RECEIVED
By BRS Document Control Officer at 11:02 am, May 28, 2019

MONSANTO

Petition for the Determination of Nonregulated Status for Insect-Protected MON 88702 Cotton

The undersigned submits this petition under 7 CFR § 340.6 to request that the Administrator make a determination that the article should not be regulated under 7 CFR Part 340

March 29, 2019

(Revised May 24, 2019)

OECD Unique Identifier: MON-887Ø2-4

Monsanto Petition number: CT273-19U1

Submitted by:
Lieselot Bertho, M.S.
Monsanto Company
700 Chesterfield Pkwy W
Chesterfield, MO 63017
Phone: (636) 737-9547
Fax: (636) 737-5390
E-mail: lieselot.bertho@bayer.com

Prepared by:

K. Adu-Tutu, Ph.D., A. Ahmad, P. Asiimwe, Ph.D., Ph.D., L.A. Burzio, Ph.D., E. Dickinson, B.S., D. Farmer, Ph.D., J. Francois, Ph.D., C. Garnaat, M.S., X. Li, M.S., M. S. Rajani, Ph.D., R. Wang, M.S., T. Werk, M.S.

Contributors and/or Principal Investigators:

L.D. Barberis, B.S., P. Bachman, Ph.D., E. Bell, C. Brown, Ph.D., Ph.D., L. Cheng, Ph.D., P. Chinnadurai, A. Deffenbaugh, M.S., M.S., R. Dobert, Ph.D., C. Duong, M.S., T. Edrington, Ph.D., J.R. Fischer, Ph.D., J. Fridley, B.S., A. Galadima, Ph.D., X. Gu Ph.D., A. Hendrickson Culler, Ph.D., G. Head, Ph.D., R. Hileman, Ph.D., J. Hua, M.D., P. Jensen, Ph.D., C. Jiang, Ph.D., C.M. Johnson, B.S., S. Joshi, Ph.D., C. Kessenich, M.S., T. Klusmeyer, Ph.D., M. Koch, Ph.D., J. Komorek, Ph.D., S. Levine, Ph.D., B. Li, Ph.D., M. Malven, M.S., C. Meng, Ph.D., J. Monken, G. Mueller, M.A., B.J., K. Robinson, Ph.D., R. Schneider, B.S., A. Silvanovich, Ph.D., J. Tan, Ph.D., J. Uffman, B.S., W. Urquhart, Ph.D., F. Zapata, M.S.

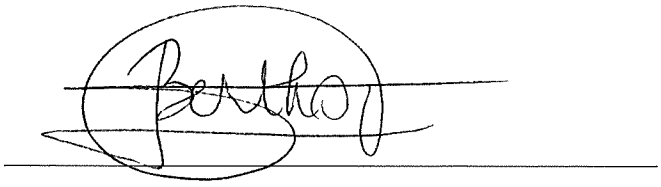
Contains No Confidential Business Information

RELEASE OF INFORMATION

Monsanto is submitting the information in this petition for review by the USDA as part of the regulatory process. Monsanto understands that the USDA complies with the provisions of the Freedom of Information Act (FOIA). In the event the USDA receives a FOIA request, pursuant to 5 U.S.C., § 552, and 7 CFR Part 1, covering all or some of the information in this petition, Monsanto expects that, in advance of the release of the document(s), USDA will provide Monsanto with a copy of the material proposed to be released and the opportunity to object to the release of any information based on appropriate legal grounds, e.g., responsiveness, confidentiality, and/or competitive concerns. Monsanto understands that a CBI-deleted copy of this information may be made available to the public in a reading room and upon individual request as part of a public comment period. Monsanto also understands that when deemed complete, a copy of the petition may be posted to the USDA-APHIS BRS website or other U.S. government websites (e.g., www.regulations.gov). Except in accordance with the foregoing, Monsanto does not authorize the release, publication or other distribution of this information without Monsanto's prior notice and consent.

CERTIFICATION

The undersigned certifies that, to the best knowledge and belief of the undersigned, this petition includes all information and views on which to base a determination, and that it includes all relevant data and information known to the petitioner that are unfavorable to the petition.



Lieselot Bertho, M.S.
Regulatory Affairs Manager

Address:
Monsanto Company
700 Chesterfield Pkwy W
Chesterfield, MO 63017

Tel: (636) 737-9547
Fax: (636) 737-5390

EXECUTIVE SUMMARY

The Animal and Plant Health Inspection Service (APHIS) of the United States (U.S.) Department of Agriculture (USDA) has responsibility under the Plant Protection Act (Title IV Pub. L. 106-224, 114 Stat. 438, 7 U.S.C. § 7701-7772) to prevent the introduction and dissemination of plant pests into the U.S.

APHIS regulation 7 CFR § 340.6 provides that an applicant may petition APHIS to evaluate submitted data to determine that a particular regulated article does not present a plant pest risk and no longer should be regulated. If APHIS determines that the regulated article does not present a plant pest risk, the petition is granted, thereby allowing unrestricted introduction of the article.

Monsanto Company is submitting this request to APHIS for a determination of nonregulated status for the new biotechnology-derived cotton product, MON 88702, any progeny derived from crosses between MON 88702 and conventional cotton, and any progeny derived from crosses of MON 88702 with biotechnology-derived cotton that have previously been granted nonregulated status under 7 CFR Part 340.

Product Description

Monsanto Company has developed insect-protected cotton MON 88702, which produces a modified Cry51Aa2 insecticidal crystal (Cry) protein derived from *Bacillus thuringiensis* (*Bt*) that protects against feeding damage caused by targeted hemipteran (*Lygus hesperus* and *Lygus lineolaris*) and thysanopteran (*Frankliniella* spp.) insect pests. The modified Cry51Aa2 protein has been assigned the unique name Cry51Aa2.834_16 (herein referred to as mCry51Aa2).

The data and information presented in this petition demonstrate that MON 88702 cotton is agronomically and phenotypically comparable to conventional cotton, with the exception of the introduced trait. Moreover, the data and information presented demonstrate that MON 88702 cotton is not expected to adversely affect non-target organisms, or to pose an increased plant pest risk, including weediness, compared to conventional cotton. The food, feed, and environmental safety of MON 88702 cotton was confirmed based on multiple, well-established lines of evidence:

- Cotton is a familiar crop that does not possess any of the attributes commonly associated with weeds and has a history of safe consumption. The conventional control used for the transformation process was included in studies to serve as an appropriate basis of comparison for MON 88702.

- A detailed molecular characterization of the inserted DNA demonstrates a single, intact copy of the T-DNA insert in a single locus within the cotton genome.

- The mode of action of mCry51Aa2 is well characterized, understood and documented in the peer-reviewed literature, and data assessing the human safety demonstrates it is unlikely to be a human toxin or allergen. These data were provided to the U.S. Environmental Protection Agency (EPA) and a permanent

exemption from the requirement for a tolerance for residues of mCry51Aa2 in food and feed products derived from cotton has been granted (U.S. EPA, 2018a).

- An assessment of potential impact to non-target organisms (NTOs), including organisms beneficial to agriculture and endangered species indicates that MON 88702 cotton is not expected to have an increased effect on non-target organisms compared to conventional cotton under standard agricultural practices.

- An assessment of anti-nutrient components supports the conclusion that MON 88702 cottonseed is equivalent to conventional cottonseed for these components. Composition data for 30 components including major nutrients of cottonseed (protein, amino acids, total fat, carbohydrates, linoleic acid, acid detergent fiber, neutral detergent fiber and ash) and anti-nutrients was submitted to the Food and Drug Administration as part of the voluntary food/feed safety and nutritional assessment for MON 88702 cotton. The FDA concluded their consultation and agreed with the conclusion that MON 88702 was compositionally equivalent and as safe and nutritious as conventional cotton with respect to its uses in human or animal food (U.S. FDA, 2018). Therefore, MON 88702 cottonseeds do not pose increased risk to non-target organisms upon consumption compared to conventional cottonseed and are not anticipated to alter the cotton plant's impact on non-target pests and beneficial insects.

- An extensive evaluation of MON 88702 cotton phenotypic and agronomic characteristics, and environmental interactions demonstrates MON 88702 has no increased plant pest risk or weediness potential compared to conventional cotton.

Cotton is a Familiar Crop Lacking Weedy Characteristics

Cotton, as a commodity crop, has a longstanding history of cultivation and its byproducts, including processed fractions, also have a history of safe use and consumption. Cotton is grown in 17 states across the southern U.S and in approximately 80 countries worldwide, and in 2018, U.S. growers planted approximately 14.1 million acres of cotton.

The commercial cotton species in the U.S. (*Gossypium hirsutum* and *Gossypium barbadense* L. Merr.) do not exhibit weedy characteristics as defined by USDA, and neither invade established ecosystems, nor outcross to weedy relatives. Cotton is not listed as a weed in major weed references, nor is it present on the lists of noxious weed species distributed by the federal or U.S. state governments (7 CFR Part 360). Cotton does not possess any of the attributes commonly associated with weeds, such as long persistence of the seed in the soil, ability to disperse, invade, or become a dominant species in new or diverse landscapes, or the ability to compete well with native vegetation. It is recognized that in some agricultural systems, cotton can volunteer in a subsequent rotational crop. However, volunteers are easily controlled through tillage or the use of appropriate herbicides with diverse modes-of-action.

In the continental U.S., wild populations of *Gossypium* species and some feral populations of cultivated variants of *G. hirsutum* exist, but those species able to cross with cultivated cotton are not known to exist in cotton growing areas. Furthermore, the

EPA imposes strict geographical restrictions on the sale and distribution of *Bt* cotton in order to mitigate the potential for gene flow to wild populations of *Gossypium* species (U.S. EPA, 2019). Importantly, MON 88702 would not be expected to confer a selective advantage to, or enhance the pest potential of progeny resulting from such a cross if it were to occur. Thus, with environmental, biological and regulatory limitations, there is limited probability for MON 88702 or any *Gossypium* species to outcross with wild or feral plants.

Conventional Cotton DP393 is an Appropriate Comparator to MON 88702

Cotton variety DP393 is the near isogenic line to MON 88702 and was used as the conventional cotton comparator to support the safety assessment of MON 88702 cotton. MON 88702 and the near isogenic conventional cotton control DP393 have similar genetic backgrounds with the exception of the *Cry51Aa2.834_16* expression cassette (herein referred to as the *mCry51Aa2* expression cassette); thus, the effect of the *mCry51Aa2* expression cassette and mCry51Aa2 protein could be evaluated.

Molecular Characterization Verified the Integrity and Stability of the Inserted DNA in MON 88702

MON 88702 cotton was developed through *Agrobacterium*-mediated transformation of cotton using a two transfer DNA (T-DNA) transformation plasmid vector. The first T-DNA, designated as T-DNA I, contains the *mCry51Aa2* expression cassette, which encodes the mCry51Aa2 protein. The second T-DNA, designated as T-DNA II, contains the *aadA* expression cassette. During transformation, both T-DNAs were inserted into the cotton genome. Subsequently, traditional breeding, segregation, selection, and screening were used to isolate those plants that contained the *mCry51Aa2* expression cassette (T-DNA I) and did not contain the *aadA* expression cassette (T-DNA II).

Characterization of the DNA insert in MON 88702 cotton was conducted using a combination of next-generation sequencing, PCR, and bioinformatics. The results of this characterization demonstrate that MON 88702 cotton contains a single copy of the intended transfer DNA (T-DNA I) with the *mCry51Aa2* expression cassette, which is stably inherited over multiple generations and segregates according to Mendelian principles. No additional backbone sequence from the transformation plasmid was detected in the transformed plant. The results of this characterization also confirm that T-DNA II is not present in MON 88702 cotton.

Data Confirms the Safety of the mCry51Aa2 Protein Safety for Food and Feed

The mCry51Aa2 protein expressed by MON 88702 is a plant incorporated protectant (PIP) and as such its safety assessment falls within the purview of the U.S. Environmental Protection Agency (EPA). The data for the food and feed safety assessment of the mCry51Aa2 protein and the food and feed derived from MON 88702 cottonseed were submitted to EPA to support the establishment of a tolerance exemption for residues of mCry51Aa2 in food and feed. A permanent exemption from the requirement for a tolerance was granted in January 2018 where it was concluded that "*there is a reasonable certainty that no harm will result to the U.S. population, including*

infants and children, from aggregate exposure to residues of the Cry51Aa2.834_16 protein derived from Bacillus thuringiensis" *(U.S. EPA, 2018a).*

A summary of the results of these analyses is presented within this petition, providing the weight-of-evidence which supports the conclusion reached by the EPA.

MON 88702 Cotton Will Not Negatively Affect NTOs Including Those Beneficial to Agriculture

The NTO assessment for MON 88702 has taken into consideration a number of characteristics of the mCry51Aa2 protein expressed in MON 88702 to evaluate potential hazards to NTOs.

The first step of this assessment was to establish the spectrum of activity of the mCry51Aa2 protein using a broad range of taxa, including multiple orders and ecological groups (Bachman et al., 2017). The activity spectrum assessment provided initial hazard identification for potential activity of the mCry51Aa2 protein. The results indicated activity within three insect orders where toxicity or protection from feeding damage was shown against targeted hemipteran (*Lygus hesperus* and *Lygus lineolaris*) and thysanopteran (*Frankliniella* spp.) species. Additionally, activity was observed against the hemipteran pest *Pseudatomoscelis seriatus*, one hemipteran predator species (*Orius insidiosus*) and two coleopteran species (*Leptinotarsa decemlineata* and *Diabrotica undecimpunctata howardi*).

The subsequent tier 1 NTO diet feeding assays, using diets containing high levels of the mCry51Aa2 protein, tested surrogate, beneficial species which were selected based on the results of the activity spectrum assessment, while ensuring representation of different taxonomic groups, habitats and functions in the cotton agro-ecosystem. These included a pollinator [honey bee larvae and adults (*Apis mellifera*)], five beneficial insect species that provide biological control functions [parasitic wasp (*Pediobius foveolatus*), ladybird beetle (*Coccinella septempunctata*), rove beetle (*Aleochara bilineata*), lacewings (*Chrysoperla carnea*), big-eyed bug (*Geocoris punctipes*), Western damsel bug (*Nabis alternatus*), leafhopper assassin bug (*Zelus renardii*) and insidious flower bug (*Orius insidiosus*)], and two representative soil biota [earthworm (*Eisenia andrei*) and Collembola (*Folsomia candida*)] that provide decomposition functions. No adverse effects of MON 88702 against any of the tested species was observed, except for the observation of activity against *Orius insidiosus* in the activity spectrum assessment, indicating that MON 88702 does not pose a risk to the functional groups where no activity was observed.

To further characterize any potential risk of MON 88702 towards *Orius* spp., several additional studies were conducted that included tri-trophic feeding studies using two herbivorous species as prey (tier 2), leaf disk assays to model different feeding scenarios representing different exposure and feeding ecology (tier 3) and a field study to assess the impact of MON 88702 in the most realistic exposure conditions (tier 4). With each higher tiered study, the exposure scenario of *Orius* spp. to the mCry51Aa2 protein was further refined to reflect increasingly more realistic conditions. The activity of the mCry51Aa2 protein against *Orius* spp. as observed in the initial activity spectrum assessment could

also be observed under specific tier 2 study conditions. However, more realistic exposure scenarios in tier 3 and tier 4 studies demonstrated the lack of an adverse effect on *Orius* spp. Therefore, considering the comprehensive data provided, MON 88702 will not pose a risk to *Orius* spp.

Predatory Hemiptera closely related to *Orius* spp: *Geocoris* spp., *Nabis* spp. and *Zelus* spp., were also tested in tier 1 diet assay studies and their abundance was also monitored in the tier 4 field study. No impacts on survival of these three species from exposure to the mCry51Aa2 protein were observed in tier 1 studies. The results of the tier 4 field study corroborate that there was no adverse effect of MON 88702 on abundance of *Geocoris* spp., *Nabis* spp. or *Zelus* spp. Therefore, considering the comprehensive data provided, MON 88702 will not pose a risk to predatory Hemiptera present in the cotton agro-ecosystem.

The mCry51Aa2 protein was administered to bobwhite quails and mice at exaggerated doses of 2500 and 5000 mg of mCry51Aa2 per kg of body weight, respectively. No adverse effects were observed in either species.

These results demonstrate that the introduction of MON 88702 cotton and the expressed mCry51Aa2 protein are unlikely to have adverse effects on NTOs beneficial to agriculture at field exposure levels, and that MON 88702 cotton is unlikely to pose a risk to NTOs.

Anti-nutrient Composition of MON 88702 is Equivalent to Conventional Cottonseed

Given that cotton plants contain low levels of known anti-nutrients which may adversely impact non-ruminant animals and non-target organisms, a subset of components, specifically anti-nutrients listed in the OECD consensus document on cottonseed composition (2009), were evaluated in MON 88702. For MON 88702 cotton, the introduced protein, mCry51Aa2, is a *Bt*-derived crystal (Cry) protein that lacks catalytic activity and is neither intended to nor expected to affect the plant's metabolism.

Compositional analysis of anti-nutrients was conducted using cottonseed harvested from MON 88702 cotton and a conventional control grown at five sites in the U.S. during 2015. There were no statistically significant differences ($p<0.05$) between MON 88702 and the conventional control for key anti-nutrients in cottonseed, namely total gossypol, free gossypol, malvalic acid, sterculic acid, and dihydrosterculic acid. These results support the conclusion that MON 88702 did not meaningfully alter key anti-nutrient levels in cottonseed. Therefore, MON 88702 cotton is not expected to have an effect on non-target organisms as a consequence of changes in anti-nutrient components.

Composition data for 30 components including major nutrients of cottonseed (protein, amino acids, total fat, carbohydrates, linoleic acid, acid detergent fiber, neutral detergent fiber and ash) and anti-nutrients were submitted to FDA which confirmed the compositional equivalence of MON 88702 and conventional cotton (U.S. FDA, 2018).

MON 88702 Does Not Change Cotton Plant Pest Potential or Environmental Interactions

Plant pest potential of a biotechnology-derived crop is assessed from the basis of familiarity that the USDA recognizes as an important underlying concept in risk assessment. The concept of familiarity is based on the fact that the biotechnology-derived plant is developed from a conventional plant hybrid or variety whose biological properties and plant pest potential are well known. Familiarity considers the biology of the plant, the introduced trait, the receiving environment, and the interactions among these factors. This approach provides a basis for comparative risk assessment between a biotechnology-derived plant and the conventional control. Thus, the phenotypic, agronomic, and environmental interaction assessment of MON 88702 cotton included the genetically similar conventional control as a comparator. This evaluation used a weight of evidence approach and considered any observed statistical differences between MON 88702 and the conventional control with respect to reproducibility, magnitude, and directionality. Comparison to a range of commercial conventional reference cotton varieties grown concurrently established the range of natural variability for cotton, and provided a context from which to further evaluate any statistical differences. Characteristics assessed included: seed dormancy and germination, pollen morphology, plant phenotypic observations, and environmental interaction evaluations conducted in the field. The phenotypic, agronomic, and environmental interaction assessment demonstrated that MON 88702 cotton is not significantly different from the conventional control in these characteristics. Thus, MON 88702 cotton is not expected to have increased weediness or plant pest potential compared to conventional cotton.

Considering the defined activity spectrum of the mCry51Aa2 protein expressed in MON 88702, a theoretical assessment was performed to determine the likelihood of an adverse effect to listed threatened and endangered hemipteran (one species), thysanopteran (no species) and coleopteran insect species (19 species). The assessment indicates there will be no adverse effect of MON 88702 expressing the mCry51Aa2 protein on threatened and endangered species in these orders due to their known geographic occurrences, lack of exposure to the mCry51Aa2 protein in the cotton agro-ecosystem and based on the specific feeding ecology of the evaluated species. The EPA has assessed the possible effect of mCry51Aa2 on threatened and endangered species and made a "no effects" determination for the hemipteran and coleopteran species listed in 2017 (U.S. EPA, 2018b).

Deregulation of MON 88702 Cotton is Not Expected to Have Negative Effects on Cotton Agronomic Practices or Land Use

An assessment of current cotton agronomic practices was conducted to determine whether the cultivation of MON 88702 has the potential to impact current cotton agronomic practices. Cotton fields are typically highly managed areas that are dedicated to crop production.

MON 88702 cotton is similar to conventional cotton in its agronomic, phenotypic, and environmental interaction characteristics, as well as its compositional characteristics, including anti-nutrients. While MON 88702 cotton offers protection against feeding

damage caused by *Lygus* spp. and *Frankliniella* spp., growers are still anticipated to incorporate current methods of crop protection into their overall insect pest management program with the potential for reduced insecticide use currently required to control these targeted hemipteran and thysanopteran insect pests. In this regard, cultivation of MON 88702 cotton is not expected to differ from typical cotton cultivation compared to current cotton management practices, except for the additional control of targeted hemipteran and thysanopteran insect pests.

Conclusion

Based on the data and information presented in this petition, it is concluded that MON 88702 cotton is unlikely to pose a plant pest risk. Therefore, Monsanto Company requests a determination from APHIS that MON 88702 cotton and any progeny derived from crosses between MON 88702 and conventional cotton or previously deregulated biotechnology-derived cotton be granted nonregulated status under 7 CFR Part 340.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS AND DEFINITIONS[1]

AA	Amino Acid
ADF	Acid Detergent Fiber
ai	Active ingredient
APHIS	Animal and Plant Health Inspection Service
BIO	Biotechnology Innovation Organization
BRS	Biotechnology Regulatory Service
Bt	*Bacillus thuringiensis*
CaMV	Cauliflower Mosaic Virus
CDS	Control Dose Substance
CFR	Coordinated Framework for Regulation
Cry	Crystal
DNA	Deoxyribonucleic Acid
Dwt	Dry Weight
E. coli	*Escherichia coli*
EEC	Expected Environmental Concentrations
ELISA	Enzyme Linked Immunosorbent Assay
EPA	Environmental Protection Agency
ERA	Ecological Risk Assessment
ETS	Excellence Through Stewardship
FA	Fatty Acid
FAO	Food and Agriculture Organization
FAW	Fall Armyworm
FDA	Food and Drug Administration (U.S.)
FID	Flame Ionization Detection
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
FOIA	Freedom of Information Act
FMV	Figwort Mosaic Virus
fwt	Fresh Weight
g	Gram
GC	Gas Chromatography
HOSU	History Of Safe Use
IPM	Integrated Pest Management
IRM	Insect Resistance Management
Kb	Kilobase
LOD	Limit of Detection
LOQ	Limit of Quantitation
MEEC	Mean Expected Environmental Concentrations
MOA	Mode of Action
MOE	Margin of Exposure
MT	Metric Ton
NDF	Neutral Detergent Fiber
NGS	Next Generation Sequencing

[1] Alred, G.J., C.T. Brusaw, and W.E. Oliu. 2003. Handbook of Technical Writing, 7th edn., pp. 2-7. Bedford/St. Martin's, Boston, MA.

NOAEL	No-Observed-Adverse-Effects Level
NOEC	No Observed Effect Concentrations
NPT II	Neomycin Phosphotransferase II
NTO	Non-Target Organism
OECD	Organisation for Economic Co-operation and Development
OSL	Over Season Leaf
PCR	Polymerase Chain Reaction
PIP	Plant Incorporated Protectant
RH	Relative Humidity
ROP	Repressor of Primer
SAP	Scientific Advisory Panel
SD	Standard Deviation
SE	Standard Error
T-DNA	Transfer Deoxyribonucleic Acid
TDS	Test Dose Substance
USDA	United States Department of Agriculture
U.S.	United States
VDS	Vehicle Dosing Solution
WHO	World Health Organization
µg	Microgram

I. RATIONALE FOR THE DEVELOPMENT OF MON 88702

I.A. Basis for the Request for a Determination of Nonregulated Status under 7 CFR § 340.6

The Animal and Plant Health Inspection Service (APHIS) of the United States (U.S.) Department of Agriculture (USDA) has responsibility, under the Plant Protection Act (Title IV Pub. L. 106-224, 114 Stat. 438, 7 U.S.C. § 7701-7772) to prevent the introduction and dissemination of plant pests into the U.S. APHIS regulation 7 CFR § 340.6 provides that an applicant may petition APHIS to evaluate submitted data to determine that a particular regulated article does not present a plant pest risk and no longer should be regulated. If APHIS determines that the regulated article does not present a plant pest risk, the petition is granted, thereby allowing unrestricted introduction of the article.

Monsanto Company is submitting this request to APHIS for a determination of nonregulated status for the new biotechnology-derived cotton product, MON 88702, any progeny derived from crosses between MON 88702 and conventional cotton, and any progeny derived from crosses of MON 88702 with biotechnology-derived cotton that have previously been granted nonregulated status under 7 CFR Part 340.

I.B. Rationale for the Development of Insect-Protected Cotton MON 88702

Cotton is a leading source of natural fiber used in the textile industry, and is also used in feed, food, cosmetics, pharmaceuticals, and animal feed. The U.S., India and China together produce the majority of cotton fiber used globally. Effective production of cotton is dependent on good management of many factors during the growing season, including selection of cotton variety to plant, quality of starting seed, seedbed preparation and planting depth, fertility, disease, weed control, and protection of the crop from feeding damage by insect pests.

Production of cotton in the U.S. is limited to the warmer, more southern states which, as a region, are referred to as the "cotton belt". Insect pest pressure in parts of the cotton belt can be severe, and cotton producers face potential economic damage from a wide range of insect pests including cotton bollworm, plant bugs, stink bugs, aphids, thrips and spider mites.

In the mid-1990s, Monsanto Company commercialized the first insect-protected cotton (MON 531 marketed as Bollgard®), which expressed the Cry1Ac protein from *Bacillus thuringiensis*. This provided cotton growers with an additional option for protecting their crop from feeding damage by insect pests from the order Lepidoptera, and in subsequent years this trait was reported to have both economic and environmental benefits (Fernandez-Cornejo et al., 2014). These include reductions in pesticide application and runoff, air pollution and waste and production costs, and improvements in grower safety, populations of beneficial insects in cotton fields, and yield (U.S. EPA, 2001).

In recent years, insects from the orders Hemiptera and Thysanoptera have become some of the most economically detrimental pests in U.S. cotton production (Williams, 2016). The tarnished plant bug (*Lygus lineolaris*) and Western tarnished plant bug (*Lygus hesperus*), which are piercing-sucking pests of the insect order Hemiptera, are widely prevalent in the mid-South and western areas of the cotton belt, respectively, and can cause severe yield reductions during the flowering stages of plant development if left unmanaged. Resistance to both pyrethroid and organophosphate insecticides has made these pests more difficult to control. As a result, growers have fewer management options to prevent yield loss from these pests, relying on more frequent applications at higher rates of insecticide (Gore et al., 2012; Mississippi State University, 2016). Thrips (*Frankliniella* spp., order Thysanoptera) are found throughout the cotton belt, and can be an extremely damaging early-season pest in cotton (Cook et al., 2011). Thrips may be controlled through a combination of seed treatment (e.g., neonicotinoids) and foliar (organophosphate) insecticides; however, greater control costs and yield losses to thrips are being reported in some areas, resulting in growers needing to make an increased number of foliar applications against thrips (Cook et al., 2011).

Beyond the use of chemical insecticides (including both seed treatments and foliar sprays) to control these hemipteran and thysanopteran insect pests, additional approaches include crop rotation, variety selection, and judicious application of fertilizer. As an additional tool for minimizing crop damage from these insect pests, Monsanto Company has developed insect-protected cotton MON 88702. MON 88702 produces a modified Cry51Aa2 insecticidal crystal (Cry) protein derived from *Bacillus thuringiensis* (*Bt*), which protects against feeding damage caused by targeted hemipteran and thysanopteran insect pests, including two species of tarnished plant bugs (*Lygus hesperus* and *Lygus lineolaris*) and thrips (*Frankliniella* spp.).

MON 88702 offers cotton growers an additional choice for insect pest management, and may be combined through traditional breeding methods with other insect-protected and herbicide-tolerant biotechnology traits. These next-generation cotton products will provide greater crop management choices for growers, to help meet the needs of U.S. and global food, feed and fiber markets.

I.C. Submissions to Other Regulatory Agencies

Under the Coordinated Framework for Regulation of Biotechnology (CFR) (USDA-APHIS, 1986), the responsibility for regulatory oversight of biotechnology-derived cottons falls primarily on three U.S. agencies: U.S. Food and Drug Administration (FDA), the United States Department of Agriculture (USDA), and in the case of plant incorporated protectants (PIPs), the Environmental Protection Agency (EPA). Deregulation of MON 88702 cotton by USDA would constitute only one component of the overall regulatory oversight and review of this product. As a practical matter, MON 88702 cannot be released and marketed until U.S. EPA (for PIPs), FDA and USDA have completed their reviews and assessments under their respective jurisdictions.

I.C.1. Submission to U.S. EPA

Substances that are pesticides, as defined under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) [7 U.S.C. §136(u)], are subject to regulation by U.S. EPA. Pesticides produced *in planta*, referred to as plant incorporated protectants (PIPs), are also subject to regulation by U.S. EPA under FIFRA. Approvals for an Experimental Use Permit for more extensive field trials of MON 88702 cotton, a permanent exemption from the requirement for a tolerance for mCry51Aa2 and a Section 3 breeding increase registration for the MON 88702 single product have been granted (U.S. EPA, 2018a; b). An additional submission was made for a Section 3 breeding increase for the MON 88702 x MON 15985 x COT102 stack product in 2018 which will be followed by the full commercial registration submission in 2019.

I.C.2. Submission to FDA

MON 88702 cotton falls within the scope of the 1992 FDA policy statement concerning regulation of products derived from new plant varieties, including those developed through biotechnology (U.S. FDA, 1992). Consistent with this policy, Monsanto has concluded its consultation with the FDA, identified under BNF 000160, and the agency agreed with the conclusion that MON 88702 does not raise any safety or regulatory issues with respect to its uses in human or animal food (U.S. FDA, 2018).

I.C.3. Submissions to Foreign Government Agencies

Consistent with Monsanto's commitments to Excellence Through Stewardship® (ETS)[2], Monsanto will meet applicable regulatory requirements for MON 88702 cotton in the country of intended production and for major import countries identified in the trade assessment process that have functioning regulatory systems to assure global compliance and support the flow of international trade. Monsanto will continue to monitor other countries that are key importers of cotton from the U.S., for the development of formal biotechnology approval processes. If new functioning regulatory processes are developed, Monsanto will re-evaluate its stewardship plans and make appropriate modifications to minimize the potential for trade disruption.

® Excellence Through Stewardship is a registered trademark of Excellence Through Stewardship, Washington, DC.

[2] http://www.excellencethroughstewardship.org/; Accessed on May 22, 2019.

II. THE BIOLOGY OF COTTON

The Organisation for Economic Co-operation and Development Consensus Document (OECD, 2008) on the biology of cotton (*Gossypium* spp.) provides key information on:

- general description of cotton biology, including taxonomy and morphology and use of cotton as a crop plant
- agronomic practices in cotton cultivation
- geographic centers of origin
- reproductive biology
- cultivated cotton as a volunteer weed
- inter-species/genus introgression into relatives and interactions with other organisms
- a summary of the ecology of cotton

Additional information on the biology and growth and development of cotton is available in the literature (Kohel and Lewis, 1984; OGTR, 2008; Smith and Cothren, 1999).

To support the evaluation of the plant pest potential of MON 88702 relative to conventional cotton, additional information regarding several aspects of cotton biology can be found elsewhere in this petition. This includes: agronomic practices for cotton in Section VIII; volunteer management of cotton in Section VIII.J; and inter-species/genus introgression potential in Section IX.F.

II.A. Cotton as a Crop

Cotton belongs to the genus *Gossypium*, which currently has approximately 50 species that are widely cultivated in tropical and subtropical regions around the world (OECD, 2008; Percival et al., 1999). There are four cultivated species that were domesticated independently, two of which account for greater than 95% of world cotton production. *Gossypium hirsutum* (often called upland, American, Mexican, or Acala cotton) accounts for 90% and *Gossypium barbadense* (often called extra-long staple, Pima, or Egyptian cotton) accounts for 5% of world cotton production. Due to the utility of the fibers for the production of textiles, human selection pressure on cotton has altered the plant from essentially perennial shrubs or trees with small impermeable seeds and sparse hairs to a compact annual row crop, yielding large, easily germinating seeds with white, thick, long, and strong fibers (Brubaker et al., 1999).

The four cultivated species, which are widely cultivated across the entire globe, are comprised of two diploid species *G. arboretum* and *G. herbaceum*, which evolved from Africa and the Middle East, and two allotetraploid species *G. barbadense* and *G. hirsutum*, which evolved in the Americas (Brubaker et al., 1999).

Improved modern varieties of *G. hirsutum* and *G. barbadense* are currently cultivated in the southern U.S., with *G. barbadense* grown primarily in the western states; and *G. hirsutum* produced throughout the 17 states comprising the U.S. cotton growing region, commonly referred to as the cotton belt. *G. hirsutum* comprises the vast majority of U.S. cotton production. Commercial cotton, including *G. hirsutum* and *G. barbadense*,

has a long history of agricultural production (Lee, 1984; USDA-AMS, 2001). Extra-long staple lint from *G. barbadense* is segregated and classed separately from *G. hirsutum* and is sold at a premium (USDA-AMS, 2001; USDA-NASS, 2012). However, cottonseed and cottonseed by-products (e.g., oil and meal) are not generally distinguished by species (OECD, 2008).

II.B. Characteristics of the Recipient Plant

The cotton variety used as the recipient for the DNA insertion to create MON 88702 was DP393, a non-transgenic *G. hirsutum* conventional upland variety developed by Delta and Pineland Technology Holding Company, LLC (Bridge et al., 2005), which was released in 2005 in the U.S.

II.C. Cotton as a Test System in Product Safety Assessment

DP393 was used as the conventional parental cotton comparator (referred to in this consultation document as the conventional control) in the safety assessment of MON 88702 cotton. MON 88702 and the conventional control have similar genetic backgrounds with the exception of the *mCry51Aa2* expression cassette; thus, the effect of the *mCry51Aa2* expression cassette and the expressed mCry51Aa2 protein can be assessed in an objective manner.

In addition, conventional commercial cotton varieties (referred to herein as commercial reference varieties) were used as reference materials to establish ranges of natural variability representative of commercial cotton varieties. The commercial reference varieties used at each field trial location were selected based on their availability and agronomic fit for the respective geographic region.

III. DESCRIPTION OF THE GENETIC MODIFICATION

This section provides a description of the transformation process and plasmid vector used in the development of MON 88702 cotton. Molecular analyses are an integral part of the characterization of cotton products with new traits introduced by methods of biotechnology. Vectors and methods are selected for transformation to achieve high probability of obtaining the trait of interest and integration of the introduced DNA into a single locus in the plant genome. This helps ensure that only the intended DNA encoding the desired trait(s) is integrated into the plant genome and facilitates the molecular characterization of the product. Information provided here allows for the identification of the genetic material present in the transformation vector delivered to the host plant and for an analysis of the data supporting the characterization of the DNA inserted in the plant found in Section IV.

III.A. Description of Transformation Plasmid PVGHIR508523

Plasmid PV-GHIR508523 was used for the transformation of conventional cotton to produce MON 88702. A map of the plasmid is shown in Figure III-1. The elements included in this plasmid vector are described in Table III-1. PV-GHIR508523 is approximately 14.6 kb and contains two separate T-DNAs, each delineated by Left and Right Border regions. The first T-DNA, designated as T-DNA I, contains the *mCry51Aa2* expression cassette. The second T-DNA, designated as T-DNA II, contains the *aadA* expression cassette; *aadA* acts as a selectable marker to allow selection of transformed plants and encodes an aminoglycoside-modifying enzyme that confers spectinomycin and streptomycin resistance (Fling et al., 1985), and allows selection of transformed tissue.

The backbone region of PV-GHIR508523, located outside both T-DNAs, contains two origins of replication for maintenance of the plasmid vector in bacteria (*ori-pRi*, *ori-pBR322*), a bacterial selectable marker gene (*nptII*), and a coding sequence for repressor of primer (ROP) protein for the maintenance of the plasmid vector copy number in *Escherichia coli*. A description of the genetic elements and their prefixes (e.g., B, E, P, TS, CS, T, and OR) in PV-GHIR508523 is provided in Table III-1.

During transformation, both T-DNAs were inserted into the cotton genome (Section III.B). Subsequently, traditional breeding, segregation, selection, and screening were used to isolate those plants that contained the *mCry51Aa2* expression cassette (T-DNA I) but did not contain the *aadA* expression cassette (T-DNA II) or plasmid backbone.

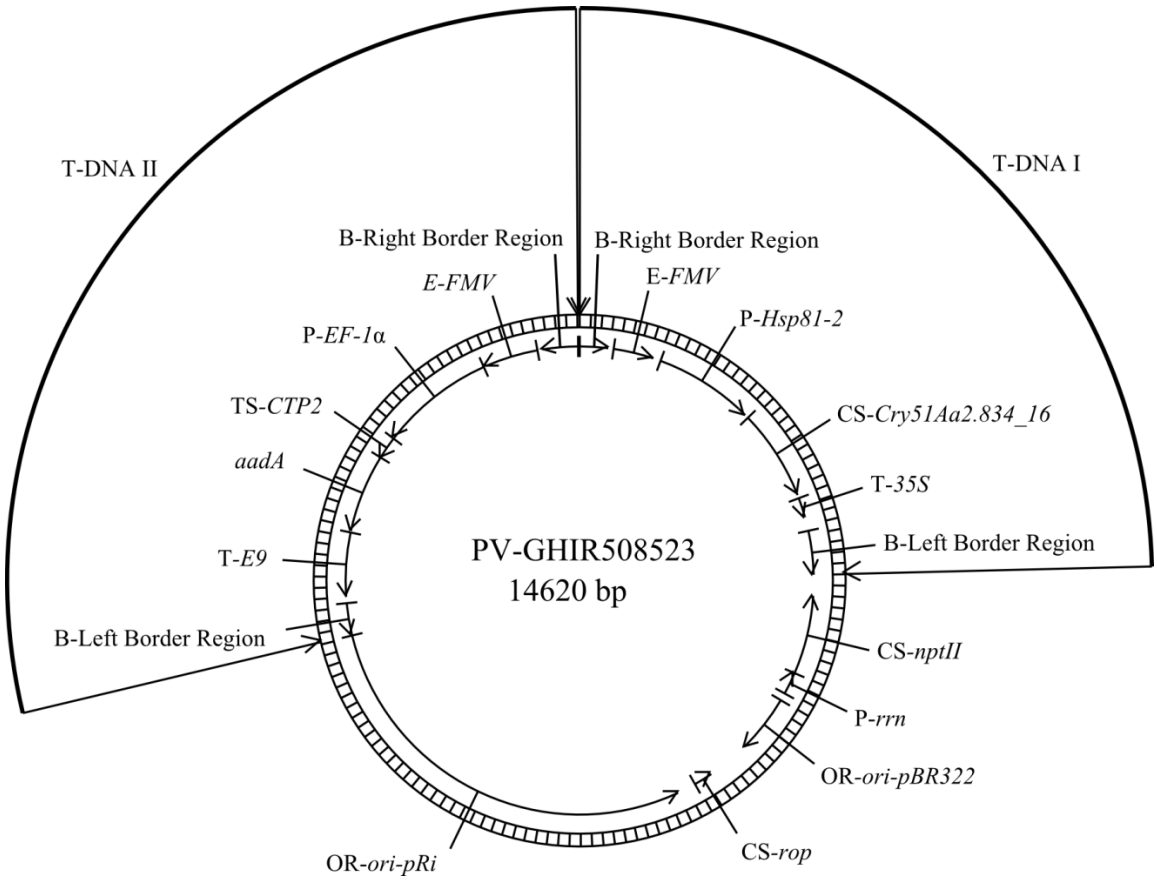


Figure III-1. Circular Map of PV-GHIR508523
A circular map of PVGHIR508523 used to develop MON 88702 cotton is shown. PVGHIR508523 contains two T–DNAs. Genetic elements are shown on the exterior of the map.

Table III-1. Summary of Genetic Elements in Plasmid Vector PV-GHIR508523

Genetic Element	Location in Plasmid Vector	Function (Reference)
T–DNA-I		
B[1]-Right Border Region	1-285	DNA region from *Agrobacterium tumefaciens* containing the right border sequence used for transfer of the T–DNA (Depicker et al., 1982; Zambryski et al., 1982).
Intervening Sequence	286-338	Sequence used in DNA cloning.
E[2]-*FMV*	339-745	Enhancer from the 35S RNA of figwort mosaic virus (FMV) (Richins et al., 1987) that enhances transcription in most plant cells (Rogers, 2000).
Intervening Sequence	746-820	Sequence used in DNA cloning.
P[3]-*Hsp81-2*	821-1828	Promoter and leader sequence for the heat shock protein 81-2 (Hsp81-2) from *Arabidopsis thaliana* that directs transcription in plant cells (Yabe et al., 1994).
Intervening Sequence	1829-1865	Sequence used in DNA cloning.
CS[4]-*Cry51Aa2.834_16*	1866-2786	Coding sequence of the modified Cry51Aa2 protein of *Bacillus thuringiensis* that provides insect resistance (Anderson et al., 2015; Baum et al., 2012; Gowda et al., 2016).
Intervening Sequence	2787-2818	Sequence used in DNA cloning
T[5]-*35S*	2819-3018	3' UTR sequence of the 35S RNA of cauliflower mosaic virus (CaMV) (Mogen et al., 1990) that directs polyadenylation in plant cells.
Intervening Sequence	3019-3156	Sequence used in DNA cloning.
B-Left Border Region	3157-3598	DNA region from *Agrobacterium tumefaciens* containing the left border sequence used for transfer of the T–DNA (Barker et al., 1983).
Vector Backbone		
Intervening Sequence	3599-3807	Sequence used in DNA cloning
CS-*nptII*	3808-4602	Coding sequence of the *neo* gene from transposon Tn*5* of *E.coli* encoding neomycin phosphotransferase II (NPT II) (Beck et al., 1982) that confers neomycin and kanamycin resistance (Fraley et al., 1983).

Table III-1. Summary of Genetic Elements in Plasmid Vector PVGHIR508523 (continued)

Genetic Element	Location in Plasmid Vector	Function (Reference)
P-*rrn*	4603-4827	Promoter of the ribosomal RNA operon from *Agrobacterium tumefaciens* (Bautista-Zapanta et al., 2002) that directs transcription in bacteria.
Intervening Sequence	4828-4903	Sequence used in DNA cloning.
OR[6]-*ori pBR322*	4904-5492	Origin of replication from plasmid pBR322 for maintenance of plasmid in *E. coli* (Sutcliffe, 1979).
Intervening Sequence	5493-5919	Sequence used in DNA cloning.
CS-*rop*	5920-6111	Coding sequence for repressor of primer protein from the ColE1 plasmid for maintenance of plasmid copy number in *E. coli* (Giza and Huang, 1989).
Intervening Sequence	6112-6299	Sequence used in DNA cloning
OR-*ori-pRi*	6300-10413	Origin of replication from plasmid pRi for maintenance of plasmid in *Agrobacterium* (Ye et al., 2011).
Intervening Sequence	10414-10420	Sequence used in DNA cloning.
T–DNA-II		
B-Left Border Region	10421-10739	DNA region from *Agrobacterium tumefaciens* containing the left border sequence used for transfer of the T–DNA (Barker et al., 1983).
Intervening Sequence	10740-10803	Sequence used in DNA cloning
T-*E9*	10804-11446	3' UTR sequence from *Pisum sativum* (pea) *rbcS* gene family encoding the small subunit of ribulose bisphosphate carboxylase protein (Coruzzi et al., 1984) that directs polyadenylation of the mRNA.
Intervening Sequence	11447-11461	Sequence used in DNA cloning.
aadA	11462-12253	Bacterial coding sequence for an aminoglycoside-modifying enzyme, 3"(9)-*O*-nucleotidyltransferase from the transposon Tn*7* (Fling et al., 1985) that confers spectinomycin and streptomycin resistance.

Table III-1. Summary of Genetic Elements in Plasmid Vector PVGHIR508523 (continued)

Genetic Element	Location in Plasmid Vector	Function (Reference)
TS[7]-*CTP2*	12254-12481	Targeting sequence of the *ShkG* gene from *Arabidopsis thaliana* encoding the EPSPS transit peptide region that directs transport of the protein to the chloroplast (Herrmann, 1995; Klee et al., 1987).
Intervening Sequence	12482-12490	Sequence used in DNA cloning
P-*EF-1α*	12491-13638	Promoter, leader, and intron sequences of the *EF–1α* gene from *Arabidopsis thaliana* encoding elongation factor EF–1α that directs transcription in plant cells (Axelos et al., 1989).
Intervening Sequence	13639-13661	Sequence used in DNA cloning.
E-*FMV*	13662-14198	Enhancer from the 35S RNA of figwort mosaic virus (FMV) (Richins et al., 1987) that enhances transcription in most plant cells (Rogers, 2000).
Intervening Sequence	14199-14248	Sequence used in DNA cloning
B-Right Border Region	14249-14605	DNA region from *Agrobacterium tumefaciens* containing the right border sequence used for transfer of the T–DNA (Depicker et al., 1982; Zambryski et al., 1982).
Vector Backbone		
Intervening Sequence	14606-14620	Sequence used in DNA cloning.

[1] B, Border
[2] E, Enhancer
[3] P, Promoter
[4] CS, Coding Sequence
[5] T, Transcription Termination Sequence
[6] OR, Origin of Replication
[7] TS, Targeting Sequence

III.B. Transformation System

MON 88702 cotton was developed through *Agrobacterium*-mediated transformation of conventional cotton, based on the method described by (Chen et al., 2014), which allows for the generation of transformed plants without the utilization of callus. Briefly, meristem tissues were excised from the embryos of germinated conventional seed. After co-culturing with *Agrobacterium* carrying the transformation plasmid, the meristems were placed on selection medium containing spectinomycin, carbenicillin disodium salt and cefotaxime sodium salt, to inhibit the growth of untransformed plant cells and excess *Agrobacterium*. The meristems were then placed in media conducive to shoot development followed by a transfer to a Jiffy Carefree® propagation plug for root development. Rooted plants with normal phenotypic characteristics were selected and transferred to soil for growth and further assessment. As demonstrated in this petition, the use of disarmed *A. tumefaciens* strain AB33, a designated plant pest, as the transformation vector has not imparted plant pest characteristics to MON 88702 cotton.

The R0 plants generated through this transformation process were self-pollinated to produce R1 seed, and the unlinked insertions of T-DNA I and T-DNA II were segregated. R1 plants that were positive for the mCry51Aa2 expression cassette (T-DNA I) and did not contain the *aadA* expression cassette (T-DNA II) were identified by a quantitative polymerase chain reaction (PCR)-based analysis. Subsequently, R1 plants homozygous for T-DNA I were selected for further development and their progenies were subjected to further molecular analysis, insect efficacy and phenotypic assessments. As is typical of a commercial event production and selection process, hundreds of different transformation events (regenerants) were generated in the laboratory using PV-GHIR508523. After careful selection and evaluation of these events in the laboratory, greenhouse and field, MON 88702 was selected as the lead event based on superior agronomic, phenotypic, and molecular characteristics. Studies on MON 88702 cotton were initiated to further characterize the genetic insertion and the expressed products, and to establish the food and feed safety and no increased plant pest risk potential compared to conventional cotton. The major development steps of MON 88702 cotton are depicted in Figure III-2.

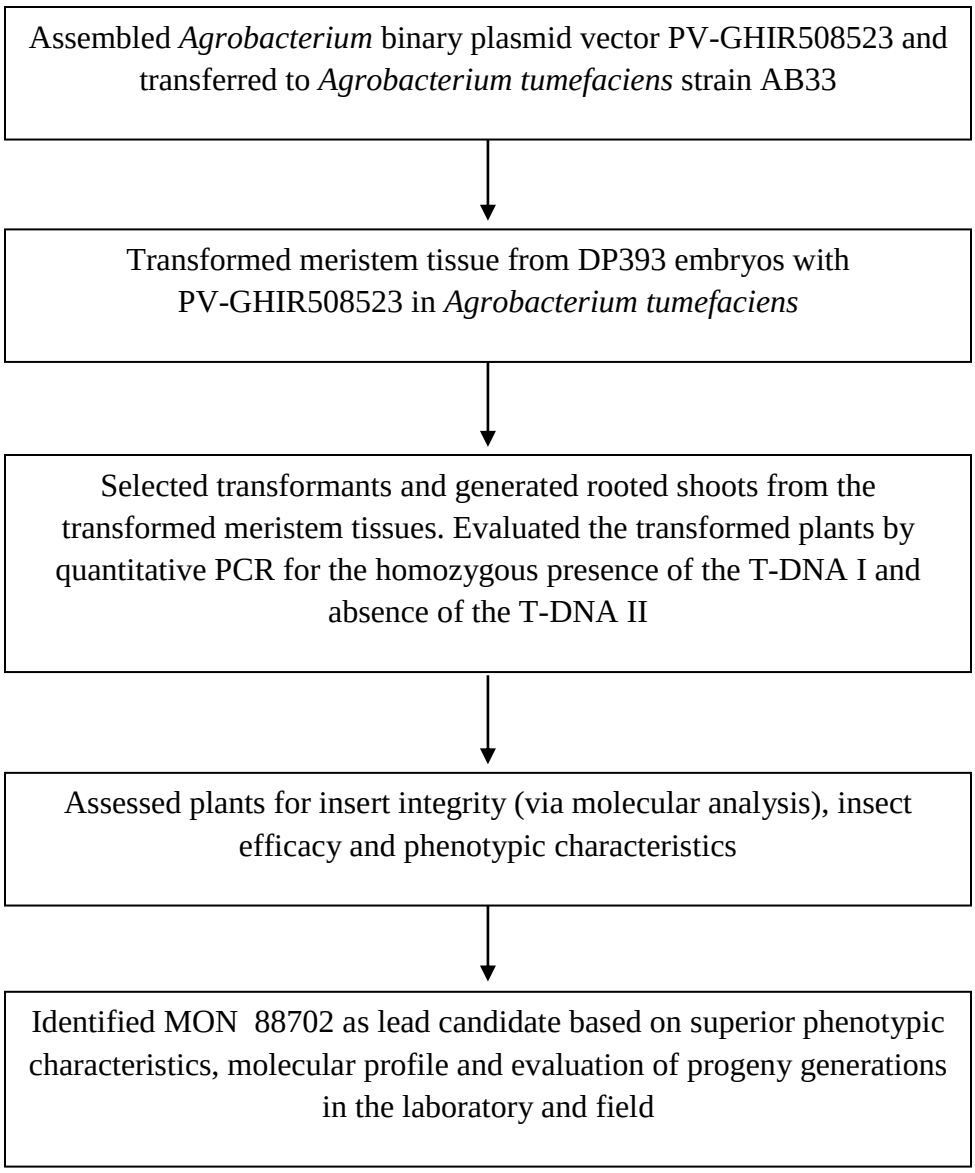


Figure III-2. Schematic of the Development of MON 88702 Cotton

III.C. The *mCry51Aa2* Coding Sequence and mCry51Aa2 Protein

The *mCry51Aa2* expression cassette in MON 88702 encodes the mCry51Aa2 protein, which consists of a single polypeptide (Figure III-3). The mCry51Aa2 protein expressed by MON 88702 is highly homologous (approximately 96% sequence similarity) to the amino acid sequence of wild-type Cry51Aa2 from *Bacillus thuringiensis*, with 8 amino acid substitutions (F46S, Y54H, S95A, F147S, Q149E, S167R, P219R, R273W) and a deletion of 3 amino acids (Δ196-198) (Gowda et al., 2016).

```
MAILDLKSLV LNAINYWGPK NNNGIQGGDF GYPISEKQID TSIITSTHPR    50
LIPHDLTIPQ NLETIFTTTQ VLTNNTDLQQ SQTVSFAKKT TTTTATSTTN   100
GWTEGGKISD TLEEKVSVSI PFIGEGGGKN STTIEANFAH NSSTTTSQEA   150
STDIEWNISQ PVLVPPRKQV VATLVIMGGN FTIPMDLMTT IDSTEHYSGY   200
PILTWISSPD NSYSGRFMSW YFANWPNLPS GFGPLNSDNT VTYTGSVVSQ   250
VSAGVYATVR FDQYDIHNLW TIEKTWYARH ATLHNGKKIS INNVTEMAPT   300
SPIKTN                                                   306
```

Figure III-3. The mCry51Aa2 Protein Sequence Encoded by MON 88702

III.D. Regulatory Sequences

The *mCry51Aa2* coding sequence in T-DNA I is under the regulation of the *FMV* enhancer, the *Hsp81-2* promoter, and the *35S CaMV* 3' untranslated region. The *FMV* sequence is a genetic element of the 35S RNA of figwort mosaic virus (FMV) (Richins et al., 1987), which enhances transcription in most plant cells (Rogers, 2000). The *Hsp81-2* sequence is the promoter for the heat shock protein *81-2* gene of *A. thaliana* (Yabe et al., 1994), which functions to direct transcription in plant cells. The *35S* 3' sequence is the 3' untranslated region of the 35S RNA of cauliflower mosaic virus (CaMV), which directs polyadenylation of mRNA in plant cells (Mogen et al., 1990).

T-DNA II contains the *aadA* coding sequence under the regulation of the *FMV* enhancer (described above), the *EF-1α* promoter, the *CTP2* targeting sequence, and the *T-E9* untranslated region. The *EF-1α* promoter consists of the leader, promoter, and intron sequences from *A. thaliana* encoding elongation factor *EF-1α (Axelos et al., 1989)*, which functions to direct transcription in plant cells. The *CTP2* targeting sequence is from the *ShkG* gene encoding the EPSPS transit peptide region in *A. thaliana* (Herrmann, 1995; Klee et al., 1987), which functions to direct transport of the protein to the chloroplast. The *E9* 3' sequence is the 3' untranslated region from *P. sativum* (pea) *rbcS* gene family encoding the small subunit of ribulose bisphosphate carboxylase protein (Coruzzi et al., 1984), which functions to direct polyadenylation of the mRNA.

III.E. T-DNA Border Regions

PV-GHIR508523 contains Left and Right Border regions (Figure III-1 and Table III-1) that were derived from *A. tumefaciens* plasmids. The border regions each contain a 24-25 bp nick site that is the site of DNA exchange during transformation (Barker et al., 1983; Depicker et al., 1982; Zambryski et al., 1982). The border regions separate the T-DNA from the plasmid backbone region and are involved in the efficient transfer of T-

DNA I into the cotton genome. Because PV-GHIR508523 is a two T-DNA vector, it contains two Left Border regions and two Right Border regions, where one border region set flanks T-DNA I and the other border region set flanks T-DNA II.

III.F. Genetic Elements Outside the T-DNA Border Regions

Genetic elements that exist outside of the T-DNA border regions are those that are essential for the maintenance or selection of PV-GHIR508523 in bacteria and are referred to as plasmid backbone. The origin of replication, *ori-pRi*, is required for the maintenance of the plasmid in *Agrobacterium* and is derived from the broad host plasmid *pRi* (Ye et al., 2011). The origin of replication, *ori-pBR322*, is required for the maintenance of the plasmid in *E. coli* and is derived from the plasmid vector pBR322 (Sutcliffe, 1979). Coding sequence *rop* encodes the repressor of primer (ROP) protein which is necessary for the maintenance of plasmid vector copy number in *E. coli* (Giza and Huang, 1989). The *rrn* promoter is the promoter for the ribosomal RNA operon from *A. tumefacien*s (Bautista-Zapanta et al., 2002). The selectable marker *nptII* is the coding sequence for an enzyme from transposon Tn5 that confers neomycin and kanamycin resistance (Fraley et al., 1983) in *E. coli* and *Agrobacterium* during molecular cloning. Because these elements are outside the border regions, they are not expected to be transferred into the cotton genome. The absence of the backbone and other unintended plasmid sequence in MON 88702 cotton was confirmed by sequencing and bioinformatic analyses (described in Section IV.B).

IV. CHARACTERIZATION OF THE GENETIC MODIFICATION

This section contains a comprehensive molecular characterization of the genetic modification present in MON 88702 cotton. It provides information on the DNA insertion into the plant genome of MON 88702 and additional information relative to the arrangement and stability of the introduced genetic material. The information provided in this section addresses the relevant factors in Codex Plant Guidelines, Section 4, paragraphs 30, 31, 32, and 33 (Codex Alimentarius, 2009).

IV.A. Description of Methodology Used to Characterize MON 88702

A schematic representation of the Next Generation Sequencing (NGS) methodology and the basis of the characterization using NGS and PCR sequencing is illustrated in Figure IV-1 below. Appendix B provides an additional overview of these techniques, their use in DNA characterization in cotton plants and the materials and methods.

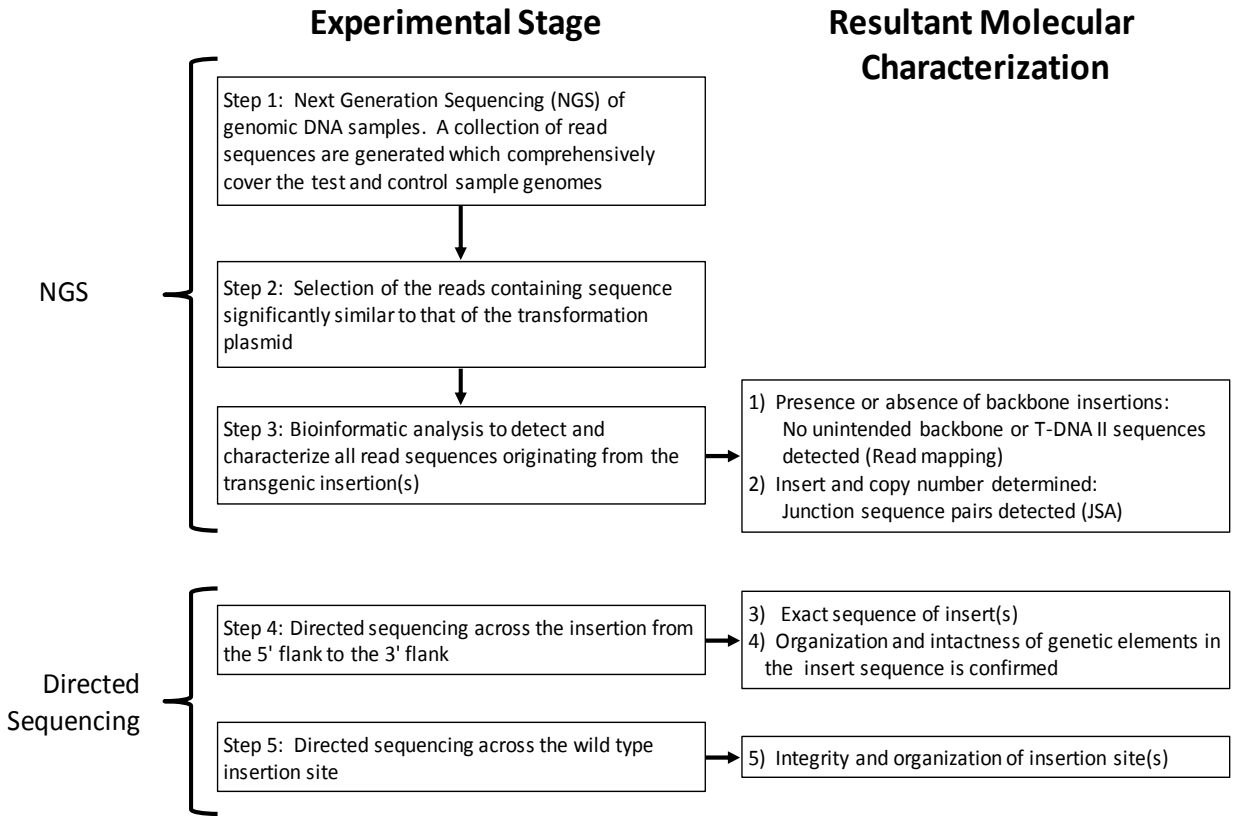


Figure IV-1. Molecular Characterization using Sequencing and Bioinformatics

Genomic DNA from MON 88702 and the conventional control was sequenced using NGS technology that produces a set of short, randomly distributed sequence reads that comprehensively cover MON 88702 and control genomes (Step 1). Utilizing these genomic sequence reads, bioinformatics searches are conducted to identify and select all sequence reads that are significantly similar to the sequence of the transformation plasmid (Step 2). These selected reads are then mapped and analyzed to determine the presence/absence of transformation plasmid backbone and/or T-DNA II sequences, identify insert junctions, and to determine the insert and copy number (Step 3). Overlapping PCR products which spanned any identified insert and the DNA regions flanking the 5' and 3' ends of the insert (Step 4 and 5, respectively) were sequenced to allow for detailed characterization of the inserted DNA and the insertion site including flanking genomic DNA.

Genomic DNA from five breeding generations of MON 88702 (Figure IV-4) and the conventional control was isolated from seed and prepared for sequencing using the KAPA Hyper Prep Kit (Kapa Biosystems). For material and method details see Appendix B. These genomic DNA libraries were used to generate short (~125 bp) randomly distributed sequencing reads of the cotton genome (see Figure IV-1).

The NGS method was used to characterize the genomic DNA from MON 88702 and the conventional control using sequencing reads generated in sufficient numbers to ensure comprehensive coverage of the sample genomes. It has been previously demonstrated that 75× coverage of the soybean genome is adequate to provide comprehensive coverage and ensure detection of inserted DNA (Kovalic et al., 2012). Similarly, it is expected that 75× will provide comprehensive coverage of the cotton genome. To confirm sufficient sequence coverage of the genome, the 125-mer sequence reads are analyzed to determine the coverage of a known single-copy endogenous gene, demonstrating the depth of coverage (the median number of times each base of the genome is independently sequenced). The level of sensitivity of this method was demonstrated by detection of a positive control spike that is randomly sampled at 1 and $1/10^{th}$ copy-per-genome equivalent; this confirms the method's ability to detect any sequences derived from the transformation plasmid that may be present in MON 88702. Bioinformatics analysis was then used to select sequencing reads that contained sequences significantly similar to the transformation plasmid, and these were analyzed in depth to determine the number of DNA inserts. NGS was run on all five generations of MON 88702 samples and the conventional control. The NGS results are shown in Sections IV.B and IV.E.

To demonstrate sufficient sequence coverage the 125-mer sequence reads were analyzed by mapping all reads to a single copy locus for acyl carrier protein (*acp1),* used as a positive control for sequencing depth, selected from the *Gossypium hirsutum* genome in each of the five breeding generations. The analysis of sequence coverage plots showed that the depth of coverage was 80× or greater for the five generations of MON 88702 (R3 through R7) and the conventional control (Appendix B, Table B-1 and Table B-2).

To demonstrate the method's ability to detect any sequences derived from the PVGHIR508523 transformation plasmid, a sample of conventional control genomic DNA spiked with PVGHIR508523 DNA, utilized as a positive control, and was analyzed by NGS and bioinformatics. The level of sensitivity of this method was demonstrated to a level of one genome equivalent and $1/10^{th}$ genome equivalent, 100% nucleotide identity was observed over 100% of PVGHIR508523. This result demonstrates that all nucleotides of PVGHIR508523 are observed by the sequencing and bioinformatic assessments performed and that a detection level of at least $1/10^{th}$ genome equivalent was achieved for the plasmid DNA sequence assessment.

The DNA inserts of MON 88702 were determined by mapping of sequencing reads relative to the transformation plasmid and identifying junctions and unpaired read mappings adjacent to the junctions. Examples of the five types of NGS reads are shown in Figure IV-2. The junctions of the DNA insert and the flanking DNA are unique for each insertion (Kovalic et al., 2012). Therefore, insertion sites can be recognized by analyzing for sequence reads containing such junctions.

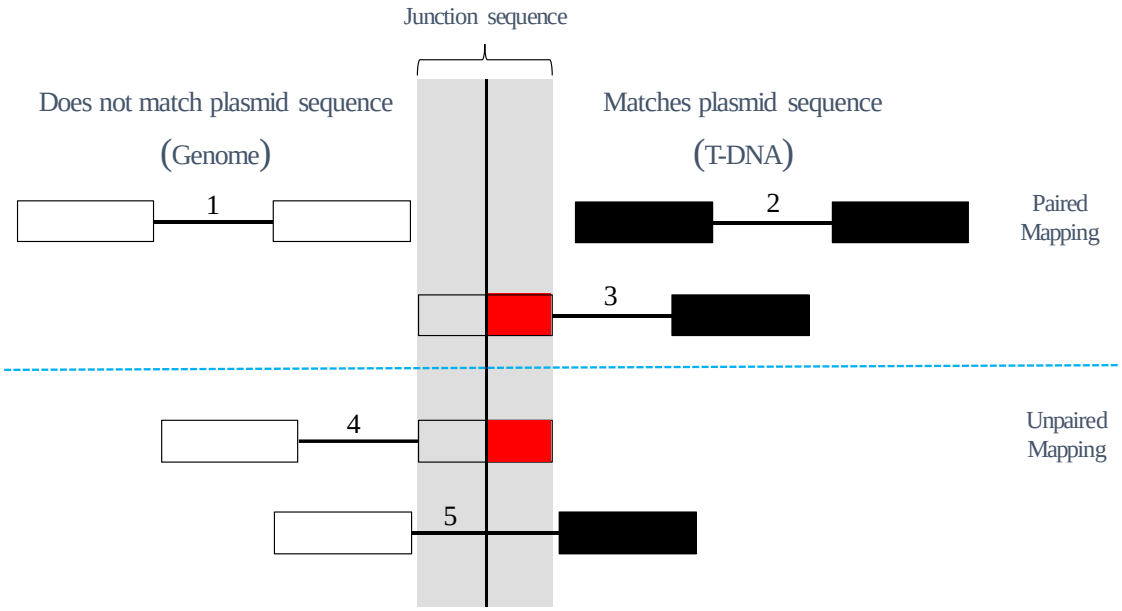


Figure IV-2. Five Types of NGS Reads

NGS sequencing yields data in the form of read pairs where sequence from each end of a size selected DNA fragment is obtained. Depicted above are five types of sequencing reads/read pairs generated by NGS sequencing which can be found spanning or outside of junction points. Sequence boxes are color-filled if it matches with plasmid sequence, and empty if it matches with genomic sequence. Grey highlighting indicates sequence reads spanning the junction. Junctions are detected by examining the NGS data for reads having portions of plasmid sequences that span less than the full read, as well as reads mapping adjacent to the junction points where their mate pair does not map to the plasmid sequence. The five types of sequencing reads/read pairs are:

1) Paired and unpaired reads mapping to genomic sequence outside of the insert, greater than 99.999% of collected reads fall into this category and are not further evaluated in this analysis.

2) Paired reads mapping entirely to the transformation plasmid sequence, such reads reveal the presence of transformation plasmid sequence *in planta*.

3) Paired reads where one read maps entirely within the inserted DNA and the other read maps partially to the insert (indicating a junction point).

4) Single read mapping partially to the transformation plasmid DNA sequence (indicating a junction point) where its mate maps entirely to the genomic flanking sequence.

5) Single read mapping to the transformation plasmid DNA sequence where its mate maps entirely to genomic flanking sequence, such reads are part of the junction signature.

Directed sequencing (locus-specific PCR and DNA sequencing analyses, Figure IV-1, Step 4) complements the NGS method. Sequencing of the insert and flanking genomic

DNA determined the complete sequence of the insert and flanks by evaluating if the sequence of the insert was identical to the corresponding sequence from the T-DNA I in PV-GHIR508523, and if each genetic element in the insert was intact. It also characterizes the flank sequence beyond the insert corresponding to the genomic DNA of the transformed cotton. Results are described in Sections IV.B and IV.C; methods are presented in Appendix B.

The stability of the T-DNA I present in MON 88702 across multiple breeding generations was evaluated by NGS as described above. This information was used to determine the number and identity of the DNA inserts in each generation. For the single copy T-DNA I insert, two junction sequences are expected. In the case of an event where a single insert is stably inherited over multiple breeding generations, two identical junction sequence classes would be detected in each of the breeding generations tested. Results are described in Section IV.E; methods are presented in Appendix B.

Segregation analysis of the T-DNA I was conducted to determine the inheritance and generational stability of the insert in MON 88702. Segregation analysis corroborates the insert stability demonstrated by NGS and independently establishes the genetic behavior of the T-DNA I. Results are described in Section IV.F.

IV.B. Determination of DNA Insert and Copy Number

The number of insertion sites of PV-GHIR508523 DNA in MON 88702 was assessed by performing NGS on MON 88702 genomic DNA using the R4 generation (Figure IV-4). A plasmid map of PV-GHIR508523 is shown in Figure III-1.

A schematic representation of the insert and flanking sequences in MON 88702 is shown in Figure IV-3. Table IV-1 provides a description of the genetic elements present in MON 88702. For full details on materials and methods see Appendix B.

Table IV-1. Summary of Genetic Elements in MON 88702

Genetic Element	Location in Sequence	Function (Reference)
Flanking DNA	1-1642	Flanking DNA
B[1]-Right Border Region*r1*	1643-1710	DNA region from *Agrobacterium tumefaciens* containing the right border sequence used for transfer of the T–DNA (Depicker et al., 1982; Zambryski et al., 1982).
Intervening Sequence	1711-1763	Sequence used in DNA cloning.
E[2]-*FMV*	1764-2170	Enhancer from the 35S RNA of figwort mosaic virus (FMV) (Richins et al., 1987) that enhances transcription in most plant cells (Rogers, 2000).
Intervening Sequence	2171-2245	Sequence used in DNA cloning
P[3]-*Hsp81-2*	2246-3253	Promoter and 5′ UTR leader sequence for the heat shock protein 81-2 (Hsp81-2) from *Arabidopsis thaliana* that directs transcription in plant cells.
Intervening Sequence	3254-3290	Sequence used in DNA cloning.
CS[4]-*Cry51Aa2.834_16*	3291-4211	Coding sequence of the modified Cry51Aa2 protein of *Bacillus thuringiensis* that provides insect resistance (Anderson et al., 2015; Baum et al., 2012; Gowda et al., 2016).
Intervening Sequence	4212-4243	Sequence used in DNA cloning.
T[5]-*35S*	4244-4443	3' UTR sequence of the 35S RNA of cauliflower mosaic virus (CaMV) (Mogen et al., 1990) that directs polyadenylation in plant cells.
Intervening Sequence	4444-4581	Sequence used in DNA cloning.
B- Left Border Region*r1*	4582-4785	DNA region from *Agrobacterium tumefaciens* containing the left border sequence used for transfer of the T–DNA (Barker et al., 1983).
Flanking DNA	4786-6748	Flanking DNA.

[1] B, Border
[2] E, Enhancer
[3] P, Promoter
[4] CS, Coding Sequence
[5] T, Transcription Termination Sequence
[r1] Superscript in Left and Right Border Regions indicate that the sequence in MON 88702 was truncated compared to the sequences in PVGHIR508523

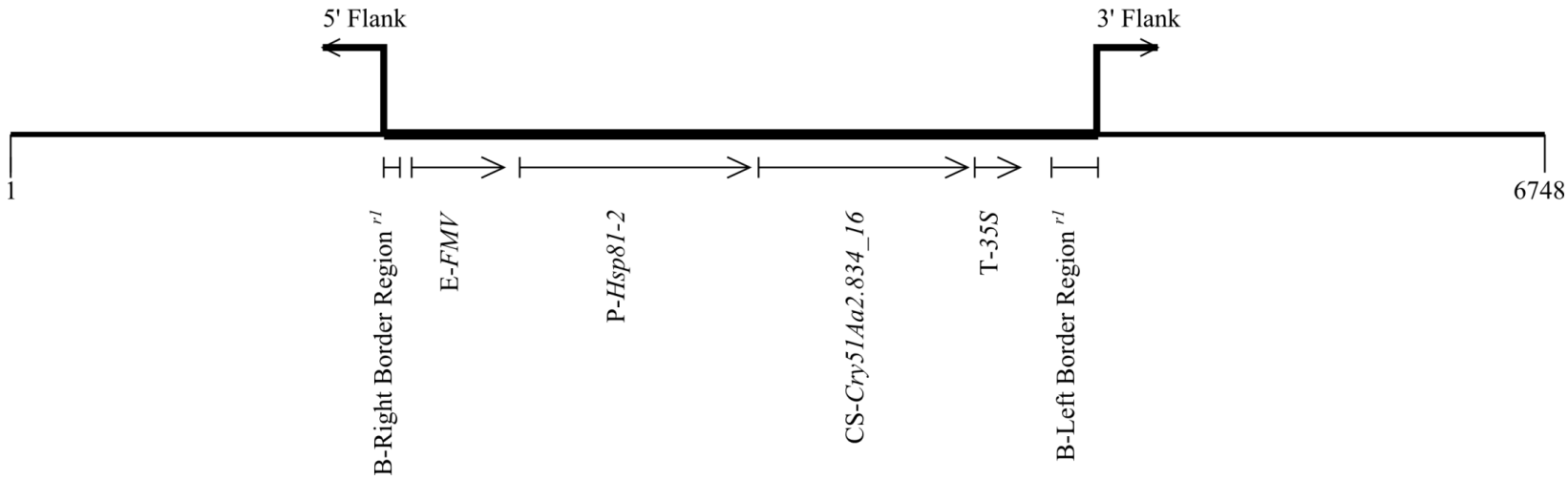


Figure IV-3. Schematic Representation of the Insert and Flanking Sequences in MON 88702

DNA derived from T-DNA of PVGHIR508523 integrated in MON 88702. Right-angled arrows indicate the ends of the integrated T-DNA and the beginning of the flanking sequence. Identified on the map are genetic elements within the insert. This schematic diagram is not drawn to scale.

[r1] Superscripts in Left and Right Border Regions indicate that the sequence in MON 88702 was truncated compared to the sequences in PVGHIR508523.

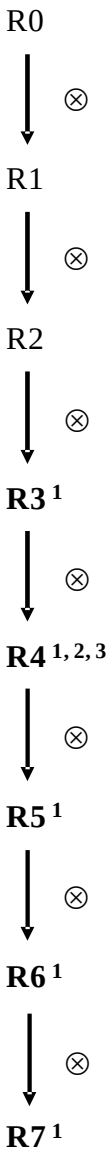


Figure IV-4. Breeding History of MON 88702

The generations used for molecular characterization and insert stability analyses are indicated in bold text. R0 corresponds to the transformed plant, ⊗ designates self-pollination.

[1]Generations used to confirm insert stability
[2] Generation used for molecular characterization
[3] Generation used for breeding commercial varieties of MON 88702

As T-DNA from plasmid PV-GHIR508523 was transformed into the parental variety DP393 to produce MON 88702, any DNA inserted into MON 88702 will consist of sequences that are similar to the PV-GHIR508523 DNA sequence. Complete analysis of only the sequence reads that have similarity to plasmid PV-GHIR508523 (Figure IV-1, Step 2) is sufficient to characterize the DNA from PV-GHIR508523 inserted in MON 88702. Any inserted transformation plasmid vector sequence, regardless of origin, either T-DNA I, T-DNA II, or backbone, can be identified by mapping sequence reads to the transformation plasmid vector sequence, while the number of inserted DNA sequences can be determined by identifying the number of junction sequences. Therefore, the NGS method described above used the entire plasmid vector sequence as a query to determine the DNA insertion site number.

Unlike the traditional Southern blot analysis that separately hybridizes T-DNA or backbone probes, NGS uses identification of sequence reads that match PV-GHIR508523 to determine T-DNA I presence and insert number, as well as the absence of backbone, T-DNA II, or unintended sequences. This alternative method can be used to reach the same conclusions regarding the number of inserts, and presence or absence of backbone or T-DNA II that can be determined using traditional Southern blots (Kovalic et al., 2012).

Using established criteria (described in Appendix B), sequence reads similar to PV-GHIR508523 were selected from MON 88702 sequence datasets and mapped relative to the transformation plasmid sequence in order to identify junction sequences (Figure IV-6). PV-GHIR508523 sequences were also compared against the conventional control sequence dataset.

No reads from the conventional control dataset were found to map with T-DNA I, T-DNA II, OR-*ori-pRi*, CS-*nptII* or P-*rrn*. However, a small number of alignments were found to align with OR-*ori-pBR322* or CS-*rop* sequences (Figure IV-5). The sporadic low level detection of plasmid sequences such as OR-*ori-pBR322* has previously been described (Zastrow-Hayes et al., 2015), and reported (see Supplemental Figure S1 in (Yang et al., 2013) and is due to the presence of environmental bacteria in tissue samples used in the preparation of genomic DNA used for library construction. Despite the low-level presence of sequence from environmental bacteria, altogether these results indicate the expected absence of inserted transformation plasmid DNA in the control.

When reads from the MON 88702 (R4) dataset were aligned with the transformation plasmid sequence, large numbers of reads mapped to T-DNA I, E-*FMV* contained in T-DNA II, and a significantly smaller number to OR-*ori-pBR322* and CS-*rop* contained in the transformation plasmid backbone.

The mapping of large numbers of reads (> 2000) from the MON 88702 (R4) dataset to T-DNA I is expected and fully consistent with the presence of the inserted DNA. Likewise, the mapping to E-*FMV* in T-DNA II is not unexpected as the E-*FMV* sequence is contained within both T-DNA I and T-DNA II. Since these sequences are identical, the mapped read mate pairs were used to distinguish their true mapping location. Since all pairs which were not fully contained within the E-*FMV* region had their mates map to T-DNA I and not T-DNA II, these reads were uniquely assigned to T-DNA I. Furthermore,

those paired reads fully contained in the E-*FMV* region that could map to either T-DNA I or T-DNA II were also uniquely assigned to T-DNA I. Consequently, no reads in the MON 88702 R4 generation dataset were identified that uniquely aligned with the plasmid backbone or T-DNA II (Figure IV-6). The small number of reads mapping with OR-*ori-pBR322* and CS-*rop* are comparable to those previously described in the conventional control dataset (Figure IV-5). As a result, it is concluded that MON 88702 (R4) does not contain inserted sequence from the transformation plasmid backbone or T-DNA II.

To determine the insert number in MON 88702 (R4), selected reads mapping to T-DNA I were analyzed to identify junctions. This bioinformatic analysis is used to identify partially matched reads at the of ends of insertions. The number of unique junctions determined by this analysis are shown in Table IV-2.

Table IV-2. Unique Junction Results

Sample	Junctions Detected
MON 88702 (R4)	2
DP393	0

The location and orientation of the flanking DNA relative to the T-DNA I insert determined for MON 88702 (as described in Section IV.C) are illustrated in Figure IV-3. There are two junctions identified in MON 88702, both contain the T-DNA I border sequence joined to adjacent flanking sequence. As such they represent the sequences at the junctions of the intended T-DNA I insert and the adjacent flanking sequence.

Complete alignment to the full flank/insert sequence confirms that both of these junctions originate from the same locus of the MON 88702 genome and are linked by contiguous, known DNA that makes up the single insert.

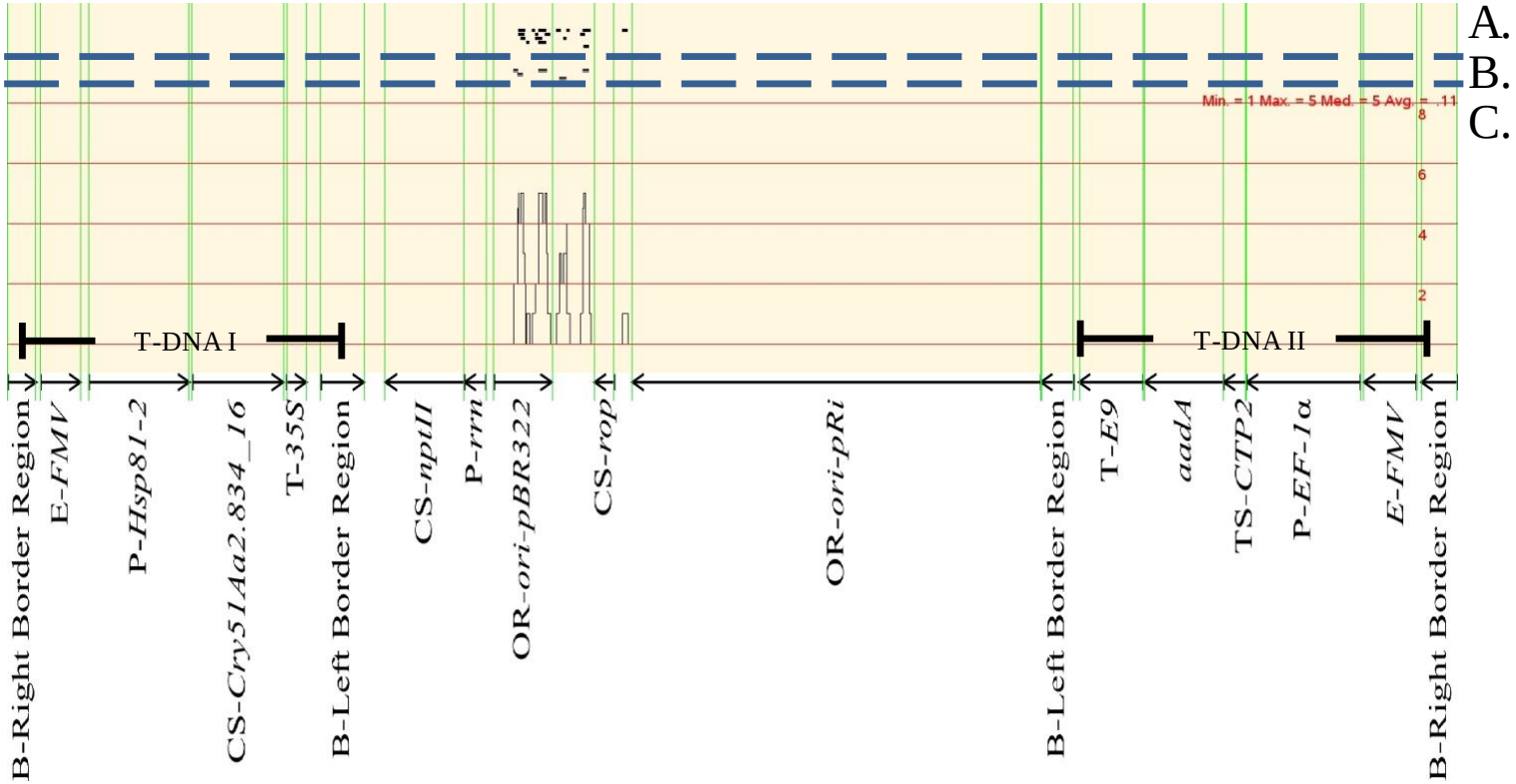


Figure IV-5. Read Mapping of Conventional Cotton Versus PV−GHIR508523

Panel A shows the location of unpaired mapped reads, Panel B shows paired mapped reads, and Panel C shows a representation of combined read depth for unpaired and paired reads.

Vertical lines show genetic element boundaries.

