washes at 65°C ± 5°C in 0.1X SSC with 0.1% SDS. Finally, the membranes were subjected to imaging using a Molecular Dynamics Storm 860® phosphorimager.

A.7. Nucleotide Sequencing of T-DNA Flanking Regions in Corn Genome

The 5′ and 3′ corn genomic sequences flanking the 5307 corn insert were previously recovered. This preliminary sequence was used to design primers for amplification of the flanking regions from 5307 corn. The flanking regions were amplified from genomic DNA extracted from 5307 corn using the Expand™ High-Fidelity PCR System. Table A-5 lists the primers used to amplify the flanking regions; Table A-6 contains the thermal cycling parameters.

Table A-5. Primers used to amplify the flanking regions of 5307 DNA insert

Region	Forward primer name	Forward primer sequence (5' to 3')	Reverse primer name	Reverse primer sequence (5' to 3')
5' flanking region	5307_F1	GCATTGGCATTTTCAT TAGCAAGCA	5307_R1	TGATTAAAGGCAGCCG ACCTAACCT
3' flanking region	5307_F2	CATCTCTTGCTAAGCT GGGAGCTCG	5307_R2	GACTTGTGTGGTTTCTC ACGGTCCA

Table A-6. PCR cycling parameters for the flanking regions of 5307 DNA insert

Cycle	Step	Temperature (°C)	Time	Number of cycles
A	1	95	5 min	1
B	1	95	15 sec	35
B	2	60	15 sec	35
B	3	72	2 min	35
C	1	72	10 min	1
D	1	4	Hold	1

The PCR fragments were cloned into pCR®4-TOPO® vector, and three colonies for each PCR product were randomly selected and grown. The plasmid DNA was then independently extracted, and the resulting plasmid preparations, which contained the PCR amplification products, were subsequently sequenced as described above (see Nucleotide Sequence of the T-DNA Insert).

A.8. Flanking Sequence Analysis to Determine if T-DNA Inserted into a Known Corn Gene

The following parameters were used for the BLASTN analysis against the National Center for Biotechnology Information (NCBI) Nucleotide Database (NCBI 2010a) to identify corn genomic sequences having identity or high similarity to sequences flanking the 5′ and 3′ ends of the 5307 T-DNA insert:

- Expect = 10. The expectation value (*E*-value) is a measure of the probability that matches between sequences occurred by chance. Search results involving comparisons between nucleotides with highly similar sequences yield *E*-values approaching zero; the probability that sequence similarities occurred by chance increases with higher *E*-values (Ponting 2001). The search identified all sequences in the database with search results yielding an *E*-value of 10 or lower; this is the conservative default search setting for this parameter.
- The scoring scheme used was the default for nucleotides: +1 for a match and -3 for a mismatch.
- The following gap penalties were used for this scoring matrix: Existence = 5 and Extension = 2. A gap is a space introduced into an alignment to compensate for insertions and/or deletions in one sequence relative to another. The introduction of a gap causes the deduction of a fixed value from the alignment score to prevent the accumulation of excessive gaps in an alignment. Extension of the gap to encompass additional nucleotides is also penalized in determining the score of an alignment. The resultant score is derived from the number of identical matches between the query sequence and the database entry, with higher scores indicating more identity between the two sequences.
- A low complexity filter was used for this search.

A.9. Amino Acid Sequence Comparison of Query Peptide to Known or Putative Toxins

The Basic Local Alignment Search Tool for Proteins (BLASTP) program, version 2.2.19, (Altschul et al. 1997) was used to compare the query peptide representing the translated sequence of the putative 243-bp ORF to all entries in the NCBI Entrez® Protein Database (containing over 10 million amino acid sequences) (NCBI 2010b). Information associated with the sequences having the highest similarity to the query sequence was examined to determine if any of the sequences was a toxin or putative toxin.

The BLASTP algorithm is optimized to identify regions of local similarity between protein sequences. This approach detects more similarities than would a search that aligns two sequences over their entire length. The following default parameters were used in the BLASTP comparisons:

- Expectation value (*E*-value) = 10.
- Word size = 3
- Gap costs: existence = 11 and extension = 1
- Similarity matrix: Blocks Substitution Matrix62 (BLOSUM62)
- No complexity filter

The *E*-value is a measure of the probability that matches between sequences occurred by chance. Search results involving comparisons between proteins with highly similar sequences yield *E*-values approaching zero; the probability that sequence similarities occurred by chance increases with higher *E*-values (Ponting 2001). The search identified all sequences in the database with search results yielding an *E*-value of 10 or lower. These sequences were evaluated for source and biological function. Any sequences described as toxins or putative toxins were identified.

A.10. Amino Acid Sequence Comparison of Query Peptide to Known or Putative Allergens

The 81-amino-acid query peptide sequence representing the translated sequence of the putative 243-bp ORF was screened for biologically relevant amino acid sequence similarity to any of the known or putative protein allergens within the FARRP AllergenOnline database (FARRP 2010). The FARRP AllergenOnline database is a curated, peer-reviewed database containing proteins identified as food allergens, respiratory allergens, allergenic venom proteins, contact allergens, gliadins, and glutenins. Entries were compiled primarily from searches of publicly available protein databases using the NCBI Entrez® search and retrieval system, most recently searched in 2009 (NCBI 2009). The NCBI dataset was screened by searches for entries associated with allergy or celiac disease; duplicate entries were removed, and additional entries were identified from publications. The list of candidate entries was then reviewed by an international panel of allergy experts who reviewed published clinical and laboratory evidence to support the candidate sequences as allergens. Proteins are classified as known or putative allergens according to predetermined criteria set by the FARRP expert review panel. The latest version of the FARRP AllergenOnline database (2010) contains 1,471 nonredundant entries. Similarity searches were performed using an exact copy of the entire list of sequences in the current version of the FARRP AllergenOnline database (2010) (maintained at Syngenta Biotechnology, Inc., Research Triangle Park, NC, USA). Two different sequence searches were performed to compare the translated junction sequence with sequences in the FARRP AllergenOnline database. In the first search, the FASTA search algorithm, version 3.45 (Pearson and Lipman 1988), was used to assess overall sequence similarity by comparing sequential 80-amino-acid peptides of the query sequence with the sequences in the FARRP

AllergenOnline database. Each successive "window" of 80 amino acids was offset from the previous window by one residue, such that each peptide overlapped the previous peptide by 79 amino acids. The default FASTA settings used include an extension penalty of two and gap creation penalty of 12. The scoring matrix for FASTA was the Blocks Substitution Matrix50 (BLOSUM50), the same scoring matrix used by the authors of the FARRP AllergenOnline database. The BLOSUM50 matrix is weighted to favor identical amino acids likely to impact protein structure. In the second search, the query sequence was screened for matches of eight or more contiguous amino acids (Hileman et al. 2002) using a program developed by Syngenta; this program compared every possible peptide of eight contiguous amino acids of the translated putative ORF with the sequences in the FARRP AllergenOnline database.

The FASTA search produces alignments between the 80-amino-acid peptides of the query sequence and the sequences in the allergen database. The evaluation of each query peptide sequence alignment utilizes the minimum criterion of 80 amino acids of alignment length with greater than 35% shared amino acid identity. Any alignments exceeding this criterion for shared sequence similarity indicate the potential for immunologically relevant sequence similarity (Codex 2009). Additionally, any match of eight (or more) identical contiguous amino acids between any query sequence and any sequence in the allergen database indicates the potential for immunologically relevant sequence similarity (Codex 2009).

Appendix B: Materials, Methods and Results of Characterization Studies on the eCry3.1Ab and PMI Proteins

This appendix presents materials, methods and detailed results of analyses of the biochemical properties and biological activity of the eCry3.1Ab and PMI proteins in 5307 corn and in the corresponding eCry3.1Ab and PMI test substances used in safety studies. A summary of the results and conclusions of these analyses is provided in Part VI of this submission, titled **Safety Assessment of the Introduced eCry3.1Ab and PMI Proteins**.

B.1. Materials and Methods for Comparison of eCry3.1Ab Produced in 5307 Corn Plants and Recombinant *E. coli*

Three preparations containing eCry3.1Ab were evaluated in this study: (1) LP5307, extracted from leaf material of 5307 corn plants; (2) IAP5307, immunopurified eCry3.1Ab derived from leaf material of 5307 corn plants; and (3) microbial test substance ECRY3.1AB-0208, prepared from a recombinant *E. coli* overexpression system. The sample designated LP5307 was used for Western blot analysis and insecticidal activity assays. The sample designated IAP5307 was used as the source of purified plant-produced eCry3.1Ab for Western blot, glycosylation, N-terminal sequencing and peptide mass mapping analyses. Nontransgenic, near-isogenic plants were used as negative control plant material, the source of the material designated LP-NEG.

Event 5307 Corn Leaf Tissue and Negative Control Corn Leaf Tissue

Young leaves from greenhouse-grown 5307 plants and nontransgenic, near-isogenic plants were collected 4-6 weeks after emergence, frozen at –80°C ± 10°C, and ground into a fine powder using a Grindomix Knife Mill (Retsch).

Extracts of 5307 Corn and Control Corn Leaf Tissue for Western Blot Analysis

Leaf powder was resuspended in extraction buffer containing 100 mM sodium borate (pH 10.0), 0.2% polyvinylpyrrolidone (PVP), 7.69 mM sodium azide, 0.5% Tween 20, and supplemented with one Complete Protease Inhibitor Cocktail tablet/50 ml of buffer (Roche). The mixture was homogenized with an Omni-Prep Homogenizer (Omni International), incubated for 30 minutes on ice and centrifuged at 3,000 rpm for 15 minutes at 4°C (Sorvall Legend RT). The resulting supernatants were stored overnight at 2°C to 8°C and then stored at -20°C ± 8°C in 4X NuPage LDS Sample Buffer (Invitrogen) containing NuPage Sample Reducing Agent (Invitrogen) for subsequent Western blot analysis. The sample extracts from the 5307 and control corn leaves were designated LP5307 and LP-NEG, respectively.

Extracts of 5307 Corn and Control Corn Leaf Tissue for Insect Bioassays

Extracts for bioassays were prepared by resuspending leaf powder from 5307 and control plants as described above, in a 10 mM ammonium bicarbonate, pH 10.0 buffer. The extraction mixtures were homogenized in a Waring blender for 45 seconds, incubated on ice for 1.5 hours and centrifuged (Sorvall RC5B) at 8,000 rpm for 30 minutes at 4°C. The resulting supernatant was filtered through cheesecloth, centrifuged for an additional 30 minutes at 8,000 rpm at 4°C, and filtered through cheesecloth again. The resulting clear supernatant was concentrated using centrifugal filter devices (Millipore). The sample was then stored overnight at 2-8°C for subsequent insect diet incorporation and ELISA analysis. The sample extracts from the 5307 and control corn leaves were designated LP5307 and LP-NEG, respectively.

Extracts of Control Leaf Tissue Fortified with Test Substance ECRY3.1AB-0208 for Western Blot Analysis

To determine whether the plant matrix affects eCry3.1Ab mobility or immunoreactivity, ECRY3.1AB-0208 was added to control leaf extract. This sample allowed for comparison of the microbially produced eCry3.1Ab and plant-produced eCry3.1Ab in the same matrix. For Western blot analysis, ECRY3.1AB-0208 was added to LP-NEG, as prepared for above Western blot analysis, such that the total protein and amount of eCry3.1Ab loaded on the gel was equivalent to that estimated for sample LP5307, as prepared for Western blot analysis. This sample was designated LP-NEG + ECRY3.1AB-0208.

Extracts of Control Leaf Tissue Fortified with Microbially Produced Test Substance ECRY3.1AB-0208 for Insect Bioassays

To determine if the plant matrix affects bioactivity, ECRY3.1AB-0208 was added to control leaf extract. This sample allowed for comparison of the microbially produced eCry3.1Ab and plant-produced eCry3.1Ab in the same matrix. For the bioassays, ECRY3.1AB-0208 was added to LP-NEG, as prepared for bioassays, such that when incorporated into the diet the concentration of eCry3.1Ab in the diet was equivalent to the eCry3.1Ab concentration in the diet containing only the ECRY3.1AB-0208 test substance. This sample was designated LP-NEG + ECRY3.1AB-0208.

Immunoaffinity-Purified Plant-Produced Protein

Leaf powder from 5307 corn, prepared as described above, was resuspended in extraction buffer (pH 7.5) containing 100 mM sodium borate, 0.2% PVP, 7.69 mM sodium azide, 1.2% concentrated hydrochloric acid, 0.5% Tween 20, supplemented with one Complete Protease Inhibitor Cocktail tablet/50 ml of buffer (Roche). The mixture was homogenized with an Omni-Prep Homogenizer and incubated for up to 2 hours on ice. The mixture was then centrifuged at approximately 2700 rpm for 10 minutes at 4°C (Sorvall Legend RT). The supernatant was filtered through cheesecloth and centrifuged at approximately 3100 rpm for

15 minutes at 4°C (Sorvall Legend RT). After a second centrifugation step (10,000 rpm for 12 minutes; Sorvall RC5B) the clarified supernatant was then loaded onto an equilibrated immunoaffinity column with mouse anti-mCry3A antibodies bound to the matrix. To remove any proteins not bound to the antibodies, the column was washed with a 50 mM sodium bicarbonate buffer pH 8.0 containing 150 mM sodium chloride. After an additional wash step with a 10 mM sodium phosphate buffer pH 6.8, eCry3.1Ab was eluted in 100 mM glycine buffer (pH 2.5), neutralized, and fractions were analyzed for eCry3.1Ab protein by ELISA. Fractions containing eCry3.1Ab protein were pooled, concentrated by ultrafiltration, and stored at 2°C to 8°C until further use. The resulting sample, designated IAP5307, was used as the source of purified plant-produced eCry3.1Ab for Western blot, glycosylation, N-terminal sequencing and peptide mass mapping analysis.