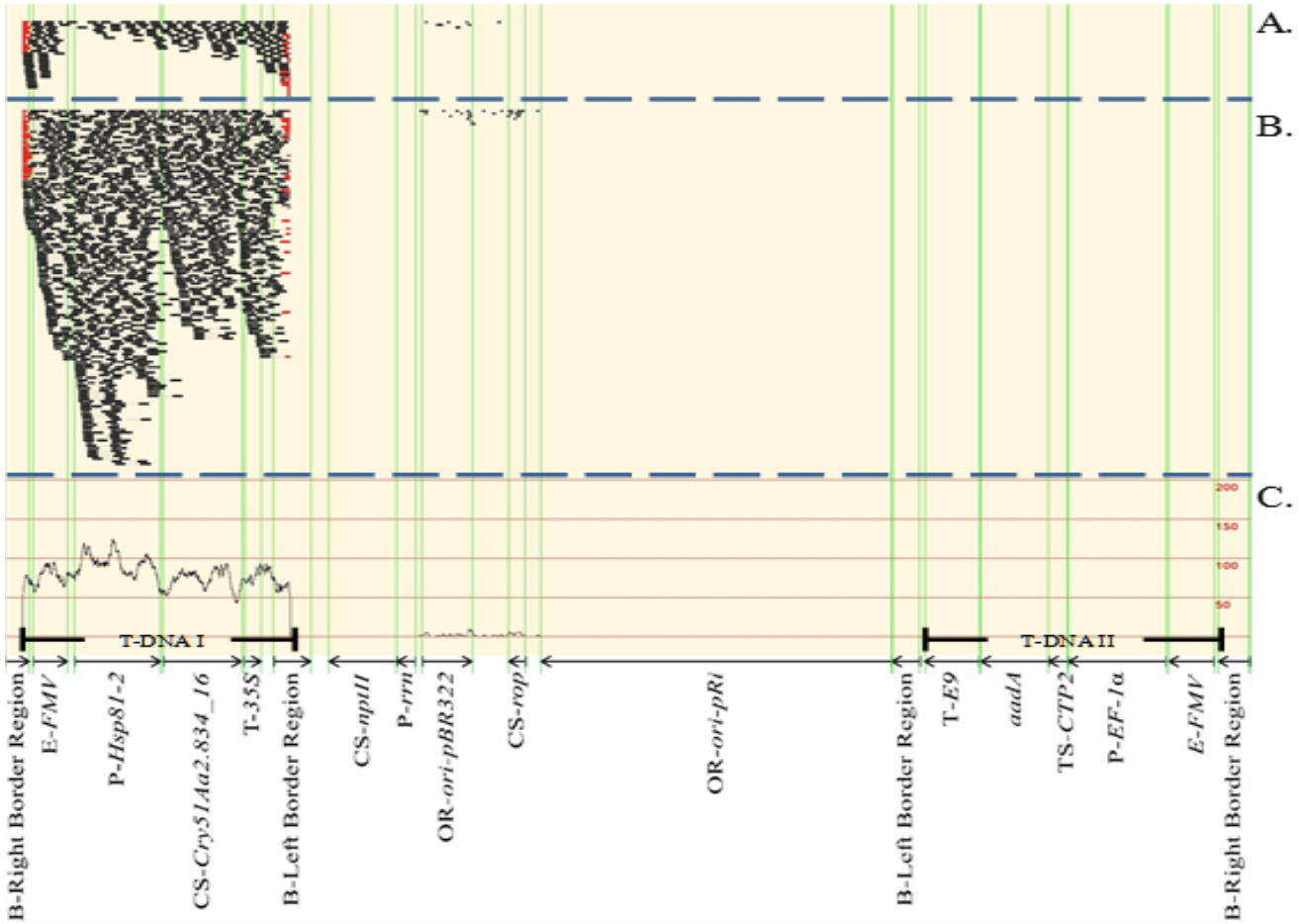


Figure IV-6. Read Mapping of MON 88702 (R4) Versus PV−GHIR508523

Panel A shows the location of unpaired mapped reads. Panel B shows paired mapped reads and Panel C shows a representation of combined read depth for unpaired and paired reads. Vertical lines show genetic element boundaries. The region of flank junction sequences that aligns with transformation plasmid is shown in red.

IV.C. Organization and Sequence of Insert and Adjacent DNA

The intactness and organization of the elements within the DNA insert in the R4 generation of MON 88702 was confirmed by using PCR to amplify and subsequently sequence two overlapping DNA amplicons that span the entire insert and the associated flanking DNA sequence. The positions of the PCR products relative to the insert, as well as the results of the PCR analyses, are shown in Figure IV-7. The amplified PCR products were subjected to DNA sequencing analyses. The results of this analysis confirm that the MON 88702 insert is 3,143 bp, that each genetic element (except for the T-DNA border regions) in the insert is intact, and the sequence of the insert is identical to the corresponding sequence in PV-GHIR508523. This analysis also shows that only T-DNA I elements (described in Table IV-1) were present. Moreover, the result, together with the conclusion of single DNA insert detected by NGS, demonstrated that no PV-GHIR508523 backbone or T-DNA II elements are present in MON 88702.

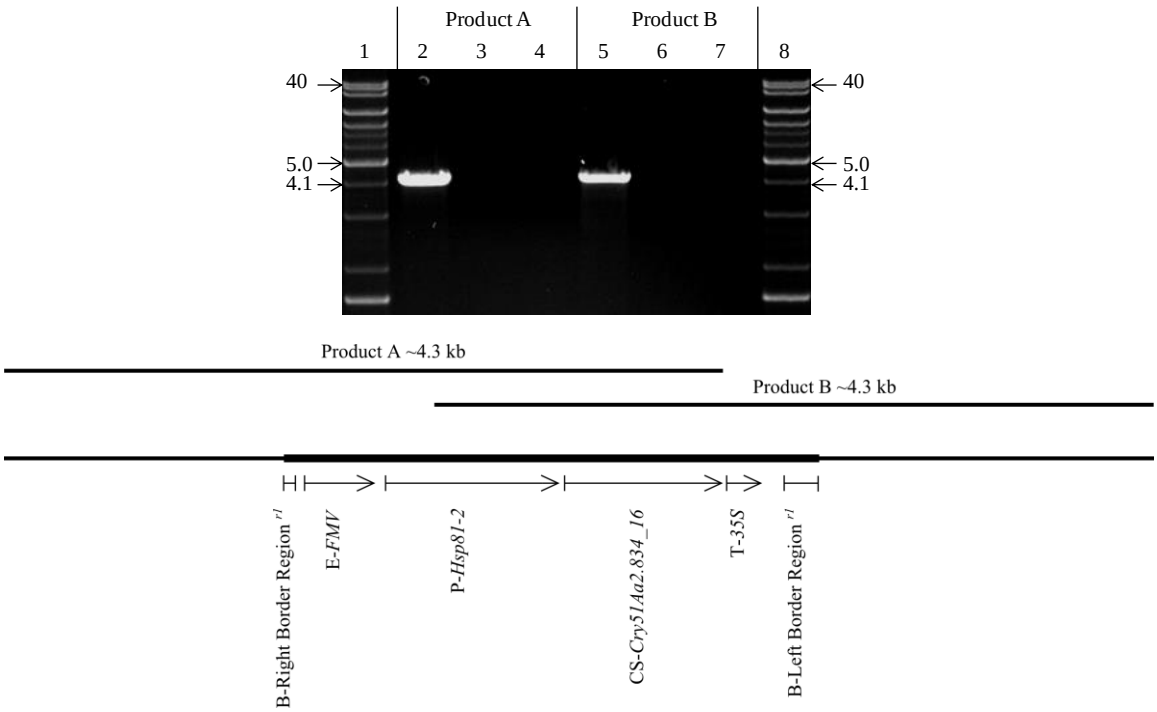


Figure IV-7. Overlapping PCR Analysis across the Insert in MON 88702

PCR was performed on both conventional control genomic DNA and genomic DNA of the R4 generation of MON 88702 using two pairs of primers to generate overlapping PCR fragments from MON 88702 for sequencing analysis. To verify the PCR products, 2 µl of each of the PCR reactions was loaded on the gel. The expected product size for each amplicon is provided in the illustration of the insert in MON 88702 that appears at the bottom of the figure. This figure is a representative of the data generated in the study. Lane designations are as follows:

Lane	
1	1Kb DNA Extension Ladder
2	MON 88702
3	Conventional Control
4	No template control
5	MON 88702
6	Conventional Control
7	No template control
8	1Kb DNA Extension Ladder

Arrows on the agarose gel photograph denote the size of the DNA, in kilobase pairs, obtained from the 1Kb DNA Extension Ladder (Invitrogen) on the ethidium bromide stained gel.

r1 Superscript in Left and Right Border Regions indicate that the sequence in MON 88702 was truncated compared to the sequences in PVGHIR508523.

IV.D. Sequence of the Insertion Site

PCR and sequence analysis were performed on genomic DNA extracted from the conventional control to examine the insertion site in conventional cotton (see Figure IV-1, Step 5). The PCR was performed with one primer specific to the genomic DNA sequence flanking the 5' end of the MON 88702 insert paired with a second primer specific to the genomic DNA sequence flanking the 3' end of the insert (Figure IV-8). A sequence comparison between the PCR product generated from the conventional control and the sequence generated from the 5' and 3' flanking sequences of MON 88702 indicates that 244 bases of cotton genomic DNA were deleted and four bases inserted in the MON 88702 3' flanking sequence during integration of the T-DNA I. The remainder of the flanks in MON 88702 are identical to the conventional control. Such changes naturally occur in plants during conventional breeding practices and are also common during plant transformation (Anderson et al., 2016). These changes presumably resulted from double stranded break repair mechanisms in the plant during *Agrobacterium*-mediated transformation process (Salomon and Puchta, 1998).

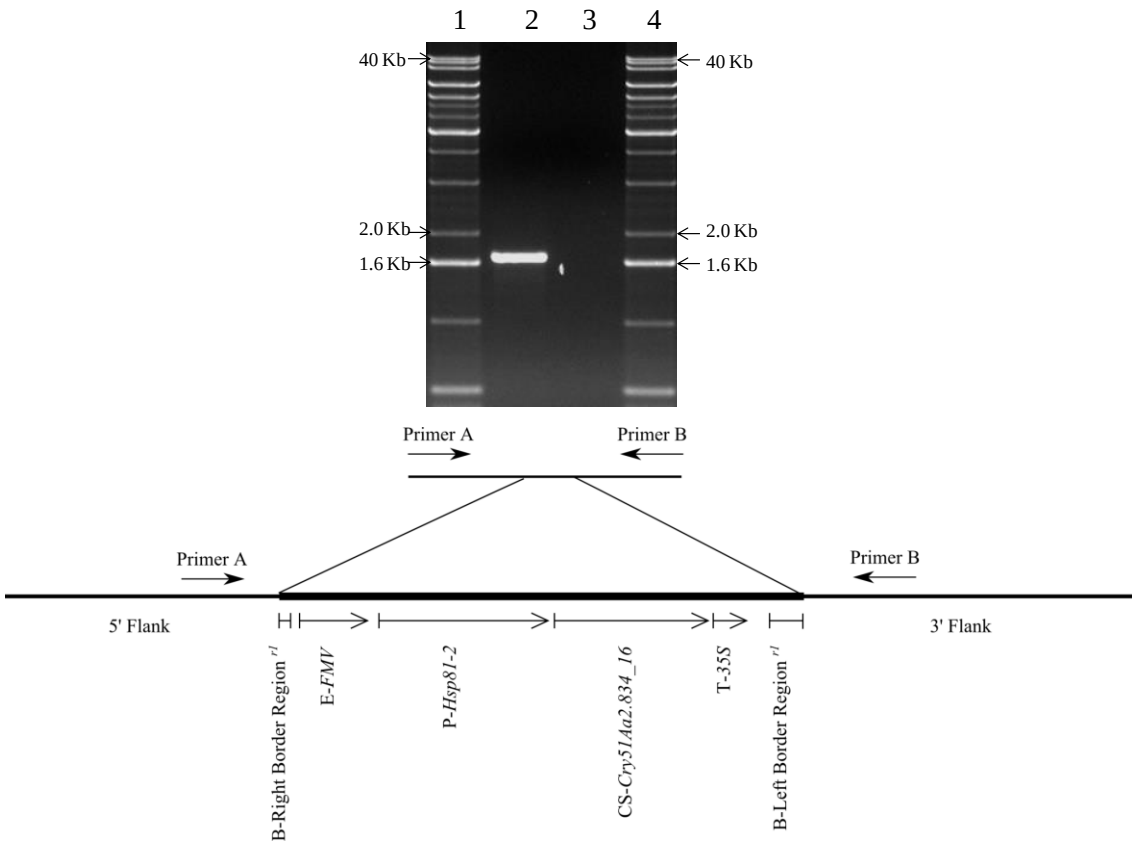


Figure IV-8. PCR Amplification of the MON 88702 Insertion Site

PCR Analysis was performed to evaluate the insertion site. PCR was performed on conventional control DNA using Primer A, specific to the 5′ flanking sequence, and Primer B, specific to the 3′ flanking sequence of the insert in MON 88702. The amplicon generated from PCR with the conventional control genomic DNA was used for sequencing analysis. This illustration depicts the MON 88702 insertion site in the conventional control (upper panel) and the MON 88702 inset (lower panel). Approximately 2 µl of each of the PCR reactions was loaded on the gel. This figure is representative of the data generated in the study. Lane designations are as follows:

Lane	
1	1Kb DNA Extension Ladder
2	Conventional Control
3	No template control
4	1Kb DNA Extension Ladder

Arrows on the agarose gel photograph denote the size of the DNA, in kilobase pairs, obtained from the 1Kb DNA Extension Ladder (Invitrogen) on the ethidium bromide stained gel.

r1 Superscript in Left and Right Border Regions indicate that the sequence in MON 88702 was truncated compared to the sequences in PVGHIR508523.

IV.E. Determination of Insert Stability over Multiple Generations

To demonstrate the stability of the T-DNA I present in MON 88702 through multiple breeding generations, NGS was performed using DNA obtained from five breeding generations of MON 88702 cotton. The breeding history of MON 88702 is presented in Figure IV-4 and the specific generations tested are indicated in the figure legend. The MON 88702 R4 generation was used for the molecular characterization analyses discussed in Sections IV.B-IV.D. To assess stability, four additional generations were evaluated by the NGS method as previously described in Section IV.B, and compared to the fully characterized R4 generation. The conventional control used for the generational stability analysis was DP393, a conventional variety with similar background genetics. Genomic DNA isolated from each of the selected generations of MON 88702 and conventional control was used for NGS.

To determine the insert number in the MON 88702 generations, the sequences were analyzed using NGS (Kovalic et al., 2012). Table IV-3 shows the number of unique junctions containing PV-GHIR508523 DNA sequence determined by this analysis.

Table IV-3. Junction Sequence Classes Detected

Sample	Junction Sequences Detected
MON 88702 R3	2
MON 88702 R4	2
MON 88702 R5	2
MON 88702 R6	2
MON 88702 R7	2
DP393	0

Alignment of the junction sequences from each of the assessed MON 88702 generations to the full flank/insert sequence determined for the MON 88702 R4 generation, confirms that the pair of junction sequences originates from the same region of the MON 88702 genome and is linked by contiguous, known and expected DNA sequence. This single identical pair of junction sequences is observed as a result of the insertion of PVGHIR508523 T-DNA I at a single locus in the genome of MON 88702. The consistency of the data across all generations tested demonstrates that this single locus was stably maintained throughout the MON 88702 breeding process, thereby confirming the stability of the insert. Based on this comprehensive sequence data and bioinformatic analysis, it is concluded that MON 88702 contains a single and stable T-DNA I insertion.

IV.F. Inheritance of the Genetic Insert Segregation Results for MON 88702 from the BC1F1, BC2F2, and BC3F1

The MON 88702 cotton breeding path, from which segregation data were generated, is described in Figure IV-9. The transformed R0 plant was self-pollinated to generate R1 seed. An individual homozygous positive plant was identified in the R1 segregating population via an End Point TaqMan® PCR assay.

The homozygous positive R1 plant was self-pollinated to give rise to R2 seed. The R2 plants were self-pollinated to produce R3 seed. The R3 plants were self-pollinated to produce R4 seed. Homozygous positive R4 plants were crossed via traditional breeding techniques to a Monsanto proprietary recurrent parent that does not contain the *mCry51Aa2* coding sequence to produce hemizygous R4F1 seed. The R4F1 plants were crossed with the recurrent parent to produce BC1F1 seed. The BC1F1 generation was tested for the presence of T DNA I by End Point TaqMan® PCR assay. The inheritance of the MON 88702 T-DNA I was assessed in the BC1F1 generation. At the BC1F1 generation, the MON 88702 T-DNA I was predicted to segregate at a 1:1 ratio (positive: negative) according to Mendelian inheritance principles.

The BC1F1 plants hemizygous for MON 88702 T-DNA I were crossed with the recurrent parent to produce the BC2F1 plants. The BC2F1 plants hemizygous for MON 88702 T-DNA I were crossed with the recurrent parent to produce the BC3F1. The inheritance of the MON 88702 T-DNA I was assessed in the BC3F1 generation. At the BC3F1 generation, the MON 88702 T-DNA I was predicted to segregate at a 1:1 ratio (positive: negative) according to Mendelian inheritance principles.

The BC2F1 plants hemizygous for MON 88702 T-DNA I were also self-pollinated to produce the BC2F2 plants. The BC2F2 generation was tested for the presence of MON 88702 T-DNA I by End Point TaqMan® PCR assay. The inheritance of the MON 88702 T-DNA I was assessed in the BC2F2 generation. At the BC2F2 generation, the MON 88702 T-DNA I was predicted to segregate at a 1:2:1 ratio (homozygous positive: hemizygous positive: homozygous negative) according to Mendelian inheritance principles.

A Pearson's chi-square (χ^2) analysis was used to compare the observed segregation ratios of the MON 88702 T-DNA I to the expected ratios. The χ^2 analysis was performed using the statistical program R Version 3.2.2 (2015-08-14).

The Chi-square was calculated as:

$$\chi^2 = \sum [(| o - e |)^2 / e]$$

where o = observed frequency of the genotype or phenotype and e = expected frequency of the genotype or phenotype. The level of statistical significance was predetermined to be 5% (α=0.05).

The results of the χ^2 analysis of the segregating progeny of MON 88702 are presented in Table IV-4. The χ^2 value in the BC1F1, BC2F2, and BC3F1 generations indicated no

statistically significant difference between the observed and expected segregation ratios of MON 88702 T-DNA I. These results support the conclusion that the MON 88702 T-DNA I resides at a single locus within the cotton genome and is inherited according to Mendelian principles of inheritance. These results are also consistent with the molecular characterization data indicating that MON 88702 cotton contains a single intact copy of the T-DNA I inserted at a single locus in the cotton genome.

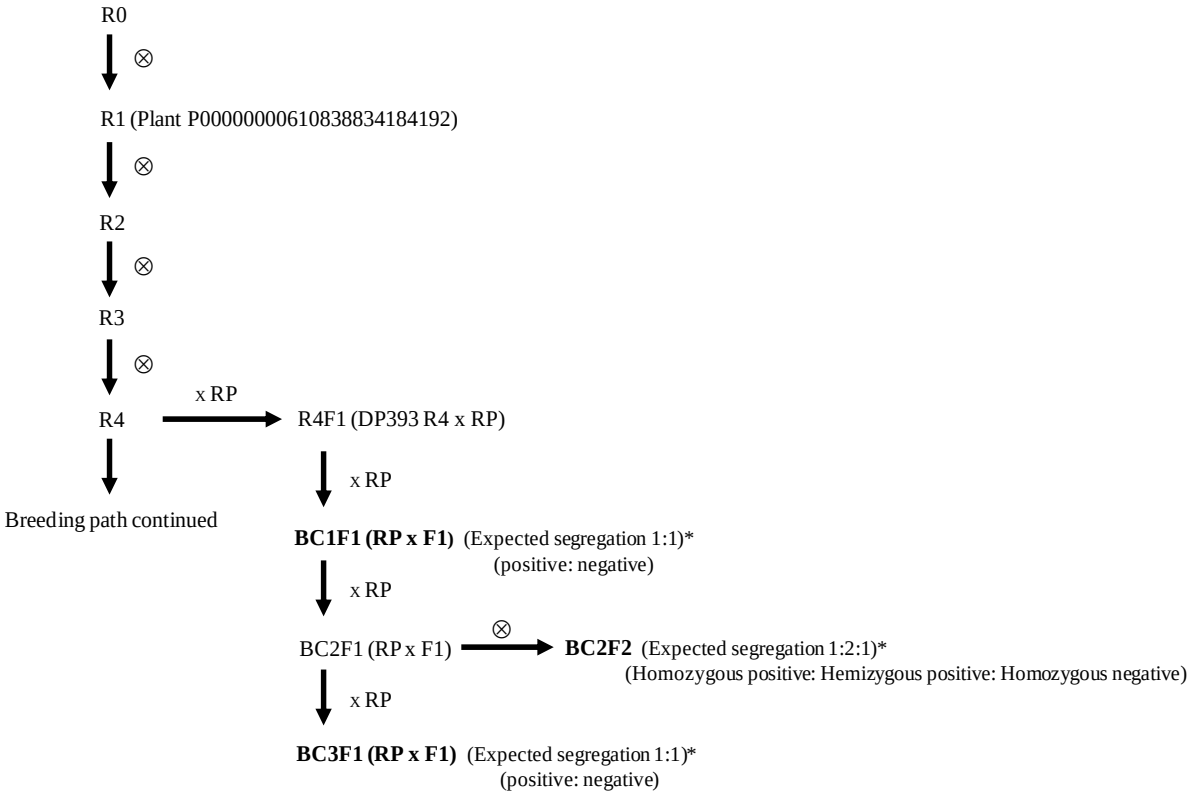


Figure IV-9. Breeding Path for Generating Segregation Data for MON 88702

* Chi-square analysis was conducted on segregation data from BC1F1, BC2F2, and BC3F1 generations (bolded text).

RP: Recurrent parent

⊗: Self- Pollinated

Table IV-4. Segregation Results for MON 88702 from the BC1F1, BC2F2, and BC3F1

				1:1 Segregation			
Generation	**Total Plants**	**Observed # Plant Positive**	**Observed # Plant Negative**	**Expected # Plant Positive**	**Expected # Plant Negative**	**χ^2**	**Probability**
BC1F1	267	138	129	133.50	133.50	0.30	0.582
BC3F1	176	86	90	88.00	88.00	0.09	0.763

					1:2:1 Segregation				
Generation	**Total Plants**	**Observed # Plant Homozygous Positive**	**Observed # Plant Hemizygous Positive**	**Observed # Plant Homozygous Negative**	**Expected # Plant Homozygous Positive**	**Expected # Plant Hemizygous Positive**	**Expected # Plant Homozygous Negative**	**χ^2**	**Probability**
BC2F2	155	38	75	42	38.75	77.50	38.75	0.37	0.832

IV.G. Characterization of the Genetic Modification Summary and Conclusion

As described above, characterization of the genetic modification in MON 88702 cotton was conducted using a combination of sequencing, PCR, and bioinformatics. The results of this characterization demonstrate that MON 88702 contains a single copy of the intended T-DNA containing the *mCry51Aa2* expression cassette that is stably integrated at a single locus and is inherited according to Mendelian principles over multiple generations. These conclusions are based on the following:

- Molecular characterization of MON 88702 by NGS demonstrated that MON 88702 contained a single DNA insert. These whole-genome sequence analyses provided a comprehensive assessment of MON 88702 to determine the presence of sequences derived from PV-GHIR508523 (Kovalic et al., 2012) and demonstrated that MON 88702 contained a single DNA insert with no detectable backbone sequences.

- Directed sequencing (locus-specific PCR, DNA sequencing and analyses) of MON 88702 which characterized the complete sequence of the single DNA insert from PV-GHIR508523, the adjacent flanking DNA, and the 5' and 3' insert-to-flank junctions. This analysis confirmed that the sequence and organization of the DNA is identical to the corresponding region in the PV-GHIR508523 T-DNA. Furthermore, the genomic organization at the insertion site was assessed by comparing the sequences flanking the T-DNA I insert in MON 88702 to the sequence of the insertion site in conventional cotton. This analysis determined that no major DNA rearrangement occurred at the insertion site in MON 88702 upon DNA integration.

- Generational stability analysis by NGS demonstrated that the single PV-GHIR508523 T-DNA I insert in MON 88702 has been maintained through five breeding generations, thereby confirming the stability of the T-DNA I in MON 88702.

- Segregation data confirm that the inserted T-DNA I segregated following Mendelian inheritance patterns, which corroborates the insert stability demonstrated by NGS and independently establishes the nature of the T-DNA I at a single chromosomal locus.

Taken together, the characterization of the genetic modification in MON 88702 cotton demonstrates that a single copy of the intended T-DNA was stably integrated at a single locus of the cotton genome and that no plasmid backbone or T-DNA II sequences are present in MON 88702 cotton.

V. CHARACTERIZATION AND SAFETY ASSESSMENT OF THE Cry51Aa2.834_16 PROTEIN EXPRESSED IN MON 88702

Characterization of the introduced protein(s) in a biotechnology-derived crop is important for establishing food, feed, and environmental safety. As described in Section IV, MON 88702 contains the *mCry51Aa2* expression cassette that, when transcribed and translated, results in the expression of the mCry51Aa2 protein.

V.A. Expression Levels of mCry51Aa2 Protein in MON 88702

The mCry51Aa2 protein expression levels in various tissues and growth stages of MON 88702 cotton (leaf, root, pollen, square and seed; described in Appendix C) were determined. These values were also used to evaluate exposure in additional studies conducted to support the deregulation of MON 88702 cotton. Being under the control of a constitutive promoter, the expression of mCry51Aa2 would be expected to vary similarly to the expression of other endogenous proteins and be impacted by the same factors that drive the variability of protein expression (Chinnadurai et al., 2018).

MON 88702 was grown in five and four field sites, representing the cotton production region in the U.S., in 2015 and 2018, respectively. Tissues of MON 88702 were collected from four replicate plots planted in a randomized complete block design at each site. OSL1, OSL4, root, pollen and seed tissues were collected from the 2015 field trials. Leaf and square tissues from various plant growth stages (OSL1, OSL2, OSL3, OSL4, Square1, Square2, Square3 and Square4) and pollen were collected in 2018 to further characterize the mCry51Aa2 expression levels in MON 88702. Within each season, the mCry51Aa2 protein levels determined for each tissue type were averaged across the sites and are summarized in Table V-1 (2015) and Table V-2 (2018).

The OSL1, OSL4 and pollen samples were collected in both seasons and the mCry51Aa2 expression levels measured in the 2018 growing season were comparable to those reported for the 2015 season. Natural variability of protein expression in different tissues can be observed in any protein, as at a given time the amount of protein determined in each tissue type will depend on transcription, stability of mRNA, translation and protein degradation (Egelkrout et al., 2012; Flavell, 1994). Protein expression can also be influenced by a variety of environmental factors including geography, temperature, soil, and rainfall among other factors which contribute significantly to variation in protein expression (Jamal et al., 2009; Nguyen and Jehle, 2009; Székács et al., 2012). This natural variability in the mCry51Aa2 protein expression can also be observed in the distribution of the individual sample values, leading to the overlapping ranges of the expression levels from the 2015 and 2018 growing season samples (Figure V-1).

Table V-1. Summary of mCry51Aa2 Protein Levels in Cotton Tissues Collected from MON 88702 Produced in United States Field Trials During 2015

Tissue Type[1]	Growth Stage[2]	Mean (SD) Range (µg/g dwt)[3]	LOQ/LOD (µg/g dwt)[4]
OSL1	2 to 6-Leaf	1200 (380) 550-1700	0.078/0.010
OSL4	Cut out	1000 (160) 700-1300	0.078/0.010
Root	Peak Bloom	190 (41) 150-290	0.078/0.028
Pollen	Peak Bloom	2.6 (0.41) 2.0-2.9	0.078/0.016
Seed	Maturity	130 (17) 91-170	0.078/0.021

[1]OSL = over season leaf

[2]The crop development stage at which each tissue was collected.

[3]Protein levels are expressed as the arithmetic mean and standard deviation (SD) as microgram (µg) of protein per gram (g) of tissue on a dry weight basis (dwt). The means, SD, and ranges (minimum and maximum values) were calculated for each tissue across all sites (n=20 except in pollen where n=5 as four replicates per site were pooled)

[4]LOQ=limit of quantitation; LOD=limit of detection.

Table V-2. Summary of mCry51Aa2 Protein Levels in Cotton Tissues Collected from MON 88702 Produced in United States Field Trials During 2018

Tissue Type[1]	Development Stage[2]	Mean (SE) Range (µg/g dwt)[3]	LOQ (µg/g dwt)[4]
OSL1	Pre-Flower	1900 (63) 1300 - 2300	0.56
OSL2	Pre-Flower	2000 (53) 1700 - 2400	0.56
OSL3	Peak Bloom	1600 (120) 790 - 2700	0.56
OSL4	Cutout	1500 (76) 1000 - 2300	0.56
Square 1	Pre-flower	2200 (88) 1500 - 2800	0.14
Square 2	Pre-flower	3000 (130) 2100 - 4000	0.14
Square 3	Peak Bloom	2600 (110) 1800 - 3600	0.14
Square 4	Cutout	2700 (110) 1900 - 3600	0.14
Pollen	Peak Bloom	4.0 (0.65) 2.8 - 5.0	0.28

[1]OSL = over season leaf

[2]The crop development stage at which each tissue was collected.

[3]Protein levels are expressed as the arithmetic mean and standard error (SE) as microgram (µg) of protein per gram (g) of tissue on a dry weight basis (dwt). The means, SE, and ranges (minimum and maximum values) were calculated for each tissue across all sites (n=16 except in OSL1 where n=15 due to one sample expressing <LOQ) and for pollen where n=3 (pooled)).

[4]LOQ=limit of quantitation; LOD=limit of detection.

Figure V-1. Distribution of Individual Sample Expression Levels

Overview of mCry51Aa2 protein expression levels measured in Over-Season Leaf tissues 1 and 4 (Panel A) and pollen (Panel B) collected during the 2015 and 2018 growing seasons. Each point represents an individual replicate sample, the middle bar represents the mean, and the top and bottom bars represent the maximum and minimum levels, respectively.

V.B. Safety Assessment of the mCry51Aa2 Protein Produced in MON 88702

V.B.1. Summary of the mCry51Aa2 Protein Safety Data

MON 88702 produces a modified Cry51Aa2 insecticidal crystal (Cry) protein derived from *Bacillus thuringiensis* (*Bt*). A history of safe use for *Bt* microbial biopesticide applications has been established through the documented use of these products for over 50 years (Hammond, 2004; OECD, 2010a). There are at least 180 registered microbial *Bt* products in the U.S. and over 120 microbial products approved in the European Union (Hammond, 2004). Applications of sporulated *Bt* have a long history of safe use for pest control in agriculture, especially in organic farming (Cannon, 1993; U.S. EPA, 1988). They have been safely and directly applied to consumed agriculture commodities including berry crops, cabbage, grapes, tomatoes, celery, lettuce, and spinach (U.S. EPA, 1988). Furthermore, extensive toxicity testing of commercial *Bt* microbial biopesticides that contain numerous Cry proteins (e.g., Cry2A, Cry1Aa, Cry1Ab, Cry1Ac, Cry1C, and Cry1F) has resulted in no evidence of adverse effects to human or animal health (Koch et al., 2015; McClintock et al., 1995; Moar et al., 2017; OECD, 2010b). Thus, *Bt* microbial formulations and the insecticidal proteins present in *Bt* microbial biopesticide formulations have been safely consumed by humans and animals for over 50 years.

The mCry51Aa2 protein, and the parent Cry51Aa2 protein, are β-pore forming proteins (β-PFPs) that belong to the ETX_MTX2 family of proteins. ETX_MTX2 family members, and the broader β-PFPs, are found in a broad range of plant, animal and bacterial species and humans and animals have documented safe exposure to members of the protein family (Moar et al., 2017). Insecticidal proteins in this family confer insect control by formation of pores in the insect intestinal tract, leading to insect death. The steps involved in pore formation of β-PFPs are generally known (Narva et al., 2017), and the mode of action (MOA) of the mCry51Aa2 protein has been well-characterized (Jerga et al., 2016) (Section V.B.2). Their specificity is mediated in part by their activation by proteases and their binding to specific receptors on the brush-border membrane within the insect midgut. Such specific receptors are not present in humans or other mammals, nor in the majority of non-target insects. This therefore limits the potential hazards related to exposure in humans, animals and the majority of non-target insects (Farmer et al., 2017).

A specific example of an Aerolysin-like protein with an established history of safe consumption by humans and animals is the Cry35Ab1 protein, which is expressed in some HERCULEX® (a registered trademark of Dow AgroSciences LLC), SmartStax®, (a registered trademark of the Bayer Group) and AcreMax® (a registered trademark of Pioneer Hi-Bred International, Inc.) biotechnology-derived corn hybrids. These corn varieties have been safely grown on millions of acres annually in the U.S. since 2006 (Moar et al., 2017). Additional sources of ETX_MTX2 family members include numerous genes, transcripts or proteins that have been identified in foods, including those that are directly consumed by humans such as fish, common crop plants and vegetables, that display structural and sequence homology to the ETX_MTX2 protein family (Moar et al., 2017). Thus, human consumption of ETX_MTX2 proteins in the diet is common, and no adverse effects have been reported.

The global use of biopesticides derived from the *Bt* subspecies *israelensis* (*Bti*) and *Lysinibacillus sphaericus*, which are used for the control mosquito and black flies and express the ETX_MTX2 proteins MTX2 and MTX3 (Hu et al., 2008; Liu et al., 1996; Thanabalu and Porter, 1996), is further evidence for safe human and animal exposure to ETX_MTX2 proteins. No adverse effects to humans or the environment have been documented to result from the use of *Bti* and *L. sphaericus* biopesticide formulations, which are often applied in highly-populated urban areas and aquatic environments, including drinking water reservoirs (Berry, 2012; Moar et al., 2017). A History Of Safe Use (HOSU) can therefore be established for many ETX_MTX2 protein family members through the extensive human and animal exposure described above.

Key differences, including sequence and structural differences in the receptor binding head region, distinguish ETX_MTX2 protein family members from each other, and from a limited number of ETX_MTX2 proteins associated with toxicity in humans and other mammals (e.g., epsilon toxin from *Clostridium perfringens*, for which the protein family partially receives its namesake) (Moar et al., 2017). The similarity between ETX_MTX2 protein family members is largely localized to the pore-forming and oligomerization protein domains, while sequence divergence is observed within the head domain that confers receptor binding specificity. Although the mCry51Aa2 protein is an ETX_MTX2 protein, it has significant sequence divergence from other members of that protein family, enabling its limited activity spectrum (Farmer et al., 2017; Moar et al., 2017).

The mCry51Aa2 protein expressed by MON 88702 cotton is a Plant Incorporated Pesticide (PIP) regulated by the U.S. Environmental Protection Agency (EPA). The data for the safety assessment of mCry51Aa2 have been provided to the EPA to support the establishment of a permanent exemption from tolerance for residues of mCry51Aa2 in food and feed. A permanent exemption from tolerance was granted by the EPA based on the data submitted in which it was concluded that "*there is a reasonable certainty that no harm will result to the U.S. population, including infants and children, from aggregate exposure to residues of the Cry51Aa2.834_16 protein derived from* Bacillus thuringiensis" (U.S. EPA, 2018a). Data to support the Section 3 breeding increase for MON 88702 were also provided to the agency that included an assessment of any impact on non-target organisms including beneficial insects, vertebrates and mammals. The registration was granted by the EPA in January 2018 (U.S. EPA, 2018b).

The weight-of-evidence of the protein safety evaluations demonstrate that mCry51Aa2 does not pose a food or feed safety concern, and support a conclusion that MON 88702 cotton is unlikely to pose an increased plant pest risk compared to conventional cotton. In brief, the data provided to U.S. EPA demonstrate:

- The *Bacillus thuringiensis* (*Bt*) donor organism from which the *mCry51Aa2* coding sequence was derived, has a long history of safe use, and is not known for human or animal pathogenicity, or allergenicity.

- Bioinformatic analysis confirmed that the mCry51Aa2 protein lacks relevant structural similarity to known protein allergens and toxins, or other proteins known to have adverse effects on mammals.

- Bioinformatic analyses also indicated that if putative translation products other than the mCry51Aa2 protein were to be produced in MON 88702, they would pose no identified concern as a potential allergen or toxin.

- Expression measurements reveal that the mCry51Aa2 protein is present in very low levels in harvested cottonseed of MON 88702, and therefore would constitute a very small portion of the total protein present in grain or other processed food and feed fractions derived from MON 88702 cotton. Furthermore, no consumption of the mCry51Aa2 protein derived from MON 88702 is expected for the general population given the nature of cottonseed fractions consumed by humans.

- The mCry51Aa2 protein rapidly degraded in the presence of pepsin and pancreatin and was not stable to heat treatment.

- The mCry51Aa2 protein demonstrated no acute oral toxicity in mice at the levels tested, which allowed for establishing a No Observed Adverse Effects Level (NOAEL) of 5,000 mg mCry51Aa2 protein/kg body weight; see Section V.B.5.2.

Taken together, this safety assessment confirms that the consumption of the mCry51Aa2 protein from MON 88702, and the consumption of cottonseed derived from MON 88702 or its progeny, is considered safe for humans and animals and as such would not pose a greater plant pest risk than that of conventional cotton. This is in line with the conclusions made by the EPA (U.S. EPA, 2018a).

V.B.2. Mode of Action of mCry51Aa2

The insecticidal mode of action of *Bt* proteins in general requires insect uptake (ingestion), proteolytic activation (which converts the inactive protoxin form of the *Bt* protein to the active toxin form), receptor binding in the insect midgut, oligomerization at the membrane interface, and membrane pore formation in the midgut cells, which in turn leads to insect death (Gill et al., 1992; Pigott and Ellar, 2007; Schnepf et al., 1998). Any of these steps can define the specificity of *Bt* proteins since the inability of a protein to progress through them will render it inactive to the insect.

The mode of action of mCry51Aa2 resulting in target pest mortality has been well characterized and follows the same general steps as other *Bt* insecticidal proteins currently in commercial use for insect crop protection. In a report characterizing the mode of action of the mCry51Aa2 protein it was described that the full-length mCry51Aa2 is a stable dimer in solution and that the activation of mCry51Aa2 occurs through exposure to Lygus saliva, which results in proteolytic cleavage at the C-terminal end of each mCry51Aa2 monomer in the dimer (Jerga et al., 2016). This C-terminal proteolytic cleavage results in the removal of amino acids 280 to 306 and the dissociation of the dimer into two separate monomers. Ligand binding immunoblotting assays indicate the activated mCry51Aa2 monomeric form displays binding to a single band of the Lygus brush border membrane proteins, and forms a membrane-associated oligomeric complex both *in vitro* and *in vivo* (Jerga et al., 2016). Immunohistochemistry analysis further

demonstrated that upon mCry51Aa2 exposure, midgut epithelium and cellular sloughing occurs (Jerga et al., 2016), which is consistent with observations of other insecticidal *Bt* Cry proteins. Finally, chemical cross-linking of the mCry51Aa2 dimer was shown to render the protein inactive, but still competent to compete for binding sites with the mCry51Aa2 protein *in vivo*. Thus, disassociation of the mCry51Aa2 dimer into sterically unhindered monomers is required for brush border membrane binding, oligomerization, and the subsequent steps which culminate in insect toxicity.

V.B.3. Protein Expression Values for Exposure

As previously indicated, tissues for protein expression analysis were collected from MON 88702 plants grown in two seasons (2015 and 2018) in U.S. fields (Section V.A); the measured mCry51Aa2 expression levels for tissues collected in 2015 and 2018 are provided in Table V-1 and Table V-2, respectively. The mCry51Aa2 expression levels were used to 1) determine the mCry51Aa2 concentration in the diets of the laboratory assays in the activity spectrum (Section V.B.4) and in the NTO assessment (Section V.B.5) and 2) provide values for exposure (Expected Environmental Concentrations (EECs)) to determine Margins of Exposure (MOEs) in the NTO assessment (Section V.B.5).

Both the laboratory assays in the activity spectrum (Section V.B.4) and the NTO assessment (Section V.B.5) were conducted prior to measuring the mCry51Aa2 expression levels in the tissues collected in 2018. Therefore, the determination of the mCry51Aa2 protein concentrations in the diets used in the activity spectrum and NTO assessments was based on the available mean, fresh weight mCry51Aa2 expression levels determined in 2015.

When defining the EEC for MOE calculations in the NTO assessment (Section V.B.5), the mCry51Aa2 expression levels determined in both 2015 and 2018 were used. More specifically, the mean mCry51Aa2 expression level was reported on a dry weight basis and from these data, the 95th percentile expression values were calculated. A fresh weight conversion factor was then used to estimate the 95th percentiles of mCry51Aa2 expression levels in fresh weight to provide conservative values for exposure; the EEC. The fresh weight conversion factor for each tissue type was experimentally determined, except in the case of pollen where the factor reported in Burke (2011) was used (Table V-3). For each tissue type, the highest fresh weight, 95th percentile expression value was used to establish the EEC, which was then applied to MOE calculations resulting in the most conservative exposure scenarios (Table V-16) (Table V-3).

Table V-3. 95th Percentile mCry51Aa2 Protein Levels in Selected Cotton Tissues used to Determine Expected Environmental Concentrations (EEC) from MON 88702

Tissue Type[1]	Developmental Stage[2]	95th Percentile (µg/g dwt)[3]	Conversion Factor (dwt to fwt)[4]	95th Percentile (µg/g fwt)[5]	Growing Season
Leaf (OSL3)	Peak bloom	2247.5	0.22	494.4	2018
Pollen	Peak bloom	4.9	0.49	2.4	2018
Root	Peak bloom	267.9	0.31	83.1	2015
Square 2	Pre-flower	3853.4	0.20	770.7	2018

[1] OSL=over season leaf
[2] The crop development stages at which each tissue was collected.
[3] The mCry51Aa2 protein levels are determined as µg of mCry51Aa2 protein per gram of tissue on a dry weight (dwt) basis (Table V-1 and Table V-2).
[4] Conversion factors were determined from tissue-specific water content data for leaf, root and square (experimentally determined) and pollen (as reported in (Burke, 2011)). In both cases, a formula was used to determine the conversion factors for each tissue: [conversion factor = 1 – (mean % water content / 100)].
[5] The mCry51Aa2 protein levels are calculated as microgram (µg) of mCry51Aa2 protein per gram (g) of tissue on a fresh weight (fwt) basis using tissue-specific conversion factor.

V.B.4. Activity Spectrum Assays for mCry51Aa2 Protein

V.B.4.1. Introduction

Numerous insect species are present in cotton fields across the U.S. The diversity of arthropod communities in cotton fields varies greatly depending on location and year (Sisterson et al., 2004). However, a much more limited set of species play key ecological roles in cotton agro-ecosystems. Several of these species can injure the plants and in some cases can be considered as persistent pests causing economic losses in cotton (Smith and Cothren, 1999) (Section VIII.F.1). Typically, insect pests are a common and continuous threat to cotton production in all regions of the U.S., with the greatest yield reductions caused by Lygus, stink bugs and thrips (Table VIII-2). A diversity of insect species are also commonly found in cotton fields, some of which are rarely considered to be pests of cotton while others are considered beneficial insects (Boyd et al., 2004).

An assessment of the activity spectrum for a PIP is typically conducted during product development and is designed to characterize the biological activity of a protein against a range of insect taxa that includes possible target organism(s) as well as a wide variety of other insects (Raybould, 2006; Romeis et al., 2013; USDA-APHIS and EPA, 2007). Therefore, the assessment of the activity spectrum of any PIP, such as mCry51Aa2, is an important component of not only evaluating the impact on target organisms, but also to assess any impacts on other organisms including known pests in cotton and other row crops, and non-target organisms including pests and beneficial species. The approach taken to assess activity is based on the mode of action of the protein and serves to characterize a hazard, while the protein expression levels define exposure. Characterization of the activity spectrum is one of several factors that can be used to inform the scope of tier 1 NTO testing for an ERA (Romeis et al., 2008; Romeis et al., 2013), which is an important component of the risk assessment, the results of which are reported in Section V.B.5 and Appendix I.

The activity spectrum for the mCry51Aa2 protein was assessed for initial hazard characterization. A broad range of species for which a valid assay was available was tested, including known economically relevant pests in cotton and/or other row crops. More specifically, 20 invertebrate species (comprising nine orders and 14 families) were screened for mCry51Aa2 protein insecticidal activity and/or protection against feeding damage of MON 88702 cotton plants in controlled environments (e.g. greenhouse/growth chamber), and/or field evaluations. The species tested in the activity spectrum assessment were selected considering the target species for mCry51Aa2, the ability to effectively test the organism in the laboratory, representation of different habitats (below ground, ground dwelling, above ground), representation of ecological groups (e.g. herbivore, predator, parasitoids, decomposers, or pollinators), taxonomic relatedness (e.g. relationship to species where insecticidal activity was observed), and economical roles in the agro-ecosystem (Figure V-3) (Raybould, 2006; Romeis et al., 2013; USDA-APHIS and EPA, 2007). The overall results of these studies have been published (Bachman et al., 2017) and, together with the results of additional studies, are presented in Table V-7. Since target species for the mCry51Aa2 protein were identified in two insect orders (Hemiptera and Thysanoptera), the range of species tested in the assessment included a diverse range

of species that generally exceeded the well-established practices utilized when conducting a risk assessment on a PIP.

The activity spectrum assessment included testing against the target species (*Lygus hesperus*, *Lygus lineolaris* and *Frankliniella* spp.). Considering that lethal effects against *L. hesperus* and *L. lineolaris* (order Hemiptera) had been described previously (Baum et al., 2012; Gowda et al., 2016), the activity of the mCry51Aa2 protein against these two species in this assessment was expected and confirmed. Thysanoptera and Hemiptera are sister orders belonging to the superorder Condylognatha (Grimaldi and Engel, 2005), and, therefore, observing activity against *Frankliniella* spp. (order Thysanoptera) was not unexpected. Given this observed cross-order activity, the potential for detecting activity of the mCry51Aa2 protein against species more closely related to the target species, e.g. other Hemiptera could not be ruled out. Therefore, the activity spectrum testing also included several hemipteran species, e.g. *Orius insidiosus* and *Pseudatomoscelis seriatus*.

Activity of native Cry51Aa1 and native Cry51Aa2 proteins against *Leptinotarsa decemlineata* from the order Coleoptera, family Chrysomelidae, has been documented previously (Baum et al., 2012; Xu et al., 2015). Both native Cry51Aa1 and Cry51Aa2 proteins have more than 95% sequence identity with mCry51Aa2 and therefore activity of the protein against *L. decemlineata* or related coleopteran species would not be unexpected. Similar cross-order activity of several *Bt* proteins has been previously observed (van Frankenhuyzen, 2009; 2013) and where present, the activity was generally lower compared to the activity against target species (van Frankenhuyzen, 2009; 2013).

The representative herbivores Euschistus heros, Diabrotica virgifera virgifera, Epilachna varivestis, Spodoptera frugiperda, Helicoverpa zea, Ostrinia nubilalis and Plutella xylostella, the dipteran species Aedes aegyptii, and representative beneficial arthropods Coleomegilla maculata, Apis mellifera, Pediobius foveolatus, Folsomia candida and Eisenia andrei were also included in the testing panel of the activity spectrum assessment.

In laboratory testing, the assays utilized for the insects tested were designed to: (1) provide continuous exposure of each test species to the mCry51Aa2 protein and (2) provide a duration of exposure sufficient to evaluate potential adverse effects of the mCry51Aa2 protein based on lethal and sublethal endpoints when possible. Field trial data are also presented for three species for which established laboratory assays are not available (assays described in Appendix H). For each of the lab-evaluated species, the assay followed a different experimental design based on available methods to evaluate activity. In some cases, depending on the species, a range of various endpoints was measured (i.e. development, growth, survival and/or reproduction) with these established methods. The results from the studies were used to determine whether the protein was active against each of the species tested, which was then indicated as yes or no (Y/N) in the results (Table V-7). Footnotes in Table V-7 indicate the percentage of survival that corresponded to an assignment of "Y" for activity. As indicated in Table V-7 and Appendix H, species were either exposed to the protein through feeding on plant tissue or treated diets. Where laboratory assays with artificial diets were available, the insects were exposed to concentrations to determine the LC_{50} (in the case of *L. hesperus*) or to concentrations of 200 and 400 µg mCry51Aa2/mL or g diet. The latter concentrations

were set based on protein concentrations of mCry51Aa2 in cotton leaf tissues available at the time of conducting the assay. At this screening stage, the observation of activity on any of the non-target species would indicate the need for further testing to characterize the dose-effect response.

V.B.4.2. Results - Target Species

The target species for the initial activity spectrum assessment of the mCry51Aa2 protein were select species in the orders Hemiptera and Thysanoptera. The hemipteran target species *Lygus hesperus* (Family: Miridae) demonstrated sensitivity in diet incorporation assays with a mean LC_{50} of 3.0 µg/mL diet (Table V-7 and Appendix H.1.1). Likewise, a growth chamber study confirmed the activity of the mCry51Aa2 protein expressed in MON 88702 cotton plants against the other hemipteran target species, *Lygus lineolaris*, in the same family as *L. hesperus* (Table V-7 and Appendix H.1.2). The MON 88702 efficacy against *L. lineolaris* was demonstrated by a 19-fold reduction in numbers of large nymphs, the economically most important stage, recovered from MON 88702 compared to conventional cotton (Bachman et al., 2017). The results of these studies with *L. hesperus* and *L. lineolaris* are consistent with the results reported previously (Gowda et al., 2016) where lethal effects of the mCry51Aa2 protein were reported for each of these species. Several publications have further documented the field efficacy of MON 88702 against *Lygus* spp. (Akbar et al., 2018; Graham and Stewart, 2018). A field study was conducted in 2018 across six sites in the U.S. cotton growing regions, with the main objective to assess potential effects of MON 88702 on abundance of beneficial Hemiptera in the field (Section V.B.5.1.2.4 and Appendix I.4). Within this study, populations of *Lygus* spp. were also collected using sweep nets (total *Lygus* spp. counts) and vertical beat sheets (*Lygus* spp. large nymph, adult, and total counts) (Table V-4). A combined-site analysis demonstrates a significant reduction in *Lygus* spp. abundance determined using the sweep nets (total counts) and vertical beat sheets (large nymph, adult and total counts) in unsprayed MON 88702 plots compared unsprayed conventional control DP393 plots (Table V-4 and Appendix I.4).

Table V-4. Mean[1] Abundance of *Lygus* spp. in Unsprayed Conventional Control DP393 and Unsprayed MON 88702 Cotton Plots

Life Stage	Collection Method	Mean (SE) DP393 Unsprayed	Mean (SE) MON 88702 Unsprayed	Power (%)[2]
Total (Nymphs + Adults)	Sweep net	5.8 (0.30)	3.8 (0.27)*	99.3
Adults	Vertical beat sheet	2.9 (0.38)	1.5 (0.25)*	37.1
Large Nymphs[3]	Vertical beat sheet	5.1 (0.35)	2.4 (0.14)*	86.2
Total	Vertical beat sheet	6.1 (0.37)	2.9 (0.16)*	90.1

[1] This table provides the arithmetic means. Least Square (LS) means were used in the statistical model for conducting pairwise comparisons and are provided in Appendix I.4.
[2] Power to detect a 50% difference in abundance.
[3] Third to fifth instar *Lygus* spp. nymphs were identified as "Large Nymphs".
* Indicates a statistically significant difference with the unsprayed DP393 entry (α=0.05).

Frankliniella spp. (commonly referred to as thrips) infest cotton plants, which can result in severe damage. Typical feeding damage by thrips is reflected in different phenotypic characteristics such as distortion, malformation and tearing of seedling leaves (Cook et al., 2011). The activity against thrips was first assessed in a field trial using MON 88702 cotton plants expressing the mCry51Aa2 protein. Within this activity spectrum study, the protection against feeding damage from thrips was demonstrated by an approximately three-fold reduction in damage on MON 88702 compared to conventional cotton (Table V-7) (Appendix H.2.1). The protection of MON 88702 plants against thrips damage was also demonstrated in field experiments conducted across different years and cotton growing regions in the U.S. (Akbar et al., 2018; Graham and Stewart, 2018). Akbar et al. (2018) demonstrated that MON 88702 plants were nearly free of thrips damage in both medium and high-pressure scenarios. Graham and Stewart (2018) demonstrated that untreated MON 88702 plants provided as good as, or better, protection against thrips compared to conventional control cotton that was treated with both a seed treatment and a foliar insecticide application registered for use in controlling thrips.

The possibility of MON 88702 having a non-preference effect on thrips, i.e. leading to the thrips preferring to feed on conventional control cotton instead of MON 88702, is described by Graham and Stewart (2018). This suggests that the mCry51Aa2 protein expressed in MON 88702 leads to deterrence of exposed thrips, resulting in reduced plant damage. Such a non-preference effect on thrips has previously been documented for imidacloprid, a neonicotinoid seed treatment frequently used by cotton growers to control insect pests (Cook et al., 2011; Huseth et al., 2017; Joost and Riley, 2005). Huseth et al. (2017) also demonstrated that several foliar and seed treatments such as cyantraniliprole and imidacloprid reduced the average eggs laid per female tobacco thrips (*F. fusca*), another indicator of non-preference effect.

Additional experiments were conducted using either caged whole plants in a greenhouse setting or leaf disk assays to characterize development and survival effects of the mCry51Aa2 protein expressed in MON 88702 on tobacco thrips (*F. fusca*) and Western flower thrips (*F. occidentalis*), two of the most common thrips species found in cotton fields across the U.S. cotton belt and both members of the *Frankliniella* spp. genus (Reay-Jones et al., 2017; Stewart et al., 2013) (Appendix H.2.2). An overview of the conducted experiments is provided in Figure V-2.

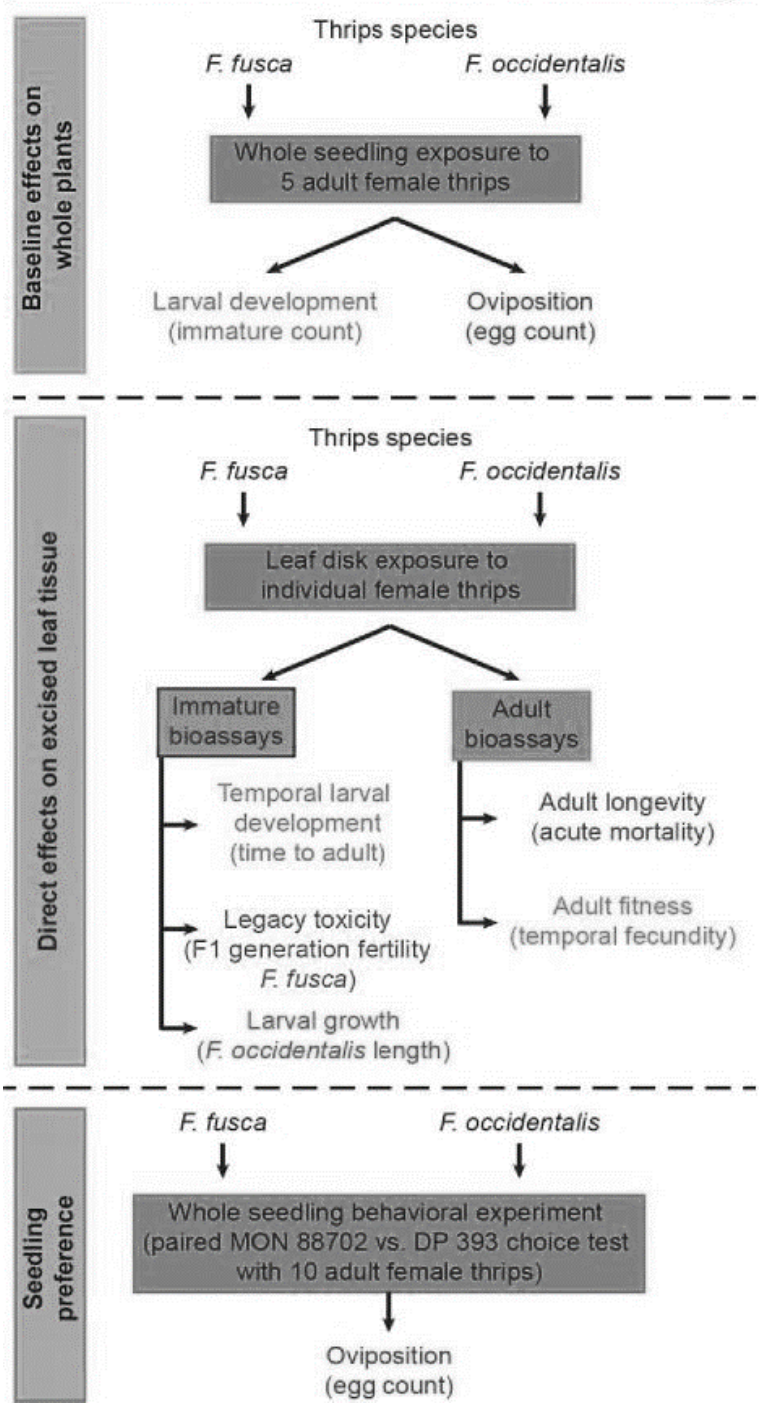


Figure V-2. Overview of Conducted Whole Plant and Leaf Disk Studies to Evaluate MON 88702 Effects on *F. fusca* and *F. occidentalis* (Appendix H.2.2)

The results of the additional studies are provided in Table V-5. Feeding on MON 88702 led to a significant reduction in immature survival and adult longevity of *F. occindentalis* together with a strong oviposition deterrence effect (Appendix H.2.2). For *F. fusca* the oviposition deterrence effect was also apparent, but a weak longevity effect was observed only for *F. fusca* adults (Appendix H.2.2). Therefore, similar to several commercialized insecticides used for thrips control, the protection against thrips species by MON 88702 is likely due to a combination of effects on survival and ovipositioning of the mCry51Aa2 protein.

Table V-5. Overview of the Effects of MON 88702 on *F. fusca* and *F. occidentalis*

Significant MON 88702 Effect Observed	***F. fusca***	***F. occidentalis***
Baseline effects on whole plants		
Decreased larval development (immature count)	Yes	Yes
Reduced oviposition (egg count)	Yes	Yes
Direct effects on excised leaf tissue		
Reduced acute adult survival	No	No
Reduced adult longevity	Yes	Yes
Delayed development to adult	Yes	Yes
Decreased potential for development to adult	No	Yes
Reduced fertility of F1 female generation	No	NA[1]
Reduced larval growth	NA[1]	Yes
Seedling preference		
Reduced oviposition in choice experiment	Yes	Yes

[1] NA = Not Applicable (endpoint was not measured for this species).

V.B.4.3. Results – Other Hemipteran Pest Species where Activity was Observed

The activity of mCry51Aa2 against the hemipteran herbivore *Pseudatomoscelis seriatus* (Family: Miridae) was tested because of its taxonomic relatedness to the target pests *Lygus* spp. In a caged field trial, MON 88702 and conventional control DP393 plants were infested with three pairs of sexually mature *P. seriatus* and abundance data of the progeny were collected after four weeks of infestation (Appendix H.3.1). The results demonstrated activity of the mCry51Aa2 protein expressed in MON 88702 cotton against this species by a significant three-fold reduction in the number of next-generation adults on MON 88702 compared to conventional cotton (Table V-7). Larger-scale field studies conducted to further explore the potential efficacy of MON 88702 against this cotton insect pest demonstrated variable efficacy of MON 88702 against *P. seriatus*. Therefore, commercial scale activity of MON 88702 against *P. seriatus* has not been confirmed at this time.

V.B.4.4. Results - *Orius insidiosus*

The activity of the mCry51Aa2 protein against a hemipteran representative from the family Anthocoridae, the insidiosus flower bug, *Orius insidiosus*, was evaluated in a direct feeding assay during the activity spectrum assessment. A significant effect on survival due to feeding on mCry51Aa2-containing diet was observed in the activity spectrum assessment at levels of 200 and 400 µg mCry51Aa2/g diet. Considering its taxonomic relatedness to the target pest *Lygus* spp., the activity of mCry51Aa2 against *Orius* spp. was not unexpected.

In the activity spectrum assay conducted with five-day old *O. insidiosus* nymphs, the total survival was 67% after 7 days of exposure to the mCry51Aa2 protein at concentrations of 200 and 400 µg/g diet (Table V-7), with all surviving nymphs developing normally into adulthood (Appendix H.7.1). To further characterize the response of *O. insidiosus* to the mCry51Aa2 protein, another assay was conducted with five concentrations of the protein in the diet ranging from 13 to 200 µg/g diet (Table V-7). In this assay, the corrected survival was approximately 60% at a concentration of 100 µg/g diet and comparable corrected survival was observed at 200 µg/g diet, the highest dose tested (Table V-7 and Appendix H.7.1). The development of surviving five-day old *O. insidiosus* nymphs was not affected by ingestion of mCry51Aa2 protein across the concentrations, as 100% adult emergence was observed in the surviving nymphs (Appendix H.7.1). A survival value equal to or less than 50% was not observed at the highest concentration tested of 400 µg mCry51Aa2/g diet, preventing the estimation of an LC_{50} for five-day old *O. insidiosus* nymphs. The results indicate that an LC_{50} should be greater than 400 µg mCry51Aa2/g diet. These results demonstrate that at an approximate concentration of 100 µg mCry51Aa2/g diet, a maximum response is observed and reaches a plateau.

This plateau effect is likely due to the affinity of certain receptors for the protein and the inability to bind sufficient proteins to form a pore and elicit a response that would allow the estimation of an LC_{50}. Similary, within pharmacology, such a plateau effect is

typically observed for partial agonists, i.e. a drug that binds to and activates a receptor, but does not elicit a full response; the maximum effect of a drug is achieved when all the receptors are occupied (Salahudeen and Nishtala, 2017). Once activated, mCry51Aa2 specifically binds to receptors in the target insect's gut and once sufficient active monomers are present they oligomerize and form pores (Jerga et al., 2016). Like what has been observed in pharmacology research, it is likely that for certain species a variety of receptors with different affinity for the mCry51Aa2 are present in the insect's midgut, resulting in binding of the protein to a point where the response becomes saturated and reaches a plateau. It is important to note that testing was done under a conservative exposure scenario of obligate and continuous consumption of the mCry51Aa2 protein by *O. insidiosus*.

Table V-6. *O. insidiosus* Survival at Day 7 for a Range of mCry51Aa2 Protein Treatments

Treatment	Test Nymphs	Survival (%)	Corrected Survival (%)[1]
Assay control	34	82.35	100
13µg mCry51Aa2/g diet	35	85.71	100
25µg mCry51Aa2/g diet	34	67.65	82
50µg mCry51Aa2/g diet	32	56.25	68
100µg mCry51Aa2/g diet	35	51.43	62
200µg mCry51Aa2/g diet	34	50.00	61
Positive control diet containing potassium arsenate at 100µg/g	35	0	0

[1] The correct survival % was calculated using a slightly modified Abbott's formula where survival was used instead of mortality.

Based on the results of the activity spectrum and because of the role of *O. insidiosus* as an important beneficial insect in the cotton agro-ecosystem, follow up studies were performed under refined exposure scenarios (Section V.B.5). (U.S. EPA, 2010b; USDA-APHIS and EPA, 2007)

V.B.4.5. Results - Coleoptera

Within Coleoptera, five different species that are members of the functional groups of herbivores and predators, were tested (three in the family Chrysomelidae and two in the family Coccinellidae) at similarly high doses of the mCry51Aa2 protein in activity spectrum studies (Table V-7). An effect on survival of *Leptinotarsa decemlineata* (Family: Chrysomelidae) and *Diabrotica undecimpunctata howardi* (Family: Chrysomelidae) larvae was observed at 200 µg/mL diet.

Leptinotarsa decemlineata (Colorado potato beetle) was selected as one of the four representative herbivores in the order Coleoptera for which an activity spectrum assay is available. *L. decemlineata* is an agronomically important pest in potatoes, but it is not a

pest in cotton fields and, therefore, not necessarily relevant to a risk assessment of a cotton product (Lawrence et al., 2008) (Table VIII-2). Wild-type Cry51Aa1 and wild-type Cry51Aa2, both of which have greater than 95% sequence identity with mCry51Aa2, have been reported to have activity against *L. decemlineata* (Baum et al., 2012; Xu et al., 2015), hence it was a candidate to be tested for mCry51Aa2 activity. In the initial activity spectrum assay, survival of 50% and 46% was observed at 200 and 400 µg mCry51Aa2/mL, respectively. Table V-7 shows the corrected survival response for the highest concentration tested for this species in that assay (400 µg mCry51Aa2/mL diet). Additional activity spectrum assays were conducted at increasing concentrations up to 800 µg mCry51Aa2/mL diet to estimate an LC_{50} for this species (Appendix H.4.1). At the highest concentration of 800 µg mCry51Aa2/mL, survival of the insects had clearly decreased when compared to control diet, confirming the initially reported activity of mCry51Aa2 against *L. decemlineata*. However, the obtained results showed variability across repeated assays, with survival ranging from 35-81% at the highest tested concentration; a consistent concentration-response curve could not be generated. The stability of the mCry51Aa2 protein in the diet was confirmed throughout the majority of the assay using the target sensitive species *L. hesperus* (Appendix H.4.1). Therefore, similar as to the results observed for *O. insidiosus* (Section V.B.4.4), even though there was activity of the mCry51Aa2 protein against *L. decemlineata*, an LC_{50} value could not be reliably estimated. Overall, these findings are similar to those of van Frankenhuyzen (2009; 2013), who showed that for the majority of the proteins with quantified cross-activities, the toxicity levels outside the order of primary specificity were several orders of magnitude lower.

Diabrotica undecimpunctata howardi (Southern corn rootworm) was also selected as a representative coleopteran herbivore for which an activity spectrum assay is established. This species is mainly a pest in corn, and, though it is widely distributed across North America, it is not a pest in cotton (Vaughn et al., 2005) (Table VIII-2). Therefore, like *L. decemlineata*, this species is not necessarily relevant to a risk assessment of a cotton product. An activity spectrum assay was conducted at increasing concentrations of mCry51Aa2 ranging from 1.6 to 200 µg mCry51Aa2/mL diet (Appendix H.4.3). The results indicate that survival below 50% was not observed at concentrations up to 200 µg mCry51Aa2/mL (Table V-7). Additional activity spectrum assays were conducted at increasing concentrations up to 800 µg mCry51Aa2/mL to estimate an LC_{50} for this species (Appendix H.4.3). The results of these additional assays demonstrated that the mCry51Aa2 protein did not impact *D. u. howardi* survival, although activity in the form of a reduced mean insect mass was observed. Considering this high survival and the absence of a consistent concentration-response curve, an LC_{50} value could not be reliably estimated for this species.

Given the lack of consistency of the concentration-response curves, the stability of the mCry51Aa2 protein in the diet for *D. u. howardi* was assessed using the same methodology as described for the *L. decemlineata* diet. A partial loss of the protein stability was observed in the *D. u. howardi* diet matrix, likely due to lack of solubility of the mCry51Aa2 protein in the diet matrix. This would explain the observation of some degree of activity both in the current studies as well as those reported by Bachman et al. (2017). In both cases, the activity is reported as a conservative characterization of hazard,

which is not significant in this case given the low impact on the survivability of *D. u. howardi* under high-dose continuous exposure conditions. Furthermore, as was described, *D. u. howardi* is a pest but not of cotton, resulting in negligible risk due to low, or no, exposure to MON 88702 cotton.

Ultimately, the activity that was identified against both *L. decemlineata* and *D. u. howardi* served to inform the scope of NTO testing regarding coleopteran species relevant to the cotton agro-ecosystem. Most importantly, beneficial NTOs present in cotton fields such as lady and rove beetles (*Coccinella septempunctata* and *Aleochara bilineata*, respectively) (Torres and Ruberson, 2005) were tested in tier 1 assays, where diets were confirmed to be active throughout the assay. The results of the tiered NTO assessment are further discussed in Section V.B.5.1.2.9 and demonstrate the absence of adverse effects of the mCry51Aa2 protein on *C. septempunctata* and *A. bilineata*.

Additional Coleoptera were also tested in activity spectrum assays (Table V-7 and Appendix H.4 and Appendix H.8). No activity of the mCry51Aa2 protein was observed against larvae of a closely related species *Diabrotica virgifera* (Appendix H.4.2), also a representative herbivore from the family Chrysomelidae, and a major pest of corn (Vaughn et al., 2005), even though this species was tested at 2.5-5 fold higher concentrations than *D. u. howardi*. Two other coleopteran species from the more basal family Coccinellidae, *Coleomegilla maculata* (Appendix H.8.1), a predator, and *Epilachna varivestis* (Appendix H.4.4), a herbivore, showed no effect from exposure to the mCry51Aa2 protein at similar concentrations.

Furthermore, as is discussed in Section VII.C.2.2, the environmental interaction assessment data demonstrate there was no difference in susceptibility between MON 88702 and conventional cotton to damage from coleopteran families typically present in cotton fields, nor was there any adverse effect of MON 88702 on non-target arthropod abundance in the field (Table VII-5 and Table VII-7, Section VII.C.2). The weight of evidence, especially under the most environmentally relevant conditions, therefore, demonstrates that MON 88702 is unlikely to pose a risk to Coleoptera present in cotton fields.

V.B.4.6. Results - Other Species where No Activity was Observed

Additional activity spectrum studies with representatives of key ecological functional groups selected according to the criteria described previously and encompassing the orders Lepidoptera, Hymenoptera, Collembola, Diptera and Haplotaxida demonstrated no adverse effects from continuous dietary exposure to the mCry51Aa2 protein (Table V-7 and Appendix H). This further supports the conclusion that the mCry51Aa2 protein is active against targeted species within the insect orders Hemiptera and Thysanoptera.

V.B.4.7. Conclusions of the Activity Spectrum Assessment

Within the activity spectrum study, twenty invertebrate species, comprising nine orders and 14 families, were screened for mCry51Aa2 protein insecticidal activity. The results demonstrate that the insecticidal activity of the mCry51Aa2 protein is selective and limited within three insect orders where toxicity or protection from feeding damage was demonstrated against targeted hemipteran (*L. hesperus* and *L. lineolaris*) and thysanopteran (*Frankliniella* spp.) insect pests. Initially, activity was demonstrated against *P. seriatus,* which has currently not been confirmed on a commercial level (Section V.B.4.3). Therefore, the product concept for MON 88702, established through the activity spectrum assays, and confirmed through other sources, is the protection of cotton from feeding damage caused by *L. hesperus*, *L. lineolaris* and *Frankliniella* spp. due to the expression of the mCry51Aa2 protein.

Activity was also observed against *O. insidiosus*, another Hemiptera. Although this was not unexpected due to its relatedness to *Lygus* spp., the detected activity against this species can be considered low according to existing definitions for activity outside of the primary insect targets (van Frankenhuyzen, 2013) and because an LC_{50} could not be reached at higher concentrations. The indication that mCry51Aa2 may be a hazard to *Orius* spp. triggered further testing to determine the risk of MON 88702 on this beneficial insect in the tiered NTO assessment (Section V.B.5.1).

Though the same is true for the activity spectrum results in two of the five coleopteran species tested, further testing under different exposure scenarios is not warranted due to the lack of relevance of these insects to the risk assessment of a cotton product. Neither *L. decemlineata* nor *D. u. howardi* are typically pests in cotton fields and therefore their exposure, and risk would be negligible. Additionally, testing of other closely related Coleoptera, as well as those that are relevant to the risk assessment of a cotton product showed no effect of mCry51Aa2 on these insects, indicating that MON 88702 does not pose a risk to coleopteran species (Sections V.B.4.5and V.B.5.1.3).

The determination of mCry51Aa2 insecticidal activity in these activity spectrum studies is not necessarily indicative of risk to tested species under field cultivation conditions for MON 88702 cotton expressing the mCry51Aa2 protein. Instead, the design of these activity spectrum studies was intended as an initial characterization of hazard by establishing which species, from a broad selection of insects, are sensitive to the mCry51Aa2 protein, and can therefore inform the ERA for PIPs (Section V.B.4). The results from the study also help inform the selection of the relevant, beneficial species for

testing in the tiered NTO assessment (Section V.B.5). The taxa from these orders in the tiered NTO assessment are especially important as they serve as key surrogate NTOs utilized for the ERA of PIPs (Romeis et al., 2013; Wach et al., 2016).

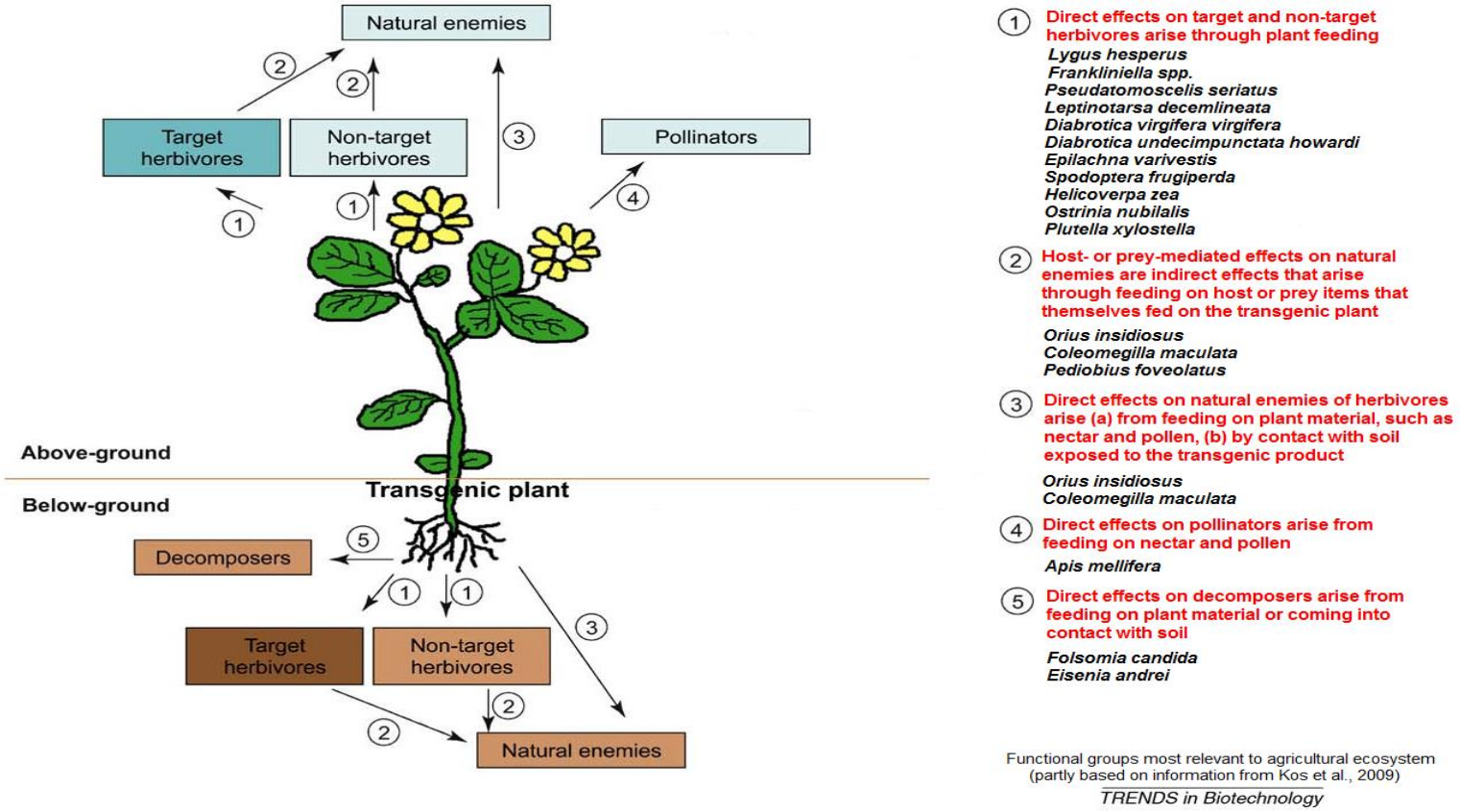


Figure V-3. Rationale for the Use of Representative Species Selected for Activity Spectrum Assessment

Table V-7. Activity Spectrum Results from Feeding Assays with the mCry51Aa2 Protein in Invertebrates Representing Target and Non-target Invertebrate Species

Order	Family	Genus species	Representative Function	Mean LC_{50} value or Maximum Concentration Tested	Activity
Hemiptera	Miridae	*Lygus hesperus*	Herbivore (Target pest)	3.009 µg/mL diet	Yes
Hemiptera	Miridae	*Lygus lineolaris*	Herbivore (Target pest)	Plant expression	Yes
Hemiptera	Miridae	*Pseudatomoscelis seriatus*	Herbivore	Plant expression	Yes
Hemiptera	Pentatomidae	*Euschistus heros*	Herbivore	5000 µg/ml	No
Thysanoptera	Thripidae	*Frankliniella* spp.	Herbivore (Target pest)	Plant expression	Yes
Coleoptera	Chrysomelidae	*Leptinotarsa decemlineata*	Herbivore	400 µg/mL diet	Yes[1]
Coleoptera	Chrysomelidae	*Diabrotica virgifera virgifera*	Herbivore	1000 µg/mL diet	No
Coleoptera	Chrysomelidae	*Diabrotica undecimpunctata howardi*	Herbivore	200 µg/mL diet	Yes[2]
Coleoptera	Coccinellidae	*Epilachna varivestis*	Herbivore	400 µg/mL diet	No
Lepidoptera	Noctuidae	*Spodoptera frugiperda*	Herbivore	400 µg/mL diet	No
Lepidoptera	Noctuidae	*Helicoverpa zea*	Herbivore	400 µg/mL diet	No
Lepidoptera	Crambidae	*Ostrinia nubilalis*	Herbivore	400 µg/mL diet	No
Lepidoptera	Plutellidae	*Plutella xylostella*	Herbivore	400 µg/mL diet	No
Hemiptera	Anthocoridae	*Orius insidiosus*	Predator	400 µg/g diet	Yes[3]
Coleoptera	Coccinellidae	*Coleomegilla maculata*	Predator	400 µg/mL diet	No
Hymenoptera	Apidae	*Apis mellifera*	Pollinator	2000 µg/mL diet	No
Hymenoptera	Eulophidae	*Pediobius foveolatus*	Parasitoid	400 µg/mL diet	No
Collembola	Isotomidae	*Folsomia candida*	Decomposer	400 µg/g diet	No
Haplotaxida	Lumbricidae	*Eisenia andrei*	Decomposer	400 µg/g soil dwt	No
Diptera	Culicidae	*Aedes aegyptii*	Decomposer	800 µg/mL diet	No

[1] The corrected survival response was near 50% in treatment concentrations from 50 to 400 µg mCry51Aa2/mL diet treatment for *L. decemlineata.*

[2] The corrected survival response was 64% in the 200 µg mCry51Aa2/mL diet treatment for *D. u. howardi*.

[3] The survival response was 67% in the 400 µg mCry51Aa2/g diet treatment which was the highest concentration tested for *O. insidiosus*.

V.B.5. Non-target Organism Safety Assessment for the mCry51Aa2 Protein Expressed in MON 88702 Cotton

Evaluation of the potential risks to NTOs is an important component of risk assessment of a biotechnology-derived crop. Assessment of the potential risks to NTOs associated with the introduction of a biotechnology-derived crop producing an insecticidal trait is based on the characteristics of the crop and the introduced trait. Since risk is a function of hazard and exposure, it is critical to determine the potential hazard and routes and levels of exposure. Selection of the test organisms and test material are important decisions that are based on the characteristics of the trait and the product (Romeis et al., 2008).

The data provided in this section describe the results of tier-based testing. The tiered NTO assessment data are an important source of information to evaluate the potential effects of mCry51Aa2 to NTOs since the taxa evaluated are tested at concentrations much higher than environmental exposure levels, therefore ensuring a sufficient margin of safety. In case an effect was observed on a species when tested under these conservative exposure levels, higher-tier studies were conducted to ensure a comprehensive understanding of any potential impact of MON 88702 on NTOs in the cotton agro-ecosystem.

V.B.5.1. Tier-based Approach for NTO Testing

V.B.5.1.1. Introduction

The testing of any impact of mCry51Aa2 expressed in MON 88702 cotton on NTOs was done according to U.S. regulatory guidelines for NTO testing and risk assessment of insect-protected crops (crops expressing PIPs). These were developed by the EPA and suggest that testing and assessment be conducted based on a tier-based system (U.S. EPA, 2010b). In this tiered approach, risk (a function of hazard and exposure) is evaluated within different levels or "tiers" that progress from worst-case scenarios to increasingly more realistic exposure scenarios, as refinement if the earlier tiered tests fail to indicate adequate certainty of acceptable risk. Additionally, the EPA has convened several Scientific Advisory Panel meetings to gather recommendations and provide guidance for NTO testing and risk assessment for PIPs (U.S. EPA, 2001; 2002; 2004; 2010b). Following the tiered approach, the tier 1 study represents a worst-case exposure scenario to estimate hazard using an exposure pathway that is usually not realistic due to the high level of continuous exposure (e.g. laboratory assays using artificial diets). Typically, an exposure factor of 10-fold, a highly conservative exposure estimate, is administered in a tier 1 test. The EPA has established that "*an endpoint of 50% mortality to be used as a trigger for additional higher tier testing. Less than 50% mortality under these conditions of extreme exposure suggest that population effects are likely to be negligible given realistic field exposure scenarios*" (U.S. EPA, 2010b).. However, if an adverse effect (i.e. less than 50% survival) is observed under these conditions, this does not necessarily indicate the PIP poses an unacceptable risk in the field, but it does trigger the need for a better understanding of potential hazards at lower test doses using more realistic exposure conditions reflective of field concentrations (e.g. 1X the amount of the

PIP expected to be available to the NTO at the field level). When these initial tests trigger the need for additional data, a higher-tier study may be conducted. The objective of a tier 2 study is then to collect further information about the impact of the PIP on the NTO under refined exposure conditions (e.g. tri-trophic feeding studies for testing of predatory NTOs). Such a study will use a design that is more complex than previous studies. Any adverse effects observed in a tier 2 study will trigger a tier 3 study (e.g. long-term laboratory and/or semi-field tests). The outcomes of this study may ultimately lead to a tier 4 study, i.e. a full season field test, that represents the most realistic exposure scenario. Such a field test may be conducted across multiple locations and may involve looking at specific (and/or groups of) insects or could be a census study where organisms from a wide variety of taxa are collected and identified (U.S. EPA, 2010b; USDA-APHIS and EPA, 2007). Conservative interpretation of these criteria may also be applied where an impact is observed and at times was deemed necessary to ensure proper characterization of the risk to NTOs.

Based on the results from the activity spectrum assessment (Section V.B.4) and known expression levels of the mCry51Aa2 protein in MON 88702 (Section V.A), an evaluation of the potential toxicity to selected NTOs was conducted. The tiered NTO studies performed for the mCry51Aa2 protein expressed in MON 88702 cotton followed either established EPA/OECD protocols or in-house protocols that were sufficiently powerful to detect adverse effects to biologically relevant endpoints that are consistent with EPA's specific protection goals (e.g. pollination services, biological control). Surrogate beneficial species used in tier 1 laboratory tests were selected based on the results of the activity spectrum assessment and to ensure representation of different taxonomic groups, habitats and functions in the agro-ecosystem (cotton fields), the characteristics of the crop (cotton), the trait (insect protection) and the availability of robust tier 1 test methods. The lack of adverse effects to an ecosystem function indicates that it will be maintained in the cotton agro-ecosystem (Figure V-3). This approach follows common practices as described in policy documents and the peer-reviewed literature (Dutton et al., 2003; Romeis et al., 2013; USDA-APHIS and EPA, 2007; Wach et al., 2016). The use of surrogate species in an NTO assessment is also in line with the strategy for tier-based testing for the effects of PIPs on non-target invertebrates described by the USDA-EPA (USDA-APHIS and EPA, 2007), in which it was stated that "*it is impossible to test all species that are potentially present…*".

Tier 1 testing to assess the hazard of MON 88702 included laboratory toxicity testing against a representative pollinator [honey bee larvae and adults (*Apis mellifera*)], eight beneficial insect species that represent biocontrol species [parasitic wasp (*Pediobius foveolatus*), lady beetle (*Coccinella septempunctata*), rove beetle (*Aleochara bilineata*), lacewing (*Chrysoperla carnea*), insidious flower bug (*Orius insidiosus*), big-eyed bug (*Geocoris punctipes*), Western damsel bug (*Nabis alternatus*), and leafhopper assassin bug, (*Zelus renardii*)], and two representative soil biota [earthworm (*Eisenia andrei*) and collembola (*Folsomia candida*)]. Ecologically relevant endpoints of survival and/or growth and development observations were assessed in the lady beetle, rove beetle, lacewing, insidious flower bug, big-eyed bug, Western damsel bug, leafhopper assassin bug, and honey bee studies, while survival and reproduction were assessed in collembola and survival and biomass in earthworm. Tests were of sufficient duration to detect

adverse effects based on the mode of action of the mCry51Aa2 protein and time to effect in sensitive species. All the studies met specific performance criteria demonstrating their validity, including protein stability (Appendix I.1).

Test concentrations in the tier 1 studies were based on the measured mCry51Aa2 protein expression in the tissue type(s) that the NTO would most likely be exposed to in the environment. A targeted, conservative margin of exposure (MOE) of ≥10 times the expected environmental concentration (EEC) typically was used to set test concentrations (U.S. EPA, 2010b). The results from these NTO studies were evaluated using the high-end EEC values derived from the MON 88702 expression levels (Section V.B.3; Table V-3). The MOE for each species was calculated based on the ratio of the No Observed Effect Concentration (NOEC) to the EEC (Table V-16). For a conservative tier 1 assessment, an MOE that is ≥10x the EEC, using a median lethal concentration (LC_{50}), is indicative of negligible risk. EPA guidance states that only adverse effects to NTOs at ≤ 1x the realistic field exposure are viewed as an environmental risk (U.S. EPA, 2010a; b; USDA-APHIS and EPA, 2007).

V.B.5.1.2. Results - Predatory Hemiptera

Cotton fields can support a diverse population of predatory arthropods that play a role as natural enemies in cotton pest management. Predation in cotton fields is primarily conducted by generalist predators (Torres and Ruberson, 2005) with the most abundant predators in cotton fields being *Orius* spp., big-eyed bugs (*Geocoris* spp.), ladybird beetles and lacewings (Naranjo, 2005a; Naranjo and Ellsworth, 2009). Also damsel bugs (*Nabis* spp.) and assassin bugs (*Zelus* spp.) are known hemipteran predators in cotton fields, although the densities of *Zelus* spp. have been documented to be relatively low (Naranjo and Ellsworth, 2009; Torres and Ruberson, 2005). Because of their generalist behavior, predators present in a MON 88702 cotton field are expected to mainly be indirectly exposed to the mCry51Aa2 protein through consumption of diverse prey items.

As described previously, one important consideration in selecting surrogate beneficial species used in tier 1 laboratory tests was the results of the initial activity spectrum assessment. This activity spectrum assessment demonstrated activity of mCry51Aa2 against the target species *Lygus* spp. and the NTO *O. insidiosus*, both of which belong to the order Hemiptera (Section V.B.4). Therefore, tiered-based testing was used to comprehensively assess the risk to *Orius* spp. from MON 88702. In addition, several other predatory Hemiptera ((big-eyed bug (*Geocoris punctipes*), Western damsel bug (*Nabis alternatus*), and assassin bug, (*Zelus renardii*)) that are related to *Orius* spp. and will likely be present in U.S. cotton fields, were also included in the tiered NTO assessment supporting the risk assessment of MON 88702.

The results of the tiered NTO assessment that characterizes the risk of MON 88702 to *Orius* spp., *G. punctipes*, *N. alternatus* and *Z. renardii* are provided below. An overview of the results of the tiered NTO assessment for these hemipteran species is provided in Figure V-5.

V.B.5.1.2.1. *Orius* spp. – Tier 1

In the activity spectrum assessment, a significant effect on survival of five-day old *O. insidiosus* nymphs was observed (Section V.B.4.4). Subsequently, a tier 1 study was conducted to establish a NOEC for five-day old *O. insidiosus* nymphs over a concentration range from 13 to 500 µg/g diet of mCry51Aa2 (Appendix I.1.8). Effects on *O. insidiosus* survival were observed in a concentration dependent manner. At an mCry51Aa2 protein concentration of 13 µg mCry51Aa2/g diet there were no significant differences to the control treatment, while concentrations of ≥32 µg mCry51Aa2/g diet showed significant effects on survival with approximately 53% survival at 500 µg mCry51Aa2/g diet, resulting in an estimated LC_{50} of >500 µg mCry51Aa2/g diet. Therefore, the NOEC for the five-day old nymphs was determined to be 13 µg mCry51Aa2/g in diet, resulting in an MOE of 5.4 when considering the exposure to pollen (EEC = 2.4 µg mCry51Aa2/g fwt pollen, Table V-3, Table V-16; Figure V-5).

Orius spp. is primarily a beneficial arthropod in cotton fields where it functions as a major predator feeding on several prey items in the cotton agro-ecosystem including thrips, mites, aphids and whiteflies (Dicke and Jarvis, 1962; Kiman and Yeargan, 1985). It has also been reported to feed on plant tissue as a source of water and supplementary nutrition (Armer et al., 1998). Of the plant tissues, pollen is the typical source of supplemental nutrition (Tan et al., 2011) and therefore the mCry51Aa2 expression levels determined in this tissue type were used as EEC. Using a conservative approach, though unlikely exposure scenario that assumes *Orius* spp. will feed exclusively on MON 88702 leaf tissue, the use of the 95th percentile expression value with the highest fresh weight expression level (OSL3, Table V-3)), would result in an MOE of 0.026, which is below the threshold of 1one, set by EPA guidance (U.S. EPA, 2010a; b; USDA-APHIS and EPA, 2007). However, with *Orius* spp. being a generalist predator, these exposure levels would be expected to be significantly lower given the decrease in the mCry51Aa2 levels through the trophic layers in cotton fields as discussed in Section V.B.5.1.2.

In order to explore the relevance of leaf tissue as an exclusive food source and potential impacts of this MON 88702 exposure scenario on *Orius* spp., a leaf disk study with one-day old *O. insidiosus* nymphs was conducted. When exposed to MON 88702 or DP393 leaf disks, none of the individuals survived past 48 hours with either leaf as its only food source (assay A in Table V-8, Appendix I.3). This result is consistent with published literature, in which some leaf feeding has been reported under obligate conditions (Hagler et al., 2004), but *Orius* spp. could not survive with leaf tissue as a sole source of nutrition (Lumbierres et al., 2012). It is, therefore, unlikely that *Orius* spp. will consume high amounts of leaf tissue and that it is an inadequate food source. In a scenario where one-day old *O. insidiosus* nymphs were exposed to MON 88702 and DP393 leaf disks in the presence of eggs of the lepidopteran Mediterranean flour moth *Ephestia kuehniella* as a food source, no effect on survival was observed in the treatments (Section V.B.5.1.2.3), further demonstrating that *O. insidiosus* is unlikely to consume high levels of mCry51Aa2 through leaf feeding.

The limited utility of vegetative tissue for developing nymphs and the importance of pollen as an alternative food source to insect prey support the use of pollen as an appropriate

tissue type for use as the EEC in this first tier NTO assessment. Therefore, an MOE of 5.4 could be established for *O. insidiosus* (Table V-16). According to U.S. EPA guidance, this would not be classified as an environmental risk because the MOE of 5.4 is based on a NOEC that included an ecologically relevant sublethal endpoint (U.S. EPA, 2010a; b; USDA-APHIS and EPA, 2007). However, under a conservative assessment scenario and in order to characterize any potential risk of MON 88702 to *Orius* spp. under more realistic exposure scenarios a tier 2, tri-trophic feeding study was conducted.

V.B.5.1.2.2. *Orius* spp. – Tier 2

Given that the laboratory assays assume a worst-case exposure scenario, a better understanding of the dynamics of how *Bt* proteins are distributed throughout the trophic layers in cotton fields becomes important in understanding the exposure to these proteins for various insects. A comprehensive field assessment of *Bt* protein distribution demonstrated that lower levels of protein were detected in herbivore insects than in the plant, and that predators contained less *Bt* proteins than herbivores, with most predators containing less than the limit of detection of the assay and a few with levels that were two to three orders of magnitude below the levels in plants; *O. insidiosus* was in this latter group (Eisenring et al., 2017). *O. insidiosus* is a predatory insect (Armer et al., 1998; Tan et al., 2011)and, therefore, a tier 2 experiment represents a more realistic route of exposure for *O. insidiosus* to the mCry51Aa2 protein in MON 88702 cotton.

The generally accepted strategy for prey selection in tri-trophic feeding studies is to select species that are not susceptible to the insecticidal protein, provided it can be demonstrated that there was uptake of the protein by the herbivore (Romeis et al., 2011). Using prey species that are sensitive to the protein has the potential to affect the quality of the prey for the predator, consequently making it difficult to distinguish prey-quality mediated effects from effects of the protein (Naranjo, 2009; Romeis et al., 2006; USDA-APHIS and EPA, 2007).

In this first tier 2 study, the objective was to assess if there were any potential adverse effects of the mCry51Aa2 protein on *O. insidiosus* when exposed through consumption of a prey item. Five-day old *O. insidiosus* nymphs were exposed to mCry51Aa2 through the consumption of a representative lepidopteran prey species *Spodoptera frugiperda* larvae. This is a widespread and important agricultural pest in many crops, and is also known as an occasional pest in cotton (Ali et al., 1990; Ali et al., 1989; Luttrell and Mink, 1999). In addition, it was deemed an appropriate prey for this assessment because it was not affected by the mCry51Aa2 protein in the activity spectrum assessment (Section V.B.4.6). Therefore, by using *S. frugiperda* larvae, the quality of the prey was not expected to change upon consumption of mCry51Aa2. The *S. frugiperda* larvae were fed with a diet containing a nominal concentration of 2500 µg/ml mCry51Aa2 protein. The dietary uptake of the protein by *S. frugiperda* was determined through an ELISA (Table V-16, Appendix I.2.1) at a concentration of 6.47 µg mCry51Aa2/g in the larvae prior to feeding to the *O. insidiosus* nymphs. This demonstrated that, when fed high concentrations, detectable quantities of the protein could be transferred to the next trophic level. No adverse effects were observed after 10 days when *O. insidiosus* fed on mCry51Aa2 protein-exposed *S. frugiperda*. This is not unexpected considering that the

levels of the mCry51Aa2 protein measured in *S. frugiperda* larvae were below the NOEC of 13 µg mCry51Aa2/g in diet for five-day old *O. insidiosus* nymphs established the tier 1 test (Figure V-5 and Section V.B.5.1.2.1). The MOE calculated for *O. insidiosus* in this tri-trophic feeding study was 4.1x the EEC (Table V-16).

(Head et al., 2001)In studies that assessed the levels of Cry proteins in different species abundant in *Bt* crop fields, spider mites have been described as a species that can contain high levels of Cry protein after feeding on *Bt* crops (Torres and Ruberson, 2008). Based on this information, a second tri-trophic feeding study was conducted using spider mites as a prey, to assess a possibly higher trophic transfer of the mCry51Aa2 protein and, therefore, higher exposure to the protein.

In a first instance, the second tri-trophic experiment used one-day old nymphs of a sister species, *Orius majusculus*, and spider mites (*Tetranychus urticae*) (Appendix I.2.2). No significant differences were observed for spider mites feeding on MON 88702 or DP393 leaves in any of the parameters measured, confirming that spider mites are a non-sensitive prey. The uptake of the mCry51Aa2 protein by the mites from MON 88702 leaf disks was confirmed by ELISA, with median protein levels ranging from 11 to 85 µg mCry51Aa2/g spider mite fwt. Based on 1) the absence of sensitivity of spider mites to the mCry51Aa2 protein and 2) the detectability of the protein in the mites after feeding on MON 88702 leaf disks, *T. urticae* was deemed to be an appropriate prey for the tri-trophic feeding experiments with *O. majusculus*. The one-day old *O. majusculus* nymphs were placed on MON 88702 or DP393 leaf disks and MON 88702- or DP393-fed spider mites were added *ad libitum* to the respective treatments. The leaf disks were replaced every 3-4 days throughout the assay duration of 16 days, until adult emergence. *O. majusculus* development and survival were significantly impacted when feeding on *T. urticae* that had fed on MON 88702 compared to DP393 (assay B in Table V-8 and Figure V-5).

To assess whether *O. majusculus* would be less sensitive to mCry51Aa2 at a later life stage, the experiment was repeated with five-day old *O. majusculus* nymphs. In this scenario, survival was not significantly different between nymphs feeding on MON 88702 and DP393 leaf disks with MON 88702- or DP393-fed spider mites, respectively. Significant sublethal effects were observed, including lower female weight and longevity, as well as a reduction in number of eggs laid on MON 88702 compared to DP393 leaf disks (defined "fecundity" in Appendix I.2.2), although no difference in the percentage of eggs hatched was observed (assay C in Table V-8). Considering the absence of impact on five-day old nymph survival, younger *O. majusculus* nymphs were clearly more sensitive to the mCry51Aa2 protein (Figure V-5), and subsequent experiments used one-day old *Orius* spp. nymphs.

To assess the potential for species-related effects, the same experiment was conducted using one-day old *O. insidiosus* nymphs (Appendix I.2.3). Determination of mCry51Aa2 levels in spider mites (25 µg/g fwt) and MON 88702 leaf disks (234-270 µg/g fwt) ensured the potential for mCry51Aa2 exposure to the nymphs throughout the 13-day assay. The results of the experiment show that approximately 22% of the *O. insidiosus* population survived on the MON 88702 spider mite/leaf treatment compared to

approximately 89% survival on the DP393 spider mite/leaf treatment (assay D in Table V-8). These survival rates are comparable to what was observed for *O. majusculus* and therefore a species-related difference in response to the MON 88702 spider mite/leaf treatment was not observed.

These tier 2 experiments indicate a development stage-dependent effect of the mCry51Aa2 protein on *Orius* spp. nymphs following exposure to spider mites containing the protein as a sole and obligate prey. *Orius* spp. is a generalist predator feeding on different prey items present in cotton fields while occasionally feeding on plant tissue to complement its diet. Therefore, to more closely represent the ecologically relevant feeding behavior of *Orius* spp., a higher tier study using alternative prey options was initiated under controlled environmental conditions.

V.B.5.1.2.3. *Orius* spp. – Tier 3

The objective of the tier 3 study was to assess the impact of MON 88702 on *Orius* spp. in further refined exposure conditions that are more representative of the field scenario. Prior to conducting the tier 3 study, two additional leaf disk experiments were conducted with *O. insidiosus* nymphs (Appendix I.3).

A first leaf disk experiment assessed the ability of *O. insidiosus* to survive on leaf tissue alone and was introduced previously in the description of the tier 1 study. The results demonstrated that *O. insidiosus* is unlikely to consume high amounts of leaf tissue since it cannot survive solely on this tissue (assay A in Table V-8 and Section V.B.5.1.2.1).

A second leaf disk study was conducted with one-day old *O. insidiosus* nymphs exposed to MON 88702 or DP393 leaves and fed with eggs from the lepidopteran Mediterranean flour moth *Ephestia kuehniella* (Section V.B.5.1.2.1). This study served to test the following two hypotheses: 1) when given a proper food source, *O. insidiosus* survival will not be impacted by the *ad libitum* consumption of MON 88702 leaf tissue; and 2) *E. kuehniella* is an appropriate prey as *Orius* spp. is known to feed on their eggs and provides an adequate nutrition source (Bernardo et al., 2017). During the study, the leaves were replaced every three days (the stability of mCry51Aa2 in the leaf disks was confirmed for this three-day period) and fresh *E. kuehniella* eggs were added at each leaf replacement. The results of the experiment demonstrated that there was no significant difference in the survival of one-day old *O. insidiosus* nymphs on the MON 88702 and DP393 leaf disks (82 and 88% survival, respectively) (assay E in Table V-8). After 11 days, all the surviving nymphs had developed into adults on both MON 88702 and DP393 leaf disks. This result supports the first hypothesis by demonstrating that, when given the opportunity to feed on leaf tissue, *O. insidiosus* does not consume enough mCry51Aa2 to impact its survival or development. This indicates that there is no risk to *O. insidiosus* from consumption of green tissue of MON 88702 since it is not its preferred dietary tissue. As discussed previously, this would imply that MOE calculations based on pollen expression levels to determine EECs are adequate for *O. insidiosus* (Section V.B.5.1.2.1). These results also confirm the second hypothesis, i.e. *E. kuehniella* eggs are a nutritious prey, for a generalist predator likes *O. insidiosus*.

With the results of the two abovementioned leaf disk assays, a tier 3 study was conducted in which the potential impact of MON 88702 on *Orius* spp. in a choice experiment was assessed.An evaluation of *O. insidiosus* development and survival was conducted when exposed to MON 88702 or DP393 leaf disks, spider mites (*T. urticae*) fed on either MON 88702 or DP393, and eggs of Mediterranean flour moth (*E. kuehniella*) as an alternative prey. The hypothesis of the tier 3 study was that when one-day old *O. insidiosus* nymphs are exposed to the mCry51Aa2 protein via different possible routes of administration, both prey and plant tissues, but allowed an alternative food source, no impact on their survival or development would be observed. An evaluation of *O. insidiosus* development and survival was conducted when exposed to MON 88702 or DP393 leaf disks, spider mites (*T. urticae*) fed on either MON 88702 or DP393, and eggs of Mediterranean flour moth (*E. kuehniella*) as an alternative prey. No differences in *O. insidiosus* development or survival were observed when exposed to *T. urticae* that had fed on MON 88702 or DP393 cotton leaves and *E. kuehniella* eggs. After 11 and 12 days on MON 88702 or DP393 leaf disks, respectively, all of the surviving *O. insidiosus* nymphs (86% for both treatments) had developed into adults (assay F in Table V-8) (Figure V-5). Importantly, as was the case in the tier 2 study with *T. urticae* as obligate prey, during the assaytier 3 study the *O. insidiosus* nymphs were observed feeding on *T. urticae*, which confirms there was exposure to the mCry51Aa2 protein. The results of the tier 3 study support the hypothesis that under more realistic exposure scenarios, where *O. insidiosus* has the opportunity to feed on a combination of different types of abundant prey and plant tissue, MON 88702 does not impact *O. insidiosus* development or survival. Therefore, due to their feeding ecology, being a generalist predator, *Orius* spp. is not expected to encounter levels of the mCry51Aa2 protein in the field that would result in an adverse biological effect and consequently these results indicate that it is unlikely that MON 88702 will pose a risk to *O. insidiosus*.

It should be noted the same three leaf disk experiments were conducted with *O. majusculus*. Preliminary results from these studies resulted in similar outcomes where no impact of mCry51Aa2 on *O. majusculus* was observed, further confirming that the effect of the mCry51Aa2 protein on *Orius* spp. observed in a tier 2 study was mitigated under a more realistic exposure scenario of the tier 3 study, and further confirms a lack of a species-specific effect.

Table V-8. Overview of the Survival Results Observed in Leaf Tissue Assays Conducted with *Orius* spp.

	Leaf Disk Assay	Testing	Species	Age	Survival (%) - MON 88702	Survival (%) - DP393	Significant Difference Between Test and Control	Reference to Study Details
A	Leaf only	NA[1]	*Orius insidiosus*	One day	0	0	No	Appendix I.3
B	Leaf + spider mites	Tier 2	*Orius majusculus*	One day	10	79	Yes	Appendix I.2.2
C	Leaf + spider mites	Tier 2	*Orius majusculus*	Five days	95	100	No[2]	Appendix I.2.2
D	Leaf + spider mites	Tier 2	*Orius insidiosus*	One day	22	89	Yes	Appendix I.2.3
E	Leaf + *E. kuehniella* eggs	NA[1]	*Orius insidiosus*	One day	82	88	No	Appendix I.3
F	Leaf + spider mites + *E. kuehniella* eggs	Tier 3	*Orius insidiosus*	One day	86	86	No	Appendix I.3

[1] These studies were conducted in preparation of the tier 3 study.

[2] A significant reduction in female weight, longevity and number of eggs laid was observed between five-day old nymphs on MON 88702 and DP393 leaf disks. No difference in their development time or the percentage of hatched eggs from the adult females was observed (Appendix I.2.2).

The tiered approach described above demonstrates that under high concentrations and continuous exposure there is an impact of the mCry51Aa2 protein on *Orius* spp., which is not observed under laboratory assay conditions that represent more realistic exposure scenarios. The tier 3 assay, which represented the closest approximation to field conditions under controlled environmental conditions, indicates that *Orius* spp. exposure to the mCry51Aa2 protein in MON 88702 will be below the NOEC resulting in negligible risk to this predator. As an added measure of conservative assessment of the potential of MON 88702 posing any risk to *Orius* spp. and other related hemipteran predators, a tier 4 field study was carried out which used the most realistic exposure scenario representing the conditions encountered in commercial cotton cultivation.

V.B.5.1.2.4. *Orius* spp. – Tier 4

This study was conducted at six locations in the U.S. cotton belt during the 2018 growing season: Pinal county, Arizona (AZMA); Yuma county, Arizona (AZYU); Rapides parish, Louisiana (LACH); Washington county, Mississippi (MSGV); Edgecombe county, North Carolina (NCRC); Uvalde county, Texas (TXUV). At each site MON 88702 and the conventional control DP393 were planted under different insecticide regimes. Five different treatments, with three replicates each, were included at all sites: 1) DP393 treated with a broad spectrum insecticide regime; 2) DP393 treated with a selective insecticide regime; 3) MON 88702 treated with a selective insecticide regime; 4) DP393 untreated; and 5) MON 88702 untreated.

The insecticide applications in treatments 1, 2 and 3 were conducted at or above local recommended thresholds for *Lygus* spp. control. Treatment 1 (DP393) received acephate applications as needed. Acephate is a broad spectrum organophosphate that is frequently used across the U.S. cotton belt as an effective option for *Lygus* spp. control and known to adversely impact beneficial arthropods (Asiimwe et al., 2014; Catchot, 2019; Ellsworth, 1999; 2004). The number of acephate applications ranged from 1-3 across sites for treatment 1 (Appendix I.4). This treatment therefore served as a positive control within the field study with the expectation that its application would depress both *Lygus* spp. and beneficial arthropod populations in these plots relative to unsprayed plots with the same material (DP393, treatment 4) thus validating the ability of the experiment to detect a difference in insect abundance when an insecticide is applied. Treatments 2 (DP393) and 3 (MON 88702) were treated with a selective insecticide regime where insecticides that are known to have a reduced-risk profile to beneficial insects were applied at threshold. The selective insecticides used were flonicamid, imidacloprid and sulfoxaflor, which are known to provide effective control against *Lygus* spp.but have minimal to no effect on beneficial arthropods (Asiimwe et al., 2014; Catchot, 2019). The number of selective insecticide applications ranged from 1-2 across the season at most sites (Appendix I.4). Treatments 2 and 3 served to assess any effects of MON 88702 on NTOs when planted under conditions that are realistic for certain areas in the U.S. cotton growing region. Finally, treatments 4 and 5 did not receive any insecticide applications throughout the season and, therefore, served as a baseline for NTO populations in the cotton fields. In addition, as discussed in Section V.B.4.2 (Table V-4), the abundance of the target pest, *Lygus* spp., was monitored and compared between entries 4 and 5 in order to confirm the ability of the field trial to detect the expected trait effect.

The five different entries were replicated three times per site in a randomized complete block design with plot sizes approximately 20m x 20m at all but one site, where the plot size was 18m x 18m. Inter-plot isolation ranged from 3-4m across all sites to minimize arthropod movements across plots. An example of a trial map is provided in Figure V-4.

Weekly samples were collected from all the plots over the course of the growing season, initiated at early squaring through cut-out for a total of 10 sampling times over the course of the season. A vertical beat sheet method was used consistently to determine nymph and adult abundance of the beneficial Hemiptera (*Orius* spp., *Geocoris* spp., *Nabis* spp., *Zelus* spp., predatory stink bug) across all sampling times and sites (Drees and Rice, 1985). All beneficial Hemiptera sampling was conducted in the center of each plot to ensure within-plot effects were captured, while minimizing edge effects. Additionally, densities of key cotton pests; *Lygus* spp., thrips, cotton fleahoppers, bollworms stink bugs, aphids, whiteflies and spider mites, were also monitored using the appropriate sampling methods for each case, which included sweep nets for *Lygus* spp., cotton fleahoppers and stink bugs, and visual leaf turns for spider mites, thrips and bollworms. Monitoring the abundance of aphids, whiteflies, *Lygus* spp., cotton fleahopper and aphids was also conducted using the vertical beat sheet method. All sampling was conducted in prior to high noon and was conducted consistently by the same people at each site, to minimize variability.

The relative abundance of each arthropod was evaluated across sites and collections using a linear mixed model (Appendix I.4). In order to enable a valid analysis of any potential treatment effect, inclusion criteria were applied for each species: 1) the mean count per plot had to be ≥ 1; 2) at least one capture from each replicated plot at each collection time. Based on the statistical model, all other data, including zero counts, were included in the analysis. All pairwise comparisons between treatments were made using Least Squares (LS) means at the $\alpha = 0.05$ level of significance. LS means are treatment means appropriately adjusted for the other effects in the model and may therefore be different from the arithmetic means (Milliken and Johnson, 2009). The arithmetic means are presented in the tables in this Section since they provide context on the actual abundance of taxa collected from field plots. Both the arithmetic and LS means for each collected species are provided in Appendix I.4.

A power analysis was conducted for arthropod population comparisons between the different treatments. The statistical power was estimated assuming a 50% difference (i.e. effect size) in the abundance of each taxonomic group. This effect size was based on several publications that have indicated a 50% difference in arthropod abundance may be considered both ecologically-relevant and practical for detection in the field (Blümel et al., 2000; Candolfi et al., 2000; de Jong et al., 2010; Perry et al., 2003); and followed the method introduced by Duan et al. (2006). Further, (Naranjo, 2005a; b) determined that a 50% effect size was appropriate based on previous studies comparing predator abundance in *Bt* and non-*Bt* cotton plots, that indicated this percent reduction in abundance of key predators in the cotton agro-ecosystem would result in a biologically meaningful reduction in the biological control function. Further details for this analysis are provided in Appendix I.4.

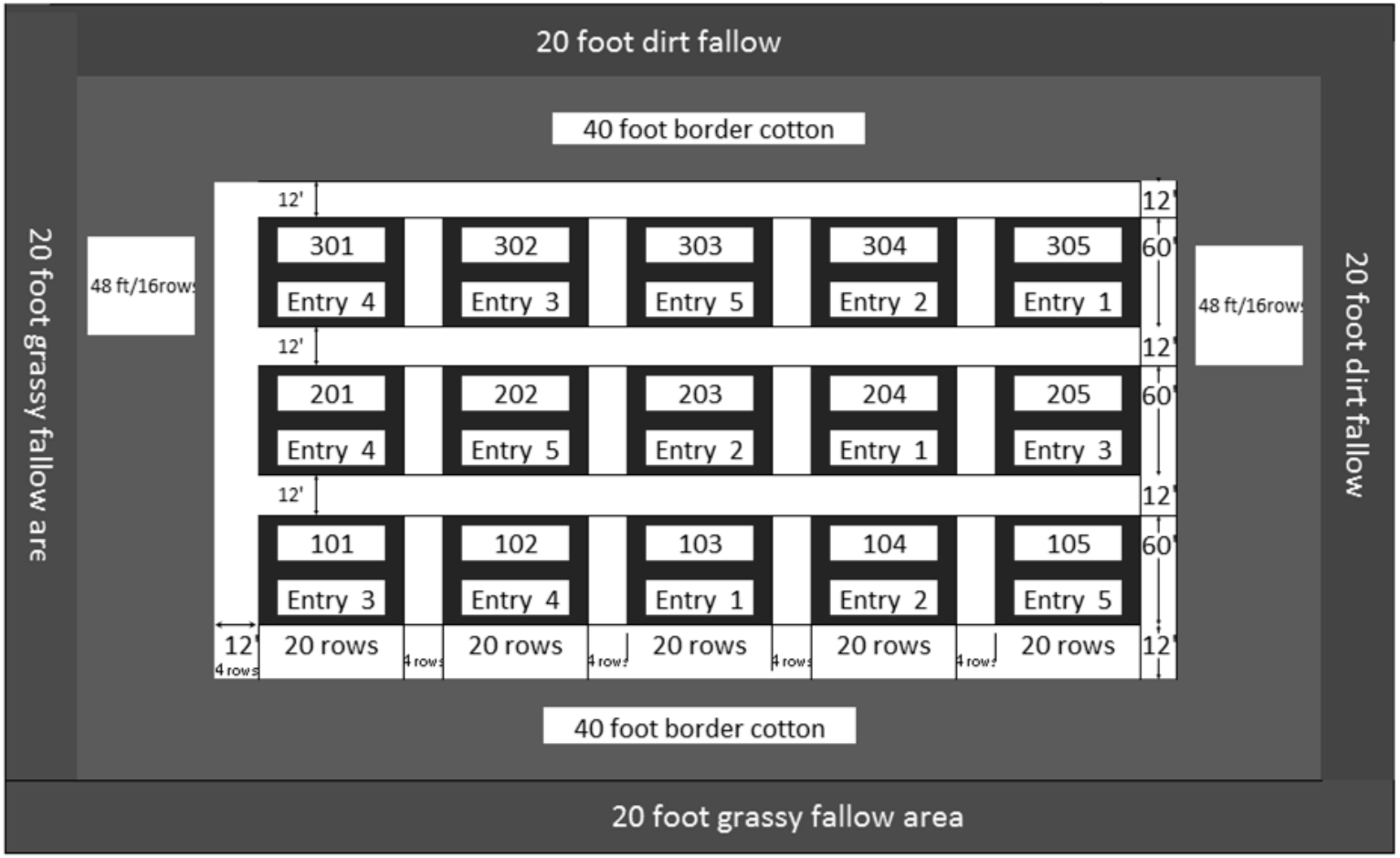


Figure V-4. Example of a Trial Map for a Site Included in the Tier 4 Field Study

A combined-site analysis provided the ability to compare *Orius* spp. abundance between different treatments with a high statistical power (Table V-9). Abundance groupings included nymphs, adults and a "total" category for both nymphs and adults. Comparing their abundance in positive control treatment 1 plots, i.e. DP393 treated with a broad spectrum insecticide, to unsprayed DP393 plots (treatment 4) in the combined-site analysis, a significant difference in *Orius* spp. nymph, adult and total abundance was detected (Table V-9). This is consistent with what has been described in available literature that documents the application of acephate significantly reduces NTO abundance (Asiimwe et al., 2014; Catchot, 2019; Ellsworth, 1999; 2004). These results demonstrate the ability of the field trial design to detect the effect of the broad spectrum insecticide spray on *Orius* spp. abundance.

In order to assess whether there were any MON 88702-related effects on nymph, adult or total abundance, *Orius* spp. abundance was compared between unsprayed DP393 (treatment 4) and unsprayed MON 88702 (treatment 5) plots in a combined-site analysis (Table V-9). This comparison did not show any statistically significant difference in *Orius* spp. nymph, adult or total abundance between the two treatments.

The individual-site abundance data for these treatments are provided in Table V-10, and *Orius* spp was abundant in all sites. An individual-site analysis shows that *Orius* spp. abundance was not consistently higher or lower in either unsprayed DP393 (treatment 4) or unsprayed MON 88702 (treatment 5) plots (Table V-10). While the majority of the sites showed higher nymph abundance on unsprayed MON 88702 plants (Table V-10), significantly lower *Orius* spp. nymph and total abundance was observed in unsprayed MON 88702 plots compared to unsprayed DP393 plots at the AZYU and MSGV sites, respectively. The mean abundance in the unsprayed MON 88702 compared to unsprayed DP393 plots in AZYU was 1.6 to 3 nymphs, respectively. The total *Orius* spp. abundance in the unsprayed MON 88702 compared to unsprayed DP393 plots in MSGV was 6.2 to 9.1, respectively. In DP393 and MON 88702 plots treated with selective insecticides (treatments 2 and 3, respectively) no significant difference in mean abundance of *Orius* spp. nymphs at AZYU or *Orius* spp. total abundance at MSGV were observed, indicating a lack of a trait-related effect under these conditions (Appendix I.4).

Based on these results, MON 88702 is not expected to reduce *Orius* spp. abundance in cotton fields (Figure V-5). This conclusion is further supported by the field environmental interaction assessment data (Section VII.C.2.2.2), a census study in which the abundance of arthropods typically present in cotton fields was monitored, including *Orius* spp. Seasonal abundance of *Orius* spp. across several sites in the U.S. cotton belt was not different in MON 88702 compared to conventional cotton.

Taking into consideration the results from the tier 2 study during obligatory feeding on MON 88702-fed spider mites (Section V.B.5.1.2.2), the populations of spider mites were monitored during the season. Abundance of spider mite eggs, nymphs and adults was determined by visual inspection of the cotton plants. Overall, their abundance was low in the majority of the sites, but sufficiently high to enable a statistical analysis in the NCRC and TXUV sites (Table V-11), which are sites at which also *Orius* spp. was abundant

(Table V-10). The simultaneous presence of *Orius* spp. and spider mites in unsprayed MON 88702 plots in the absence of a significant difference in *Orius* spp. abundance compared to unsprayed DP393 plots at this location supports the conclusion from the tier 3 study, i.e. that MON 88702 is unlikely to impact *Orius* spp. abundance in a field scenario where a variety of prey items, including spider mites, is available.

Table V-9. Combined-site Mean[1] Abundance of *Orius* spp. in Sprayed and Unsprayed DP393 and Unsprayed MON 88702 Cotton Plots

Life Stage	Mean (SE) DP393 Sprayed with Broad Spectrum Insecticide	Mean (SE) DP393 Unsprayed	Mean (SE) MON 88702 Unsprayed	Power (%)[2]
Adults	11.5 (0.92)*	15.0 (0.68)	16.2 (0.27)	99.4
Nymphs	10.8 (1.19)*	11.4 (0.78)	12.4 (0.73)	99.7
Total	19.3 (1.70)*	22.7 (1.11)	24.3 (0.73)	100.0

[1] This table provides the arithmetic means. Least Square (LS) means were used in the statistical model for conducting pairwise comparisons and are provided in Appendix I.4.
[2] Power to detect a 50% difference in abundance.
* Indicates a statistically significant difference with the unsprayed DP393 entry (α=0.05).

Table V-10. Individual-Site Mean[1] Abundance of *Orius* spp. in Unsprayed DP393 and Unsprayed MON 88702 Cotton Plots

Site[2]	Life Stage	Mean (SE) DP393 Unsprayed	Mean (SE) MON 88702 Unsprayed	Power (%)[3]
AZMA	Adults	4.0 (0.41)	3.6 (0.61)	72.6
	Nymphs	3.7 (0.10)	4.4 (0.62)	41.9
	Total	5.9 (0.30)	5.8 (0.92)	84.3
AZYU	Adults	3.4 (0.38)	2.7 (0.38)	60.7
	Nymphs	3.0 (0.19)	1.6 (0.33)*	54.8
	Total	6.5 (0.2)	4.2 (0.72)	79.7
LACH	Adults	NA[4]	NA[4]	NA[4]
	Nymphs	2.7 (0.35)	3.1 (0.36)	27.5
	Total	3.3 (0.44)	3.1 (0.18)	48.8
MSGV	Adults	4.3 (0.55)	2.9 (0.24)	47.0
	Nymphs	5.6 (1.40)	3.8 (0.61)	50.7
	Total	9.1 (0.83)	6.2 (0.29)*	74.7
NCRC	Adults	2.9 (0.43)	4.0 (0.40)	53.7
	Nymphs	4.2 (0.46)	4.6 (0.99)	67.8
	Total	7.1 (0.90)	8.6 (0.77)	89.7
TXUV	Adults	60.5 (5.80)	67.6 (1.38)	99.9
	Nymphs	49.0 (8.97)	56.7 (7.80)	91.4
	Total	104.2 (13.67)	118.0 (7.81)	100

[1] This table provides the arithmetic means. Least Square (LS) means were used in the statistical model for conducting pairwise comparisons and are provided in Appendix I.4.

[2] Site codes: AZMA = Pinal county, AZ; AZYU = Yuma county, AZ; LACH = Rapides parish, LA; MSGV = Washington County, MS; NCRC = Edgecombe County, NC; TXUV = Uvalde County, TX.

[3] Power to detect a 50% difference in abundance.

[4] Abundance was below the inclusion criterion and was therefore not statistically analyzed.

* Indicates a statistically significant difference with the unsprayed DP393 entry (α=0.05).

Table V-11. Individual-site Mean[1] Abundance of Spider Mites in Unsprayed DP393 and Unsprayed MON 88702 Cotton Plots

Site[2]	Life Stage	Mean (SE) DP393 Unsprayed	Mean (SE) MON 88702 Unsprayed	Power (%)[3]
NCRC	Eggs	NA[4]	NA[4]	NA[4]
	Nymphs + adults	2.3 (0.25)	5.8 (1.82)	15.6
	Total	4.5 (1.67)	6.4 (2.19)	23.2
TXUV	Eggs	12.3 (3.38)	24.3 (8.52)	42.5
	Nymphs + adults	13.7 (3.46)	20.8 (7.94)	59.0
	Total	23.3 (5.96)	40.1 (14.45)	58.7

[1] This table provides the arithmetic means. Least Square (LS) means were used in the statistical model for conducting pairwise comparisons and are provided in Appendix I.4.
[2] Site codes: NCRC = Edgecombe County, NC; TXUV = Uvalde County, TX.
[3] Power to detect a 50% difference in abundance.
[4] Abundance was below the inclusion criterion and was therefore not statistically analyzed.
[*] Indicates a statistically significant difference with the DP393 entry grown under the same conditions (α=0.05).

V.B.5.1.2.5. *Orius* spp. – Conclusion

A comprehensive assessment was conducted to characterize the potential risk that MON 88702 could pose to *Orius* spp. The assessment used a tiered approach where each higher tier further refined the exposure of *Orius* spp. to the mCry51Aa2 protein to reflect more realistic field-based exposure and risk.

After evidence of hazard was determined in an activity screening assay, a tier 1 diet feeding assay demonstrated significantly reduced survival of five-day old *O. insidiosus* nymphs at mCry51Aa2 protein concentrations > 13 µg/g diet (NOEC). This represented a worst-case scenario and, despite the inability to establish an LC_{50} for five-day old *O. insidiosus* nymphs, prompted tier 2 assays to assess risk under feeding scenarios representative of predatory insects.

In tier 2 studies, tri-trophic feeding assays with different prey items were used to characterize risk. In a first assay, five-day old *O. insidiosus* nymphs were exposed to mCry51Aa2-fed *S. frugiperda*. No adverse effects were observed likely because the protein concentration in *S. frugiperda* was below the NOEC established for five-day old *O. insidiosus* nymphs. In a second tier 2 study, one-day old *Orius* spp. nymphs were exposed to MON 88702-fed *T. urticae* and significant effects on nymph survival and development were observed, likely due to the higher mCry51Aa2 protein concentration in *T. urticae*. This feeding assay was repeated with five-day old *O. majusculus* nymphs and survival was not significantly different between MON 88702-fed *T. urticae* exposed nymphs and those not exposed to mCry51Aa2. This demonstrates the one-day old nymphs were more sensitive to the mCry51Aa2 protein than the five-day old nymphs. Therefore, further refinements used one-day old nymphs as a more sensitive indicator of hazard and incorporated other food sources to establish whether the impact on survival could be mitigated in a tier 3 feeding assay.

Before carrying out the tier 3 assay, the viability of one-day old *O. insidiosus* nymphs with different food sources was assessed. It was first established that leaves alone did not provide sufficient nutrition because *O. insidiosus* could not survive on leaves of either MON 88702 or DP393 alone. When *Ephestia kuehniella* eggs were added to leaf disks, *O. insidiosus* survived and developed normally, establishing *E. kuehniella* eggs as a good food source for *O. insidiosus*. Additionally, these results demonstrated that *O. insidiosus* exposure to the mCry51Aa2 protein in MON 88702 plant tissue was not significant in the presence of *E. kuehniella* eggs.

The tier 3 study was a tri-trophic feeding study that provided one-day old *O. insidiosus* nymphs with two prey items in a leaf disk assay, i.e. MON 88702-fed *T. urticae* and *E. kuehniella* eggs. This refinement reflected more realistic exposure scenarios for a generalist predator such as *Orius* spp. No adverse effects of MON 88702 on one-day old *O. insidiosus* nymphs were observed in this assay, indicating that the exposure to mCry51Aa2 was reduced when feeding on a combination of different abundant prey types and MON 88702 plant tissue. Though many additional prey combinations would be possible, the ultimate refinement to characterize the risk of MON 88702 to *Orius* spp. is a tier 4 assay where the field provides a variety of real-world feeding options and exposure scenarios.

The tier 4 field study represented a range of environmental and agronomic conditions under which MON 88702 is expected to be cultivated. Hemipteran predators, including *Orius* spp., were present and abundant at multiple sites, and were able to feed on any available food source (prey or plant tissue). The combined-site analysis showed no differences in abundance for *Orius* spp. (nymphs or adults, sampled throughout the entire growing season) between MON 88702 and DP393 cotton. These results demonstrated a lack of adverse effect of MON 88702 on *Orius* spp., confirming the tier 3 observations. Therefore, the comprehensive tiered assessment conducted with *Orius* spp. demonstrates that MON 88702 is unlikely to pose a risk to *Orius* spp. in cotton fields (Figure V-5).

V.B.5.1.2.6. Other Predatory Hemiptera - Introduction

Additional studies were conducted with predatory Hemiptera that are present in the cotton agro-ecosystem and taxonomically closely related to *Orius* spp. (Asiimwe et al., 2014; Naranjo and Ellsworth, 2009). The taxa assessed were big-eyed bug (*G. punctipes*), Western damsel bug (*N. alternatus*) and assassin bug (*Z. renardii*).

Big-eyed bugs (*Geocoris* spp.) feed on a wide range of prey items, where bollworm and budworm eggs, small caterpillars, thrips, mites, aphids, plant bugs and whiteflies are preferred. They are known to complement their diet by feeding on cotton nectar, leaves and plant fluids (Asiimwe et al., 2011; Torres and Ruberson, 2005; Vyavhare et al., 2017), although this species requires feeding on prey to support its proper development and fecundity (Stoner, 1970). *Geocoris* spp. is also, along with *Orius* spp, typically, the most abundant predators in the cotton fields (Naranjo, 2005a; Naranjo and Ellsworth, 2009).

Damsel bugs (*Nabis* spp.) can feed on a diversity of prey, including moth eggs, small larvae, aphids, cotton fleahoppers, Lygus, whiteflies, mites and occasionally other predatory insects (Naranjo, 2005a; Vyavhare et al., 2017). Limited feeding of *Nabis* spp. on cotton leaf tissue for moisture has been documented, but considering its role as a predator in the ecosystem, its development depends on the consumption of prey (Bellows et al., 1999; Lattin, 1989). In experiments where *Nabis* spp. nymphs were only fed plant tissue without prey, no development of the nymphs into adults was observed (Stoner, 1975).

The assassin bug (*Zelus* spp.), is one of the largest in terms of body size, though they are relatively low abundant predators in the cotton fields. It feeds on a wide variety of arthropods, including other predators (Cisneros and Rosenheim, 1998) and its prey preference is dependent on its development stage, with the prey size increasing as the predator body size increases (Cisneros and Rosenheim, 1997). The most common herbivores consumed by *Zelus* spp. are caterpillars, budworms and bollworms, beet armyworms, Lygus nymphs and lacewing larvae (Brown et al., 2011). Considering its role as an intraguild predator, *Zelus* spp. also feeds on key predators in the cotton agro-ecosystem (Naranjo and Ellsworth, 2009). Feeding on extrafloral nectaries has also been observed for this species (Vyavhare et al., 2017), while feeding on leaf is expected to be minimal considering *Zelus* spp. cannot develop on this tissue (Stoner, 1975).

V.B.5.1.2.7. Other Predatory Hemiptera – Tier 1

One-day old nymphs of *Geocoris punctipes*, *Nabis alternatus* and *Zelus renardii* were initially exposed to concentrations several-fold higher than the EEC observed across all plant tissues, using the conservative assumption that, despite their major role as predators, they would occasionally feed on developing fruiting structures. As indicated in Section V.B.3, the mCry51Aa2 protein concentration used in the diet assays to estimate a 10-fold exposure was based on the available, mean, fresh weight mCry51Aa2 expression levels determined in 2015.

The results of these studies demonstrated that there was no difference in survival compared to a buffer control for *G. punctipes*, *N. alternatus* and *Z. renardii* when fed with diet that contained a dose of 4000 µg mCry51Aa2 protein/g diet compared to when feeding on control diet (Table V-12) (Figure V-5). All surviving nymphs developed to adulthood under this chronic and conservative high exposure scenario. Since there were no effects on survival when feeding on mCry51Aa2 treatment diet, no LC_{50} could be estimated for these species (Table V-16). However, sublethal effects were observed at this concentration, including a longer development time for the three taxa and a significant decrease in adult biomass observed for *N. alternatus* and *Z. renardii*, when feeding on test compared to control diets.

To further characterize the response of these three Hemiptera to the mCry51Aa2 protein, additional assays were conducted using a concentration of 400 µg mCry51Aa2 protein/g diet. As expected, there was no difference in survival when compared to the control diet and all the surviving nymphs developed into adults. It took the *G. punctipes* and *N. alternatus* nymphs approximately one day longer to develop into adults when feeding on the test diet compared to the control diet (Table V-12). Although this difference was statistically significant, it is unlikely that this development time delay would result in a biologically relevant effect on populations of *N. alternatus* and *G. punctipes* in the field. No difference in adult biomass was observed for *N. alternatus* and *G. punctipes*. At this same concentration, a statistically significant difference between test and control diet for adult biomass and development time was observed for *Z. renardii*. The biomass of the surviving adults feeding on diet containing 400 µg mCry51Aa2 protein/g diet was significantly reduced compared to control diet. Although all nymphs were able to develop into adults, it took approximately 12.7 days longer when feeding on test diet (Table V-12).

It is important to note that this exposure scenario assumes worst-case, chronic and obligate feeding on MON 88702 plant tissue by these predators. However, under more realistic field conditions the exposure to the mCry51Aa2 protein would primarily happen through a variety of prey items that contain orders of magnitude less Cry protein (Section V.B.5.1.2.2 and Section V.B.5.1.2.5). Even in the case of those herbivores that tend to contain relatively high levels of Cry proteins, like spider mites, the levels are typically an order of magnitude below those measured in plant tissue. In the tier 2 studies completed with *Orius* spp. (Section V.B.5.1.2.2), it was demonstrated that spider mites accumulated at most 85 µg mCry51Aa2 protein/g fwt. Considering *Geocoris* spp. and *Nabis* spp. will spend the majority of their time feeding on herbivores present in cotton fields, exposure to the mCry51Aa2 protein will be several-fold lower than the tested concentration of

400 µg mCry51Aa2 protein/g diet. Even if these predators were to exclusively consume spider mites, the exposure to mCry51Aa2 would be 5-fold less than the lowest concentration tested. Consequently, MON 88702 is not expected to have any adverse effects on their abundance in the cotton agro-ecosystem.

In the case of *Zelus* spp., exposure to the protein is expected to be even lower than for *Orius* spp., *Geocoris* spp. and *Nabis* spp. considering its role as an intraguild predator (Naranjo and Ellsworth, 2009). Predators contain lower levels of Cry proteins than herbivores and in most cases they are not detected (Eisenring et al., 2017). This was also demonstrated for the mCry51Aa2 protein within the leaf disk assays conducted with *Orius* spp. (Appendix I.2.2). In this assay, the median protein levels in MON 88702-fed spider mites ranged from 11-85 µg/g fwt. The mCry51Aa2 levels measured in *Orius* spp. that had consumed MON 88702-fed spider mites were also determined, resulting in median concentrations ranging from 0.13-0.30 µg/g fwt, and therefore several-fold lower compared to the concentrations measured in spider mites. The feeding behavior of *Z. renardii* was studied by Cisneros and Rosenheim (1998); it was demonstrated that herbivores comprise a greater fraction of its diet in the nymphal stage while adults spend most of their time feeding on other predators. More specifically, the diet of second instar *Z. renardii* comprised for 93% of herbivores and for 7% of predators, whereas this was a 29 to 71% ratio for adults. Based on the documented feeding ecology and the mCry51Aa2 expression levels measured in herbivores (spider mites) and predators (*Orius* spp.), the protein concentration to which *Z. renardii* is expected to be exposed to could be calculated. Using the highest median protein levels measured in spider mites and *Orius* spp. and the diet composition values from Cisneros and Rosenheim (1998), a weighted exposure concentration of 79.1 µg/g fwt to second instar *Z. renardii* nymphs, and 24.9 µg/g fwt to adults was calculated. This confirms that the dose of 400 µg mCry51Aa2 protein/g diet administered in the assay represents a worst-case scenario and it is unlikely the species will be exposed to these levels of mCry51Aa2 protein in the field.

Based on the above, MON 88702 is not expected to pose a risk to *Zelus* spp. or any of the other Hemiptera tested when considering field exposure scenarios, nor to the overall predatory function of these Hemiptera in the cotton agro-ecosystem. However, a further assessment was conducted to evaluate any potential impact of MON 88702 on predatory Hemiptera under realistic and relevant environmental exposure conditions. Therefore, their populations were monitored within the tier 4 field study.

Table V-12. Summary of the Tier 1 Study Results with *G. punctipes, N. alternatus* and *Z. renardii*

		4000 µg mCry51Aa2 protein/g diet[1]	Buffer control[2]	400 µg mCry51Aa2 protein/g diet[3]	Buffer control[4]
G. punctipes	Survival (%)	96.55	89.66	97.5	100
	Time to adult (days±SE)	28.71 (±0.45)*	25.31 (±0.34)	30.33 (±0.30)*	29.13 (±0.17)
	Adult body mass (mg±SE)	3.78 (±0.15)	3.99 (±0.11)	4.26 (±0.14)	4.32 (±0.14)
N. alternatus	Survival (%)	100	100	97.37	90
	Time to adult (days±SE)	22.06 (±0.71)*	16.84 (±0.21)	21.41 (±0.42)*	20.28 (±0.34)
	Adult body mass (mg±SEM)	4.39 (±0.17)*	6.03 (±0.28)	6.35 (±0.26)	6.24 (±0.29)
Z. renardii	Survival (%)	60	72.41	65.52	NA[5]
	Time to adult (days±SE)	62.06 (±1.95)*	46.71 (±1.40)	59.42 (±2.23)*	NA[5]
	Adult body mass (mg±SE)	16.48 (±0.68)*	20.48 (±0.82)	17.63 (±0.73)*	NA[5]

1 The number of *G. punctipes*, *N. alternatus* and *Z. renardii* nymphs tested was 29, 32 and 30, respectively.
2 The number of *G. punctipes*, *N. alternatus* and *Z. renardii* nymphs tested was 29, 37 and 29, respectively.
3 The number of *G. punctipes*, *N. alternatus* and *Z. renardii* nymphs tested was 40, 38 and 29, respectively.
4 The number of *G. punctipes* and *N. alternatus* nymphs tested was 39 and 40, respectively. respectively.
5 For *Z. renardii* the treatments were tested in one set and therefore the same buffer control was used to compare the results from the two test diets against.
* Indicates a significant difference between the test and control diet (α=0.05).

V.B.5.1.2.8. Other Predatory Hemiptera – Tier 4

In order to assess any potential impacts of MON 88702 on *Geocoris* spp., *Nabis* spp. and *Zelus* spp. in a field scenario, their abundance was monitored within the tier 4 field study. Abundance groupings for these taxa included nymphs, adults and a "total" category for both nymphs and adults. In order to enable a valid analysis of any potential treatment effect, inclusion criteria were applied for each species (Section V.B.5.1.2.4). Based on these inclusion criteria, the abundance of separate nymphs and adults of *Nabis* spp., as well as *Zelus* spp. adults was too low to allow for a statistical comparison. A comparison of their total abundance (nymphs + adults) and the separate *Zelus* spp. nymph abundance could be conducted. *Geocoris* spp. was sufficiently abundant to enable an analysis of nymph, adult and total abundance.

In the combined-site analysis, the DP393 plots treated with a broad spectrum insecticide (treatment 1) significantly reduced the abundance of the predatory Hemiptera compared to the unsprayed DP393 plots (treatment 4), thus demonstrating the suitability of the study design to detect differences in their abundance when a broad spectrum insecticide is applied (Table V-13).

In a combined-site analysis, no significant differences in *Geocoris* spp. nymph, adult, and total abundance were observed between unsprayed DP393 (treatment 4) and unsprayed MON 88702 (treatment 5) plots (Table V-13).

Additionally, the individual-site analysis demonstrates that *Geocoris* spp. abundance was generally similar in these plots (Table V-14), and *Geocoris* spp. was abundant in all sites. One significant difference was observed for total *Geocoris* spp. abundance at one site (TXUV) (Table V-14), where the mean abundance in unsprayed DP393 and unsprayed MON 88702 plots was 5.4 and 4.2, respectively. However, under selective insecticide regimes, DP393 (treatment 2) and MON 88702 (treatment 3) plots showed no significant difference in mean abundance of total *Geocoris* spp. at TXUV, with mean levels of 2.8 and 3.5, respectively (Appendix I.4). Therefore, the single detected difference for total *Geocoris* spp. in the TXUV site was not considered meaningful in the overall assessment of an impact of MON 88702 and differences were not detected in the combined-site analysis. Similar results were observed in the environmental interaction census study (Section VII.C.2.2.2), in which *Geocoris* spp. could be collected from 10 of the 12 sampled sites and no statistically significant difference in their abundance in MON 88702 and DP393 plots was observed. These results therefore confirm the findings of the tier 1 study and support the conclusion that MON 88702 is unlikely to negatively impact *Geocoris* spp. abundance in cotton fields and, therefore, it does not pose a risk to this species (Figure V-5).

Due to its low abundance, only a statistical comparison for total *Nabis* spp. (nymphs + adults) was possible. In a combined-site analysis, no significant difference in their abundance was observed in unsprayed DP393 and unsprayed MON 88702 plots (Table V-13). In an individual-site analysis, no significant differences were detected for total *Nabis* spp. abundance between unsprayed DP393 and unsprayed MON 88702 plots (Table V-14). The results indicate a lack of a trait-related effect on the abundance of this species.

In a combined-site analysis, *Zelus* spp. nymph and total abundance in unsprayed DP393 and unsprayed MON 88702 plots was not significantly different (Table V-13 and Figure V-5). *Zelus* spp. could only be collected from two sites, AZMA and TXUV (Table V-14). In an individual-site analysis there were no significant differences between unsprayed DP393 and unsprayed MON 88702 plots for total *Zelus* spp. and *Zelus* spp. nymph abundance in the AZMA and TXUV sites, respectively. *Zelus* spp. nymph and total abundance in unsprayed MON 88702 plots was significantly lower compared to that in unsprayed DP393 plots in the AZMA and TXUV sites, respectively (Table V-14). When comparing the abundance in DP393 and MON 88702 plots that were treated with selective insecticides (treatment 2 and treatment 3, respectively), the significant differences at these sites were not observed, indicating a lack of a trait-related effect on the abundance of *Zelus* spp. under these conditions (Appendix I.4).

It should be noted that, due to their low abundance, the power of the field trial to detect a difference for *Zelus* spp. was relatively low compared to *Geocoris* spp. or *Orius* spp. The relative abundance of predators in controlled field experiments can be used to infer their overall contribution to biological control (Naranjo and Ellsworth, 2009). For *Zelus* spp., the relatively low abundance of this predator across the sites indicates that it does not contribute significantly to the overall biological control function in the cotton agro-ecosystem. This relatively low abundance has been observed in other studies monitoring predator abundance in cotton that have also demonstrated that *Orius* spp. and *Geocoris* spp. are considered to be the major hemipteran contributors to predation and consequently the biological control function (Asiimwe et al., 2014; Torres and Ruberson, 2005). Low abundance of *Zelus* spp. was also observed in the field environmental interaction census study (Section VII.C.2.2.2), in which arthropods in U.S. cotton fields were monitored. Within that study, *Orius* spp. was abundant in all of the twelve fields monitored in two growing seasons, while *Geocoris* spp. was abundant in ten of the twelve monitored fields. In contrast, *Zelus* spp. was only found in two out of the twelve sites. In addition, *Zelus* spp. has been demonstrated to negatively impact predation through intra-guild predation of the more common predators in the field (Cisneros and Rosenheim, 1997). Therefore, despite the differences in *Zelus* spp. observed in the individual-site analysis of the tier 4 study, it is unlikely that this would impact the overall biological control function in the cotton agro-ecosystem in the U.S.

Overall, MON 88702 is not expected to pose a risk to any of the Hemiptera tested when considering field exposure scenarios, nor to the overall predatory function of these Hemiptera in the cotton agro-ecosystem (Figure V-5). Therefore, based on the lack of effects on survival in tier 1 testing and the lack of significant abundance impacts on predatory Hemiptera in the tier 4 field study it is unlikely that MON 88702 will pose a risk to these beneficial insects in the cotton agro-ecosystem.

Table V-13. Combined-site Mean[1] Abundance of *Geocoris* spp., *Nabis* spp. and *Zelus* spp. in Sprayed and Unsprayed DP393 and Unsprayed MON 88702 Cotton Plots

Life Stage	Mean (SE) DP393 Sprayed with Broad Spectrum Insecticide	Mean (SE) DP393 Unsprayed	Mean (SE) MON 88702 Unsprayed	Power (%)[2]
		***Geocoris* spp.**		
Adults	1.7 (0.13)*	4.3 (0.33)	3.7 (0.24)	89.5
Nymphs	1.9 (0.23)*	6.2 (0.51)	5.0 (0.37)	84.0
Total	2.8 (0.19)*	8.0 (0.43)	6.8 (0.35)	93.3
		***Nabis* spp.**		
Total	0.8 (0.22)*	2.1 (0.42)	1.5 (0.38)	27.9
		***Zelus* spp.**		
Nymphs	3.1 (0.62)	5.6 (0.50)	3.9 (0.51)	19.6
Total	2.9 (0.60)*	6.0 (0.44)	3.8 (0.51)	25.3

[1] This table provides the arithmetic means. Least Square (LS) means were used in the statistical model for conducting pairwise comparisons and are provided in Appendix I.4.
[2] Power to detect a 50% difference in abundance.
* Indicates a statistically significant difference with the unsprayed DP393 entry (α=0.05).

Table V-14. Individual-site Mean[1] Abundance of *Geocoris* spp., *Nabis* spp. and *Zelus* spp. in Unsprayed DP393 and Unsprayed MON 88702 Cotton Plots

Site[2]	Life Stage	Mean (SE) DP393 Unsprayed	Mean (SE) MON 88702 Unsprayed	Power (%)[3]
		***Geocoris* spp.**		
AZMA	Adults	3.4 (0.38)	2.4 (0.34)	64.1
	Nymphs	6.4 (0.94)	5.6 (0.27)	74.1
	Total	9.8 (0.94)	8.0 (0.26)	92.2
AZYU	Adults	5.0 (0.43)	4.4 (1.18)	72.9
	Nymphs	8.2 (1.86)	6.3 (2.09)	70.2
	Total	13.2 (1.79)	10.7 (3.27)	90.1
LACH	Adults	NA[4]	NA[4]	NA[4]
	Nymphs	NA[4]	NA[4]	NA[4]
	Total	1.6 (0.64)	2.3 (0.35)	21.2
MSGV	Adults	5.9 (1.01)	5.2 (0.20)	78.3
	Nymphs	10.2 (1.28)	7.9 (0.70)	85.9
	Total	15.1 (1.29)	12.4 (0.83)	93.2
NCRC	Adults	NA[4]	NA[4]	NA[4]
	Nymphs	2.5 (0.67)	3.2 (0.67)	37.8
	Total	3.0 (0.50)	3.3 (0.38)	60.0
TXUV	Adults	3.1 (0.84)	3.0 (0.21)	47.5
	Nymphs	3.7 (0.96)	1.8 (0.38)	22.7
	Total	5.4 (1.20)	4.2 (0.06)*	73.0
		***Nabis* spp.**		
AZYU	Total	2.9 (0.74)	1.8 (0.64)	32.6
MSGV	Total	1.3 (0.43)	1.3 (0.43)	16.2
		***Zelus* spp.**		
AZMA	Nymphs	6.8 (0.92)	4.6 (0.34)*	62.3
	Total	7.4 (0.91)	5.0 (0.63)	70.9
TXUV	Nymphs	4.4 (0.48)	3.1 (1.11)	21.9
	Total	4.6 (0.33)	2.7 (0.82)*	36.6

[1] This table provides the arithmetic means. Least Square (LS) means were used in the statistical model for conducting pairwise comparisons and are provided in Appendix I.4.

[2] Site codes: AZMA = Pinal county, AZ; AZYU = Yuma county, AZ; LACH = Rapides parish, LA; MSGV = Washington County, MS; NCRC = Edgecombe County, NC; TXUV = Uvalde County, TX.

[3] Power to detect a 50% difference in abundance.

[4] Abundance was below the inclusion criterion and was therefore not statistically analyzed.

* Indicates a statistically significant difference with the unsprayed DP393 entry (α=0.05).

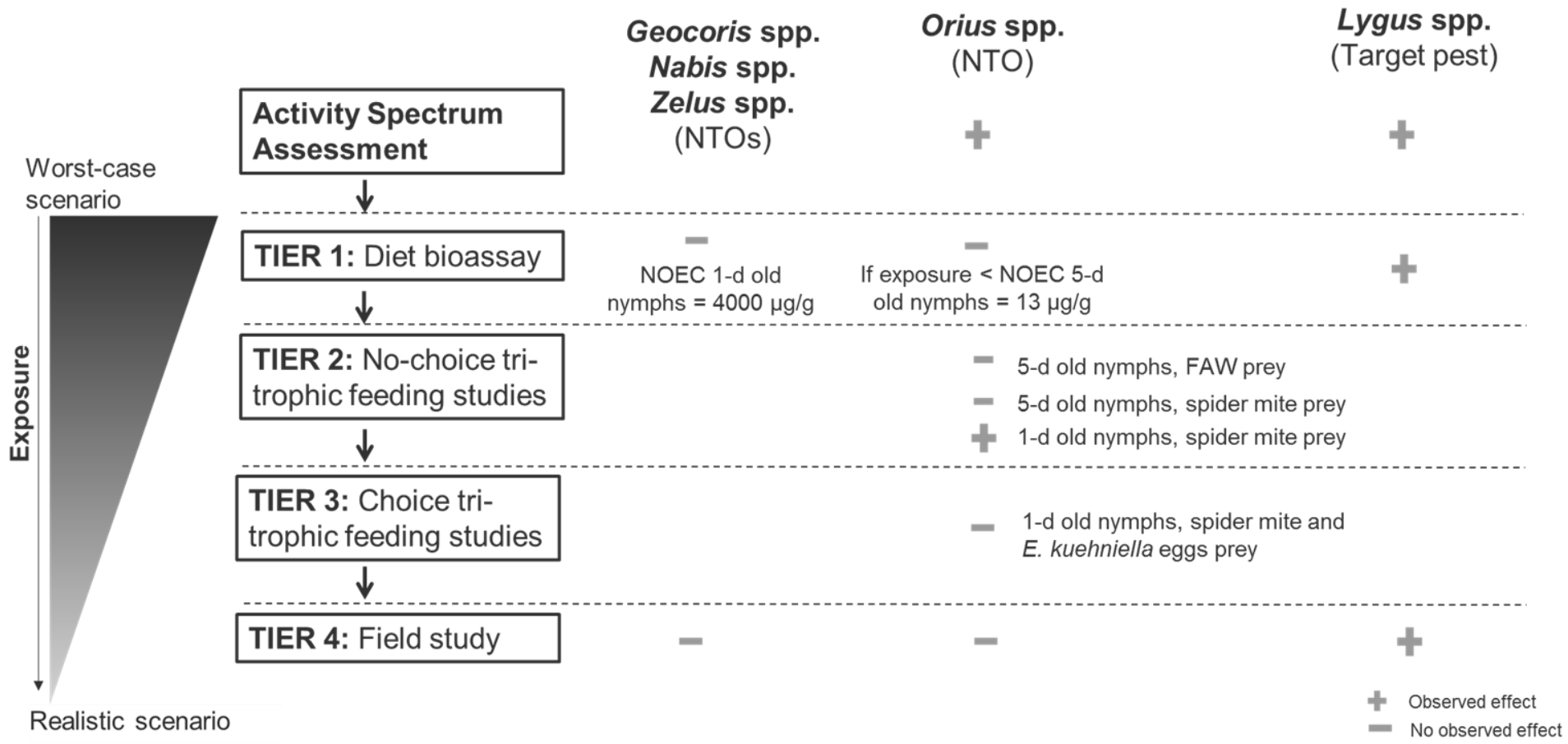


Figure V-5. Overview of the Results of the Tiered NTO Assessment with *Orius* spp., *Geocoris* spp., *Nabis* spp. and *Zelus* spp.

V.B.5.1.2.9. Spider Mite Abundance in the Tier 4 Field Study

When assessing the impact of MON 88702 on *Orius* spp. in the presence of spider mites, no significant differences were observed in *Orius* spp. abundance (Section V.B.5.1.2.4). Given that any impacts on the predatory function could also lead to increased abundance of herbivores, and specifically of spider mites in this case (Section V.B.5.1.2.2), the abundance of spider mites was assessed across the different sites of the tier 4 field study. It should be noted that in previously described studies, no impact of MON 88702 was observed on spider mites which allowed them to be used as a non-sensitive prey in tri-trophic feeding studies (Section V.B.5.1.2.2).

Within the tier 4 field study, spider mites were sufficiently abundant to allow for a statistical analysis in two of the six sites monitored; TXUV (eggs, nymphs and adults, and total) and NCRC (nymphs and adults, and total), though the TXUV site had considerably higher spider mite abundance than the NCRC site (Table V-15). A combined-site analysis of spider mite nymph and adult abundance showed a statistically significant difference between unsprayed DP393 (treatment 4) and unsprayed MON 88702 (treatment 5) plots (Table V-15). This does not correlate to the abundance of predators in cotton, which, in the combined-site analysis, was not significantly different for the most (*Orius* spp. and *Geocoris* spp.) and less (*Nabis* spp. and *Zelus* spp.) abundant predators measured in this field study (Sections V.B.5.1.2.4 and V.B.5.1.2.8). Furthermore, the difference in nymph and adult spider mite abundance does not correlate with thrips abundance, which was also monitored in the tier 4 field study and, in general, their abundance was low (Appendix I.4). The combined-site differences in thrips abundance were not significant between unsprayed DP393 and unsprayed MON 88702 plots. This indicates that the difference in spider mite abundance was not due to changes in predatory functions between MON 88702 and the conventional control.

In an individual-site analysis, this lack of statistical difference was also observed for the abundance of spider mites. In the TXUV site, where spider mite abundance was highest, there was no significant difference between the abundance of spider mite eggs, nymphs and adults, or total spider mites between the unsprayed DP393 and unsprayed MON 88702 plots. Furthermore, no significant difference was observed between DP393 and MON 88702 plots under a selective insecticide regime (treatments 2 and 3, respectively), where mean spider mite abundance was similar (Table V-15). Though the abundance of spider mites was considerably lower in the NCRC site, a lack of significant differences between unsprayed DP393 and unsprayed MON 88702 plots was also observed, as was the case for the DP393 and MON 88702 under a selective insecticide regime.

No difference in thrips abundance between unsprayed MON 88702 and DP393 plots was observed in the combined-site analysis, though the abundance was low in the limited sites where they were observed. This would indicate that they did not affect the levels of spider mites. Considering MON 88702 does not impact the populations of the most (*Orius* spp. and *Geocoris* spp.) and less (*Nabis* spp. and *Zelus* spp.) abundant hemipteran predators monitored in this study, the lack of an effect on the predatory function due to MON 88702 is supported.

Table V-15. Combined-site and Individual-site Mean[1] Abundance of Spider Mites in Sprayed and Unsprayed DP393 and MON 88702 Cotton Plots

Site[1]	Life Stage	Mean (SE) DP393 Unsprayed	Mean (SE) MON 88702 Unsprayed	Mean (SE) DP393 Sprayed with Selective Insecticide	Mean (SE) MON 88702 Sprayed with Selective Insecticide
Combined-site	Eggs	12.3 (3.38)	24.3 (8.52)	27.0 (6.45)	29.0 (7.06)
	Nymphs + adults	8.0 (1.31)*	13.3 (3.45)	14.4 (3.24)	14.7 (1.67)
	Total	13.9 (2.70)	23.3 (5.89)	26.0 (5.40)	26.3 (1.20)
NCRC	Eggs	NA[3]	NA[3]	NA[3]	NA[3]
	Nymphs + adults	2.3 (0.25)	5.8 (1.82)	5.5 (1.42)	3.0 (0.66)
	Total	4.5 (1.67)	6.4 (2.19)	6.9 (2.54)	3.1 (0.57)
TXUV	Eggs	12.3 (3.38)	24.3 (8.52)	27.0 (6.45)	29.0 (7.06)
	Nymphs + adults	13.7 (3.46)	20.8 (7.94)	23.3 (7.75)	26.4 (4.06)
	Total	23.3 (5.96)	40.1 (14.45)	45.1 (12.73)	49.4 (2.83)

[1] This table provides the arithmetic means. Least Square (LS) means were used in the statistical model for conducting pairwise comparisons and are provided in Appendix I.4.

[2] Site codes: NCRC = Edgecombe County, NC; TXUV = Uvalde County, TX.

[3] Abundance was below the inclusion criterion and was therefore not statistically analyzed.

*Indicates a statistically significant difference with the DP393 entry grown under the same conditions (α=0.05).

V.B.5.1.3. Results - Coleoptera

The activity spectrum assessment demonstrated measurable activity on two related species in the order Coleoptera, within the Chrysomelidae family, *Leptinotarsa decemlineata* and *Diabrotica undecimpunctata howardi* (Section V.B.4.5). This was not unexpected due to previously reported results; wild type Cry51Aa1 and wild type Cry51Aa2, both of which have greater than 95% sequence identity with mCry51Aa2, were reported to have activity against *L. decemlineata* (Baum et al., 2012; Xu et al., 2015). However, no effect of the mCry51Aa2 protein was observed toward larvae of a closely related species *Diabrotica virgifera* within the same genus and/or family (Chrysomelidae), or toward two other coleopteran species from the more basal family Coccinellidae, *Coleomegilla maculata* and *Epilachna varivestis*. These activity spectrum results demonstrate that mCry51Aa2 has limited activity in the insect order Coleoptera and is restricted to some herbivorous members of the Chrysomelidae family. These conclusions are further supported by the results from the tier 1 NTO and environmental interaction assessments.

Based on the activity spectrum results, additional species were tested to assess the susceptibility of beneficial Coleoptera to the mCry51Aa2 protein expressed in MON 88702. Since risk is a function of hazard and exposure, it is critical to take into consideration the potential routes and levels of exposure. Data show that species in the order Coleoptera are not an important insect pest in cotton fields. More importantly, the two species that showed susceptibility to the mCry51Aa2 protein in the activity spectrum assessment are not pests in U.S. cotton fields but are mainly pests of potatoes and corn (Lawrence et al., 2008; Vaughn et al., 2005). Therefore, exposure of this order of insects to MON 88702 is expected to be minimal, significantly reducing any risk from the mCry51Aa2 protein. As described in Section V.B.5.1, the species selection for the tier 1 NTO assessment takes into consideration the cotton agro-ecosystem, selecting insects abundant in cotton fields or those that serve as their surrogates to be assessed. Therefore, two species from the order Coleoptera were tested in the NTO assessment, rove beetle (Family: Staphylinidae) and lady beetle (Family: Coccinellidae) (Table V-16 and Table IX-2), both representing the functional group of the predators within the cotton agro-ecosystem.

Predatory rove beetles consume herbivorous prey and will have an indirect exposure to the mCry51Aa2 protein expressed in MON 88702 cotton. Therefore, a conservative exposure scenario assumes that the 95th percentile expression value with the highest fresh weight expression level in leaf (OSL3) is consumed directly by the predator and was used to calculate the MOE (Table V-3). The calculated MOEs for rove beetles was ≥5.1 (Table V-16). As documented in Section V.B.3, the expression levels of mCry51Aa2 in square tissue were determined in a 2018 field trial. Using a conservative approach that assumes rove beetles will feed on MON 88702 square tissue, the use of the 95th percentile expression value with the highest fresh weight expression level (square 2, Table V-3) would still result in an MOE of 3.2, which is above the threshold of 1 set by EPA guidance (U.S. EPA, 2010a; b; USDA-APHIS and EPA, 2007). As a predator, these exposure levels would be expected to be significantly lower given the decrease in the

mCry51Aa2 levels through the trophic layers in cotton fields as discussed in Section V.B.5.1.2.

Pollen was used as the route of exposure for facultative predators such as the lady beetle, since pollen feeding represents the main direct route of exposure for this species (Lundgren, 2009) (Table V-3). The calculated MOE for lady beetles was ≥1041.7 (Table V-16), providing margins of safety well above any guidance thresholds (U.S. EPA, 2010a; b; USDA-APHIS and EPA, 2007).

The results from this assessment demonstrate that for most coleopteran species tested, the mCry51Aa2 protein was not a hazard, since no effects were detected in activity spectrum or NTO assessments at exposure levels, conservatively, above three-fold the highest tissue expression levels. Additionally, considering their feeding ecology, geographical distribution, and overall exposure, the coleopteran species are not expected to encounter levels of the mCry51Aa2 protein in the field that would result in an adverse biological effect and consequently the results of the tiered NTO assessment demonstrate that MON 88702 is unlikely to pose a risk to Coleoptera.

These conclusions are confirmed by the field environmental interaction assessment data (Section VII.C.2.2) that represent a realistic exposure scenario for all insects present in cotton fields, beneficial or not, across all functional groups and across all insect orders. The results indicate that there was no difference in susceptibility between MON 88702 and conventional cotton to damage from coleopteran families typically present in cotton fields, nor was there any adverse effect of MON 88702 on the arthropod abundance in the fields. An overview of the coleopteran species tested in all assessments and the functional group they represent is given in Table IX-2. The weight of evidence, especially under the most environmentally relevant conditions, therefore, demonstrates that MON 88702 is unlikely to pose a risk to Coleoptera.

V.B.5.1.4. Results - Species Representing Other Ecological Functions

As described above, the approach for the selection of species tested in this assessment follows common practices as described in policy documents and the peer-reviewed literature (Dutton et al., 2003; Romeis et al., 2013; USDA-APHIS and EPA, 2007; Wach et al., 2016). In addition to the hemipteran and coleopteran species described in the previous sections, the function of pollinators (honey bee), facultative predators (lacewing) and parasitoids (parasitic wasp) were further represented in the tier 1 assessment. Pollen was utilized as the route of exposure for pollinators (honey bee), facultative predators (lacewing) and parasitoids (parasitic wasp) that could use pollen as a supplementary or life-stage specific food source. EECs for these species were based on the highest 95th percentile fresh weight mCry51Aa2 protein expression level in pollen (Table V-3). For all of these functional NTOs the calculated MOEs were more than 200 orders of magnitude above any guidance thresholds (Table V-16), indicating negligible risk to these species (U.S. EPA, 2010a; b; USDA-APHIS and EPA, 2007).

For soil-dwelling organisms, such as earthworms and Collembola, the most ecologically relevant route of exposure was considered to be from decomposing late season plant tissue in the soil environment. Consequently, the EECs were based on the highest 95th percentile fresh weight mCry51Aa2 protein expression level in cotton roots (Table V-3). For soil-dwelling NTOs the calculated MOEs were more than 30 (Table V-16), which is well above any guidance thresholds, indicating negligible risk to these species (U.S. EPA, 2010a; b; USDA-APHIS and EPA, 2007).

V.B.5.1.5. Predatory Thrips

Following the criteria for species selection as described above, additional predatory thrips were not included in the tiered NTO assessment mainly due to limited relevance as a key predator in the cotton agro-ecosystem in the southern U.S. (Torres and Ruberson, 2005). In this evaluation, Torres and Ruberson (2005) did not record predatory thrips in an assessment of natural enemies in cotton and this is consistent with other studies reporting few thrips beyond the typical pest species (Section V.B.4 and Section VIII.E). Taken together, thrips have not been found to be major contributors to the predation function, and ultimately biological control, in the majority of the cotton agro-ecosystem in the U.S. Where predation has been attributed to thrips, they are considered opportunistic predators because they do not rely on prey as their primary nutritional source for survival and reproduction (Mound, 2005; Trichilo and Leigh, 1986; Zhi et al., 2006). Therefore, any potential loss of predation pressure due to control of thrips by MON 88702 cultivation is unlikely to have an impact on the overall biological control function as there is functional redundancy in the cotton agro-ecosystem. Through this built-in redundancy, several of the known major predators (e.g. *Orius* spp., *Geocoris* spp., ladybird beetles and lacewings) (Section V.B.5.1.2) compensate for any potential loss in contribution from an occasional predator to the overall biological control function (Naranjo and Ellsworth, 2009) (Naranjo, 2005a). Taking into consideration the factors of relevance, availability and reliability when selecting surrogate species for NTO assessment (Romeis et al., 2013), the species tested included the four aforementioned major predators in cotton fields (Section V.B.5.1.2). All the predators tested in the tiered NTO assessment therefore represented the overall predation/biological control function in the cotton agro-ecosystem, making it unnecessary to test additional thrips species.) compensate for any potential loss in contribution from an occasional predator to the overall biological control function (Naranjo, 2005a; Naranjo and Ellsworth, 2009). Taking into consideration the factors of relevance, availability and reliability when selecting surrogate species for NTO assessment (Romeis et al., 2013), the species tested included the four aforementioned major predators in cotton fields (Section V.B.5.1.2). All the predators tested in the tiered NTO assessment therefore represented the overall predation/biological control function in the cotton agro-ecosystem, making it unnecessary to test additional thrips species.

Table V-16. Expected Environmental Concentrations (EECs), No Observed Effect Concentrations (NOECs) from NTO Studies with Terrestrial Beneficial Invertebrate Species and MOEs for mCry51Aa2 Protein

Test Organism	Common Name	Order	Representative Function	Testing	EEC[1]	NOEC[2]	MOE[3]
Coccinella septempunctata	Lady Beetle	Coleoptera	Predator	Tier 1	2.4 µg/g fwt pollen	≥2500 µg/g	≥1041.7
Aleochara bilineata	Rove Beetle	Coleoptera	Predator	Tier 1	494.4 µg/g fwt leaf [4]	≥2500 µg/g	≥5.1
Apis mellifera larvae	Honey Bee	Hymenoptera	Pollinator	Tier 1	0.0048 µg/g fwt pollen[5]	≥5.6 µg/larvae[6]	≥1166.7
Apis mellifera adult	Honey Bee	Hymenoptera	Pollinator	Tier 1	2.4 µg/g fwt pollen	≥500 µg/g	≥208.3
Chrysoperla carnea	Lacewing	Neuroptera	Predator	Tier 1	2.4 µg/g fwt pollen	≥2500 µg/g	≥1041.7
Pediobius foveolatus	Parasitic Wasp	Hymenoptera	Parasitoid	Tier 1	2.4 µg/g fwt pollen	≥2500 µg/mL	≥1041.7
Orius insidiosus	Insidious Flower Bug	Hemiptera	Predator	Tier1	2.4 µg/g fwt pollen	13 µg/g	5.4
Orius insidiosus	Insidious Flower Bug	Hemiptera	Predator	Tier 2	1.58 µg/g fwt leaf [7]	6.47 µg/g FAW	4.1
Geocoris punctipes	Big-eyed Bug	Hemiptera	Predator	Tier 1	770.7 µg/g fwt square	≥4000 µg/g	≥5.2
Nabis alternatus	Damsel Bug	Hemiptera	Predator	Tier 1	770.7 µg/g fwt square	≥4000 µg/g	≥5.2
Zelus renardii	Assassin Bug	Hemiptera	Predator	Tier 1	770.7 µg/g fwt square	≥4000 µg/g	≥5.2
Eisenia andrei	Earthworm	Haplotaxida	Decomposer	Tier 1	83.1 µg/g fwt root[8]	≥2500 µg/g dry	≥30.1
Folsomia candida	Springtail	Collembola	Decomposer	Tier 1	83.1 µg/g fwt root[8]	≥2500 µg/g	≥30.1

[1] 95th percentile expression levels determined from MON 88702 tissues across five and four sites in 2015 and 2018, respectively Table V-3.

[2] NOECs reflect nominal test substance concentrations.

[3] MOE values were calculated based on the ratio of the NOEC to EEC. The MOE was determined based on the 95th percentile expression level of the mCry51Aa2 protein in the tissue from MON 88702 deemed most relevant to the NTO exposure.

[4] The 95th percentile expression value from the leaf development stage (OSL3) with the highest expression level was used to represent worst-case-scenario for a predator consuming a herbivorous prey.

[5] EEC based upon mean quantity of mCry51Aa2 protein expressed in 2 mg of MON 88702 pollen fresh weight (fwt). The average consumption of pollen by honey bee larvae is 2 mg during development (Babendreier et al., 2004). The EEC was calculated as follows: (2 mg pollen × (2.4 µg mCry51Aa2 protein /1000 mg pollen).

[6] The NOEC represents a single dose of 10 µl of 500 µg/mL solution added to each larval cell. The total mass added and consumed in each larval cell was 5.6 µg mCry51Aa2 protein/cell. The concentration of 500 µg/g mCry51Aa2 protein in the diet solution is calculated based on the density of the 30% sucrose/water (w/v) solution of 1.127 g/mL.

[7] In the tri-trophic study (tier 2), the *S. frugiperda* (Fall armyworm – FAW) were fed artificial diet containing 1999 µg/mL mCry51Aa2 protein, resulting in 6.47 µg/g mCry51Aa2 protein concentration (0.32%) in the prey used as food source for *O. insidiosus* nymphs. The EEC was determined as 0.32% of 494 µg/g fwt leaf to represent worst-case-scenario of biotransfer of mCry51Aa2 protein between trophic levels.

[8] The 95th percentile expression value from the root development stage (peak bloom) was used to represent worst-case-scenario for a soil dwelling invertebrate.

V.B.5.2. Other Non-Target Organisms: Avian and Mammalian Species

The specificity of the *Bt* Cry proteins activity is dependent upon their binding to specific receptors present in the insect midgut (OECD, 2007; Pigott and Ellar, 2007). These specific receptors are not present in vertebrate taxa, therefore Cry proteins are not expected to adversely affect wild mammals and no adverse effects have been reported in mammals or avian species (OECD, 2007; Schnepf et al., 1998).

For an evaluation of potential hazard to an insectivorous avian species, a 14-day acute study was conducted with northern bobwhite quail (*Colinus virginianus*) following EPA's test guideline OPPTS 850.2200. There were no biologically relevant differences for any of the endpoints (mortality, signs of toxicity, and abnormal behavior) to bobwhite quail dosed with the mCry51Aa2 protein from MON 88702 as compared to those in the control groups. Based on the results, the NOEC of ≥2500 mg mCry51Aa2 protein/kg body weight was established for the northern bobwhite quail.

An evaluation of potential hazard to mammalian species was conducted by an acute oral gavage study with mice (*Mus musculus*) which was performed following EPA's test guideline OPPTS 870.1100. No mortality occurred during the study and no test substance-related clinical findings were observed. There were no mCry51Aa2-related differences in body weight, body weight gain, food consumption, or gross necropsy findings. Based on the lack of adverse effects observed for 14 days post-dosing, an NOAEL of 5,000 mg mCry51Aa2 protein/kg body weight was established for the mouse.

The results from these studies demonstrated the lack of toxicity in both representative vertebrate species exposed to mCry51Aa2 protein at doses well above the maximum levels anticipated in cotton, based upon the expression values of this protein. Based on the narrow spectrum of activity of mCry51Aa2 protein and the lack of receptors, the likelihood of adverse effects to non-target terrestrial vertebrates from cultivation of MON 88702 cotton is concluded to be extremely low. These conclusions are supported by the outcome of the EPA's assessment of the previously described studies as part of the establishment of an exemption from the requirement of a tolerance for mCry51Aa2, indicating that "*the submitted studies indicate the safety and lack of toxicity from exposure to the mCry51Aa2 protein in cotton*" (U.S. EPA, 2018a).

V.B.5.3. Conclusions of the mCry51Aa2 Protein NTO Assessment

The assessment of the risk of mCry51Aa2 expressed in MON 88702 cotton on NTOs was conducted according to regulatory guidelines, following a tiered approach when necessary, resulting in a thorough evaluation of invertebrate NTOs relevant to the cotton agro-ecosystem. In each assessment, various measures of conservative exposure scenarios were incorporated (e.g. the use of 95th percentile protein expression levels for exposure estimations), including considerations for predatory insects feeding directly on plant tissues. Across all NTOs assessed, the MOEs were ≥4.1 (Table V-16), above the threshold that would indicate an environmental risk for NTOs (U.S. EPA, 2010a; b; USDA-APHIS and EPA, 2007).

In the activity spectrum assessment, a hazard was identified to *Orius insidiosus*. The recommended tiered approach was utilized to conduct a comprehensive assessment of the risk of MON 88702 against *Orius* spp. This assessment included tri-trophic feeding studies (tier 2), leaf disk assays (tier 3) and a field study (tier 4) and demonstrated that there is a low risk for adverse effects to *Orius* spp under more realistic exposure conditions to the mCry51Aa2 protein expressed in MON 88702 cotton. Additional predatory Hemiptera closely related to *Orius* spp. were also tested within the NTO assessment, demonstrating negligible risk to those species (Figure V-5). Therefore, MON 88702 is unlikely to pose a risk to the predatory function in cotton fields.