Microbially Produced Test Substance ECRY3.1AB-0208

Test substance ECRY3.1AB-0208 was prepared from an *E. coli* overexpression system. The eCry3.1Ab protein in test substance ECRY3.1AB-0208 is identical to that expressed in 5307 corn except that it contains one additional methionine and six histidine residues at the N-terminus. The intended additional seven amino acids aid in purification from the *E. coli* overexpression system. The *ecry3.1Ab* gene used for microbial expression was linked to the bacterial *tac* promoter in a vector derived from pET24a (Novagen) and transformed into *E. coli* strain DH5α (New England Biolabs). ECRY3.1AB-0208 was prepared from pooled batches of *E. coli* cell paste. *E. coli* cells were ruptured and the cell debris removed by centrifugation. The soluble material was filtered, applied to an immobilized metal affinity column (GE Healthcare Nickel Sepharose Fast Flow column), and eluted using an imidazole step gradient. Fractions containing the eCry3.1Ab protein were then further purified via anion exchange chromatography and eCry3.1Ab was eluted with a sodium chloride gradient. The eluted eCry3.1Ab-containing fractions were pooled, concentrated and the buffer was exchanged. The solution was lyophilized and designated ECRY3.1AB-0208. The test substance was stored at -20°C ± 8°C until further use. ECRY3.1AB-0208 was determined to contain 89.6% eCry3.1Ab by weight and the intact mass of the eCry3.1Ab protein, as measured by mass spectrometry, was 74.8 kDa.

eCry3.1Ab Quantification

The Beacon Analytical Systems (BAS) eCry3.1Ab ELISA kit was used as described in Appendix C., section C.4., to quantify eCry3.1Ab.

Total Protein Determination

Total protein in samples, LP5307, LP-NEG + ECRY3.1AB-0208, and LP-NEG as prepared for Western blot analysis, was quantified via the bicinchoninic acid method (Hill and Straka 1988), using bovine serum albumin as the reference protein standard. The results were

analyzed with BioMetallics DeltaSoft PC Microplate Analysis Software, v. 1.71.2, using a four-parameter fit of the standard curve.

Immunoreactivity and Molecular Weight Determination

Western blot analysis was used to investigate the integrity of eCry3.1Ab in ECRY3.1AB-0208, LP5307, LP-NEG + ECRY3.1AB-0208 and IAP5307. Aliquots containing 10 ng of eCry3.1Ab prepared in NuPage LDS Sample Buffer (Invitrogen) were subjected to SDS polyacrylamide gel electrophoresis (SDS-PAGE) with a NuPAGE 4-12% Bis-Tris polyacrylamide gradient gel (Invitrogen) using 3-(N-morpholino)propane-sulfonic acid (MOPS) running buffer (Bio-Rad). An aliquot of the control plant sample LP-NEG, equivalent in total protein to the amount loaded on the gel for LP5307 (29.6 µg total protein), was included in the analysis as a negative control. The molecular-weight standard was SeeBlue Plus2 pre-stained standard (Invitrogen). After electroblotting, the membrane was probed with polyclonal goat antibodies capable of detecting eCry3.1Ab protein. Alkaline phosphatase-conjugated donkey anti-goat IgG (Jackson ImmunoResearch) diluted to 1:3,000 in Tris-buffered saline with Tween 20 (Sigma-Aldrich) and 5% normal donkey serum was used to bind to the primary antibody and was visualized by development with 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (BCIP/NBT) alkaline phosphatase substrate solution (Sigma-Aldrich). The Western blot was examined for the presence of intact immunoreactive eCry3.1Ab or immunoreactive eCry3.1Ab fragments.

Insecticidal Activity

The insecticidal activity of eCry3.1Ab was assessed in feeding assays with freshly hatched first-instar Colorado potato beetles *(L. decemlineata)* in three independent bioassays. The insect diet was prepared by blending a boiling mixture of 2.6 grams of agar and 169 ml of Milli-Q water with 28.1 grams of Colorado potato beetle diet powder mix and 1 gram of potassium hydroxide as per the manufacturer's instructions (Bio-Serv). The diet mixture was cooled to approximately 55°C in a water bath. Antibacterial and antifungal agents were each added to the cooled diet.

Bioassay treatments consisted of (1) test substance ECRY3.1AB-0208, (2) eCry3.1Ab extracted from 5307 corn leaves; LP5307, (3) control leaf extract fortified with test substance ECRY3.1AB-0208; LP-NEG + ECRY3.1AB-0208, and (4) control corn leaf tissue extract; LP-NEG. A a stock solution of ECRY3.1AB-0208 was prepared in 10 mM ammonium bicarbonate buffer (pH 10.0) to a concentration of 5 mg eCry3.1Ab/ml. From this, a 50 µg eCry3.1Ab/ml solution was prepared and serially diluted 1:1 (v/v) in 10 mM ammonium bicarbonate buffer (pH 10.0) to produce eight solutions with concentrations ranging from 50 to 0.390 µg eCry3.1Ab/ml. The dilution series was then mixed 1:1 (v/v) with the freshly prepared Colorado potato beetle diet to produce eight diets with eCry3.1Ab concentrations ranging from 25 to 0.195 µg/ml diet. Additional treatments containing

eCry3.1Ab extracted from 5307 corn leaves, LP5307 (treatment 2), control leaf extract fortified with the microbially-produced test substance, LP-NEG + ECRY3.1AB-0208 (treatment 3) and control leaf tissue extract LP-NEG (treatment 4) as prepared for bioassays were also serially diluted 1:1 (v/v) in 10 mM ammonium bicarbonate buffer (pH 10.0) and subsequently mixed 1:1 (v/v) with freshly prepared Colorado potato beetle diet. Each of these treatments was analyzed as a series of eight dilutions. Water and buffer controls were prepared in the same manner for each bioassay. The water control was prepared by mixing 1:1 (v/v) of purified water with freshly prepared Colorado potato beetle diet. The buffer control was prepared by mixing 1:1 (v/v) of 10 mM ammonium bicarbonate buffer (pH 10.0) with freshly prepared Colorado potato beetle diet.

The bioassays were conducted in 24-well culture plates. Each well contained one freshly hatched *L. decemlineata* insect larva and 100 µl of insect diet. Larvae were transferred to each well manually using a small paint brush. The wells were covered with silicone stoppers and stored at ambient laboratory conditions. Mortality readings were taken periodically starting at 72 hours and continued until at least 144 hours.

Glycosylation Analysis

To determine whether eCry3.1Ab in ECRY3.1AB-0208 and eCry3.1Ab immuno-affinity purified from 5307 corn leaf extract (IAP5307) were glycosylated, aliquots equivalent to 1 and 2 µg of eCry3.1Ab were analyzed with the DIG Glycan Detection Kit (Roche), in accordance with the manufacturer's instructions. The positive control was transferrin (a glycosylated protein) at 100, 50, 25 and 10 ng, and the negative control was creatinase (a nonglycosylated protein) at 2 µg. Samples were separated by SDS-PAGE with a NuPAGE4-12% Bis-Tris polyacrylamide gradient gel and NuPAGE MES SDS running buffer and electroblotted to nitrocellulose membrane (Invitrogen). While on the membrane, glycan moieties were oxidized with periodate, labeled with digoxigenin (DIG), and detected with an alkaline-phosphatase-linked anti-DIG antibody.

Peptide Mass Mapping Analysis

eCry3.1Ab purified from an extract of 5307 corn leaves (IAP5307) and from ECRY3.1AB-0208 were analyzed by peptide mass mapping. Aliquots containing 2.5 to 5 µg of eCry3.1Ab purified from 5307 corn leaf extract (IAP5307) and from test substance ECRY3.1AB-0208 were subjected to SDSPAGE using a NuPAGE 4-12% Bis-Tris polyacrylamide gradient gel and NuPAGE MES SDS running buffer. The gel was stained with Coomassie G250 (Invitrogen), the protein band corresponding to the molecular weight of eCry3.1Ab was excised from the gel, and the protein was reduced, alkylated with iodoacetamide, and independently digested with trypsin and chymotrypsin. The mass analysis of the eCry3.1Ab-produced peptides was performed using a quadrupole time-of-

flight mass spectrometer (Waters/Micromass Q-TOF Premier) connected to a Waters CapLC capillary liquid chromatography instrument. The detected peptide masses were searched using Mascot Software (Matrix Science) against a protein database containing the eCry3.1Ab protein sequence. The Mascot search parameters included likely N-terminal modifications, which have previously been reported to occur in plants. Specifically, the modifications investigated included α-N-acetylation, protein N-formylation and protein N-methylation.

N-Terminal Amino Acid Sequence Analysis

To determine the N-terminal amino acid sequence of eCry3.1Ab from test substance ECRY3.1AB-0208 and eCry3.1Ab purified from 5307 corn leaf extract (IAP5307) were both subjected to SDS-PAGE followed by electroblotting to a PVDF membrane. The blot was stained with amido black, and the band corresponding to eCry3.1Ab was excised and subjected to N-terminal amino acid sequence analysis using automated Edman-based chemistry (Brauer et al. 1984).

Statistical Methods

The LC_{50} values determined in the insecticidal activity assay were calculated using the U.S. EPA Probit Analysis Program, version 1.5.

B.2. Results of Comparison of eCry3.1Ab Produced in 5307 Corn Plants and Recombinant *E. coli*

Immunoreactivity and Molecular Weight

Western blot analysis of eCry3.1Ab in test substance ECRY3.1AB-0208, LP-NEG + ECRY3.1AB-0208, LP5307, and IAP5307 revealed immunoreactive bands consistent with the predicted molecular weight[1] of 74.8 kDa for samples containing eCry3.1Ab from ECRY3.1AB-0208 (Figure B-1, Lanes 2, 3 and 6) and 73.7 kDa for samples containing plant-produced eCry3.1Ab (Lanes 4 and 5).

[1] Although the eCry3.1Ab protein band showed slightly higher mobility (and therefore an apparent lower molecular weight [mw]) in comparison to the mw standards on the Western blot (Figure B-1), the difference between the expected and observed mw on the gels can be explained by the limitations of SDS-PAGE for accurate determination of mw. Dube and Flynn (1988) have reviewed the reliability of SDS-PAGE for mw determinations and concluded that the apparent mw of a protein by this method is typically within 10% of its true mw. This depends greatly on the similarity between the properties of the protein of interest and the proteins in the standard set (Sadeghi *et al*., 2003). Additionally, the intact mass of eCry3.1Ab in ECRY3.1AB-0208 waspreviously measured as 74.8 kDa.

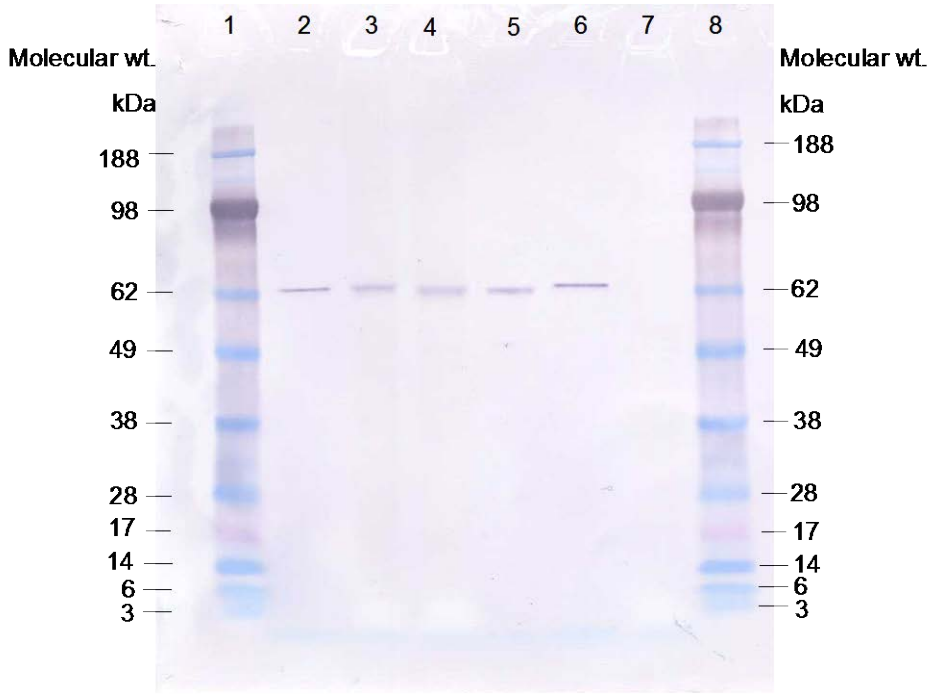


Figure B-1. Western blot analysis of eCry3.1Ab in test substance ECRY3.1AB-0208, LP-NEG + ECRY3.1AB-0208, LP5307, IAP5307 and the negative control LP-NEG.