Finally, the results from acute toxicity studies conducted with representative vertebrate species demonstrated the lack of toxicity to the mCry51Aa2 protein at doses well above the maximum exposure levels anticipated from cotton, indicating negligible risk to non-target terrestrial vertebrates from cultivation of MON 88702 cotton is extremely low.

V.B.6. Environmental Fate of MON 88702 Products Expressing mCry51Aa2

Soil organisms may be exposed to the mCry51Aa2 protein from MON 88702 cotton by contact with roots, or with above-ground plant biomass deposited on or tilled into the soil. In addition, feeding on living or dead crop biomass or ingesting or absorbing the Cry proteins after their release into the soil may represent a route of exposure to soil-dwelling organisms.

Several soil factors (e.g., pH and clay content) can influence the degradation rate of Cry proteins. Published studies suggest that Cry proteins may bind to the clay components of soil and become more resistant to degradation by soil microorganisms (Fiorito et al., 2008; Stotzky, 2004). Laboratory and field studies, however, show that only a very small fraction of the Cry protein derived from post-harvest residues persists long enough to be stabilized by soil colloids or clay minerals (Hopkins and Gregorich, 2005). In addition, soil pH near or above neutrality substantially increases the degradation rate of Cry proteins (Tapp and Stotzky, 1998). A soil pH of 5.8 - 8.0 is recommended to maximize nutrient availability for cotton cultivation. Some experts recommend soil amendments if soil pH falls beyond this range and advise that soil pH below 5.2 or above 8.5 can be very detrimental to cotton production (Advancing Cotton Education, 2017).

Many laboratory soil degradation studies have been conducted with Cry proteins from a variety of biotechnology-derived crops (*e.g.*, Cry1Ab, Cry1Ac, Cry1A.105, Cry2Ab2, Cry3Bb1, Cry1F, Cry34/35); the weight of evidence indicates that Cry proteins do not persist in soil (Herman et al., 2002; Icoz and Stotzky, 2008b; a; Sims and Holden, 1996; Sims and Ream, 1997). Furthermore, a number of field monitoring studies have been conducted to assess the dissipation of Cry proteins following several years of sustained maize or cotton production. These studies have shown no persistence or accumulation of Cry proteins in fields where maize expressing Cry1Ab protein (Dubelman et al., 2005) or Cry3Bb1 protein (Ahmad et al., 2005) and cotton expressing Cry1Ac protein (Head et al., 2002) were grown continuously for several years.

A laboratory soil degradation study demonstrated that the mCry51Aa2 protein expressed in MON 88702 dissipated rapidly in different representative agricultural soils that ranged

in pH from 5.7 to 7.1 and contained significant proportions of clay (up to 36%) or organic matter (up to 6.1%). Purified mCry51Aa2 protein and a mixture of cotton root and shoot tissues expressing mCry51Aa2 protein were added to each soil in excess relative to the maximum concentration possible under cotton growing conditions. Elevated levels of mCry51Aa2 protein were used to improve the accuracy of the analytical quantifications, and to ensure that the DT_{50} and DT_{90} (time to 50% and 90% dissipation of the mCry51Aa2 protein) could be calculated from measurable values (*i.e.*, above the limit of detection). The excess mCry51Aa2 protein also provided an increased margin of environmental safety to include any currently unforeseen routes of protein introduction into soil. Soil extracts were analyzed for mCry51Aa2 concentrations using ELISA. Results indicated a maximum estimated DT_{50} of 4.7 days, and a maximum estimated DT_{90} of 74.5 days.

Therefore, these results indicate that the mCry51Aa2 protein produced in MON 88702 cotton will not persist or accumulate under cotton production conditions, indicating negligible exposure to NTOs and persistence in the environment.

V.C. Conclusions on the Characterization and Safety Assessment of the Cry51Aa2.834_16 Protein Expressed in MON 88702

The mCry51Aa2 protein levels were determined in tissues collected from U.S. fields planted in the 2015 and 2018 growing seasons. Additional safety information on the mCry51Aa2 protein was summarized and the weight of evidence demonstrates that the mCry51Aa2 protein expressed in MON 88702 cotton is safe for humans and animals. The full dataset was submitted to the EPA, which agreed with these conclusions, granting a permanent exemption from the requirement for a tolerance for residues of the mCry51Aa2 protein (U.S. EPA, 2018b).

The specificity of the protein was established for targeted hemipteran (*Lygus hesperus* and *Lygus lineolaris*) and thysanopteran (*Frankliniella* spp.) insect pests.

Given the activity of the mCry51Aa2 against the target pests and initial activity observed in a beneficial species (*O. insidiosus*) and two coleopteran species (*L. decemlineata* and *D. u. howardi*), a thorough NTO assessment including multiple species and representatives of different functional groups was conducted. Risk was assessed to various invertebrate and vertebrate NTOs, most of which showed no impact from mCry51Aa2. Risk to *Orius* spp. and other hemipteran predators was assessed under various exposure scenarios, across various tiers, and demonstrated no risk to these beneficial insects, especially under the most realistic environmental exposure scenarios. Exposure levels are further reduced for soil inhabiting organisms due to the short persistence of mCry51Aa2 in the environment, further reducing risk to these NTOs. The results from this assessment lead to the conclusion that MON 88702 does not pose a risk.

VI. COMPOSITIONAL ASSESSMENT OF KEY ANTI-NUTRIENTS IN MON 88702

Safety assessments of biotechnology-derived crops follow the comparative safety assessment process (Codex Alimentarius, 2009) in which the composition of grain and/or other raw agricultural commodities of the biotechnology-derived crop are compared to the appropriate conventional control that has a history of safe use. For cotton, assessments are performed using the general principles outlined in the OECD consensus document for cotton composition (OECD, 2009). For MON 88702, the introduced protein, mCry51Aa2, is a *Bt*-derived crystal (Cry) protein that lacks catalytic activity and is neither intended to nor expected to affect the plant's metabolism. Given the nature of this introduced trait and the overall lack of meaningful unintended compositional characteristics observed for biotechnology-derived products characterized to date (Herman and Price, 2013; Venkatesh et al., 2015), compositional changes that would affect the levels of components in MON 88702 cotton were not expected.

Cotton is known to contain certain anti-nutrients that may impact animal health (OECD, 2009). In addition, the anti-nutrient gossypol is reported to deter cotton pests, as glandless cotton, with reduced levels of gossypol, is highly susceptible to insect pests, herbivory and disease (OGTR, 2008). The cyclopropenoid fatty acid anti-nutrients are reported to protect plants from herbivory (Yu et al., 2011) and pathogens (Schmid and Patterson, 1988). Thus, comparative assessment of anti-nutrient levels in cottonseed of MON 88702 and the conventional control is provided to assess the potential for an effect of MON 88702 on the levels of these anti-nutrients.

Monsanto has completed a consultation with the Food and Drug Administration following their policy, "Foods Derived from New Plant Varieties," on the food and feed safety of insect-protected cotton MON 88702 (BNF 000160) where other key components were also measured. Thirty components including major nutrients of cottonseed (protein, amino acids, total fat, carbohydrates, linoleic acid, acid detergent fiber (ADF), neutral detergent fiber (NDF) and ash), as well as the anti-nutrients included here were assessed. The findings from that assessment support the conclusion that MON 88702 did not meaningfully alter component levels in cottonseed and confirms the compositional equivalence of MON 88702 to conventional cotton. The FDA therefore agreed with the conclusion that MON 88702 does not raise any safety or regulatory issues with respect to its uses in human or animal food (U.S. FDA, 2018).

VI.A. Results from Compositional Analyses of Key Anti-Nutrients in MON 88702 Cottonseed

This section provides analyses of levels of key anti-nutrients in cottonseed of MON 88702 compared to that of a conventional control cotton variety grown and harvested under similar conditions. The production of materials for compositional analyses used diverse field trial sites, robust field designs (randomized complete block design with four blocks at each of the five sites), and sensitive analytical methods that allow accurate measurements of these components over a range of environmental conditions under which MON 88702 cotton is expected to be grown.

As discussed, compositional analyses of acid-delinted cottonseed samples are reported for a subset of components listed in the cottonseed OECD consensus document (2009), specifically anti-nutrients (Table VI-1). Moisture values for cottonseed were measured for conversion of components to dry weight, but were not statistically analyzed.

The statistical comparisons of MON 88702 cotton and the conventional control were based on compositional data combined across all field sites. Statistically significant differences were evaluated at the 5% level (α=0.05).

There were no statistically significant differences ($p<0.05$) for any of the anti-nutrients analyzed (Table VI-1). These results support the overall conclusion that MON 88702 did not meaningfully alter anti-nutrient levels in cottonseed and confirmed the compositional equivalence of MON 88702 to the conventional control in levels of these components. Therefore, MON 88702 is not expected to have a different effect on human or animal health when compared to conventional cotton as a consequence of changes in plant composition. Furthermore, a consultation was completed with FDA in which the agency agreed with the conclusion that, based on the provided data, "*MON 88702 cotton does not raise any safety or regulatory issues with respect to its uses in human or animal food*" (U.S. FDA, 2018).

Table VI-1. Summary of Cottonseed Anti-Nutrients for MON 88702 and the Conventional Control

Component	MON 88702 Mean (S.E.)[1] Range	Control Mean (S.E.) Range	Control Range Value[2]	Difference (MON 88702 minus Control) Mean (S.E.)	Difference (MON 88702 minus Control) p-Value
Total gossypol (% dwt)[3]	1.01 (0.12) 0.55 - 1.44	1.01 (0.12) 0.59 - 1.46	0.87	-0.0064 (0.032)	0.852
Free gossypol (% dwt)	0.62 (0.093) 0.26 - 0.93	0.61 (0.093) 0.23 - 0.89	0.66	0.0074 (0.014)	0.614
Malvalic acid (% Total FA)[4]	0.52 (0.046) 0.41 - 0.73	0.55 (0.046) 0.38 - 0.74	0.36	-0.034 (0.025)	0.246
Sterculic acid (% Total FA)	0.24 (0.015) 0.19 - 0.29	0.24 (0.015) 0.20 - 0.31	0.10	0.00063 (0.0058)	0.919
Dihydrosterculic acid (% Total FA)	0.38 (0.038) 0.30 - 0.54	0.37 (0.038) 0.31 - 0.56	0.25	0.0068 (0.0078)	0.437

[1] Mean (S.E.) = least-square mean (standard error)
[2] Maximum value minus minimum value for the control cotton variety
[3] dwt=dry weight
[4] FA=Fatty Acid

VII. PHENOTYPIC, AGRONOMIC, AND ENVIRONMENTAL INTERACTIONS ASSESSMENT

This section provides a comparative assessment of the phenotypic, agronomic, and environmental interaction characteristics of MON 88702 cotton compared to the conventional control. The data support a conclusion that MON 88702 cotton is not meaningfully different in plant pest risk from the conventional control. These conclusions are based on the results of multiple evaluations from laboratory and field assessments. Phenotypic, agronomic, and environmental interaction characteristics of MON 88702 cotton were evaluated in a comparative manner to assess plant pest potential. These assessments included evaluation of seed germination characteristics, plant growth and development characteristics, observations of plant responses to abiotic stress, plant-disease and plant-arthropod interactions, and pollen characteristics. Results from these assessments demonstrate that MON 88702 cotton does not possess a) increased weediness characteristics; b) increased susceptibility or tolerance to specific abiotic stresses, diseases, or arthropods; or c) characteristics that would confer a plant pest risk compared to the conventional control.

VII.A. Characteristics Measured for Assessment

In the phenotypic, agronomic, and environmental interactions assessment of MON 88702, data were collected to evaluate altered plant pest potential. A detailed description of the regulated article phenotype is requested as part of the petition for determination of nonregulated status in 7 CFR § 340.6, including differences from the unmodified recipient organism that would "substantiate that the regulated article is unlikely to pose a greater plant pest risk than the unmodified organism from which it was derived". As part of the characterization of MON 88702, data were collected to provide a detailed description of the phenotypic, agronomic, and environmental interaction characteristics of MON 88702. A subset of these data was included in an evaluation of specific characteristics related to altered plant pest potential (*e.g.*, seed dormancy, fruit retention, and environmental interactions data).

The plant characterization of MON 88702 cotton encompassed five general data categories: 1) seed germination, dormancy, and emergence; 2) vegetative growth; 3) reproductive development (including pollen characteristics); 4) fruit retention on the plant; 5) plant response to abiotic stress and interactions with diseases and arthropods. An overview of the characteristics assessed is presented in Table VII-1.

The phenotypic, agronomic, and environmental interactions data were evaluated from a basis of familiarity (OECD, 1993) and comprised a combination of laboratory and field studies conducted by scientists who are familiar with the production and evaluation of cotton. In each of these assessments, MON 88702 cotton was compared to an appropriate conventional control that had a genetic background similar to MON 88702 cotton but did not possess the insect-protection trait. In addition, multiple conventional commercial cotton varieties developed through conventional breeding and selection (see Appendix F and Table F-1 through Table F-3) were included to provide a range of comparative values for each characteristic that are representative of the variability in existing commercial

cotton varieties. Data collected for the various characteristics from the commercial reference varieties provide context for interpreting experimental results.

Table VII-1. Phenotypic, Agronomic, and Environmental Interaction Characteristics Evaluated in United States Field Trials and Laboratory Studies

Data category	Characteristics measured (associated section where discussed)	Evaluation timing ((Setting of evaluation)	Evaluation description (measurement endpoints)
Dormancy, germination, and emergence	Normal germinated (VII.C.1)	Day 4 and12: 20/30°C (Laboratory)	Seedlings that exhibited normal developmental characteristics and possessed both a root and a shoot
	Abnormal germinated (VII.C.1)	Day 12: 20/30°C (Laboratory)	Seedlings that could not be classified as normal germinated seed (e.g., germinated but had insufficient root and shoot development, lacked a shoot, shoot with deep cracks or lesions, or exhibited mechanical damage)
	Germinated (VII.C.1)	Day 4, Day 12: 10, 20, 30, 10/20 and 10/30°C (Laboratory)	Seedlings with a radical protruding through the seed coat and greater than 1 mm in length
	Dead (VII.C.1)	Day 4 and12: 10, 20, 30, 10/20, 10/30, and 20/30°C Day 18: 10, 20, 30, 10/20 and 10/30°C (Laboratory)	Seeds that had visibly deteriorated and had become soft to the touch
	Hard[1] (VII.C.1)	Day 12: 20/30°C Day 18: 10, 20, 30, 10/20 and 10/30°C (Laboratory)	Seeds that did not imbibe water and remained hard to the touch
	Firm-swollen[1] (VII.C.1)	Day 12: 20/30°C Day 18: 10, 20, 30, 10/20 and 10/30°C (Laboratory)	Seeds that were visibly swollen (imbibed water) and were firm to the touch but lacked any evidence of growth
	Early stand count (VII.C.2.1)	30 days after planting (Field)	Number of plants in two rows
	Final stand count (VII.C.2.1)	Harvest (Field)	Number of plants in two rows
Vegetative growth	Plant height (VII.C.2.1)	Harvest (Field)	Distance from the cotyledonary nodes to the main stem terminal

Table VII-1. Phenotypic, Agronomic and Environmental Interaction Characteristics Evaluated in U.S. Field Trials and Laboratory Studies (continued)

Data category	Characteristics measured (associated section where discussed)	Evaluation timing (Setting of evaluation)	Evaluation description (measurement endpoints)
Reproductive growth	Days to first flower (VII.C.2.1)	Flowering (Field)	Number of days from planting to five visible white or pink flowers per two rows.
	Pollen viability (VII.C.3)	Flowering (Laboratory)	Percentage of viable pollen based on pollen grain staining characteristics.
	Pollen morphology (VII.C.3)	Flowering (Laboratory)	Diameter of viable pollen grains along two perpendicular axes
	Seedcotton yield (VII.C.2.1)	Harvest (Field)	Harvested seedcotton.
	Seed Index (VII.C.2.1)	Harvest (Laboratory)	Mass of 100 delinted seeds.
Fruit retention	First position fruit retention (VII.C.2.1)	Harvest (Field)	First position fruit as a percentage of first position fruiting sites
Environmental interactions	Abiotic stress response, disease damage, and arthropod damage (VII.C.2.2)	Four times during the growing season: vegetative, squaring, bloom, post-cutout (field).	Qualitative assessments made using a categorical scale of increasing severity (none, slight, moderate, severe)
	Heliothine damage (VII.C.2.2)	Four times during the two growing seasons: starting at early squaring and every two weeks thereafter.	Quantitative assessment on ten plants per plot by counting number of total and damaged fruiting bodies.
	Stink bug damage (VII.C.2.2)	Four times during the two growing seasons: starting at the second week of bloom and every week thereafter.	Quantitative assessment on twenty bolls per plot by cracking and inspecting the bolls for internal injury.
	Arthropod abundance (VII.C.2.2)	Six collection times during two growing seasons (field).	Quantitative assessment of arthropod abundance via a vertical beatsheet method.

[1] Viability of hard and firm-swollen seed were determined by a tetrazolium test (AOSA/SCST, 2010).

VII.B. Interpretation of Phenotypic and Environmental Interaction Data

Plant pest risk assessments for biotechnology-derived crops are comparative assessments and are considered from a basis of familiarity. The concept of familiarity is based on the fact that the biotechnology-derived crop is developed from a well-characterized conventional variety whose biological properties and plant pest potential are well-known. Familiarity considers the biology of the crop, the introduced trait, the receiving environment and the interaction of these factors, and provides a basis for comparative environmental risk assessment between a biotechnology-derived plant and its conventional counterpart.

Expert knowledge and experience with conventionally bred cotton was the basis for selecting appropriate endpoints and estimating the range of responses that would be considered typical for cotton. As such, MON 88702 cotton was compared to the conventional control in the assessment of phenotypic, agronomic, and environmental interaction characteristics. An overview of the characteristics assessed is presented in Table VII-1. A subset of the data relating to well-understood weediness characteristics (*e.g.*, seed dormancy, fruit retention, and environmental interactions data) was used to assess whether there was an increase in weediness potential of MON 88702 cotton compared to a conventional cotton variety. Evaluation of environmental interaction characteristics (*e.g.*, plant abiotic stress, plant-disease, and plant-arthropod interactions) was also considered in the plant pest assessment. Prior to analysis, the overall dataset was evaluated for possible evidence of biologically-relevant changes and unexpected plant responses. No unexpected observations or issues were identified. Based on the data collected, an assessment was made to determine if MON 88702 cotton could be expected to pose an increased plant pest risk compared to conventional cotton.

VII.B.1. Interpretation of Detected Differences Criteria

Comparative plant characterization data between a biotechnology-derived crop and the conventional control are interpreted in the context of contributions to increased plant pest/weed potential. Under the framework of familiarity, characteristics for which no differences are detected support a conclusion of no increased plant pest/weed potential. Characteristics for which differences are detected are considered in a step-wise assessment process to determine whether the difference would increase the crop's plant pest/weed potential (Figure VII-1). Ultimately, a weight of evidence approach considering all characteristics and data is used for the overall risk assessment of differences and evaluation of their significance.

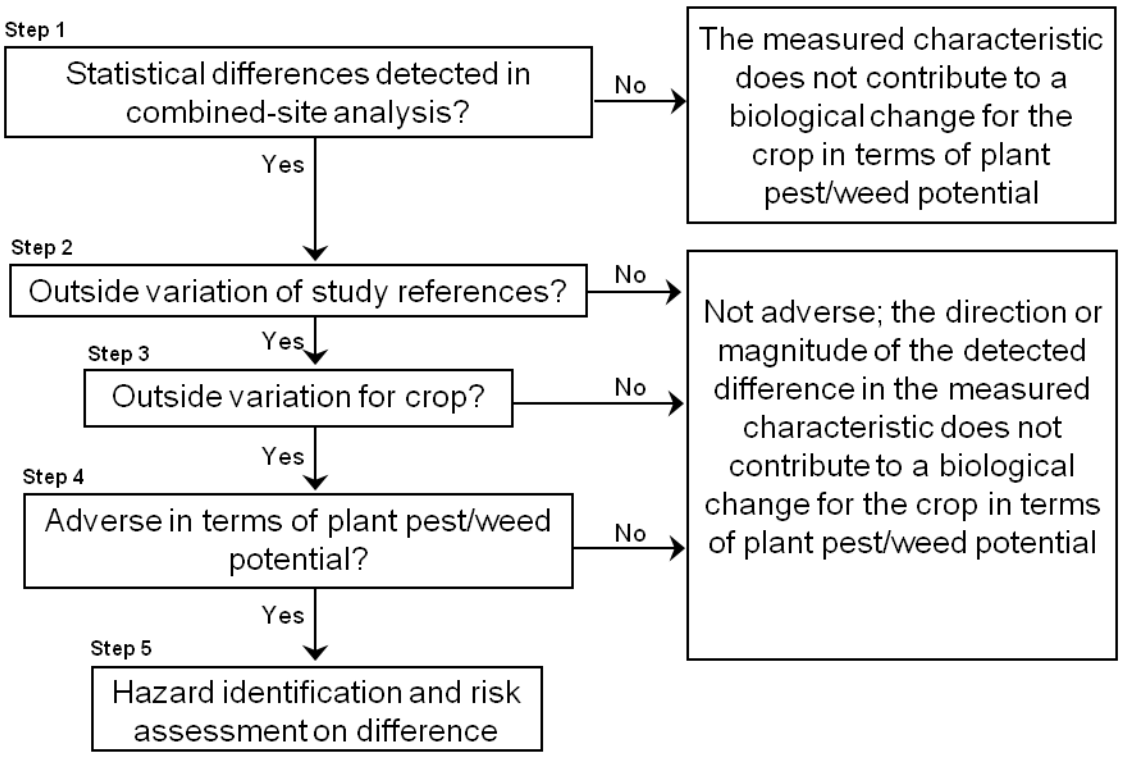


Figure VII-1. Schematic Diagram of Phenotypic Data Interpretation Methods

Steps 1 – Evaluate Detected Statistically Significant Differences

Data on each measured characteristic are statistically analyzed in a combined-site analysis, in which the data are pooled among sites. All statistically significant differences are evaluated and considered in the context of a change in plant pest/weed potential. Any difference detected in the combined-site analysis is further assessed.

Step 2 – Evaluate Differences in the Context of Commercial Reference Materials Included in the Study

If a difference for a characteristic is detected in the combined-site analysis across multiple environments, then the mean value of the biotechnology-derived crop for the characteristic is assessed relative to the range of variation of the commercial reference materials included in the study (*e.g.*, reference range).

Step 3 – Evaluate Differences in the Context of the Crop

If the mean value of the characteristics for a biotechnology-derived crop is outside the variation of the commercial reference materials included in the study, the mean value of the biotechnology-derived crop is assessed relative to known values common for the crop (*e.g.*, published values).

Step 4 – Relevance of Difference to Plant Pest/Weed Potential

If the mean value of the characteristics for a biotechnology-derived crop is outside the range of values common for the crop, the difference is then assessed for whether or not it is meaningful in terms of plant pest/weed potential.

Step 5 – Conduct Risk Assessment on Identified Hazard

If an adverse effect (hazard) is identified, risk assessment on the difference is conducted. The risk assessment considers contributions to enhanced plant pest/weed potential of the crop itself, the impact of differences detected in other measured characteristics, and potential for and effects of trait introgression into any populations growing outside of cultivated environments or into a sexually-compatible species.

VII.B.1.1. Interpretation of Environmental Interactions Data

Comparative environmental interactions data between a biotechnology-derived crop and the conventional control are interpreted in the context of contributions to increased plant pest potential.

For the qualitative assessments of abiotic stress response, arthropod damage, and disease damage, the biotechnology-derived crop and conventional control are considered different in susceptibility or tolerance if the ranges of injury symptoms do not overlap between the biotechnology-derived crop and the conventional control across four replications within a site. Any observed differences between the biotechnology-derived crop and conventional control are assessed for biological significance in the context of the range of the references, and for consistency in other observation times and sites. Differences are not considered biologically meaningful in terms of plant pest potential if the biotechnology-derived crop stress responses and damage ratings are within the reference range or are not consistently observed in multiple environments in which the same stressor occurred.

Quantitative assessments of heliothine and stink bug damage are analyzed within individual sites for each of the two growing seasons. Statistically significant differences detected between the biotechnology-derived crop and conventional controls are evaluated using the method outlined in Figure VII-1.

Quantitative assessments of arthropod abundance are analyzed within each individual site of the individual growing seasons (Table F-19 and Table F-20). Statistically significant differences between the biotechnology-derived crop and conventional control are assessed for biological significance in the context of the range of the commercial reference hybrids, and for consistency in other collection times and collection sites and in the context of pest potential. Differences that are not consistently detected in multiple environments are considered not biologically meaningful in terms of plant pest potential. In addition, quantitative assessments of arthropod abundance analyzed within each individual site are pooled across sites in a combined-site analysis for each individual year and a combined-site across years in a combined-site and combined-year analysis (Table F-21 and Table VII-7). Statistically significant differences between the biotechnology-derived crop and conventional control are assessed for biological significance in the context of the range of the commercial reference hybrids.

VII.C. Comparative Assessments of the Phenotypic, Agronomic, and Environmental Interaction Characteristics of MON 88702

This section provides the results of comparative assessments conducted in replicated laboratory and/or multi-site field experiments to provide a detailed phenotypic, agronomic, and environmental interactions description of MON 88702 cotton. The characteristics for MON 88702 cotton evaluated in these assessments included: seed dormancy and germination characteristics (Section VII.C.1), plant phenotypic, agronomic, and environmental interaction observations under field conditions (Section VII.C.2), and pollen characteristics (Section VII.C.3). Additional details for each assessment are provided in Appendix E, Appendix F and Appendix G, respectively.

VII.C.1. Seed Dormancy and Germination Characteristics

Seed germination and dormancy mechanisms vary with species and their genetic basis tends to be complex. Seed dormancy (*e.g.*, hard seed) is an important characteristic that is often associated with plants that are considered weeds (Anderson, 1996; Lingenfelter and Hartwig, 2007). Information on germination and dormancy characteristics is therefore useful when assessing a plant for increased weediness potential. To assess germination characteristics, standardized germination assays are available and routinely used. The Association of Official Seed Analysts (AOSA), an internationally recognized seed testing organization, recommends an alternating temperature regime of 20/30°C as optimal for testing the germination and dormancy characteristics of cotton seed (AOSA, 2016a; b; AOSA/SCST, 2010). Additional temperature regimes were also evaluated and details on the materials and experimental methods used in this evaluation are presented in Appendix F.

A comparative assessment of seed germination and dormancy characteristics was conducted for MON 88702 and the conventional control. The seed of MON 88702, the conventional control, and the reference varieties (four per site, seven unique references across all locations) were produced in replicated field trials during 2015 in Perquimans County, North Carolina; San Patricio County, Texas and Uvalde County, Texas. The geographic locations used represent a broad range of environmental conditions for cotton production. The experiments were arranged as separate split-plot experiments with eight replications for each temperature regime. Descriptions of the evaluated germination and dormancy characteristics and the timing of the evaluations for all temperature regimes are listed in Table VII-1.

No statistically significant differences (α=0.05) in the combined-site analysis were detected between MON 88702 and the conventional control for any characteristic at the temperature regimes of 30°C or alternating 10°C and 30°C (Table VII-2). Ten statistically significant differences (α=0.05) were detected out of 25 statistical comparisons at the additional temperature regimes of 10°C and 20°C and alternating 10°C/20°C and 20°C/30°C (Table VII-2). Compared to the conventional control, MON 88702 had lower germinated seed at 20°C (91.7 vs. 96.0%), alternating 10°C/20°C (66.2 vs. 75.7%), and alternating 20°C/30°C (86.9 vs. 91.3%). MON 88702 had higher percent hard seed compared to the conventional control at 10°C (16.9 vs. 4.0%),

alternating 10°C/20°C (3.8 vs. 1.6%), and alternating 20°C/30°C (0.5 vs. 0.0%). MON 88702 had higher percent dead seed compared to the conventional control at 20°C (7.2 vs. 3.6%) and alternating 20°C/30°C (8.6 vs. 5.3%). In addition, MON 88702 had differences in percent viable firm-swollen seed compared to the conventional control at 10°C (67.0 vs. 79.0%) and alternating 10°C/20°C (22.2 vs. 15.6%). The mean values for all characteristics, except % viable hard seed and % viable firm-swollen seed at 10°C, were within the reference range in this study (Figure VII-1, Step 3, "no" answer). The mean values for % viable hard seed and % viable firm-swollen seed at 10°C were outside of the reference ranges in this study. However, the values are within the reference ranges Monsanto previously presented for % viable hard seed and % viable firm-swollen seed at 10°C in its petitions for Dicamba and Glufosinate-tolerant cotton MON 88701 (Petition #12-185-01p, page 147) and Glyphosate-tolerant cotton MON 88913 (Petition #04-086-01p, tables C-2 through C-4, pages 175-177), incorporated here by reference (Monsanto, 2004; Monsanto Company, 2012). The reference ranges reported from previous studies provide evidence that the mean values of % viable hard seed and % viable firm-swollen seed at 10°C for MON 88702 are within typical parameters associated with conventional cotton varieties (Figure VII-1, Step 4, "no" answer). It has been demonstrated that cotton is not likely to germinate when the seed or seedling temperature is less than 14.4ºC. If cold temperature occurs during the most sensitive stage of cotton growth, when the seed is absorbing water, seed viability will be lost or else the taproot will be permanently impaired (Hake et al., 1996). Therefore, it is not a common agronomic practice to plant cotton where the ambient temperature is so low. The results from the agronomic and phenotypic assessment (Section VII.C.2.1), in which MON 88702 was grown in fields representative for commercial cotton production, show there was no statistically significant difference in characteristics such as early stand count, final stand count or yield, supporting the conclusion that the dormancy and germination characteristics of MON 88702 are not different compared to conventional cotton. In all cases, the mean values for MON 88702 were either within the respective reference ranges included in this study, or, were within the reference range reported from previous dormancy and germination studies for cotton. Therefore, the differences are unlikely to be biologically meaningful in terms of pest/weed potential of MON 88702 compared to conventional cotton.

No statistical comparisons could be made for one additional comparison (for viable hard seed at alternating 10/30°C) due to lack of variability in the data. For these data, the values for MON 88702 and the conventional control were identical, indicating no differences.

The dormancy and germination characteristics evaluated in this study were used to assess the pest/weed potential of cotton MON 88702 compared to the conventional control. Seed characteristics were thoroughly evaluated using six temperature regimes including the AOSA industry standard conditions for cotton. In this study, ten differences were observed in the seed characteristics. In all cases, the mean values for MON 88702 were either within the respective reference ranges included in this study, or, were within the reference range reported from previous dormancy and germination studies for cotton. Based on the assessed characteristics, the results of this study demonstrate that there were no differences in the dormancy and germination characteristics indicative of increased

plant pest/weed potential of MON 88702 compared to conventional cotton. Therefore, the introduction of the *mCry51Aa2* expression cassette is not expected to result in increased plant pest/weed potential of MON 88702 compared to conventional cotton.

Table VII-2. Germination Characteristics of MON 88702 and the Conventional Control

Temperature		Mean % (S.E.)[1]			
(°C)	Characteristic	MON 88702	DP393	Reference Range[2]	P-value
10	Germinated [4]	0.4 (0.17)	0.1 (0.08)	0.0 – 1.0	0.218
	Viable Hard [3]	16.9 (2.75)*	4.0 (0.95)	0.0 – 12.8	0.000
	Dead [3]	15.7 (1.83)	16.7 (1.58)	10.0 – 19.1	0.350
	Viable Firm-Swollen [3]	67.0 (3.37)*	79.0 (1.61)	70.0 – 88.5	0.000
20	Germinated [3]	91.7 (1.85)*	96.0 (0.94)	85.8 – 97.5	0.005
	Viable Hard [4]	0.2 (0.17)	0.1 (0.08)	0.0 – 1.2	0.568
	Dead [3]	7.2 (1.66)*	3.6 (0.78)	2.5 – 11.7	0.020
	Viable Firm-Swollen [4]	1.0 (0.40)	0.3 (0.20)	0.0 – 1.2	0.071
30	Germinated [3]	91.6 (1.79)	93.3 (1.43)	87.8 – 97.0	0.161
	Viable Hard [4]	0.1 (0.08)	0.0 (0.00)	0.0 – 0.8	1.000
	Dead [3]	8.0 (1.7)	6.2 (1.22)	3.0 – 11.6	0.198
	Viable Firm-Swollen [4]	0.3 (0.16)	0.5 (0.42)	0.0 – 1.8	0.548
10/20	Germinated [3]	66.2 (4.67)*	75.7 (2.92)	62.5 – 94.0	0.000
	Viable Hard [4]	3.8 (1.15)*	1.6 (0.65)	0.0 - 6.8	0.001
	Dead [3]	7.9 (1.64)	7.2 (1.01)	3.6 - 12.5	0.806
	Viable Firm-Swollen [3]	22.2 (3.18)*	15.6 (2.54)	0.5 – 31.5	0.002
10/30	Germinated [3]	92.2 (1.78)	94.9 (0.93)	87.8 - 96.8	0.175
	Viable Hard [5]	0.0 (0.00)	0.0 (0.00)	0.0 - 0.3	-
	Dead [3]	7.8 (1.78)	4.9 (0.88)	3.3 - 12.3	0.159
	Viable Firm-Swollen [4]	0.0 (0.00)	0.2 (0.17)	0.0 - 0.5	0.250
20/30	Normal Germinated [3]	86.9 (2.44)*	91.3 (1.40)	81.8 - 96.5	0.043
	Abnormal Germinated [4]	2.9 (0.52)	2.9 (0.43)	1.3 – 3.8	1.000
	Viable Hard [4]	0.5 (0.28)*	0.0 (0.00)	0.0 - 0.8	0.031
	Dead [3]	8.6 (1.91)*	5.3 (1.06)	2.0 – 14.5	0.038
	Viable Firm-Swollen [4]	1.0 (0.38)	0.4 (0.21)	0.0 – 1.5	0.142

Note: The experimental design was a split-plot with eight replicates.

[1] MON 88702 and the conventional control values represent means with standard error (S.E.) in parentheses.

[2] Reference range is the minimum and maximum mean values observed among the commercially available conventional reference materials.

[3] Indicates statistical comparisons were performed using ANOVA.

[4] Indicates statistical comparisons were performed using Fisher's Exact Test.

[5] No statistical comparisons were made because test and control values were 0.

* Indicates a statistically significant difference between test and the control (α=0.05).

VII.C.2. Field Phenotypic, Agronomic, and Environmental Interactions Characteristics

Phenotypic and agronomic characteristics, and environmental interactions were evaluated under field conditions as part of the plant characterization assessment of MON 88702 cotton. These data were developed to provide USDA-APHIS with a detailed description of MON 88702 cotton relative to the conventional control and reference varieties. Specific characteristics that are related to weediness, (*e.g.*, seed dormancy, fruit retention, and environmental interactions data), were used to assess whether there is a potential increase in weediness of MON 88702 compared to conventional cotton. Environmental interactions including plant response to abiotic stress, disease damage, arthropod-related damage, and arthropod abundance were also assessed as an indirect indicator of changes to MON 88702 cotton and are also considered in the assessment.

The results of the assessments of agronomic and phenotypic characteristics demonstrated that the introduction of the insect-protection trait did not meaningfully alter the plant pest/weed potential of MON 88702 compared to conventional cotton. Furthermore, the lack of meaningful differences in plant response to abiotic stress, disease damage, arthropod-related damage, and arthropod abundance also support the conclusion that the introduction of the insect-protection trait is not expected to result in increased plant pest/weed potential for MON 88702 compared to conventional cotton.

VII.C.2.1. Field Phenotypic and Agronomic Characteristics

Field trials were established at eight sites to evaluate phenotypic and agronomic characteristics of MON 88702 cotton compared to the conventional control (Table VII-3). The trial sites provided a range of environmental and agronomic conditions representative of commercial cotton production areas in North America. The planted plot dimensions varied between sites, due to variability in available planting equipment and the number of rows required for data collection (Appendix F). All plots of MON 88702, the conventional control, and the reference varieties at each site were uniformly managed, in order to assess whether the introduction of the insect-protected trait altered the phenotypic and agronomic characteristics of MON 88702 compared to the conventional control. No statistically significant differences were detected in the combined-site analysis between MON 88702 and the conventional control for seven of eight characteristics: early stand count, days to first flower, final stand count, plant height, total bolls, seed cotton yield, and seed index (Table VII-3). One statistically significant difference was detected between MON 88702 and the conventional control. MON 88702 had higher first position fruit retention (59.2 vs. 51.6%) compared to the conventional control. However, the mean value for MON 88702 for first position fruit retention was within the respective reference variety range. Thus, the difference was not considered biologically meaningful in terms of increased plant pest potential (Figure VII-1, step 2 “not outside variation of study references”).

The lack of biologically meaningful differences in phenotypic characteristics supports the conclusion that the introduction of the insect-protected trait in MON 88702 cotton is not expected to pose increased plant pest/weed potential compared to conventional cotton.

Table VII-3. Field Phenotypic Evaluation Sites for MON 88702 during 2015

Site Code	Year	County, State
CASN	2015	Fresno, California
GACH	2015	Tift, Georgia
LACH	2015	Rapides, Louisiana
MSLE	2015	Washington, Mississippi
NCBD	2015	Perquimans, North
TXLV	2015	Hockley, Texas
TXPO	2015	San Patricio, Texas
TXUV	2015	Uvalde, Texas

Table VII-4. Combined-Site Comparison of MON 88702 to Conventional Control for Phenotypic and Agronomic Characteristics During 2015

Characteristic (units)	Mean (Standard Error)[1]		Reference Range[2]	
	MON 88702	Control	Minimum	Maximum
Early Stand Count	247.4 (3.15)	255.7 (3.10)	179.6	259.8
Days to first flower	56.7 (0.91)	57.3 (0.94)	50.9	66.3
Final Stand Count	232.6 (3.37)	236.3 (3.17)	168.5	236.6
Plant height (cm)	84.1 (2.56)	87.6 (2.25)	66.1	95.2
Total bolls	9.5 (0.54)	9.3 (0.46)	7.1	13.1
First position fruit retention (%)	59.2 (2.53) *	51.6 (2.48)	35.1	61.3
Yield (kg/ha)	4239.6 (242.06)	4209.3 (201.62)	2345.9	5248.3
Seed Index (g/100 seed)	7.8 (0.10)	8.0 (0.11)	6.3	9.8

Notes: The experimental design was a randomized complete block with four replicates per site.

*Indicates a statistically significant difference between MON 88702 and the conventional control (α=0.05) using ANOVA.

[1] N = 32 for means, except where noted in Appendix F, Table F-9.

[2] Minimum and maximum mean values among 12 reference varieties, where each mean was combined over all the sites at which the reference variety was planted.

VII.C.2.2. Environmental Interaction Characteristics

Evaluations of environmental interactions were conducted as part of the plant characterization for MON 88702 cotton. In the eight 2015 U.S. field trials conducted to evaluate the phenotypic and agronomic characteristics of MON 88702 cotton, data were also collected on plant response to abiotic stress (e.g., drought, wind, nutrient deficiency, etc.), disease damage and arthropod-related damage (Table F-12 through Table F-14). Quantitative data on arthropod damage and abundance were collected from six 2015 and six 2016 U.S. fields (Table F-15 through Table F-21 and Table VII-7). These data were used as part of the environmental analysis (Section IX) to assess effect of MON 88702 compared to the conventional control and provide confirmatory data for the conclusion of tiered NTO testing data (Section V.B.5.1.). The results of the field evaluations showed that the insect-protected trait did not unexpectedly alter the assessed environmental interactions of MON 88702 cotton compared to the conventional control. The lack of significant biological differences in plant responses to abiotic stress, disease damage, arthropod-related damage, and pest- and beneficial arthropod abundance support the conclusion that the introduction of the insect-protected trait is not expected to result in increased plant pest potential of MON 88702 compared to conventional cotton.

VII.C.2.2.1. Qualitative Environmental Interaction Assessments

Plant responses to abiotic stressors, disease damage, and arthropod damage were assessed at natural levels, *i.e.*, no artificial infestation or imposed abiotic stress; therefore, these levels typically varied between observations at a site and among sites. Plant responses to abiotic stress, disease damage, and arthropod damage data were collected from each plot using a categorical scale (none, slight, moderate, and severe) of increasing severity of observed damage for each stressor. This scale was utilized to allow for the evaluation of the wide variety of potential abiotic stressors, disease damage, and arthropod damage symptoms potentially occurring across the season and across sites. These data were categorical and therefore were summarized and not subjected to statistical analysis (ANOVA). For a particular stressor, all comparisons of the range of responses for MON 88702 cotton to the range of responses for the conventional control across all observation times and sites are reported.

Descriptions of the evaluated environmental interactions characteristics and the timing of the evaluations are listed in Table VII-1. The materials, methods and additional details concerning the qualitative environmental interactions assessments are presented and discussed in Appendix F (Tables F-12 through F-14).

No differences were observed between MON 88702 cotton and the conventional control for any of the 96 comparisons for the assessed abiotic stressors: cold, drought, flooding, heat, mineral toxicity, nutrient deficiency, sandstorm, soil compaction, sun scald, wet soil, and wind (Table VII-5 and Appendix F; Table F-12).

No differences were observed between MON 88702 cotton and the conventional control for any of the 96 comparisons for the assessed diseases: bacterial blight, boll rot, Fusarium wilt, leaf spot, Phymatotrichum root rot, Phytophthora root rot, Pythium,

Rhizoctonia, rust, seedling blight, Stemphylium leaf spot, target spot, Verticillium wilt, and wet weather blight (Table VII-5 and Appendix F; Table F-14).

No differences were observed between MON 88702 cotton and the conventional control for 94 of the 95 comparisons for the assessed arthropods: aphids, armyworms, bollworms, cutworms, grape colaspis, grasshoppers, Japanese beetles, loopers, spider mites, stink bugs, striped flea beetles, and whiteflies (Table VII-5 and Appendix F; Table F-13). A single difference was observed in the arthropod damage assessment in which MON 88702 exhibited lower bollworm severity damage compared to the conventional control at site TXUV (none vs. slight rating) (Table VII-5 and Appendix F; Table F-13). However, the rating for MON 88702 was within the reference range and this difference was not observed consistently across observations and/or sites where bollworms occurred. Thus, the difference was not considered biologically meaningful in terms of increased plant pest potential and/or adverse environmental impact. Furthermore, the assessment of damage to MON 88702 and conventional cotton by several other members of the order Hemiptera (aphids (Family: Aphididae) and whiteflies (Family: Aleyrodidae)) also indicated that MON 88702 was not more or less susceptible to damage from other hemipteran species. The results further demonstrate that MON 88702 was not more or less susceptible than conventional cotton to damage from these coleopteran species. This result further supports the conclusions from the activity spectrum and the tiered NTO assessment, demonstrating that the activity of the mCry51Aa2 protein is limited to certain species in the family Chrysomelidae that was determined under laboratory conditions which were not relevant to field scenarios.

The lack of biologically meaningful differences in abiotic stress response, arthropod damage, and disease damage supports the conclusion that MON 88702 cotton is not expected to pose an increased risk potential compared to conventional cotton.

Table VII-5. Summary of Qualitative Environmental Interactions Assessments during 2015

Stressor	Number of observations across all sites	Number of observations with no differences between MON 88702 and the conventional control across all sites
Abiotic stressors	96	96
Disease damage	96	96
Arthropod-related damage	95	94
Total	287	286

Note: The experimental design was a randomized complete block with four replicates per site.
No differences were observed between MON 88702 and the conventional control during any observation for damage caused by any of the assessed abiotic stressors or diseases.
One difference was observed between MON 88702 and the conventional control for Bollworm damage at the TXUV site (MON 88702 = none; control = slight; reference range = none – slight). Categorical data were summarized across sites and observation times.

VII.C.2.2.2. Quantitative Environmental Interaction Assessments

Quantitative arthropod assessments of heliothine damage, stink bug damage and arthropod abundance were conducted at six sites planted in the 2015 and 2016 growing seasons (Table VII-6). Heliothine and stink bug damage was assessed up to four times during the growing seasons at all sites. Arthropod abundance was assessed from collections performed six times, encompassing the cotton growing season at these sites, using the vertical beat sheet method.

Descriptions of the quantitative, evaluated environmental interactions characteristics and the timing of the evaluations are listed in Table VII-1. The materials, methods, additional details concerning the specific arthropod damage assessments, and detailed results of the individual-site data comparisons for heliothine and stink bug damage as well as arthropod abundance are presented and discussed in Appendix F (Table F-15 through Table F-21). A summary of the results together with those of the combined-site and combined-year analysis of the arthropod abundance data are provided below.

In the assessment of heliothine and stink bug damage, no statistically significant differences were detected between MON 88702 and the conventional control for 34 of 39 and for 36 of 39 comparisons in the 2015 and 2016 growing seasons, respectively (Table F-15 through Table F-18). For these detected differences, the mean damage values for MON 88702 were either inside the respective reference ranges, and in cases where they were outside of the reference ranges, the detected differences were not consistent across observation times or sites. Thus, these differences in arthropod damage were not indicative of a consistent response associated with the trait and are not considered biologically meaningful in terms of adverse environmental impact of MON 88702 compared to conventional cotton. Lack of variability in the data precluded statistical comparisons between MON 88702 and the conventional control for eight additional comparisons; however, the means for MON 88702 and the conventional control were the same value for these comparisons, indicating no biologically meaningful differences.

Arthropod abundance was assessed from collections performed using the vertical beat sheet method at six sites in 2015 and 2016, of which four were the same across the two years. Statistical analyses and significance testing of differences between MON 88702 and the conventional control material were only performed for the arthropods present in sufficient numbers to estimate the material mean arthropod counts and the variation of the means. An inclusion criterion was established where a given arthropod must have an average count per plot per collection time (across all materials) of ≥ 1 (Ahmad et al., 2015). Also, a collection in a site was excluded in the across-collection calculation and subsequent analysis if the average capture per collection (across materials and replicates) was less than one. The combined-site analysis for the individual years, as well as the combined-site, combined-year analysis included a power analysis which demonstrates the power estimated for detecting a 50% difference in the abundance of each taxonomic group. Factors contributing to the amount of power to detect significant differences for any taxa include variability, abundance, and sample size (Duan et al., 2006). Additional details of the arthropod abundance assessments and detailed results of the individual-site

and combined-site analyses for the individual years are provided in Appendix F (Table F-19 through Table F-21).

In the combined-site and combined-year analysis, no significant differences were detected for 15 of the 16 taxa assessed (Table VII-7). Abundance of ladybird beetles was significantly higher in MON 88702 fields compared to the conventional control ($p=0.027$). This difference was primarily driven by a similarly observed statistical difference in abundance of this species in the 2015 growing season (Table F-21). An overview of all the coleopteran species tested in the activity spectrum and NTO assessments as well as the functional group they represent is provided in Table IX-2. Based on the weight of evidence provided, it can be concluded that MON 88702 is unlikely to pose a risk to Coleoptera.

It is important to highlight that the results demonstrate that no difference in *Orius* spp. abundance between MON 88702 and conventional cotton fields was detected (Table VII-7). *Orius* spp. is an important predator in the cotton agro-ecosystem and it is therefore not surprising that it was abundant in all 12 sites assessed throughout the two growing seasons. The estimated power to detect a 50% difference in abundance was 100% (Table VII-7). Therefore, these results support the conclusion from the tiered NTO assessments conducted with this species, indicating that MON 88702 is unlikely pose a risk to *Orius* spp.

Other predatory Hemiptera were abundant within the fields. Big-eyed bugs (*Geocoris* spp.) were observed in five out of six sites during each growing season (Table F-19 and Table F-20). No difference in abundance of this important predator in MON 88702 and conventional cotton fields was detected in the combined-site and combined-year analysis, and the estimated power to detect a 50% difference was 97.7% (Table VII-7). Also damsel bugs (*Nabis* spp.) were abundant in three and two out of six sites in the 2015 and 2016 growing seasons, respectively (Table F-19 and Table F-20). The analysis across sites and years demonstrated that there was no difference in their abundance between test and control field, with a power to detect a 50% difference of 89.8% (Table VII-7). Assassin bugs (*Zelus* spp.) were only found in two out of the six sites in the 2015 growing season but were absent in all the monitored sites in 2016. Consequently, they were not included in the combined-site, combined-year analysis. Their low abundance is not surprising since it has been documented in literature that assassin bugs are one of the less common hemipteran predators found in cotton fields compared to *Orius* spp. and *Geocoris* spp. (Section V.B.5.1.2).

The environmental interactions evaluated were used to characterize the plant and its interactions with the environment. Results from these evaluations aid in the environmental risk assessment to reduce uncertainty of unintended effects through collection of in planta data and provide additional weight of evidence to the conclusions from the multi-tiered laboratory and field NTO testing data (Section V.B.5). The results confirm that MON 88702 does not adversely affect arthropod communities representing the ecological functions of herbivores, predators and parasitoids in cotton agro-ecosystems.

Table VII-6. Quantitative Environmental Interaction Sites for MON 88702 During 2015 and 2016

Site Code	Year	County, State
GACH	2016	Tift, Georgia
GATI	2015	Tift, Georgia
LACH	2015, 2016	Rapides, Louisiana Georgia
MSLE	2015, 2016	Washington, Mississippi
NCRC	2015, 2016	Edgecombe, North Carolina
SCEK	2016	Barnwell, South Carolina
TXPO	2015	San Patricio, Texas
TXUV	2015, 2016	Uvalde, Texas

Table VII-7. Mean (SE) Arthropod Abundance Across 2015 and 2016 Vertical Beat Sheet Collections on MON 88702 Compared to the Conventional Control

Arthropod[1]	Number of sites[2]	MON 88702	Control	Power (%)[3]
		Order Araneae		
Spiders	12	10.4 (0.88)	10.3 (0.91)	100.0
		Order Coleoptera		
Ant-like flower beetles (Family: Anthicidae)	9	2.9 (0.23)	2.4 (0.26)	88.2
Click beetles (Family: Elateridae)	2	1.8 (0.41)	2.3 (0.33)	53.2
Ladybird beetles (Family: Coccinellidae)	10	7.6 (1.15) *	6.2 (0.91)	100.0
		Order Hemiptera		
Aphids (Family: Aphididae)	9	20.7 (4.62)	19.5 (4.30)	89.5
Assassin bugs (Family: Reduviidae)	2	4.1 (1.18)	3.8 (0.67)	46.8
Big-eyed bugs (Family: Geocoridae)	10	9.3 (1.73)	10.2 (1.79)	97.7
Damsel bugs (Family: Nabidae)	5	1.9 (0.27)	2.8 (0.45)	89.8
Leafhoppers (Family: Cicadellidae)	4	3.1 (0.43)	2.6 (0.32)	75.1
Minute pirate bugs (Family: Anthocoridae)	12	5.4 (0.42)	5.3 (0.48)	100.0
Stink bugs (Family: Pentatomidae)	4	3.3 (0.56)	4.5 (0.95)	97.5
Whiteflies (Family: Aleyrodidae)	5	108.1 (42.74)	129.1 (52.98)	87.3

Table VII-7. Mean (SE) Arthropod Abundance Across 2015 and 2016 Vertical Beat Sheet Collections on MON 88702 Compared to the Conventional Control (continued)

Arthropod[1]	Number of sites[2]	MON 88702	Control	Power (%)[3]
Order Hymenoptera				
Ants (Family: Formicidae)	11	7.2 (1.04)	6.6 (0.88)	94.2
Parasitic wasps	8	5.1 (0.94)	5.6 (1.13)	98.6
Order Lepidoptera				
Heliothines (Family: Noctuidae)	3	2.5 (0.28)	2.2 (0.24)	81.3
Order Neuroptera				
Lacewings (Family: Chrysopidae)	8	1.9 (0.20)	2.1 (0.24)	99.5

* Indicates statistically significant difference between MON 88702 and the conventional control (α=0.05) using ANOVA.
Note: The experimental design was a randomized complete block with four replications.

[1] Arthropods were collected six times at each site starting at early squaring and bi-weekly thereafter.

[2] Number of sites where each taxon occurred pooled across both years.

[3] Power to detect a 50% change in arthropod abundance between MON 88702 and the conventional control given an RCBD and α=0.05

VII.C.3. Pollen Characteristics

The potential for gene flow and introgression of the biotechnology-derived trait(s) into sexually compatible plants and wild relatives can be used to evaluate the potential for increased weedy or invasive characteristics of the receiving species. In addition, morphological characterization of pollen produced by MON 88702 cotton and the conventional control is relevant to the plant pest risk assessment because it adds to the detailed description of the phenotype of MON 88702 cotton compared to the conventional control.

The viability and morphology of pollen collected from MON 88702 cotton compared to that of the conventional control were assessed. Pollen was collected from MON 88702, the conventional control, and four commercial reference varieties grown under similar agronomic conditions at a field site in Washington County, Mississippi, a geographic area that represents environmentally relevant conditions for cotton production for this product. The study was arranged in a randomized complete block design with four replications. Once all plants across the replications reached the flowering stage, pollen was collected from three non-systematically selected plants per plot and stained for assessment. The details of the materials and experimental methods used in this evaluation are presented in Appendix G.

No statistically significant differences (α=0.05) were detected between MON 88702 and the conventional control for percent viable pollen or pollen grain diameter (Table VII-8). Furthermore, no visual differences in general pollen morphology were observed between MON 88702 and the conventional control (Figure VII-2).

The pollen characterization data contribute to the detailed phenotypic description of MON 88702 compared to the conventional control. Based on the assessed characteristics, the results support a conclusion that neither pollen viability nor morphology of MON 88702 were altered compared to conventional cotton.

Table VII-8. Pollen Characteristics of MON 88702 Compared to the Conventional Control during 2015

	MON 88702	Conventional Control	Reference Range[1]	
Pollen Characteristic (unit)	Mean (SE)[2]	Mean (SE)[2]	Minimum	Maximum
Viability[3] (%)	98.5 (0.10)	99.3 (0.10)	98.5	99.1
Diameter[4] (µm)	95.7 (0.36)	95.7 (0.49)	94.8	98.6

No significant differences were detected between the MON 88702 and the conventional control (α=0.05) using analysis of variance (ANOVA).

[1] Reference range is the minimum and maximum mean value observed among the four reference cotton varieties.

[2] SE = Standard Error.

[3] Evaluated for three subsamples per replication of MON 88702, the conventional control, and reference varieties.

[4] Evaluated for 10 representative viable pollen grains per replication.

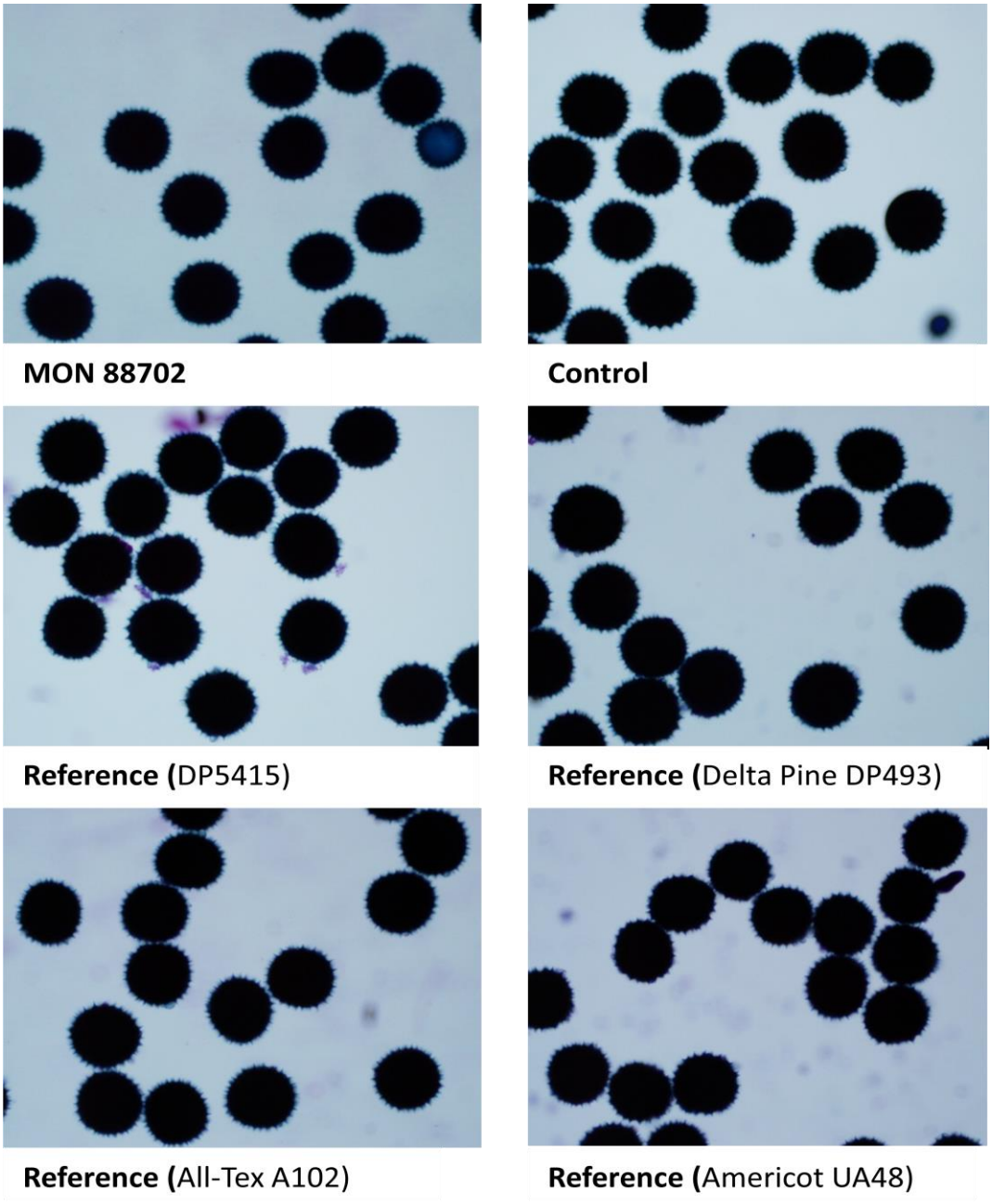


Figure VII-2. General Morphology of Pollen from Cotton MON 88702, Conventional Control, and Reference Materials under 200X Magnification

Pollen was stained with Alexander's stain diluted 1:5 with distilled water (Alexander, 1980). Viable pollen grains stain purple and appear round. In cases of non-viability, pollen grains tend to stain light blue to green and appear round to collapsed depending on the degree of hydration.

VII.D. Conclusions for Phenotypic, Agronomic, and Environmental Interactions Evaluation

Comparative plant characterization data between a biotechnology-derived crop and the conventional control are interpreted in the context of contributions to increased plant pest potential as assessed by USDA-APHIS. Under the framework of familiarity, characteristics for which no differences are detected support a conclusion of no increased plant pest potential of the biotechnology-derived crop compared to the conventional crop. Ultimately, a weight of evidence approach that considers all characteristics and data is used for the overall risk assessment of differences and their significance.

An extensive set of agronomic, phenotypic, and environmental interactions data, including specific weedy characteristics, were used to assess whether the introduction of the insect-protection trait altered the plant pest potential of MON 88702 cotton compared to the conventional control, considered within the context of the variation among the reference varieties. These assessments included five general data categories: 1) seed germination, dormancy, and emergence; 2) vegetative growth; 3) reproductive development (including pollen characteristics); 4) fruit retention on the plant; and 5) plant response to abiotic stress and interactions with diseases and arthropods. Within these data categories, data relevant to understanding specific characteristics associated with weediness were also assessed to determine whether there was a potential increase in weediness of MON 88702 compared to conventional cotton.

Results from these assessments comparing MON 88702 and the conventional control demonstrate that MON 88702 cotton does not possess: 1) increased weediness characteristics; 2) increased susceptibility or tolerance to specific abiotic stress, diseases, or arthropods; or 3) characteristics that would confer a plant pest risk compared to conventional cotton. Therefore, based on the results of multiple assessments discussed above and presented in the appendices, the weight of evidence indicates that MON 88702 cotton is not meaningfully different from conventional cotton with the exception of the insect-protection trait and is not expected to pose a plant pest risk compared to conventional cotton.

VIII. U.S AGRONOMIC PRACTICES

VIII.A. Introduction

As part of the plant pest assessment required by 7 CFR § 340.6(c)(4), impacts to agricultural and cultivation practices must be considered. This section provides a summary of current agronomic practices in the U.S. for producing cotton and is included in this petition as a baseline to assess possible impacts to agricultural practices due to the cultivation of MON 88702. Discussions include cotton production, seed production, plant growth and development, general management practices during the season, management of insects, diseases and weeds, cotton rotational crops, and volunteer cotton management. Information presented in the previous sections demonstrated that MON 88702 is no more susceptible to diseases or pests and is phenotypically equivalent to conventional cotton and, therefore, is not expected to pose a plant pest risk compared to commercially cultivated cotton. While MON 88702 cotton offers protection against feeding damage caused by targeted hemipteran and thysanopteran insect pests including two species of tarnished plant bugs (*Lygus hesperus* and *Lygus lineolaris*) and thrips (*Frankliniella* spp.), growers are still anticipated to incorporate current methods of crop protection into their overall insect pest management program. In this regard, cultivation of MON 88702 cotton is not expected to differ from typical cotton cultivation, with regards to current cotton management practices, except for the additional control of targeted hemipteran and thysanopteran insect pests that may result in reduced number of crop protection applications (i.e., insecticides) compared to commercially available cotton. Thus, there are no likely impacts to the majority of the agronomic practices employed for the production of cotton from the introduction of MON 88702. Agronomic practices that may be influenced from the deregulation in whole of MON 88702 cotton are discussed.

VIII.B. Background

The CEQ Implementing Regulations encourage agencies to consider previously published final environmental impact statements (EIS) as a reference to eliminate repetitive discussions of the same issues and to focus on the issues that are ripe for discussion at each level of environmental review (Mandelker, 2012). Specifically, 40 CFR §1502.20 encourages agencies to "tier" (i.e., reference) to their previous EISs. The regulations at 40 CFR §1508.28 indicate tiering (referencing) is appropriate when the sequence of analyses are (a) from a program, plan, or policy environmental impact statement to a program, plan, or policy statement or ***analysis of lesser scope*** [emphasis added], and (b) from an environmental impact statement on a specific action to supplement at an early stage (which is preferred) or a subsequent statement or analysis at a later stage. Tiering (referencing) in such cases is appropriate when it helps the agency to focus on the issues which are ripe for decision and exclude from consideration issues already decided. Additionally, the CEQ Implementing Regulations, at 40 CFR §1500.4, indicate an agency shall reduce paperwork by using program, policy, or plan environmental impact statements and tiering (referencing) from these to eliminate repetitive discussions of the same issue.

Therefore, to avoid repetitive and duplicative analyses, the MON 88702 cotton agronomic practices section incorporates by reference the agronomic practices discussions in Monsanto's Petition for the Determination of Nonregulated Status for Dicamba and Glufosinate-Tolerant Cotton MON 88701 (petition #12-185-01p) that was assessed by APHIS in its EIS for Dicamba and Glufosinate-tolerant cotton MON 88701: Final Environmental Impact Statement – Monsanto Petitions (10-188-01p and 12-185-01p) for Determinations of Nonregulated Status for Dicamba-Resistant Soybean and Cotton Varieties. Because APHIS's EIS was based on the nationwide effects of cotton cultivation, much of the agronomic practices analysis therein will be applicable to MON 88702 cotton.

VIII.C. Overview of U.S. Cotton Production

VIII.C.1. Cotton Production

The majority of the value of the producer's cotton crop is based on the quality and quantity of the lint produced, with the exception of contracted acres for planting seed production. Little consideration is given by growers to the disposition of the cottonseed and its by-products. Most of the world's cotton production (123.5 million bales annually) is grown in India (29.0 million bales), China (27.5 million bales), the U.S. (20.9 million bales), Brazil (9.2 million bales) and Pakistan (8.2 million bales). Values are from the 2017/2018 cotton season (USDA-FAS, 2019b). In 2017/2018, the U.S. supplied over 15.8 million bales of the world's cotton exports, accounting for approximately 39% of the total world export market for cotton (USDA-FAS, 2019b). Bangladesh, Vietnam, China, Turkey, Indonesia and Pakistan are major importers of cotton (USDA-FAS, 2019b). The largest customers for U.S. cotton are Asian countries and Mexico, due to the prevalence of textile manufacturing (NCCA, 2010; USDA-FAS, 2019b).

Cottonseed is of less value to the cotton farmer and is mainly consumed domestically. Cottonseed production globally was approximately 45 million metric tons (MMT) in 2017/2018 (USDA-FAS, 2019a) with India (12.3 MMT) and China (10.8 MMT) being the largest producers. The U.S. produced approximately 13% of the world's cottonseed (5.8 MMT), while Pakistan, Brazil and Uzbekistan produce the remainder of the world majority. Of that total production, the U.S. only exported 254,165 metric tons (MT) or 4.4% of production. Of this small percentage, most the exports of cottonseed went to South Korea (107,644 MT) and a considerably smaller amount was exported to Mexico and Japan (USDA-FAS, 2019a).

Gossypium hirsutum (upland cotton) cultivars account for more than 90% of the world's annual cotton crop and approximately 98% of the U.S. cotton production in 2018 (Smith and Cothren, 1999) (USDA-NASS, 2018f). *G. barbadense*, known as extra-long staple, Pima, or Egyptian cotton, is also grown in the U.S., and accounts for approximately 2% of the acreage in the U.S. (USDA-NASS, 2018f). The long, strong, fine fibers produced by Pima are ideal for specialized uses, but due to the geographic limitation for optimum production it is economically less viable than the *G. hirsutum* cultivars in the U.S. Pima cotton requires a longer growing season than upland cotton, and production is limited to the Southwestern states.

Cotton (*Gossypium* spp.) is grown in the U.S. across southern states where the climate is warmer and the season is longer (Figure VIII-1 and Figure VIII-2). The total U.S. cotton acreage in the past 10 years has varied from approximately 8.5 to 14.7 million planted acres (Table VIII-1). Average cotton yields have varied from 766 to 905 pounds per acre over this same time period. Total annual cotton production ranged from 12.19 to 20.92 million bales (480 pounds/bale) over the past ten years. The variations observed in cotton acreage and production is driven by current market conditions, rather than agronomic considerations. According to data from USDA-NASS (USDA-NASS, 2018a, 2019) cotton was planted on approximately 14 million acres in the U.S. in 2018, producing approximately 18 million bales of cotton (Table VIII-1).

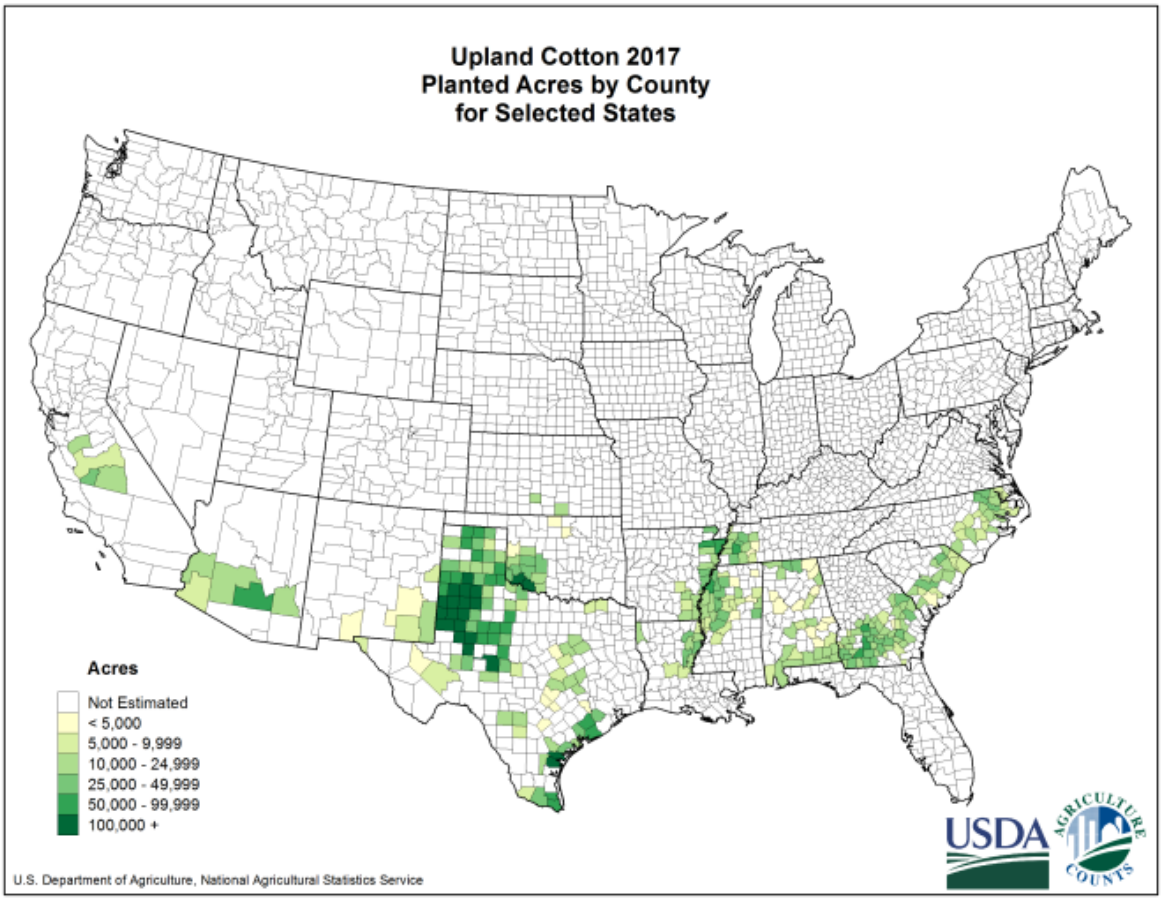


Figure VIII-1. Planted Upland Cotton Acres by County in the U.S. in 2017

(USDA-NASS, 2018e)

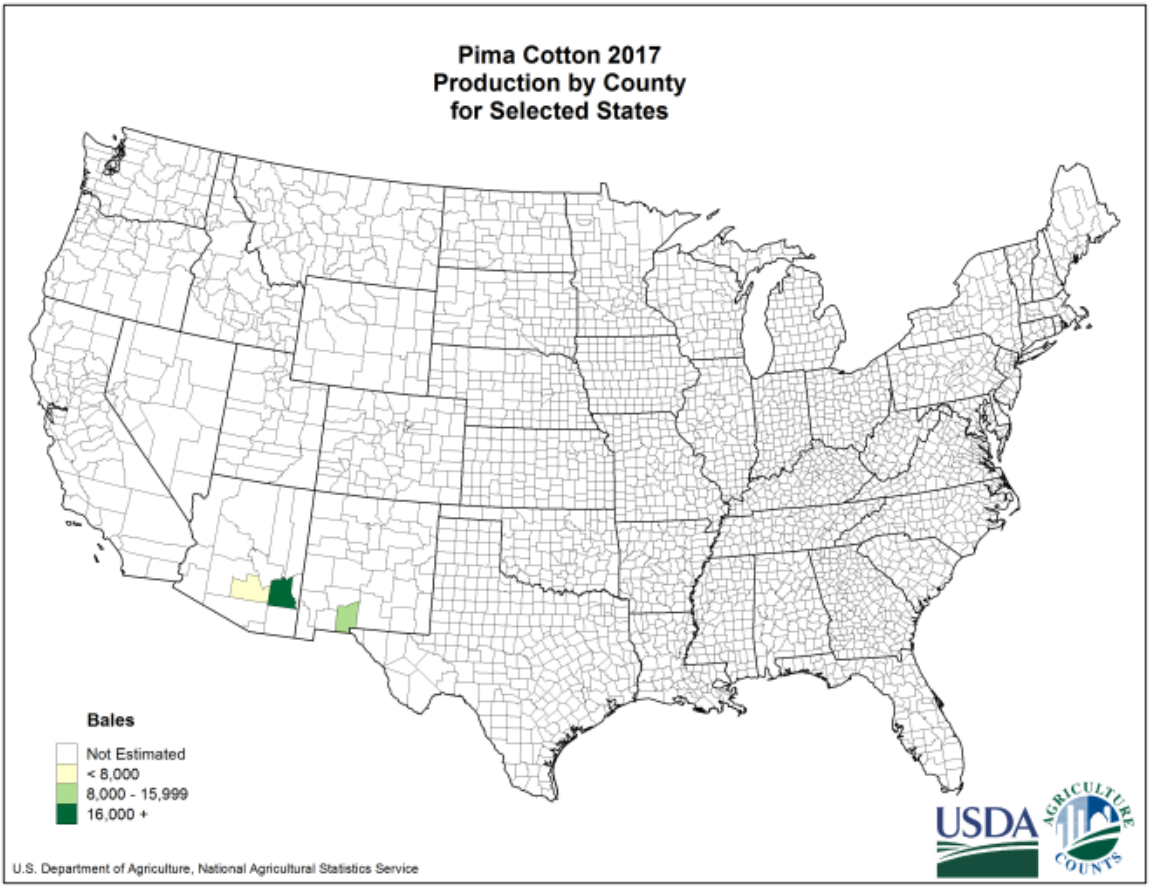


Figure VIII-2. Planted Pima Cotton Acres by County in the U.S. in 2017

(USDA-NASS, 2018d)

Table VIII-1. Cotton Production in the U.S., 2008-2018[1]

Year	Acres Planted (×1000)	Acres Harvested (×1000)	Average Yield (lbs./acre)	Total Production (480 lb. bales)	Value (billions $)
2018	14,099	10,531	838	18,390,000	NA[2]
2017	12,612	11,349	899	21,263,000	7.227
2016	10,073	9,508	867	17,169,900	5.814
2015	8,581	8,075	766	12,888,000	3.989
2014	11,037	9,347	838	16,319,400	5.147
2013	10,407	7,544	821	12,909,200	5.192
2012	12,264	9,322	892	17,313,800	6.292
2011	14,735	9,461	790	15,573,200	6.986
2010	10,974	10,699	812	18,101,800	7.347
2009	9,150	7,534	776	12,183,000	3.787
2008	9,471	7,569	813	12,825,400	3.024

[1] (USDA-NASS, 2018c; 2019)

[2] NA denotes “not available”.

VIII.C.2. Cotton Seed Production

Monsanto summarized major considerations associated with the seed production management of cotton in its petition for nonregulated status for Dicamba and Glufosinate-tolerant cotton MON 88701 (Petition #12-185-01p). None of the information on this subject has changed in any substantive way and Section VIII.B.2 (pages 175 to 176) is incorporated here by reference (Monsanto Company, 2012).

VIII.D. Production Management Considerations

Monsanto summarized major considerations associated with the production management of cotton in its petition for nonregulated status for Dicamba and Glufosinate tolerant cotton MON 88701 (Petition #12-185-01p). None of the information on this subject has changed in any substantive way and Section VIII.C. (pages 176 to 179) is incorporated here by reference (Monsanto Company, 2012).

Other than the specific insertion of the coding sequence for mCry51Aa2 that provides protection against targeted hemipteran and thysanopteran insect pests, including two species of tarnished plant bugs (*Lygus hesperus* and *Lygus lineolaris*) and thrips (*Frankliniella* spp.), MON 88702 is comparable to other cotton varieties in terms of its production management requirements. USDA has deregulated numerous crop plants that express Cry proteins to protect against insect pests and approved the first insect-protected cotton in 1995[3]. Given the widespread use of insect-protected crops, production of MON 88702 cotton is not expected to differ from typical cotton production management practices, except for the potential of a reduced number of insecticide applications due to the additional control of targeted hemipteran and thysanopteran insect pests.

VIII.E. Management of Insect Pests

Insect and mite pests are a common and continuous threat to cotton production in all regions of the U.S., leading to decreased yield and quality. Generally, only 40 insect pests are considered persistent problems causing economic losses in cotton (Smith and Cothren, 1999). The susceptibility of cotton plants to insect pests varies across and within the various production regions. Insect and mite pests affect cotton production by decreasing yield and reducing quality. Nearly every phenological stage of cotton is susceptible to injury by one or more insect pests during the growing season. Therefore, cotton fields must be monitored regularly to detect the presence of insect pests. The susceptibility of cotton plants to economic yield losses from insect pests is influenced by pest population density, timing of infestations as related to plant phenology, local environmental conditions, and agronomic practices (Smith and Cothren, 1999).

---

[3] https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/permits-notifications-petitions/petitions/petition-status

Numerous insect species are observed in cotton fields across the U.S., but only a few are considered of economic importance. Yield loss and treatment costs for the most common insect pests in cotton in 2016 are shown in Table VIII-2. These data are estimates collected from surveys of county agents, extension specialists, private consultants, and research entomologists. Insect damage resulted in yield losses of approximately 775,000 bales of cotton in 2016 or a 2.6% yield loss which represented an average loss of $23.99 per acre. The Lygus insects caused the greatest yield reductions followed by stink bugs and thrips. Thrips infested more acres in 2016 than any other insect in cotton. However, this insect ranked third in yield reductions, due to the damage occurring early in the growing season before the development of fruiting structures.

Insect pests are best managed through the use of integrated pest management (IPM) programs where all viable control strategies are considered and appropriate strategies are selected for use against specific insect pests (Bradley et al., 2013). Pre-plant tillage and crop rotation are important agronomic or cultural practices utilized to reduce insect populations prior to planting cotton. Other agronomic practices are utilized to promote early maturity and reduce that period of time the crop is susceptible to insect and mite pests, and to increase the probability that an acceptable yield can be produced before insect pest densities exceed economic threshold levels (Smith and Cothren, 1999).

Applications of insecticide for control of various cotton insect pests have been commonly used and in 2017 approximately 43% of acres were treated with an insecticide (Table VIII-3) (USDA-NASS, 2018a), though in general, overall insecticide application has gone down over the past 20 years (Fernandez-Cornejo et al., 2014). Numerous insecticidal active ingredients are registered for use in cotton for the control of insect pests. However, three insecticide active ingredients [acephate (25%), bifenthrin (15%) and imidacloprid (11%)] represent the large majority of the total treated cotton acres in the U.S. (Table VIII-3). These insecticides are effective in managing many insect pests that may cause damage to cotton from emergence through maturity. Given the diversity of insect pests and the development of resistance to some insecticides currently in use a rotational strategy is sometimes recommended to protect cotton from planting to cut out and ensure maximum yield (Mississippi State University, 2016).

Thrips infestations have varied in magnitude across the U.S. over the years, but are typically within the top ten causes of yield loss (Cook et al., 2011). Currently, control is solely based on insecticide applications with treatment methodologies varying geographically across cotton growing states. Adults and larvae will infest seedlings and feed on plant epidermal cells causing early developmental damage which may result in yield losses (Cook et al., 2011). Studies have shown that, depending on the geography, foliar and seed treatment applications can result in greater than 400 lbs of lint per acre compared to non-treated controls (Herbert et al., 2007). Control of thrips is generally attempted with at-planting prophylactic application of insecticides, either in-forrow or as seed treatments; where the risk of early cotton seedling damage dominates that the majority of insecticide use is seed treatment (approximately 8 million acres) and a minor use is in-furrow spray (approximately 800,000 acres). Aldicarb and acephate are widely used for in-furrow sprays while neonicotinoids (imidacloprid and thiamethoxam) have been widely adopted as seed treatments (Cook et al., 2011). Supplemental foliar

treatments are also applied when cool temperatures slow the growth rate of cotton seedlings. Most states recommend acephate, dicrotophos or dimethoate for foliar applications (Mississippi State University, 2016; Vyavhare et al., 2017).

Lygus is an important cotton pest, with two species being the most damaging in the U.S. The tarnished plant bug, *Lygus lineolaris*, is an important pest mainly in the mid-south and the south-east of the U.S. (parts of LA, AR, MS and TX), while the western tarnished plant bug, *Lygus hesperus*, is more abundant in the western part of the U.S. (CA, AZ and NM). They prefer to feed on small to medium sized squares resulting in abscission of the square and yield losses (Layton, 2000). Feeding on larger squares may also occur which results in abnormal flowers and possibly small abnormal bolls. Though not historically a major cotton pest, the advent of *Bt* cotton to control other lepidopteran pests (see below) resulted in reduced spraying of insecticides, where previous uses patterns had a secondary effect of controlling Lygus populations (Gore et al., 2012). Historically, Lygus have been controlled by broad spectrum insecticides such as organophosphates, carbamates and pyrethroids; although resistance to these insecticides has increased in recent years (Gore et al., 2012). Resistance to pyrethroids and organophosphates, especially acephate, is substantial in many areas of the mid-south (Gore et al., 2012; Mississippi State University, 2016), although Lygus are still considered susceptible to these insecticides in certain areas of the U.S. (Vyavhare et al., 2017). With increased resistance to pyrethroids and organophosphates, neonicotinoid insecticides have become important for Lygus control during pre-flowering and flowering stages. Imidacloprid and thiamethoxam are the most widely used neonicotinoids, while newer insect growth regulators (novaluron) and a pyridine (carboxamide flunicamid) are also currently labeled for control of Lygus on cotton. Although, these newer insecticides are slower acting than the traditional pyrethroids and organophosphates (Gore et al., 2012).

Nematodes are considered another major pest in cotton in the southern U. S. and have the potential to cause significant yield loss, reduction in fiber quality, and impact crop maturity. Yield losses in cotton from nematodes alone exceed 672,000 bales in 2016 in the U.S.(Lawrence et al., 2017). Management decisions for controlling nematodes must be made prior to or at planting since few control options are available during the season. Complicating this decision is sampling for nematodes and characterizing the levels of infestation is difficult (Overstreet et al., 2014) and application of nematicides are typically made across entire fields (Ortiz et al., 2012). Seed treatments have also been applied to help protect against damage from nematodes.

As indicated above, nearly every phenological stage of cotton is susceptible to injury by one or more insect pests during the growing season. Therefore, approximately 43% of the cotton acres planted in 2017 were treated with an insecticide (Table VIII-2). The use of several broad-spectrum insecticides often times leads to the reduction of the amount of target pests together with a wide array of other, non-target species (Asiimwe et al., 2014; Ellsworth and Barkley, 2005; Mississippi State University, 2016; Naranjo et al., 2004). It has been demonstrated that minor negative effects of planting *Bt* crops are inconsequential in comparison to using insecticides as an alternative crop protection measure (Naranjo, 2009; Wolfenbarger et al., 2008). Natural enemies present in cotton have been demonstrated to be more abundant in fields that have *Bt* cotton planted

compared to sprayed conventional cotton fields (Naranjo, 2009). The meta-analysis conducted by Wolfenbarger et al. (2008) showed that the abundance of predators as well as herbivores and mixed-guild taxa was significantly higher in untreated *Bt* cotton compared to treated conventional cotton. The adoption of *Bt* cotton has reduced the active ingredient use globally with 288 million kg since 1996 (Brookes and Barfoot, 2017). The reduction in the use of broad-spectrum insecticides by cultivation of *Bt* cotton therefore has the potential to conserve populations of non-target arthropods present in cotton fields. Based on the above, it is important that insecticides should be integrated in a comprehensive IPM program.

VIII.E.1. Plant Incorporated Protectants to Control Insects

The introduction of biotechnology-derived insect-protected *Bt* cotton has offered growers alternative and highly effective solutions for control of major insect pests in cotton. Though no longer the most economically important pest, bollworms have traditionally been an important pest in cotton. The Bollgard® line of biotechnology-derived cotton was first introduced in 1996 to protect cotton from damaging lepidopteran insect pests (USDA-APHIS, 1995). Approximately 94% of the total U.S. cotton acres in 2017 were planted with cotton varieties containing biotechnology-derived traits, including traits expressing the insecticidal crystal (Cry) proteins derived from *Bt* (USDA-NASS, 2018f) to protect against damage from insect pests, including bollworms, budworms and armyworm (Table VIII-2).

VIII.F. Introduction of Insect Protected Cotton MON 88702

VIII.F.1. MON 88702 Cotton Product Concept

MON 88702 cotton produces a modified Cry51Aa2 insecticidal crystal (Cry) protein derived from *Bacillus thuringiensis* (*Bt*) that protects against feeding damage caused by targeted hemipteran and thysanopteran insect pests, more specifically two species of tarnished plant bugs (*Lygus hesperus* and *Lygus lineolaris*) and thrips (*Frankliniella* spp.). MON 88702 cotton will provide protection against two of the currently most important cotton pests in terms of crop damage and number of infested acres. While MON 88702 cotton offers protection against feeding damage caused by the targeted hemipteran and thysanopteran insect pests, growers are still anticipated to incorporate current methods of crop protection into their overall insect pest management program with the potential for a reduced number of insecticide applications currently required to control these targeted hemipteran and thysanopteran insect pests. MON 88702 cotton was developed to offer cotton growers an additional biotechnology derived tool for insect pest management and will be combined through traditional breeding methods with other insect-protected and herbicide-tolerant biotechnology traits commercially available in cotton to build upon current *Bt* protein-based cotton products.

® Bollgard is a registered trademark of Bayer Group.

Table VIII-2. Insect Losses in U.S. Cotton in 2016

Insect Pest	% Yield Reduction	Cotton Acres Infested	Cotton Acres Treated	Treatment Cost ($/Acre)	Cotton Bales Lost
Thrips	0.423%	9,477,763	3,340,547	$2.14	120,286
Cotton Fleahopper	0.091%	6,229,625	1,355,471	$0.76	16,439
Lygus	0.734%	4,906,100	2,374,603	$6.62	260,154
Stink Bugs	0.640%	4,390,201	2,623,231	$2.95	164,558
Bollworm/Budworm	0.413%	3,709,377	1,480,156	$1.86	117,118
Aphids	0.017%	3,054,545	498,525	$0.31	5,949
Spider Mites	0.120%	2,066,204	687,779	$1.03	40813
Grasshoppers	0.000%	1,597,856	81,983	$0.04	19
Brown Stink bug	0.005%	705,959	76,415	$0.00	1,962
Fall Armyworm	0.062%	571,926	140,556	$0.10	21,604
Banded Winged Whitefly	0.027%	495,141	5,621	$0.01	5,631
Silverleaf Whitefly	0.037%	465,999	214,807	$0.78	12,338
Clouded Plant bugs	0.023%	363,648	104,340	$0.05	8,141
Cutworms	0.000%	305,759	494,839	$0.18	100
Leaf footed bugs	0.000%	252,778	7,140	$0.00	42
Verde Plant Bugs	0.002%	157,780	75,460	$0.05	52
Beet Armyworm	0.000%	130,088	934	$0.00	29
Loopers	0.000%	122,325	0	$0.00	0
Boll Weevils	0.000%	68,600	0	$0.00	0
Saltmarsh Caterpillar	0.000%	32,104	982	$0.00	68
Pale-striped Flea Beetles	0.000%	14,475	3,244	$0.00	85
Cotton Leafperforator	0.000%	6,648	0	$0.00	0
Darkling Beetle	0.000%	2,906	0	$0.00	0
Crickets	0.000%	982	0	$0.00	0
Total	**2.6**			**16.89**	**775,389**

Source: (Williams, 2016)

Table VIII-3. Insecticide Applications in U.S. Cotton in 2017

Insecticide	Target Insects and Application Method	Chemical Family	Mode of Action (MOA)	Percent Cotton Acres Treated	Total Quantity Applied
Bifenthrin[1]	Lygus and thrips, foliar	Pyrethroids	Sodium channel modulators	15%	267
Cypermethrin	-			1%	15
λ-Cyhalothrin	-			9%	53
Z-Cypermethrin	-			>1%	1
Acephate[2]	Thrips, foliar	Organophosphates	Acetylcholine-esterase inhibitors	25%	3,101
Dicrotophos	-			9%	525
Imidacloprid[3]	Thrips, seed treatment	Neonicotinoid	Nicotine Acetylcholine receptor antagonists	11%	204
Sulfoxaflor	-			4%	36
Thiamethoxam[3]	Thrips, seed treatment			6%	56
Abamectin	-	Lactone	GABA stimulator	2%	3
Novaluron	-	Benzoylphenyl Urea	Chitin formation inhibitor	4%	34

(USDA-NASS, 2018b)

[1] Restricted Use Pesticides; may be fatal if swallowed LD_{50} 10-25 mg/kg.

[2] Hazardous to humans and animals; Toxic to wildlife.

[3] Effectiveness diminishing due to insect resistance.

[4] Restricted Use Pesticides; may be fatal if swallowed LD_{50} <10 mg/kg.

VIII.F.2. Insect Resistance Management

A critical component for the long-term durability of biotechnology-derived crops containing *Bt* insecticidal proteins is to implement Insect Resistance Management (IRM) programs to prevent or delay the onset of resistance in the target insect species. Research by industry as well as academic scientists over the past decade has improved understanding and gained broad agreement for the major elements of IRM plans for *Bt* crops. The core element of an IRM plan is the use of a refuge to ensure an adequate population of susceptible insects of the target species is available to mate with any resistant insects that survive exposure to the Cry protein produced by the crop. This refuge may include wild host plants, other crops, or non-*Bt* plantings of the host crop.

MON 88702 cotton will not be offered for commercial use as a stand-alone single-event product. Thus, no specific IRM program is being developed for MON 88702 cotton alone. Instead MON 88702 cotton is intended to be commercialized as combined-trait breeding stacks with previously deregulated biotechnology-derived insect protected traits. As these selected insect-protected combined-trait products are identified and developed, Monsanto (or other developers) will develop appropriate IRM program(s) for these products and submit them to the U.S. EPA as part of a registration package(s) and will be implemented as the product is commercialized. U.S. EPA has required IRM proposals for each of the insect-protected cotton products it has previously registered. IRM plans, which are included as conditions of U.S. EPA registration, are designed to delay the development of insect resistance to specific PIPs and prolong the useful life of these products.

VIII.G. Management of Disease and Other Pests

Monsanto summarized major considerations associated with disease and other pest management in cotton cultivation in its petition for nonregulated status for Dicamba and Glufosinate-tolerant cotton MON 88701 (Petition #12-185-01p). None of the information on this subject has changed in any substantive way and, therefore, Section VIII.E. (page 182) of that petition is incorporated here by reference (Monsanto Company, 2012).

Disease management is essential in cotton production to achieve optimum yields and economic returns. Plant pathologists estimate that diseases cause annual losses in cotton production of 1.8 million bales or a yield reduction of approximately 9.0 % in the U.S. (Blasingame et al., 2008). Seedling diseases, fungal wilts, root rots, and foliar diseases constitute the major disease complex in cotton (Smith and Cothren, 1999). These types of infestation can result in yield losses of as much as 20% without any awareness of the root infections by soil-borne pathogens.

As demonstrated in Section VII.C.2., MON 88702 cotton is not significantly different from conventional cotton in terms of susceptibility to disease. Therefore, commercial cotton containing MON 88702 is not expected to impact current agronomic practices used to manage disease in U.S. cotton production.

VIII.H. Weed Management

Monsanto summarized major considerations associated with weed management in cotton cultivation in its petition for nonregulated status for Dicamba and Glufosinate-tolerant cotton MON 88701 (Petition #12-185-01p). None of the information on this subject has changed in any substantive way and, therefore, Sections VIII.F. and VIII.G (pages 183 to 214) of that petition is incorporated here by reference (Monsanto Company, 2012).

Weed management practices include mechanical tillage, crop rotations, cultural practices (e.g., planting clean seed, cleaning tillage and harvesting equipment), and herbicide application. Numerous selective herbicides are utilized for preplant, preemergence, and postemergence control of annual and perennial weeds in cotton. In 2017, approximately 91`% of the cotton acreage in the U.S. received a herbicide application (USDA-NASS, 2018a). Herbicide-tolerant cotton was grown on 91% of U.S. cotton acres in 2018 (USDA-NASS, 2018f) .

MON 88702 cotton is not herbicide tolerant and, therefore, is no different from other non-herbicide tolerant cotton in terms of its weed management considerations. Commercial cotton containing MON 88702 combined with deregulated herbicide-tolerant cotton traits will have weed management practices consistent with those of the deregulated herbicide-tolerant cotton.

VIII.I. Crop Rotation Practices in Cotton

Monsanto summarized major considerations associated with crop rotation practices in cotton cultivation in its petition for nonregulated status for Dicamba and Glufosinate-tolerant cotton MON 88701 (Petition #12-185-01p). None of the information on this subject has changed in any substantive way and, therefore, Section VIII.H. (pages 215 to 227) of that petition is incorporated here by reference (Monsanto Company, 2012). MON 88702 cotton is not expected to differ from commercially available cotton in terms of crop rotation practices.

The rotation of cotton with other crops is an integral part of most farm management programs across the southern U.S. cotton growing region but is not a practice that is used in the management of cotton insect pests.

VIII.J. Cotton Volunteer Management

Monsanto summarized major considerations associated with volunteer management in cotton cultivation in its petition for nonregulated status for Dicamba and Glufosinate-tolerant cotton MON 88701 (Petition #12-185-01p). None of the information on this subject has changed in any substantive way and, therefore, Section VIII.H.1. (pages 228 to 230) of that petition is incorporated here by reference (Monsanto Company, 2012).

Volunteer cotton refers to plants that have germinated, emerged and established unintentionally from the previous year’s cotton crop (Roberts et al., 2002). Volunteer cotton plants generally come from seed that falls to the ground as a result of preharvest losses due to adverse weather condition or losses during the harvesting operation.

Volunteer cotton will compete with the rotational crop and potentially cause yield loss and act as early host plants for pests such as spider mites and aphids (Roberts et al., 2002). An integrated weed management system of tillage and herbicides has traditionally been a common method of volunteer cotton control (Alford et al., 2002; Murdock et al., 2002; Roberts et al., 2002).

MON 88702 cotton is agronomically, phenotypically, and compositionally comparable to conventional cotton. Additionally, the seed dormancy data did not show a notable change in dormancy, indicating that MON 88702 cotton does not have an increased volunteer potential. Therefore, the introduction of MON 88702 in the U.S. cotton production system is not expected to impact the management of cotton volunteer plants in rotational crops, including the major rotational crops such as corn, soybean, sorghum, and wheat.

VIII.K. Stewardship of MON 88702 Cotton

Monsanto develops effective biotechnology-derived crop products and technologies and is committed to assuring that its products and technologies are safe, efficacious and used in an environmentally responsible manner. Monsanto demonstrates this commitment by implementing product stewardship processes throughout the lifecycle of a product and by participation in the Excellence Through Stewardship^{SM} (ETS) Program (BIO, 2010). These policies and practices include rigorous field compliance and quality management systems and verification through auditing. Monsanto's Stewardship Principles are also articulated in Technology Use Guides (Bayer, 2019) and Monsanto Technology Stewardship Agreements that are signed by growers who utilize Monsanto branded traits, to ensure stewardship compliance.

As an integral action of fulfilling this stewardship commitment, Monsanto will seek biotechnology regulatory approvals for MON 88702 cotton in all important cotton import countries with a functioning regulatory system to assure global compliance and support the flow of international trade in cotton and cotton by-products. These actions will be consistent with the Biotechnology Industry Organization (BIO) Policy on Product Launches (BIO, 2010). Monsanto continues to monitor other countries that are key importers of cotton from the U.S., for the development of formal biotechnology approval processes. If new functioning regulatory processes are developed, Monsanto will make appropriate and timely regulatory submissions. In addition, Monsanto actively interacts with and participates in cotton industry groups, such as the National Cotton Council, state grower boards, Farm Bureau, Cotton Inc., and trade affiliates, to obtain input on market trends to ensure awareness of the current key markets for whole cottonseed and cottonseed by-products.

Monsanto also commits to industry best practices on seed quality assurance and control to ensure the purity and integrity of MON 88702 cottonseed. As with all of Monsanto's products, before commercializing MON 88702 cotton in the U.S., a MON 88702 detection method will be made available to cotton producers, processors, and buyers.

VIII.L. Impact of the Introduction of MON 88702 Cotton on Agronomic Practices

Cotton fields are typically highly managed agricultural areas that are dedicated to crop production. MON 88702 cotton is anticipated to be cultivated in common crop rotation patterns on land previously used for agricultural purposes. Certified seed production will continue to use well-established industry practices to deliver high quality seed containing MON 88702 to growers. While MON 88702 cotton offers protection against feeding damage caused by targeted hemipteran and thysanopteran insect pests, more specifically two species of tarnished plant bugs (*Lygus hesperus* and *Lygus lineolaris*) and thrips (*Frankliniella* spp.), growers are still anticipated to incorporate current methods of crop protection into their overall insect pest management program. In this regard, cultivation of MON 88702 cotton is not expected to differ from current cotton cultivation, with regards to cotton management practices, except for the potential of a reduced number of crop protection (i.e., insecticides) applications due to the additional control of targeted hemipteran and thysanopteran insect pests. Thus, no significant change in agronomic practices or land use is anticipated to occur with the cultivation of MON 88702 cotton.

MON 88702 cotton is similar to conventional cotton in its agronomic, phenotypic, and compositional characteristics, and has susceptibility to damage by non-target insect pests and diseases comparable to conventional cotton. Based on this assessment, the introduction of MON 88702 cotton is not likely to impact current U.S. cotton agronomic or cultivation practices, other than the intended insect-protection benefits from targeted hemipteran and thysanopteran insect pests.

IX. PLANT PEST ASSESSMENT

IX.A. Introduction

This section provides a brief review and assessment of the plant pest potential of MON 88702 cotton and its impact on agronomic practices and the environment. USDA-APHIS has responsibility, under the Plant Protection Act (PPA) (7 U.S.C. § 7701-7772), to prevent the introduction and dissemination of plant pests into the U.S. Regulation 7 CFR § 340.6 provides that an applicant may petition APHIS to evaluate submitted data to determine that a particular regulated article does not present a plant pest risk and should no longer be regulated. If APHIS determines that the regulated article is unlikely to pose a plant pest risk, the petition is granted, thereby allowing unrestricted introduction of the article.

According to the PPA, the definition of "plant pest" is the living stage of any of the following that can directly or indirectly injure, damage, or cause disease in any plant or plant product: (A) a protozoan; (B) a nonhuman animal; (C) a parasitic plant; (D) a bacterium; (E) a fungus; (F) a virus or viroid; (G) an infectious agent or other pathogens; or (H) any article similar to or allied with any of the articles specified in the preceding subparagraphs (7 U.S.C. § 7702[14]). The regulatory endpoint under the PPA for biotechnology-derived crop products is not zero risk, but rather a determination that deregulation of the article in question is not expected to pose a plant pest risk. Information in this petition related to plant pest risk characteristics includes: 1) mode of action 2) composition; 3) expression and characteristics of the gene product; 4) potential for weediness of the regulated article; 5) impacts to NTOs; 6) disease and pest susceptibilities; 7) impacts on agronomic practices; and 8) impacts on the weediness of any other plant with which it can interbreed, as well as the potential for gene flow. Using the assessment above, the data and analysis presented in this petition lead to a conclusion that MON 88702 cotton is not expected to be a plant pest, and therefore should no longer be subject to regulation under 7 CFR § 340.

IX.B. Plant Pest Assessment of MON 88702 and the Expressed mCry51Aa2 Protein

IX.B.1. Characteristics of the Genetic Insert

As described in Section IV.D, MON 88702 cotton was developed by *Agrobacterium*-mediated transformation of cottonseed embryos using plasmid vector PV-GHIR508523. Characterization of the DNA insert in MON 88702 was conducted using a combination of sequencing, PCR, and bioinformatics methods. The results of this characterization demonstrate that MON 88702 contains one copy of the intended transfer DNA (T-DNA) containing the *mCry51Aa2* expression cassette that is stably integrated at a single locus and is inherited according to Mendelian principles over multiple breeding generations. These methods also confirmed that no vector backbone or other unintended plasmid sequences are present in MON 88702. Additionally, the genomic organization at the insertion site was assessed by comparing the sequences flanking the T-DNA insert in MON 88702 to the sequence of the insertion site in conventional cottonseed. This

analysis determined that no major DNA rearrangement occurred at the insertion site in MON 88702 upon DNA integration.

IX.B.2. Characteristics of the Expressed Protein

IX.B.2.1. Mode of Action of mCry51Aa2

The mode of action for mCry51Aa2 has been well assessed, and follows the same general steps as other *Bt* insecticidal proteins currently in commercial use for insect crop protection (Jerga et al., 2016). The uptake, activation, receptor binding, and pore formation steps that lead to insect toxicity are conserved in mCry51Aa2. The receptor binding of mCry51Aa2 confers specificity to the targeted hemipteran (*Lygus hesperus* and *Lygus lineolaris*) and thysanopteran (*Frankliniella* spp.) insect pests.

IX.B.2.2. mCry51Aa2 Expression Levels

Expression levels of the mCry51Aa2 protein in MON 88702 cotton were determined in different plant tissue types from trials conducted in 2015 and 2018 in the U.S. (Section V.A). OSL1, OSL4, root, pollen and seed tissues were analyzed from the 2015 field trials (Table V-1), while additional development stages of leaf and square tissues (OSL1, OSL2, OSL3, OSL4, Square1, Square2, Square3 and Square4) and pollen were analyzed in 2018 (Table V-2). The expression levels measured in common tissues were comparable amongst both growing seasons (Figure V-1).

IX.B.2.3. Safety of the mCry51Aa2 Protein Expressed in MON 88702

The mCry51Aa2 protein expressed by MON 88702 cotton is a PIP regulated by the U.S. Environmental Protection Agency (EPA). The data for the safety assessment of mCry51Aa2 and MON 88702 were provided to EPA to support the establishment of an exemption from tolerance for residues of mCry51Aa2 in food and feed. These data demonstrate the history of safe use of the *B. thuringiensis* donor organism, the absence of significant structural similarity of mCry51Aa2 to known allergens and toxins, that mCry51Aa2 constitutes a very small portion of the protein present in grain or other processed food and feed fractions from MON 88702, the rapid degradation of mCry51Aa2 in pepsin and pancreatin, the lack of stability to heat treatment, and the absence of oral toxicity in mice.

The weight-of-evidence of these analyses indicates that mCry51Aa2 would not pose a food or feed safety concern. This was documented within the conclusions of the EPA assessment, which expressly indicated that ”*there is a reasonable certainty that no harm will result to the U.S. population, including infants and children, from aggregate exposure to residues of the Cry51Aa2.834_16 protein derived from* Bacillus thuringiensis”. A permanent exemption from the requirement of a tolerance was granted by the EPA based on the safety data submitted (U.S. EPA, 2018a).

IX.B.3. Impact on Non-target Organisms, Including Those Beneficial to Agriculture

Conservative exposure scenarios were estimated using the 95th percentile expression values for fresh weight derived from the highest values reported per relevant tissues across both years of field data collected (Section V.A and Section V.B.3). These conservative values for NTO ecological exposure to establish the environmental exposure concentration (EEC) was then applied to margin of exposure (MOE) calculations to add an additional margin of safety (Section V.B.5).

For the NTO assessment of MON 88702, the mode of action, spectrum of insecticidal activity, and exposure levels to the mCry51Aa2 protein produced in MON 88702 cotton, were described to meet the requirements of 7 CFR Part 340.6 for the evaluation of plant pest potential. The information is presented in Section V.B.2 through Section V.B.5.

The activity spectrum of the mCry51Aa2 protein was described in Section V.B.4. As presented, the rationale for species selected for testing in this assessment follows commonly established practices as described in the literature (Raybould, 2006; Romeis et al., 2013; USDA-APHIS and EPA, 2007). Based on the results, activity was identified against the target species in the insect orders of the Hemiptera (*L. hesperus*, and *L. lineolaris*) and Thysanoptera (thrips (*Frankliniella* spp.)). Activity against the hemipteran pest species *P. seriatus* was detected in a caged field study, however, this activity was not consistently observed within larger-scale field trials and has not been confirmed at a commercial level. Activity spectrum testing also revealed impacts on the hemipteran species *O. insidiosus* and two distantly related coleopteran species (*L. decemlineata* and *D. u. howardi*). In the case of these three species, an LC_{50} could not be reached at the levels tested. Activity outside of the target pest species activity is not uncommon and, as characterized, considered low as defined in the literature (van Frankenhuyzen, 2013). Additionally, the activity spectrum assessment showed no activity of the mCry51Aa2 protein against other herbivores *E. heros, D. v. virgifera*, *E. varivestis, S. frugiperda*, *H. zea*, *O. nubilalis* and *P. xylostella*, the dipteran species *A. aegyptii*, and beneficial arthropods *C. maculata, A. mellifera, P. foveolatus, F. candida* and *E. andrei*. Therefore, the activity spectrum assays demonstrate that mCry51Aa2 has specific, primary activity against *L. hesperus*, *L. lineolaris* and *Frankliniella* spp. The two coleopteran species where activity was observed against (*L. decemlineata* and *D. u. howardi*) are pests but are not commonly present in the cotton agro-ecosystem, therefore their exposure to MON 88702 would be negligible. Other Coleoptera tested showed no activity of mCry51Aa2, indicating that MON 88702 would not pose a risk to species from this insect order. The impact observed on *O. insidiosus* was further explored using the standard approach for tiered NTO testing (Section V.B.5.1.2).

The use of a tiered approach allowed for the assessment of any potential impact on NTOs within different levels or "tiers" that progressed from worst-case exposure scenarios to increasingly more realistic exposure scenarios, as refinement if the earlier tiered tests failed to indicate adequate certainty of acceptable risk. The lowest tier 1 studies represent a worst-case exposure scenario to estimate hazard using an exposure pathway that is usually not realistic. The EPA has established that an endpoint of 50% mortality is to be

used as a trigger for additional higher tier testing (U.S. EPA, 2010b). In the tiered NTO assessment several species were selected for initial tier 1 testing, based on the results from the activity spectrum assessment and following common practices as described in policy documents and peer-reviewed literature (Dutton et al., 2003; Romeis et al., 2013; USDA-APHIS and EPA, 2007; Wach et al., 2016).

Considering the activity of the mCry51Aa2 protein observed against *O. insidiosus*, the panel of species for tier 1 testing included this species in order to further characterize the hazard and establish a NOEC; which was determined to be 13 µg/g for five-day old *O. insidiosus* nymphs. This resulted in an MOE of 5.4 under the relevant EEC of pollen expression levels, which is above the thresholds established for acceptable margins of safety (Table V-16, Figure V-5). Considering the importance of *Orius* spp. as a predator in the cotton agro-ecosystem, a conservative approach was taken to further test this NTO in higher tiered tests to ensure that MON 88702 does not pose a risk to *Orius* spp.

First, higher order tier 2 studies were conducted to further assess the impact of mCry51Aa2 on *Orius* spp. from a more ecologically relevant perspective considering the predatory function of *Orius* spp. Feeding studies using non-susceptible prey are common practice in risk assessment (Naranjo, 2009; Romeis et al., 2006; USDA-APHIS and EPA, 2007) and in a first study, *S. frugiperda* (fall armyworm (FAW)) was chosen as the prey species. In spite of being fed high concentrations of the mCry51Aa2 in a direct feeding assay, the concentration of the protein in FAW did not reach levels above the NOEC and, as expected, did not impact the survivability of five-day old *O. insidiosus* nymphs (Table V-16). Follow-up studies used spider mites as prey, since they have been described as a species that can contain high levels of Cry protein after feeding on *Bt* crops (Torres and Ruberson, 2008). These tri-trophic feeding studies were conducted with one-day old *Orius* spp. nymphs and MON 88702- and DP393-fed spider mites as prey on MON 88702 and DP393 leaf disks, respectively. In this scenario, a higher potential for trophic transfer of the mCry51Aa2 protein to the prey was observed resulting in higher *Orius* spp. exposure to the protein. The results demonstrated a significant decrease in survival of the one-day old *Orius* spp. nymphs in the MON 88702 spider mite/leaf treatment, whereas the same experiment with five-day old *Orius* spp. nymphs showed no effects on survival although significant sublethal effects were apparent. This experiment also demonstrated higher sensitivity of younger *Orius* spp. nymphs (Section V.B.5.1.2.2, Table V-8, Figure V-5, Appendix I.2).

Considering these results, a tier 3 study was conducted to assess the impact of MON 88702 on the more sensitive, one-day old *O. insidiosus* nymphs under further refined exposure conditions that are more representative of a field scenario. Within this study, one-day old *O. insidiosus* nymphs were exposed to MON 88702-fed spider mites as prey on MON 88702 leaf disks and *E. kuehniella* eggs, which were introduced as an alternative prey. Under these conditions where *O. insidiosus* had a food choice, there were no significant differences in one-day old nymph survival between the MON 88702- and conventional control DP393-exposed groups, nor were there any differences in development (Section V.B.5.1.2.3; Table V-8, Appendix I.3). This indicates that under more realistic exposure scenarios, where *O. insidiosus* has the choice to feed on a combination of different types of abundant prey and plant tissue, MON 88702 does not

impact *O. insidiosus* development or survival (Figure V-5). This final tier 3 assay, which is the closest approximation to a field scenario under controlled conditions, clearly indicates that, due to their feeding ecology of being a generalist predator, *Orius* spp. is not expected to encounter levels of the mCry51Aa2 protein in the field that would result in an adverse effect.

As an added measure of conservative assessment of the potential risk of MON 88702 to *Orius* spp., as one of the major predators in the cotton agro-ecosystem, a tier 4 field study was conducted to assess the most realistic exposure scenario; representing the conditions encountered in commercial cotton cultivation. This study was set up at six locations in the U.S. cotton belt during the 2018 growing season (Section V.B.5.1.2.4). No significant differences were observed in *Orius* spp. nymph, adult or total abundance in MON 88702 compared to conventional control DP393 plots. Importantly, also no significant difference in their abundance was observed at locations where spider mites were abundant. The results from the study confirm that MON 88702 is not expected to have any adverse effect on *Orius* spp. populations in the cotton agro-ecosystem and that MON 88702 is unlikely to pose a risk to this species (Figure V-5).

Additional studies were conducted with other predatory Hemiptera that are present in the cotton agro-ecosystem and taxonomically closely related to *Orius* spp., namely: *Geocoris punctipes*, *Nabis alternatus* and *Zelus renardii*. Results of tier 1 tests, direct feeding assays, established that no effect on survival was observed in one-day old nymphs of these three species fed at 4000 µg mCry51Aa2/g diet, resulting in an MOE of ≥5.2 under the conservative EEC of square expression levels (Table V-16, Figure V-5, Section V.B.5.1.2.7).

At the tested concentrations of 400 and 4000 µg mCry51Aa2/g diet, the development time from nymph to adult was significantly increased for all species. At the highest concentration of 4000 mCry51Aa2/g diet, a significant decrease in adult biomass was observed for *N. alternatus* and *Z. renardii*, although this decrease was no longer observed for *N. alternatus* at a concentration of 400 mCry51Aa2/g diet. The tier 1 test represented a worst-case, high concentration, chronic and obligate feeding on MON 88702 plant tissue by *G. punctipes*, *N. alternatus* and *Z. renardii*. Considering their role as predators in the cotton agro-ecosystem, under field conditions, the exposure to the mCry51Aa2 protein would primarily happen through feeding on a variety of herbivorous, and in the case of *Z. renardii* also predatory, prey items that contain orders of magnitude less mCry51Aa2 protein than those found in leaf or square tissue (Section V.B.5.1.2). Consequently, MON 88702 is not expected to have any adverse effects on their abundance in the cotton agro-ecosystem.

To assess any impact from MON 88702 on predatory Hemiptera under realistic and relevant environmental exposure conditions, populations of *Geocoris* spp., *Nabis* spp. and *Zelus* spp. were monitored within the tier 4 field study. While the separate *Nabis* spp. nymph and adult abundance, as well as the adult *Zelus* spp. abundance was too low to conduct a statistical analysis, no differences were observed in the abundance of *Geocoris* spp. nymph, adult or total abundance, the *Nabis* spp. total abundance, or *Zelus* spp nymphs or total abundance between MON 88702 and conventional control DP393 plots, indicating no trait-related effect on these species. *Zelus* spp. is typically a

less abundant predator in cotton fields, where *Orius* spp., *Geocoris* spp., ladybird beetles and lacewings are considered the major contributors to predation and consequently the biological control function. This was also reflected in the tier 4 field study collections, where *Zelus* spp. abundance and consequently the power to detect a significant difference was low. Therefore, *Zelus* spp. is not a major contributor to the predation function, and ultimately biological control, in the majority of the cotton agro-ecosystem of the U.S. (Asiimwe et al., 2014; Torres and Ruerson, 2005).

Based on the comprehensive dataset developed for *Orius* spp. and other predatory Hemiptera, MON 88702 is not expected to pose a risk to any of the predatory Hemiptera tested when considering field exposure scenarios, nor to the overall predatory function in the cotton agro-ecosystem (Figure V-5).

In the order Coleoptera, the initial activity spectrum assays showed activity of the mCry51Aa2 protein on two species (*L. decemlineata* and *D. u. howardi*), both from the same family (Chrysomelidae). The two species that were impacted are known pests, although not in cotton fields. However, in the same assessment, additional Coleoptera were tested and found not to be impacted by MON 88702 or the mCry51Aa2 protein. More specifically, no activity was detected against the closely related species *Diabrotica virgifera* within the same genus and/or family (Chrysomelidae), or against Coccinellidae when both a herbivore (*Epilachna varivestis)* and a predator (*Coleomegilla maculata*) were tested at 400 µg/mL of diet (Table V-7), indicating that the activity of the mCry51Aa2 protein was limited to *L. decemlineata* and *D. u. howardi*.

Additional coleopteran species representing key ecosystem functions within the cotton agro-ecosystem present in cotton fields were tested in the tier 1 NTO assessment; (*Coccinella septempunctata* (Coccinellidae)) and *Aleochara bilineata* (Staphylinidae)) (Section V.B.5.1.2.9). No impact on either species was observed and, using the pollen and leaf expression levels as EEC, MOEs of ≥1041.7 and ≥5.1 were determined for *C. septempunctata* and *A. bilineata*, respectively (Table V-16) providing margins of safety well above guidance thresholds (U.S. EPA, 2010a; b; USDA-APHIS and EPA, 2007).

Since risk is a function of hazard and exposure, it is critical to take into consideration the potential routes and levels of exposure for non-target coleopteran pest species. In 2016, the major cotton losses caused by insects in the U.S. were caused by infestations of fields with Lygus, stink bugs, thrips, fleahopper, bollworm/budworm and spider mites (Table VIII-2). From these data, it can be concluded that Coleoptera are not an important insect pest in cotton fields and therefore exposure of MON 88702 to this order of insects will be limited; the beneficial coleopteran predators that were tested were not impacted by the mCry51Aa2 protein. Importantly, the two species to which the mCry51Aa2 protein demonstrated activity against in the activity spectrum assessment are not present in the list of most common pests in U.S. cotton fields. As also described by the USDA-EPA (USDA-APHIS and EPA, 2007), "*it is impossible to test all species that are potentially present…*". Following this approach and taking into consideration the aspect of economical relevance, no additional coleopteran species were tested in the NTO assessment.

These conclusions are further supported by the field environmental interaction assessment data (Section VII.C.2.2.2), a census study in which the abundance of arthropods typically present in cotton fields was monitored. Seasonal abundance of NTOs across several sites in the U.S. cotton belt were not different in MON 88702 compared to conventional cotton, confirming that MON 88702 is unlikely to have an adverse effect on NTOs under realistic field exposure scenarios.

An overview of the hemipteran and coleopteran species tested in all assessments and the functional group they represent is given in Table IX-1 and Table IX-2, respectively. The weight of evidence, especially under the most environmentally relevant conditions, therefore, demonstrates that MON 88702 cotton will not negatively impact NTOs, including those insects that are beneficial to agriculture, vertebrates and mammals, and is unlikely to pose a risk to NTOs.

Table IX-1. Overview of Hemipteran Species Tested in the Activity Spectrum, Tiered NTO and Environmental Interaction Assessments

Species	Family	Study Type	Effect Detected	Functional Group
Whiteflies	Aleyrodidae	Qualitative EI	N	Herbivore
		Quantitative EI	N	
		Tier 4	N	
Orius insidiosus	Anthocoridae	Activity spectrum	Y	Predator
		NTO tier 1 (five-day old nymphs)	N[1]	
		NTO tier 2 (FAW prey, five-day old nymphs)	N	
		NTO tier 2 (spider mite prey, one-day old nymphs)	Y	
		NTO tier 3	N	
		NTO tier 4	N	
Orius majusculus	Anthocoridae	NTO tier 2 (spider mite prey, one-day old nymphs)	Y	Predator
		NTO tier 3	N	
Orius tristicolor	Anthocoridae	NTO tier 4	N	Predator
		Quantitative EI	N	
Aphids	Aphididae	Qualitative EI	N	Herbivore
		Quantitative EI	N	
		NTO tier 4	N	
Leafhoppers	Cicadellidae	Quantitative EI	N	Herbivores
Geocoris spp. Big-eyed bugs	Geocoridae	NTO tier 1	N[2]	Predator
		NTO tier 4	N	
		Quantitative EI	N	
Lygus hesperus Western Tarnished Plant Bug	Miridae	Activity spectrum	Y	Herbivore (Target pest)
		NTO tier 4	Y	
Lygus lineolaris Tarnished Plant Bug	Miridae	Activity spectrum	Y	Herbivore (Target pest)
		NTO tier 4	Y	
Pseudatomoscelis seriatus Cotton Fleahopper	Miridae	Activity spectrum	Y	Herbivore
		NTO tier 4	N[3]	
Damsel bugs	Nabidae	NTO tier 1	N[2]	Predator
		NTO tier 4	N	
		Quantitative EI	N	

Table IX-1. Overview of Hemipteran Species Tested in the Activity Spectrum, Tiered NTO and Environmental Interaction Assessments (continued)

Species	Family	Study Type	Effect Detected	Functional Group
Stink bugs	Pentatomidae	Qualitative EI	N	Herbivore
		Quantitative EI	N	
		Activity spectrum	N	
Assassin bugs	Reduviidae	NTO tier 1	N[2]	Predator
		NTO tier 4	N	
		Quantitative EI	N	

[1] No effects were observed at an mCry51Aa2 concentration below the NOEC (13 µg/g).
[2] This study indicated there was no impact on survival, though a delay in development time and adult biomass was observed.
[3] No consistent effect of MON 88702 on *P. seriatus* abundance was observed.

Table IX-2. Overview of Coleopteran Species Tested in the Activity Spectrum, Tiered NTO and Environmental Interaction Assessments

Species	Family	Study Type	Effect Detected	Functional Group
Ant-like Flower Beetles	Anthicidae	Quantitative EI	N	Predator
Colaspis brunnea Grape colaspis	Chrysomelidae	Qualitative EI Quantitative EI	N N	Herbivore
Leptinotarsa decemlineata Colorado Potato Beetle	Chrysomelidae	Activity spectrum	Y	Herbivore
Diabrotica virgifera Western Corn Rootworm	Chrysomelidae	Activity spectrum	N	Herbivore
Diabrotica undecimpunctata howardi Southern Corn Rootworm	Chrysomelidae	Activity spectrum	Y	Herbivore
Phyllotreta spp. Striped Flea Beetles	Chrysomelidae	Qualitative EI	N	Herbivore
Coleomegilla maculata Pink Spotted Lady Beetle	Coccinellidae	Activity spectrum	N	Predator
Epilachna varivestis Mexican Bean Beetle	Coccinellidae	Activity spectrum	N	Herbivore
Coccinella septempunctata Lady Beetle	Coccinellidae	NTO tier 1	N	Predator
Ladybird Beetles	Coccinellidae	Quantitative EI	N	Predator
Click Beetles	Elateridae	Quantitative EI	N	Herbivore
Popillia japonica Japanese Beetles	Scarabaeidae	Qualitative EI	N	Herbivore
Aleochara bilineata Rove Beetle	Staphylinidae	NTO tier 1	N	Predator

IX.B.3.1. Environmental Fate of mCry51Aa2 Expressed in MON 88702 Cotton

Soil organisms may be exposed to the mCry51Aa2 protein from MON 88702 cotton by contact with roots, or with above-ground plant biomass deposited on the soil or tilled into the soil. In addition, feeding on living or dead crop biomass or ingesting or absorbing the Cry proteins after their release into the soil may represent a route of exposure to soil-dwelling organisms. Results presented here demonstrated that the mCry51Aa2 protein expressed in MON 88702 cotton dissipated rapidly in different representative agricultural soils resulting in a maximum estimated DT_{50} of 4.7 days, and a maximum estimated DT_{90} of 74.5 days (Section V.B.6).

Commercial experience with the cultivation of biotechnology-derived insect protected crops expressing Cry proteins for more than 15 years, as well as the results cited here, indicate that the mCry51Aa2 protein produced in MON 88702 cotton will not persist or accumulate under cotton production conditions, thereby indicating negligible exposure to NTOs and persistence in the environment.

IX.B.4. Impact on Threatened and Endangered Species

As discussed in Sections V.B.4 and V.B.5, evaluations with the mCry51Aa2 protein were conducted assessing its activity against a range of target and non-target arthropods. The information used in these evaluations demonstrated that the mCry51Aa2 protein is specific with primary insecticidal activity against targeted insects in the orders Hemiptera and Thysanoptera, and secondary activity observed against a limited number of coleopteran insects (Bachman et al., 2017).

Because of the activity spectrum of the mCry51Aa2 protein, assessment of any impacts on endangered species is restricted to the orders Hemiptera, Thysanoptera and Coleoptera. Currently, there are no listed Thysanoptera on the threatened and endangered species list (USFWS, 2019a). There is one listed hemipteran species, the threatened Ash Meadows Naucorid (*Ambrysus amargosus*) and 19 listed members of the Coleoptera (USFWS, 2019a).

For the single listed hemipteran species, the threatened Ash Meadows Naucorid (*Ambrysus amargosus*) occurs in a single county in Nevada and no cotton cultivation occurs in this county, nor this state (USDA-NASS, 2017; USFWS, 2019a). Therefore, no exposure to the mCry51Aa2 protein is anticipated which means no risk exists for the Ash Meadows Naucorid from the cultivation of MON 88702 cotton.

Currently, there are 19 listed Coleoptera on the threatened and endangered species list (Table IX-3) (USFWS, 2019a). Of these 19 species, 14 occur in counties where cotton is grown. Six of the 14 listed coleopteran species that may be present in cotton producing counties live in subterranean environments where exposure to the cotton plants cultivated in general, and specifically MON 88702, will not occur (Table IX-3). The remaining eight species are present in either terrestrial or aquatic habitats and they are habitat specialists that are largely excluded from agricultural areas due to their specific habitat requirements (Table IX-3).

As discussed in Sections V.B.4 and V.B.5, the activity of the mCry51Aa2 protein produced by MON 88702 cotton has only been observed in two species in the family Chrysomelidae out of the many coleopterans tested. None of the 19 listed threatened and endangered coleopteran species are members of the family Chrysomelidae (Table IX-3). Further testing of additional coleopteran species in the family Chrysomelidae and the families of the Scarabaeidae, Coccinellidae, Staphylinidae, Elateridae, and Anthicidae did not demonstrate any activity of the mCry51Aa2 protein (Section V.B.4). The phylogenetically closest listed species, the Valley Elderberry Longhorn Beetle (*Desmocerus californicus dimorphus)*, while a member of same Superfamily as the Chrysomelidae (Chrysomelidae), resides within the taxonomically distinct Cerambicidae family (Hunt et al., 2007). In addition to being outside of the Chrysomelidae family, the Valley Elderberry Longhorn Beetle has an obligate relationship with its host, the elderberry (*Sambucus* spp.) that is associated with riparian forests along rivers and streams (USFWS, 2006b). Therefore, the specific habitat requirements would greatly limit any potential exposure to MON 88702 cotton (Table IX-3) (USFWS, 2006b).

Aquatic threatened and endangered beetles will not encounter MON 88702 cotton tissues, as none of these endangered beetles will occur in or near cotton fields and the possibility of movement of the mCry51Aa2 protein to these habitats is negligible. Cotton pollen is known to be "sticky" (i.e., to form clumps) and too heavy to be dispersed by wind, therefore little pollen is expected to move off field and provide a potential exposure route for listed species. Previously, the U.S. EPA has determined that only small amounts of cotton plant matter are expected to move off field and enter aquatic systems. Additionally, the high potential for proteins to adsorb to soil, and rapid degradation of soluble free protein in the environment (Section V.B.6) would severely limit the entry of this protein into the aquatic environment. Therefore, exposure of listed aquatic beetles to the mCry51Aa2 protein through erosion of soil containing bound protein, surface runoff containing soluble protein, aerial deposition of pollen, and aerial deposition of crop dust can largely be ruled out. Therefore, exposure to mCry51Aa2 is not expected to occur and MON 88702 does not pose a risk to these aquatic beetle species.

An analysis of the county level distributions of terrestrial threatened or endangered coleopteran species indicates that the potential concern regarding range overlap with cotton production is restricted to a single species, the American burying beetle (*Nicrophorus americanus*, Silphidae). The American burying beetle is the largest carrion beetle in North America (USFWS, 1991) and is only found in limited areas encompassing parts of ten[4] states, including the cotton growing states of Arkansas, Kansas, Oklahoma, and Texas (USFWS, 2019b). Adults feed on carrion and occasionally other insects (USFWS, 1991), while larvae feed exclusively on buried carrion or carrion regurgitations provided by their parents (U.S. EPA, 2010b; USFWS, 2008). The American burying beetle's habitat is variable and includes mature forests, shrub-covered areas, some grassland habitats and the beetle's preferred habitat has been correlated with an

[4] An experimental population that is listed as non-essential exists in Missouri .

abundance of small vertebrate biomass (USFWS, 2008). Considering that both larvae and adult beetles are carrion feeders, exposure to the mCry51Aa2 protein produced by MON 88702 cotton is highly unlikely due to their feeding ecology. Therefore, exposure to mCry51Aa2 is not expected and MON 88702 does not pose a risk to the American burying beetle.

Based on the current assessment, the cultivation of MON 88702 cotton, which provides protection against feeding damage in cotton from targeted hemipteran and thysanopteran insect pests through expression of the mCry51Aa2 protein, will have no adverse effect on listed threatened and endangered hemipteran and coleopteran species or their critical habitat. This conclusion is supported by: (1) endangered hemipteran and coleopteran species are largely excluded from cotton agricultural areas due to their specific habitat requirements, (2) the specificity of mCry51Aa2 protein for specific coleopteran species further limits potential risk, and (3) where listed species and cotton production overlap, exposure via consumption of MON 88702 cotton tissue is not anticipated due to specific food requirements and feeding ecology of the listed species and lack of off-field movement of cotton tissues.

Finally, as part of the registration decision on mCry51Aa2 expressed in MON 88702, the EPA assessed the possible effect of mCry51Aa2 on threatened and endangered species in these three insect orders (U.S. EPA, 2018b). The agency conducted proximity analysis for the species identified and made a “no effects” determination for the hemipteran and coleopteran species listed as threatened and endangered, and their designated critical habitats (U.S. EPA, 2018b).

Table IX-3. Threatened or Endangered Hemipteran and Coleopteran Species and Their Habitats and Presence in Cotton Production Counties[1]

Common Name	Species Name	Family	Habitat	Feeding Ecology	Counties with Cotton (per Ag Census)[2]	Exposure to MON 88702
Ash Meadows Naucorid	*Ambrysus amargosus*	Naucoridae	Aquatic. Endemic to the Ash Meadows habitat in a National Wildlife Refuge, NV; extremely restricted habitat to Point of Rocks Springs; likely to feed upon insect larvae. (USFWS, 1990)	Predator. Likely to feed upon insect larvae.(USFWS, 1990)	Counties: 0 No cotton grown in state (NV) where found	**No exposure** to MON 88702 because there is no cotton cultivation where species is present.
American burying beetle	*Nicrophorus americanus*	Silphidae	Terrestrial. Variable including oak-hickory forest and grasslands and forest/pasture ecotone and open pasture. (USFWS (1991))	Carrion Feeder. Small carrion (birds and small mammals) and live insects. (USFWS (1991))	Counties: 13 AR: Miller KS: Elk OK: Bryan, Garvin, Kay, McClain, McCurtain, Muskogee, Payne TX: Bowie, Kleberg, Lamar, Red River	**No exposure** to MON 88702 expected due to specific species habitat requirements and feeding ecology.
Casey's June Beetle	*Dinacoma caseyi*	Scarabidae	Terrestrial. Associated with native Sonoran (Coloradan) desert vegetation located on desert alluvial fans and bajadas (compound alluvial fans) at the base of the San Jacinto Mountains. These areas include sandy dry washes with ephemeral flow, and dry upland areas associated with soil deposition from extreme flood events. Habitat with minimal perturbations is preferred. (USFWS, 2007)	Herbivore/Detritivore. Likely plant roots or plant detritus and associated decay organisms.(USFWS, 2007)	Counties: 1 CA: Riverside	**No exposure** to MON 88702 expected due to specific species habitat requirements.
Coffin Cave mold beetle	*Batrisodes texanus*	Staphylinidae	Terrestrial cave dweller. Caves and mesocavernous voids in karst limestone (a terrain characterized by landforms and subsurface features, such as sinkholes and caves) in Williamson County, TX. (USFWS, 2009a)	Detritivore/Omnivore. Leaf litter fallen or washed in, animal droppings, and animal carcasses. (USFWS, 2009a)	Counties: 1 TX: Williamson	**No exposure** to MON 88702 due to subterranean habitat.

Table IX-3. Threatened or Endangered Hemipteran and Coleopteran Species and Their Habitats and Presence in Cotton Production Counties (continued)

Common Name	Species Name	Family	Habitat	Feeding Ecology	Counties with Cotton (per Ag Census)[2]	Exposure to MON 88702
Comal Springs dryopid beetle	*Stygoparnus comalensis*	Dryopidae	Aquatic. Subterranean and inhabits air-filled voids in springs. Restricted in distribution to spring sites in Comal and Hays counties, TX. . (USFWS, 1997)	Not well defined	Counties: 5 TX: Bexar, Blanco, Comal, Hays, Kendall	**No exposure** to MON 88702 expected due to subterranean habitat and being an aquatic species.
Comal Springs riffle beetle	*Heterelmis comalensis*	Elmidae	Aquatic. Gravel substrate and shallow riffles. Restricted in distribution to spring sites in Comal and Hays counties, TX (USFWS, 1997)	Not well defined	Counties: 5 TX: Bexar, Blanco, Comal, Hays, Kendall	**No exposure** to MON 88702 expected, aquatic species.
Delta green ground beetle	*Elaphrus viridis*	Carabidae	Terrestrial. Grassland interspersed with vernal pools usually within 1.5 m of the water's edge where soil conditions are very moist and very low growing vegetation provides cover. (USFWS, 2005b)	Predator. Generalized predators that prey upon insects and other small invertebrates. (USFWS, 2005b)	Counties: 3 CA: San Joaquin, Solano, Yolo	**No exposure** to MON 88702 expected due to specific species habitat requirements.
Helotes mold beetle	*Batrisodes venyivi*	Staphylinidae	Terrestrial cave dweller. Caves and mesocavernous voids in karst limestone (a terrain characterized by landforms and subsurface features, such as sinkholes and caves) in Bexar County, TX. (USFWS, 2011)	Detritivore/Omnivore. Nutrient sources include leaf litter fallen or washed in, animal droppings, and animal carcasses.(USFWS, 2011)	Counties: 1 TX: Bexar	**No exposure** to MON 88702 due to subterranean habitat.
Hungerford's crawling water beetle	*Brychius hungerfordi*	Halipidae	Aquatic. Clear cool streams with well-aerated riffle segments, a cobble bottom, an underlying sand substrate, and alkaline water conditions. Often found downstream from culverts, dams, and impoundments.(USFWS, 2006a)	Herbivore. Likely feeds on algae and periphyton. (USFWS, 2006a)	Counties: 0 No cotton grown in state (MI) where found	**No exposure** to MON 88702 because there is no cotton cultivation where species is present.
Kretschmarr Cave mold beetle	*Texamaurops reddelli*	Staphylinidae	Terrestrial cave dweller. Dark zones of caves. (USFWS, 1994b)	Invertivore and thought to be a Predator. NatureServe, 2017)	Counties: 1 TX: Travis	**No exposure** to MON 88702 due to subterranean habitat.

Table IX-3. Threatened or Endangered Hemipteran and Coleopteran Species and Their Habitats and Presence in Cotton Production Counties (continued)

Common Name	Species Name	Family	Habitat	Feeding Ecology	Counties with Cotton (per Ag Census)[2]	Exposure to MON 88702
Miami Tiger Beetle	*Cicindelidia floridana*	Carabidae	Terrestrial. found exclusively in bare or sparsely vegetated sandy areas in pine rockland habitat in Miami-Dade County, Florida. (USFWS, 2017)	Predator. Feeds primarily on ants and other small arthropods. (USFWS, 2017)	Counties: 0 No cotton grown in single county (Miami-Dade, FL) where found	**No exposure** to MON 88702 because there is no cotton cultivation where species is present.
Mount Hermon June Beetle	*Polyphylla barbata*	Scarabidae	Terrestrial and primarily subterranean. Only found in Zayante Sandhills of Santa Cruz, CA. Loose sandy soil with preferred habitat of widely spaced ponderosa pines and barren open-sand understory. (USFWS, 2009b)	Herbivore. Larvae forage on multiple plant species, mostly on subterranean parts. Likely that adults do not feed. (USFWS, 2009b)	Counties: 0 No cotton grown in single county (Santa Cruz, CA) where found	**No exposure** to MON 88702 because there is no cotton cultivation where species is present.
Northeastern beach tiger beetle	*Cicindela dorsalis dorsalis*	Carabidae	Terrestrial. Historically occurred on beaches along the Atlantic Coast, from Cape Cod to central New Jersey, and along Chesapeake Bay beaches in Maryland and Virginia. Currently, only two small populations remain on the Atlantic Coast. (USFWS, 1994a)	Predator. Small amphipods, flies, or other beach arthropods. (USFWS, 1994a)	Counties: 3 VA: Accomack, Northampton, Northumberland,	**No exposure** to MON 88702 expected due to specific species habitat requirements.
Ohlone Tiger beetle	*Cicindela ohlone*	Carabidae	Terrestrial. Coastal terraces supporting patches of native grassland habitat found primarily in Santa Cruz County, CA. (USFWS, 2009c)	Predator. Preys upon a variety of small arthropods. (USFWS, 2009c)	Counties: 1 CA: Santa Clara	**No exposure** to MON 88702 expected due to specific species habitat requirements.
Puritan tiger beetle	*Cicindela puritana*	Carabidae	Terrestrial. Shorelines along the Connecticut River and along the Chesapeake Bay(USFWS, 1993)	Predator. Preys upon a variety of small arthropods(USFWS, 1993)	Counties: 0 No cotton grown in states (CT, VT, MA, NH, MD) where found	**No exposure** to MON 88702 because there is no cotton cultivation where species is present.
Salt Creek tiger beetle	*Cicindela nevadica lincolniana*	Carabidae	Terrestrial. Limited to segments of Little Salt Creek and adjacent remnant saline wetlands in northern Lancaster County, NE. (USFWS, 2016)	Predator. Preys upon a variety of small arthropods. (USFWS, 2016)	Counties: 0 No cotton grown in state (NE) where found	**No exposure** to MON 88702 because there is no cotton cultivation where species is present.

Table IX-3. Threatened or Endangered Hemipteran and Coleopteran Species and Their Habitats and Presence in Cotton Production Counties (continued)

Common Name	Species Name	Family	Habitat	Feeding Ecology	Counties with Cotton (per Ag Census)[2]	Exposure to MON 88702
Tooth Cave ground beetle	*Rhadine persephone*	Carabidae	Terrestrial cave dweller. Dark zones of caves. Found only underground in caves and karst features in Williamson and Travis Counties, TX. (USFWS, 2005a)	Invertivore. Known to feed on cricket eggs. (NatureServe, 2019)	Counties: 2 TX: Travis, Williamson	**No exposure** to MON 88702 due to subterranean habitat.
[unnamed] Ground Beetle	*Rhadine infernalis*	Carabidae	Terrestrial and subterranean. Caves and mesocavernous voids in karst limestone (a terrain characterized by landforms and subsurface features, such as sinkholes and caves) in Bexar County, TX. (USFWS, 2011)	Detritivore/Omnivore. Nutrient sources include leaf litter fallen or washed in, animal droppings, and animal carcasses.(USFWS, 2011)	Counties: 1 TX: Bexar	**No exposure** to MON 88702 due to subterranean habitat.
[unnamed] Ground Beetle	*Rhadine exilis*	Carabidae	Terrestrial and subterranean. Caves and mesocavernous voids in karst limestone (a terrain characterized by landforms and subsurface features, such as sinkholes and caves) in Bexar County, TX. (USFWS, 2011)	Detritivore/Omnivore. Nutrient sources include leaf litter fallen or washed in, animal droppings, and animal carcasses. (USFWS, 2011)	Counties: 1 TX: Bexar	**No exposure** to MON 88702 due to subterranean habitat.
Valley elderberry longhorn beetle	*Desmocerus californicus dimorphus*	Cerambycidae	Terrestrial. Elderberry trees (*Sambucus* spp.) associated with riparian forests along rivers and streams in California's Central Valley.	Herbivore. Obligate feeder on elderberry (*Sambucus* spp.).	Counties: 15 CA: Butte, Colusa, Fresno, Glenn, Kern, Kings, Madera, Merced, San Joaquin, Santa Clara, Solano, Stanislaus, Sutter, Tulare, Yolo	**No exposure** to MON 88702 expected due to specific species habitat requirements.

[1] USFWS, (https://www.fws.gov/endangered/), access date March 23, 2019. Historic (pre-1977) and extirpated locations were not included. The county-level locations for these endangered species were also cross-referenced with NatureServe's Explorer website (http://explorer.natureserve.org/index.htm) accessed on March 23, 2019 and any omitted counties were included.

[2] Counties listing "cotton acres harvested-total". Data source: USDA 2002, 2007 and 2012 Census of Agriculture (http://www.nass.usda.gov/Census_of_Agriculture/index.asp), access date March 23, 2019. No new census data for more recent years were available when accessed on March 23, 2019. State Abbreviations are based on the Official United States Postal Service Abbreviations for States.

IX.C. Compositional Characteristics

The compositional analysis provided a comprehensive, comparative assessment of the levels of key anti-nutrients, including total gossypol, free gossypol, malvalic acid, sterculic acid, and dihydrosterculic acid, which are considered relevant to cottonseed (OECD, 2009) (Section VI). The statistical comparisons of MON 88702 to the conventional control were based on data combined across all field sites. Statistically significant differences were evaluated at the 5% level (α=0.05).

There were no statistically significant differences in the levels of any of the key anti-nutrients analyzed. These results support the overall conclusion that MON 88702 did not meaningfully alter anti-nutrient levels in cottonseed and confirmed the compositional equivalence of MON 88702 to the conventional control in levels of these components. Additional composition data were submitted to FDA (BNF 000160) supporting the conclusion that MON 88702 did not meaningfully alter component levels in cottonseed and confirming the compositional equivalence of MON 88702 to conventional cotton. The FDA therefore agreed with the conclusion that MON 88702 does not raise any safety or regulatory issues with respect to its uses in human or animal food (U.S. FDA, 2018).

IX.D. Phenotypic, Agronomic, and Environmental Interaction Characteristics

An extensive set of comparative plant characterization data were used to assess whether the introduction of the trait altered the plant pest potential of MON 88702 cotton compared to the conventional control (Section VI). Phenotypic, agronomic, and environmental interaction characteristics of MON 88702 cotton were evaluated and compared to those of the conventional control.

No statistically significant differences were detected in the combined-site analysis between MON 88702 cotton and the conventional control for seven of eight phenotypic characteristics: early stand count, days to first flower, final stand count, plant height, total bolls, seed cotton yield, and seed index (Table VII-4). Only one statistically significant difference was detected where MON 88702 had higher first position fruit retention (59.2 vs. 51.6%) compared to the conventional control. However, the mean value for MON 88702 for first position fruit retention was within the respective reference variety range indicating that the difference was not biologically meaningful in terms of increased plant pest potential. No biologically meaningful differences in plant responses to abiotic stress, disease damage, arthropod-related damage, and arthropod abundance were observed during comparative field observations between MON 88702 cotton and the conventional control. A comparative assessment of seed germination and dormancy characteristics was conducted on MON 88702 cotton and the conventional control; ten differences were observed in the seed characteristics, at additional temperature regimes of 10°C and 20°C and alternating 10°C/20°C and 20°C/30°C. In all cases, the mean values for MON 88702 were either within the respective reference ranges included in the study, or, were within the reference range reported from previous dormancy and germination studies for cotton. Finally, no statistically significant differences were detected between

MON 88702 cotton and the conventional control for percent viable pollen, pollen grain diameter, and no visual differences were observed in general pollen morphology.

Therefore, the results from all phenotypic, agronomic, and environmental interaction assessments demonstrated that MON 88702 cotton does not possess weedy characteristics, or increased susceptibility or tolerance to specific diseases, insects, or abiotic stressors compared to the conventional control. Taken together, the results of the analysis support a determination that MON 88702 cotton is no more likely to pose a plant pest risk than conventional cotton.

IX.E. Weediness Potential of MON 88702 Cotton

Cotton is not listed as a weed in the major weed references (Crockett, 1977; Holm et al., 1997), nor is it present on the lists of noxious weed species distributed by the federal government (7 CFR Part 360). The United States Department of Agriculture has previously determined that "cotton is not considered to be a serious, principal or common weed pest in the U.S." (USDA-APHIS, 1995). Commercial *Gossypium* species in the U.S. are not considered weeds and are not effective in invading established ecosystems. Cotton is not considered to have weedy characteristics in the U.S. and does not possess attributes commonly associated with weeds, such as long soil persistence, the ability to invade and become a dominant species in new or diverse landscapes, or the ability to compete well with native vegetation. It is recognized that in some agricultural systems, cotton can volunteer in a subsequent rotational crop. However, volunteers are easily controlled through tillage or use of appropriate herbicides (Alford et al., 2002; Murdock et al., 2002; Roberts et al., 2002).

In comparative studies between MON 88702 cotton and the conventional control, phenotypic, agronomic, and environmental interaction data were evaluated (Section VI) for changes that would impact the plant pest potential and in particular, plant weed potential. Results of these evaluations show that there is no biologically meaningful difference between MON 88702 cotton and the conventional control for characteristics potentially associated with weediness. Furthermore, comparative field observations between MON 88702 cotton and its conventional control in their response to abiotic stressors, such as drought, heat stress, and high winds, indicated no biologically meaningful differences and, therefore, no increased weed potential. Data on environmental interactions also indicate that MON 88702 does not confer any biologically meaningful increased susceptibility or tolerance to specific diseases or insect pests. Collectively, these findings support the conclusion that MON 88702 cotton has no increased weediness compared to commercially cultivated cotton.

Volunteer MON 88702 cotton, like volunteer conventional cotton, would compete poorly with any succeeding crops, making it extremely unlikely to have any prolonged negative effects. Volunteer MON 88702 cotton would also not be difficult to manage because it can be controlled easily with herbicides and other mechanical means (Alford et al., 2002; Murdock et al., 2002; Roberts et al., 2002).

IX.F. Potential for Pollen-Mediated Gene Flow and Introgression

Pollen-mediated gene flow (often referred to as cross-pollination) occurs when pollen of one plant fertilizes ovules of a second sexually-compatible plant. Pollen-mediated gene flow is affected by both biotic and abiotic factors such as plant biology, pollen biology/volume, plant phenology, overlap of flowering times, proximity of the pollen source and sink, ambient conditions such as temperature and humidity, and field architecture. Pollen-mediated gene flow is a natural biological process, and, therefore, does not constitute an environmental risk in and of itself.

Introgression is a process whereby one or more genes successfully incorporate into the genome of a recipient plant. Pollen-mediated gene flow and gene introgression must be considered in the context of the transgenes inserted into the biotechnology-derived plant, and the likelihood that the presence of the transgenes and their subsequent transfer to recipient plants and plant populations will result in increased plant pest potential. The potential for gene flow and introgression from deregulation of MON 88702 cotton is discussed in greater detail below.

IX.F.1.Hybridization with Cultivated cotton

Although natural crossing can occur, cotton is normally considered to be a self-pollinating crop (Niles and Feaster, 1984). There are no morphological barriers to cross-pollination based on flower structure. However, the pollen is heavy and sticky and transfer by wind is limited. Pollen is transferred instead by insects, in particular by various wild bees, bumble bees (*Bombus* sp.), and honeybees (*Apis mellifera*) (Van Deynze et al., 2005). Numerous studies on cotton cross-pollination have been conducted, and the published results, with and without supplemental pollinators, are summarized in Table IX-4. Literature on cotton shows that the frequency of cross-pollination decreases with distance from the pollen source. McGregor (1976) traced movement of pollen by means of fluorescent particles and found that, even among flowers located only 150 to 200 feet from a cotton field that was surrounded by a large number of bee colonies to ensure ample opportunity for transfer of pollen, fluorescent particles were detected on only 1.6% of the flowers. In a 1996 study with various field designs, Llewellyn and Fitt (1996) also found low levels of cross-pollination in cotton. At one meter from the source they observed cross-pollination frequencies of 0.15 to 0.4%, decreasing to below 0.3% at 16 meters from the source. Umbeck et al. (1991) used a selectable marker to examine cross-pollination from a 30 × 136 meter source of biotechnology-derived cotton. Cross-pollination decreased from 5 to less than 1% from one to seven meters, respectively, away from the source plot. A low level of cross-pollination (less than 1%) was sporadically detected at the furthest sampling distance of 25 meters. Berkey et al. (2002) reported that cross-pollination between fields separated by a 13 foot road decreased from 1.89% in the row nearest the source to 0% in the 24th row. Van Deynze et al. (2005) conducted a two year study on pollen-mediated gene flow with high and low pollinator activity. In the presence of high pollinator activity, the pollination frequency was 7.65% at 0.3 meters and less than 1% at greater than nine meters whereas the pollination frequency in the presence of low pollinator activity was below 1% at just over a meter.

In a 2008 study, pollination frequencies of 5% and 0% were demonstrated at one and eight meters, respectively (Kairichi et al., 2008).

The potential for outcrossing and gene introgression from MON 88702 cotton to cultivated cotton in the U.S. is low since cotton pollen movement by wind is limited due to it is large and sticky nature, and several studies have demonstrated that cross-pollination, even in the presence of high pollinator activity, is limited by distance. Therefore, the pollen transfer from MON 88702 to other cotton or related *Gossypium* species is considered to be negligible.

Table IX-4. Summary of Published Literature on Cotton Cross-Pollination

Distance from Pollen Source (meters)	Cross-Pollination (%)	Comments	Reference
45-61	1.60%	Used fluorescent particles to follow pollinator movement in cotton fields over one season.	(McGregor, 1976)
1 4 16	0.15-0.4% <0.08% <0.03%	Used a selectable marker to examine cross-pollination in the progeny of buffer row plants over one season.	(Llewellyn and Fitt, 1996)
1 1-25	5% <1%	Used a selectable marker to examine cross-pollination from a 20 x 136 meter source of biotechnology-derived cotton over one season.	(Umbeck et al., 1991)
5 10.5 17 25	1.89% 0.77% 0.13% 0.00%	Used herbicide bioefficacy to examine pollen flow between fields separated by a 13 foot road over one season.	(Berkey et al., 2002)
0.3 >9 >1 1625	7.65% * < 1% * < 1% ** 0.04% **	Used herbicide bioefficacy confirmed by DNA testing to measured pollen-mediated gene flowing in four directions over 2 years.	(Van Deynze et al., 2005)
1 2-7 8	5.00% 2.00% 0.00%	Used ELISA strips to examine pollen-mediated gene flow in four directions from *Bt* source over a period of one season.	(Kairichi et al., 2008)

* High pollinator activity

** Low pollinator activity

IX.F.2.Hybridization with Wild and Feral *Gossypium* Species

Based on cytological evidence, seven genomic types, A through G, many with subtypes, have been identified for the genus *Gossypium* (Endrizzi et al., 1984). The domesticated species *G. hirsutum* and *G. barbadense* are allotetraploid (AADD, 2n=4x=52), while *G. thurberi* is a diploid (DD, 2n=2x=26), and *G. tomentosum* is an allotetraploid (AADD, 2n=4x=52). *G. tomentosum* is capable of crossing with domesticated cotton to produce fertile offspring (Waghmare et al., 2005). However, Hawaii is the only U.S. region where *G. tomentosum* is found and domesticated cotton is not grown commercially in Hawaii, with the exception of potential counter-season breeding nurseries where appropriate isolation distances and practices are required. Thus, the potential for gene flow to these wild relatives is limited. Importantly, MON 88702 would not be expected to confer a selective advantage to, or enhance the pest potential of, progeny resulting from such a cross if it were to occur. Any potential gene exchange between *G. thurberi* and domesticated cotton, if it were to occur, would result in triploid (ADD, 3x=39), sterile plants because *G. hirsutum* and *G. barbadense* are allotetraploids (AADD, 2n=4x=52) and *G. thurberi* is a diploid (DD, 2n=2x=26). Such sterile hybrids have not been observed to persist in the wild. Fertile allohexaploids (6x=78) have not been reported in the wild either.

Only two 'wild' *Gossypium* species related to cultivated cotton are known to be present in the U.S., *G. thurberi* Todaro, which is known to be found in Arizona (Fryxell, 1984), and feral populations of cultivated *G. hirsutum* and 'wild' populations of *G. hirsutum* are known to occur in South Florida and Puerto Rico (Brubaker et al., 1999). Both of these species would be capable of crossing with cultivated cotton, but they are not known to exist in cotton growing areas. Furthermore, the EPA imposes strict geographical restrictions on the sale and distribution of *Bt* cotton in order to mitigate the potential for gene flow to wild populations of *Gossypium* species (U.S. EPA, 2019). Importantly, MON 88702 would not be expected to confer a selective advantage to, or enhance the pest potential of, progeny resulting from such crosses if they were to occur.

Importantly, the environmental consequences of pollen transfer from MON 88702 cotton to other cotton or related *Gossypium* species is considered to be negligible due to the plant biology and limited movement of cotton pollen, the safety of the introduced protein, and the lack of any selective advantage by the insect-protection trait that might be conferred on a recipient plant of feral or wild cotton, or a wild relative.

IX.F.3.Transfer of Genetic Information to Species with which Crop Cannot Interbreed (Horizontal Gene Flow)

Monsanto is unaware of any reports regarding the unaided transfer of genetic material from cotton species to other sexually-incompatible plant species. The likelihood for horizontal gene flow to occur is exceedingly small. Therefore, potential ecological risk associated with horizontal gene flow from MON 88702 cotton due to the presence of the insect-protection trait is not expected. The consequence of horizontal gene flow of the insect-protection trait into other plants that are sexually-incompatible is negligible since, as data presented in this petition confirm, the insect-protection genes and trait confer no

increased plant pest potential to cotton. Thus, in the highly unlikely event that horizontal gene transfer was to occur, the presence of the insect-protection trait would not be expected to increase plant pest potential in the recipient species.

IX.G. Potential Impact on Cotton Agronomic Practices

An assessment of current cotton agronomic practices was conducted to determine whether cultivation of MON 88702 cotton has the potential to impact current cotton and insect management practices (Section VIII). Cotton fields are typically highly managed agricultural areas that are dedicated to crop production. MON 88702 cotton is likely to be used in common rotations on land previously used for agricultural purposes. Certified seed production will continue to use well-established industry practices to deliver high quality seed containing MON 88702 to growers. Cultivation of MON 88702 cotton is not expected to differ from typical cotton cultivation, with the exception of the added benefit of the insect-protection trait against targeted hemipteran and thysanopteran insect pests that may result in a reduction of the total insecticide applications used by growers to produce commercially acceptable cotton.

MON 88702 cotton is similar to conventional cotton in its agronomic, phenotypic, composition, and has susceptibility to damage by non-target arthropods and diseases comparable to conventional cotton. Based on this assessment, the introduction of MON 88702 cotton is not likely to impact current U.S. cotton agronomic or cultivation practices, other than the intended insect-protection benefits as discussed in Section VIII.

IX.H. Conventional Breeding with Other Biotechnology-Derived or Conventional Cotton

Several biotechnology-derived cotton products have been deregulated or are under consideration for deregulation. Once deregulated, MON 88702 cotton may be bred using conventional breeding techniques with these deregulated biotechnology-derived cotton products, as well as with conventional cotton. APHIS has determined that none of the individual biotechnology-derived cotton products it has previously deregulated displays increased plant pest characteristics. APHIS has also concluded that any progeny derived from crosses of these deregulated biotechnology-derived cotton products with conventional or previously deregulated biotechnology-derived cotton are unlikely to exhibit new plant pest properties. This presumption, that combined-trait biotechnology products are unlikely to exhibit new characteristics that would pose new plant pest risks or potential environmental impacts not observed in the single event biotech product, is based upon several facts. Namely: 1) stability of the genetic inserts is confirmed in each approved biotech-derived cotton product across multiple generations (See Section IV.E for MON 88702 data); 2) stability of each of the introduced traits is continually and repeatedly assessed as new combined-trait varieties are created by plant breeders and tested over multiple seasons prior to commercialization; 3) combined-trait products are developed using the well-established process of conventional breeding that has been safely used for thousands of years to generate new varieties (Cellini et al., 2004; NRC, 2004; WHO, 1995); 4) worldwide organizations, such as World Health Organization, Food and Agriculture Organization/World Health Organization, International Seed

Federation, CropLife International and U.S. FDA, conclude that the safety of the combined-trait product can be based on the safety of the parental GE events (CLI, 2015; FAO-WHO, 1996; ISF, 2005; U.S. FDA, 2001; WHO, 1995); and 5) practical applications in the field have shown that two unrelated biotechnology traits combined together by conventional breeding do not display new characteristics or properties distinct from those present in the single event biotech products (Brookes and Barfoot, 2012; James, 2010; Lemaux, 2008; Pilacinski et al., 2011; Sankula, 2006).

Therefore, based on the considerations above and the conclusion that MON 88702 cotton is unlikely to pose a plant pest risk and it can be concluded that any progeny derived from crosses between MON 88702 and conventional cotton or deregulated biotechnology-derived cotton are no more likely to pose a plant risk than conventional cotton.

IX.I. Summary of Plant Pest Assessments

A plant pest, as defined in the PPA, is the living stage of any of the following that can directly or indirectly injure, damage, or cause disease in any plant or plant product: (A) a protozoan; (B) a nonhuman animal; (C) a parasitic plant; (D) a bacterium; (E) a fungus; (F) a virus or viroid; (G) an infectious agent or other pathogens; or (H) any article similar to or allied with any of the articles specified in the preceding subparagraphs (7 U.S.C. § 7702[14]). Characterization data presented in Sections IV through VI of this petition confirm that MON 88702 cotton, with the exception of the insect-protection trait, is not fundamentally different from conventional cotton, in terms of plant pest potential. Monsanto is not aware of any study results or observations associated with MON 88702 cotton that would suggest an increased plant pest risk would result from its introduction.

The plant pest assessment was based on multiple lines of evidence developed from a detailed characterization of MON 88702 cotton compared to conventional cotton, followed by a risk assessment on detected differences. The plant pest risk assessment in this petition was based on the following lines of evidence: 1) insertion of a single functional copy of the *mCry51Aa2* expression cassette; 2) characterization of the expressed product; 3) negligible risk to NTOs including organisms beneficial to agriculture in the field; 4) anti-nutrient compositional equivalence of MON 88702 compared to a conventional control; 5) phenotypic, agronomic, and environmental characteristics demonstrating no increased plant pest potential compared to conventional cotton; 6) familiarity with cotton as a cultivated crop and 7) no greater likelihood to impact agronomic practices, including land use, cultivation practices, or the management of weeds, diseases and insects, than conventional cotton.

Based on the data and information presented in this petition, it is concluded that, like conventional cotton and previously deregulated biotechnology-derived cotton, MON 88702 is unlikely to pose a plant pest risk. Results also support a conclusion of no increased weediness potential of MON 88702 cotton compared to conventional cotton. Therefore, Monsanto Company requests a determination from APHIS that MON 88702 cotton and any progeny derived from crosses between MON 88702 and other cotton be granted nonregulated status under 7 CFR part 340.

X. ADVERSE CONSEQUENCES OF INTRODUCTION

Monsanto knows of no study results or observations associated with MON 88702 cotton indicating that there would be adverse consequences from its introduction. As demonstrated by field results and laboratory tests, the only phenotypic difference between MON 88702 and conventional cotton is protection from feeding by targeted hemipteran and thysanopteran insect pests.

The data and information presented herein demonstrate that MON 88702 cotton is unlikely to pose a plant pest risk. This conclusion is reached based on multiple lines of evidence developed from a detailed characterization of the product compared to conventional cotton, followed by risk assessment on detected differences. The characterization evaluations included molecular analyses, which confirmed the insertion of one copy of the intended DNA containing the *mCry51Aa2* expression cassette that is stably integrated at a single locus and is inherited according to Mendelian principles over multiple generations.

The comprehensive NTO assessment demonstrated that MON 88702 cotton is not expected to have an adverse impact on beneficial or non-target organisms, including threatened or endangered species. The analysis of key anti-nutrients demonstrate that MON 88702 is equivalent to conventional cotton with regard to these components. The phenotypic evaluations, including an assessment of seed germination and dormancy characteristics, plant growth and development characteristics, pollen characteristics, ecological interaction characteristics, symbiont interactions and environmental interactions also indicated MON 88702 is unchanged compared to conventional cotton. Therefore, based on the lack of increased pest potential compared to conventional cotton, the risks for humans, animals, and other NTOs from MON 88702 cotton are negligible.

The introduction of MON 88702 cotton will not adversely impact cultivation practices or the management of weeds, diseases, and insects in cotton production systems. Farmers familiar with insect-protected products currently available will be advised to continue to employ the same crop rotational practices currently in place for these products.

Successful integration of MON 88702 cotton into commercially available insect-protected cotton systems will provide cotton growers with a new and effective insect control management tool for targeted hemipteran, including two species of tarnished plant bugs (*Lygus hesperus* and *Lygus lineolaris*) and thysanopteran insects pests, thrips (*Frankliniella* spp.). This insect-protected trait along with labeled applications of crop protection agents will provide cotton growers with an effective insect pest control system necessary for cotton production yields to meet the growing needs of the food, feed and industrial markets.

REFERENCES

Advancing Cotton Education. 2017. Integrated crop management. National Cotton Council of America, The Cotton Foundation. http://www.cotton.org/tech/ace/educational-opportunities.cfm [Accessed June 6, 2017].

Ahmad, A., I. Negri, W. Oliveira, C. Brown, P. Asiimwe, B. Sammons, M. Horak and C. Jiang. 2015. Transportable data from non-target arthropod field studies for the environmental risk assessment of genetically modified maize expressing an insecticidal double-stranded RNA. Transgenic Research 24:1-17.

Ahmad, A., G.E. Wilde and K.Y. Zhu. 2005. Detectability of coleopteran-specific Cry3Bb1 protein in soil and its effect on nontarget surface and below-ground arthropods. Environmental Entomology 34:385-394.

Akbar, W., A. Gowda, J.E. Ahrens, J.W. Stelzer, R.S. Brown, S.L. Bollman, J.T. Greenplate, J. Gore, A.L. Catchot, G. Lorenz, S.D. Stewart, D.L. Kerns, J.K. Greene, M.D. Toews, D.A. Herbert, D.D. Reisig, G.A. Sword, P.C. Ellsworth, L.D. Godfrey and T.L. Clark. 2018. First transgenic trait for control of plant bugs and thrips in cotton. Pest Managment Science 75:867-877.

Alexander, M.P. 1980. A versatile stain for pollen fungi, yeast and bacteria. Stain Technology 55:13-18.

Alford, J.L., R.M. Hayes, T.C. Mueller and G.N. Rhodes. 2002. Roundup Ready® soybean (*Glycine max*) and cotton (*Gossypium hirsutum* L.) control in Roundup Ready® cotton (*Gossypium hirsutum* L.). Pages 2-3 in 2002 Proceedings of the Southern Weed Science Society, Atlanta, Georgia.

Ali, A., R.G. Luttrell and H.N. Pitre. 1990. Feeding sites and distribution of fall armyworm (Lepidoptera: Noctuidae) larvae on cotton. Environmental Entomology 19:1060-1067.

Ali, A., R.G. Luttrell, H.N. Pitre and F.M. Davis. 1989. Distribution of fall armyworm (Lepidoptera: Noctuidae) egg masses on cotton. Enviornmental Entolology 18:881-885.

Anderson, H.M., D.J. Bowen, C.A. Chay, S. Flasinski, U.R. Kesanapalli, J.S. Milligan, R.N. Slightom and Y. Yin. 2015. Pesticidal toxin active against Coleopteran and/or Hemipteran insects. Patent 20150047076, U.S. Patent Office, Washington, D.C.

Anderson, J.E., J.M. Michno, T.J. Kono, A.O. Stec, B.W. Campbell, S.J. Curtin and R.M. Stupar. 2016. Genomic variation and DNA repair associated with soybean transgenesis: A comparison to cultivars and mutagenized plants. BMC Biotechnology 16:41.

Anderson, W.P. 1996. Weed ecology. Pages 27-38 in Weed Science: Principles and Applications. Third Edition. West Publishing Company, St. Paul, Minnesota.

AOSA. 2016a. AOSA Rules for testing seeds. Volume 1: Principles and procedures. Association of Official Seed Analysts, Washington, D.C.

AOSA. 2016b. AOSA Rules for testing seeds. Volume 4: Seedling evaluation. Association of Official Seed Analysts, Washington, D.C.

AOSA/SCST. 2010. Tetrazolium testing handbook. Association of Official Seed Analysts and the Society of Commercial Seed Technologists, Ithaca, New York.

Armer, C.A., R.N. Wiedenmann and D.R. Bush. 1998. Plant feeding selection on soybean by the facultatively phytophagous predator *Orius insidiosus*. Entomologia Experimentalis et Applicata 86:109-118.

Asiimwe, P., L. Brown, T. Vandervoet, P. Ellsworth and S.E. Naranjo. 2011. Big-eyed bugs have big appetite for pests. University of Arizona. Arizona Cooerative Extension.

Asiimwe, P., S.E. Naranjo and P.C. Ellsworth. 2014. Effects of irrigation levels on interactions among *Lygus hesperus* (Hemiptera: Miridae), insecticides, and predators in cotton. Environmental Entomology 43:263-273.

Axelos, M., C. Bardet, T. Liboz, A. Le Van Thai, C. Curie and B. Lescure. 1989. The gene family encoding the *Arabidopsis thaliana* translation elongation factor EF-1α: Molecular cloning characterization and expression. Molecular and General Genetics 219:106-112.

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.

Bachman, P.M., A. Ahmad, J.E. Ahrens, W. Akbar, J.A. Baum, S. Brown, T.L. Clark, J.M. Fridley, A. Gowda, J.T. Greenplate, P.D. Jensen, G.M. Mueller, M.L. Odegaard, J. Tan, J.P. Uffman and S.L. Levine. 2017. Characterization of the activity spectrum of MON 88702 and the plant-incorporated protectant Cry51Aa2.834_16. PLoS One 12:1-20.

Barker, R.F., K.B. Idler, D.V. Thompson and J.D. Kemp. 1983. Nucleotide sequence of the T-DNA region from the *Agrobacterium tumefaciens* octopine Ti plasmid pTi15955. Plant Molecular Biology 2:335-350.

Baum, J.A., U.R. Sukuru, S.R. Penn, S.E. Meyer, S. Subbarao, X. Shi, S. Flasinski, G.R. Heck, R.S. Brown and T.L. Clark. 2012. Cotton plants expressing a hemipteran-active *Bacillus thuringiensis* crystal protein impact the development and survival of *Lygus hesperus* (Hemiptera: Miridae) nymphs. Journal of Economic Entomology 105:616-624.

Bautista-Zapanta, J.-n., K. Suzuki and K. Yoshida. 2002. Characterization of four ribosomal RNA operons in the genome of *Agrobacterium tumefaciens* MAFF301001. Nucleic Acids Research S2:91-92.

Bayer. 2019. U.S. technology use guide.

Beck, E., G. Ludwig, E.A. Auerswald, B. Reiss and H. Schaller. 1982. Nucleotide sequence and exact localization of the neomycin phosphotransferase gene from transposon Tn*5*. Gene 19:327-336.

Bellows, T.S., T.W. Fisher, L.E. Caltagirone, D.L. Dahlsten, G. Gordh and C.B. Huffaker. 1999. Principles and applications of biological control. Handbook of Biological Control. Academic Press,.

Berkey, D.A., B.R. Savoy, S.R. Miller and P.G. Johnson. 2002. Pollen dissemination from adjacent fields of genetically enhanced cotton in the Mississippi Delta. 2002 Proceedings of the Beltwide Cotton Conference, Atlanta, Georgia.

Bernardo, A.M.G., C.M. de Oliverira, R.A. Oliveira, H.E. Vacacela, M. Venzon, A. Pallini and A. Janssen. 2017. Performance of *Orius insidious* on alternative foods. Journal of Applied Entomology 141:702-707.

Berry, C. 2012. The bacterium, *Lysinibacillus sphaericus*, as an insect pathogen J Invertebr Pathol 109:1-10.

BIO. 2010. Product launch stewardship policy. Biotechnology Industry Organization, Washington, D.C. http://www.bio.org/articles/product-launch-stewardship-policy [Accessed May 31, 2012].

Blasingame, D., M.V. Patel, W. Gazaway, M. Olsen, T. Kirkpatrick, M. Davis, R.K. Sprenkel, B. Kemerait, P. Colyer, A. Wrather, N. Goldberg, S. Koenning, J.C. Banks, J. Muller, M. Newman, J. Woodward and P. Phipps. 2008. Cotton disease loss estimate committee report: Table 1. Estimated reduction in 2007 cotton yield resulting from diseases. National Cotton Council of America, Cordova, Tennessee.

Blümel, S., S. Aldershof, F.M. Bakker, B. Baier, E. Boller , K. Brown, D. Bylemans, M.P. Candolfi, B. Huber, C. Linder, F. Louis, J. Müther, K.M. Nienstedt, C. Oberwalder, B. Reber, K.J. Schirra, A. Ufer and H. Vogt. 2000. Guidance document to detect side effects of plant protection products on predatory mites (Acari: Phytoseiidae) under field conditions: vineyards and orchards. Guidance to evaluate side-effects of plant protection products to non-target arthropods.

Boyd, M.L., B.J. Phipps and J.A. Wrather. 2004. Cotton pests scouting & management. I.P. Management. University of Missouri-Columbia Extension.

Bradley, K.W., L.E. Sweets, W.C. Bailey and J.A. Wrather. 2013. Pest management guide: Corn, grain sorghum, soybean, winter wheat, rice, cotton. University of Missouri Extension, Columbia, Missouri.

Bridge, R., J.M. Bridge and R. McGowen. 2005. Cotton cultivar DP 393. Patent US 6,930,228 B2, U.S. Patent Office, Washington, D.C.

Brookes, G. and P. Barfoot. 2012. GM crops: Global socio-economic and environmental impacts 1996-2010. PG Economics Ltd, Dorchester, United Kingdom.

Brookes, G. and P. Barfoot. 2017. Environmental impacts of genetically modified (GM) crop use 1996-2016: Impacts on pesticide use and carbon emissions. GM Crops & Food 9:109-139.

Brown, L., T. Vandervoet, P. Ellsworth and S.E. Naranjo. 2011. Assassin bugs top the food web. University of Arizona. Arizona Cooperative Extension.

Brubaker, C.L., F.M. Bourland and J.F. Wendel. 1999. The origin and domestication of cotton. Pages 3-31 in Cotton: Origin, History, Technology, and Production. C.W. Smith and J.T. Cothren (eds.). John Wiley & Sons, Inc., New York, New York.

Burke, J.J. 2011. Cotton flowers: Pollen and petal humidity sensitivities determine reproductive competitiveness in diverse environments. Pages 25-36 in Cotton Physiology. D.M. Oosterhuis (ed.). The Cotton Foundation, Cordova, T.N.