Lane 1: Molecular-weight markers
Lane 2: Microbially produced test substance ECRY3.1AB-0208 (10 ng eCry3.1Ab)
Lane 3: Control corn leaf extract, LP-NEG (29.6 µg total protein) with the addition of test substance ECRY3.1AB-0208 (10 ng eCry3.1Ab)
Lane 4: 5307 corn leaf extract, LP5307 (10 ng eCry3.1Ab/29.6 µg total protein)
Lane 5: Immunopurified eCry3.1Ab protein from 5307 corn leaf extract, IAP5307 (10 ng eCry3.1Ab)
Lane 6: Test substance ECRY3.1AB-0208 (10 ng eCry3.1Ab)
Lane 7: Control corn leaf extract, LP-NEG (29.6 µg total protein)
Lane 8: Molecular-weight markers

The slight difference in molecular weight between the microbial and plant sources of eCry3.1Ab was consistent with the presence of the seven additional N-terminal amino acids in the microbially produced protein. As expected, no immunoreactive bands were observed in the plant-produced control substance, LP-NEG (Figure B-1, Lane 7).

Insecticidal Activity

The results of the insect bioassays were presented in Table VI-1 and discussed in Part VI.C of this submission.

Glycosylation Analysis

The positive control protein, transferrin, at 10 ng generated a clearly visible band (Figure B-2, Lane 4). Transferrin has a molecular weight of approximately 80,000 Da and contains approximately 5% glycan moieties by weight. This corresponds to approximately 25 glucose equivalents per molecule (based on a calculated molecular weight of 162 Da for the

glycan moiety). Of the 10 ng of transferrin loaded on the gel, 0.5 ng could be attributed to glycan moieties and was clearly detectable. The highest concentration of eCry3.1Ab from both plant and microbial sources (IAP5307 and ECRY3.1AB-0208) loaded on the blot was 2 µg (2,000 ng). If 0.5 ng of glycan were detected in eCry3.1Ab, this would correspond to 0.025% by weight (0.5/2,000 ng), or 0.115 glucose equivalents per molecule. In other words, if eCry3.1Ab bands were stained as strongly as 10 ng of transferrin in Lane 4, this would indicate glycosylation of about 1 in 8.7 of the eCry3.1Ab molecules. No bands corresponding to glycosylated eCry3.1Ab were visible for the sample prepared from the microbially produced ECRY3.1AB-0208 test substance (Figure B-2, Lanes 9 and 10) or immunopurified plant-produced eCry3.1Ab, IAP5307 (Lanes 7 and 8). Therefore, the results indicate that neither the microbially produced nor the plant-produced eCry3.1Ab protein was glycosylated.

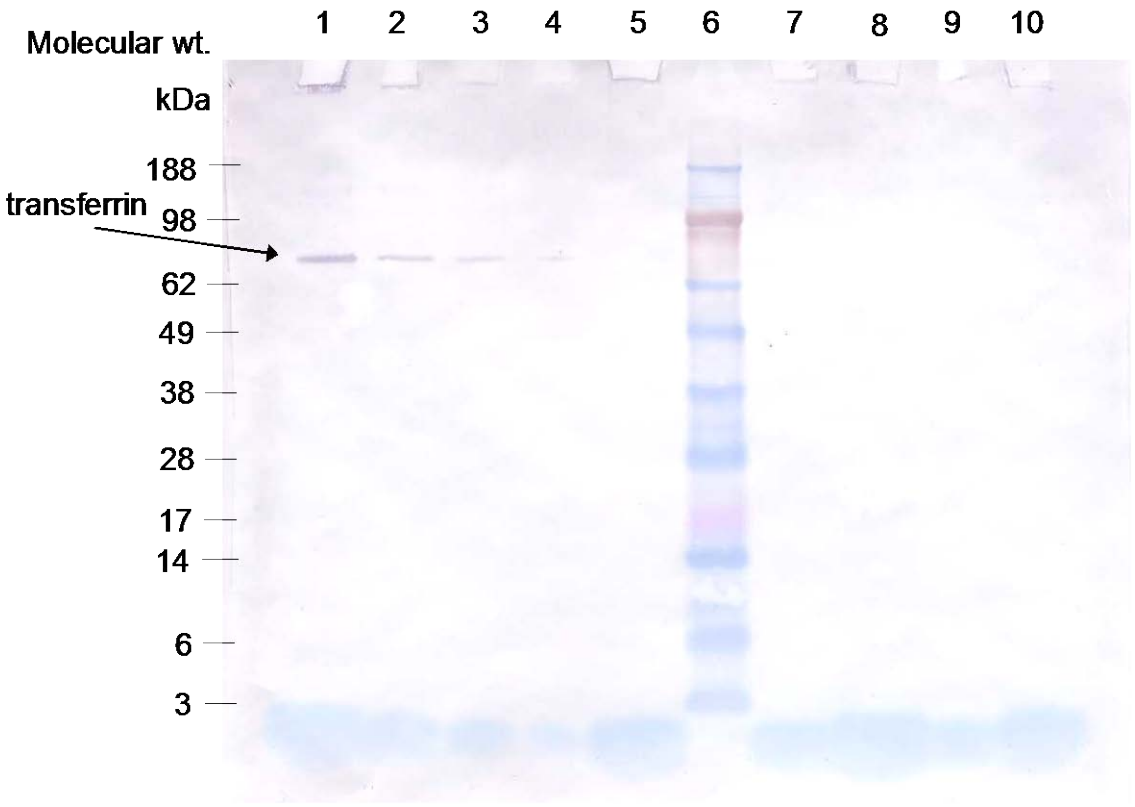


Figure B-2. Glycosylation analysis of eCry3.1Ab in IAP5307 (immunopurified plant-produced protein) and ECRY3.1AB-0208 (microbially produced test substance).

Lane 1: Transferrin (positive control), 100 ng
Lane 2: Transferrin (positive control), 50 ng
Lane 3: Transferrin (positive control), 25 ng
Lane 4: Transferrin (positive control), 10 ng
Lane 5: Creatinase (negative control), 2 µg.
Lane 6: Molecular-weight markers.
Lane 7: Immunopurified eCry3.1Ab protein from Event 5307 corn leaf extract, IAP5307; 1 µg
Lane 8: Immunopurified eCry3.1Ab protein from Event 5307 corn leaf extract, IAP5307; 2 µg
Lane 9: eCry3.1Ab from microbially produced test substance ECRY3.1AB-0208; 1 µg
Lane 10: eCry3.1Ab from microbially produced test substance ECRY3.1AB-0208; 2 µg.

Peptide Mass Mapping

Analysis of the plant-produced eCry3.1Ab yielded coverage equivalent to 76% of the total predicted eCry3.1Ab amino acid sequence, as shown in Figure B-3. Analysis of the microbially produced eCry3.1Ab yielded coverage equivalent to 87% of the total predicted eCry3.1Ab amino acid sequence, as shown in Figure B-4. The identified peptides corresponded to regions throughout the sequence of eCry3.1Ab including the N-termini of both proteins. The results of the peptide mass mapping analysis confirmed the identity of the purified proteins from both sources as eCry3.1Ab.

Figure B-3. Predicted amino acid sequence of eCry3.1Ab and sequence identified by peptide mass mapping analysis of eCry3.1Ab from immunopurified plant-produced sample IAP5307.

Identified eCry3.1Ab protein fragments are bold and underlined.

M**TSNGRQCAGIRPY**DGRQQHR**GLDSSTTKDVIQK**GISVVGDLLGVVGFPFGGALVSFYTNF
LNTIWPSEDPW**KAFMEQVEALMDQK**IADYAKNKALAELQGLQNNVEDYVSALSSWQK**NPAA**
PFRNPHSQGRIRELFSQAESHFRNSMPSFAISGYEVLFLTTYAQAANTHLFLLKDAQIYGE
EWGYEKEDIAEFYKRQLK**LTQEYTDHCVKWYNVGLDKLRGSSYESWVNFNR**YRR**EMTLTVL**
DLIALFPLYDVRLYPKEVKTELTRDVLTDPIVGVNNLRGYGTTFSNIENYIRKPHLFDYLH
RIQF**HTRFQPGYY**GNDSFNYWSGNY**VSTRPSIGSNDIITSPFYGNKSSEPVQNLEFNGEK**V
Y**RAVANTNLAVWPSAVYSGVTKVEFSQYNDQTDEASTQTYDSKR**NVGAVSWDSIDQLPPET
TDEPLEK**GYSHQLNYVMCFLMQGSRGTIPVLTWTHKSVDFFNMIDSKKITQLPLTKSTNLG**
SGTSVVKGPGFTGGDILRR**TSPGQISTLRVNITAPLSQR**YRVRIR**YASTTNLQFHTSIDGR**
PINQGNFSATMSSGSNLQSGSFRTVGFTTPFNFSNGSSVF**TLSAHVFNSGNEVYIDR**IEF**V**
PAEVTFEAEY**DLERAQKAVNELFTSSNQIGLKTDVTDYHID**QV

Figure B-4. Predicted amino acid sequence of eCry3.1Ab and sequence identified by peptide mass mapping analysis of eCry3.1Ab from microbially produced test substance ECRY3.1AB-0208.

Identified eCry3.1Ab protein fragments are bold and underlined.

MHHHHHHMTSNGRQCAGIRPYDGRQQHR**GLDSSTTKDVIQK**GISVVGDLLGVVGFPFGGAL
VSFYTNFLNTIWPSEDPW**KAFMEQVEALMDQK**IADY**AKNKALAELQGLQNNVEDYVSALSS**
WQKNPAAPFRNPHSQGRIRELFSQAESHFRNSMPSFAISGYEVLFLTTYAQAANTHLFLLK
DAQIYGEEWGYEKEDIAEFYKRQLK**LTQEYTDHCVKWYNVGLDKLRGSSYESWVNFNR**YRR
EMTLTVLDLIALFPLYDVRLYPKEVKTELTRDVLTDPIVGVNNLRGYGTTFSNIENYIRKP
HLFDYLHRIQFHTRFQPGYYGNDSFNYWSGNY**VSTRPSIGSNDIITSPFYGNKSSEPVQNL**
EFNGEKVYR**AVANTNLAVWPSAVYSGVTKVEFSQYNDQTDEASTQTYDSKRNVGAVSWDSI**
DQLPPETTDEPLEKGYSHQLNYVMCFLMQGSRGTIPVLTWTHKSVDFFNMIDSKKITQLPL
TKSTNLGSGTSVVKGPGFTGGDILRRTSPGQISTLRVNITAPLSQRYRVRIR**YASTTNLQF**
HTSIDGRPINQGNFSATMSSGSNLQSGSFRTVGFTTPFNFSNGSSVFTLSAHVFNSGNEVY
IDRIEFVPAEVTFEAEYDLERAQKAVNELFTSSNQIGLKTDVTDYHIDQV

The peptide mass analysis provided two additional results regarding the structure of the proteins. Firstly, the intact N-terminus of the plant-produced protein could be identified. The analysis showed that the N-terminal methionine was removed leaving the penultimate amino acid, threonine, at the N-terminus of the eCry3.1Ab protein. This is a common process for many proteins occurring during translation (Walling 2006). Secondly, the nature of the N-terminal block found for the plant-produced protein, as described under **N-Terminal Amino Acid Sequence Analysis**, below, was identified. The analysis of the N-terminal peptide of the plant-produced protein suggested the addition of an acetyl-residue at the primary amino group of the N-terminal threonine. This is a common modification known for plant-expressed proteins (Martinez et al. 2008).

N-Terminal Amino Acid Sequence Analysis

The N-terminal sequence results confirmed that eCry3.1Ab in the microbially produced test substance ECRY3.1AB-0208 had the predicted N-terminal amino acid sequence:

Predicted sequence:	MHHHHHHMTS
eCry3.1Ab in ECRY3.1AB-0208:	MHHHHHHMTS

N-terminal sequencing analysis of eCry3.1Ab immunoaffinity-purified from 5307 corn leaves (IAP5307) revealed that the majority of the protein was naturally blocked at the N-terminus. However, the N-terminal peptide was identified by peptide mass mapping (see **Peptide Mass Mapping**, above) and confirmed the expected sequence for the eCry3.1Ab protein, starting at threonine as described above. The analysis of the N-terminal peptide of the plant-produced protein suggested the addition of an acetyl residue at the primary amino group of the N-terminal threonine.

B.3. Materials and Methods for Comparison of PMI Produced in 5307 Corn Plants and Recombinant *E. coli*

The purpose of these analyses was to compare PMI from 5307 corn plants with PMI from test substance PMI-0105, which was produced by overexpressing the gene *pmi* in recombinant *E. coli*. The PMI proteins from both sources were compared biochemically and functionally to justify use of PMI in PMI-0105 as a surrogate for PMI in 5307 corn plants, for safety testing purposes. Both sources of PMI were predicted to have the identical amino acid sequence, because the gene *pmi* in both the plant and *E. coli* transformation vectors encoded the same PMI enzyme.

The conclusions of these analyses are described in Part VI.Iof this submission, Characterization of the Plant- and *Escherichia coli*-produced PMI Proteins.