Candolfi, M.P., F. Bigler, D. Forti, R. Forster, A. Gathmann, S. Hassan, M. Mead-Briggs, M. Melandri, P. Neumann, E. Pasqualini, W. Powell, J.-N. Reboulet, P. Campbell, K. Romijn, B. Sechser, T. Thieme, A. Ufer, C. Vergnet, H. Vogt, U. Heimbach, R. Schmuck, G. Angeli, F. Bakker, K. Brown, G. Carli and A. Dinter. 2000. Principles for regulatory testing and interpretation of semi-field and field studies with non-target arthropods. Anzeiger fur Schadlingskunde 76:141-147.

Cannon, R.J.C. 1993. Prospects and progress for *Bacillus thuringiensis*-based pesticides. Pesticide Science 37:331-335.

Catchot, A. 2019. Insect control guide for agronomic crops. Mississippi State University Extension, Mississippi State, MS.

Cellini, F., A. Chesson, I. Colquhoun, A. Constable, H.V. Davies, K.H. Engel, A.M.R. Gatehouse, S. Kärenlampi, E.J. Kok, J.-J. Leguay, S. Lehesranta, H.P.J.M. Noteborn, J. Pedersen and M. Smith. 2004. Unintended effects and their detection in genetically modified crops. Food and Chemical Toxicology 42:1089-1125.

Chen, Y.R., A. Rivlin, A. Lange, X.D. Ye, Z. Vaghchhipawala, E. Eisinger, E. Dersch, M. Paris, B. Martinell and Y.C. Wan. 2014. High throughput *Agrobacterium*

tumefaciens-mediated germline transformation of mechanically isolated meristem explants of cotton (*Gossypium hirsutum* L.). Plant Cell Reports 33:153-164.

Chinnadurai, P., D. Stojšin, K. Liu, G.E. Frierdich, K.C. Glenn, T. Geng, A. Schapaugh and K. Huang. 2018. Variability of CP4 EPSPS expression in genetically engineered soybean (Glycine max L. *Merrill*). Transgenic Research 22.

Cisneros, J.J. and J.A. Rosenheim. 1997. Ontogenetic change of prey preference in the generalist predator *Zelus renardii* and its influence on predator-predator interactions. Ecological Entomology 22:399-407.

Cisneros, J.J. and J.A. Rosenheim. 1998. Changes in the foraging behavior, within-plant vertical distribution, and microhabitat selection of a generalist insect predator: an age analysis. Population Ecology 27:949-957.

CLI. 2015. Position paper: Regulation of plant biotechnology-derived breeding stacks, January 2015. CropLife International, Brussels, Belgium.

Codex Alimentarius. 2009. Foods derived from modern biotechnology. Volume Second Edition. Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, Food and Agriculture Organization of the United Nations, Rome, Italy.

Cook, D., A. Herbert, D.S. Akin and J. Reed. 2011. Biology, crop injury, and management of thrips (Thysanoptera: Thripidae) infesting cotton seedlings in the United States. Journal of Integrated Pest Management 2:1-9.

Coruzzi, G., R. Broglie, C. Edwards and N.-H. Chua. 1984. Tissue-specific and light-regulated expression of a pea nuclear gene encoding the small subunit of ribulose-1, 5-bisphosphate carboxylase. EMBO Journal 3:1671-1679.

Crockett, L. 1977. Contents. Pages vii-ix in Wildly Successful Plants: A Handbook of North American Weeds. Macmillan Publishing Co., Inc., New York, New York.

de Jong, F.M.W., F.M. Bakker, K. Brown, C.J.T.J. Jilesen, C.J.A.M. Posthuma-Doodeman, C.E. Smit, J.J.M. van der Steen and G.M.A. van Eekelen. 2010. Guidance for summarising and evaluating field studies with non-target arthropods: A guidance document of the Dutch platform for the assessment of higher tier studies. RIVM. National Institute for Public Health and the Environment, The Netherlands.

Depicker, A., S. Stachel, P. Dhaese, P. Zambryski and H.M. Goodman. 1982. Nopaline synthase: Transcript mapping and DNA sequence. Journal of Molecular and Applied Genetics 1:561-573.

Dicke, F.F. and J.L. Jarvis. 1962. The habits and seasonal abundance of *Orius insidiosus* (Say) (Hemiptera-Heteroptera: Anthocoridae) on corn. Journal of the Kansas Entomological Society 35:339-344.

Drees, B.M. and M.E. Rice. 1985. The vertical beat sheet: A new device for sampling soybeans insects. Journal of Economic Entomology 78:1507-1510.

Duan, J.J., C. Jiang, G.P. Head, M.A. Bhatti, D.P. Ward, S.L. Levine, T.E. Nickson and M.A. Nemeth. 2006. Statistical power analysis of a 2-year field study and design of experiments to evaluate non-target effects of genetically modified *Bacillius thuringiensis* corn. Ecological Entomology 31:521-531.

Dubelman, S., B.R. Ayden, B.M. Bader, C.R. Brown, C.J. 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., J. Romeis and F. Bigler. 2003. Assessing the risks of insect resistant transgenic plants on entomophagous arthropods: Bt-maize expressing Cry1Ab as a case study. Biocontrol 48:611-636.

Egelkrout, E., V. Rajan and J.A. Howard. 2012. Overproduction of recombinant proteins in plants. Plant Science 184:83-101.

Eisenring, M., J. Romeis, S.E. Naranjo and M. Meissle. 2017. Multitrophic Cry-protein flow in a dual-gene Bt-cotton field. Agriculture, Ecosystems & Environment 247:283-289.

Ellsworth, P.C. 1999. Evaluation of chemical controls of Lygus hesperus in Arizona. Pages 1-116 in Arizona Cotton Report University of Arizona.

Ellsworth, P.C. 2004. Search for effective chemical controls for Lygus bugs and whiteflies in Arizona cotton. Pages 1-138 in Arizona cotton report. University of Arizona, Barkley, Virginia.

Ellsworth, P.C. and V. Barkley. 2005. Transitioning Lygus chemical controls to more selective options for Arizona cotton. Cotton: A College of Agriculture and Life Sciences Report.

Endrizzi, J.E., E.I. Turcotte and R.J. Kohel. 1984. Qualitative genetics, cytology, cytogenetics. Pages 81-129 in Cotton. R.J. Kohel and C.F. Lewis (eds.). American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Society of America, Madison, Wisconsin.

FAO-WHO. 1996. Joint FAO/WHO expert consultation on biotechnology and food safety. Paper 61. Food and Agriculture Organization of the United Nations, World Health Organization, Rome, Italy.

Farmer, D.R., T.C. Edrington, C.R. Kessenich, C. Wang and J.S. Petrick. 2017. Improving insect control protein activity for GM crops: A case study demonstrating that

increased target insect potency can be achieved without impacting mammalian safety. Regulatory Toxicology and Pharmacology 89:155-164.

Fernandez-Cornejo, J., S. Wechsler, M. Livingston and L. Mitchell. 2014. Genetically engineered crops in the united states. USDA.

Fiorito, T.M., I. Icoz and G. Stotzky. 2008. Adsorption and binding of the transgenic plant proteins, human serum albumin, ß-glucuronidase, and Cry3Bb1, on montmorillonite and kaolinite: Microbial utilization and enzymatic activity of free and clay-bound proteins. Applied Clay Science 39:142-150.

Flavell, R.B. 1994. Inactivation of gene expression in plants as a consequence of specific sequence duplication. Proceedings of the National Academy of Sciences of the United States of America 91:3490-3496.

Fling, M.E., J. Kopf and C. Richards. 1985. Nucleotide sequence of the transposon Tn*7* gene encoding an aminoglycoside-modifying enzyme, 3"(9)-*O*-nucleotidyltransferase. Nucleic Acids Research 13:7095-7106.

Fraley, R.T., S.G. Rogers, R.B. Horsch, P.R. Sanders, J.S. Flick, S.P. Adams, M.L. Bittner, L.A. Brand, C.L. Fink, J.S. Fry, G.R. Galluppi, S.B. Goldberg, N.L. Hoffmann and S.C. Woo. 1983. Expression of bacterial genes in plant cells. Proceedings of the National Academy of Sciences of the United States of America 80:4803-4807.

Fryxell, P.A. 1984. Taxonomy and germplasm resources. Pages 27-57 in Cotton. R.J. Kohel and C.F. Lewis (eds.). American Society of Agronomy, Inc., Crop Science Society of America, Inc., Soil Science Society of America, Inc., Madison, Wisconsin.

Gill, S.S., E.A. Cowles and P.V. Pietrantonio. 1992. The mode of action for *Bacillus thuringiensis* endotoxins. Annu Rev Entomol 37:615-636.

Giza, P.E. and R.C.C. Huang. 1989. A self-inducing runaway-replication plasmid expression system utilizing the Rop protein. Gene 78:73-84.

Gore, J., A. Catchot, F. Musser, J. Greene, B.R. Leonard, D.R. Cook, G.L. Snodgrass and R. Jackson. 2012. Development of a plant-based threshold for tarnished plant bug (Hemiptera: Miridae) in cotton. Journal of Economic Entomology 105:2007-2014.

Gowda, A., T.J. Rydel, A.M. Wollacott, R.S. Brown, W. Akbar, T.L. Clark, S. Flasinski, J.R. Nageotte, A.C. Read, X. Shi, B.J. Werner, M.J. Pleau and J.A. Baum. 2016. A transgenic approach for controlling *Lygus* in cotton. Nature Communications 7:12213.

Graham, S.H. and S.D. Stewart. 2018. Field study investigating Cry51Aa2.834_16 in cotton for control of thrips (Thysanoptera: Thripidae) and tarnished plant bugs (Hemiptera: Miridae). Journal of Economic Entomology XX:1-10.

Grimaldi, D. and M.S. Engel. 2005. Table 4.1. Hierarchical classification of class insecta. Page 147 in Evolution of the Insects. Science (ed.). Cambridge University Press.

Hagler, J.R., C.G. Jackson and I.R. Machtley. 2004. Foraging behavior and prey interactions by a guild of predators on various lifestages of *Bemisia tabaci*. Journal of Insect Science 4:13.

Hake, S.J., T.A. Kerby and K.D. Hake. 1996. Preparation for the new crop season - Fall/winter. Pages 6-14 in Cotton Production Manual. S.J. Hake, T.A. Kerby, and K.D. Hake (eds.). University of California Division of Agriculture and Natural Resources, Davis, California.

Hammond, B. 2004. A review of the food/feed safety and benefits of *Bacillus thuringiensis* protein containing insect-protected crops. Pages 103-123 in Agricultural Biotechnology: Challenges and Prospects. M.K. Bhalgat, W.P. Ridley, A.S. Felsot, and J.N. Seiber (eds.), American Chemical Society, Washington, D.C.

Head, G., C.R. Brown, M.E. Groth and J.J. Duan. 2001. Cry1Ab protein levels in phytophagous insects feeding on transgenic corn: Implications for secondary exposure risk assessment. Entomologia Experimentalis et Applicata 99:37-45.

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 transgenic *Bt* cotton (Bollgard) use. Environmental Entomology 31:30-36.

Herbert, D.A., J. Bacheler, S. Malone and D. Mott. 2007. Thrips control options in Virginia/North Carolina: Overviews, insights and updates. Beltwide Cotton Conferences, New Orleans, Lousiana.

Herman, R.A. and W.D. Price. 2013. Unintended compositional changes in genetically modified (GM) crops: 20 years of research. Journal of Agricultural and Food Chemistry 61:11695-11701.

Herman, R.A., J.D. Wolt and W.R. Halliday. 2002. Rapid degradation of the Cry1F insecticidal crystal protein in soil. Journal of Agricultural and Food Chemistry 50:7076-7078.

Herrmann, K.M. 1995. The shikimate pathway: Early steps in the biosynthesis of aromatic compounds. The Plant Cell 7:907-919.

Holm, L., J. Doll, E. Holm, J. Pancho and J. Herberger. 1997. *Chenopodium murale* L. Pages 178-182 in World Weeds: Natural Histories and Distribution. John Wiley & Sons, Inc., New York, New York.

Hopkins, D.W. and E.G. Gregorich. 2005. Decomposition of residues and loss of the δ-endotoxin from transgenic (*Bt*) corn (*Zea mays* L.) in soil. Canadian Journal of Soil Science 85:19-26.

Hu, X., W. Fan, B. Han, H. Liu, D. Zheng, Q. Li, W. Dong, J. Yan, M. Gao, C. Berry and Z. Yuan. 2008. Complete genome sequence of the mosquitocidal bacterium *Bacillus sphaericus* C3-41 and comparison with those of closely related Bacillus species. Journal of Bacteriology 190:2892-2902.

Hunt, T., J. Bergsten, Z. Levkanicova, A. Papadopoulou, O. St. John, R. Wild, R.M. Hammond, D. Ahrens, M. Balke, M.S. Caterino, J. Gomez-Zurita, I. Ribera, T.G. Barraclough, M. Bocakova, L. Bocak and A.P. Vogler. 2007. A comprehensive phylogeny of beetles reveals the evolutionary origins of a superradiation. Science 318:1913-1916.

Huseth, A.S., D.A. D'Ambrosio and G.G. Kennedy. 2017. Response of neonicotinoid resistant and susceptible *Frankliniella fusca* life stages to multiple insecticide groups in cotton. Pest Managment Science 73:2118-2130.

Icoz, I. and G. Stotzky. 2008a. Fate and effects of insect-resistant *Bt* crops in soil ecosystems. Soil Biology and Biochemistry 40:559-586.

Icoz, I. and G. Stotzky. 2008b. Cry3Bb1 protein from *Bacillus thuringiensis* in root exudates and biomass of transgenic corn does not persist in soil. Transgenic Research 17:609-620.

ISF. 2005. Genetically modified crops and plant breeding. International Seed Foundation, Nyon, Switzerland.

Jamal, A., K. Ko, H.-S. Kim, Y.-K. Choo, H. Joung and K. Ko. 2009. Role of genetic factors and environmental conditions in recombinant protein production for molecular farming. Biotechnology Advances 27:914-923.

James, C. 2010. Global status of commercialized biotech/GM crops: 2010. Brief 42. International Service for the Acquisition of Agri-Biotech Applications, Ithaca, New York.

Jerga, A., D. Chen, C. Zhang, J. Fu, J.-L.K. Kouadio, Y. Wang, S.M.G. Duff, J.E. Howard, T.J. Rydel, A.G. Evdokimov, P. Ramaseshadri, A. Evans, R. Bolognesi, Y. Park and J.A. Haas. 2016. Mechanistic insights into the first Lygus-active β-pore forming protein. Archives of Biochemistry and Biophysics 600:1-11.

Joost, P.H. and D.G. Riley. 2005. Imidacloprid effects on probing and settling behavior of *Frankliniella fusca* and *Frankliniella occidentalis* (Thysanoptera: Thripidae) in tomato. Journal of Economic Entomology 98:1622-1629.

Kairichi, M.N., B.W. Waswa, C.N. Waturu, W. Wiesel, R.G. Ngigi, G.K. Njenga and S.M. Njinju. 2008. Assessment of pollen-mediated gene flow of *Bt*-cotton to local commerical variety, Hart 89m in Kari-Mwea Station. Page 257 in Proceedings of the 1st All African Congress on Biotechnology, Nairobi, Kenya.

Kiman, K.B. and K.V. Yeargan. 1985. Development and reproduction of the predator *Orius insidiosus* (Hemiptera: Anthocoridae) reared on diets of selected plant material and arthropod prey. Annals of the Entomological Society of America 78:464-467.

Klee, H.J., Y.M. Muskopf and C.S. Gasser. 1987. Cloning of an *Arabidopsis thaliana* gene encoding 5-enolpyruvylshikimate-3-phosphate synthase: Sequence analysis and manipulation to obtain glyphosate-tolerant plants. Molecular and General Genetics 210:437-442.

Koch, M.S., J.M. Ward, S.L. Levine, J.A. Baum, J.L. Vicini and B.G. Hammond. 2015. The food and environmental safety of *Bt* crops. Front Plant Sci 6:283.

Kohel, R.J. and C.F. Lewis. 1984. Cotton. American Society of Agronomy, Inc., Crop Science Society of America, Inc., Soil Science Society of America, Inc., Madison, Wisconsin.

Kovalic, D., C. Garnaat, L. Guo, Y. Yan, J. Groat, A. Silvanovich, L. Ralston, M. Huang, Q. Tian, A. Christian, N. Cheikh, J. Hjelle, S. Padgette and G. Bannon. 2012. The use of next generation sequencing and junction sequence analysis bioinformatics to achieve molecular characterization of crops improved through modern biotechnology. The Plant Genome 5:149-163.

Lattin, J.D. 1989. Bionomics of the nabidae. Annual Reviews of Entomology 34:383-400.

Lawrence, K., A. Hagan, T. Faske, R. Hutmacher, J. Muller, D. Wright, I. Small, B. Kemerait, C. Overstreet, P. Price, G. Lawrence, T. Allen, S. Atwell, A. Jones, S. Thomas, N. Goldberg, R. Boman, J. Goodson, H. Kelly, J. Woodward and H.L. Mehl. 2017. Cotton disease loss estimate committee report, 2016. Pages 150-152 in 2017 Beltwide Cotton Conferences, Dallas, TX.

Lawrence, S.D., N.G. Novak, C.J.T. Ju and J.E.K. Cooke. 2008. Potato, *Solanum Tuberosum*, Defense Against Colorado Potato Beetle, *Leptinotarsa Decemlineata* (Say): Microarray Gene Expression Profiling of Potato by Colorado Potato Beetle Regurgitant Treatment of Wounded Leaves. Journal of Chemical Ecology 34:1013-1025.

Layton, M.B. 2000. Biology and damage of the tarnished plant bug, *Lygus lineolaris*, in cotton. Southwestern Entomologist Suppl. No. 23:7-20.

Lee, J.A. 1984. Cotton as a world crop. Pages 1-25 in Cotton. R.J. Kohel and C.F. Lewis (eds.). American Society of Agronomy, Inc., Crop Science Society of America, Inc., Soil Science Society of America, Inc., Madison, Wisconsin.

Lemaux, P.G. 2008. Genetically engineered plants and foods: A scientist's analysis of the issues (Part I). Annual Review of Plant Biology 59:771-812.

Lingenfelter, D.D. and N.L. Hartwig. 2007. Introduction to weeds and herbicides. Penn State University Agricultural Research and Cooperative Extension, University Park, Pennsylvania.

Liu, J.-W., A.G. Porter, B.Y. Wee and T. Thanabalu. 1996. New gene from nine *Bacillus sphaericus* strains encoding highly conserved 35.8-kilodalton mosquitocidal toxins. Applied and Environmental Microbiology 62:2174-2176.

Llewellyn, D. and G. Fitt. 1996. Pollen dispersal from two field trials of transgenic cotton in the Namoi Valley, Australia. Molecular Breeding 2:157-166.

Lumbierres, B., R. Albajes and X. Pons. 2012. Positive effect of Cry1Ab-expressing Bt maize on the development and reproduction of the predator *Orius majusculus* under laboratory conditions. Biological Control 63:150-156.

Lundgren, J.G. 2009. Relationships of natural enemies and non-prey foods. Springer, Brookings, SD.

Luttrell, R.G. and J.S. Mink. 1999. damage to cotton fruiting structures be the fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Journal of Cotton Science 3:35-44.

Mandelker, D.R. 2012. NEPA law and litigation: Environmental law series. Second Edition. Thompson Reuters West Group, Eagan, Minnesota.

McClintock, J.T., C.R. Schaffer and R.D. Sjoblad. 1995. A comparative review of the mammalian toxicity of *Bacillus thuringiensis*-based pesticides. Pesticide Science 45:95-105.

McGregor, S.E. 1976. Insect pollination of cultivated crop plants. Agricultural Handbook No. 496. U.S. Department of Agriculture, Agricultural Research Service, Washington, D.C.

Milliken, G.A. and D.E. Johnson. 2009. 10.6 Population marginal means and least squares means. Analysis of messy data: Designed experiments. Volume 1. 2nd Edition. CRC Press.

Mississippi State University. 2016. 2016 insect control guide for agronomic crops. Volume 2471. Mississippi State University Extension, Mississippi State, Mississippi.

Moar, W.J., A.J. Evans, C.R. Kessenich, J.A. Baum, D.J. Bowen, T.C. Edrington, J.A. Haas, J.-L.K. Kouadio, J.K. Roberts, A. Silvanovich, Y. Yin, B.E. Weiner, K.C. Glenn and M.L. Odegaard. 2017. The sequence, structural, and functional diversity within a protein family and implications for specificity and safety: The case for ETX_MTX2 insecticidal proteins. J Invertebr Pathol 142:50-59.

Mogen, B.D., M.H. MacDonald, R. Graybosh and A.G. Hunt. 1990. Upstream sequences other than AAUAAA are required for efficient messenger RNA 3'-end formation in plant. The Plant Cell 2:1261-1272.

Monsanto. 2004. Petition for the determination of nonregulated status for roundup ready flex cotton MON 88913. Monsanto, St. Louis, Missouri.

Monsanto Company. 2012. Petition for determination of nonregulated status of Dicamba and Glufosinate resistant MON 88701 cotton. Monsanto Company, St. Louis, Missouri.

Mound, L.A. 2005. Thysanoptera: Diversity and interactions. Annu Rev Entomol 50:247-269.

Murdock, E.C., M.A. Jones and R.F. Graham. 2002. Control of volunteer glyphosate (Roundup)-tolerant cotton and soybean in Roundup Ready cotton. 2002 Proceedings of the Beltwide Cotton Conference, National Cotton Council, Atlanta, Georgia.

Naranjo, S.E. 2005a. Long-term assessment of the effects of transgenic *Bt* cotton on the abundance of nontarget arthropod natural enemies. Environmental Entomology 34:1193-1210.

Naranjo, S.E. 2005b. Long-term assessment of the effects of transgenic *Bt* cotton on the function of the natural enemy community. Environmental Entomology 34:1211-1223.

Naranjo, S.E. 2009. Impacts of *Bt* crops on non-target invertebrates and invertebrates and insecticide use patterns CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 4:1-23.

Naranjo, S.E. and P.C. Ellsworth. 2009. Fifty years of the integrated control concept: Moving the model and implementation forward in Arizona. Pest Managment Science 65:1267-1286.

Naranjo, S.E., P.C. Ellsworth and J.R. Hagler. 2004. Conservation of natural enemies in cotton: Role of insect growth regulators in management of *Bemisia tabaci*. Biological Control 30:52-72.

Narva, K.E., N.X. Wang and R. Herman. 2017. Safety considerations derived from Cry34Ab1/Cry35Ab1 structure and function. J Invertebr Pathol 142:27-33.

NatureServe. 2019. Kretschmarr cave mold beetle. http://explorer.natureserve.org/servlet/NatureServe?searchSciOrCommonName=Kretschmarr+Cave+Mold+Beetle+&x=6&y=6 [Accessed March 26, 2019].

NCCA. 2010. Cotton: From field to fabric. National Cotton Council of America, Atlanta, Georgia.

Nguyen, H.T. and J.A. Jehle. 2009. Expression of Cry3Bb1 in transgenic corn MON88017. Journal of Agricultural and Food Chemistry 57:9990-9996.

Niles, G.A. and C.V. Feaster. 1984. Breeding. Pages 201-231 in Cotton. R.J. Kohel and C.F. Lewis (eds.). American Society of Agronomy, Inc., Crop Science Society of America, Inc., Soil Science Society of America, Inc., Madison, Wisconsin

NRC. 2004. New approaches for identifying unintended changes in food composition. Pages 73-102 in Safety of Genetically Engineered Foods: Approaches to Assessing Unintended Health Effects. Institute of Medicine and National Research Council, National Academies Press, Washington, D.C.

OECD. 1993. Safety considerations for biotechnology: Scale-up of crop plants. Organisation for Economic Co-operation and Development, Paris, France. www.biosafety.be/CU/BSL_Ressources/PDF/M00034525.pdf [Accessed January 9, 2009].

OECD. 2007. Consensus document on safety information on transgenic plants expressing *Bacillus thuringiensis*-derived insect control proteins. ENV/JM/MONO(2007)14. Organisation for Economic Co-operation and Development, Paris, France.

OECD. 2008. Consensus document on the biology of cotton (*Gossypium* spp.). ENV/JM/MONO(2008)33. Organisation for Economic Co-operation and Development, Paris, France.

OECD. 2009. Consensus document on the compositional considerations for new varities of cotton (*Gossypium hirsutum* and *Gossypium barbadense*): Key food and feed nutrients and anti-nutrients. ENV/JM/MONO(2004)16. Organisation for Economic Co-operation and Development, Paris, France.

OECD. 2010a. 6.2 Human health assessment. Pages 234-237 in Safety Assessment of Transgenic Organisms. Volume 4. Organisation for Economic Co-operation and Development, Paris, France.

OECD. 2010b. Section 1. Cotton (*Gossypium* spp.). Safety Assessment of Transgenic Organisms. Volume 4. Organisation for Economic Co-operation and Development, Paris, France.

OGTR. 2008. The biology of *Gossypium hirsutum* L. and *Gossypium barbadense* L. (cotton). Australian Government, Department of Health and Ageing, Office of the Gene Technology Regulator, Canberra, Australia.

Ortiz, B.V., C. Perry, D. Sullivan, P. Lu, R. Kemerait, R.F. Davis, A. Smith, G. Vellidis and R. Nichols. 2012. Variable rate application of nematicides on cotton fields: A promising site-specific management strategy. Journal of Nematology 44:31-39.

Overstreet, C., E.C. McGawley, A. Khalilian, T.L. Kirkpatrick, W.S. Monfort, W. Henderson and J.D. Mueller. 2014. Site specific nematode management-development and success in cotton production in the united states. Journal of Nematology 46:309-320.

Percival, A.E., J.F. Wendel and J.M. Stewart. 1999. Taxonomy and germplasm resources. Pages 33-63 in Cotton: Origin, History, Technology, and Production. W.C. Smith and J.T. Cothren (eds.). John Wiley & Sons, Inc., New York, New York.

Perry, J.N., P. Rothery, S.J. Clark, M.S. Heard and C. Hawes. 2003. Design, analysis and statistical power of the farm-scale evaluations of genetically modified herbicide-tolerant crops. Journal of Applied Ecology 40:17-31.

Pigott, C.R. and D.J. Ellar. 2007. Role of receptors in *Bacillus thuringiensis* crystal toxin activity. Microbiology and Molecular Biology Reviews 71:255-281.

Pilacinski, W., A. Crawford, R. Downey, B. Harvey, S. Huber, P. Hunst, L.K. Lahman, S. MacIntosh, M. Pohl, C. Rickard, L. Tagliani and N. Weber. 2011. Plants with genetically modified events combined by conventional breeding: An assessment of the need for additional regulatory data. Food and Chemical Toxicology 49:1-7.

Raybould, A. 2006. Problem formulation and hypothesis testing for environmental risk assessments of genetically modified crops. Environmental Biosafety Research 5:119-125.

Reay-Jones, F.P.F., J.K. Greene, D.A. Herbert, A.L. Jacobson, G.G. Kennedy, D.D. Reisig and P.M. Roberts. 2017. Within-plant distribution and dynamics of thrips species (Thysanoptera: Thripidae) in cotton. Journal of Economic Entolology 110:1563-1575.

Richins, R.D., H.B. Scholthof and R.J. Shepherd. 1987. Sequence of figwort mosaic virus DNA (caulimovirus group). Nucleic Acids Research 15:8451-8466.

Roberts, G., S. Kerlin and M. Hickman. 2002. Controlling volunteer cotton. Page F4 in WEEDpak - A Guide for Integrated Management of Weeds in Cotton. Cotton Catchment Communities CRC, Australian Cotton Research Institute, Narrabri, New South Wales.

Rogers, S.G. 2000. Promoter for transgenic plants. Patent 6,018,100, U.S. Patent Office, Washington, D.C.

Romeis, J., D. Bartsch, F. Bigler, M.P. Candolfi, M.M.C. Gielkens, S.E. Hartley, R.L. Hellmich, J.E. Huesing, P.C. Jepson, R. Layton, H. Quemada, A. Raybould, R.I. Rose, J. Schiemann, M.K. Sears, A.M. Shelton, J. Sweet, Z. Vaituzis and J.D. Wolt. 2008. Assessment of risk of insect-resistant transgenic crops to nontarget arthropods. Nature Biotechnology 26:203-208.

Romeis, J., R.L. Hellmich, M.P. Candolfi, K. Carstens, A. De Schrijver, A.M.R. Gatehouse, R.A. Herman, J.E. Huesing, M.A. McLean, A. Raybould, A.M. Shelton and A. Waggoner. 2011. Recommendations for the design of laboratory studies on non-target arthropods for risk assessment of genetically engineered plants. Transgenic Research 20:1-22.

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

Romeis, J., A. Raybould, F. Bigler, M.P. Candolfi, R.L. Hellmich, J.E. Huesing and A.M. Shelton. 2013. Deriving criteria to select arthropod species for laboratory tests to assess the ecological risks from cultivating arthropod-resistant genetically engineered crops. Chemosphere 90:901-909.

Salahudeen, M.S. and P.S. Nishtala. 2017. An overview of pharmacodynamic modelling, ligand-binding approach and its application in clinical practice. Saudi Pharmaceutical Journal 25:165-175.

Salomon, S. and H. Puchta. 1998. Capture of genomic and T-DNA sequences during double-strand break repair in somatic plant cells. EMBO Journal 17:6086-6095.

Sankula, S. 2006. Quantification of the impacts on US agriculture of biotechnology-derived crops planted in 2005. National Center for Food and Agricultural Policy, Washington, D.C.

Schmid, K.M. and G.W. Patterson. 1988. Effects of cyclopropenoid fatty acids on fungal growth and lipid composition. Lipids 3:248-252.

Schnepf, E., N. Crickmore, J. van Rie, D. Lereclus, J. Baum, J. Feitelson, D.R. Zeigler and D.H. Dean. 1998. *Bacillus thuringiensis* and its pesticidal crystal proteins. Microbiology and Molecular Biology Reviews 62:775-806.

Sims, S.R. and L.R. Holden. 1996. Insect bioassay for determining soil degradation of *Bacillus thuringiensis* subsp. *kurstaki* Cry1A(b) protein in corn tissue. Environmental Entomology 25:659-664.

Sims, S.R. and J.E. Ream. 1997. Soil inactivation of the *Bacillus thuringiensis* subsp *kurstaki* CryIIA insecticidal protein within transgenic cotton tissue: Laboratory microcosm and field studies. Journal of Agricultural and Food Chemistry 45:1502-1505.

Sisterson, M.S., R. Biggs, W., C. Olson, Y. Carriere, T.J. Dennehy and B.E. Tabashnik. 2004. Arthropod abundance and diversity in Bt and non-Bt cotton fields. Environmental Entomology 33:921-929.

Smith, C.W. and J.T. Cothren. 1999. Cotton: Origin, history, technology, and production. John Wiley and Sons, Inc., New York, New York.

Stewart, S.D., S.D. Akin, J. Reed, J. Bacheler, A. Catchot, D. Cook, J. Gore, J. Greene, A. Herbert, R.E. Jackson, D.L. Kerns, B.R. Leonard, G.M. Lorenz, S. Micinski, D. Reisig, P. Roberts, G. Studebaker, K. Tindall and M. Toews. 2013. Arthropod management & applied ecology: Survey of thrips species infesting cotoon across the southern U.S. cotton belt. The Journal of Cotton Science 17:263-269.

Stoner, A. 1970. Plant feeding by a predaceous insect, *Geocoris punctipes*. Journal of Economic Entomology 63:1911-1915.

Stoner, A. 1975. Plant feeding by Reduviidae, a Predaceous family (Hemiptera). Journal of the Kansas Entomological Society 48:185-188.

Stotzky, G. 2004. Persistence and biological activity in soil of the insecticidal proteins from *Bacillus thuringiensis,* especially from transgenic plants. Plant and Soil 266:77-89.

Sutcliffe, J.G. 1979. Complete nucleotide sequence of the *Escherichia coli* plasmid pBR322. Cold Spring Harbor Symposia on Quantitative Biology 43:77-90.

Székács, A., G. Weiss, D. Quist, E. Takács, B. Darvas, M. Meier, T. Swain and A. Hilbeck. 2012. Inter-laboratory comparison of Cry1Ab toxin quantification in MON 810 maize by enzyme-immunoassay. Food and Agricultural Immunology 23:99-121.

Tan, J., M.S. Paradise, S.L. Levine, P.M. Bachman, J.P. Uffman, C. Jiang and D.B. Carson. 2011. Development and survival of *Orius insidiosus* (Say) nymphs on encapsulated bee pollen-based diet in a Tier-I toxicity assay. Environmental Entomology 40:1613-1621.

Tapp, H. and G. Stotzky. 1998. Persistence of the insecticidal toxin from *Bacillus thuringiensis* subsp. *kurstaki* in soil. Soil Biology and Biochemistry 30:471-476.

Thanabalu, T. and A.G. Porter. 1996. A *Bacillus sphaericus* gene encoding a novel type of mosquitocidal toxin of 31.8 kDa. Gene 170:85-89.

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 34:1242-1256.

Torres, J.B. and J.R. Ruberson. 2008. Interactions of *Bacillus thuringiensis* Cry1Ac toxin in genetically engineered cotton with predatory heteropterans. Transgenic Research 17:345-354.

Trichilo, P.J. and T.F. Leigh. 1986. Predation on spider mite eggs by the western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripdae), an opportunist in a cotton agroecosystem. Enviornmental Entolology 15:821-825.

U.S. EPA. 1988. Guidance for the reregistration of pesticide products containing *Bacillus thuringiensis* as the active ingredient. 540/RS-89-023. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 2001. Sets of scientific issues being considered by the Environmental Protection Agency regarding: Bt plant-pesticides risk and benefit assessments. FIFRA Scientific Advisory Panel meeting, October 18-20, 2000. SAP Report No. 2000-07. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 2002. A set of scientific issues being considered by the Environmental Protection Agency regarding: Corn rootworm plant-incorporated protectant non-target insect and insect resistance management issues. FIFRA Scientific Advisory Panel meeting, August 27-29, 2002. SAP Meeting Minutes No. 2002-05. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 2004. A set of scientific issues being considered by the U.S. Environmental Protection Agency regarding: Product characterization, human health risk, ecological risk, and insect resistance management for *Bacillus thuringiensis* (*Bt*) cotton products. FIFRA Scientific Advisory Panel meeting, June 8-10, 2004. SAP Report No. 2004-05. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 2010a. Biopesticides registration action document: *Bacillus thuringiensis* Cry1A.105 and Cry2Ab2 insecticidal proteins and the genetic material necessary for their production in corn [PC Codes 006515 (Cry2Ab2), 006514 (Cry1A.105)]. U.S. Environmental Protection Agency, Office of Pesticide Programs, Biopesticides and Pollution Prevention Division, Washington, D.C.

U.S. EPA. 2010b. Biopesticides registration action document: *Bacillus thuringiensis* Cry3Bb1 protein and the genetic material necessary for its production (Vector PV-ZMIR13L) in MON 863 corn (OECD Unique Identifier: MON-ØØ863-5); and *Bacillus thuringiensis* Cry3Bb1 protein and the genetic material necessary for its production (Vector PV-ZMIR39) in MON 88017 Corn (OECD Unique Identifier: MON-88Ø17-3). U.S. Environmental Protection Agency, Office of Pesticide Programs, Biopesticides and Pollution Prevention Division, Washington, D.C.

U.S. EPA. 2018a. Pages 3601-3603 in *Bacillus thuringiensis* Cry51Aa2.834_16; Exemption From the Requirement of a Tolerance. U.S. Enviornmental Protection Agency, Washington, D.C.

U.S. EPA. 2018b. Registration Decision for the New Active Ingredient Bacillus thuringiensis Cry51 Aa2.834_ 16 and the Genetic Material (vector PV-GHIR508523) Necessary for its Production in MON 88702 Cotton (OECD Unique Identifier: MON-88702-4). U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 2019. Introduction to biotechnology regulation for pesticides. U.S. Environmental Protection Agency. Washington, D.C. https://www.epa.gov/regulation-biotechnology-under-tsca-and-fifra/introduction-biotechnology-regulation-pesticides [Accessed March 26, 2019].

U.S. FDA. 1992. Statement of policy: Foods derived from new plant varieties. Federal Register 57: 22984-23005.

U.S. FDA. 2001. Premarket notice concerning bioengineered foods. Federal Register 66:4706-4738.

U.S. FDA. 2018. Biotechnology consultation - Note to the file biotechnology notification file No. 000160. U.S. Food and Drug Administration, Washington, D.C.

Umbeck, P.F., K.A. Barton, E.V. Nordheim, J.C. McCarty, W.L. Parrott and J.N. Jenkins. 1991. Degree of pollen dispersal by insects from a field test of genetically engineered cotton. Journal of Economic Entomology 84:1943-1950.

USDA-AMS. 2001. The classification of cotton. Agricultural Handbook 566. U.S. Department of Agriculture, Agricultural Marketing Service, Washington, D.C.

USDA-APHIS. 1986. Coordinated framework for regulation of biotechnology. Federal Register 51:23302.

USDA-APHIS. 1995. USDA/APHIS determination on a petition 94-308-01p of Monsanto Agricultural Company seeking nonregulated status of lepidopteran-resistant cotton lines 531, 757, 1076: Environmental assessment and finding of no significant impact. U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Washington, D.C.

USDA-APHIS and U.S. EPA. 2007. White paper on tier-based testing for the effects of proteinaceous insecticidal plant-incorporated protectants on non-target arthopods for regulatory risk assessments. U.S. Department of Agriculture, Animal and Plant Health Inspection Service and U.S. Environmental Protection Agency, Biotechnology Regulatory Services and Biopesticides and Pollution Prevention Division, Washington, D.C.

USDA-FAS. 2019a. World agricultural production: Australia cotton: Production estimate down 48 percent from last year. U.S. Department of Agriculture, Foreign Agricultural Service, Washington, D.C.

USDA-FAS. 2019b. Cotton: World market and trade. USDA 2019/20 outlook shows higher global production and stocks. U.S. Department of Agriculture, Foreign Agricultural Service, Washington, D.C.

USDA-NASS. 2012. Quick stats: Cotton, acres planted, Pima - acres planted, Pima - price received, Upland acres. U.S. Department of Agriculture, National Agricultural Statistics Service, Washington, D.C. http://quickstats.nass.usda.gov/data/printable/2550EF99-0495-3D73-97B4-C71FFC453437 [Accessed April 4, 2012].

USDA-NASS. 2017. 2012 Census of agriculture, state profile. U.S. Department of Agriculture, National Agricultural Statistics Service, Washington, D.C.

USDA-NASS. 2018a. 2017 agricultural chemical use survey: Cotton. U.S. Department of Agriculture, National Agricultural Statistics Service, Washington, D.C.

USDA-NASS. 2018b. USDA-NASS Quick Stats. U.S. Department of Agriculture, National Agricultural Statistics Service. https://quickstats.nass.usda.gov/data/printable/75C56012-A673-3BB5-8898-C4734905C364 [Accessed March 21, 2019].

USDA-NASS. 2018c. Crop protection historical track records. U.S. Department of Agriculture, National Agricultural Statistics Service, Washington, D.C.

USDA-NASS. 2018d. Prima cotton 2017: Planted acres by county for selected states. U.S. Department of Agriculture, National Agricultural Statistics Service, Washington, D.C.

USDA-NASS. 2018e. Upland cotton: Planted acreage by county. U.S. Department of Agriculture, National Agricultural Statistics Service, Washington, D.C.

USDA-NASS. 2018f. Acreage (June 2018). U.S. Department of Agriculture, National Agricultural Statistics Service, Washington, D.C.

USDA-NASS. 2019. Crop Production. U.S. Department of Agricultre, National Agricultural Statictis Services, Washington, D.C.

USFWS. 1990. Recovery plan for the endangered and threatened speices of ash meadows, Nevada. U.S. Fish and Wild Life Services, Reno, Nevada.

USFWS. 1991. American burying beetle (*Nicrophorus americanus*) recovery plan. U.S. Fish and Wildlife Service, Newton Corner, Massachusetts.

USFWS. 1993. Puritan tiger beetle (*Cicindela puritana*) recovery plan. U.S. Fish and Wildlife Service, Northeast Region, Washington, D.C.

USFWS. 1994a. Northeastern beach tiger beetle (*Cincindela dorsalis dorsalis* Say) recovery plan. United States Fish and Wildlife Service, Hadley, MA.

USFWS. 1994b. Endangered Karst invertebrates (Travis and Williamson Counties, Texas) recovery plan. U.S. Fish and Wildlife Service, Washington, D.C.

USFWS. 1997. Endangered and threatened wildlife and plants; Final rule to list three aquatic invertebrates in Comal and Hays Counties, TX, as endangered. Federal Register 62:66295-66304.

USFWS. 2005a. Pages 48191-48192 in Endangered and threatened wildlife and plants; 5-year review of tooth cave ground beetle. Federal Registry.

USFWS. 2005b. Recovery plan for vernal pool ecosystems of California and Southern Oregon. U.S. Fish and Wildlife Service, Washington, D.C.

USFWS. 2006a. Hungerford's crawling water beetle (*Brychius hungerfordi*) recovery plan. Department of the Interior USFWS, Fort Snelling, MN.

USFWS. 2006b. Valley elderberry longhorn beetle (*Desmocerus californicus dimorphus*). 5-year review: Summary and Evaluation. U.S. Fish and Wildlife Service, Sacramento Fish and Wildlife Office, Sacramento, California.

USFWS. 2007. Endangered and threatened wildlife and plants; 12-month finding on a petition to list the Casey's June Beetle (Dinacoma caseyi) as endangered with critical habitat. Federal Register.

USFWS. 2008. American burying beetle (*Nicrophorus americanus*) 5-year review: summary and evaluation. U.S. Fish and Wildlife Service, New England Field Office, Concord, NH.

USFWS. 2009a. Coffin Cave Mold Beetle (Batrisodes texanus); 5- year review: Summary and evaluation. U.S. Fish and Wildlife Service, Austin, Texas.

USFWS. 2009b. Zayante band-winged grasshopper (*trimerotropis infantalis*) and Mount Hermon June Beetle (*Polyphylla barabata*) 5-Year review: Summary and evaluation. U.S. Fish and Wildlife Service, Ventura, California.

USFWS. 2009c. Ohlone tiger beetle (*Cicindela ohlone*) 5-Year review: Summary and evaluation. U.S. Fish and Wildlife Service, Ventura, California.

USFWS. 2011. *Rhadine exilis* (no common name), *Rhadine infernalis* (no common name), Madla Cave meshweaver (*Cicurina madla*), Braken Bat Cave meshweaver (*C. venii*), Government Canyon Bat Cave meshweaver (*C. vespera*), Robber Baron Cave meshweaver (*C. baronia*), Cokendolpher cave harvestman (*Texella cokendolpheri*),

Government Canyon Bat Cave spider (*Neoleptoneta microps*), and Helotes mold beetle (*Batrisodes venyivi*): 5- year review: Summary and evaluation. U.S. Fish and Wildlife Service, Austin, Texas.

USFWS. 2016. Recovery plan for the Salt Creek tiger beetle (*Cicindela nevadica lincolniana*). U.S. Fish and Wildlife Service, University of Nebraska, Lincoln,.

USFWS. 2017. Miami tiger beetle (Cicindelidia floridana). U.S. Fish and Wildlife Service, Environmental Conservation Online System, Washington, D.C. https://ecos.fws.gov/ecp0/profile/speciesProfile?spcode=I0XA [Accessed March 24, 2019].

USFWS. 2019a. Threatened and endangered animals. U.S. Fish and Wildlife Service, Washington, D.C. https://www.fws.gov/endangered/ [Accessed March 26, 2019].

USFWS. 2019b. American burying beetle (Nicrophorus americanus). U.S. Fish and Wildlife Service, Environmental Conservation Oline System, Washington, D.C. https://ecos.fws.gov/ecp0/profile/speciesProfile?spcode=I028 [Accessed March 24, 2019].

Van Deynze, A.E., F.J. Sundstrom and K.J. Bradford. 2005. Pollen-mediated gene flow in California cotton depends on pollinator activity. Crop Science 45:1565-1570.

van Frankenhuyzen, K. 2009. Insecticidal activity of *Bacillus thuringiensis* crystal proteins. J Invertebr Pathol 101:1-16.

van Frankenhuyzen, K. 2013. Cross-order and cross-phylum activity of *Bacillus thuringiensis* pesticidal proteins. J Invertebr Pathol 114:76-85.

Vaughn, T., T. Cavato, G. Brar, T. Coombe, T. DeGooyer, S. Ford, M. Groth, A. Howe, S. Johnson, K. Kolacz, C. Pilcher, J. Purcell, C. Romano, L. English and J. Pershing. 2005. A method of controlling corn rootworm feeding using a *Bacillus thuringiensis* protein expressed in transgenic maize. Crop Science 45:931-938.

Venkatesh, T.V., G.G. Harrigan, T. Perez and S. Flint-Garcia. 2015. Compositional assessments of key maize populations: B73 hybrids of the Nested Association Mapping founder lines and diverse landrace inbred lines. Journal of Agricultural and Food Chemistry 63:5282-5295.

Vyavhare, S.S., D. Kerns, C. Allen, R. Bowling, M. Brewer and M. Parajulee. 2017. Managing cotton insects in Teaxs. Texas A&M.

Wach, M., R.L. Hellmich, R. Layton, J. Romeis and P.G. Gadaleta. 2016. Dynamic role and importance of surrogate species for assessing potential adverse environmental impacts of genetically engineered insect-resistant plants on non-target organisms. Transgenic Research Online:1-7.

Waghmare, V.N., J. Rong, C.J. Rogers, G.J. Pierce, J.F. Wendel and A.H. Paterson. 2005. Genetic mapping of a cross between *Gossypium hirsutum* (cotton) and the Hawaiian endemic, *Gossypium tomentosum*. Theoretical and Applied Genetics 111:665-676.

WHO. 1995. Application of the principles of substantial equivalence to the safety evaluation of foods or food components from plants derived by modern biotechnology. World Health Organization, Geneva, Switzerland.

Williams, M.R. 2016. Cotton insect losses 2016. Mississippi State University, Mississippi State, Mississippi. http://www.entomology.msstate.edu/resources/croplosses/2016loss.asp [Accessed April 12, 2017].

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:e2118.

Xu, C., U. Chinte, L. Chen, Q. Yao, Y. Meng, D. Zhou, L.-J. Bi, M.J. Adang, B.-C. Wang, Z. Yu and M. Sun. 2015. Crystal structure of Cry51Aa1: A potential novel insecticidal aerolysin-type β-pore-forming toxin from *Bacillus thuringiensis*. Biochemical and Biophysical Research Communications 462:184-189.

Yabe, N., T. Takahashi and Y. Komeda. 1994. Analysis of tissue-specific expression of *Arabidopsis thaliana* HSP90-family gene *HSP81*. Plant and Cell Physiology 35:1207-1219.

Yang, L., C. Wang, A. Holst-Jensen, D. Morisset, Y. Lin and D. Zhang. 2013. Characterization of GM events by insert knowledge adapted re-sequencing approaches. Scientific Reports 3:2839.

Ye, X., E.J. Williams, J. Shen, S. Johnson, B. Lowe, S. Radke, S. Strickland, J.A. Esser, M.W. Petersen and L.A. Gilbertson. 2011. Enhanced production of single copy backbone-free transgenic plants in multiple crop species using binary vectors with a pRi replication origin in *Agrobacterium tumefaciens*. Transgenic Research 20:773-786.

Yu, X.-H., R. Rawat and J. Shanklin. 2011. Characterization and analysis of the cotton cyclopropane fatty acid synthase family and their contribution to cyclopropane fatty acid synthesis. BMC Plant Biology 11:97.

Zambryski, P., A. Depicker, K. Kruger and H.M. Goodman. 1982. Tumor induction by *Agrobacterium tumefaciens*: Analysis of the boundaries of T-DNA. Journal of Molecular and Applied Genetics 1:361-370.

Zastrow-Hayes, G.M., H. Lin, A.L. Sigmund, J.L. Hoffman, C.M. Alarcon, K.R. Hayes, T.A. Richmond, J.A. Jeddeloh, G.D. May and M.K. Beatty. 2015. Southern-by-sequencing: A robust screening approach for molecular characterization of genetically modified crops. The Plant Genome 8:1-15.

Zhi, J., D.C. Margolies, J.R. Nechols and J.E.J. Boyer. 2006. Host-plant-mediated interaction between populations of a true omnivore and its herbivorous prey. Entomologia Experimentalis et Applicata 121:59-66.

APPENDICES

Appendix A. USDA Notifications and Permits

Field trials of MON 88702 cotton have been conducted in the U.S. since 2011. The protocols for these trials include field performance, breeding and observation, agronomics, and generation of field materials and data necessary for this petition. In addition to the MON 88702 cotton phenotypic assessment data, observational data on pest and disease stressors were collected from these product development trials. The majority of the final reports have been submitted to the USDA. However, some final reports, mainly from the 2011-2018 seasons, are still in preparation. A list of trials conducted under USDA notifications or permits and the status of the final reports for these trials are provided in Table A-1.

Table A-1. USDA Notifications and Permits Approved for MON 88702 and Status of Trials Planted under These Notifications

Field Trial Year	USDA No.	Effective Date	Trial Status	Release State	Sites
2011	11-129-101rm	9/1/2011	Submitted to USDA	PR	1
2012	11-341-101rm	3/17/2012	Submitted to USDA	AZ	4
				CA	1
				MS	2
				PR	1
				TX	9
	12-086-101n	4/25/2012	Submitted to USDA	AR	3
				AZ	1
				CA	2
				IL	1
				LA	2
				MO	1
				MS	2
				NC	1
				SC	1
				TN	1
				TX	1
2013	12-312-110rm	3/28/2013	Submitted to USDA	PR	1
	13-058-104n	3/29/2013	Submitted to USDA	AR	2
				MO	1
				MS	1
				TN	2
				TX	9

Table A-1. USDA Notifications and Permits Approved for MON 88702 and Status of Trials Planted under These Notifications (continued)

Field Trial Year	USDA No.	Effective Date	Trial Status	Release State	Sites
2013	13-059-101n	3/30/2013	Submitted to USDA	AR	3
				AZ	2
				CA	2
				LA	2
				MS	1
				NC	1
				SC	1
				TX	3
	13-113-102rm	7/22/2013	Submitted to USDA	PR	1
2014	13-277-102n	11/15/2013	Submitted to USDA	PR	1
	13-288-101rm	2/22/2014	Submitted to USDA	AR	2
				MS	4
				TX	8
	14-049-101n	3/19/2014	Submitted to USDA	AR	3
				AZ	2
				CA	1
				LA	2
				MS	2
				NC	2
				SC	2
				TN	2
				TX	5
	14-072-107n	4/12/2014	Submitted to USDA	AL	3
				GA	1
				VA	1
	14-091-108n	5/1/2014	Submitted to USDA	PR	2
	14-114-102rm	9/1/2014	Submitted to USDA	PR	1
	14-283-103n	11/9/2014	Submitted to USDA	PR	1

Table A-1. USDA Notifications and Permits Approved for MON 88702 and Status of Trials Planted under These Notifications (continued)

Field Trial Year	USDA No.	Effective Date	Trial Status	Release State	Sites
2015	14-283-103n	11/9/2014	Submitted to USDA	PR	1
	15-048-102n	3/19/2015	Submitted to USDA	AR	1
				AZ	1
				GA	1
				LA	2
				MS	2
				SC	1
				TN	1
				TX	2
				VA	1
	15-062-106n	4/2/2015	Submitted to USDA	AL	1
				AR	1
				AZ	2
				CA	1
				GA	2
				LA	2
				MS	2
				NC	2
				SC	1
				TX	3
	15-089-103n	4/29/2015	Submitted to USDA	TX	1
	15-097-105n	5/7/2015	Submitted to USDA	PR	2
	15-281-104n	11/23/2015	Submitted to USDA	PR	1
2016	15-281-104n	11/23/2015	Submitted to USDA	PR	1
	16-033-101n	3/3/2016	Submitted to USDA	MS	2
				TN	2
				TX	1
				VA	1

Table A-1. USDA Notifications and Permits Approved for MON 88702 and Status of Trials Planted under These Notifications (continued)

Field Trial Year	USDA No.	Effective Date	Trial Status	Release State	Sites
2016	16-063-103n	4/2/2016	Submitted to USDA	AZ	1
				CA	1
				GA	1
				LA	2
				MS	2
				NC	2
				SC	1
				TX	4
	16-103-101n	5/12/2016	Submitted to USDA	PR	1
	16-281-101n	11/7/2016	Submitted to USDA	PR	1
2017	16-281-101n	11/7/2016	Submitted to USDA	PR	2
	17-046-104n	3/17/2017	Submitted to USDA	GA	1
	17-100-105n	5/15/2017	Submitted to USDA	PR	2
	17-283-102n	11/15/2017	Submitted to USDA	PR	1
2018	17-283-102n	11/15/2017	Submitted to USDA	PR	1
	17-303-101rm	3/1/2018	In Progress	PR	1
	18-050-101n	3/16/2018	In Progress	AZ	1
	18-061-104n	4/1/2018	In Progress	AR	3
				GA	1
				MO	1
				MS	3
				NC	1
				SC	1
				TX	4

Table A-1. USDA Notifications and Permits Approved for MON 88702 and Status of Trials Planted under These Notifications (continued)

Field Trial Year	USDA No.	Effective Date	Trial Status	Release State	Sites
2018	18-065-102n	4/5/2018	In Progress	AZ	2
				GA	1
				LA	1
				MS	1
				NC	1
				SC	1
				TX	1
	18-073-104n	4/12/2018	In Progress	AL	1
				AR	1
				AZ	1
				GA	2
				LA	2
				MS	3
				NC	1
				SC	1
				TN	2
				TX	2
				VA	1
	18-082-101n	4/19/2018	In Progress	NC	1
	18-113-101rm	9/1/2018	In Progress	PR	2
	18-264-101rm	11/14/2018	In Progress	TX	1
				AR	1
2019	18-113-101rm	9/1/2018	In Progress	PR	1

Appendix B. Overview, Materials, Methods and Supplementary Results for Molecular Analyses of MON 88702

B.1. Test Substance

The test substance in this study was MON 88702. Five breeding generations of MON 88702 were used to assess the stability of the T-DNA I insert. Genomic DNA for use in this study was extracted from seed tissue listed in the table below.

Generation	Seed Lot Number
R3	11411259
R4	11411260
R5	11411261
R6	11411262
R7	11411263

B.2. Control Substance

The control substance is the conventional cotton variety DP393 that has a similar genetic background as the MON 88702 generations. Genomic DNA for use in this study was extracted from seed tissue listed in the table below.

Control Substance	Seed Lot Number
DP393	11408073

B.3. Reference Substance

The reference substance was plasmid vector PV-GHIR508523, which was used to develop MON 88702. Whole plasmid DNA and its sequence served as a positive control for sequencing and bioinformatic analyses. The identity of the reference plasmid was confirmed by sequencing within the study. Documentation of the confirmation of the plasmid vector identity was archived with the raw data. Appropriate molecular weight markers from commercial sources were used for size estimations on agarose gels. The unique identity of the molecular weight markers was documented in the raw data.

B.4. Characterization of Test, Control, and Reference Substances

The identities of the test substance and the conventional control substance were confirmed by the sequencing in the study. The Study Director reviewed the chain of custody documentation to confirm the identity of the test and control substances prior to the use of these materials in the study.

Test, control and reference DNA substance were considered stable during storage if they yielded interpretable signals in sequencing experiments and/or did not appear visibly degraded on the stained gels.

B.5. Genomic DNA Isolation

For sequencing library construction and PCR reactions, genomic DNA was isolated from seed tissues of the test and control substances. First the seeds were decontaminated by vigorously agitating them by hand for 30 seconds with 0.05% (v/v) Tween-20, followed by a tap water rinse. The seeds were then vigorously agitated with 0.5% (w/v) NaOCl, allowed to stand for one minute at room temperature, and rinsed with tap water. The seeds were then vigorously agitated with 1% (v/v) HCl, allowed to stand for one minute at room temperature, and rinsed with tap water. The 1% (v/v) HCl rinse was repeated one time, and then the seeds were rinsed with distilled water and placed in a drying oven at 80°C to dry. The dried seeds were ground to a fine powder in a Harbil paint shaker. Genomic DNA was extracted using a hexadecyltrimethylammonium bromide (CTAB) extraction protocol. Briefly, 16 ml CTAB buffer (1.5% (w/v) CTAB, 75 mM Tris HCl (pH 8.0), 100 mM EDTA (pH 8.0), 1.05 M NaCl, and 0.75% (w/v) PVP) and RNase A was added to ground seed tissue. The samples were incubated at 64°C-66°C for 25-35 minutes with intermittent mixing. The samples were cooled to room temperature and subjected to multiple rounds of chloroform:isoamyl alcohol (24:1) extraction. An additional round of extraction with 10% CTAB solution (10% (w/v) CTAB and 0.7 M NaCl) and chloroform:isoamyl alcohol (24:1) was performed. Genomic DNA was precipitated by adding ~1.5-2× volumes of CTAB precipitation buffer (1% (w/v) CTAB, 50 mM Tris HCl (pH 8.0), 10 mM EDTA (pH 8.0) to the samples, followed by resuspension in high salt TE buffer (10 mM Tris HCl (pH 8.0), 1 mM EDTA (pH 8.0), 1 M NaCl). Genomic DNA was precipitated again with 3 M sodium acetate (pH 5.2) and 100% (v/v) ethanol, washed with 70% ethanol, air dried and resuspended in TE buffer (10 mM Tris HCl, 1 mM EDTA, pH 8.0). All extracted DNA was stored in a 4°C refrigerator.

B.6. DNA Quantification

PV-GHIR508523 DNA and extracted genomic DNA were quantified using a Qubit® Fluorometer (Invitrogen) or a Nanodrop™ Spectrophotometer (Thermo Scientific).

B.7. Agarose Gel Electrophoresis

After quantification, approximately 0.5 µg of the extracted DNA for NGS library construction was run on a 1% (w/v) agarose gel to check the quality.

B.8. Shearing of DNA

Approximately 1 µg of DNA from the test, control and reference substances were sheared using a Covaris S-220 ultrasonicator. The DNA was diluted to ~20 ng/µl in Buffer EB (Qiagen Inc.) and fragmented using the following settings to create approximately 400 bp fragments with 3′ or 5′ overhangs: duty cycle of 10; peak incident power of 175; intensity of 5.0, 200 bursts per cycle, in the frequency sweeping mode at ~3-10°C for 90 seconds for test and control DNA or 60 seconds for reference DNA.

B.9. Bioanalyzer Analysis

One microliter of sheared genomic DNA was diluted 1:10 in Buffer EB and run on a DNA High Sensitivity chip on an Agilent 2100 Bioanalyzer (Agilent Technologies) to check the quality of the shearing. After preparing the chip, 1 µl of each diluted DNA sample or water was added to individual wells and the chip was run on the Bioanalyzer using the dsDNA, High Sensitivity Assay.

B.10. Paired End Library Preparation

Paired end genomic DNA libraries were prepared for the test, control, and reference substances using the KAPA Hyper Prep kit (Kapa Biosystems) and a Sage Science PippinHT DNA Size Selection system (Sage Science Inc.) was used to size select the DNA fragments.

First, the 3′ and 5′ overhangs of the DNA fragments generated by the shearing process were converted into blunt ends and adenylated by following the manufacturer's KAPA Hyper Prep kit instructions (Kapa Biosystems).

Next, adaptors were ligated to the end repaired and A-tailing reaction product by following the manufacturer's KAPA Hyper Prep kit instructions. Following adaptor ligation, an AMPure XP (Beckman Coulter) cleanup was performed on the libraries which were then resuspended in 25 µl of Buffer EB prior to PCR amplification of the libraries. A five cycle PCR amplification of the libraries were carried out following the manufacturer's KAPA Hyper Prep kit instructions. A second AMPure XP cleanup was performed on the libraries which were then resuspended in 22.5 µl of Qiagen Elution Buffer (EB) and stored at 4°C.

The libraries were run on the Sage Science PippinHT Size Selection system using 1.5% agarose gel cassettes and following the manufacturer's PippinHT Quick Guide instructions. After elution of the desired size range (~500 bp +/- ~58 bp) of DNA fragments, the DNA sample in the elution chamber of the cassette was removed from the cassette by pipette and transferred into PCR strip tubes.

After removal from the Pippin Prep, the libraries were again purified with the AMPure XP cleanup procedure and resuspended in 32.5 µl of Buffer EB. Finally, 1 µl of each DNA library was diluted 1:10 in Buffer EB for running in a DNA High Sensitivity chip on an Agilent 2100 Bioanalyzer as described above in Section B.9. All purified library DNA was stored in a -20°C freezer.

B.11. Next-Generation Sequencing

The library samples described above were sequenced by Monsanto's Sequencing Technologies using Illumina HiSeq technology that produces short sequence reads (~125 bp long). Sufficient numbers of these sequence fragments were obtained to comprehensively cover the entire genomes of the test samples and the conventional control (Kovalic et al., 2012). Furthermore, a transformation plasmid spike was sequenced to >75× to assess method sensitivity through modeling of $1/10^{th}$ and one full genome equivalent plasmid spike.

B.12. Read Mapping and Junction Identification

High-throughput sequence reads were captured by aligning to the PV-GHIR508523 transformation plasmid sequence using the read alignment software Bowtie (V2.2.3) (Langmead and Salzberg, 2012) in order to collect all reads that were sourced from the plasmid as well as reads with sequences representing integration points. Captured reads were subsequently mapped using FASTA (V36.3.6) local alignment program (Pearson, 2000).

Captured reads from both test and control samples were mapped to the complete PV-GHIR508523 transformation plasmid sequence in order to detect junction sequences using the FASTA (V36.3.6) local alignment program. Reads with partial matches to the transformation plasmid of at least 30 bases and 96.6% or greater identity were also collected as potential junction sequences (Kovalic et al., 2012).

Table B-1. Summary NGS Data for the Control and Test Substances

Sample	Total Nucleotide Number (Gb)	Effective Median Depth of Coverage (×fold)
DP393	321.8	80×
MON 88702 (R3)	257.5	89×
MON 88702 (R4)	255.3	82×
MON 88702 (R5)	255.8	90×
MON 88702 (R6)	282.3	89×
MON 88702 (R7)	321.3	105×

Table B-2. Summary NGS Data for the Reference Substance

	0.1 Genome Equivalent (8× coverage)	1 Genome Equivalent (80× coverage)
Extent of coverage[1] of PV-GHIR508523	100%	100%
Percent identity of coverage[2] of PV-GHIR508523	100%	100%

[1] Extent of coverage is calculated as the percent of all PV-GHIR508523 bases observed in random sampling of reads.

$$\text{extent of coverage} = \frac{\text{number of spike in bases detected}}{\text{total length (bp)of spike in plasmid}} \times 100$$

[2] Percent identity of coverage is calculated as the percent of all PV-GHIR508523 bases observed in random sampling of reads.

$$\text{Percent identity of coverage} = \frac{\text{number of identical bases (spike in vs. plasmid sequence) detected}}{\text{total length (bp)of spike in plasmid detected}} \times 100$$

B.13. PCR and DNA Sequence Analyses to Examine the Insert and Flanking Sequences in MON 88702

Overlapping PCR products, denoted as Product A and Product B were generated that span the insert and adjacent 5′ and 3′ flanking DNA sequences in MON 88702. For each fragment generation, experimental conditions were chosen to successfully produce on-target amplifications. These products were analyzed to determine the nucleotide sequence of the insert in MON 88702, as well as that of the DNA flanking the 5′ and 3′ ends of the insert.

The PCR analyses for both Product A and Product B were conducted using 100ng of genomic DNA template in a 50µl reaction volume. The reaction contained a final concentration of 0.2µM of each primer, 0.2mM of each dNTP, and 1.25 units/reaction of PrimeSTAR GXL Polymerase (TaKaRa Bio Inc.).

The PCR amplification of both Product A and Product B was performed under the following cycling conditions: 30 cycles at 98°C for 10 seconds; 64°C for 15 seconds; and 68°C for 5 minutes.

Aliquots of each PCR product were separated on a 1.0% (w/v) agarose gel and visualized by ethidium bromide staining to verify that the products were the expected sizes. Each PCR product was purified with ExoSAP-IT PCR Product Cleanup (Affymetrix). The treated PCR products were sequenced using multiple primers, including primers used for PCR amplification. All sequencing was performed by Sequencing Technologies using BigDye terminator chemistry (Applied Biosystems).

A consensus sequence was generated by compiling sequences from multiple sequencing reactions performed on the overlapping PCR products. This consensus sequence was aligned to the PV-GHIR508523 sequence to determine the integrity and organization of the integrated DNA and the 5′ and 3′ insert-to-flank DNA junctions in MON 88702.

B.14. PCR and DNA Sequence Analyses to Examine the Integrity of the DNA Insertion Site in MON 88702

To examine the MON 88702 T-DNA I insertion site in conventional cotton, PCR and sequence analyses were performed on genomic DNA from the conventional control.

The primers used in this analysis were designed from the DNA sequences flanking the insert in MON 88702. A forward primer specific to the DNA sequence flanking the 5′ end of the insert was paired with a reverse complement primer specific to the DNA sequence flanking the 3′ end of the insert.

The PCR reactions were conducted using 100ng of genomic DNA template in a 50µl reaction volume. The reaction contained a final concentration of 0.2µM of each primer, 0.2mM of each dNTP, and 1.25 units/reaction of PrimeSTAR GXL Polymerase (TaKaRa Bio Inc.). The PCR amplification was performed under the following cycling conditions: 30 cycles at 98°C for 10 seconds; 64°C for 15 seconds; and 68°C for 5 minutes.

Aliquots of PCR product were separated on a 1.0% (w/v) agarose gel and visualized by ethidium bromide staining to verify that the PCR product of the expected size was produced. Each PCR product was purified with ExoSAP-IT PCR Product Cleanup (Affymetrix). The treated PCR product was sequenced using multiple primers, including primers used for PCR amplification. All sequencing was performed by Sequencing Technologies using BigDye terminator chemistry (Applied Biosystems).

A consensus sequence was generated by compiling sequences from multiple sequencing reactions performed on the verified PCR product. This consensus sequence was aligned to the 5′ and 3′ sequences flanking the MON 88702 insert to determine the integrity and any rearrangement of the insertion site.

References for Appendix B

Kovalic, D., C. Garnaat, L. Guo, Y. Yan, J. Groat, A. Silvanovich, L. Ralston, M. Huang, Q. Tian, A. Christian, N. Cheikh, J. Hjelle, S. Padgette and G. Bannon. 2012. The use of next generation sequencing and junction sequence analysis bioinformatics to achieve molecular characterization of crops improved through modern biotechnology. The Plant Genome 5:149-163.

Langmead, B. and S.L. Salzberg. 2012. Fast gapped-read alignment with Bowtie 2. Nature Methods 9:357-359.

Appendix C. Materials and Methods for the Analysis of the Levels of mCry51Aa2 Protein in MON 88702

C.1. Materials

Tissue samples from five and four sites in the U.S. were harvested from MON 88702 during the 2015 and 2018 growing seasons, respectively. *Bacillus thuringiensis*-produced mCry51Aa2 was used as the analytical reference standard.

C.2. Characterization of the Materials

The identities of the test substances were confirmed by analysis of the starting seed DNA by an event-specific polymerase chain reaction method.

C.3. Field Design and Tissue Collection

Field trials were initiated during the 2015 and 2018 planting season to generate tissues of MON 88702 at various cotton growing locations in the U.S. In 2015, leaf (OSL1 and OSL4), root, pollen, and seed tissue samples from the following field sites were analyzed: Graham County, Arizona (AZSA); Rapides County, Louisiana (LACH); Washington County, Mississippi (MSLE); Perquimans County, North Carolina (NCBD); San Patricio County, Texas (TXPO). In 2018, leaf (OSL1, OSL2, OSL3 and OSL4), square (Square1, Square2, Square3 and Square4) and pollen tissue samples from the following field sites were analyzed: Yuma County, Arizona (AZSA); Rapides County, Louisiana (LACH); Washington County, Mississippi (MSGV); Barnwell County, South Carolina (SCEK). At each site, four replicated plots of plants containing MON 88702 were planted using a randomized complete block field design. Tissue samples were collected from each replicated plot at all field sites. See Table V-1 and Table V-2 for detailed descriptions of when the samples were collected.

C.4. Tissue Processing and Protein Extraction

The mCry51Aa2 protein was extracted from each tissue by adding the appropriate volume of extraction buffer and beads, and shaking in a Genogrinder (SPEX, NJ). The extracted samples were clarified by centrifugation. The protein extracts were aliquotted and stored frozen in a -80°C freezer until analysis. The tissue extraction parameters are described in Table C-1.

Table C-1. MON 88702 mCry51Aa2 Protein Extraction Parameters for Tissue Samples[1]

Sample Type	Tissue to Buffer Ratio[3]	Extraction Buffer
Leaf[1]	1:100	TB[2]
Root	1:100	TB
Seed	1:100	TB
Square	1:100	TB
Pollen	1:100	TB

[1]Over- season leaf (OSL1, OSL2, OSL3 and OSL4)
[2]Trisborate buffer (pH 7.8) [0.1 M Tris, 0.1 M $Na_2B_4O_7$, 0.005 M $MgCl_2$, 0.05% (v/v) Tween20]
[3]Ratio +/- 10%

Extraction efficiency is a parameter determined during the validation of every immunoassay method and as its name implies it determines the efficiency of the extraction of the protein to be analyzed from each specific tissue. This parameter depends on the protein, the tissue matrix, the buffer and conditions used which can influence the level of extraction; every effort is made to extract the maximum amount of protein within a buffer system compatible with an immunoassay. A recent guidance (EFSA, 2018), has led to an approach whereby the extraction of a protein under native conditions needs to be followed by extraction and analysis under denaturing conditions; the proteins are extracted using a harsh buffer and analysis is done by western blot. This new process has the potential of changing the extraction efficiencies for each tissue and further requires the calculation and application of a correction factor as follows:

$$\text{Correction Factor} = \frac{100}{\%\ \text{Extraction Efficiency}}$$

$$\text{Corrected value} = \text{Expressed value} \times \text{Correction Factor}$$

Therefore, in compliance with this guidance, the extraction efficiencies of the mCry51Aa2 protein in leaf, square, pollen and root tissue, together with the corresponding correction factors, were determined according to the method described by EFSA (Table C-2). The results demonstrate that the extraction efficiency for mCry51Aa2 in these tissues is well within the acceptable limits of extraction efficiency and would not be distinguishable from the typical variation in any immunoassay. Considering the high extraction efficiencies reported in Table C-2, the application of any of the correction factors is not expected to significantly impact the existing protein expression values. Considering the use of these tissues for exposure assessment to non-target organisms (NTOs), it can be concluded that there is no impact on the safety assessment from the application of this correction factor and they were therefore not applied to the reported expression levels.

Table C-2. Extraction Efficiencies for the mCry51Aa2 Protein in MON 88702 Cotton Tissues Determined Following the EFSA Recommendations (EFSA, 2018)

Tissue Type	Extraction Efficiency (%)	Correction Factor
Leaf	91	1.10
Square	91	1.10
Pollen	96	1.04
Root	80	1.25

C.5. Antibody

Mouse anti-mCry51Aa2 clone 3-7.2.5 was purified using Protein A affinity chromatography. The concentration of the purified antibody was determined to be 3.28 mg/mL by spectrophotometric methods. The purified antibody was stored in phosphate buffered saline (0.001 M KH_2PO_4, 0.01 M Na_2HPO_4, 0.137 M NaCl, 0.0027 M KCl) with 15mM NaN3.

Goat polyclonal antibodies specific for the mCry51Aa2 protein were purified using Protein G affinity chromatography. The purified antibodies were coupled with biotin (Thermo Fisher Scientific), according to the manufacturer's instructions. The detection reagent was NeutrAvidin conjugated to horseradish peroxidase (Thermo Fisher Scientific) for the ELISA method (samples from the 2015 growing season) and streptavidin conjugated to R-Phycoerythrin (Thermo Fisher Scientific) for the immunoassay method (samples from the 2018 growing season).

C.6. Immunoassay Methods

Immunoassays were used for analysis of samples from 2015 and 2018, respectively. These assays are equivalent antibody based methods for determining protein expression (Yeaman et al., 2016).

C.6.1. ELISA Method

For ELISA analysis of 2015 samples, mouse anti-mCry51Aa2 capture antibody was diluted in a coating buffer (0.015 M Na_2CO_3 and 0.035 M $NaHCO_3$ with 150mM NaCl) and immobilized onto 384-well microtiter plates at 4µg/mL followed by incubation in a 4°C refrigerator for ≥8 h. Prior to each step in the assay, plates were washed with 1× phosphate buffered saline containing 0.05% (v/v) Tween 20). Plates were blocked with the addition of 50µl per well of blocking buffer, 5% (w/v) bovine serum albumin in 1× phosphate buffered saline containing 0.05% (v/v) Tween 20 for 60 to 90 minutes at 37°C. mCry51Aa2 protein standard or sample extract was added at 25µl per well and incubated for 60 to 70 minutes at 37°C. Biotinylated goat anti-mCry51Aa2 antibodies were added at 25 µl per well and incubated for 60 to 70 minutes at 37 C. NeutrAvidin-horseradish peroxidase conjugate was added at 25µl per well and incubated for 60 to 70 minutes at

37°C. Plates were developed by adding 25µl per well of horseradish peroxidase substrate, 3, 3', 5, 5' tetramethylbenzidine (TMB, Sigma-Aldrich). The enzymatic reaction was terminated by the addition of 25µl per well of 6 M H_3PO_4. Quantification of the mCry51Aa2 protein was accomplished by interpolation from a mCry51Aa2 protein standard curve.

C.6.2. Multiplexed Immunoassay Method

For the multiplexed immunoassay analysis of 2018 samples, capture antibodies were covalently coupled to xMAP beads (Luminex Corp., Austin, TX) using the Antibody Coupling Kit (Luminex Corp., Austin, TX) as per the manufacturer's instructions. Antibody-coupled xMAP beads specific to the mCry51Aa2 protein were diluted in sample buffer (1 × PBS containing 1% BSA (w/v)) to a final concentration of 50 beads/µl. Standards, QCs, and tissue samples were added to wells as appropriate followed by diluted beads and incubated for 30-60 minutes (min) at room temperature (RT) while shaking on a plate shaker set at ~800. Plates were washed with 1 × PBS containing 0.05 % (v/v) Tween 20 (1 × PBST). Biotinylated secondary antibodies were prepared in sample buffer containing 0.5% non fat dry milk (NFDM) (w/v) and 0.5 mg/ml of mouse, rabbit, and goat IgG. The biotinylated antibody was added and incubated for 30-60 min at RT while shaking on a plate shaker set at ~800. Plates were washed with 1 × PBST. Streptavidin RPE conjugate was added at a final concentration of 4 µg/ml and incubated for 30-60 min at RT by shaking on a plate shaker set at ~800. Plates were washed with 1 × PBST. The beads were re-suspended by adding sample buffer and shaking on a plate shaker set at 800 for at least 10 min at RT. Quantification of the mCry51Aa2 protein was accomplished by interpolation from the protein standard curve.

C.7. Data Analyses

All ELISA plates were analyzed on a SPECTRAmax Plus 384 (Molecular Devices) microplate spectrophotometer, using a dual wavelength detection method. Protein concentrations were determined by optical absorbance at a wavelength of 450 nm with a simultaneous reference reading of 620 nm. Data reduction analyses were performed using Molecular Devices SOFTmax PRO GxP software. Absorbance readings and protein standard concentrations were fitted with a five-parameter logistic curve fit.

Multiplexed immunoassay plates were analyzed on the FLEXMAP 3D (Luminex Corp., Austin, TX). Plates were run as a batch on FLEXMAP 3D using appropriate protocol, standards and control definitions. Data reduction analyses were performed using Milliplex Analyst software. Protein standard concentrations for each of the reference standards were fitted to a curve by the software using a best fit analysis (i.e. multiple models are fitted and the statistical best fit is used).

Milliplex analyst software mathematically determined MinDC (experimental lower limit of quantification, LLOQ) and MaxDC (experimental upper limit of quantification, ULOQ) for each individual curve where sufficiently accurate determinations of concentration can be determined. Tissue LODs (limitation of detection) were determined

by establishing the mean apparent concentrations of negative samples and adding three standard deviations. The final LOQ is defined as the tissue LOD value, or the MinDC value determined by the software for an individual run, whichever is greater.

Following the interpolation from the standard curve, for data that were greater than or equal to the LOQ, the protein levels (ng/mL) in the tissues were converted to a µg/g dw value utilizing a sample dilution factor and a tissue-to-buffer ratio.

For the samples analyzed from the 2015 field trial, Microsoft Excel 2007 (Microsoft) was used to calculate the protein levels in cotton tissues. The sample means, standard deviations (SDs), and ranges were also calculated by Microsoft Excel 2007. All protein expression levels were rounded to two significant figures.

For the samples analyzed from the 2018 field trial, Core Informatics Laboratory Information Management System (LIMS, version 5.1.28) was used to calculate the protein levels in cotton tissues. The sample means, standard errors (SE), and ranges were calculated using a .NET Core application. All protein expression levels were rounded to two significant figures.

References for Appendix C

EFSA (European Food Safety Authority), Paraskevopoulos K, Ramon M, Dalmay T, du Jardin P, Casacuberta J, Guerche P, Jones H, Nogué F, Robaglia C, Rostoks, N 2018. Explanatory note on the determination of newly expressed protein levels in the context of genetically modified plant applications for EU market authorisation. EFSA supporting publication 2018:EN-1466. 13 pp. doi:10.2903/sp.efsa.2018.EN-1466

Yeaman, G., S. Paul, I. Nahirna, Y. Wang, A.E. Deffenbaugh, Z.L. Liu and K.C. Glenn. 2016. Development and Validation of a Fluorescent Multiplexed Immunoassay for Measurement of Transgenic Proteins in Cotton (Gossypium hirsutum). Journal of Agricultural and Food Chemistry. 64(24):5117-5127.

Appendix D. Materials and Methods for Compositional Analysis of MON 88702 Cottonseed

Compositional comparisons between MON 88702 and the conventional control cotton were performed using the principles outlined in the OECD consensus documents for cotton composition (OECD, 2009). These principles are accepted globally and have been employed previously in assessments of cotton products derived through biotechnology. The compositional assessment was conducted on cottonseed harvested from a single growing season conducted in the U.S. during 2015 under typical agronomic practices.

D.1. Materials

Harvested cottonseed from MON 88702 and a conventional control that has similar genetic background to that of MON 88702 were compositionally assessed.

D.2. Characterization of the Materials

The identities of MON 88702 and the conventional control were confirmed prior to use in the compositional assessment.

D.3. Field Production of Samples

Cottonseed samples were harvested from MON 88702 and the conventional control grown at five locations in the U.S. during the 2015 season. The field sites were planted in a randomized complete block design with four replicates per site. MON 88702 and the conventional control were grown under normal agronomic field conditions for their respective growing regions.

D.4. Summary of Analytical Methods

Anti-nutrients assessed in cottonseed included total gossypol, free gossypol, malvalic acid, sterculic acid and dihydrosterculic acid. Moisture was also assessed for the purpose of converting each component from fresh weight to dry weight for statistical analysis and final reporting; however, moisture was not statistically analysed.

D.4.1. Moisture

Subsamples of ground cottonseed were dried to a constant weight in a vacuum oven at 100°C and at least 25 inches of mercury pressure for at least 15 hours. Moisture content was determined gravimetrically. There was no analytical reference standard for this analysis. The reporting limit was 0.01%. Moisture results were reported on a percent fresh weight basis.

D.4.2. Cyclopropenoid Fatty Acids

The amount of cyclopropenoid fatty acids in ground cottonseed was determined by Gas Chromatography (GC) with Flame Ionization Detection (FID) following microwave-

assisted fat extraction and derivatization of the fatty acids into methyl esters with sodium methoxide/methanol. The following analytical reference standards were used:

D.4.3. Reference Standards

Methyl dihydrosterculate – lot number 23608 with purity >98%. The reporting limits for malvalic acid, dihydrosterculic acid and sterculic acid were 0.00302%.

D.4.4. Internal Standards

The fatty acid analytical reference standards were purchased from Nu-Chek Prep, Inc. with the exception of methyl dihydrosterculate which was purchased from Matreya, LLC. The fatty acid results were reported on a percent fresh weight basis.

D.5. Free Gossypol

Free gossypol was extracted from ground cottonseed samples using an acetone/DI water solution. The extracted gossypol was reacted with aniline to form dianilinogossypol. The percentage of free gossypol was determined by spectrophotometric measurement of the absorbance of dianilinogossypol. The gossypol acetic acid analytical reference standard was purchased from Sigma-Aldrich. The lot number was 085M4201V with a purity of 95.53%. The reporting limit was 0.0250%. Free gossypol results were reported on a percent fresh weight basis.

D.6. Total Gossypol

Free and bound forms of gossypol were extracted with 3-amino-1-propanol in dimethylformamide to form a diaminopropanol complex. This complex was reacted with aniline to form dianilinogossypol which was measured by spectrophotometric absorbance. The same gossypol acetic acid analytical reference standard used in free gossypol determination was also used in total gossypol determination. The reporting limit was 0.0500%. Total gossypol results were reported on a percent fresh weight basis.

D.7. Data Processing and Analytics

After compositional analyses were performed, data spreadsheets containing individual values for each analysis were sent to Monsanto Company for review. Data were then transferred to Certus International, Inc., where they were converted into the appropriate units and statistically analysed. The following formulas were used for re-expression of composition data for statistical analysis (Table D-1):

Table D-1. Re-expression Formulas for Statistical Analysis of Composition Data

Component	From (X)	To	Formula[1]
Free Gossypol, Total Gossypol	% fw	% dw	X/d
Dihydrosterculic Acid, Malvalic Acid, Sterculic Acid (Fatty Acids = FA)	% fw	% Total FA	$(100)X_j/\Sigma X$, for each antinutrient FA_j listed, where ΣX is over all the FA and antinutrients
[1]'X' is the individual sample value; d is the fraction of the sample that is dry matter.			

In order to complete a statistical analysis for a compositional constituent in this compositional assessment, at least 50% of all the values for an analyte in cottonseed had to be greater than the assay limit of quantitation (LOQ). No analytes with more than 50% of observations below the assay LOQ were observed.

The following randomized complete block design model was used for the combined-site analysis for each component.

$$Y_{ijk} = \mu + S_i + R(S)_{j(i)} + M_k + (SM)_{ik} + \varepsilon_{ijk}$$

where:

Y_{ijk} is the observed response for the k_{th} substance in the j_{th} replicate of the i_{th} site;

μ is the overall mean;

S_i is the random effect of the i_{th} site;

$R(S)_{j(i)}$ is the random effect of the j_{th} replicate nested with the i_{th} site;

M_k is the fixed effect of the k_{th} substance;

$(SM)_{ik}$ is the random effect of the interaction between the i_{th} site and k_{th} substance;

ε_{ijk} is the residual error.

SAS PROC MIXED was used to fit model (1) separately for each component to conduct an analysis of variance (ANOVA). Studentized residuals were obtained to detect potential outliers in the dataset. Studentized residuals tend to have a standard normal distribution when outliers are absent. Thus, most values are expected to be between ± 3. Data points

that are outside of the ± 6 studentized residual ranges are considered as potential outliers. No value had a studentized residual outside of the ± 6 range for this study.

The ANOVA model (1) assumes that the experimental errors are independent, normally-distributed, and have common variance. In this analysis, independence of the errors was controlled by the randomized complete block design. The normality and common variance assumptions were checked by visual examination of residual plots and histograms. No extreme violations were observed for any characteristic.

References for Appendix D

OECD. 2009. Consensus document on the compositional considerations for new varieties of cotton (*Gossypium hirsutum* and *Gossypium barbadense*): Key food and feed nutrients and anti-nutrients. ENV/JM/MONO(2004)16. Organisation for Economic Co-operation and Development, Paris, France.

Appendix E. Materials and Methods for Seed Dormancy and Germination Assessment of MON 88702

E.1. Materials

Seed germination and dormancy characteristics were assessed on seed from MON 88702, the conventional control, and reference varieties produced in replicated field trials during 2015 at the following sites: Perquimans County, North Carolina; San Patricio County, Texas and Uvalde County, Texas (Table E-1).

E.2. Characterization of the Materials

The identities of the MON 88702 and the conventional control starting seed were verified by event-specific PCR analyses. During the growing season, the field planting order of MON 88702 and the conventional control plots was confirmed by event-specific PCR analyses. Chain-of-custody documentation for all starting seed for this germination and dormancy study was maintained from harvest through shipment to the performing laboratory with the use of packaging labels and plant sample transfer forms.

E.3. Germination Testing Facility and Experimental Methods

Dormancy and germination evaluations were conducted at Eurofins BioDiagnostics, Inc. in River Falls, Wisconsin. The Principal Investigator was qualified to conduct seed dormancy and germination testing consistent with the standards established by the Association of Official Seed Analysts (AOSA, 2016a, b; AOSA/SCST, 2010).

The seed lots of MON 88702, the conventional control, and four reference varieties from each location were tested under six different temperature regimes. Six germination chambers were used in this study, and each chamber was maintained dark under one of the following six temperature regimes: constant temperature of approximately 10, 20, or 30 °C or alternating temperatures of approximately 10/20, 10/30, or 20/30 °C. The alternating temperature regimes were maintained at the lower temperature for approximately 16 hours and the higher temperature for approximately eight hours. The temperature inside each germination chamber was monitored and recorded throughout the duration of the study.

Approximately 50 seeds each of MON 88702, the conventional control, and the reference hybrids were placed on pre-moistened germination towels. Additional pre-moistened germination towels were placed on top of the seed. The germination towels were then rolled up in a wax cover. All rolled germination towels were labeled and placed into an appropriately labeled bucket. Each bucket within a temperature regime represented a replication per site. There were eight replications per site for a total of 12 buckets for each temperature regime. Each bucket contained one towel per entry. Buckets were then placed in the appropriate germination chambers. Each temperature regime constituted a separate split-plot experiment with eight replications. A description of each germination characteristic evaluated and the timing of evaluations is presented in Table VII-2. The types of data collected depended on the temperature regime. Each rolled germination

towel in the AOSA-recommended temperature regime (i.e., alternating 20/30 °C) was assessed periodically during the study for normally germinated, abnormally germinated, hard (viable and non-viable), dead, and firm swollen (viable and non-viable) seed as defined by AOSA guidelines (AOSA, 2016a, b; AOSA/SCST, 2010). AOSA only provides guidelines for testing seed under a single regime of optimal temperature regime (20/30 °C), whereas five additional temperature regimes were included to test diverse environmental conditions. Therefore, each rolled germination towel in the additional temperature regimes (i.e., 10, 20, 30, alternating 10/20, and alternating 10/30 °C) was assessed periodically during the study for germinated, hard (viable and non-viable), dead, and firm swollen (viable and non-viable) seed. Because temperature extremes could affect the development of seedlings, AOSA standards were not applied, and no distinction was made between normal or abnormal germinated seed. Therefore, any seedling with a radicle of 1 mm or more was classified as germinated.

The calculation of percent seed in each assessment category was based on the actual number of seeds evaluated (e.g., 99 or 101). Across temperature regimes, the total number of seeds evaluated from each germination towel was approximately 100.

Within both AOSA and the additional temperature regimes, hard and firm-swollen seeds remaining at the final evaluation date were subjected to a tetrazolium (Tz) test for evaluation of viability according to AOSA standards (AOSA, 2016a, b; AOSA/SCST, 2010). The number of non-viable hard and non-viable firm swollen seed was added to the number of dead seed counted on both collection dates to determine the total percent dead seed. Total counts for percent viable hard and viable firm swollen seed were determined from the Tz test.

E.4. Statistical Analysis

An ANOVA was conducted according to a split-plot design using SAS® (SAS, 2012) to compare MON 88702 to the conventional control for the dormancy and germination characteristics listed in Section VII, Table VII-2. If analysis of variance assumptions were not satisfied, Fisher's Exact test was conducted using SAS® (SAS, 2012). Comparisons of MON 88702 to the conventional control were conducted in a combined-site analysis. The level of statistical significance was predetermined to be 5% (α=0.05). MON 88702 and the conventional control were not statistically compared to the reference materials, nor were comparisons made across temperature regimes. The reference range for each seed dormancy and germination characteristic was determined from the minimum and maximum mean values among the seven references, where each mean was combined over all the sites.

®SAS is a registered trademark of SAS Institute, Inc.

Table E-1. Starting Seed of MON 88702, Conventional Control and Commercial cotton Reference varieties Used in Dormancy Assessment

Site Code	Material Name	Regulatory Lot Number	T/C/R[1]
NCBD	DP393	11460828	C
NCBD	Americot UA48	11460829	R
NCBD	SG125	11460830	R
NCBD	DP5415	11460831	R
NCBD	ST 474	11460832	R
NCBD	MON 88702	11460833	T
TXPO	DP393	11460834	C
TXPO	Delta Pine DP399	11460835	R
TXPO	Delta Pine DP493	11460836	R
TXPO	Coker 130	11460837	R
TXPO	SG125	11460838	R
TXPO	MON 88702	11460839	T
TXUV	DP393	11460840	C
TXUV	SG125	11460841	R
TXUV	DP5415	11460842	R
TXUV	ST 474	11460843	R
TXUV	Coker 130	11460844	R
TXUV	MON 88702	11460845	T

[1]T/C/R = test (T), control (C), conventional reference (R) starting material.

References for Appendix E

AOSA. 2016a. AOSA Rules for testing seeds. Volume 1: Principles and procedures, Association of Official Seed Analysts, Ithaca, New York.

AOSA. 2016b. AOSA Rules for testing seeds. Volume 4: Seedling evaluation, Association of Official Seed Analysts, Ithaca, New York.

AOSA/SCST. 2010. Tetrazolium testing handbook. Association of Official Seed Analysts and the Society of Commercial Seed Technologists, Ithaca, New York.

SAS. 2012. Software Release 9.4 (TS1M1). Copyright 2002-2012 by SAS Institute, Inc., Cary, North Carolina.

Appendix F. Materials and Methods for the Phenotypic, Agronomic, and Environmental Interaction Assessment of MON 88702 under Field Conditions

F.1. Materials

Agronomic, phenotypic, and environmental interaction characteristics were assessed for test material MON 88702, the conventional control, and the reference varieties grown under similar agronomic conditions (Table F-1 through Table F-3). The control material was the conventional cotton variety DP393, which had a genetic background similar to the test material but without the biotechnology-derived trait. For the agronomic/phenotypic/qualitative environmental interaction and the quantitative environmental interaction assessments, twelve and eleven commercial cotton varieties, developed through conventional breeding and selection, were included, respectively, in this study across locations to provide a range of comparative values for each characteristic that are representative of the variability in existing commercial cotton varieties. Four references were evaluated at each site.

F.2. Characterization of the Materials

The presence or absence of the MON 88702 event in the starting seed of MON 88702 and the conventional control was verified by event-specific PCR analyses. No molecular analyses were performed on the reference starting seed.

F.3. Field Sites and Plot Design

For the agronomic/phenotypic/qualitative environmental interaction assessments, field trials were established in 2015 at eight sites (Section VII, Table VII-3). For the quantitative environmental interaction assessments, field trials were established in 2015 and 2016 at six sites (Table VII-6). All the sites provided a range of environmental and agronomic conditions representative of U.S. cotton growing regions. The Principal Investigator at each site was familiar with the growth, production, and evaluation of cotton characteristics.

The study was established at each site in a randomized complete block design with four replications. Plot and row dimensions are described in Table F-4 through Table F-6.

F.4. Planting and Field Operations

Planting information, soil description, and cropping history of the study area are listed in Table F-4 through Table F-6. The Principal Investigator at each site followed local agronomic practices including proper seed bed preparation and trial maintenance such as application of agricultural chemicals, fertilizer, and irrigation. General trial maintenance, such as agricultural chemicals, fertilizer, irrigation, and other management practices were applied as necessary throughout the season. All maintenance operations were performed uniformly across all plots within each site. Insecticide applications in the quantitative environmental interaction assessments were applied uniformly across plots in order to isolate the trait effect, and they generally used reduced risk chemistries whenever

possible. The number of applications was also minimized to ensure the crop was maintained but allowing for above threshold applications. Additionally all environmental interaction data were collected either prior to insecticide applications or no less than 7 days after an application. The insecticide applications for all the sites used in the quantitative environmental interaction assessments in the 2015 and 2016 growing seasons are listed in Table F-7.

Table F-1. Starting Seed for Phenotypic, Agronomic, and Qualitative Environmental Interaction Assessment of MON 88702

Site Code[1]	Material Name	Regulatory Lot Number	Phenotype	Material Type
All	MON 88702[2]	11408074	Insect Protected	Test
All	DP393 [2]	11408073	Conventional	Control
CASN	PhytoGen PHY 72 ACALA	11299331	Conventional	Reference
CASN	All-Tex 7A21	11406987	Conventional	Reference
CASN	DP5415	11299234	Conventional	Reference
CASN	Acala 1517-08	11406998	Conventional	Reference
GACH	Coker 130	11299233	Conventional	Reference
GACH	Americot UA48	11406994	Conventional	Reference
GACH	DP5415	11299234	Conventional	Reference
GACH	Acala 1517-08	11406998	Conventional	Reference
LACH	Delta Pine DP493	11406992	Conventional	Reference
LACH	All-Tex LA 122	11406988	Conventional	Reference
LACH	All-Tex A102	11406989	Conventional	Reference
LACH	Acala 1517-08	11406998	Conventional	Reference
MSLE	DP5415	11299234	Conventional	Reference
MSLE	Delta Pine DP493	11406992	Conventional	Reference
MSLE	All -Tex A102	11406989	Conventional	Reference
MSLE	Americot UA48	11406994	Conventional	Reference
NCBD	Americot UA48	11406994	Conventional	Reference
NCBD	SG125	11299231	Conventional	Reference
NCBD	DP5415	11299234	Conventional	Reference
NCBD	ST 474	11266156	Conventional	Reference
TXLV	All -Tex A102	11406989	Conventional	Reference
TXLV	Delta Pine DP399	11406991	Conventional	Reference
TXLV	All -Tex 7A21	11406987	Conventional	Reference
TXLV	Acala 1517-08	11406998	Conventional	Reference
TXPO	Delta Pine DP399	11406991	Conventional	Reference
TXPO	Delta Pine DP493	11406992	Conventional	Reference
TXPO	Coker 130	11299233	Conventional	Reference
TXPO	SG125	11299231	Conventional	Reference
TXUV	SG125	11299231	Conventional	Reference
TXUV	DP5415	11299234	Conventional	Reference
TXUV	ST 474	11266156	Conventional	Reference
TXUV	Coker 130	11299233	Conventional	Reference

[1] Site codes: CASN = Fresno County, CA; GACH = Tift County, GA; LACH = Rapides Parish, LA; MSLE = Washington County, MS; NCBD = Perquimans County, NC; TXLV = Hockley County, TX; TXPO = San Patricio County, TX; TXUV = Uvalde County, TX.

[2] Starting seed of test and control materials were produced in Juana Diaz, Puerto Rico in 2015.

Table F-2. Starting Seed for Quantitative Environmental Interaction Assessment of MON 88702 Conducted in the 2015 Growing Season

Site Code[1]	Material Name	Regulatory Lot Number	Phenotype	T/C/R/[2]
All	DP393	11408073	Conventional	C
GATI	Delta Pine DP493	11406992	Conventional	R
GATI	UA 48	11355395	Conventional	R
GATI	Coker 130	11299233	Conventional	R
GATI	PhytoGen PHY 72 ACALA	11299331	Conventional	R
LACH	All-Tex 7A21	11406987	Conventional	R
LACH	All-Tex A102	11406989	Conventional	R
LACH	ST 474	11299235	Conventional	R
LACH	Delta Pine DP399	11406991	Conventional	R
MSLE	Delta Pine DP493	11406992	Conventional	R
MSLE	SG125	11299231	Conventional	R
MSLE	All-Tex LA122	11406988	Conventional	R
MSLE	All-Tex 7A21	11406987	Conventional	R
NCRC	Delta Pine DP399	11406991	Conventional	R
NCRC	SG125	11299231	Conventional	R
NCRC	ST 474	11299235	Conventional	R
NCRC	PhytoGen PHY 72 ACALA	11299331	Conventional	R
TXPO	Delta Pine DP399	11406991	Conventional	R
TXPO	DP5415	11299234	Conventional	R
TXPO	UA 48	11355395	Conventional	R
TXPO	All-Tex A102	11406989	Conventional	R
TXUV	DP5415	11299234	Conventional	R
TXUV	All-Tex LA122	11406988	Conventional	R
TXUV	Coker 130	11299233	Conventional	R
TXUV	ST 474	11299235	Conventional	R
All	MON 88702	11408074	Insect Protected	T

[1] Site code: GATI = Tift county, GA; LACH = Rapides Parish, LA; MSLE = Washington County, MS; NCRC = Edgecombe County, NC; TXPO = San Patricio County, TX; TXUV = Uvalde County, TX.

[2] T/C/R=Test/Control/Reference

Table F-3. Starting Seed for Quantitative Environmental Interaction Assessment of MON 88702 Conducted in the 2016 Growing Season

Site Code[1]	Material Name	Regulatory Lot Number	Phenotype[2]	Material Type
All	MON 88702	11427706	IR	Test
All	DP393	11427700	Conventional	Control
GACH	Delta Pine DP399	11427261	Conventional	Reference
GACH	DP 5415	11427696	Conventional	Reference
GACH	Americot UA48	11406994	Conventional	Reference
GACH	All-Tex A102	11406989	Conventional	Reference
LACH	Delta Pine DP493	11427260	Conventional	Reference
LACH	SG125	11427695	Conventional	Reference
LACH	DP 6166	11427699	Conventional	Reference
LACH	All-Tex 7A21	11406987	Conventional	Reference
MSLE	DP 5415	11427696	Conventional	Reference
MSLE	All-Tex LA122	11406988	Conventional	Reference
MSLE	SG501	11427693	Conventional	Reference
MSLE	DP 6166	11427699	Conventional	Reference
NCRC	Delta Pine DP399	11427261	Conventional	Reference
NCRC	SG125	11427695	Conventional	Reference
NCRC	DP 6166	11427699	Conventional	Reference
NCRC	Acala 1517-08	11406998	Conventional	Reference
SCEK	Delta Pine DP493	11427260	Conventional	Reference
SCEK	Americot UA48	11406994	Conventional	Reference
SCEK	SG501	11427693	Conventional	Reference
SCEK	Acala 1517-08	11406998	Conventional	Reference
TXUV	All-Tex LA122	11406988	Conventional	Reference
TXUV	Delta Pine DP399	11427261	Conventional	Reference
TXUV	Delta Pine DP493	11427260	Conventional	Reference
TXUV	DP 5415	11427696	Conventional	Reference

[1] Site code: GACH = Tift county, GA; LACH = Rapides Parish, LA; MSLE = Washington County, MS; NCRC = Edgecombe County, NC; SCEK = Barnwell County, SC; TXUV = Uvalde County, TX.

[2] Phenotype abbreviations: IR = insect resistant

Table F-4. Field and Planting Information for Phenotypic, Agronomic, and Qualitative Environmental Interaction Assessment of MON 88702

Site Code[1]	Planting Date[2]	Harvest Date[3]	Planting Rate (seeds/m^2)	Rows/ Plot	Inter-row Distance (m)	Row Length (m)	Plot Size (m^2)	Soil Type	Organic Matter (%)	Previous Crop 2014
CASN	05/14/2015	11/10/2015-11/12/2015	16.1	6	1.02	9.1	55.5	Clay	1.3	Fallow
GACH	06/01/2015	10/30/2015-10/31/2015	17.9	6	0.92	9.1	50.1	Sand	0.6	Maize
LACH	05/06/2015	09/25/2015-09/30/2015	16.1	6	1.02	9.1	55.5	Silt loam	1.3	Soybean/Maize
MSLE	06/05/2015	10/23/2015	17.0	6	0.97	9.1	52.8	Sandy loam	1.3	Cotton
NCBD	05/20/2015	10/22/2015-10/27/2015	17.0	6	0.97	9.1	52.8	Loamy sand	24.7	Maize
TXLV	06/04/2015	11/20/2015	16.1	6	1.02	9.1	55.5	Sandy loam	0.8	Fallow
TXPO	05/03/2015	09/08/2015	21.5	6	0.77	9.1	41.9	Sandy clay	1.3	Sorghum
TXUV	05/01/2015	09/28/2015	16.1	6	1.02	9.1	55.5	Clay	2.3	Sorghum

Note: Planting Rate and plot / row dimensions are approximate.

[1] Site codes: CASN = Fresno County, CA; GACH = Tift County, GA; LACH = Rapides Parish, LA; MSLE = Washington County, MS; NCBD = Perquimans County, NC; TXLV = Hockley County, TX; TXPO = San Patricio County, TX; TXUV = Uvalde County, TX.

[2] Date format = mm/dd/yyyy.

[3] Harvest date of agronomic phenotypic rows.

Table F-4. Field and Planting Information for Phenotypic, Agronomic, and Qualitative Environmental Interaction Assessment of MON 88702 (continued)

Site Code[1]	Planting Date[2]	Harvest Date[3]	Planting Rate (seeds/m^2)	Rows/ Plot	Inter-row Distance (m)	Row Length (m)	Plot Size (m^2)	Soil Type	Organic Matter (%)	Previous Crop 2014
CASN	05/14/2015	11/10/2015-11/12/2015	16.1	6	1.02	9.1	55.5	Clay	1.3	Fallow
GACH	06/01/2015	10/30/2015-10/31/2015	17.9	6	0.92	9.1	50.1	Sand	0.6	Maize
LACH	05/06/2015	09/25/2015-09/30/2015	16.1	6	1.02	9.1	55.5	Silt loam	1.3	Soybean/Maize
MSLE	06/05/2015	10/23/2015	17.0	6	0.97	9.1	52.8	Sandy loam	1.3	Cotton
NCBD	05/20/2015	10/22/2015-10/27/2015	17.0	6	0.97	9.1	52.8	Loamy sand	24.7	Maize
TXLV	06/04/2015	11/20/2015	16.1	6	1.02	9.1	55.5	Sandy loam	0.8	Fallow
TXPO	05/03/2015	09/08/2015	21.5	6	0.77	9.1	41.9	Sandy clay	1.3	Sorghum
TXUV	05/01/2015	09/28/2015	16.1	6	1.02	9.1	55.5	Clay	2.3	Sorghum

Note: Planting Rate and plot / row dimensions are approximate.

[1] Site codes: CASN = Fresno County, CA; GACH = Tift County, GA; LACH = Rapides Parish, LA; MSLE = Washington County, MS; NCBD = Perquimans County, NC; TXLV = Hockley County, TX; TXPO = San Patricio County, TX; TXUV = Uvalde County, TX.

[2] Date format = mm/dd/yyyy.

[3] Harvest date of agronomic phenotypic rows.

Table F-5. Field Information for Quantitative Environmental Interaction Assessment of MON 88702 Conducted in the 2015 Growing Season

Site[1]	Planting Date[2]	Approximate Planting Rate (seeds/m)	Rows per Plot	Approximate Plot Size (m x m)	Soil Type	Organic Matter (%)	Previous Crop 2014
GATI	5/27/2015	10.8	16	9.1 × 14.6	Loamy sand	1.2	Peanut
LACH	5/6/2015	13.1	16	9.1 × 16.2	Silt loam	1.2	Soybean and Maize
MSLE	6/5/2015	13.1	16	9.1 × 15.5	Silt loam	1.5	Cotton
NCRC	5/31/2015	12.5	16	9.8 × 14.6	Sandy loam	4	Peanuts
TXPO	5/3/2015	13.1	16	9.1 × 12.2	Sandy clay	1.3	Grain sorghum
TXUV	5/1/2015	13.1	16	9.1 × 16.2	Clay	2.2	Grain sorghum

[1] Site code: GATI = Tift county, GA; LACH = Rapides Parish, LA; MSLE = Washington County, MS; NCRC = Edgecombe County, NC; TXPO = San Patricio County, TX; TXUV = Uvalde County, TX.

[2] Planting Date = mm/dd/yyyy

Table F-6. Field Information for Quantitative Environmental Interaction Assessment of MON 88702 Conducted in the 2016 Growing Season

Site Code[1]	Planting Date[2]	Harvest Date[2]	Planting Rate (seeds/m^2)	Rows / Plot	Inter-row Distance[3] (m)	Row Length (m)	Plot Size (m^2)	Soil Type	% Organic Matter	Previous Crop 2015
GACH	06/25/2016	N/A	18.1	16	0.92	9.1	132.9	Loamy sand	-	Cotton
LACH	06/01/2016	N/A	16.3	16	1.02	9.1	147.4	Silt loam	1.5	Corn, Sorghum
MSLE	05/24/2016	N/A	17.1	16	0.97	9.1	140.1	Silt loam	1.1	Cotton
NCRC	05/25/2016	N/A	18.1	16	0.92	9.1	132.9	Sandy loam	4.0	Cotton
SCEK	05/16/2016	N/A	18.1	16	0.92	9.1	132.9	Sand	1.1	Soybean
TXUV	05/05/2016	N/A	16.3	16	1.02	9.1	147.4	Clay	2.4	Sorghum

[1] Site code: GACH = Tift county, GA; LACH = Rapides Parish, LA; MSLE = Washington County, MS; NCRC = Edgecombe County, NC; SCEK = Barnwell County, SC; TXUV = Uvalde County, TX.
[2] Planting Date = mm/dd/yyyy.

Table F-7. Summary of Insecticide Applications in the Quantitative Environmental Interaction Assessment of MON 88702 in 2015 and 2016 U.S. Field Trials

Year	Site	Insecticide Applied	Date Applied
2015	LACH	Spinetoram	22-May-2015
		Sulfoxaflor	20-Jun-2015
	MSLE	Spinetoram	24-Jun-2015
		Acetamiprid	14-Aug-2015
	NCRC	Spinetoram	10-Jun-2015
	TXPO	Imidacloprid & β-cyfluthrin	19-May-2015
		Thiamethoxam	05-Jun-2015
	TXUV	Dicrotophos	10-Jun-2015
		Imidacloprid	09-Jul-2015
		Etoxazole	21-Jul-2015
2016	GACH	Pyriproxyfen	07-Sep-2016
	SCEK	Spinetoram	27-May-2016
	TXUV	Spinetoram	24-May-2016
		Etoxazole	06-Jul-2016

F.5. Phenotypic and Agronomic Observations

Phenotypic and agronomic characteristics assessed and the timing of each assessment are presented in Section VII, Table VII-1.

F.6. Environmental Observations

Environmental interactions (i.e., interactions between the crop plants and their receiving environment) were used to characterize MON 88702 by evaluating plant response to abiotic stressors, disease damage, and arthropod-related damage using qualitative methods described in Section F.6.1 In addition, specific arthropod damage (heliothine and stink bug damage) and arthropod abundance were evaluated using the quantitative methods described in Section F.6.2. The results from the quantitative arthropod damage and abundance were subject to an individual-site analysis (Table F-18 through Table F-20, whereas a combined-site and across-year analysis was conducted for the arthropod abundance data (Table VII-7).

F.6.1. Plant Response to Abiotic Stress, Disease Damage, and Arthropod-related Damage

MON 88702, the conventional control, and reference varieties were evaluated at all sites for plant response to abiotic stressors, disease damage, and arthropod damage. A target of three abiotic stressors, three diseases, and three arthropod pests were evaluated four times during the following four intervals: vegetative stage, squaring stage, bloom stage, and after cut-out.

The field cooperator at each site chose abiotic stressors, diseases, and arthropod pests that were either actively causing plant injury in the study area or were likely to occur in cotton during a given observation period. Abiotic stressors, diseases, and arthropod pests that were assessed often varied between observations at a site and among sites.

Abiotic stressor, disease damage, and arthropod damage observations were collected from each plot using the following categorical scale of increasing severity of observed damage for each stressor. This scale was utilized to allow for the evaluation of the wide variety of potential abiotic stressors, disease damage, and arthropod damage symptoms potentially occurring across the season and across sites.

Category	Severity of plant damage
None	No symptoms observed
Slight	Symptoms not damaging to plant development (e.g. minor feeding or minor lesions); mitigation likely not required
Moderate	Intermediate between slight and severe; likely requires mitigation
Severe	Symptoms damaging to plant development (e.g. stunting or death); mitigation unlikely to be effective

F.6.2. Specific Arthropod Damage and Arthropod Abundance

Heliothine damage was evaluated up to four times, starting at approximately early squaring and every two weeks thereafter, by examining ten non-systematically selected plants in each plot. The total number of fruiting bodies (flower buds, flowers and bolls) and number of damaged fruiting bodies were evaluated on the top seven nodes of each plant. Heliothine damage was expressed as a percentage of the total number of fruiting bodies observed in each plot.

Stink bug damage was evaluated four times starting at approximately the second week of bloom over a four to five week period, by examining twenty non-systematically selected bolls of approximately one inch diameter in each plot. Damage was assessed by cracking and inspecting the bolls for internal injury. A boll was considered damaged if any warts were present in the internal wall and/or stained lint was present. Stink bug damage was expressed as a percentage of the total number of bolls evaluated in each plot.

Arthropods were collected using vertical beat sheets six times during the growing seasons starting at early squaring and up to two weeks thereafter. At each collection, a total of six sub-samples (one from each designated row) were collected from each plot and pooled into one sample per plot. Collections were made by vigorously shaking the plants to dislodge arthropods into the collecting trough, which were then gently swept into a sample container. Samples from the 2015 growing season were then sent to Monsanto Company, St. Louis Missouri; whereas samples from the 2016 growing season were sent to the University of Arkansas, Fayetteville, Arkansas, for arthropod identification and enumeration.

F.7. Data Assessment

Experienced scientists familiar with the experimental design and evaluation criteria were involved in all components of data collection, summarization, and analysis. Study personnel ensured that measurements were taken properly, data were consistent with expectations based on experience with the crop, and the study was carefully monitored. Prior to analysis, the datasets were evaluated by the Lead Scientist or Environmental Interactions Scientist for evidence of biologically relevant changes and for possible evidence of unexpected plant responses. Any unexpected observations or issues identified during the study that might impact the study objectives were noted by the Lead Scientist or Environmental Interactions Scientist. Data were then subjected to summarization or statistical analysis as indicated below.

F.8. Statistical Analysis/Data Summarization

F.8.1. Agronomic and Phenotypic Data

An ANOVA was conducted according to a randomized complete block design using SAS® (2012) to compare MON 88702 to the conventional control for the phenotypic characteristics listed in Section VII, Table VII-1. The level of statistical significance was predetermined to be 5 % (α=0.05). Comparisons of MON 88702 to the conventional

control were conducted in a combined-site analysis. The reference range for each phenotypic characteristic was determined from the minimum and maximum mean values among the 12 references, where each mean was combined over all the sites at which the reference was planted.

Data excluded from the study and the reasons for their exclusion are listed in Table F-8. Exclusion of these data did not adversely affect the quality of the study.

F.8.2. Qualitative Environmental Interaction Data

The environmental interactions data consisting of plant response to abiotic stressors, arthropod damage, and disease damage are categorical and were summarized as ranges of injury symptoms for each stressor within observation times and sites.

F.8.3. Quantitative Environmental Interaction Data: Individual-site Analysis

An ANOVA was conducted according to a randomized complete block design using SAS® (SAS, 2012) for heliothine damage, stink bug damage, and arthropod abundance. The level of statistical significance was predetermined to be 5 % (α=0.05). MON 88702 was statistically compared to the conventional control while the reference range for each measured characteristic was determined from the minimum and maximum mean values from the four reference cotton varieties planted at each site. Data excluded from the study and the reasons for their exclusion are listed in Table F-9 and Table F-10.

For the arthropod abundance data, an across-collection analysis was performed within each site, with six repeated collections from vertical beat sheets. Statistical analyses and significance testing of differences between MON 88702 and the conventional control material were only performed for the arthropods present in sufficient numbers to estimate the material mean arthropod counts and the variation of the means. An inclusion criterion was established where a given arthropod must have an average count per plot per collection time (across all materials) of ≥ 1 (Ahmad et al. 2016). All collection times that met the inclusion criterion for a given arthropod were pooled within the site and subjected to across-collection analysis.

F.8.4. Quantitative Environmental Interaction Data: Combined-site Analysis

To ensure a valid analysis of the material effect on arthropod abundance, sites were excluded if the mean count per plot per collection was less than one (Ahmad et al., 2016). Also, a collection in a site was excluded in the across-collection calculation and subsequent analysis if the average capture per collection (across materials and replicates) was less than one.

After applying the above criteria, SAS PROC MEANS was used to calculate the by-replicate sample mean of the arthropod counts (across sampling) for each taxon, site, year and material. SAS PROC MEANS was used again to calculate the across-site-replicate mean and standard error of the by-replicate means for each taxon, material, and year. SAS PROC MEANS was also used to calculate the by-replicate sample mean of the arthropod counts for each taxon, site, and material across years. SAS PROC MEANS was

used again to calculate the across-site-replicate mean and standard error of the by-collection means for each taxon and material across year. Three separate analyses were performed for the arthropod abundance data.

F.8.4.1 Statistical Analysis By-Year

The following linear mixed model was used to conduct an across-site-collection analysis:

$$y_{ijkl} = \mu + s_i + r(s)_{j(i)} + t_k + d_{l(i)} + (ts)_{ik} + e_{ijkl} \qquad \text{(Model 1)}$$

where :
y_{ijkl} = square-root of the observed count;
μ = overall mean;
s_i = random site effect;
$r(s)_{j(i)}$ = random within-site replicate effect;
t_k = fixed material effect;
$d_{l(i)}$ = random within-site collection effect;
$(ts)_{ik}$ = random interaction effect of site and material;
e_{ijkl} = residual error.

SAS PROC MIXED was used to fit model (1) separately for each combination of taxon and year. Taxa that were observed in only one site in either year were excluded from the across-site analysis. A square-root variance stabilizing transformation was used to account for the count nature of the data. Residual plots were visually inspected, and assumptions of normality and variance homogeneity were found to be satisfied after the square-root transformation. Pairwise comparisons between the test and control materials were defined within the model and tested using t-tests and the α=0.05 level of significance.

F.8.4.2 Statistical Analysis Across Sites and Years

The across-year analysis was performed by replacing the random site effect in model (1) with a concatenated site-year factor, i.e.:

$$y_{ijkl} = \mu + sy_i + r(sy)_{j(i)} + t_k + d_{l(i)} + (tsy)_{ik} + e_{ijkl} \qquad \text{(Model 2)}$$

where:

y_{ijkl} = square-root of the observed count;
μ = overall mean;
sy_i = random site-year effect;
$r(sy)_{j(i)}$ = random within-site-year replicate effect;
t_k = fixed material effect;
$d_{l(i)}$ = random within-site-year collection effect;
$(tsy)_{ik}$ = random interaction effect of site-year and material;
e_{ijkl} = residual error.

SAS PROC MIXED was used to fit model (2) separately for each arthropod taxon. A square-root variance stabilizing transformation was applied to account for the count nature of the data. Pairwise comparisons between the test and control materials were defined within the model and tested using t-tests and the α=0.05 level of significance.

F.8.4.3 Power Analysis

Statistical power was estimated assuming a 50% difference (i.e., effect size) in the abundance of each taxonomic group (Blumel et al. 2000; Perry et al. 2003), following the method introduced by Duan et al. (2006). Let x_1 and x_2 represent the observed insect counts for the control and the test materials, respectively, and μ_{x1} and μ_{x2} represent the expected mean counts. Then, the detectable difference (d_x) relative to the control implies $d_x = \mu_{x1} - \mu_{x2} = 0.5\mu_{x1}$ when $\mu_{x1} > \mu_{x2}$ or $d_x = -0.5\mu_{x1}$ when $\mu_{x1} < \mu_{x2}$. Let y represents the square root of x. The corresponding difference in y, i.e. d_y, can be obtained from the following equations:

$$\begin{cases} d_{ya} = \mu_{y1} - 0.5\sqrt{4\mu_{y1}^2 - 2\left(\mu_{y1}^2 + \sigma_y^2\right)} & \text{for } d_y > 0 \\ d_{yb} = \mu_{y1} - 0.5\sqrt{4\mu_{y1}^2 + 2\left(\mu_{y1}^2 + \sigma_y^2\right)} & \text{for } d_y < 0 \end{cases} \qquad \text{(Equation 1)}$$

where μ_{y1} and σ_y^2 are the control mean and the total variance of all random effects in model (1), respectively, on the square-root scale. The power calculation used $d_y = \min(d_{ya}, -d_{yb})$, where min represents the minimum of the two quantities in parenthesis.

A customized SAS algorithm executing the following steps was used calculate statistical power for detecting a 50% difference in the test mean count relative to the control at the α=0.05 level of significance. Results for the across-site-collection analysis and across-site-year-collection analysis are provided in Table F-20 and Table VII-7, respectively.

- Estimate μ_{y1} (Least Square mean of the control transformed) and σ_y^2 (relevant random variation of the control mean including site-by-material interaction [or site-year-by-material] and the residual) from the LSMEANS and COVPARMS statements after fitting the mixed model (1 or 2);
- Substitute the estimated μ_{y1} and σ_y^2 into equation (1) to estimate d_y;
- Estimate $\sigma_{d_y}^2$ and the degrees of freedom (ν) using the LSMEANS DIFFERENCE statement;
- Estimate power using the above estimates in the following equation:

  $$\delta_y = \frac{d_y}{\sigma_{d_y}}$$

  $$Power = Prob\left\{T_{\nu,\delta_y} > t_{0.975,\nu}\right\} + Prob\left\{T_{\nu,\delta_y} < t_{0.025,\nu}\right\}$$

  where T_{ν,δ_y} denotes the non-central t-distribution with degrees of freedom ν and non-centrality parameter δ_y, $t_{0.025,\nu}$ and $t_{0.975,\nu}$ represents the 2.5th and 97.5th percentile of the t-distribution with ν degrees of freedom.

Results of comparisons between test and control and significant differences are denoted by '*' beside the test mean.

F.9. Agronomic and Phenotypic and Environmental Interaction Results and Discussion for MON 88702

F.9.1. Agronomic and Phenotypic Assessment

In Section VII.C.2.1 it was demonstrated that there was no biologically meaningful difference in phenotypic characteristics between MON 88702 and the conventional control. Therefore, MON 88702 is not expected to pose increased plant pest/weed potential compared to conventional cotton.

F.9.2. Environmental Interaction Assessments for MON 88702

F.9.2.1 Qualitative Environmental Interaction Assessment: Plant Response to Abiotic Stressor, Disease Damage, and Arthropod-related Damage

No differences were observed between MON 88702 cotton and the conventional control for any of the 96 comparisons for the assessed abiotic stressors: cold, drought, flooding, heat, mineral toxicity, nutrient deficiency, sandstorm, soil compaction, sun scald, wet soil, and wind (Table F-11).

No differences were observed between MON 88702 cotton and the conventional control for 94 of the 95 comparisons for the assessed arthropods: aphids, armyworms, bollworms, cutworms, grape colaspis, grasshoppers, Japanese beetles, loopers, spider mites, stink bugs, striped flea beetles, and whiteflies (Table F-12). A single difference was observed in the arthropod damage assessment in which MON 88702 exhibited lower bollworm severity damage compared to the conventional control at site TXUV (none vs. slight rating). However, the rating for MON 88702 was within the reference range and this difference was not observed consistently across observations and/or sites where bollworms occurred. Thus, the difference was not considered biologically meaningful in terms of increased plant pest potential and/or adverse environmental impact.

No differences were observed between MON 88702 cotton and the conventional control for any of the 96 comparisons for the assessed diseases: bacterial blight, boll rot, Fusarium wilt, leaf spot, Phymatotrichum root rot, Phytophthora root rot, Pythium, Rhizoctonia, rust, seedling blight, Stemphylium leaf spot, target spot, Verticillium wilt, and wet weather blight (Table F-13).

F.9.2.2 Quantitative Environmental Interaction Data: Individual-site Analysis

F.9.2.2.1 Heliothine Damage and Stink Bug Damage for MON 88702

In the individual-site analysis of heliothine and stink bug damage for the fields planted in the 2015 growing season, no statistically significant differences were detected between MON 88702 and the conventional control for 34 of 39 comparisons (Table F-14 and Table F-16). Lack of variability in the data precluded statistical comparisons between

MON 88702 and the conventional control for eight additional comparisons. For the five detected differences, MON 88702 had less percent damage from stink bugs compared to the conventional control during observation two at the MSLE site (27.5% vs. 50.0%), and observations two, three and four at the NCRC site (28.8% vs. 63.8%, 36.3% vs. 78.8% and 37.5% vs. 72.5%, respectively). MON 88702 had higher percent damage from stink bugs compared to the conventional control during observation two at the TXPO site (63.8% vs. 41.3%). For these differences, the mean percent damage for MON 88702 was outside the range of the commercial reference varieties but were not consistently detected across observations and sites. Thus, these differences were not indicative of a consistent plant response associated with the trait and are unlikely to be biologically meaningful in terms of increased pest potential and adverse environmental impact of MON 88702 compared to conventional.

In the individual-site analysis of heliothine and stink bug damage for the fields planted in the 2016 growing season, no statistically significant differences were detected between MON 88702 and the conventional control for 36 of 39 comparisons (Table F-15 and Table F-17). Lack of variability in the data precluded statistical comparisons between MON 88702 and the conventional control for nine additional comparisons. For the three detected differences, heliothine damage was lower on MON 88702 compared to the conventional control during observation three at the TXUV site (0.6% vs. 3.2%) and stink bug damage was lower on MON 88702 compared to the conventional control during observation one at both the MSLE site (10.0% vs. 18.8%) and the NCRC site (46.3% vs. 75.0%). For these differences, the mean percent damage for MON 88702 was within the range of the commercial reference varieties. Thus, these differences were not indicative of a consistent plant response associated with the trait and are unlikely to be biologically meaningful in terms of increased pest potential and adverse environmental impact of MON 88702 compared to conventional cotton.

F.9.2.2.2 Arthropod Abundance for MON 88702

For the collections made in the 2015 growing season, the relative abundance and total number of all arthropod taxa collected from vertical beat sheet collections are presented in Table F-18. The following taxa met the minimum abundance criteria (Section F.8.3) required for inclusion in the statistical analysis for significance testing; ant-like flower beetles *Notoxus* spp. (Coleoptera: Anthicidae); ants, several spp. (Hymenoptera: Formicidae); aphids, several spp. (Hemiptera: Aphididae); big-eyed bugs, *Geocoris* spp. (Hemiptera: Geocoridae); grape colaspis, *Colaspis brunnea* (Coleoptera: Chrysomelidae); click beetles, several spp. (Coleoptera: Elateridae); heliothines (Lepidoptera: Noctuidae); lacewings, *Chrysoperla carnea* (Neuroptera: Chrysopidae); ladybird beetles, several spp. (Coleoptera: Coccinellidae); leafhoppers, several spp. (Hemiptera: Cicadellidae); minute pirate bugs, *Orius* spp. (Hemiptera: Anthocoridae); damsel bugs, *Nabis* spp. (Hemiptera: Nabidae); parasitic wasps, several spp. (Hymenoptera); assassin bugs, several spp. (Hemiptera: Reduviidae); spiders, several spp. (Araneae); stink bugs, several spp. (Hemiptera: Pentatomidae) and whiteflies, *Bemisia tabaci* (Hemiptera: Aleyrodidae).

In an across-collection analysis of arthropod abundance data, no statistically significant differences were detected between MON 88702 and the conventional control for 65 out of 69 comparisons (Table F-18). The four differences detected for arthropod abundance

involved ant-like flower beetles, aphids, and ladybird beetles. The mean abundance of ant-like flower beetles was higher on MON 88702 than the conventional control at one of the five sites where they were observed (NCRC site: 4.5 vs. 2.3 per plot). The mean abundance of aphids was lower on MON 88702 than the conventional control at one of the five sites where they were observed (MSLE site: 76.5 vs. 84.9 per plot). The mean abundance of ladybird beetles was higher on MON 88702 than the conventional control at two of the six sites where they were observed (MSLE site: 25.1 vs. 19.1; NCRC site: 15.4 vs. 12.2 per plot)

The mean abundance on MON 88702 was within the respective range of commercial reference varieties for one of the four detected differences. The mean abundance of Ladybird beetles on MON 88702 was within the reference range at the MSLE site (MON 88702 mean = 25.1 per plot; 25.0 – 26.4 per plot) and slightly higher than the reference range at the NCRC site (MON 88702 mean = 15.4 per plot; reference range = 12.0 – 14.5 per plot). The mean abundance value of ant-like flower beetles on MON 88702 was slightly higher than the reference range for the detected difference at the NCRC site (MON 88702 mean = 4.5 per plot; reference range = 3.0 – 4.2 per plot). The mean abundance of aphids on MON 88702 was slightly lower than the reference range for the detected difference at the MSLE site (MON 88702 mean = 76.5 per plot; reference range = 77.3 – 177.9 per plot). However, these differences were not consistently detected across sites.

For the collections made in the 2016 growing season, the relative abundance and total number of all arthropod taxa collected from vertical beat sheet collections are presented in Table F-19. The following taxa met the minimum abundance criteria (Section F.8.3) required for inclusion in the statistical analysis for significance testing; ant-like flower beetles *Notoxus* spp. (Coleoptera: Anthicidae); ants, several spp. (Hymenoptera: Formicidae); aphids, several spp. (Hemiptera: Aphididae); big-eyed bugs, *Geocoris* spp. (Hemiptera: Geocoridae); heliothines (Lepidoptera: Noctuidae); lacewings, *Chrysoperla carnea* (Neuroptera: Chrysopidae); ladybird beetles, several spp. (Coleoptera: Coccinellidae); minute pirate bugs, *Orius* spp. (Hemiptera: Anthocoridae); damsel bugs, *Nabis* spp. (Hemiptera: Nabidae); parasitic wasps, several spp. (Hymenoptera); spiders, several spp. (Araneae); stink bugs, several spp. (Hemiptera: Pentatomidae) and whiteflies, *Bemisia tabaci* (Hemiptera: Aleyrodidae).

In an across-collection analysis of arthropod abundance data, no statistically significant differences were detected between MON 88702 and the conventional control for 45 out of 46 comparisons (Table F-19). For the detected difference, the mean abundance of big-eyed bugs was lower on MON 88702 than the conventional control at one of the five sites where they were observed (GACH site: 4.3 vs. 10.0 per plot). At this site, the mean abundance of big-eyed bugs on MON 88702 was slightly lower than the reference range (MON 88702 mean = 4.3 per plot; reference range = 4.4 – 8.6 per plot). However, these differences were not consistently detected across sites.

The results from the individual-site analyses for the 2015 and 2016 growing seasons support a conclusion that the detected differences in arthropod abundance were not indicative of a consistent response associated with the trait and are not considered

biologically meaningful in terms of increased plant pest potential or adverse environmental impact of MON 88702 compared to conventional cotton.

F.9.2.3 Quantitative Environmental Interaction Data: Combined-site and Combined-year Analysis

In across year, across site analyses, no significant differences were detected for 15 of the 16 taxa assessed (Table VII-7). Abundance of ladybird beetles was significantly higher in MON 88702 compared to the conventional control ($p=0.027$). This difference was driven by a similarly observed statistical difference in this taxon in 2015 (Table F-20).

In the power analyses across years, all but three taxa had ≥80% power to detect a 50% difference between the abundance in MON 88702 and the conventional control (Table VII-7). A 50% difference in arthropod abundance between treatments has been found to be relevant ecologically and biologically in field studies (Perry et al. 2003; Naranjo 2005; Duan et al 2006) at the standard Type 1 error rate of 5%. Taxa representing key ecologically relevant functions of herbivory, predation and parasitism including the hemipteran families of Aphididae, Geocoridae, Nabidae, Anthocoridae, Pentatomidae and Aleyrodidae had ≥80% power. Additional taxa in the Coleoptera, Araneae, Neuroptera and Hymenoptera orders had ≥80% power to detect a 50% difference in the across year analyses (Table VII-7). The lack of differences is further evidence that MON 88702 is unlikely to pose a risk to or have adverse effects on relevant arthropod communities in cotton agro-ecosystems.

Table F-8. Data Missing or Excluded from Phenotypic, Agronomic, and Qualitative Environmental Interaction Assessment Study

Site Code[1]	Material Name	Material Type	Plots	Characteristics	Reason for Exclusion
CASN	Acala 1517-08	Reference	203	Early stand count, Final stand count	Data collection error
NCBD	Americot UA48	Reference	105	Early stand count, Final stand count	Data collection error
	Americot UA48	Reference	401		
TXUV	MON 88702	Test	101	Early stand count, EI observation 1 (vegetative stage), EI observation 2 (squaring stage), Date of first flower	Characteristics observed from rows for which material identity was not confirmed

[1] Site codes: CASN = Fresno County, CA; NCBD = Perquimans County, NC; TXUV = Uvalde County, TX.

Table F-9. Data Missing or Excluded from Quantitative Environmental Interaction Assessment Study Conducted in the 2015 Growing Season

Site Code[1]	Material Name	Material Type	Plots	Characteristics	Reason for Exclusion
GATI	All	All	All	Environmental interaction evaluation #4 (Heliothis)	Improper selection of stressor: Data was being collected quantitatively
GATI	All	All	All	Heliothine damage (Observation 1)	Data not collected: field cooperator oversight
GATI	Delta Pine DP493, PhytoGen PHY 72 ACALA	Conventional	106, 206	Arthropod abundance (Collection 5)	Missing samples
TXPO	All	All	All	Environmental interaction evaluation #4 (Verde Plant bug)	Improper selection of stressor: Plant bugs are target pests

[1] Site code: GATI = Tift county, GA; LACH = Rapides Parish, LA; MSLE = Washington County, MS; NCRC = Edgecombe County, NC; TXPO = San Patricio County, TX; TXUV = Uvalde County, TX.

Table F-10. Data Missing or Excluded from Quantitative Environmental Interaction Assessment Study Conducted in the 2016 Growing Season

Site Code[1]	Material Name	Material Type	Plots	Characteristics	Reason for Exclusion
SCEK	All	All	All	Environmental interaction evaluation #3 and 4 (Stink bugs)	Improper selection of stressor: Data not collected according to protocol

[1] Site code: GACH = Tift county, GA; LACH = Rapides Parish, LA; MSLE = Washington County, MS; NCRC = Edgecombe County, NC; SCEK = Barnwell County, SC; TXUV = Uvalde County, TX.

Table F-11. Summary of Abiotic Stress Response Evaluations for MON 88702 and the Conventional Control in 2015 U.S. Field Trials

Abiotic Stressor	Total observations across sites	Number of observations without differences between MON 88702 and the conventional control
Total	96	96
Cold	1	1
Drought	13	13
Flooding	4	4
Heat	22	22
Mineral toxicity	3	3
Nutrient deficiency	18	18
Sandstorm	4	4
Soil compaction	4	4
Sun scald	4	4
Wet soil	7	7
Wind	16	16

Notes: The experimental design was a randomized complete block with four replicates per site.
No differences were observed between MON 88702 and the conventional control during any observation for damage caused by any of the assessed abiotic stressors. Categorical data were summarized across sites and observation times.

Table F-12. Summary of Arthropod Damage Evaluations for MON 88702 and the Conventional Control in 2015 U.S. Field Trials

Arthropod	Total observations across sites	Number of observations without differences between MON 88702 and the conventional control
Total	95	94
Aphids (Aphididae)	20	20
Armyworms (Noctuidae)	7	7
Bollworms (Noctuidae) *	4	3
Cutworms (Noctuidae)	5	5
Grape colaspis (Chrysomelidae)	5	5
Grasshoppers (*Melanoplus* spp.)	5	5
Japanese beetles (*Popillia japonica*)	3	3
Loopers (Noctuidae)	5	5
Spider mites (*Tetranychus* spp.)	17	17
Stink bugs (Pentatomidae)	9	9
Striped flea beetles (*Phyllotreta* spp.)	2	2
Whiteflies (Aleyrodidae)	13	13

Notes: The experimental design was a randomized complete block with four replicates per site.
* One difference was observed between MON 88702 and the conventional control for Bollworm damage at the TXUV site (MON 88702 = none; control = slight; reference range = none – slight). Categorical data were summarized across sites and observation times.

Table F-13. Summary of Disease Damage Evaluations for MON 88702 and the Conventional Control in 2015 U.S. Field Trials

Disease	Total observations across sites	Number of observations without differences between MON 88702 and the conventional control
Total	96	96
Bacterial blight	19	19
Boll rot	5	5
Fusarium wilt	17	17
Leaf spot	14	14
Phymatotrichum (Texas) root rot	5	5
Phytophthora root rot	1	1
Pythium	1	1
Rhizoctonia	2	2
Rust	5	5
Seedling blight	6	6
Stemphylium leaf spot	3	3
Target spot	11	11
Verticillium wilt	6	6
Wet weather blight	1	1

Notes: The experimental design was a randomized complete block with four replicates per site.
No differences were observed between MON 88702 and the conventional control during any observation for damage caused by any of the assessed diseases. Categorical data were summarized across sites and observation times.

Table F-14. Heliothine Damage (Percent damage/plot) to MON 88702 Compared to the Conventional Control in 2015 U.S. Field Trials

Site[1]	Observation	Mean (S.E.)[2]		Reference Range[3]
		MON 88702	Control	
GATI	1	–	–	–
	2	1.7 (0.78)	1.2 (1.18)	0.2 – 1.4
	3	1.8 (0.75)	2.2 (0.67)	0.6 – 3.1
	4	1.6 (0.50)	1.0 (0.43)	1.5 – 3.0
LACH	1	0.0 (0.00) †	0.0 (0.00)	0.0 - 0.0
	2	0.0 (0.00) †	0.0 (0.00)	0.0 - 0.0
	3	0.8 (0.80) †	0.0 (0.00)	0.2 - 1.0
	4	0.0 (0.00) †	0.0 (0.00)	0.0 - 1.0
MSLE	1	0.0 (0.00) †	0.0 (0.00)	0.0 – 0.6
	2	34.7 (5.76)	40.0 (6.12)	31.8 – 54.3
	3	13.5 (2.54)	12.8 (3.55)	17.1 - 21.7
	4	9.3 (2.68)	12.1 (3.11)	7.5 - 12.5
NCRC	1	0.0 (0.00) †	0.0 (0.00)	0.0 – 0.7
	2	7.1 (2.47)	3.0 (1.05)	0.6 - 4.6
	3	22.0 (3.03)	23.1 (4.85)	20.0 - 28.3
	4	39.0 (4.70)	29.9 (2.50)	26.2 - 41.7
TXPO	1	0.0 (0.00) †	1.6 (1.56)	0.0 – 0.6
	2	4.7 (1.15)	4.7 (1.69)	3.6 - 4.5
	3	11.2 (1.98)	6.5 (0.40)	5.4 - 9.6
	4	1.9 (1.85)	0.7 (0.69)	0.0 - 3.8
TXUV	1	4.6 (2.02)	4.8 (1.44)	3.6 - 9.2
	2	1.2 (0.74)	1.9 (1.08)	0.3 - 6.5
	3	4.6 (3.59)	9.7 (1.47)	6.8 - 12.3
	4	4.9 (1.98)	3.9 (1.76)	1.8 - 7.9

Note: The experimental design was a randomized complete block with four replications. Observations were started at early squaring and approximately every two weeks thereafter.

[1] Site code: GATI = Tift county, GA; LACH = Rapides Parish, LA; MSLE = Washington County, MS; NCRC = Edgecombe County, NC; TXPO = San Patricio County, TX; TXUV = Uvalde County, TX.

[2] MON 88702 and conventional control values represent means with standard error in parentheses (N = 4).

[3] Reference range is obtained from the minimum and maximum mean reference values among all reference materials at each site.

† p-values could not be generated due to a lack of or insufficient within-material variability in the data.

– Information not available

Table F-15. Heliothine Damage (Percent damage/plot) to MON 88702 Compared to the Conventional Control in 2016 U.S. Field Trials

Site[1]	Observation	Mean (S.E.)[2]		Reference Range[3]
		MON 88702	Control	
GACH	1	0.0 (0.00)†	0.0 (0.00)	0.0 - 0.0
	2	0.2 (0.18)†	0.2 (0.18)	0.2 - 1.7
	3	0.0 (0.00)†	0.2 (0.19)	0.0 - 0.2
	4	0.7 (0.47)	1.1 (0.46)	0.8 - 1.7
LACH	1	1.9 (0.89)†	0.0 (0.00)	0.0 - 1.3
	2	1.9 (0.67)	2.2 (1.51)	0.5 - 1.9
	3	3.4 (0.88)	2.1 (1.20)	1.0 - 3.0
	4	0.5 (0.45)†	1.1 (0.66)	0.0 - 1.7
MSLE	1	0.0 (0.00)†	1.1 (0.68)	0.0 - 3.3
	2	3.4 (1.56)	1.1 (0.62)	0.9 - 4.9
	3	13.8 (3.88)	7.9 (2.12)	3.0 - 19.8
	4	10.7 (3.12)	10.7 (3.92)	2.9 - 15.0
NCRC	1	1.2 (1.19)†	0.0 (0.00)	0.0 - 16.7
	2	8.7 (2.96)	3.8 (1.76)	2.6 - 6.7
	3	25.7 (4.32)	28.9 (7.40)	23.1 - 49.3
	4	24.9 (4.80)	6.9 (4.72)	16.1 - 67.1
SCEK	1	1.3 (1.27)	0.8 (0.77)	0.5 - 3.2
	2	0.9 (0.94)	1.4 (1.15)	0.2 - 2.9
	3	9.5 (1.29)	7.8 (3.11)	1.8 - 9.4
	4	5.4 (2.47)	4.1 (2.40)	0.4 - 4.0
TXUV	1	1.2 (0.95)	0.6 (0.57)	0.6 - 1.7
	2	2.0 (1.20)	8.7 (4.56)	1.5 - 4.7
	3	0.6 (0.58)*	3.2 (1.36)	0.0 - 1.3
	4	0.0 (0.00)†	0.0 (0.00)	0.0 - 1.3

* Indicates statistically significant difference between MON 88702 and the conventional control ($\alpha = 0.05$) using ANOVA

Note: The experimental design was a randomized complete block with four replications.
Observations were started at early squaring and approximately every two weeks thereafter.

[1] Site code: GACH = Tift county, GA; LACH = Rapides Parish, LA; MSLE = Washington County, MS; NCRC = Edgecombe County, NC; SCEK = Barnwell County, SC; TXUV = Uvalde County, TX.

[2] MON 88702 and conventional control values represent means with standard error in parentheses (N = 4).

[3] Reference range is obtained from the minimum and maximum mean reference values among all reference materials at each site.

† p-values could not be generated due to a lack of or insufficient within-material variability in the data.

Table F-16. Stink bug Damage (Percent damage/plot) to MON 88702 cotton Compared to the Conventional Control in 2015 U.S. Field Trials

Site[1]	Observation	Mean (S.E.)[2]		Reference Range[3]
		MON 88702	Control	
GATI	1	3.8 (3.75)	3.8 (2.39)	3.8 - 6.3
	2	30.0 (12.42)	21.3 (5.15)	17.5 - 23.8
	3	37.0 (6.27)	37.8 (12.30)	22.5 - 47.8
	4	53.8 (7.18)	67.5 (9.46)	51.3 - 65.0
LACH	1	1.3 (1.25)	2.5 (1.44)	3.8 - 11.3
	2	7.5 (3.23)	12.5 (4.33)	10.0 - 31.3
	3	17.5 (4.79)	26.3 (5.54)	28.8 - 42.5
	4	30.0 (4.56)	45.0 (13.07)	47.5 - 57.5
MSLE	1	11.3 (3.75)	20.0 (7.91)	10.0 - 20.0
	2	27.5 (6.61)*	50.0 (10.21)	30.0 - 48.8
	3	45.0 (8.42)	48.8 (8.26)	42.5 - 52.5
	4	45.0 (10.21)	56.3 (10.28)	52.5 - 63.8
NCRC	1	23.8 (7.18)	22.5 (2.50)	23.8 - 41.3
	2	28.8 (5.54)*	63.8 (6.57)	31.3 - 70.0
	3	36.3 (5.54)*	78.8 (2.39)	61.3 - 78.8
	4	37.5 (4.79)*	72.5 (7.22)	61.3 - 76.3
TXPO	1	25.0 (6.12)	17.5 (3.23)	27.5 - 42.5
	2	63.8 (7.18)*	41.3 (2.39)	47.5 - 62.5
	3	91.3 (3.15)	86.3 (1.25)	81.3 - 95.0
	4	100.0 (0.00) †	100.0 (0.00)	95.0 – 100.0
TXUV	1	2.5 (1.44)	5.0 (3.54)	0.0 - 8.8
	2	1.3 (1.25)	5.0 (2.04)	0.0 - 2.5
	3	2.5 (2.50)	3.8 (2.39)	0.0 - 6.3
	4	2.5 (1.44)	3.8 (2.39)	0.0 - 3.8

* Indicates statistically significant difference between MON 88702 and the conventional control (α=0.05) using ANOVA.

Note: The experimental design was a randomized complete block with four replications.
Observations were started at second week of bloom and weekly thereafter.

[1] Site code: GATI = Tift county, GA; LACH = Rapides Parish, LA; MSLE = Washington County, MS; NCRC = Edgecombe County, NC; TXPO = San Patricio County, TX; TXUV = Uvalde County, TX.

[2] MON 88702 and conventional control values represent means with standard error in parentheses (N = 4.).

[3] Reference range is obtained from the minimum and maximum mean reference values among all reference materials at each site.

† p-values could not be generated due to a lack of or insufficient within-material variability in the data.

Table F-17. Stink Bug Damage (Percent damage/plot) to MON 88702 cotton Compared to the Conventional Control in 2016 U.S. Field Trials

Site[1]	Observation	Mean (S.E.)[2]		Reference Range[3]
		MON 88702	Control	
GACH	1	0.0 (0.00)†	1.3 (1.25)	0.0 - 5.0
	2	8.8 (2.39)	6.3 (1.25)	3.8 - 15.0
	3	12.5 (5.95)	13.8 (2.39)	11.3 - 23.8
	4	15.0 (2.04)	13.8 (5.15)	10.0 - 25.0
LACH	1	20.0 (6.12)	25.0 (7.36)	25.0 - 31.3
	2	31.3 (10.08)	41.3 (8.98)	26.3 - 33.8
	3	36.3 (7.18)	42.5 (6.61)	38.8 - 45.0
	4	47.5 (7.22)	52.5 (6.61)	42.5 - 60.0
MSLE	1	10.0 (2.04)*	18.8 (3.15)	10.0 - 22.5
	2	23.8 (3.15)	21.3 (5.54)	22.5 - 32.5
	3	37.5 (1.44)	28.8 (5.54)	26.3 - 43.8
	4	42.5 (7.22)	36.3 (6.88)	42.5 - 48.8
NCRC	1	46.3 (6.57)*	75.0 (9.35)	40.0 - 70.0
	2	68.8 (5.15)	75.0 (5.00)	65.0 - 82.5
	3	86.3 (1.25)	87.5 (6.29)	87.5 - 93.8
	4	91.3 (3.15)	92.5 (4.33)	88.8 - 96.3
SCEK	1	7.5 (2.50)	6.3 (4.73)	3.8 - 15.0
	2	20.0 (6.12)	28.8 (7.74)	25.0 - 40.0
	3	27.5 (6.29)	33.8 (8.00)	20.0 - 30.0
	4	20.0 (4.08)	26.3 (3.15)	25.0 - 32.5
TXUV	1	1.3 (1.25)	10.0 (3.54)	6.3 - 8.8
	2	6.3 (1.25)	10.0 (2.04)	12.5 - 26.3
	3	23.8 (5.91)	21.3 (8.26)	21.3 - 28.8
	4	17.5 (4.79)	18.8 (2.39)	15.0 - 26.3

* Indicates statistically significant difference between MON 88702 and the conventional control ($\alpha = 0.05$) using ANOVA. Note: The experimental design was a randomized complete block with four replications. Observations were started at second week of bloom and approximately weekly thereafter.

[1]Site code: GACH = Tift county, GA; LACH = Rapides Parish, LA; MSLE = Washington County, MS; NCRC = Edgecombe County, NC; SCEK = Barnwell County, SC; TXUV = Uvalde County, TX.

[2] MON 88702 and conventional control values represent means with standard error in parentheses (N = 4).

[3] Reference range is obtained from the minimum and maximum mean reference values among all reference materials at each site.

† p-values could not be generated due to a lack of or insufficient within-material variability in the data.

Table F-18. Arthropod Abundance (Mean/Plot) from Vertical Beat Sheets Collections in MON 88702 Compared to the Conventional Control in 2015 U.S. Field Trials

Arthropod[1]	Primary Function	Site[2]	Mean (S.E.)[3]		Reference Range[4]
			MON 88702	Control	
		Order Araneae			
Spiders	Predator	GATI	8.4 (1.40)	7.2 (1.16)	9.0 - 10.7
		LACH	10.5 (0.48)	12.7 (2.06)	11.5 - 14.9
		MSLE	7.9 (1.26)	8.4 (1.47)	8.2 - 8.5
		NCRC	10.8 (1.05)	10.7 (0.96)	9.7 - 11.8
		TXPO	7.2 (0.38)	6.4 (1.07)	6.8 - 7.3
		TXUV	15.2 (1.51)	15.2 (2.47)	12.6 - 14.9
		Order Coleoptera			
Ant-like flower beetles (Family: Anthicidae)	Predator	GATI	3.6 (0.80)	4.9 (1.03)	4.0 – 5.3
		LACH	2.1 (0.33)	1.8 (0.48)	1.9 - 4.3
		MSLE	2.3 (0.63)	0.8 (0.25)	1.3 - 3.5
		NCRC	4.5 (0.38) *	2.3 (0.24)	3.0 - 4.2
		TXUV	2.9 (0.22)	2.8 (0.28)	2.2 - 3.5
Grape colaspis (Family: Chrysomelidae)	Herbivore	LACH	1.8 (0.28)	3.3 (0.34)	2.6 - 3.9
Click beetles (Family: Elateridae)	Herbivore	GATI	1.4 (0.80)	1.9 (0.51)	1.4 - 8.9
		LACH	2.1 (0.28)	2.6 (0.39)	2.5 - 3.8

Table F-18. Arthropod Abundance (Mean/Plot) from Vertical Beat Sheets Collections in MON 88702 Compared to the Conventional Control in 2015 U.S. Field Trials (continued)

Arthropod[1]	Primary Function	Site[2]	Mean (S.E.)[3]		Reference Range[4]
			MON 88702	Control	
Ladybird beetles (Family: Coccinellidae)	Predator	GATI	8.1 (1.95)	6.4 (0.65)	6.1 - 9.0
		LACH	4.7 (0.53)	3.5 (0.31)	4.4 - 5.7
		MSLE	25.1 (1.90) *	19.1 (2.73)	25.0 - 26.4
		NCRC	15.4 (0.60) *	12.2 (2.12)	12.0 - 14.5
		TXPO	3.3 (0.48)	1.8 (0.49)	1.8 - 3.4
		TXUV	6.8 (0.67)	4.8 (0.78)	5.1 - 8.1
		Order Hemiptera			
Aphids (Family: Aphididae)	Herbivore	GATI	16.5 (7.01)	10.1 (2.14)	8.6 - 27.2
		LACH	10.6 (2.58)	14.3 (9.86)	7.1 - 77.4
		MSLE	76.5 (28.55) *	84.9 (9.10)	77.3 - 177.9
		NCRC	5.8 (1.10)	5.6 (0.35)	6.3 - 7.8
		TXUV	18.3 (6.30)	17.8 (4.47)	12.5 - 29.3
Big-eyed bugs (Family: Geocoridae)	Predator	GATI	20.7 (2.61)	20.5 (2.20)	19.4 - 23.3
		LACH	4.7 (0.38)	4.1 (0.53)	4.5 - 5.8
		MSLE	34.5 (6.82)	38.0 (4.62)	35.1 - 61.5
		NCRC	6.5 (0.58)	9.1 (1.90)	8.4 - 17.1
		TXUV	10.4 (2.32)	8.7 (2.39)	8.6 - 13.7

Table F-18. Arthropod Abundance (Mean/Plot) from Vertical Beat Sheets Collections in MON 88702 Compared to the Conventional Control in 2015 U.S. Field Trials (continued)

Arthropod[1]	Primary Function	Site[2]	Mean (S.E.)[3]		Reference Range[4]
			MON 88702	Control	
Leafhoppers (Family: Cicadellidae)	Herbivores	GATI	2.6 (0.24)	1.8 (0.49)	1.8 - 4.7
		MSLE	1.5 (0.59)	1.6 (0.06)	1.1 - 1.8
		TXPO	2.7 (0.33)	3.2 (0.35)	2.9 - 3.4
		TXUV	5.6 (0.41)	4.0 (0.59)	4.1 - 4.9
Minute pirate bugs (Family: Anthocoridae)	Predator	GATI	2.8 (0.32)	2.1 (0.20)	2.2 - 2.6
		LACH	11.0 (1.78)	11.8 (2.32)	8.4 - 14.1
		MSLE	6.5 (0.44)	8.5 (0.44)	6.8 - 9.2
		NCRC	6.8 (0.92)	8.0 (0.39)	5.8 - 8.4
		TXPO	1.8 (0.42)	1.8 (0.46)	2.3 - 3.5
		TXUV	4.6 (0.75)	5.2 (1.04)	4.8 - 6.0
Damsel bugs (Family: Nabidae)	Predator	GATI	1.4 (0.33)	1.1 (0.28)	1.3 - 2.9
		MSLE	3.7 (0.63)	4.6 (0.78)	4.7 - 7.8
		NCRC	2.0 (0.41)	3.8 (1.49)	3.0 - 5.3
Assassin bugs (Family: Reduviidae)	Predator	GATI	3.1 (1.13)	3.0 (0.54)	1.9 - 3.3
		TXUV	5.0 (2.16)	4.6 (1.17)	2.2 - 6.3
Stink bugs (Family: Pentatomidae)	Herbivore	GATI	0.9 (0.18)	1.4 (0.55)	0.9 - 1.2
		MSLE	5.1 (0.32)	7.0 (1.04)	4.8 - 7.2
		NCRC	5.5 (0.43)	8.1 (1.86)	5.8 - 7.6

Table F-18. Arthropod Abundance (Mean/Plot) from Vertical Beat Sheets Collections in MON 88702 Compared to the Conventional Control in 2015 U.S. Field Trials (continued)

Arthropod[1]	Primary Function	Site[2]	Mean (S.E.)[3]		Reference Range[4]
			MON 88702	Control	
Whiteflies (Family: Aleyrodidae)	Herbivore	MSLE	37.6 (24.40)	31.3 (12.31)	12.7 - 84.2
		TXUV	13.1 (0.74)	12.5 (4.21)	10.8 - 16.2
Order Hymenoptera					
Ants (Family: Formicidae)	Predator	GATI	6.2 (2.01)	7.4 (0.95)	6.0 - 11.0
		LACH	15.2 (5.73)	14.9 (3.02)	10.8 - 16.4
		MSLE	3.5 (1.18)	3.3 (1.51)	4.2 - 7.0
		NCRC	1.3 (0.75)	0.8 (0.48)	0.8 - 2.3
		TXPO	5.1 (1.12)	6.9 (1.81)	4.5 - 6.9
		TXUV	19.3 (2.91)	13.0 (3.44)	11.8 - 28.5
Parasitic wasps	Parasitoid	GATI	2.2 (0.37)	1.8 (0.21)	2.4 - 3.1
		LACH	1.8 (0.26)	2.0 (0.39)	1.0 - 2.3
		MSLE	14.5 (1.05)	18.5 (1.73)	13.3 - 24.1
		NCRC	2.3 (0.39)	2.3 (0.62)	2.7 - 3.8
		TXPO	3.5 (0.51)	2.8 (0.74)	2.4 - 3.6
		TXUV	13.4 (0.83)	13.7 (0.66)	12.7 - 15.3
Order Lepidoptera					
Heliothines (Family: Noctuidae)	Herbivore	MSLE	2.7 (0.22)	2.8 (0.16)	1.3 - 2.7
		NCRC	1.9 (0.46)	1.5 (0.36)	1.3 - 2.1

Table F-18. Arthropod Abundance (Mean/Plot) from Vertical Beat Sheets Collections in MON 88702 Compared to the Conventional Control in 2015 U.S. Field Trials (continued)

Arthropod[1]	Primary Function	Site[2]	Mean (S.E.)[3]		Reference Range[4]
			MON 88702	Control	
		Order Neuroptera			
Lacewings (Family: Chrysopidae)	Predator	GATI	1.3 (0.40)	1.5 (0.30)	1.0 - 2.1
		MSLE	1.5 (0.14)	2.9 (1.21)	1.3 - 1.3
		NCRC	3.9 (0.36)	4.0 (0.62)	3.4 - 5.2
		TXPO	1.6 (0.47)	1.8 (0.28)	1.2 - 1.7
		TXUV	1.1 (0.38)	1.0 (0.34)	1.3 - 1.6

*Indicates statistically significant difference between MON 88702 and the conventional control (α=0.05) using ANOVA.
Note: The experimental design was a randomized complete block with four replications.
[1] Arthropods were collected six times starting at early squaring and bi-weekly thereafter.
[2] Site code: GATI = Tift county, GA; LACH = Rapides Parish, LA; MSLE = Washington County, MS; NCRC = Edgecombe County, NC; TXPO = San Patricio County, TX; TXUV = Uvalde County, TX.
[3] MON 88702 and conventional control values represent means with standard error in parentheses (N = 4).
[4] Reference range is obtained from the minimum and maximum mean reference values among all reference materials at each site

Table F-19. Arthropod Abundance (Mean/Plot) from Vertical Beat Sheets Collections in MON 88702 Compared to the Conventional Control in 2016 U.S. Field Trials

Arthropod[1]	Primary Function	Site[2]	Mean (S.E.)[3]		Reference Range[4]
			MON 88702	Control	
		Order Araneae			
Spiders	Predator	GACH	5.5 (0.83)	6.3 (1.17)	5.0 - 8.3
		LACH	27.5 (1.98)	27.1 (0.81)	24.5 - 32.8
		MSLE	6.3 (0.24)	7.9 (1.88)	5.0 - 8.2
		NCRC	8.7 (0.98)	8.6 (1.03)	8.8 - 12.4
		SCEK	6.5 (0.34)	5.0 (0.48)	4.3 - 5.8
		TXUV	10.6 (1.16)	8.0 (0.50)	8.5 - 9.3
		Order Coleoptera			
Ant-like flower beetles (Family: Anthicidae)	Predator	GACH	1.9 (0.64)	2.0 (0.25)	0.8 - 1.4
		NCRC	4.3 (0.66)	3.8 (0.90)	3.3 - 4.5
		SCEK	2.2 (0.56)	2.4 (0.81)	0.9 - 2.8
		TXUV	2.4 (0.60)	1.4 (0.18)	1.0 - 2.5

Table F-19. Arthropod Abundance (Mean/Plot) from Vertical Beat Sheets Collections in MON 88702 Compared to the Conventional Control in 2016 U.S. Field Trials (continued)

Arthropod[1]	Primary Function	Site[2]	Mean (S.E.)[3]		Reference Range[4]
			MON 88702	Control	
Ladybird beetles	Predator	GACH	5.2 (0.77)	7.6 (0.85)	6.7 - 12.5
		MSLE	2.8 (0.62)	3.3 (0.46)	2.6 - 3.4
		NCRC	2.1 (0.25)	1.5 (0.57)	1.6 - 2.7
		SCEK	2.2 (0.45)	2.0 (0.57)	1.5 - 2.4
Order Hemiptera					
Aphids (Family: Aphididae)	Herbivore	GACH	26.2 (2.83)	19.5 (5.47)	17.2 - 64.2
		LACH	15.8 (2.46)	13.7 (3.86)	11.1 - 246.2
		MSLE	1.9 (0.08)	1.4 (0.17)	0.8 - 1.8
		SCEK	14.5 (2.74)	8.3 (0.76)	9.1 - 42.8
Big-eyed bugs (Family: Geocoridae)	Predator	GACH	4.3 (0.68)*	10.0 (0.57)	4.4 - 8.6
		LACH	1.9 (0.70)	2.8 (0.63)	1.2 - 2.8
		MSLE	2.5 (0.41)	2.3 (0.28)	1.8 - 2.7
		SCEK	3.8 (0.42)	4.1 (0.51)	3.0 - 4.2
		TXUV	3.4 (1.20)	2.7 (0.79)	3.5 - 4.3

Table F-19. Arthropod Abundance (Mean/Plot) from Vertical Beat Sheets Collections in MON 88702 Compared to the Conventional Control in 2016 U.S. Field Trials (continued)

Arthropod[1]	Primary Function	Site[2]	Mean (S.E.)[3]		Reference Range[4]
			MON 88702	Control	
Minute pirate bugs (Family: Anthocoridae)	Predator	GACH	3.0 (0.27)	2.5 (0.22)	2.8 - 4.1
		LACH	4.6 (1.56)	3.9 (0.54)	3.4 - 7.9
		MSLE	7.9 (0.36)	7.8 (0.20)	5.8 - 8.1
		NCRC	3.6 (0.43)	3.3 (0.46)	2.3 - 3.5
		SCEK	6.2 (1.10)	4.8 (0.79)	3.6 - 7.1
		TXUV	6.3 (0.79)	4.4 (0.65)	5.3 - 7.6
Damsel bugs (Family: Nabidae)	Predator	GACH	1.7 (0.28)	3.1 (0.73)	2.8 - 5.2
		NCRC	0.8 (0.31)	1.5 (0.27)	1.5 - 2.2
Stink bugs (Family: Pentatomidae)	Herbivore	NCRC	1.8 (0.68)	1.4 (0.38)	0.9 - 2.5
Whiteflies (Family: Aleyrodidae)	Herbivore	GACH	474.7 (31.67)	587.3 (32.62)	604.6 - 723.8
		MSLE	13.9 (0.95)	13.5 (2.87)	9.0 - 17.0
		TXUV	1.2 (0.31)	1.1 (0.37)	1.2 - 1.7

Table F-19. Arthropod Abundance (Mean/Plot) from Vertical Beat Sheets Collections in MON 88702 Compared to the Conventional Control in 2016 U.S. Field Trials (continued)

Arthropod[1]	Primary Function	Site[2]	Mean (S.E.)[3]		Reference Range[4]
			MON 88702	Control	
		Order Hymenoptera			
Ants (Family: Formicidae)	Predator	GACH	12.3 (0.89)	7.5 (3.66)	13.4 - 30.5
		LACH	5.9 (1.46)	9.8 (2.71)	11.0 - 23.0
		MSLE	1.4 (0.47)	1.0 (0.35)	0.3 - 1.7
		SCEK	2.6 (0.91)	1.8 (0.59)	2.1 - 5.1
		TXUV	7.0 (1.61)	6.5 (0.69)	7.9 - 11.8
Parasitic wasps	Parasitoid	GACH	1.9 (0.34)	2.3 (0.52)	2.7 - 3.2
		LACH	1.3 (0.22)	1.5 (0.28)	0.9 - 1.4
		Order Lepidoptera			
Heliothines (Family: Noctuidae)	Herbivore	NCRC	3.0 (0.62)	2.5 (0.44)	1.2 - 3.3

Table F-19. Arthropod Abundance (Mean/Plot) from Vertical Beat Sheets Collections in MON 88702 Compared to the Conventional Control in 2016 U.S. Field Trials (continued)

Arthropod[1]	Primary Function	Site[2]	Mean (S.E.)[3]		Reference Range[4]
			MON 88702	Control	
Order Neuroptera					
Lacewings (Family: Chrysopidae)	Predator	MSLE	2.1 (0.53)	1.8 (0.39)	0.9 - 2.0
		MCRC	2.8 (0.25)	2.7 (0.47)	2.3 - 2.7
		SCEK	1.0 (0.18)	1.3 (0.27)	0.8 - 1.5

* Indicates statistically significant difference between MON 88702 and the conventional control ($\alpha = 0.05$) using ANOVA.
Note: The experimental design was a randomized complete block with four replications.

[1] Arthropods were collected six times starting at early squaring and approximately bi-weekly thereafter.

[2] Site code: Site code: GACH = Tift county, GA; LACH = Rapides Parish, LA; MSLE = Washington County, MS; NCRC = Edgecombe County, NC; SCEK = Barnwell County, SC; TXUV = Uvalde County, TX.

[3] MON 88702 and conventional control values represent means with standard error in parentheses (N = 4).

[4] Reference range is obtained from the minimum and maximum mean reference values among all reference materials at each site.

Table F-20. Mean (SE) Arthropod Abundance in 2015 and 2016 Vertical Beat Sheet Collections on MON 88702 Compared to the Conventional Control

Arthropod[1]	2015				2016			
	Number[2] of Sites	MON 88702	Control	Power (%)[3]	Number of Sites[2]	MON 88702	Control	Power (%)[3]
Order Araneae								
Spiders	6	10.0 (0.68)	10.1 (0.87)	100.0	6	10.8 (1.65)	10.5 (1.62)	100.0
Order Coleoptera								
Ant-like flower beetles (Family: Anthicidae)	5	3.1 (0.29)	2.5 (0.38)	55.3	4	2.7 (0.36)	2.4 (0.36)	51.1
Click beetles (Family: Elateridae)	2	1.8 (0.41)	2.3 (0.33)	53.2	-	-	-	-
Ladybird beetles (Family: Coccinellidae)	6	10.6 (1.63) *	8.0 (1.35)	100.0	4	3.1 (0.41)	3.6 (0.68)	76.3
Order Hemiptera								
Aphids (Family: Aphididae)	5	25.6 (8.00)	26.6 (7.22)	86.4	4	14.6 (2.46)	10.7 (2.29)	80.8
Assassin bugs (Family: Reduviidae)	2	4.1 (1.18)	3.8 (0.67)	46.8	-	-	-	-
Big-eyed bugs (Family: Geocoridae)	5	15.4 (2.88)	16.1 (3.00)	79.8	5	3.2 (0.35)	4.4 (0.70)	75.0
Damsel bugs (Family: Nabidae)	3	2.4 (0.38)	3.1 (0.68)	61.2	2	1.2 (0.25) *	2.3 (0.46)	62.0
Leafhoppers (Family: Cicadellidae)	4	3.1 (0.43)	2.6 (0.32)	75.1	-	-	-	-
Minute pirate bugs (Family: Anthocoridae)	6	5.6 (0.71)	6.2 (0.84)	99.9	6	5.3 (0.47)	4.4 (0.40)	96.1

Table F-21. Mean (SE) Arthropod Abundance in 2015 and 2016 Vertical Beat Sheet Collections on MON 88702 Compared to the Conventional Control

Arthropod[1]	2015				2016			
	Number[2] of Sites	MON 88702	Control	Power (%)[3]	Number of Sites[2]	MON 88702	Control	Power (%)[3]
Order Hemiptera								
Stink bugs (Family: Pentatomidae)	3	3.8 (0.65) *	5.5 (1.11)	96.1	-	-	-	-
Whiteflies (Family: Aleyrodidae)	2	25.4 (12.21)	21.9 (6.98)	17.0	3	163.3 (67.10)	200.6 (83.05)	63.8
Order Hymenoptera								
Ants (Family: Formicidae)	6	8.4 (1.70)	7.7 (1.29)	90.1	5	5.8 (0.99)	5.3 (1.14)	57.0
Parasitic wasps	6	6.3 (1.16)	6.9 (1.43)	97.2	2	1.6 (0.22)	1.9 (0.31)	66.2
Order Lepidoptera								
Heliothines (Family: Noctuidae)	2	2.3 (0.28)	2.1 (0.30)	24.1	-	-	-	-
Order Neuroptera								
Lacewings (Family: Chrysopidae)	5	1.9 (0.27)	2.2 (0.36)	96.9	3	2.0 (0.29)	1.9 (0.26)	59.0

Note: The experimental design was a randomized complete block with four replications.

* Indicates statistically significant difference between MON 88702 and the conventional control ($\alpha = 0.05$) using ANOVA.

\- Indicates no taxa found or taxon was found in a single site, in either year.

[1] Arthropods were collected six times at each site starting at early squaring and bi-weekly thereafter.

[2] Number of sites where each taxon occurred within each year.

[3] Power to detect a 50% change in arthropod abundance between MON 88702 and the conventional control given an RCBD and $\alpha=0.05$.

References for Appendix F

Ahmad, A., Negri, I., Oliveira, W., Brown, C., Asiimwe, P., Sammons, B. Horak, M., Jiang, C., and Carson, D. 2016. Transportable data from non-target arthropod field studies for the environmental risk assessment of genetically modified maize expressing an insecticidal double-stranded RNA. Transgenic Research 25:1-17.

Blumel, S., Aldershof, S., Bakker, F.M., Baier, F., Boller, E., Brown, K., Bylemans, D., Candolfi, M.P., Huber, B., Linder, C., Louis, F., Muther, J., Nienstedt, K.M., Oberwalder, C., Reber, B., Schirra, K.J., Ufer, A., & Vogt, H. (2000). Guidance document to detect side effects of plant protection products on predatory mites (Acari: Phytoseiidae) under field conditions: vineyards and orchards. Guidelines to Evaluate Side-effects of Plant Protection Products to Non-target Arthropods. IOBC, BART and EPPO Joint Initiative. IOBC/WPRS, Gent, pp. 145 – 153.

Duan, J.J., Jiang, C., Head, G.P., Bhatti, M.A. *et al.* (2006) Statistical power analysis of a 2-year field study and design of experiments to evaluate non-target effects of genetically modified *Bacillius thuringiensis* corn. *Ecological Entomology*, **31**, 521–531.

Perry, J.N., Rothery, P., Clark, S.J., Heard, M.S., & Hawes, C. (2003). Design, analysis and statistical power of the farm-scale evaluation of genetically modified herbicide-tolerant crops. Journal of Applied Ecology, 40, 17 – 31.

SAS. 2012. Software Release 9.4 (TS1M1). Copyright 2002-2012 by SAS Institute, Inc., Cary, North Carolina.

Appendix G. Materials and Methods for Morphology and Viability Assessment of Pollen from MON 88702

G.1. Plant Production

MON 88702, the conventional control, and four commercially available conventional reference materials were grown in a randomized complete block design with four replications (Table G-1). The pollen collection study site was in Washington County, Mississippi. All plants were produced under similar agronomic conditions.

G.2. Flower Collection and Pollen Sample Preparation

Three flowers (one flower from each of three plants), each opened less than 24 hours, were used for pollen sampling from each plot. The pollen collected from an individual flower comprised a subsample of the plot and was placed immediately after pollen collection in a uniquely labeled vial containing approximately 600 µl of Alexander's stain diluted 1:5 with distilled water (Alexander 1980). The vials containing pollen samples were transported to the lab and were allowed to stain at ambient temperatures for at least 20 hours before assessments.

G.3. Data Collection

Slides were prepared by aliquoting suspended pollen/stain solution onto a slide. Pollen characteristics were assessed under an Olympus BX53 light microscope equipped with an Olympus DP72 digital color camera. The microscope and camera were connected to a computer running Microsoft Windows 7 and installed with an Olympus cellSens 1.4.1 software.

G.4. Pollen Viability

When pollen grains were exposed to the staining solution, viable pollen grains stained red to purple due to the presence of living cytoplasmic content. Non-viable pollen grains stained light blue to green or colorless or/and may have appeared round to collapsed in shape, depending on the degree of hydration. For each pollen sample, the number of viable and non-viable pollen grains was counted from a random field of view under the microscope. A minimum of 75 pollen grains were counted for each of the three subsamples per plot. Dense clusters of pollen or pollen grains adhering to flower parts were not counted because they may not have absorbed the staining solution uniformly.

G.5. Pollen Diameter

Micrographs of at least 10 representative, viable pollen grains from each replicate were taken at 200X magnification and imported into the imaging software. The software was used to measure pollen grain diameter along two perpendicular axes for each of the 10 representative viable pollen grains. Mean pollen diameter for each replicate was calculated from the 20 total measurements.

G.6. General Pollen Morphology

General pollen morphology was observed from micrographs of the test, control, and reference materials that were also used for pollen diameter measurements.

G.7. Data Assessment

Experienced scientists familiar with the experimental design and evaluation criteria were involved in all components of data collection, summarization, and analysis. Study personnel ensured that measurements were taken properly, data were consistent with expectations based on experience with the crop, and the experiment was carefully monitored. Prior to analysis, the overall dataset was evaluated by the Lead Scientist for evidence of biologically relevant changes and for possible evidence of an unexpected plant response. Any unexpected observations or issues during the study that would impact the study objectives were noted by the Lead Scientist.