Event 5307 Corn Leaf Tissue and Negative Control Corn Leaf Tissue

Young leaves from greenhouse-grown 5307 plants and nontransgenic, near-isogenic plants were collected 4-6 weeks after emergence, frozen at –80°C ± 10°C, and ground into a fine powder using a Grindomix Knife Mill (Retsch). The leaf powder was then lyophilized and stored at -80 ± 10°C.

Extracts of 5307 and Control Corn Leaf Tissue

Approximately 100 mg of lyophilized leaf powder was suspended in 3 ml extraction buffer (pH 7.0) containing 50 mM Tris, 2 mM DTT), 1 mM AEBSF, and 1 µM leupeptin. The mixture was then homogenized using an Omni-Prep Homogenizer (Omni International) and centrifuged for 15 minutes at 10,000 rpm at approximately 4°C. The supernatant was desalted using PD-10 columns (GE Healthcare Life Sciences) and eluted in extraction buffer. The resulting supernatant was then concentrated using the Millipore Amicon Ultra centrifugal filter devices (Millipore) and Tween 20 was added to achieve a final concentration of 0.1%. The samples derived from the 5307 and control corn tissues were designated LP5307 and LP-NEG, respectively. They were stored at 2-8°C overnight prior to ELISA and enzymatic activity assays.

Extract of Control Leaf Tissue Fortified with Test Substance PMI-0105

PMI from test substance PMI-0105 was added to the extraction buffer with an aliquot of control leaf tissue to determine if the plant matrix or extraction procedure has an effect on PMI. For this sample preparation, 600 ng PMI from test substance PMI-0105 was added to 3 ml of extraction buffer containing 100 mg of control leaf tissue prior to homogenization. The sample was then homogenized, centrifuged, desalted and concentrated as described above (see **Extracts of 5307 and Control Corn Leaf Tissue)**. This sample was designated LP-NEG + PMI-0105.

PMI Protein Quantification

The concentration of PMI in the plant extract samples (LP5307), negative control samples (LP-NEG), and the negative control sample fortified with test substance PMI-0105 (LP-NEG + PMI-0105) was determined by ELISA (Tijssen 1985). Polyclonal rabbit antibody generated against PMI protein was diluted to 2 µg/ml in a buffer containing 35 mM sodium bicarbonate and 15 mM sodium carbonate (pH 9.5) and used to coat a Nunc MaxiSorp 96-well plate (ThermoFisher Scientific) at a volume of 100 µl/well. The plates were incubated overnight at 5°C ± 3°C. The plate contents were then emptied and tapped on paper towels to remove residual solution. The plates were blocked with phosphate buffered saline (PBS) pH 7.4 containing 1% nonfat milk for at least 30 minutes at room temperature. The plates were washed five times with PBS containing 0.05% Tween 20 and incubated for 2 hours at 20°C ± 2°C with diluted plant extract samples and PMI-0105 standards at a volume of 100 µl/well. Dilutions were made in ELISA dilution buffer (PBS plus 1% nonfat milk and 0.05% Tween 20). The plant extract samples and standards were assayed in triplicate. After washing, the plates were incubated with 100 µl/well monoclonal mouse antibody (1 µg/ml) generated against PMI protein diluted in ELISA dilution buffer for 1 hour at 20°C ± 2°C. The plates were washed and subsequently incubated with 100 µl/well rabbit anti-mouse IgG conjugated with horseradish peroxidase (Sigma-Aldrich) diluted 1:20,000 in ELISA dilution buffer for 1 hour at 20°C ±2°C. The plates were washed again and incubated in 0.1 mg/ml TMB substrate solution (Sigma-Aldrich) in citrate buffer (24 mM citric acid monohydrate, 60 mM dibasic sodium phosphate, pH 5.0 containing 0.006% hydrogen peroxide) at a volume of 100 µl/well. Color was allowed to develop for 30 minutes in the dark at room temperature. The reaction was stopped by the addition of 50 µl 3 M sulfuric acid per well, and absorbance at 450 nm was measured with a Tecan Sunrise microplate reader (Tecan US). The results were analyzed with BioMetallics DeltaSoft PC Microplate Analysis Software, v. 1.71.2 using a four-parameter algorithm.

Total Protein Determination

Total protein of the plant extract samples (LP5307, LP-NEG + PMI-0105 and LP-NEG) were quantified via the bicinchoninic acid (BCA) method (Hill and Straka 1988), using bovine serum albumin as the reference protein standard. The results were analyzed with BioMetallics DeltaSoft PC Microplate Analysis Software, v. 1.71.2 using a 4-parameter fit of the standard curve.

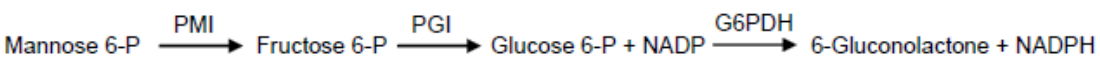
Immunoreactivity and Molecular Weight Determination

Western blot analysis was used to investigate the integrity of PMI in the plant extract sample LP5307, the control leaf extract fortified with the microbially produced test substance (LP-NEG + PMI-0105) and the microbially produced test substance PMI-0105. Aliquots equivalent to 5 ng PMI from sample LP5307, LPNEG + PMI-0105 and test substance PMI-0105 diluted in 10X Sample Buffer (as described by Laemmli (1970) containing 8%

glycerol, 1% BME, 2% SDS, 65 mM Tris, and 0.01% bromophenol blue) were subjected to SDS polyacrylamide gel electrophoresis (SDS-PAGE) with a NuPAGE 4-12% Bis-Tris gel (Invitrogen) and NuPAGE MES SDS running buffer (Invitrogen). An aliquot of the negative control sample (LP-NEG); equivalent to 92.8 µg total protein (the total amount of protein loaded on the gel for LP5307), was included in the analysis as a negative control. Additionally, based on total protein, LP-NEG + PMI-0105 was supplemented with additional LP-NEG extract to equal 86.8 µg total protein. The molecular-weight standard was SeeBlue Plus2 pre-stained standard (Invitrogen). After electroblotting, the polyvinylidene difluoride (PVDF) membrane was probed with immunoaffinity-purified polyclonal goat antibody generated against PMI diluted to 1 µg/ml in Tris-buffered saline, pH 8.0 with 3% nonfat milk. Alkaline phosphatase-conjugated donkey anti-goat IgG (Jackson ImmunoResearch) diluted 1:3000 in Tris-buffered Saline with Tween 20, pH 8.0) was used to bind to the primary antibody and was visualized by development with BCIP/NBT alkaline phosphatase substrate solution (Sigma-Aldrich). The Western blot was examined for the presence of intact immunoreactive PMI or immunoreactive PMI fragments.

Enzymatic Activity

The enzymatic activity of PMI was measured in triplicate using a continuous coupled spectrophotometric assay based on the method described by Gracy and Noltmann (1968) and Gill et al. (1986). PMI activity was measured by linking the formation of fructose 6-phosphate (resulting from the isomerization of mannose 6-phosphate) to the reduction of NADP via phosphoglucose isomerase and glucose 6-phosphate dehydrogenase (as shown in the diagram below). The molar reduction of NADP in this system can be directly converted into the molar isomerization of mannose 6-phosphate via PMI. The enzymatic reactions were conducted in 96-well plates. Microbially-produced and plant produced PMI were diluted in 50 mM Tris buffer, pH 7.0 containing 0.1% Tween 20. The reaction was initiated by adding 5 ng PMI from triplicate plant extract samples LP5307, LP-NEG + PMI-0105, or PMI from test substance PMI-0105 to an assay mixture containing 10 mM mannose 6-phosphate (Sigma-Aldrich), 1 mM β-nicotinamide adenine dinucleotide phosphate (NADP) sodium salt hydrate (Sigma-Aldrich), 2 U/ml phosphoglucose isomerase (Sigma-Aldrich) and 2 U/ml glucose 6-phosphate dehydrogenase (G6PDH)(Sigma-Aldrich) in 50 mM Tris buffer, pH 7.0. The total volume of the reaction mixture was 200 µl.



PMI = Phosphomannose isomerase, PGI = Phosphoglucose isomerase, G6PDH = Glucose 6-phosphate dehydrogenase

The assay mixture was preincubated at 25°C ± 2°C for 10 minutes prior to the addition of PMI. Following the 10 minute preincubation, PMI was added to the assay mixture and the plate was read by a SpectraMax Plus384 spectrophotometer (Molecular Devices) to determine the pathlength of the sample in each well. The reduction of NADP was monitored spectrophotometrically at 340 nm at 25ºC ± 2ºC over 10 minutes with readings taken every 15 seconds. The change in absorbance over time was monitored using SoftMax Pro version 5.2. The extinction coefficient of NADPH, 6.22 cm^{-1} mM^{-1}, was used for calculating the amount of NADPH formed. One unit (U) of PMI activity is defined as the amount of enzyme required to catalyze the conversion of 1 µmol of mannose 6-phosphate to fructose 6-phosphate per min (equivalent to 1 µmol NADP reduced per min) under the described reaction conditions. Results are reported as the mean and standard deviation of triplicate enzymatic assays.

B.4. Results of Comparison of PMI Produced in 5307 Corn and Recombinant *E. coli*

Immunoreactivity and MolecularWeight Determination

Western blot analysis of PMI in the plant extract sample LP5307, the control leaf extract fortified with PMI test substance PMI-0105 (LP-NEG + PMI-0105), and test substance PMI-0105 revealed a dominant immunoreactive band consistent with the predicted molecular weight of PMI (Figure B-5, Lanes 2, 3, and 4). The intensity of the PMI bands in the presence of the plant matrix (Lanes 2 and 3) was diminished compared to the intensity of the PMI band in the absence of the matrix (Lane 4). However, there was similar intensity between the PMI bands from test substance PMI-0105 in the presence of plant matrix (Figure B-5, Lane 3) and PMI from 5307 corn extract (Lane 2). Thus, the relatively diminished intensity of the PMI bands in the presence of the plant matrix was most likely due to matrix effects. The matrix effect can be attributed to the nature of the sample. Crude plant extract preparations contain all soluble cell proteins of varying molecular weights that may interfere with the mobility of the analyzed protein and with antibody binding. The co-migration of the matrix with PMI or PMI fragments may have limited access of the antibody to epitopes of the target protein and therefore diminished the signal.

The Western blot analysis also revealed some faint bands combined with a diffuse background signal in the molecular weight range above 62 kDa in both LP5307 and LP-NEG + PMI-0105 (Figure B-5, Lanes 2 and 3). As this effect (with exactly the same band pattern) was also detected in the negative plant extract sample LP-NEG (Lane 5) it is not related to PMI protein and most likely represents a nonspecific response on the Western blot. As expected, no immunoreactive band corresponding to the molecular weight of PMI was observed in the negative plant extract sample LP-NEG (Lane 5). The Western blot analysis also revealed a very faint protein band with a molecular weight of approximately 30 kDa in the microbially produced test substance PMI-0105. Because this protein cross-reacted with the anti-PMI antibody it is most likely a PMI degradation product. The degradation product was not visible in the sample with the control leaf extract fortified with the microbially produced test substance (LP-NEG + PMI-0105) due to the matrix effect described above.

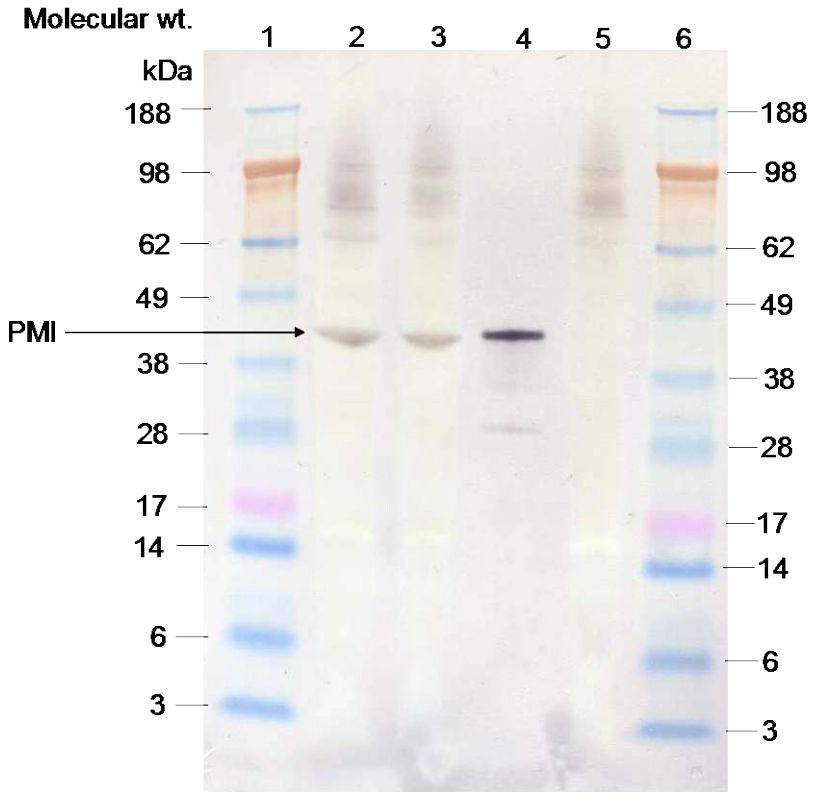


Figure B-5. Western blot analysis for PMI in LP5307, LP-NEG + PMI-0105, test substance PMI-0105, and LP-NEG.

Lane 1: Molecular weight standard SeeBlue Plus2 (Invitrogen)
Lane 2: Plant extract sample LP5307
Lane 3: Plant extract sample LP-NEG + PMI-0105
Lane 4: Microbially-produced test substance PMI-0105
Lane 5: Plant extract sample LP-NEG
Lane 6: Molecular weight standard SeeBlue Plus2

The average PMI enzymatic activity was 455.67 U/mg PMI in the triplicate plant extract samples LP5307 and 526.26 U/mg PMI in the microbially produced test substance PMI-0105 (Table B-1). The control leaf extract fortified with the microbially produced test substance (LP-NEG + PMI-0105) had a specific activity of 518.81 U/mg PMI (Table B-1), demonstrating that the plant matrix had little effect on the enzymatic activity of PMI. As expected, no PMI activity was detected in the negative control plant extract sample, LP-NEG (Table B-1).

Table B-1. Enzymatic activity of PMI in 5307 corn leaf extract, control corn leaf extract fortified with test substance PMI-0105, test substance PMI-0105, and control corn leaf extract.

LP5307 is leaf extract of 5307 corn plants. LP-NEG + PMI-0105 is control corn leaf extract fortified with microbially produced PMI test substance PMI-0105. LP-NEG is control corn leaf extract.

	Assay Replicate	PMI Activity[1] (U/mg PMI)	Average PMI Activity of Assay Replicates (U/mg PMI)	SD of Assay Replicate s (U/mg PMI)	CV of Assay Replicates (%)	Overall Average PMI Activity (U/mg PMI)	Overall SD (U/mg PMI)	Overall CV (%)
LP5307-1[2]	1	469.58	479.60	10.23	2.1%	455.67	30.37	6.7%
	2	490.03						
	3	479.21						
LP5307-2	1	478.77	464.39	21.67	4.7%			
	2	474.93						
	3	439.47						
LP5307-3	1	418.44	423.03	23.32	5.5%			
	2	448.30						
	3	402.34						
LPNEG + PMI-0105	1	514.24	518.81	13.82	2.7%			
	2	534.33						
	3	507.86						
PMI-0105	1	519.00	526.26	6.59	1.3%			
	2	531.87						
	3	527.90						
LPNEG	1	below LOD	below LOD	not applicable	not applicable			
	2	below LOD						
	3	below LOD						

[1] One unit of PMI activity is defined as the amount of enzyme required to catalyze the conversion of 1 µmol of mannose 6-phosphate to fructose 6-phosphate per min (equivalent to 1 µmol NADP reduced per min) under the described reaction conditions.

[2] LP5307-1, -2, and -3 represent three independent extractions.

Appendix C: Quantification of eCry3.1Ab and PMI in 5307 Corn

This appendix provides details of the materials and methods used to determine the concentrations of eCry3.1Ab and PMI in the tissues of 5307 plants at various growth stages. Refer to Part VI.N.1 of this submission for a description of the study and results.

C.1. Plant Tissue Production

Plants were grown for the collection of tissues in Bloomington, IL; Sadorus, IL; Shirley, IL; and Stanton, MN in two plots per location; one for the 5307 hybrid and one for the control hybrid.

C.2. Plant Tissue Processing

In the presence of dry ice, the leaf, kernel and whole-plant samples were individually processed to a fine powder. A subsample from each homogeneous, powdered sample was lyophilized and stored at –80 ºC ± 10ºC. The percent dry weight of each sample was determined from the sample weight before and after lyophilization.

C.3. Tissue Extraction and Analysis

Protein extractions were performed on representative aliquots of the lyophilized leaf, kernel, and whole-plant samples. The extracts were analyzed by ELISA (Tijssen 1985) to quantify the amount of eCry3.1Ab and PMI in each sample. Sample extracts were analyzed in triplicate, and standard curves were generated with known amounts of the corresponding reference protein. Standard curves were generated for each ELISA plate. Nontransgenic plant tissue extracts were analyzed in parallel to evaluate any impact of the plant matrix on the ELISA.

C.4. eCry3.1Ab Quantification – Extraction and ELISA Procedures

Buffers

The buffers used for extraction and ELISA analysis of eCry3.1Ab are listed in the following table:

Name of buffer	Constituents
Phosphate-buffered saline (PBS)	140 mM sodium chloride, 8.24 mM sodium phosphate dibasic, 1.81mM sodium phosphate monobasic, pH 6.75
Borate buffer (leaves, kernels, whole plants)	0.1 M Sodium tetraborate decahydrate, 0.2% PVP-360, 7.69 mM sodium azide, 0.5% Tween 20; titrated to pH 10.0. Complete Protease Inhibitor Cocktail (Roche Applied Science) added on day of extraction
Dilution buffer	PBS, 0.05% Tween 20, 1% BSA, 0.02% sodium azide
Wash buffer	10 mM Tris, 0.05% Tween 20, 0.02% sodium azide

eCry3.1Ab Extraction from Leaves, Kernels, and Whole Plants

A ratio of 3 ml of borate buffer pH 10.0 was added to 100 mg of lyophilized tissue. The samples were vortexed, placed on wet ice for at least 30 minutes, homogenized using an Omni-Prep Homogenizer, and centrifuged at 2°C to 8°C to form a pellet. The supernatants were removed and stored at 2°C to 8°C until analysis.

eCry3.1Ab Quantitation

The eCry3.1Ab ELISA kit was manufactured at Beacon Analytical Systems (BAS), Portland, ME. The assay is a double-antibody sandwich assay in which the eCry3.1Ab protein is affixed to the wells of a microtiter plate using a monoclonal, anti-mCry3A antibody that binds to the mCry3A domains of the eCry3.1Ab protein. The primary antibody was diluted and added to each well of a 96-well microtiter plate. The plate was then blocked using a proprietary method. Dilutions of each tissue extract and appropriate serial dilutions of eCry3.1Ab reference protein (ECRY3.1AB-0208), prepared in dilution buffer, were applied to the pre-coated plates at a total volume of 100 µl/well. The plates were incubated at room temperature on a titre plate shaker at 400 rpm for 1 hour. The plates were washed five times with wash buffer in a BioTek ELx405 Microplate Washer. After washing the plates, a secondary, rabbit polyclonal anti-Cry1Ab antibody (provided in the kit) was then used to bind the Cry1Ab domain of the eCry3.1Ab protein at 100 µl/well. The plates were incubated at room temperature on a titre plate shaker at 400 rpm for one hour and washed five times as described above.

After the plates were washed, a tertiary donkey anti-rabbit conjugated with alkaline phosphatase diluted in dilution buffer was added to each of the wells (100 µl/well) and incubated at room temperature on a titre plate shaker at 400 rpm for one hour. The plates were then washed five times as described above, and alkaline phosphatase substrate solution

provided in the kit was added at a volume of 100 µl/well. The plates were incubated for 30 minutes at room temperature on a titre plate shaker at 400 rpm. The reaction was stopped by the addition of 3N sodium hydroxide (100 µl/well), and absorbance of the reaction was measured at a dual wavelengths (405 and 492 nm) with a Tecan Sunrise Microplate Reader. The results were analyzed with BioMetallics DeltaSoft PC Microplate Analysis Software, v. 1.71.2, using a four-parameter algorithm. The results for kernel samples were analyzed with SoftMax Pro, v. 5.2, using a four-parameter algorithm.

The lower limit of quantification (LOQ) or limit of detection (LOD) of eCry3.1Ab is shown on Table VI-2 (Part VI.C.) for any tissue type in which eCry3.1Ab could not be quantified or detected, respectively. Extraction efficiencies ranged from 77% to 88% across the tissue types analyzed; the reported tissue concentrations have been adjusted for extraction efficiency.

C.5. PMI Quantification –Extraction and ELISA Procedures

Buffers

The buffers used for extraction and ELISA analysis of PMI are listed in the following table:

Name of buffer	Constituents
PBS	138 mM sodium chloride, 2.7 mM potassium chloride, 10.1 mM disodium phosphate, 1.8 mM potassium dihydrogen phosphate, pH 7.4
Blocking buffer	PBS, 1% powdered milk
Borate buffer	0.1 M sodium tetraborate decahydrate, 0.2% PVP-360, 7.69 mM sodium azide, 1.2% concentrated hydrochloric acid, 0.5% Tween 20; pH will be approximately 7.5. Complete Protease Inhibitor Cocktail (Roche Applied Science) added on day of extraction
Carbonate-bicarbonate buffer	34.9 mM sodium bicarbonate, 15.0 mM sodium carbonate, pH 9.5
Citrate-phosphate buffer	23.8 mM citric acid, 59.9 mM disodium phosphate, pH 5.0
Dilution buffer	PBS, 0.05% Tween 20, 1% powdered milk
Wash buffer	PBS, 0.05% Tween 20

PMI Extraction from Leaves, Kernels, andWhole Plants

A ratio of 3 ml of borate buffer, pH 7.5 was added to 100 mg of lyophilized tissue. The samples were mixed, placed on wet ice for at least 30 minutes, homogenized using an Omni-Prep Homogenizer, and centrifuged at 2°C to 8°C to form a pellet. The supernatants were removed and stored at 2°C to 8°C until analysis.

PMI Quantification

Rabbit polyclonal anti-PMI antibody was diluted in carbonate-bicarbonate buffer and added to each well of a 96-well microtiter plate at a volume of 100 µl/well. The plates were stored overnight in a refrigerator set at 2°C to 8°C. The antibody solution was removed and blocking buffer was added to the plate at a volume of 250 µl/well and then incubated at room temperature for at least 30 minutes. After blocking incubation, the plates were washed five times with wash buffer in a BioTek ELx405 microplate washer and dilutions of each tissue extract and appropriate serial dilutions of PMI reference protein (PMI-0105) prepared in dilution buffer were applied to the plates at a total volume of 100 µl/well. The plates were incubated at 18°C to 22°C for 2 hours. After incubation, plates were washed five times as described above and a monoclonal anti-PMI antibody diluted in dilution buffer was added to the plate at a volume of 100 µl/well and incubated at 18°C to 22°C for one hour.

The plates were washed five times after incubation and a horseradish-peroxidase-conjugated rabbit anti-mouse immunoglobin diluted in dilution buffer was added at a volume of 100 µl/well and incubated at 18°C to 22°C for one hour. After incubation, the plates were washed five times, and TMB substrate solution was added at a volume of 100 µl/well (one

tablet per 10 ml of citrate-phosphate buffer) and incubated at room temperature in the dark for 30 minutes. The reaction was stopped by addition of 3 M sulfuric acid at a volume of 50 µl/well, and the absorbance of the reaction was measured at 450 nm with a Tecan Sunrise microplate reader. The results were analyzed with BioMetallics DeltaSoft PC Microplate Analysis Software, v. 1.71.2, using a four-parameter algorithm.

The lower limit of quantification (LOQ) or limit of detection (LOD) of PMI is shown on Table VI-3 (Part VI.C.) for any tissue type in which PMI could not be quantified or detected, respectively. PMI extraction efficiencies ranged from 72% to 80% across the tissue types analyzed; the reported tissue concentrations have been adjusted for extraction efficiency.

Appendix D: Compositional Analysis of 5307 Forage and Grain

This appendix describes the methods used to conduct the compositional analysis study described in Part VII.Cof this submission, wherein the results are also provided. References cited for individual methods in this appendix are listed at the end of the appendix.

Study Design

Forage and grain for compositional analyses were harvested from multiple locations planted in the U.S. in 2008. The locations chosen were representative of major corn producing regions in the country. For all locations, trials were planted with a 5307 hybrid and near-isogenic, nontransgenic hybrid in a randomized complete block design with three replicated plots, and were managed following local agronomic practices. The plants were self-pollinated by hand and the developing ears were bagged to avoid cross-pollination. Trials were planted in eight locations in an effort to ensure that grain and forage from at least six locations could be harvested in the event of loss due to adverse environmental conditions (early freeze, drought, etc.). Six locations that produced sufficient grain and forage were selected for this study (see Part VII.C.1).

Forage Sampling and Processing

For each genotype, the entire above-ground portion of five plants from each of the three replicate plots at each location was harvested at dough stage (R4), the stage at which silage typically is prepared. Plants were pooled to create a composite sample for each replicate plot, then ground using a chipper-shredder. A subsample from each well-mixed composite sample was shipped overnight on ice packs to Syngenta Crop Protection, Inc. (Greensboro, NC). The samples were stored at –20°C ± 10°C, then finely ground and shipped on dry ice to a contract research laboratory, where they were stored at –20°C ± 10°C until they were analyzed.

Grain Sampling and Processing

For each genotype, ears were collected from 15 plants from each replicate plot at each location. Ears were harvested after reaching physiological maturity (R6) and then mechanically dried to approximately 9% to 12% moisture content. (Mechanical drying after harvest is standard agronomic practice for improving storage characteristics of corn grain.) Each sample consisted of grain shelled from ears collected from 15 plants from one replicate plot. A well-mixed subsample of approximately 500 g of grain from each plot was shipped at ambient temperature to Syngenta Crop Protection, Inc., where it was stored at –20°C ± 10°C, then finely ground and shipped on dry ice to the contract testing facililty. The samples were stored at –20°C ± 10°C until they were analyzed.

Compositional Analyses

As detailed in Table VII-1 (Part VII of this submission), forage was analyzed for proximates and the minerals calcium and phosphorus. Grain was analyzed for major constituents (proximates, including starch), minerals, amino acids, fatty acids, vitamins, and selected anti-nutrients and secondary metabolites.

All compositional analyses were conducted using methods published and approved by AOAC International, or other industry-standard analytical methods, described below. Based on the moisture content of each sample, analyte levels were converted to equivalent units of dry weight.

Analytical Methods and Reference Standards for Compositional Analyses

2-Furaldehyde (Albala-Hurtado et al. 1997)

The ground sample was extracted with 4% trichloroacetic acid and injected directly on a high-performance liquid chromatography system for quantitation of free furfurals by ultraviolet detection. The limit of quantitation (LOQ) for this study was 0.500 ppm, calculated on a fresh-weight basis.

Reference Standard: Acros 2-Furaldehyde, 99.7%, Lot Number A0219180

Acid Detergent Fiber (ADF) (USDA 1970)

The sample was washed with acetone to remove fats and pigments. It was then placed in a filter bag and positioned in an Ankom analyzer where it was washed with an acidic boiling detergent solution that dissolved the protein, carbohydrate, and ash. The lignocellulose fraction remaining was determined gravimetrically. The LOQ for this study was 0.100%, calculated on a fresh-weight basis.

Amino Acid Composition (AOAC 2005k)

Total aspartic acid (including asparagine)
Total threonine
Total serine
Total glutamic acid (including glutamine)
Total proline
Total glycine
Total alanine
Total valine
Total isoleucine
Total leucine
Total tyrosine
Total phenylalanine
Total histidine

Total lysine
Total arginine
Total tryptophan
Sulfur-containing amino acids: Total methionine
Total cystine (including cysteine)

The sample was assayed by three methods to obtain the full profile. Tryptophan required a base hydrolysis with sodium hydroxide. The sulfur-containing amino acids required an oxidation with performic acid prior to hydrolysis with hydrochloric acid. Analysis of the samples for the remaining amino acids was accomplished through direct acid hydrolysis with hydrochloric acid. Once hydrolyzed, the individual amino acids were then quantitated using an automated amino acid analyzer. The LOQ for each amino acid assay was 0.100 mg/g, calculated on a fresh-weight basis.

Reference Standards: ThermoScientific K18, 2.5 µmol/mL per constituent except cystine (1.25 µmol/mL), Lot Number JK126327
Sigma, L-Tryptophan, 100%, Lot Number 076K0075
Sigma/BioChemika, L-Cysteic Acid Monohydrate, >99% (used as 100%), Lot Number 1305674
Sigma, L-Methionine Sulfone, 100%, Lot Number 047K1321

Ash (AOAC 2005b)

The sample was placed in an electric furnace at 550°C and ignited to drive off all volatile organic matter. The nonvolatile matter remaining was quantitated gravimetrically and calculated to determine percent ash. The LOQ for this study was 0.100%, calculated on a fresh-weight basis.

Beta-Carotene (AOAC 2005e; Quackenbush 1987)

The sample was saponified and extracted with hexane. The sample was then injected on a reverse phase high-performance liquid chromatography system with ultraviolet light detection. Quantitation was achieved with a linear regression analysis. The LOQ for beta-carotene was 0.0200 mg/100 g, calculated on a fresh-weight basis.

Reference Standard: Sigma-Aldrich, Beta-carotene, Type I, 100% (stock standard concentration determined spectrophotometrically), Lot Number 068K2561

Carbohydrates (USDA 1973)

The total carbohydrate level was calculated by difference using the fresh weight-derived data and the following equation:

% carbohydrates = 100 % - (% protein + % fat + % moisture + % ash)

The LOQ for this study was 0.100%, calculated on a fresh-weight basis.

Fat by Acid Hydrolysis (AOAC 2005a)

The sample was hydrolyzed with hydrochloric acid at an elevated temperature. The fat was extracted with ether and hexane. The extract was evaporated on a steambath, redissolved in hexane and filtered through a sodium sulfate column. The hexane extract was then vaporated again on a steambath under nitrogen, dried, and weighed. The LOQfor this study was 0.1%, calculated on a fresh weight basis.

Fatty Acids (AOAC 2005l; AOCS 1997b and 2001)

The lipid was extracted and saponified with 0.5N sodium hydroxide in methanol. The saponification mixture was methylated with 14% boron trifluoride in methanol. The resulting methyl esters were extracted with heptane containing an internal standard. The methyl esters of the fatty acids were analyzed by gas chromatography using external standards for quantitation. The LOQ was 0.00500%, calculated on a fresh-weight basis.

Reference Standards:

Nu Chek Prep GLC Reference Standard Hazelton No. 1, Lot Number AU18-S
Nu Chek Prep GLC Reference Standard Hazelton No. 2, Lot Number M13-O
Nu Chek Prep GLC Reference Standard Hazelton No. 3, Lot Number MA18-S
Nu Chek Prep GLC Reference Standard Hazelton No. 4, Lot Number JA16-T
Nu Chek Prep Methyl Gamma Linolenate, used as 100%, Lot Number U-63M-JY12-R
Nu Chek Prep Methyl Tridecanoate, used as 100%, Lot Number N-13M-JA16-T

Folic acid (AOAC 2005i; Infant Formula Council 1985)

The sample was hydrolyzed in a potassium phosphate buffer with the addition of ascorbic acid to protect the folic acid during autoclaving. Following hydrolysis by autoclaving, the sample was treated with a chicken-pancreas enzyme and incubated approximately 18 hours to liberate the bound folic acid. The amount of folic acid was determined by comparing the growth response of the sample, using the bacteria *Lactobacillus casei*, with the growth response of a folic acid standard. This response was measured turbidimetrically. The LOQ was 0.00600 mg/100 g, calculated on a fresh-weight basis.

Reference Standard: USP, Folic acid, 98.9%, Lot Number Q0G151

ICP Emission Spectrometry (AOAC 2005m)

The sample was dried, precharred, and ashed overnight in a muffle set to maintain 500°C. The ashed sample was re-ashed with nitric acid, treated with hydrochloric acid, taken to dryness, and put into a solution of 5% hydrochloric acid. The amount of each element was determined at appropriate wavelengths by comparing the emission of the unknown sample, measured on the inductively coupled plasma spectrometer, with the emission of the standard solutions. The LOQs (Table D-1) were calculated on a fresh-weight basis.

Table D-1. Inorganic Ventures Reference Standards and Limits of Quantitation

Mineral	Lot Numbers	Calibration Standard Concentration (µg/ml)	LOQ (ppm)
Calcium	B2-MEB280039 B2-MEB266040	200 1000	20.0
Copper	B2-MEB280039 B2-MEB280036	2 10	0.50
Iron	B2-MEB280039 B2-MEB280035	10 50	2.00
Magnesium	B2-MEB280039 B2-MEB280036	50 250	20.0
Manganese	B2-MEB280039 B2-MEB280036	2 10	0.30
Phosphorus	B2-MEB280039 B2-MEB266040	200 1000	20.0
Potassium	B2-MEB280039 B2-MEB266040	200 1000	100
Sodium	B2-MEB280039 B2-MEB266040	200 1000	100
Zinc	B2-MEB280039 B2-MEB280036	10 50	0.40

ICP-Mass Spectrometry (AOAC 2005o)

The sample was wet-ashed with nitric acid using microwave digestion. Using inductively coupled plasma mass spectrometry, the amount of each element was determined by comparing the counts generated by the unknowns to those generated by standard solutions of known concentrations. The LOQ for this study was 50.0 ppb, calculated on a fresh-weight basis.

Reference Standard: SPEX, Selenium, 100 mg/L, Lot Number 6-74GS

Inositol (Infant Formula Council 1985b; Atkins et al. 1943)

The inositol sample was extracted with dilute hydrochloric acid at a high temperature. The amount of inositol was determined by comparing the growth response of the sample, using the yeast *Saccharomyces carlsbergenesis,* with the growth response of an inositol standard. The response was measured turbidimetrically. The LOQ for this study was 40.0 µg/g, calculated on a fresh-weight basis.

Reference Standard: Sigma-Aldrich, Myo-Inositol, 100%, Lot Number 065K0018

Moisture (AOAC 2005c)

The sample was dried in a vacuum oven at approximately 100°C to a constant weight. The moisture weight loss was determined and converted to percent moisture. The LOQ for this study was 0.100%, calculated on a fresh-weight basis.

Neutral Detergent Fiber (NDF), Enzyme Method (AACC 1998; USDA 1970)

The sample was washed with acetone to remove fats and pigments. It was then placed in a filter bag and positioned in an Ankom analyzer where it was washed with a neutral boiling detergent solution that dissolved the protein, carbohydrate, enzyme, and ash. The remaining hemicellulose, cellulose, and lignin fractions were determined gravimetrically. The LOQ for this study was 0.100%.

Niacin (AOAC 2005g)

The sample was hydrolyzed with sulfuric acid and the pH was adjusted to remove interferences. The amount of niacin was determined by comparing the growth response of the sample, using the bacteria *Lactobacillus plantarum,* with the growth response of a niacin standard. This response was measured turbidimetrically. The LOQ for this study was 0.0300 mg/100 g.

Reference Standard: USP, Niacin, 99.8%, Lot Number I0E295

p-Coumaric Acid and Ferulic Acid (Hagerman and Nicholson 1982)

The sample was extracted with methanol using ultrasonication, hydrolyzed using 4N sodium hydroxide, buffered using acetic acid/sodium hydroxide, acidified with 3N hydrochloric acid, and filtered. The levels of p-coumaric and ferulic acids in the extract were determined by reverse phase high-performance liquid chromatography with ultraviolet detection. The LOQ for p-coumaric acid and ferulic acid was 50.0 ppm, calculated on a fresh-weight basis.

Reference Standards: Acros Organics, 4-Hydroxy-3-methoxycinnamic acid (ferulic acid), 99.4%, Lot Number A0248008

Acros Organics, p-Hydroxycinnamic acid (p-coumaric Acid), 99.4%, Lot Number A0236839

Phytic Acid (Lehrfeld 1989 and 1994)

The sample was extracted using 0.5 M HCl with ultrasonication. Purification and concentration were accomplished on a silica-based anion-exchange column. The sample was analyzed on a polymer high-performance liquid chromatography column PRP-1, 5 µm (150 x 4.1 mm) with a refractive index detector. The LOQ for this study was approximately 0.100%, calculated on a fresh-weight basis.

Reference Standard: Aldrich, Phytic Acid, Dodecasodium Salt Hydrate, 95%, Lot Number 077K0693

Protein (AOAC 2005h; Bradstreet 1965; Kalthoff and Sandell 1948)

Nitrogenous compounds in the sample were reduced in the presence of boiling sulfuric acid and a mercury catalyst mixture to form ammonia. The acid digest was made alkaline. The ammonia was distilled and then titrated with a previously standardized acid. The percent nitrogen was calculated and converted to protein using the factor 6.25. The LOQ for this study was 0.100%, calculated on a fresh-weight basis.

Pyridoxine Hydrochloride (AOAC 2005j; Atkins et al. 1943)

The sample was hydrolyzed with dilute sulfuric acid in the autoclave and the pH was adjusted to remove interferences. The amount of pyridoxine was determined by comparing the growth response of the sample, using the yeast *Saccharomyces carlsbergensis*, with the growth response of a pyridoxine standard. The response was measured turbidimetrically. Results were reported as pyridoxine hydrochloride. The LOQ for this study was 0.00700 mg/100 g, calculated on a fresh-weight basis.

Reference Standard: USP, Pyridoxine hydrochloride, 99.8%, Lot Number Q0G409

Raffinose (Brobst 1972; Mason and Stover 1971)

The sample was extracted with deionized water and the extract treated with a hydroxylamine hydrochloride solution in pyridine, containing phenyl-β-D-glucoside as an internal standard. The resulting oximes were converted to silyl derivatives by treatment with hexamethyldisilazane and trifluoracetic acid and analyzed by gas chromatography using a flame ionization detector. The acceptable LOQ for this study was 0.100%, calculated on a fresh weight basis.

Reference Standards: Sigma, D(+)-Raffinose Pentahydrate, 99% (84.0% after correction for degree of hydration), Lot Number 037K1059

Starch (AOAC 2005p)

The sample was extracted with alcohol to remove carbohydrates other than starch, i.e. sugars. Then it was hydrolyzed into glucose with α -amylase and amyloglucosidase. Glucose was oxidized with glucose oxidase to form peroxide, which reacted with a dye in the presence of peroxidase to give a stable colored product proportional to glucose concentration. The glucose concentration was quantitated by measurement on a spectrophotometer at 540 nm. Percent starch was then calculated from the glucose concentration. The LOQ for this study was 0.05%, calculated on a fresh-weight basis.

Reference Standard: Sigma D(+)-Glucose, 99.9%, Lot Number 123K0095

Thiamine Hydrochloride (AOAC 2005f)

The sample was autoclaved under weak acid conditions to extract the thiamine. The resulting solution was incubated with a buffered enzyme solution to release any bound thiamine. The solution was purified on a cation-exchange column. An aliquot was reacted with potassium ferricyanide to convert thiamine to thiochrome. The thiochrome was extracted into isobutyl alcohol, measured on a fluorometer, and quantitated by comparison to a known standard. The limit of quantitation was calculated and reported on a fresh weight basis. The LOQ for this study was 0.01 mg/100 g. Results were reported as thiamine hydrochloride.

Reference Standard: USP, Thiamine Hydrochloride, Purity 99.8% (used as 95.9% after correction for moisture content), Lot Number 01F236

Total Dietary Fiber (TDF)(AOAC 2005n)

Duplicate samples were gelatinized with α-amylase and digested with enzymes to break down starch and protein. Ethanol was added to each sample to precipitate the soluble fiber. The samples were filtered, and the residue was rinsed with ethanol and acetone to remove starch and protein degradation products and moisture. Protein content was determined for one of the duplicates; ash content was determined for the other. The total dietary fiber in the sample was calculated using the protein and ash values. The LOQ for this study was 1.00%, calculated on a fresh-weight basis.

Trypsin Inhibitor (AOCS 1997a)

The sample was ground and defatted with petroleum ether. A sample of matrix was extracted with 0.01N sodium hydroxide. Varying aliquots of the sample suspension were exposed to a known amount of trypsin and benzoy1-DL-arginine~p~nitroanilide hydrochloride. The sample was allowed to react for 10 minutes at 37°C. After 10 minutes, the reaction was halted by the addition of acetic acid. The solution was centrifuged, then the absorbance was determined at 410 nm. Trypsin inhibitor activity was determined by photometrically measuring the inhibition of trypsin's reaction with benzoyl-DL-arginine~p~nitroanilide hydrochloride. The LOQ for this study was 1.00 Trypsin Inhibitor Units (TIU)/mg, calculated on a fresh-weight basis.

Vitamin B2 (Riboflavin) (AOAC 2005d; US Pharmacopeia 2005)

The sample was hydrolyzed with dilute hydrochloric acid and the pH was adjusted to remove interferences. The amount of riboflavin was determined by comparing the growth response of the sample, using the bacteria *Lactobacillus casei*, with the growth response of multipoint riboflavin standards. The growth response was measured turbidimetrically. The LOQ for this study was 0.0200 mg/100 g, calculated on a fresh-weight basis.

Reference Standard: USP, Riboflavin, 100%, Lot Number N0C021

Vitamin E (Cort et al. 1983; McMurray et al. 1980; Speek et al. 1983)

The sample was saponified to break down any fat and release vitamin E. The saponified mixture was extracted with ethyl ether and then quantitated by high-performance liquid chromatography using a silica column. The LOQ for this study was 0.500 mg/100 g, calculated on a fresh weight basis.

Reference Standard: USP, Alpha-Tocopherol, 100%, Lot Number M

References for Appendix D

AACC. 1998. *Approved Methods of the American Association of Cereal Chemists*, 9th Ed., Method 32.20. AACC International: St. Paul, Minnesota.

Albala-Hurtado S, Veciana-Nogues MT, Izquierdo-Pulido M, Vidal-Carou MC. 1997. Determination of free and total furfural compounds in infant milk formulas by high-performance liquid chromatography. *Journal of Agricultural and Food Chemistry* 45: 2128-2133.

AOAC. 2005a. *Official Methods of Analysis of AOAC INTERNATIONAL*, 18th Ed., Methods Methods 922.06 and 954.02. AOAC International: Gaithersburg, Maryland.

AOAC. 2005b. *Official Methods of Analysis of AOAC INTERNATIONAL*, 18th Ed., Method 923.03. AOAC International: Gaithersburg, Maryland.

AOAC. 2005c. *Official Methods of Analysis of AOAC INTERNATIONAL*, 18th Ed., Methods 926.08 and 925.09. AOAC International: Gaithersburg, Maryland.

AOAC. 2005d. *Official Methods of Analysis of AOAC INTERNATIONAL*, 18th Ed., Methods 940.33 and 960.46. AOAC International: Gaithersburg, Maryland.

AOAC. 2005e. *Official Methods of Analysis of AOAC INTERNATIONAL*, 18th Ed., Method 941.15. AOAC International: Gaithersburg, Maryland.

AOAC. 2005f. *Official Methods of Analysis of AOAC INTERNATIONAL*, 18th Ed., Methods 942.23, 953.17, and 957.17. AOAC International: Gaithersburg, Maryland.

AOAC. 2005g. *Official Methods of Analysis of AOAC INTERNATIONAL*, 18th Ed., Method 944.13. AOAC International: Gaithersburg, Maryland.

AOAC. 2005h. *Official Methods of Analysis of AOAC INTERNATIONAL*, 18th Ed., Methods 955.04 and 979.09. AOAC International: Gaithersburg, Maryland.

AOAC. 2005i. *Official Methods of Analysis of AOAC INTERNATIONAL*, 18th Ed., Methods 960.46 and 992.05. AOAC International: Gaithersburg, Maryland.

AOAC. 2005j. *Official Methods of Analysis of AOAC INTERNATIONAL*, 18th Ed., Method 961.15. AOAC International: Gaithersburg, Maryland.

AOAC. 2005k. *Official Methods of Analysis of AOAC INTERNATIONAL*, 18th Ed., Method 982.30. AOAC International: Gaithersburg, Maryland.

AOAC. 2005l. *Official Methods of Analysis of AOAC INTERNATIONAL*, 18th Ed., Method 983.23. AOAC International: Gaithersburg, Maryland.

AOAC. 2005m. *Official Methods of Analysis of AOAC INTERNATIONAL*, 18th Ed., Methods 984.27 and 985.01. AOAC International: Gaithersburg, Maryland.

AOAC. 2005n. *Official Methods of Analysis of AOAC INTERNATIONAL*, 18th Ed., Method 985.29. AOAC International: Gaithersburg, Maryland.

AOAC. 2005o. *Official Methods of Analysis of AOAC INTERNATIONAL*, 18th Ed., Method 993.14. AOAC International: Gaithersburg, Maryland.

AOAC. 2005p. *Official Methods of Analysis of AOAC INTERNATIONAL*, 18th Ed., Method 996.11. AOAC International: Gaithersburg, Maryland.

AOCS. 1997a. *Official Methods and Recommended Practices of the AOCS*, 5th Ed. Method Ba 12-75. American Oil Chemists' Society: Champaign, Illinois.

AOCS. 1997b. *Official Methods and Recommended Practices of the AOCS*, 5th Ed. Method Ce 2-66. American Oil Chemists' Society: Champaign, Illinois.

AOCS. 2001. *Official Methods and Recommended Practices of the AOCS*, 5th Ed. Method Ce 1e-92. American Oil Chemists' Society: Champaign, Illinois.

Atkins L, Schultz AS, Williams WL, Frey CN. 1943. Yeast microbiological methods for determination of vitamins. *Industrial and Engineering Chemistry, Analytical Edition* 15:141-144.

Bradstreet RB. 1965. *The Kjeldahl Method for Organic Nitrogen.* Academic Press: New York, New York.

Brobst KM. 1972. Gas-liquid chromatography of trimethylsilyl derivatives. In: *Methods in Carbohydrate Chemistry, Volume 6: General Carbohydrate Methods*. RN Whistler and JN BeMiller, Eds. Academic Press: New York, New York.

Cort WM, Vincente TS, Waysek EH, Williams BD. 1983. Vitamin E content of feedstuffs determined by high-performance liquid chromatographic fluorescence. *Journal of Agricultural and Food Chemistry.* 31:1330-1333.

Hagerman AE, Nicholson RL. 1982. High-performance liquid chromatographic determination of hydroxycinnamic acids in maize mesocotyl. *Journal of Agricultural and Food Chemistry* 30:1098-1102

ILSI. 2008. *International Life Sciences Institute Crop Composition Database,* v. 3.0. http://www.cropcomposition.org/cgi-perl/search_ora.cgi; search parameters: crop type = "CORN_FIELD," tissue type = "FORAGE" or "GRAIN," crop year = "All", country = "All," and state = "All"; searching not restricted by analysis method or value range (accessed February 9, 2010).

Infant Formula Council. 1985a. *Methods of Analysis for Infant Formulas.* Section C-2. Atlanta, Georgia.

Infant Formula Council. 1985b. *Methods of Analysis for Infant Formulas.* Section C-7. Atlanta, Georgia.

Kalthoff IM, Sandell EB. 1948. *Quantitative Inorganic Analysis.* MacMillan: New York.

Kenward MG, Roger JH. 1997. Small sample inference for fixed effects from restricted maximum likelihood. *Biometrics* 53:983–997.

Lehrfeld J. 1989. High-performance liquid chromatography analysis of phytic acid on a pH-stable, macroporous polymer column. *Cereal Chemistry* 66:510-515.

Lehrfeld J. 1994. HPLC separation and quantitation of phytic acid and some inositol phosphates in foods: Problem and solutions. *Journal of Agricultural and Food Chemistry* 42:2726-2731.

Mason BS, Slover HT. 1971. A gas chromatographic method for the determination of sugars in foods. *Journal of Agricultural and Food Chemistry.* 19:551-554.

McMurray CH, Blanchflower WJ, Rice DA. 1980. Influence of extraction techniques on determination of alpha-tocopherol in animal feedstuffs. *Journal of the Association of Official Analytical Chemists* 63:1258-1261.

Quackenbush FW. 1987. Reverse phase HPLC separation of cis- and trans-carotenoids and its application to β-carotenes in food materials. *Journal of Liquid Chromatography* 10:643-653.

Speek AJ, Schijver J, Schreurs WHP. 1983. Vitamin E composition of some seed oils as determined by high-performance liquid chromatography with fluorometric quantitation. *Journal of Food Science* 50:121-124.

USDA. 1970. Forage fiber analyses. *Agriculture Handbook No. 379*. United States Department of Agriculture: Washington, DC.

USDA. 1973. Energy value of foods. *Agriculture Handbook No. 74*, pp. 2-11. United States Department of Agriculture: Washington, DC.

US Pharmacopeia. 2005. *The United States Pharmacopeia*, Twenty-Ninth Revision, p. 1913, United States Pharmacopeial Convention, Inc.: Rockville, Maryland.

Appendix C
Data Tables

Table C-1: Representative Conventional Insecticide Products to Control Corn Rootworm

Product	Active Ingredient(s); % of Formulation	Signal Word/Classification	Environmental Hazards and/or Reasons for Restricted Use Classification
Ambush® Insecticide	permethrin; 25.6%	WARNING/Restricted Use	Extremely toxic to fish and aquatic invertebrates, highly toxic to bees.
Avicta® Duo Nematicide/Insecticide (seed treatment)	abamectin; 12.4% thiamethoxam; 28.1%	WARNING/Restricted Use	Toxic to fish and wildlife, highly toxic to aquatic invertebrates, exposed treated seeds may be hazardous to wildlife.
Aztec® 2.1% Granular Insecticide	tebupirimfos; 2.0% cyfluthrin;0.1%	WARNING/Restricted Use	Toxic to fish and wildlife.
Baythroid® 2 Emulsifiable Pyrethroid Insecticide	cyfluthrin; 25%	DANGER/Restricted Use	Extremely toxic to fish and aquatic invertebrates, highly toxic to bees.
Capture® 2EC Insecticide/Miticide	bifenthrin;25.1%	WARNING/Restricted Use	Extremely toxic to fish and aquatic invertebrates, highly toxic to bees
Capture® LFR Insecticide	bifenthrin; 17.15%	WARNING/Restricted Use	Extremely toxic to fish and aquatic invertebrates, highly toxic to bees.
Counter® 15G Systemic Insecticide Nematicide	terbufos; 15%	DANGER/Restricted Use	Highly toxic to fish and wildlife, known to cause fish kills, birds and mammals may be killed if granules not covered with soil. Fatal if swallowed, inhaled or absorbed through skin, corrosive, causes irreversible eye damage.
Counter 20G Systemic Insecticide - Nematicide	terbufos; 20%	DANGER/Restricted Use	Highly toxic to fish and wildlife, known to cause fish kills, birds and mammals may be killed if granules not covered with soil. Acute dermal, oral and inhalation toxicity.
Cruiser® 5FS (seed treatment)	thiamethoxam; 47.6%	CAUTION	Toxic to wildlife, highly toxic to aquatic invertebrates, treated seeds exposed on soil surface may be hazardous to wildlife.
DuPont Asana®XL Insecticide - 0.66 emulsible concentrate	esfenvalerate; 8.4%	WARNING/Restricted Use	Extremely toxic to fish and aquatic invertebrates, highly toxic to bees.
Declare® Emulsifiable Insecticide Concentrate	methyl parathion; 45.11%	DANGER/Restricted Use	Fatal if swallowed, inhaled or absorbed through skin, highly toxic to aquatic invertebrates and wildlife, highly toxic to bees.
Dimethoate 4 EC Systemic Insecticide	dimethoate; 44.8%	WARNING	Toxic to wildlife and aquatic invertebrates, highly toxic to bees.
Dimethoate 400 Systemic Insecticide -Miticide	dimethoate; 43.5%	WARNING	Toxic to wildlife and aquatic invertebrates, highly toxic to bees.
Force® 3G Insecticide	tefluthrin; 3%	CAUTION/Restricted Use	Very highly toxic to freshwater and estuarine fish and invertebrates.
Force® ST Insecticide for corn seed treatment	tefluthrin; 26.8%	CAUTION	Very highly toxic to freshwater and estuarine fish and invertebrates; exposed treated seeds may be hazardous to birds and other wildlife.
Force® CS Insecticide	tefluthrin; 23.4%	WARNING/Restricted Use	Very highly toxic to freshwater and estuarine fish and invertebrates.
Fortress® 2.5G granular insecticide	chlorethoxyfos; 2.5%	WARNING/Restricted Use	Toxic to wild mammals, birds, fish and aquatic invertebrates; harmful if absorbed through the skin.

Product	Active Ingredient(s); % of Formulation	Signal Word/Classification	Environmental Hazards and/or Reasons for Restricted Use Classification
Fortress® 5G granular insecticide	chlorethoxyfos; 5%	DANGER/Restricted Use	Toxic to wild mammals, birds, fish and aquatic invertebrates; fatal if swallowed; may be fatal if inhaled; harmful if absorbed through skin.
Furadan® 4F insecticide/ nematicide	carbofuran; 44%	DANGER/Restricted Use	Toxic to fish, birds and other wildlife; highly toxic to bees; can seep or leach through soil and can contaminate groundwater; poisonous if swallowed or inhaled; may be fatal or harmful as a result of skin or eye contact or breathing spray mist.
Furadan® LFR insecticide/ nematicide	carbofuran; 40.64%	DANGER/Restricted Use	Toxic to fish, birds and other wildlife; highly toxic to bees; can seep or leach through soil and can contaminate groundwater; poisonous if swallowed or inhaled; may be fatal or harmful as a result of skin or eye contact or breathing spray mist.
Gaucho® 600 Flowable (seed treatment)	imidacloprid; 48.7%	CAUTION	Highly toxic to birds and aquatic invertebrates.
Gaucho® 75 ST Insecticide (seed treatment)	imidacloprid; 75%	CAUTION	Highly toxic to birds and aquatic invertebrates.
Lambda 25 CS	lam bda-cyhalothrin; 23.6%	WARNING/Restricted Use	Extremely toxic to fish and aquatic invertebrates; toxic to wildlife; highly toxic to bees
Lannate® LV insecticide	methomyl; 29%	DANGER/Restricted Use	Fatal if swallowed, toxic to fish, aquatic invertebrates and mammals, highly toxic to bees, known to leach through soil into groundwater.
Lannate® SP insecticide	methomyl; 90%	DANGER/Restricted Use	Fatal if swallowed, may cause blindness, toxic to fish, aquatic invertebrates and mammals, highly toxic to bees, known to leach through soil into groundwater.
Lorsban® 15G Granular Insecticide	chlorpyrifos; 15%	CAUTION	Toxic to fish, aquatic invertebrates, birds and small mammals, highly toxic to bees.
Lorsban® -4E Insecticide	chiorpyrifos; 44.9%	WARNING/Restricted Use	Toxic to fish, aquatic invertebrates, birds and small mammals, highly toxic to bees.
Lorsban® 75WG Insecticide	chlorpyrifos; 75%	WARNING	Toxic to fish, aquatic invertebrates, birds and small mammals, highly toxic to bees.
Mocap® 15% Granular Nematicide/ Insecticide	ethoprop; 15%	DANGER/Restricted Use	Toxic to aquatic organisms and wildlife; extremely toxic to birds; toxic to bees; fatal if absorbed through skin; may be fatal if swallowed; causes irreversible eye damage; harmful if inhaled.
Penncap-M® Microencapsulated Insecticide	methyl parathion; 22.0%	WARNING/Restricted Use	Highly toxic to aquatic invertebrates and wildlife.
Phorate 20 G Organophosphate Insecticide	phorate; 20.0%	DANGER/Restricted Use	Very highly toxic to fish and wildlife. Birds and mammals may be killed if granules are not properly covered with soil. Fatal if swallowed, inhaled or absorbed through the skin; corrosive; causes irreversible eye damage.

Product	Active Ingredient(s); % of Formulation	Signal Word/Classification	Environmental Hazards and/or Reasons for Restricted Use Classification
Poncho® 600 (seed treatment)	clothianidin; 48%	CAUTION	Toxic to aquatic invertebrates.
Pounce® 3.2 EC Insecticide	permethrin; 38.4%	CAUTION/Restricted Use	Extremely toxic to fish and aquatic invertebrates, highly toxic to bees.
Pounce® 25 WP Insecticide	permethrin; 25%	WARNING/Restricted Use	Extremely toxic to fish and aquatic invertebrates, highly toxic to bees.
Sewn® 80 Solupak	carbaryl; 80%	WARNING	Extremely toxic to aquatic invertebrates, highly toxic to bees.
Sevin® brand XLR PLUS Carbaryl Insecticide	carbaryl; 44.1%	CAUTION	Extremely toxic to aquatic invertebrates, highly toxic to bees.
Tundra® Max Insecticide	chlorpyrifos; 28.6% bifenthrin; 9.0%	WARNING/Restricted Use	Extremely toxic to fish, aquatic invertebrates, small mammals, and birds. Highly toxic to bees.
Warrior II with Zeon Technology® Insecticide	lambda-cyhalothrin; 22.8%	WARNING/Restricted Use	Extremely toxic to fish and aquatic organisms and toxic to wildlife, highly toxic to bees.
Zeta-Cype 0.8EW Insecticide	zeta-cy perm ethrin; 9.2%	WARNING/Restricted Use	Extremely toxic to fish, aquatic invertebrates, oysters and shrimp; highly toxic to bees.

Source: Vlachos and Ward. 2011.

Table C-2: Corn Diseases

Disease Name	Pathogen	Conditions	Management
Seed Rot	Fungi and Bacteria. *Pythium, Fusarium, Diplodia, Rhizoctonia, Penicillium* spp., various soil borne bacteria	Favored by prolonged wet and cold soil conditions in the spring. Soil temperatures 50 F or lower favor seed rots	Fungicide seed treatment. Plant when soil conditions are warmer and drier, use the proper planting depth
Gray Leaf Spot	Fungus. *Cercospora zeae-maydis*	Infection is favored by extended warm, wet, humid weather	Select hybrids with resistance (tolerance based on risk), two year crop rotation, cleanly plow under infected residue
Anthracnose Leaf Blight	Fungus. *Colletotrichum graminicola*	Favored by cool to warm, wet, humid weather, continuous corn with reduced tillage.	Resistant hybrids, rotate corn with nongrass crops. Cleanly plow under infected residue.
Common Corn Rust	Fungus. *Puccinia sorghi*	Disease favored by cool (66 F optimum) humid weather	Resistant hybrids. Foliar fungicides may be useful in seed production fields
Eyespot	Fungus. *Kabatiella zeae*	Disease favored by cool moist conditions.	Crop rotation, tillage practices, resistant genotypes
Brown Spot	Fungus. *Physoderma maydis*	Favored by hot, humid tropical conditions	Crop rotation and conventional tillage
Southern Corn Rust	Fungus. *Puccinia polysora*	Favored by high humidity and temperatures around 80 F.	Resistant hybrids. Foliar fungicides may be useful in seed production fields.
Northern Corn Leaf Blight	Fungus. *Exserohilum turcicum*	Favored by extended wet, cool, humid weather, minimum tillage, continuous corn. Usually occurs during or after pollination	Resistant hybrids. Foliar fungicides may be useful in seed production fields. Cleanly plow under infected residue
Northern Leaf Spot	Fungus. *Helminthosporium carbonum* (Race 3)	Favored by moderate temperatures and high relative humidity, minimum tillage, continuous corn	Resistant hybrids. Disease is primarily a problem in seed production fields with certain highly susceptible inbreds. Foliar fungicides may be useful in seed production fields. Cleanly plow under infected residue
Southern Corn Leaf Blight	Fungus. *Bipolaris maydis*	Favored by extended warm, wet, humid weather, minimum tillage, and continuous corn.	Resistant hybrids. Foliar fungicides may be useful in seed production fields. Cleanly plow under infected residue
Stewart's Wilt	Bacterium. *Erwinia stewartii*	Favored by high infestation levels of flea beetles in April through late June	Apply insecticides early to control corn flea beetles, resistant hybrids

Table C-2: Corn Diseases (continued)

Disease Name	Pathogen	Conditions	Management
Anthracnose Leaf Blight	Fungus. *Colletotrichum graminicola*	Favored by cool to warm, wet, humid weather, minimum tillage, continuous corn, stresses that result in early senescence	Resistant hybrids (full season hybrids tend to have more resistance than short season), two year crop rotation with non-grass crops, cleanly plow under infected residue, balanced soil fertility.
Goss's Wilt	Bacteria. *Clavibacter michiganenis*	Temperatures between 12°C to 40°C (ideal temperature 27°C)	Deep plowing of residue following harvest and crop rotation.
Common Smut	Fungus. *Ustilago maydis*	Rainy and Humid Conditions. Use of high levels of nitrogen or barnyard manure	Crop rotation, fungicide treatment, resistant hybrids.
Head Smut	Fungus. Sphacelotheca reliana	Favored by soil temperatures of 21 to 28°C and low soil moisture under dryland conditions, sandy soils	Fungicide seed treatment, in furrow treatment, application of ammonium sulphate or urea fertilizer
Sorghum Downy Mildew	Fungus. *Peronosclerospora sorghi*	Period of free moisture or saturated atmosphere of 4 hours needed for germination and infection	Fungicide seed treatment
Diplodia Stalk Rot	Fungus. *Diplodia maydis*	Warm, moist weather in late summer (2-3 wks after silking), stresses that result in early senescence.	Resistant hybrids (full season hybrids tend to have more resistance than short season hybrids), balanced soil fertility, recommended plant population.
Gibberella Stalk Rot	Fungus. *Gibberella zeae*	Warm, moist weather in late summer (2-3 wks after silking). More prevalent when plants are subjected to stresses that result in early senescence and a reduction of sugar to roots and stalks.	Resistant hybrids. Full season hybrids tend to have more resistance than short season. Balanced soil fertility. Do not exceed recommended plant population
Fusarium Stalk Rot	Fungus. *Fusarium moniliforme*	Warm, moist weather shortly after pollination. More prevalent when plants are subjected to stresses (such as dry weather) that result in early senescence and a reduction of sugar to roots and stalks.	Resistant hybrids. Full season hybrids tend to have more resistance than short season. Balanced soil fertility. Do not exceed recommended plant populations
Stenocarpella Stalk Rot	*Stenocarpella maydis and S.macrospora*	Warm, moist conditions	Crop rotation, tillage and resistant hybrids

Table C-2: Corn Diseases (continued)

Disease Name	Pathogen	Conditions	Management
Charcoal Rot	*Macrophomina phaseolina*	Post flowering drought stress with soil temperatures between 30-42°C	Management practices that reduce crop stress and reduce soil temperatures
Diplodia Ear Rot	Fungus. *Diplodia maydis*	Dry weather prior to silking, followed by wet conditions within first 30 days after silking.	Resistant hybrids, crop rotation, clean plowing, harvest early to prevent weathering. Dry corn to 15% moisture content and below to prevent further mold growth in storage.
Gibberella Ear Rot	Fungus. *Gibberella zeae*	Cool wet weather within first 21 days after silking favors the development of this disease.	Resistant hybrids, crop rotation, harvest early to prevent continued mold growth in the field, clean plowing. Dry corn to 15% moisture content and below to prevent further mold growth in storage.
Fusarium Ear Rot	Fungus. *Fusarium moniliforme*	Dry, warm weather. Infection occurs through injury by insects or environmental stress.	Resistant hybrids (avoid sowing hybrids with weak seed coats or poor husk cover), crop rotation, clean plowing, harvest grain early, dry corn to 15% moisture content and below to prevent further mold growth in storage
Aspergillus Ear Rot	Fungi. *Aspergillus flavus, A. glaucus, A. niger*	*A. flavus* is more common in Indiana following hot, dry weather, injury by drought stress and insect damage. Mold growth in storage when moisture is higher than 18%. *A. flavus* can produce a carcinogenic secondary metabolite known as aflatox	In storage, controlled by drying corn to a moisture content below 15% as soon after harvest as possible. In the field, avoid insect or mechanical damage to ears.
Maize Dwarf Mosaic	Virus. Maize dwarf mosaic virus (MDMV) strain A or B	Those favorable to aphids and growth of Johnson grass in fields	Resistant hybrids, control rhizome Johnsongrass or other overwintering weed hosts.
Maize Chlorotic Dwarf	Virus. Maize chlorotic dwarf virus (MCDV)	Those that favor leafhopper reproduction and growth of Johnson grass in fields.	Resistant varieties. Sow early in the growing season to avoid large leaf hopper populations. Control perennial Johnson

Table C-2: Corn Diseases (continued)

Disease Name	Pathogen	Conditions	Management
Crazy Top	Fungus. *Scleropthora macrospora*	Saturated soil conditions for 24-48 hours	Provide adequate soil drainage, control grassy weeds, avoid sowing in low, wet spots.
Maize Chlorotic Mottle	Virus. MCMV	-	Crop rotation, resistant hybrids
High Plains Disease	Virus. High plains virus and wheat streak mosaic virus	Disease occurs when corn is planted adjacent to small grains field that are beginning to dry down	Modification in planting dates, Resistant hybrids

Sources: Ruhl 2007 and Smith *et al*. 2004.