G.8. Statistical Analysis

An analysis of variance was conducted according to a randomized complete block design using SAS (2012). The level of statistical significance was predetermined to be 5% (α=0.05). MON 88702 was compared to the conventional control material for percent viable pollen and pollen grain diameter. MON 88702 and conventional control were not statistically compared to the reference materials. Minimum and maximum mean values were calculated for each characteristic from the four conventional commercial reference varieties. General pollen morphology was qualitative; therefore, no statistical analysis was conducted on these observations.

Table G-1. Starting Seed for Pollen Morphology and Viability Assessment

Entry ID	Site Code[1]	Material Name	Regulatory Lot Number	Phenotype[2]	Material Type[3]
1	MSLE	DP393	11408073	Conventional	C
2	MSLE	DP5415	11299234	Conventional	R
3	MSLE	DP493	11406992	Conventional	R
4	MSLE	All-Tex A102	11406989	Conventional	R
5	MSLE	Americot UA48	11406994	Conventional	R
6	MSLE	MON 88702	11408074	IP	T

[1] Site Code: MSLE = Washington County, Mississippi
[2] Phenotype: IP = Insect Protected.
[3] Material Type = test (T), conventional control (C), conventional reference (R) starting material.

References for Appendix G

Alexander, M.P. 1980. A versatile stain for pollen fungi, yeast and bacteria. Stain Technology 55:13-18.

SAS. 2012. Software Release 9.4 (TS1M1). Copyright 2002-2012 by SAS Institute, Inc., Cary, North Carolina.

Appendix H. Materials and Methods for the Assessment of the Activity Spectrum of the mCry51Aa2 Protein

This appendix provides a summary of the studies conducted to characterize the spectrum of biological activity of the mCry51Aa2 protein. Twenty invertebrate species (comprising nine invertebrate orders and 14 families) were screened for mCry51Aa2 protein insecticidal activity and/or protection of MON 88702 cotton plants against feeding damage in laboratory, controlled environments (e.g. greenhouse/growth chamber), and/or field evaluations.

For the diet assays, the mCry51Aa2 protein was produced in and purified from *Bt* cultures for use in insect diet assays. In total, 6 lots of mCry51Aa2 were used in the laboratory based activity spectrum screening studies (Table H-1). All lots were confirmed to be equipotent using *Lygus hesperus* assays. All lots of mCry51Aa2 used in the activity spectrum screening were confirmed for identity and purity. The mCry51Aa2 *Bt*-produced proteins were evaluated to ensure that they were physicochemically equivalent based on the following analytical tests: sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) to estimate the purity and approximate molecular weight, and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) to establish protein identity by peptide mapping. Each lot of the mCry51Aa2 received from Monsanto Biotechnology was evaluated for biological activity against *L. hesperus* and shown to be biologically active. Preliminary work has shown comparable functional equivalence between *Bt*-produced protein and the mCry51Aa2 expressed by MON 88702.

For the majority of the insects tested, continuous direct feeding assays were conducted with diet incorporation methodology that has historically produced homogenous diets and stability of the *Bt* test substance. assays followed established Monsanto guidelines, published methodologies in the peer-reviewed literature, or methods developed at Monsanto that have been accepted by regulatory agencies for previous products. Exposures typically began with neonates and were extended, when possible through one or more life-stages.

The definitive *in planta* expression of mCry51Aa2 was unknown at the time the majority of the assays was conducted. Therefore, the dose setting for most of the activity spectrum studies were set based on the protein concentrations of mCry51Aa2 in leaf tissues available at the time of conducting the assays.

Field studies: MON 88702 seeds were grown for thrips and cotton fleahopper activity spectrum studies using the untransformed cotton variety DP393 as a comparator. MON 88702 and DP393 seeds were treated with Acceleron® which contains fungicides, the insecticide Imidacloprid, and the nematicide Thiodicarb. Further details regarding the field trial conducted with *L. lineolaris* can be found in Bachman (2017).

In the following sections summaries of the experimental design, assay methodology, and results for each test species are described.

Table H-1. mCry51Aa2 Lots used in Laboratory Activity Spectrum Assays

Lot	Serial ID	Concentration (mg/mL)	Purity (%)
TIC834_16-1	15231838	3.1225	100
TIC834_16-1	15453396	10.5	100
TIC834_16-5	15281356	4.235	100
11412546	NA	4.63	100
11427633	NA	33.0	100
11418808	NA	4.93	99

H.1. Target species – Order Hemiptera

H.1.1. Western Tarnished Plant Bug (WTP), *Lygus hesperus* (Family: Miridae)

Experimental Design: *L. hesperus* were tested in 6-day continuous-feeding diet-incorporation assays to characterize the concentration-effect relationship and to estimate the LC_{50} value for mCry51Aa2. Three independent assays, each using a separate batch of insects, were performed. For each assay, the dose series was expected to elicit a response from *L. hesperus* nymphs that would allow for estimation of the LC_{50} value.

Insects: *L. hesperus* eggs were obtained from USDA-ARS (Stoneville, MS). Insect eggs were incubated at temperatures targeted at 10ºC and 27ºC, depending on desired hatch times.

Assays: The biological activity of the mCry51Aa2 protein on *L. hesperus* nymphs was evaluated with a geometric series of six treatment concentrations that included the following treatment levels: 0 (buffer control), 0.38, 0.78, 1.5, 3.0, 6.0 and 12.0 µg mCry51Aa2 protein/mL of diet (APS lot 11412546, 4.63 mg/mL). The buffer control treatment level contained a volume of buffer control that was equivalent to the volume of buffer in the highest mCry51Aa2 dose level. For each treatment level, the treated diet mixture was dispensed in 150 µL aliquots into a 48-well diet sachet created by a vacuum manifold. The sachet was heat sealed with mylar and cut into individual diet domes. Thirty-two of the 48 diet domes for each treatment were selected based on completeness of seal (i.e. not leaking) and placed into individual wells of a 128-well tray. Each well was targeted to be infested with a single acclimated *L. hesperus* nymph ($\leq$ 30 hours after first observation of hatching). Nymphs were allowed to feed for a period of six days in an environmental chamber programmed at 27ºC, 60% relative humidity, and a lighting regime of 14 hours light: 10 hours dark. The number of insects infested and the number of surviving insects were recorded for each treatment level at the end of the six-day incubation period.

Concentration response modeling: Probit analysis under PROC PROBIT in SAS, version 9.4 was used to estimate the dietary concentration required for 50% mortality (LC_{50}).

Results: The estimated LC_{50} values from three replicate WTP assays are presented in Table H-2. The mean six-day LC_{50} value for WTP exposed to diet incorporated with *Bt*-produced mCry51Aa2 protein was determined to be 3.009 µg mCry51Aa2/mL diet with a standard deviation of 0.389 µg mCry51Aa2/mL diet.

Table H-2. *L. hesperus* LC_{50} Estimates at Day 6 for the mCry51Aa2 Protein

Assay Replicate	LC_{50} Estimate (µg/mL diet)[1]	95% CI (µg/mL diet)
1	3.297	2.247 - 4.640
2	3.165	1.394 - 6.613
3	2.567	1.211 - 3.660

[1] Mean LC_{50} value of 3.009 µg mCry51Aa2/mL diet with a standard deviation of 0.389 µg mCry51Aa2/mL diet.

H.1.2. Tarnished Plant Bug, *Lygus lineolaris* (Family: Miridae) (From Bachman et al (2017))

Experimental Design: Cotton plants of MON 88702 and DP393 were established in 25 cm pots in a controlled environment growth chamber (16 h light at 32 ± 1°C and 8 h dark at 23 ± 1°C). At the peak squaring stage (~ 40 days after planting), plants were selected for use in the experiment that were approximately uniform in vigor, height, and growth stage. Individual plants were enclosed in a cage (98 cm x 140 cm) made from breathable plastic pollination sheets (Vilutis and Company Inc, Frankfort, IL) and then arranged in a completely randomized design with 5 replications (total 5 plants/treatment).

Insects: Two pairs of sexually mature male and female *L. lineolaris* adults from laboratory culture (reared on organic beans) were released into each cage. Insects were allowed to mate, reproduce, and develop for 21 days.

Cage trial: the numbers of next generation insects and their developmental stages were recorded on each plant. Nymphs younger or equal to 3rd instar were recorded as small nymphs and 4th and 5th instars were recorded as large nymphs.

Results: A high level of efficacy was recorded for MON 88702 when tested against *L. lineolaris*. The majority of next generation *L. lineolaris* on MON 88702 were small nymphs (3rd instar or younger) whereas on DP393 plants more large nymphs (4th and 5^{th} instars) were observed from Bachman et al (2017). There was a 19-fold reduction in numbers of large nymphs, the economically most important stage, recovered from MON 88702 compared to DP393 ($F = 16.35$; $df = 1, 4$; $p = 0.016$). Differences were not detected in small nymphs ($F = 3.92$; $df = 1, 4$; $p = 0.119$) and adults ($F = 1.5$; $df = 1, 4$; $p = 0.29$) between MON 88702 and DP393; however, the comparison of total number of next generation of L. lineolaris (sum of all developmental stages) also revealed 15-fold reduction on MON 88702 plants ($F = 11.25$; $df = 1, 4$; $p = 0.028$).

H.2. Target species - Order Thysanoptera

H.2.1. Thrips, *Frankliniella* spp.

Experimental Design: For the trial, MON 88702 and untransformed cotton variety DP393 were grown in eight row plots replicated four times in the field. One hundred and twenty seeds treated

with Acceleron® which contains fungicides, the insecticide Imidacloprid, and the nematacide Thiodicarb, were planted per 30-foot row (four seeds per row foot).

Insects: Because of natural variation in thrips pressure in the field trials, locations were categorized as low, medium, or high pressure sites. Low, medium, and high natural thrips pressure is defined by the highest damage rating score recorded for the untransformed variety at any rating time at that particular location. Low thrips pressure corresponds to a damage rating below two. Medium thrips pressure corresponds to a damage rating of equal to or great than two and less than four. High thrips pressure corresponds to a damage rating of equal to or greater than four.

Damage Assessments: Damage to the cotton plants caused by thrips was assessed during the 2-4 true leaf stage using a damage rating scale of 0-5. A damage rating score of zero corresponded to no damage and no thrips observed, while a damage rating of five corresponded to plant death, severe stunting, stacked internodes, reduced leaf area and terminal bud abortion of most plants.

Results: As demonstrated in Table H-3, the cotton event MON 88702 consistently had lower damage rating scores in field trials compared to DP393 regardless of the natural thrips pressure. These results provide evidence to support resistance against thrips (*Frankliniella* spp).

Table H-3. Average Thrips Damage Ratings for MON 88702 and DP393 in 2014 Field Trials

Natural Thrips Pressure	Location	MON 88702	DP393
High	MS	1.3	4
Medium	TN	0.9	3
Medium	VA	0.3	2.7

H.2.2. Thrips, *Frankliniella* spp. – Data on Oviposition Deterrence Effects (North Carolina State University)

The information below was provided by North Carolina State University as part of a collaboration with Monsanto. The information describes the data generated on the occurrence of oviposition deterrence effects for *F. fusca* and *F. occidentalis* due to MON 88702, which will be part of a future publication from North Carolina State University.

As indicated in Figure V-2, oviposition of *F. fusca* and *F. occidentalis* was assessed as part of the experiments on baseline effects on whole plants (top panel of Figure V-2) and seedling preference (bottom panel of Figure V-2).

Baseline effects on whole plants:

A completely balanced treatment design included the following: MON 88702, DP393, MON 88702+imidacloprid, and DP393+imidacloprid. Individual seeds were planted at a depth

of one inch into sterile soil mix (2 parts loam, 2 peat, 1 sand) in 6-inch clay pots. To assess oviposition deterrence, 50 plants of each treatment were grown. Seedlings were grown for seven days under individual thrips-proof cages prior to infestation.

For *F. fusca*, the average number of eggs per female thrips released onto cotton seedlings as adults was significantly affected by both MON 88702, imidacloprid seed treatment, and the trait by insecticide interaction (Figure H-1A). In the absence of imidacloprid, MON 88702 significantly reduced the number of eggs laid when compared to DP393 alone (Welch two sample t-test: $t = 18.93$; df = 89.87; $p < 0.001$).

For *F. occidentalis*, the average number of eggs per female thrips released onto cotton seedlings as adults was significantly affected by both MON 88702, imidacloprid seed treatment, and the trait by insecticide interaction (Figure H-1B). In the absence of imidacloprid, MON 88702 significantly reduced the number of eggs laid when compared to DP393 alone (Welch two sample t-test: t = 8.04; df = 63.01; $p < 0.001$).

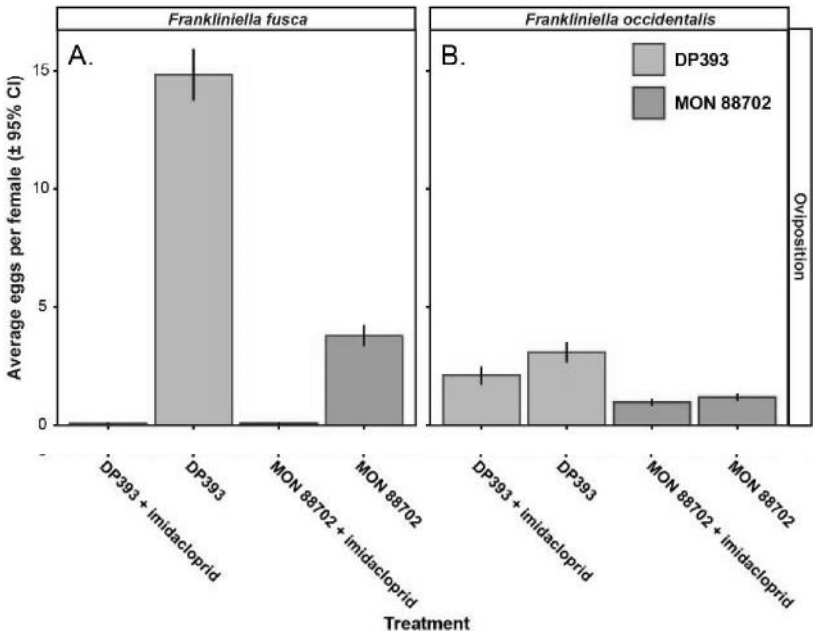


Figure H-1. Baseline toxicity on growing MON 88702 seedlings. (A, B) Percentage reduction in average number of eggs per female on cotton seedlings 72 hours after seedlings were infested with five adult female thrips per seedling at seven days after planting.

Seedling preference:

To understand if thrips prefer to oviposit on a specific host type in a choice situation, a pairwise choice experiment with ten *F. fusca* and *F. occidentalis* females per cage was conducted in greenhouse cages using whole seedlings. Forty replicated cages of MON 88702 and DP393 seedlings for each species were used.

When *F. fusca* was given a choice between MON 88702 and DP393 seedlings as oviposition hosts, 13.9 times more eggs were laid on DP393 than MON 88702. Mean egg production was

18.35 eggs per DP393 seedling (13.64-23.05) and 1.32 eggs per MON 88702 seedling (0.6-2.02) (Figure H-2). The effect of plant type (MON 88702 vs. DP393) was statistically significant (paired t-test; t=7.13; df=39; p-value<0.001). The sample estimate mean differences between plant types was 17.03 eggs (12.19-21.85; 95% CI).

When *F. occidentalis* was given a choice between MON 88702 and DP393 seedlings as oviposition hosts, 5.7 times more eggs were laid on DP393 than MON 88702. Mean egg production was 6.8 eggs per DP393 seedling (3.8-9.8) and 1.2 eggs per MON 88702 seedling (0.8-1.7) (Figure H-2). The effect of plant type (MON 88702 vs. DP393) was statistically significant (paired t-test; t=3.6; df=39; p-value<0.001). The sample estimate mean differences between plant types was 5.5 eggs (2.4-8.6; 95% CI).

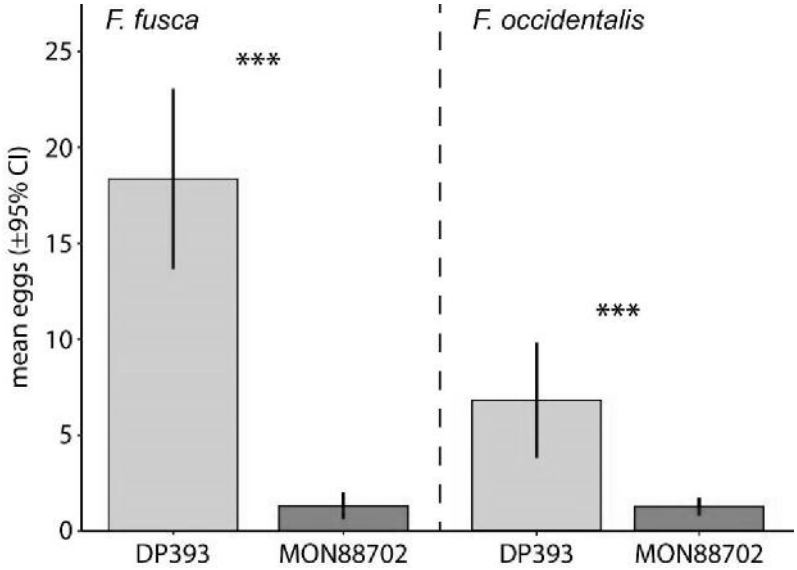


Figure H-2. Oviposition preference for adult *F. fusca* and *F. occidentalis* females provided a pairwise choice between MON 88702 and DP393 cotton seedlings. Results show significant oviposition preference for DP393.

H.3. Herbivores – Order Hemiptera

H.3.1. Cotton Fleahopper (CFH), *Pseudatomoscelis seriatus* (Family: Miridae)

Experimental Design: Cage trials were performed in the field in Texas during the 2014 cotton growing season, and plants were assessed for protection against feeding damage from cotton fleahopper. Two row plots of either MON 88702 or the untransformed DP393 negative control cotton were grown using 120 seeds per 30-foot row (four seeds per row foot). Seeds were treated with Acceleron® which contains fungicides, the insecticide Imidacloprid, and the nematacide Thiodicarb.

Insect Cage Infestations: At the second week of squaring, which occurs around 35-40 days after planting, 10 randomly selected plants (5 from each row) were enclosed in a cage made from white solid voile fabric material (JoAnne Fabrics, Item Number 8139875). Each plant was then infested with two pairs of cotton fleahopper male and female adults. The adults were released

into each individual cage and then the cage was securely closed ensuring the insects would not escape. The insects were allowed to mate and the plants were kept in the cage for 30 days.

Insect Counts: After 30 days, the plants were cut below the cages and moved to a laboratory, where the insects were collected for each plant and counted. Before opening the cage, the plants were vigorously shaken to ensure all of the insects fell off from their feeding sites to the base of the cage. Then the cage base was opened. The plant was then thoroughly inspected to recover any remaining insects. The numbers of insects and their developmental stage were recorded for each plant.

Results: Field data for cotton fleahopper trials are presented in Table H-4. The mean numbers of next generation small nymphs (prior to 3rd instar), large nymphs (4th and 5th instars) and adults is presented.

The total number of cotton fleahopper insects recovered from the cages for cotton event MON 88702 was approximately half the number found in the untransformed DP393 control. Additionally, the untransformed DP393 had approximately four-fold more adults compared to event MON 88702, suggesting that over the course of development, fewer nymphs reach adulthood when feeding on MON 88702.

Table H-4. Average Next Generation Insect Counts for MON 88702 and DP393 Infested with Cotton Fleahopper (*P. seriatus*) in TX, 2014 Field Caging Trial. "SEM" Indicates the Standard Error of the Mean and "N" is the Number of Plants Caged and Sampled

Event	N	Small Nymphs	Large Nymphs	Total Nymphs	Adults	Total CFH	Total CFH SEM
MON 88702	8	4.5	2.5	7	3.5	10.5	5.59
DP393	7	6.43	1.43	7.86	13	20.86	5.23

H.3.2. Neotropical Brown Stink Bug (NBSB), *Euschistus heros* (F.) (Family: Pentatomidae)

Experimental Design: Second instar *E. heros* nymphs (<24 hours posterior to molt) were exposed to the mCry51Aa2 protein overlaid onto the surface of diet at two concentrations of 2500 and 5000 µg mCry51Aa2 protein/mL overlay solution. An untreated (purified water) control, two buffer controls containing the buffer solution at the same level as the mCry51Aa2 protein treatment at 2500 and 5000 µg mCry51Aa2 protein/mL, respectively, and a positive control were also included in the study.

Insects: *E. heros* eggs were obtained from Monsanto insectary in Waterman, IL where the *E. heros* colonies are maintained. Upon receipt in the laboratory, *E. heros* eggs were maintained in an incubator targeting 27 °C, relative humidity 80% and a photoperiod of 16L:8D for egg hatching. Newly-hatched nymphs were maintained in the incubator until the second nymphal instar by providing sterile water with triple-antibiotic solution of Ciprofloxacin (130 µg/mL), Colistin (200 µg/mL), and Tobramycin (200 µg/mL) in a sterile cotton wick. The second instar nymphs (<24 hours posterior to molt) were surface sterilized with 1% bleach solution for 1 minute before they were used in the bioassay. Feeding the newly-hatched nymphs with the

triple-antibiotic solution and treatment of the second instar nymphs with a 1% bleach solution minimized the possibility for fungal or bacterial contamination during the assay.

Assay: The insect bioassay consisted of two treatment concentrations of 2500 and 5000 µg mCry51Aa2 protein/mL of the test material mCry51Aa2 (Lot# 11427633) which was suspended in 10 mM $NaCO_3/Na_2CO_3$ pH 10.25. When not in use the test substance was stored in a -80°C freezer or on dry ice. The buffer control solution was 10 mM $NaCO_3/Na_2CO_3$ pH 10.25 which was used to suspend the protein. The buffer solution was used to prepare buffer control overlay solution at the same rate as in the mCry51Aa2 protein treatment overlay solution at 2500 and 5000 µg mCry51Aa2 protein/mL overlay solution. The untreated control (UTC) substance was purified water. The UTC treatment was also referred to as the water control treatment in this study. The water control treatment was used to prepare the base diet and the overlay solution of the positive control, buffer control and mCry51Aa2 treatments. A stock solution of a proprietary insecticidal toxin from *B. thuringiensis* (TIC 810 in combination with ET29, described in Patent No. U.S. 9,121,035 B2) was used to prepare the positive control treatment solution at 2000 µg/mL.

Artificial agar-based diet was prepared and loaded into 48-wells plates at 250 µl each well using Scinomix machine. The diet plates were dried for 6 minutes using an automated plate drier prior to loading 50 µl of treatment solution onto the diet surface for each well. Each treatment consisted of 24 wells (one column on 4 separate 48 well plates) with three replicates. The water control treatment contained an additional three replicates of 24 wells. All treatment plates were maintained in an incubator at 4 °C overnight to allow the overlay solution to set on the diet. On the day of nymph infestation, all treatment plates were dried for 6 minutes prior to overlaying with 75 µl of 2% low melt agar (Sigma A9414-100G) solution to generate an agar film on top of the treated diet. All nymphs were from a single incubation tub to ensure uniformity of cohorts among all treatments. Once a plate was fully infested, the plate was completely sealed using a ThermalSeal RTS silicone adhesive sealing film (Sigma Z734438-100EA) and 6 holes were punched in each well for ventilation. Plates were maintained in an incubator targeting 27 °C, relative humidity 80% and a photoperiod of 16L:8D for 7 days. Observations for survival of nymphs, number of surviving nymphs developed into third instar, and combined body weight for the surviving nymphs were recorded for each treatment at the end of 7-days incubation.

Statistical analysis: The data for measurements of survival of nymphs, number of surviving nymphs that developed into 3rd instar, and body mass of the surviving nymphs were analyzed. PROC MEANS in SAS (SAS 2012) was used to calculate the means and standard errors for each treatment group in each measurement. Statistical analysis of each measurement in the six treatment groups was conducted using a linear mixed model with the replicate as a random effect. PROC MIXED in SAS (SAS 2012) was used to fit this model for each measurement. Within each model, all treatment comparisons were done using t-tests at a 0.05 level of significance. A p-value < 0.05 represented statistical significance.

Results: Less than 20 % test nymphs died at Day 7 in water control and buffer control at 2500 µg/mL and at 5000 µg/ml, confirming that the bioassay was acceptable. In the positive control treatment, 12.5 % test nymphs survived, 5.6 % nymphs could develop to the third instar,

and the growth of nymphs were significantly inhibited. These results confirm the effectiveness of the bioassay in detecting the toxicity of test substance against *E. heros* nymphs.

In buffer control treatments at both levels of 2500 µg/mL and 5000 µg/mL, the measurements of survival, percentage of nymphs surviving to third instar, and mean body mass of surviving nymphs were not significantly different from the water control treatment, indicating that buffer solution at level of 2500 µg/mL and 5000 µg/mL has no effect on test nymphs.

The overall survival, percentage of nymphs surviving to third instar and mean body mass of surviving nymphs in the mCry51Aa2 protein treatments at both the 2500 and 5000 µg/mL levels showed no significant differences from either the water control treatment or the buffer control (Table H-5). The results indicate that the mCry51Aa2 protein at dose level 2500 and 5000 µg/mL overlay solution has no toxic effects on stink bug nymphs for survival, development and growth.

Table H-5. *E. heros* Nymph Survival, Development and Body Mass at Day 7 for Control and mCry51Aa2 Protein Treatments

Endpoint	Survival			Development			Body Mass		
Treatment	% of Surviving Nymphs (±SE)	P-value (Compared to WC)	P-value (Compared to BC)	% of Nymphs Surviving to Third Instar (±SE)	P-value (Compared to WC)	P-value (Compared to BC)	Mean Body Mass of Surviving Nymphs (±SE)	P-value (Compared to WC)	P-value (Compared to BC)
Water control	90.7 ± 2.5			85.4 ± 2.4			4.4 ± 0.2		
Buffer control 2500 µg/ml	93.0 ± 2.8	0.6143	0.6049	87.5 ± 4.8	0.6590	0.2122	4.7 ± 0.2	0.5119	0.4784
mCry51Aa2 2500 µg/ml	95.8 ± 0.03	0.2795		94.4 ± 1.4	0.0709		4.4 ± 0.4	0.8686	
Buffer control 5000 µg/ml	88.1 ± 1.8	0.5846	0.9052	83.6 ± 3.7	0.6952	0.8480	4.7 ± 0.4	0.5119	0.0554
mCry51Aa2 5000 µg/ml	88.8 ± 2.7	0.6810		84.6 ± 4.9	0.8639		3.7 ± 0.3	0.1014	
Positive control	12.5 ± 7.2	<0.001*		5.6 ± 3.7	<0.001*		3.1 ± 1.3	0.0070*	

Note: WC: water control; BC: buffer control; *Indicates a statistically significant difference (α=0.05).

H.4. Herbivores – Order Coleoptera

H.4.1. Colorado Potato Beetle (CPB), *Leptinontarsa decemlineata* (Family: Chrysomelidae)

Experimental Design: First instar *L. decemlineata* larvae were exposed to mCry51Aa2 protein incorporated into base diet at seven concentrations for a period of 12 to 13 days. Observations were recorded for each insect at the termination of exposure.

Insects: *L. decemlineata* eggs were obtained from French Agricultural Research, Inc., (Lamberton, MN) where established methods are used to culture adults for egg production. The *L. decemlineata* eggs were incubated within environmental chambers targeting 27°C, 60% relative humidity, and a photo period of 14L:10D. Following methods validated within our laboratory, *L. decemlineata* have been successfully utilized in dietary exposure studies and assays.

Assays: Two separate assays were performed using *L. decemlineata*. In the first screening assay, first instar CPB larvae were exposed to mCry51Aa2 (3.1225 mg TIC834_16-1/mL) protein incorporated into base diet at concentrations of 200 and 400 µg/mL diet and observations were made for survival. The second assay was performed to provide support for a dose dependent response and observations were made for survival. For the second assay, first instar larvae (≤ 30 hours from hatching) of *L. decemlineata* were used to initiate dietary exposures to the test material mCry51Aa2 protein (4.235 mg TIC834_16-5/mL) incorporated into an artificial agar-based CPB diet (Bio-serv, Frenchtown, NJ) at six nominal concentrations ranging from 6.0 to 200 µg/mL of diet. A buffer control diet was included in both assays, in which the storage buffer of the test material (carrier) was incorporated at a level equivalent to carrier in the highest concentration tested in the test diet for each assay. Exposures to each of the treatment diets were initiated with a target total of 40 and 32 insects per diet treatment for the first and second assays, respectively. The larvae were housed individually in 128-well assay trays (BAW128, BioServe, Frenchtown, NJ). Into each well, 0.5 mL and 0.75 mL of diet was dispensed for the first and second assay, respectively, which allowed the *L. decemlineata* larvae to feed *ad libitum*. On Day 13 and Day 12 of the first and second assays, respectively, observations for survival were recorded for each insect. Throughout the in-life phase of each assay the *L. decemlineata* larvae were incubated in an environmental chamber at a target temperature of 27 °C, a target relative humidity of 60%, and a 14L:10D regime.

Statistical analysis: A statistical comparison was not required for interpretation of survival results in either assay since differences in survival greater than 10% were apparent between several treatments and the buffer control.

Results: In the screening assay with 200 and 400 µg mCry51Aa2/mL diet the survival of CPB larvae fed the untreated control and buffer control was 92.1 and 92.3%, respectively indicating a negligible background effect. The total survival in the mCry51Aa2 protein treatments was 50.0% and 46.2% for concentrations of 200 and 400 µg mCry51Aa2/mL diet, respectively.

In the second assay, larvae exposed to the assay control diet had 97% survival, indicating a negligible background effect. The survival in the mCry51Aa2 protein treatments decreased with the increase of mCry51Aa2 concentration in the diet (Table H-6). Both the 50 µg mCry51Aa2/mL and 100 µg mCry51Aa2/mL diet treatments elicited corrected survival responses near 50%.

Considering the results from both assays an estimated LC_{50} value for *L. decemlineata* larvae. the estimation is based on the impact on survival which again appears to plateau at around 50 µg mCry51Aa2/mL diet coming close to 50%.

Table H-6. *L. decemlineata* Survival of Larvae at Day 12 for a Range of mCry51Aa2 Protein Treatments

Treatment	Test Larvae	Survival (%)	Corrected Survival (%)
Buffer control	31	96.8	100
6.0 µg mCry51Aa2/mL	32	78.1	80.1
13 µg mCry51Aa2/mL	32	71.9	74.3
25 µg mCry51Aa2/mL	32	65.6	67.8
50 µg mCry51Aa2/mL	32	46.9	48.4
100 µg mCry51Aa2/mL	32	46.9	48.4
200 µg mCry51Aa2/mL	31	38.7	40.0

In order to further characterize the activity of the mCry51Aa2 protein against *L. decemlineata*, additional activity spectrum assays were conducted at concentrations exceeding those described above, where 800 µg mCry51Aa2/mL diet was the highest concentration tested.

Experimental Design: First instar *L. decemlineata* larvae (≤ 30 hours from dispersal after hatching) were used to initiate dietary exposures to the test material mCry51Aa2 in accordance with the current version of ME-0044-04.

Insects: Eggs were obtained from Monsanto Company, Waterman, IL, where established methods are used to culture adults for egg production. The CPB eggs were incubated within environmental chambers targeting 27 °C, 60% relative humidity, and a photo period of 14L:10D. Following methods validated within our laboratory, *L. decemlineata* have been successfully utilized in dietary exposure studies and assays.

Assays:

L. decemlineata Assay

Doses were prepared by diluting the protein with purified water and incorporating the dilution into an agar-based *L. decemlineata* diet (Frontier Agricultural Services Newark, DE). The dose series was aimed to assess the potential to elicit a response from *L. decemlineata* larvae that would allow for estimation of an LC_{50} value. The insect assay consisted of a geometric series of protein standard dilutions at the following treatment levels: 12.5 25, 50, 100, 200, 400, and 800 µg mCry51Aa2 protein/ml of diet. Two mCry51Aa2 protein lots were used: Lot# 11427633 which was suspended in 10 mM sodium carbonate/bicarbonate, pH 10.25 buffer and Lot# 11418808 which was suspended in 50 mM sodium carbonate/bicarbonate, pH 10.8 buffer. Three replicates of a water control and two buffer controls, which were prepared by incorporating the same amount of buffer as required to prepare the highest dose tested for each protein lot, were included in the study. Each treated diet mixture was dispensed in 0.5 mL aliquots into 24 wells per treatment level in a 128-well tray. Each well was targeted to be infested with a single *L. decemlineata* larva ≤ 30 hours after first observation of hatching. Larvae fed *ad libitum* for a period of 7 days in an environmental chamber programmed at 27ºC, at 60% relative humidity, and a photo period of 14L:10D. The number of insects infested and the number of surviving insects were recorded for each treatment level at the end of the 7-day incubation period. The assay was replicated multiple times to account for variability in the results.

Diet analysis for Confirmation of Dose and Stability of the Test Substance

A diet analysis was performed to confirm that the test substance, mCry51Aa2 (Lot# 11418808) was incorporated in the treatment diet at the nominal concentration and was stable in the treatment diet during storage and under the test conditions for the duration of the assay. Test and buffer control treatment diet samples were taken from the *L. decemlineata* assays described above at four different assay timepoints and stored at -80°C (+/- 10°C) until use. The biological activity of the mCry51Aa2 test substance in the diet samples that contained the highest concentration of the protein (800 µg mCry51Aa2/ml diet) was assessed in a six-day diet-incorporation assay using a target species, *Lygus hesperus* (Hemiptera: Miridae). Biological activity of the test treatment

diets and the reference standard was evaluated in accordance with Monsanto Method ME-0044-04. The reference standard was purified *Bt*-produced mCry51Aa2 analytical protein standard Lot # 11418808 dissolved in a buffer solution containing 50 mM sodium carbonate/bicarbonate, pH 10.8 Treatments were prepared by mixing 2 ml dosing solutions that contained an aliquot of the test and/or control treatment diet or reference standard with *L. hesperus* diet (Frontier Scientific, Newark, DE) to achieve a final volume of 10 ml. There were no known contaminants in the insect diet expected to interfere with the results of the assay. Six dose levels (0.38, 0.75, 1.5, 3.0, 6.0, and 12.0 µg mCry51Aa2/ ml diet) that bracket the expected LC_{50} value of the mCry51Aa2 protein for *L. hesperus* were included for the dose-response analyses for the reference standard and the test treatment diets. A two-fold separation factor between dose levels was generated for the reference standard and test treatment diets. Using additional buffer or buffer control diet, the test treatment diet and reference standard contained an equivalent amount of *L. decemlineata* diet matrix and buffer, which was equivalent to that incorporated into the highest treatment level of the test treatment diet. Additionally, three buffer control diet treatments were included that contained an equivalent amount of *L. decemlineata* diet matrix and buffer as incorporated into the highest test treatment diet. Each treatment dose level contained a target number of 32 individually housed nymphs. Assay trays were incubated at a target temperature of 27 ºC (+/-5°C), 60% (+/-10%), and a photo period of 14L:10D for six days. The number of insects infested, and the number of survivors was recorded at assay termination.

Statistical analysis:

L. decemlineata Assay

To determine the activity of the mCry51Aa2 protein against *L. decemlineata*, a statistical comparison was not required for interpretation of survival results in either *L. decemlineata* assay since differences in survival greater than 10% were apparent between several treatments and the buffer control.

Diet analysis for Confirmation of Dose and Stability of the Test Substance

A statistical analysis was conducted to determine the LC_{50} for *L. hesperus* in the diet analysis assay.

Results:

L. decemlineata Assay

The results from three assays are provided in Table H-7. A consistent activity of mCry51Aa2 against *L. decemlineata* was observed, confirming the initially reported activity of the protein against this species. The survival rate ranged from 35-81% at the highest concentration tested (800 µg mCry51Aa2/ml diet). Due to the variability of the results, a consistent dose-response curve could not be generated which prevented the estimation of an LC_{50} value.

Diet analysis for Confirmation of Dose and Stability of the Test Substance

The *L. hesperus* LC_{50} values and their 95% confidence intervals for the reference standard and test treatment diets are summarized in Table H-8. Each dose series with the *L. decemlineata* test diets at the four different timepoints and the reference standard resulted in the expected dose-dependent decrease in *L. hesperus* survival. The six-day *L. hesperus* assay LC_{50} values for the mCry51Aa2 *L. decemlineata* test diets collected on Day 0 and Day 3 and Day 5 were 1.44, 1.65, and 1.59 µg mCry51a2/ml diet, respectively, and were comparable to the LC_{50} value for the reference standard (1.92 µg mCry51Aa2/ml diet). The LC_{50} value of the Day 0 test diet falls within the 95% confidence interval (CI) of the reference standard, thereby confirming the dose of the mCry51Aa2 in the *L. decemlineata* diet. Additionally, the LC_{50} of the Day 3 and Day 5 test diet fall within the 95% CI of the Day 0 test diet, confirming the mCry51Aa2 diet was stable under *in situ* conditions for five days. The LC_{50} value of the Day 7 test diet (2.33 µg mCry51a2/ml diet) did not fall within the 95% CI of the Day 0 test diet which suggests loss of protein stability towards the end of the assay. Despite the reduction in stability at Day 7, the diet was stable for greater than 70% of the assay duration. The observed activity, indicates that a feeding duration of 5 days is sufficient to detect any impacts on *L. decemlineata*. Additionally, the LC_{50} observed after 7 days was similar to previously published LC_{50} for *L. hesperus* (Bachman et al 2017) indicating that the protein remained active despite the reduction in stability.

Table H-7. Survival and Corrected Survival of *L. decemlineata* Larvae After 7 Days of Exposure to a Range of mCry51Aa2 Test and Control Treatments

Assay Replicate[2]	Treatment Diet Description	Test Larvae	Survival (%)	Corrected Survival (%)[3]
1	Buffer Control[1]	59	100	-
1	12.5 µg mCry51Aa2/ml diet	24	95.8	-
1	25 µg mCry51Aa2/ml diet	22	81.8	-
1	50 µg mCry51Aa2/ml diet	22	77.3	-
1	100 µg mCry51Aa2/ml diet	21	81.0	-
1	200 µg mCry51Aa2/ml diet	21	85.7	-
1	400 µg mCry51Aa2/ml diet	24	83.3	-
1	800 µg mCry51Aa2/ml diet	21	81.0	-
2	Buffer Control[1]	72	97.2	100.00
2	200 µg mCry51Aa2/ml diet	24	45.8	47.12
2	400 µg mCry51Aa2/ml diet	24	33.3	34.26
2	800 µg mCry51Aa2/ml diet	23	34.8	35.80
3	Buffer Control[1]	71	98.6	100.00
3	25 µg mCry51Aa2/ml diet	25	96.0	97.36
3	50 µg mCry51Aa2/ml diet	23	69.6	70.59
3	100 µg mCry51Aa2/ml diet	24	70.8	71.81
3	200 µg mCry51Aa2/ml diet	24	83.3	84.48
3	400 µg mCry51Aa2/ml diet	22	59.1	59.94
3	800 µg mCry51Aa2/ml diet	24	50.0	50.71

[1] The buffer control treatment includes the combined results of the three replicates.

[2] Assay replicate 1 used mCry51Aa2 protein Lot# 11427633 and replicates 2 and 3 used protein Lot# 11418808.

[3] The corrected survival % was calculated using a slightly modified Abbott's formula where survival was used instead of mortality.

Table H-8. LC_{50} Values and 95% Confidence Intervals for the Reference Standard and Test Treatment Diets in a Six-day *L. hesperus* Diet-Incorporation Assay

Treatment Diet Description	LC_{50} Value (µg mCry51Aa2/ml Diet)	95% Confidence Interval (µg mCry51Aa2/ml Diet)
Day 0 Test Diet	1.44	0.98 – 1.91
3 Day Test Diet	1.65	1.11 – 2.20
5 Day Test Diet	1.59	1.05 – 2.14
7 Day Test Diet	2.33	1.36 – 3.31
Reference Standard	1.92	1.26 – 2.59

H.4.2. Western Corn Rootworm (WCR), *Diabrotica virgifera virgifera* (Family: Chrysomelidae)

Experimental Design: First instar WCR larvae were tested in 12-day continuous-feeding diet-incorporation assays to characterize the concentration-effect relationship for mCry51Aa2. Observations for survival were recorded on Day 12.

Insects: WCR eggs were obtained from Crop Characteristics, Inc. (Farmington, MN). Insect eggs were incubated at temperatures targeting 10° C and 25° C, depending on the desired hatch times.

Assays: First instar larvae (≤ 30 hours from hatching) of *D. v. virgifera* were used to initiate dietary exposure to mCry51Aa2 protein (10.5 mg TIC834_16-1/mL) incorporated into an artificial agar-based WCR diet (Bio-Serv) at six concentrations ranging from 31.3 to 1000 µg mCry51Aa2/mL of diet. The buffer control diet, in which the storage buffer of the test material (carrier) was incorporated, was tested at a level equivalent to the carrier in the 1000 µg/mL test diet. The treated diet mixture was dispensed in 0.25 mL aliquots into 36 wells per treatment level in 48-well plates (Becton Dickson Labware) which allowed the *D. v. virgifera* larvae to feed *ad libitum*. Exposures to each of the test diets were initiated with a target total of 36 insects per diet treatment. Observations for survival were recorded for each insect on Day 12. Throughout the in-life phase the *D. v. virgifera* were incubated in an environmental chamber at a target temperature of 25 °C, 70% relative humidity, and 24 hour darkness.

Statistical analysis: A statistical analysis was not required for interpretation of the survival endpoint since results were similar for the mCry51Aa2 protein treatments and the buffer control.

Results: *D. v. virgifera* larvae exposed to the buffer control diet had 74% survival, indicating a background effect from the assay >20% which generally does not meet assay acceptance criteria. This result is not unexpected, as Western corn rootworm tends to have higher background mortality in assay than other pest species typically used to test biological activity (i.e. Southern corn rootworm, lepidopteran species). Although the

buffer control survival was <80%, the survival in the mCry51Aa2 protein treatments was similar or greater than the buffer control for all treatment levels except 250 µg/mL. As survival in the 250 µg/mL treatment was within 10% of the control survival and there was no evidence of a concentration dependent effect at the higher concentrations (500 and 1000 µg/mL), the result for the 250 µg/mL treatment is attributed to the high background mortality characteristic of the Western corn rootworm assay (Table H-7).

The results for the continuous dietary exposure to mCry51Aa2 protein demonstrate no adverse effects on the survival of *D. v. virgifera* larvae up to the highest tested dose of 1000 µg mCry51Aa2/mL diet.

Table H-9. *D. v. virgifera* Survival of Larvae at Day 12 for a Range of mCry51Aa2 Protein Treatments

Treatment	Test Larvae	Survival (%)	Corrected Survival (%)
Buffer Control[1]	85	74.12	100
31.3 µg mCry51Aa2/mL	34	73.53	99.2
62.5 µg mCry51Aa2/mL	34	91.18	100
125 µg mCry51Aa2/mL	33	84.85	100
250 µg mCry51Aa2/mL	32	68.75	92.75
500 µg mCry51Aa2/mL	27	81.84	100
1000 µg mCry51Aa2/mL	35	78.13	100

[1]The buffer control treatment includes the combined results of the three replicates.

H.4.3. Southern Corn Rootworm (SCR), *Diabrotica undecimpunctata howardi* (Family: Chrysomelidae)

Experimental Design: First instar *D. u. howardi* larvae were tested in 12-day continuous-feeding diet-incorporation assays to characterize the concentration-effect relationship for mCry51Aa2. Observations for survival for each insect were recorded on Day 12.

Insects: *D. u. howardi* eggs were obtained from Crop Characteristics, Inc.(Farmington, MN). Insect eggs were incubated at temperatures targeting 10° C to 27° C, depending on the desired hatch times.

Assays: Newly hatched larvae (≤ 30 hours from hatching) of *D. u. howardi* were used to initiate dietary exposures to mCry51Aa2 protein (4.235 mg TIC834_16-5/mL) incorporated into an artificial agar-based *D. u. howardi* diet (BioServ) to allow for estimation of functional activity at a range of treatment levels from 1.6 to 200 µg mCry51Aa2/mL of diet. The buffer control diet, in which the storage buffer of the test material (carrier) was incorporated, was tested at a level equivalent to the carrier in the 200 µg/mL test diet. The treated diet mixture was dispensed in 0.25 mL aliquots into 32 wells per treatment level in 48-well plates (Becton Dickson Labware) which allowed the

D. u. howardi larvae to feed *ad libitum*. Exposures to each of the test diets were initiated with a target total of 32 insects per diet treatment. Observations for survival for each insect per treatment were recorded on Day 12. Throughout the in-life phase the *D. u. howardi* were incubated in an environmental chamber at a target temperature of 27 °C, 70% relative humidity, and 24 hour darkness.

Statistical analysis: A statistical comparison was not required for interpretation of survival since differences in survival greater than 10% were apparent between several treatments and the buffer control. The mCry51Aa2 protein elicited a larval development concentration-response; therefore, the development results were analyzed using a 3-parameter logistics model. Statistical Analysis was performed using GraphPad PRISM ®, Prism 6 for Windows, Version 6.03, September 2, 2013, © 1992-2013 GraphPad Software, Inc.

Results: *D. u. howardi* survival at Day 12 in the buffer control was 85.1% and included the combined results from three assay replicates, indicating an acceptable background effect. The survival in the mCry51Aa2 protein treatments decreased with the increase of mCry51Aa2 concentration in the diet (Table H-10), indicating that the effect of mCry51Aa2 protein on the survival of SCR larvae was concentration dependent. A corrected survival response equal to or less than 50% was not recorded at the highest concentration tested and therefore an estimated LC_{50} for *D. u. howardi* was not calculated but would likely be greater than 200 µg mCry51Aa2/mL diet.

Table H-10. *D. u. howardi* Survival of Larvae at Day 12 for a Range of mCry51Aa2 Protein Treatments

Treatment	Test Larvae	Survival (%)	Corrected Survival (%)
Buffer control	94	85.1	100
1.6 µg mCry51Aa2/mL	25	80.0	94.0
3.2 µg mCry51Aa2/mL	31	90.3	100
6.3 µg mCry51Aa2/mL	32	71.9	84.5
12.5 µg mCry51Aa2/mL	33	78.8	92.6
25 µg mCry51Aa2/mL	29	62.1	73.0
50 µg mCry51Aa2/mL	28	75.0	88.1
100 µg mCry51Aa2/mL	31	51.6	60.1
200 µg mCry51Aa2/mL	31	54.8	64.4

In order to further characterize the activity of the mCry51Aa2 protein against *D. u. howardi*, additional activity spectrum assays were conducted at concentrations exceeding those described above, where 800 µg mCry51Aa2/mL diet was the highest concentration tested.

Experimental design: First instar *D. u. howardi* larvae (≤ 30 hours after hatching) were used to initiate dietary exposures to the test material mCry51Aa2 in accordance with Monsanto method ME-0044-04.

Insects: Eggs were obtained from Crop Characteristics, Farmington, MN, where established methods are used to culture adults for egg production. The *D. u. howardi* eggs were incubated within environmental chambers targeting 25 to 27 °C, 70% relative humidity, and a photo period of 24 hours of darkness. Following methods validated within our laboratory, *D. u. howardi* have been successfully utilized in dietary exposure studies and bioassays.

Bioassays:

D. u. howardi Assay

Doses were prepared by diluting the protein with purified water and incorporating the dilution into an agar-based *D. u. howardi* diet (Frontier Agricultural Services Newark, DE). The dose series aimed to assess the potential to elicit a response from *D. u. howardi* larvae that would allow for determination of the LC_{50} or EC_{50} value. Two insect bioassay replicates were conducted specifically looking at survival as the end point and two bioassays were conducted looking at mean insect mass as the endpoint. The insect bioassay consisted of a geometric series of protein standard dilutions at the following treatment levels: 12.5 25, 50, 100, 200, 400, and 800 µg mCry51Aa2 protein/ml of diet. Two mCry51Aa2 protein lots were used: Lot# 11427633 which was suspended in 10 mM sodium carbonate/bicarbonate, pH10.25 buffer and Lot# 11418808 which was suspended in 50 mM sodium carbonate/bicarbonate, pH10.8 buffer. Three replicates of a water control and three buffer controls, which were prepared by incorporating the same amount of buffer as required to prepare the highest dose tested for each protein lot, were included in the study. Each treated diet mixture was dispensed in 0.25 mL aliquots into 32 wells per treatment level in a 48-well tissue culture plate. Each well was targeted to be infested with a single *D. u. howardi* larva ≤ 30 hours after first observation of hatching. Larvae fed *ad libitum* for a period of 7 days in an environmental chamber programmed at 27 ºC, at 70% relative humidity, and a photo period of 24 hours of darkness. For the bioassays looking at survival, number of insects infested, and the number of surviving insects were recorded for each treatment level at the end of the 7-day incubation period. For the bioassay looking at insect mass, the number of insects infested, the number of surviving insects, and the mass of the surviving insects were recorded for each treatment level at the end of the 7-day incubation period. Bioassays were replicated multiple times to account for variability in the results.

Diet analysis for Confirmation of Dose and Stability of the Test Substance

A diet analysis was performed to confirm that the test substance, mCry51Aa2 (Lot# 11418808) was incorporated in the treatment diet at the nominal concentration and was stable in the treatment diet during storage and under the test conditions for the duration of

the assay. Test and buffer control treatment diet samples were taken from the *D. u. howardi* bioassays described above at four different bioassay timepoints and stored at -80°C (+/-10°C) until use. The biological activity of the mCry51Aa2 test substance in the diet samples that contained the highest concentration of the protein (800 µg mCry51Aa2/ml diet) was assessed in a six-day diet-incorporation bioassay using a target species, Western tarnished plant bug (WTP), *Lygus hesperus* (Hemiptera: Miridae). Biological activity of the test treatment diets and the reference standard was evaluated in accordance with Monsanto Method ME-0044-04. The reference standard was purified *Bt*-produced mCry51Aa2 analytical protein standard Lot# 11418808 dissolved in a buffer solution containing 50 mM sodium carbonate/bicarbonate, pH 10.8. Treatments were prepared by mixing 2 ml dosing solutions that contained an aliquot of the test and/or control treatment diet or reference standard with WTP diet (Frontier Scientific, Newark, DE) to achieve a final volume of 10 ml. There were no known contaminants in the insect diet expected to interfere with the results of the bioassay. Six dose levels (0.38, 0.75, 1.5, 3.0, 6.0, and 12.0 µg mCry51Aa2/ ml diet) that bracket the expected LC_{50} value of the mCry51Aa2 protein for *L. hesperus* were included for the dose response analyses for the reference standard and the test treatment diets. A two-fold separation factor between dose levels was generated for the reference standard and test treatment diets. All treatment doses were normalized for both buffer and diet matrix. Additionally, three buffer control diet treatments were included that contained an equivalent amount of *D. u. howardi* diet matrix and buffer as incorporated into the highest test treatment diet. Each treatment dose level contained a target number of 32 individually housed nymphs. Bioassay plates were incubated at a target temperature of 27ºC (+/-5°C), 60% (+/-10%), and a photo period of 14L:10D for six days. The number of insects infested, and the number of survivors were recorded at bioassay termination.

Statistical analysis:

D. u. howardi Assay

A statistical analysis was not required for interpretation of the survival endpoint since results were similar for the mCry51Aa2 protein treatments and the buffer control. The mCry51Aa2 protein elicited a larval development dose response; therefore, the development results were statistically analyzed to estimate an EC_{50} value.

Diet analysis for Confirmation of Dose and Stability of the Test Substance

A statistical analysis was conducted to determine the LC_{50} for *L. hesperus* in the diet analysis assay.

Results:

D. u. howardi Assay

D. u. howardi survival at Day 7 in the buffer control was 97.9%, indicating an acceptable background effect. The results for survival of *D. u. howardi* that fed on mCry51Aa2-containing diet are provided in Table H-11 and the results for body mass are provided in Table H-12. The survival in the mCry51Aa2 protein treatment was comparable to the buffer control at Day 7 and for all concentrations (Table H-11). A consistent dose-

response curve could not be generated which prevented the estimation of an LC_{50} value. The assessments documenting mean insect mass of the surviving larvae demonstrated this endpoint was concentration dependent (Table H-12). Based on these data, the combined EC_{50} value of the two assay replicates was 76.69 µg mCry51Aa2/ml diet. The EC_{50} value and 95% confidence interval for both *D. u. howardi* assay replicates are summarized in Table H-13.

Diet analysis for Confirmation of Dose and Stability of the Test Substance

The *L. hesperus* LC_{50} values and their 95% confidence intervals for the reference standard and test treatment diets are summarized in Table H-14. Each dose series with the *D. u. howardi* test diets at the four different timepoints and the reference standard resulted in the expected dose-dependent decrease in *L. hesperus* survival. The six-day *L. hesperus* bioassay LC_{50} value for the mCry51Aa2 *D. u. howardi* test diets collected on Day 0 was 1.21 and was comparable to the LC_{50} value for the reference standard (1.29 µg mCry51Aa2/ml diet). The LC_{50} value of the Day 0 test diet falls within the 95% confidence interval (CI) of the reference standard, thereby confirming the dose of the mCry51Aa2 in the *D. u. howardi* diet at this timepoint. However, the LC_{50} of the Day 3, Day 5 and Day 7 test diets do not fall within the 95% CI of the Day 0 test diet.

Table H-11. Survival and Corrected Survival of *D. u. howardi* Larvae After 7 Days of Exposure to a Range of mCry51Aa2 Test and Control Treatments

Bioassay Replicate[2]	Treatment Diet Description	Number of Test Larvae Survived	Survival (%)	Corrected Survival (%)[3]
1	Buffer Control[1]	98	97.9	100.0
1	12.5 µg mCry51Aa2/ml diet	28	87.5	89.4
1	25 µg mCry51Aa2/ml diet	29	87.9	89.8
1	50 µg mCry51Aa2/ml diet	29	87.9	89.8
1	100 µg mCry51Aa2/ml diet	31	93.9	95.9
1	200 µg mCry51Aa2/ml diet	31	96.9	99.0
1	400 µg mCry51Aa2/ml diet	30	93.8	95.0
1	800 µg mCry51Aa2/ml diet	29	90.6	92.5
2	Buffer Control[1]	95	96.9	100.0
2	12.5 µg mCry51Aa2/ml diet	31	100.0	100.0
2	25 µg mCry51Aa2/ml diet	30	100.0	100.0
2	50 µg mCry51Aa2/ml diet	34	100.0	100.0
2	100 µg mCry51Aa2/ml diet	30	93.8	96.80
2	200 µg mCry51Aa2/ml diet	31	96.9	100.0
2	400 µg mCry51Aa2/ml diet	30	96.8	99.9
2	800 µg mCry51Aa2/ml diet	32	97.0	100.0

[1] The buffer control treatment includes the combined results of the three replicates.

[2] Both bioassay replicates used mCry51Aa2 protein lot# 11427633.

[3] The correct survival % was calculated using a slightly modified Abbott's formula where survival was used instead of mortality.

Table H-12. Mean Insect Mass of *D. u. howardi* Larvae After 7 Days of Exposure to a Range of mCry51Aa2 Test and Control Treatments

Bioassay Replicate[2]	Treatment Diet Description	Number of Test Larvae Survived	Mean Insect Mass (mg)
1	Buffer Control[1]	78	1.91
1	200 µg mCry51Aa2/ml diet	24	0.74
1	400 µg mCry51Aa2/ml diet	25	0.59
1	800 µg mCry51Aa2/ml diet	18	0.49
2	Buffer Control[1]	83	1.48
2	12.5 µg mCry51Aa2/ml diet	28	1.06
2	25 µg mCry51Aa2/ml diet	26	1.01
2	50 µg mCry51Aa2/ml diet	28	0.85
2	100 µg mCry51Aa2/ml diet	27	0.83
2	200 µg mCry51Aa2/ml diet	27	0.74
2	400 µg mCry51Aa2/ml diet	26	0.65
2	800 µg mCry51Aa2/ml diet	28	0.54

[1] The buffer control treatment includes the combined results of the three replicates.
[2] Both bioassay replicates used mCry51Aa2 protein lot# 11418808.

Table H-13. Combined EC_{50} Value and 95% Confidence Interval for mCry51Aa2 in Seven-day *D. u. howardi* Diet-incorporation Bioassays

EC_{50} Value (µg mCry51Aa2/ml Diet)[2]	95% Confidence Interval (µg mCry51Aa2/ml Diet)
76.69	0.00[1] – 193.70

[1] 95%LL was set to zero where the estimated values less than zero
[2] Both bioassays used protein lot# 11418808.

Table H-14. LC_{50} Values and 95% Confidence Intervals for the Reference Standard and Test Treatment Diets in a Six-day *L. hesperus* Diet-Incorporation Bioassay

Treatment Diet Description	LC_{50} Value (µg mCry51Aa2/ml Diet)[2]	95% Confidence Interval (µg mCry51Aa2/ml Diet)
Day 0 Test Diet	1.21	0.73 – 1.68
3 Day Test Diet	6.05	0.54 – 11.56
5 Day Test Diet	4.98	2.38 – 7.58
7 Day Test Diet	6.93	3.42 – 10.44
Reference Standard	1.29	0.80 – 1.78

[2]Bioassays used protein lot# 11418808.

H.4.4. Mexican Bean Beetle (MBB), *Epilachna varivestis* (Family: Coccinellidae)

Experimental Design: First instar MBB larvae (≤ 24 hours from the first observed hatching) were exposed to mCry51Aa2 protein in a laboratory agar-based diet at two concentrations. MBB were continuously exposed for a period of 28 days following methods previously validated within our laboratory. Observations for survival were recorded for each insect on test days 7, 14, 21, and 28.

Insects: MBB were obtained from the New Jersey Department of Agriculture, Philip Alampi Beneficial Insect Rearing Facility (Trenton, NJ). Eggs were incubated at 27 °C, 70% relative humidity and a photo period of 14L: 10D.

Assay: First instar larvae (≤ 24 hours from hatching) of *E. varivestis* were used to initiate dietary exposures to the test material mCry51Aa2 protein (4.235 mg TIC834_16-5/mL) incorporated into an artificial agar-based diet at two limit concentrations of 200 µg/mL and 400 µg/mL of diet. An untreated control diet, prepared by incorporation of purified water into the artificial diet, was included in the study, along with a buffer control diet in which the storage buffer of the test material (carrier) was incorporated at a level equivalent to the carrier in the 400 µg/mL test diet. Two positive control diets were also included, in which potassium arsenate (KH_2AsO_4) was incorporated at the nominal concentrations of

14 µg/g diet and 28 µg/g diet. Exposures to each of the six diets were initiated with a target total of 40 insects per diet treatment. The larvae were housed individually in 128-well assay trays (BAW128, BioServe, Frenchtown, NJ). Into each well 0.25 mL of diet was dispensed which allowed the *E. varivestis* larvae to feed *ad libitum*. Fresh diet was provided to each surviving larva every 7 days by careful transfer to a new well of a assay tray containing the respective diet treatment. Observations for survival were recorded for each insect on test days 7, 14, 21, and 28. Throughout the in-life phase the *E. varivestis* were incubated in an environmental chamber at a target temperature of 27 °C, a target relative humidity of 70%, and a 14L:10D regime.

Statistical analysis: A statistical analysis was not required for interpretation of the survival endpoint since results were similar for the mCry51Aa2 protein treatment and the buffer control.

Results: MBB larvae in the assay control had acceptable survival at Day 7 and 14, but survival in the assay control was too low (<80%) to be considered acceptable at day 21 and 28. MBB larvae in the buffer control diet had 97.5, 92.5% survival at day 7 and 14, indicating a negligible background effect. The survival in the mCry51Aa2 protein treatment was greater than or equal to the buffer control and assay control at Day 7 and 14 for both treatment levels. In contrast, in the positive control treatment of 28 µg KH_2AsO_4/g diet had 2.5% survival by day 14, confirming the effectiveness of the test system to detect toxic effects.

These results demonstrate no adverse effects on the survival of MBB larvae with continuous chronic dietary exposure over 14 days to mCry51Aa2 protein at the highest concentration tested, 400 µg mCry51Aa2/mL of diet.

Table H-15. *E. varivestis* Survival of Larvae at Day 7, 14, 21, and 28 for Control and mCry51Aa2 Protein Treatments

Treatment	Test Larvae	Day 7 Survival (%)	Day 14 Survival Day (%)	Day 14 Corrected Survival (%)	Day 21 Survival at (%)	Day 28 Survival (%)
Assay Control	40	100	90.0	100	77.5	37.5
Buffer Control	40	97.5	92.5	100	82.5	40.0
200 µg/mL mCry51Aa2	40	100	97.5	100	85.0	52.5
400 µg/mL mCry51Aa2	40	100	95.0	100	77.5	40.0
14 µg/g Positive Control	40	77.5	40.0	44.4	17.5	12.5
28 µg/g Positive Control	40	72.5	2.5	2.8	0	0

H.5. Herbivores – Order Lepidoptera

H.5.1. Fall Armyworm (FAW), *Spodoptera frugiperda* (Family: Noctuidae)

Experimental Design: First instar larvae of *S. frugiperda* were used to initiate dietary exposures to mCry51Aa2 protein incorporated into an artificial agar-based diet at a single limit concentration and observations made for survival at Day 7.

Insects: FAW eggs were obtained from Monsanto Union City Facility, (Union City, TN). Insect eggs were incubated at temperatures targeted at 10 ºC and 27 ºC, depending on desired hatch times.

Assay: First instar larvae (≤ 30 hours from hatching) of *S. frugiperda* were used to initiate dietary exposures to mCry51Aa2 protein (10.4 mg TIC834_16-1/mL) incorporated into an artificial agar-based diet at a concentration of 400 µg/mL of diet. An untreated control diet, prepared by incorporation of purified water into the artificial diet, was included in the study, along with a buffer control diet in which the storage buffer of the test material (carrier) was incorporated at a level equivalent to the carrier in the 400 µg/mL test diet. Exposures to each of the diets were initiated with a target total of 16 insects per diet treatment. The mCry51Aa2 protein treated diet at 400 µg/mL, was replicated two times for a target total of 32 larvae for each of the test diets. The larvae were housed individually in 128-well assay trays (BAW128, Frontier Agricultural Services, Newark, Delaware). Into each well 1.0 mL of diet was dispensed which allowed the *S. frugiperda* larvae to feed *ad libitum*. Observations for survival were recorded for each insect per treatment on Day 7. Throughout the in-life phase the *S. frugiperda* were incubated in an environmental chamber at a target temperature of 27 °C, a target relative humidity of 60%, and a 14L: 10D regime.

Statistical analysis: A statistical analysis was not required for interpretation of the survival endpoint since results were similar for the mCry51Aa2 protein treatment and the buffer control.

Results: Survival was 100%, 100%, and 93.8% in the mCry51Aa2 treatment, the untreated control, and the buffer control at Day 7, respectively. Survival in the mCry51Aa2 treatment was greater than in the buffer control and supports a lack of adverse effects on the survival of *S. frugiperda* larvae from continuous dietary exposure over 7 days at the nominal concentration of 400 µg mCry51Aa2/mL diet.

H.5.2. Corn earworm, *Helicoverpa zea* (Family: Noctuidae)

Experimental Design: First instar larvae of *H. zea* were used to initiate dietary exposures to the test material mCry51Aa2 protein incorporated into an artificial agar-based multiple species diet at two limit concentrations. Observations for survival were recorded for each insect on Day 7.

Insects: *H. zea* eggs were obtained from Benzon Research Inc. (Carlisle, PA) where *H. zea* colonies are maintained. The *H. zea* eggs were incubated within environmental chambers targeting 10 °C and 27 °C, 60% relative humidity, and a photoperiod of 14L:10D.

Assay: First instar larvae (≤ 30 hours from hatching) of *H. zea* were used to initiate dietary exposures to the test material mCry51Aa2 protein (4.235 mg TIC834_16-5/mL) incorporated into an artificial agar-based multiple species diet (Southland, Lake Village, AR) at two limit concentrations of 200 µg/mL and 400 µg/mL of diet. An untreated control diet, prepared by incorporation of purified water into the artificial diet, was included in the study, along with a buffer control diet in which the storage buffer of the test material (carrier) was incorporated at a level equivalent to the carrier in the 400 µg/mL test diet. Exposures to each of the four diets were initiated with a target total of 16 insects per diet treatment. The mCry51Aa2 protein treated diets, at 200 and 400 µg/mL, were replicated two times for a target total of 32 larvae for each of the test diets. The larvae were housed individually in 128-well assay trays (BAW128, BioServe, Frenchtown, NJ). Into each well 1.0 mL of diet was dispensed which allowed the *H. zea* larvae to feed *ad libitum*. Observations for survival were recorded for each insect on Day 7. Throughout the in-life phase the *H. zea* were incubated in an environmental chamber at a target temperature of 27 °C, a target relative humidity of 60%, and a 14L: 10D regime.

Statistics: A statistical analysis was not required for interpretation of the survival endpoint since results were similar for the mCry51Aa2 protein treatments and the buffer control.

Results: Survival was 96.8%, 100%, 100%, and 93.8% in the 400 µg/mL and 200 µg/mL mCry51Aa2 treatment, the buffer control, and the untreated control on Day 7, respectively. The survival in both mCry51Aa2 treatments was similar to the buffer control and greater than the untreated control. These results support a lack of adverse effects on the survival of *H. zea* larvae from continuous dietary exposure over 7 days at the highest concentration tested, 400 µg mCry51Aa2/mL diet.

H.5.3. European Corn Borer (ECB), *Ostrinia nubilalis* (Family: Crambidae)

Experimental Design: Newly hatched ECB larvae were exposed to mCry51Aa2 protein in an agar-based multiple species diet (Southland) at a single limit concentration. Observations for survival were recorded for each insect on Day 7.

Insects: ECB eggs were obtained from Monsanto Union City Facility, (Union City, TN). Insect eggs were incubated at temperatures targeted at 10 ºC and 27 ºC, depending on desired hatch times.

Assay: First instar larvae (≤24 hours from hatching) of *O. nubilalis* were used to initiate dietary exposures to mCry51Aa2 protein (4.235 mg TIC834_16-5/mL) incorporated into an artificial agar-based diet at a concentration of 400 µg/mL of diet. An untreated control diet, prepared by incorporation of purified water into the artificial diet, was included in the study, along with a buffer control diet in which the storage buffer of the test material (carrier) was incorporated at a level equivalent to the carrier in the 400 µg/mL test diet. Exposures to each of the three diets were initiated with a target total of 16 insects per diet treatment. The mCry51Aa2 protein treated diet at 400 µg/mL, was replicated two times for a target total of 32 larvae for each of the test diets. The larvae were housed individually in 128-well assay trays (BAW128, Bio-Serv, Flemington, NJ). Into each

well 0.5 mL of diet was dispensed which allowed the *O. nubilalis* larvae to feed *ad libitum*. Observations for survival or mortality were recorded for each insect per treatment on Day 7. Throughout the in-life phase the *O. nubilalis* were incubated in an environmental chamber at a target temperature of 27 °C, a target relative humidity of 60%, and a 14L:10D regime.

Statistical analysis: A statistical analysis was not required for interpretation of the survival endpoint since results were similar for the mCry51Aa2 protein treatment and the buffer control.

Results: Survival was 96.7%, 100%, and 100% in the 400 µg/mL mCry51Aa2 treatment, the untreated control, and the buffer control at Day 7, respectively. The mCry51Aa2 treatment survival was similar to the buffer control and untreated control and these results support a lack of adverse effects on the survival of *O. nubilalis* larvae from continuous dietary exposure over 7 days at the nominal concentration of 400 µg mCry51Aa2/mL diet.

H.5.4. Diamondback Moth (DBM), *Plutella xylostella* (Family: Plutellidae)

Experimental Design: Newly hatched DBM larvae were exposed to mCry51Aa2 protein in an agar-based multiple species diet (Southland) at a single limit concentration. Observations for survival were recorded for each insect on Day 7.

Insects: DBM eggs were obtained from Benzon Research (Carlisle, PA) where established methods are used to culture field-collected adults for egg production. The *P. xylostella* insect eggs were incubated at temperatures targeted at 10ºC and 27ºC, depending on desired hatch time.

Assay: First instar larvae (≤ 30 hours from hatching) of *P. xylostella* were used to initiate dietary exposures to mCry51Aa2 protein (10.4 mg TIC834_16-1/mL) incorporated into an artificial agar-based diet at a concentration of 400 µg/mL of diet. An untreated control diet, prepared by incorporation of purified water into the artificial diet, was included in the study, along with a buffer control diet in which the storage buffer of the test material (carrier) was incorporated at a level equivalent to the carrier in the 400 µg/mL test diet. Exposures to each of the three diets were initiated with a target total of 32 insects per diet treatment. The mCry51Aa2 protein treated diet at 400 µg/mL, was replicated two times for a target total of 64 larvae. The larvae were housed individually in 128-well assay trays (BAW128, Frontier Agricultural Services, Newark, Delaware). Into each well 0.5 mL of diet was dispensed which allowed the *P. xylostella* larvae to feed *ad libitum*. Observations for survival were recorded for each insect per treatment on Day 7. Throughout the in-life phase the *P. xylostella* were incubated in an environmental chamber at a target temperature of 27 °C, a target relative humidity of 60%, and a 14: 10, light: dark regime.

Statistical analysis: A statistical analysis was not required for interpretation of the survival endpoint since results were 100% for the mCry51Aa2 protein treatment and the buffer control.

Results: Survival was 100% in the 400 µg/mL mCry51Aa2 treatment, the untreated control, and the buffer control at Day 7. The mCry51Aa2 treatment survival was similar to the buffer control and untreated control and these results support a lack of adverse effects on the survival of *P. xylostella* larvae from continuous dietary exposure over 7 days at the nominal concentration of 400 µg mCry51Aa2/mL diet.

H.6. Decomposer – Order Diptera

H.6.1. Yellow Fever Mosquito (YFM), *Aedes aegypti* (Family: Culicidae)

Experimental Design:

First instar *A. aegypti* larvae (≤ 24 hours from dispersal after hatching) were used to initiate dietary exposures to mCry51Aa2 protein incorporated into an artificial diet at 400 and 800 µg/mL diet concentration. Observations for survival were recorded for each insect on test Day 4.

Insects: Eggs were obtained from Benzon Research, Carlisle, PA, where established methods are used to culture adults for egg production. The *A. aegypti* eggs were incubated in deoxygenated water at room temperature. Following validated methods, *A. aegypti* have been successfully utilized in dietary exposure studies and assays.

Assay: Doses were prepared by diluting the protein with purified water and incorporating the dilution into a base *A. aegypti* artificial diet mixture. The insect assay consisted of two treatment concentration levels 400 and 800 µg mCry51Aa2/mL diet of the test material mCry51Aa2 Lot# 11427633 which was suspended in 10 mM sodium carbonate/bicarbonate, pH10.25 buffer. For the buffer control treatment diet, the same volume of buffer solution was added into the base diet to obtain an equivalent amount of buffer as in the mCry51Aa2 protein treatment diet at 800 µg/mL diet. A stock solution of potassium arsenate was used to prepare positive control treatment diet at 200 µg/mL diet. Each treated diet mixture was dispensed in 200 µL aliquots into 32 wells per treatment level (8 wells per plate) in flat-bottom 96-well plates. Each well was targeted to be infested with a single *A. aegypti* larva ≤ 24 hours after first observation of hatching. Larvae fed *ad libitum* for a period of 4 days in an environmental chamber programmed at 27 ºC, at 70% relative humidity, and at a lighting regime of 24-hour darkness. The number of insects infested, and the number of surviving insects were recorded for each treatment level at the end of the 4-day incubation period.

Statistical analysis: A statistical analysis was not required for interpretation of the survival endpoint since results for the mCry51Aa2 protein treatment and the buffer control were comparable.

Results: The results from the assay are provided in Table H-16. There was no contamination observed in any treatment and survival in the water and buffer control was over 90%. Additionally, there were no surviving insects in the positive control treatment. Survival was 93.75%, 100%, 90.63%, and 93.55% in the 400 and 800 µg mCry51Aa2/mL diet, the buffer control, and the water control on Day 4, respectively. These results support a lack of adverse effects on the survival of *A. aegypti* larvae from

continuous dietary exposure for 4 days at the nominal concentrations of 400 and 800 µg mCry51Aa2/mL diet.

Table H-16. Survival and Corrected Survival of *A. aegypti* After 4 Days of Exposure to mCry51Aa2 Test and Control Treatments

Treatment Diet Description	Survival # / Initial Insect #	% Survival After Day 4 (%)	Corrected % Survival[1]
Water Control	29/31	93.55	100.00
Buffer Control	29/32	90.63	96.87
400 µg mCry51Aa2/ ml Diet	30/32	93.75	100.00
800 µg mCry51Aa2/ ml Diet	32/32	100.00	100.00
Potassium Arsenate at 200 µg/ ml Diet	0/31	0.00	0.00

[1] The correct survival % was calculated using a slightly modified Abbott's formula where survival was used instead of mortality.

H.7. Beneficial Arthropods – Order Hemiptera

H.7.1. Insidious Flower Bug (IFB), *Orius Insidiosus* (Family: Anthocoridae)

Experimental Design: Five-day old IFB nymphs were exposed to mCry51Aa2 protein incorporated into base diet over a range of concentrations for a period of eleven days following the methods presented in Tan et al. (2011). Daily observations were made for survival.

Insects: Adult IFB were received from Koppert Biological Systems (Romulus, MI). Upon receipt, the adult IFB were reared in the lab to produce nymphs according to the methods described in Tan et al. (2011).

Assays: Two separate assays were performed using IFB. In the first screening assay, five-day old IFB nymphs were exposed to mCry51Aa2 (3.1 mg TIC834_16-1/mL) protein incorporated into base diet at concentrations of 200 and 400 µg/g diet. The second assay was performed to provide support for a dose dependent response. Five-day old IFB nymphs were exposed to mCry51Aa2 protein (4.235 mg TIC834_16-5/mL) protein incorporated into base diet at five concentrations ranging from 13 to 200 µg/g diet. Both assays were conducted for a period of eleven days following the methods presented in Tan et al. (2011). The base diet consisted of 25% bee pollen, 25% *Ephestia kuehniella* eggs, and 50% water and was encapsulated in domes. Potassium arsenate (KH_2AsO_4) at a concentration of 100 µg/g diet was used as the positive control to demonstrate the effectiveness of the dietary exposure system to detect toxic effects. Additionally, an assay control containing the same amount of buffer solution as in the mCry51Aa2 treatment diet was also included in this study as a negative control. Each treatment consisted of more than 30 nymphs individually housed in petri dishes and fed *ad libitum* until all nymphs developed to adults or died at the same environmental conditions as described in Tan et al. (2011). All nymphs were impartially included in each treatment and hatched on the same day from the same batch of eggs. The encapsulated diets were replaced every 48 hours and daily observations were made for survival.

Statistical analysis: A statistical comparison was not made for either assay since differences in survival greater than 10% were apparent between treatments and the assay control.

Results: In the screening assay with 200 and 400 µg mCry51Aa2/g diet nymphs exposed to assay control diet had 98% survival and all survived nymphs developed to normal adults, indicating a negligible background effect. The total survival in the mCry51Aa2 protein treatments was 67% for both concentrations of 200 and 400 µg/g diet. The development of surviving *O. insidiosus* nymphs was not affected by ingestion of mCry51Aa2 protein, as both the mCry51Aa2 protein treatment and the assay control treatment exhibited 100% adult emergence from the surviving nymphs. In contrast, in the positive control treatment of 100 µg KH_2AsO4/g diet, mortality was observed at Day 3 and reached 95% by Day 7 confirming the effectiveness of the test system to detect toxic effects.

In the second assay with five treatment concentrations, IFB nymphs exposed to the assay control diet had 82% survival and all surviving nymphs developed to normal adults, indicating a negligible background effect. The survival in the mCry51Aa2 protein treatments decreased with the increase of mCry51Aa2 concentration in the diet (Table V-6), indicating that the effect of mCry51Aa2 protein on the survival of five-day old *O. insidiosus* nymphs was concentration dependent. Survival in the 13 µg mCry51Aa2/g diet treatment was not different from the assay control treatment.

A corrected survival response equal to or less than 50% was not recorded at the highest concentration tested in either of the two assays. While an estimated LC_{50} value for five-day old *O. insidiosus* nymphs could not be generated, these results indicate that it could be greater than 400 µg mCry51Aa2/g diet.

In contrast, in the positive control treatment of 100 µg KH_2AsO_4/g diet, reduced survival was observed at Day 3 and reached 0% by Day 7, confirming the effectiveness of the test system to detect toxic effects.

H.8. Beneficial Arthropods – Order Coleoptera

H.8.1. Pink Spotted Lady Beetle, *Coleomegilla maculate* (Family: Coccinellidae)

Experimental Design: First instar larvae (≤ 36 hours from the first observation of hatching) of *C. maculata* were exposed in the laboratory to mCry51Aa2 protein in an agar-based pollen diet at two nominal concentrations. *C. maculata* larvae were continuously exposed for a period of 12-17 days (until pupation) following methods previously developed for plant incorporated protectants (PIPs) at Monsanto. Observations for survival were made.

Insects: *C. maculata* eggs were obtained from a culture at the USDA (Beltsville, MD) and were incubated at temperatures targeted at 27° C, 70% relative humidity and a photo period of 14L:10D.

Assay: Newly hatched *C. maculata* larvae were acclimated for approximately 24 hours. During this period the larvae were fed *Helicoverpa zea* eggs to reduce cannibalism prior to test initiation. Following methods previously validated within our laboratory, *C. maculata* has been successfully utilized in dietary exposure studies. First instar larvae (≤ 36 hours from hatching) of *C. maculata* were used to initiate dietary exposures to mCry51Aa2 protein (3.1 mg TIC834_16-1/mL) incorporated into an artificial agar-based pollen diet at two concentrations of 200 µg/mL and 400 µg/mL of diet. An untreated control diet prepared by incorporation of purified water into the artificial diet, was included in the study, along with a buffer control diet in which the storage buffer of the test material (carrier) was incorporated at a level equivalent to the carrier in the 400 µg/mL test diet. A positive control was also included in which potassium arsenate (KH_2AsO_4) was incorporated at concentration of 100 µg/g diet. Exposures to each of the five diets were initiated with a target total of 40 insects per diet treatment. The larvae were housed individually in petri dish test arenas and fed *ad libitum* with ~ 0.15 mL of respective test or control diet administered every 48 hours. Observations for survival were made within 32 hours of adult eclosion (~16 days). Throughout all stages of the in-

life phase, the *C. maculata* were incubated in an environmental chamber at a target temperature of 27 °C, a target relative humidity of 70%, and a 14L:10D regime.

Statistical analysis: A statistical analysis was not required for interpretation of the survival endpoint since results were similar for the mCry51Aa2 protein treatment and the buffer control.

Results: *C. maculata* larvae in the buffer control diet had 82% survival, indicating an acceptable background effect for the assay. The survival in the mCry51Aa2 protein treatments was greater than the buffer control for both treatment levels (Table H-17). In contrast, in the positive control treatment of 100 µg KH_2AsO_4/g diet, survival to adult eclosion was 0%, confirming the effectiveness of the test system to detect toxic effects.

These results demonstrate no adverse effects on the survival of *C. maculata* larvae through adult eclosion with continuous chronic dietary exposure to mCry51Aa2 protein at the highest concentration tested, 400 µg mCry51Aa2/mL of diet.

Table H-17. *C. maculata* Survival at Adult Eclosion (~Day 16) for Control and mCry51Aa2 Protein Treatments

Treatment	Test Larvae	Survival (%)	Corrected Survival (%)
UTC	40	75.0	100
Buffer Control[1]	39	82.1	100
mCry51Aa2 200 µg/mL	40	87.5	100
mCry51Aa2 400 µg/mL	40	85.0	100
Positive Control	40	0	0

[1]The buffer control treatment includes the combined results of the three replicates.

H.9. Beneficial Arthropods – Order Hymenoptera

H.9.1. Honey bee, *Apis mellifera* (Family: Apidae)

Experimental Design: Approximately 2-day old *A. mellifera* larvae were exposed to mCry51Aa2 protein in a sucrose diet solution at a single limit concentration. Observations for survival were made at 6 days and 12 days, and adult emergence time (development) was evaluated on day 13 through 17 after dosing.

Insects: The larval stage of the honey bee, *A. mellifera* spp. was used in this study. Adult honey bee queens were of the "Italian" variety derived from colonies at Honeybee Genetics (Vacaville, CA) and purchased by the contract research organization that performed the study, California Agricultural research, Inc. (CAR). The stock was comprised of primarily *Apis mellifera ligustica* species. In addition, some cross breeding may also occur at the CAR site with local bees including Italian honey bees (*Apis mellifera carnica).* Age determination and genus/species of the bees was based on previous experience and an appropriate reference (Winston, 1987).

Bee hives were maintained at the CAR bee yard for the duration of the study. Each hive was acclimated in the CAR bee yard for a minimum of 30 days prior to actual treatment administration. Prior to hive selection, all hives were inventoried for their general health, vigor, and brood conditions. An assessment of general health, vigor, and brood conditions was repeated on June 17, 2015 to identify those hives able to provide adequate larval brood frames for study purposes.

Assay: Larvae of *A. mellifera* were exposed to mCry51Aa2 protein (10.5 mg TIC834_16-1/mL) at a concentration of 2 mg/mL diet solution in 10 µL (final volume) for each larva. In addition, an assay control, a buffer control, and positive control were included.

Each treatment consisted of two replicates and each replicate tested 20 honey bee larvae approximately 2 days old. In Replicate 1, two frames were selected for the treatment of mCry51Aa2 protein at 2 mg/mL diet solution, assay control, positive control (KH_2AsO_4) at 2.08 mg/mL. A frame from a separate hive was used for buffer control. The treatments in Replicate 2 followed the same pattern using the frames from separate hives. Each side of the two frames from each hive was impartially assigned to each of the treatments. Upon completion of diet solution administration, frames were held in the insulated container (e.g., an ice chest, without ice) for at least 30 minutes before being returned to their original hive.

Observations were made at 6 days after dosing by removing the treated frames from their respective hives and evaluating for capping. Once the evaluation was completed the frames were returned to their respective hives. On Day 12 after dosing, the frames were once again removed from their hives, capping was rechecked and the frames placed in a growth chamber. All frames were then moved into a screened hive box and placed in a growth chamber under a 0:24 hour light: dark photoperiod. Temperature and relative humidity were from 74.8 to 87.3 ºF and 50.7% to 63.3%, respectively. All the conditions were monitored and recorded. On Day 13 through Day 17 after dosing, daily evaluations were conducted for adult emergence; this observation interval encompassed the adult emergence of all surviving larvae.

Statistics: A statistical analysis was not required for interpretation of the results since there was 100% survival in mCry51Aa2 treatment, assay control, and buffer control and the mean development time for the mCry51Aa2 treatment was less than the buffer control.

Results: Survival was 100% in the mCry51Aa2 protein treatment at a concentration of 2 mg/mL diet solution, as well as the treatment of assay control (30% sucrose) and buffer control. The positive control treatment of potassium arsenate at 2.08 mg/mL had 0% survival confirming the effectiveness of the test system to detect toxic effects.

The average development time and its standard error was estimated to be 14.28 ±0.22 days in the treatment of mCry51Aa2, 14.25±0.15 days in assay control treatment, and 14.73±0.78 days in buffer control treatment. Since the development time in the mCry51Aa2 treatment was less than the development time for the buffer control, no statistical analysis was required to interpret the results showing a lack of an adverse effect by 2 mg/mL mCry51Aa2 on this endpoint.

H.9.2. Eulophid Wasp, *Pediobius foveolatus* (Family: Eulophidae)

Experimental Design: Adults of *P. foveolatus* were exposed to mCry51Aa2 protein incorporated into diet at concentrations of 200 µg/mL and 400 µg/mL for a period of 20 days following methods previously validated within our laboratory. All wasps in all treatments were observed for mortality every two days.

Insects: The wasps were obtained from the New Jersey Department of Agriculture, Phillip Alampi Beneficial Insect Laboratory (Trenton, NJ) as larvae and/or pupae in parasitized Mexican bean beetle (MBB, *Epilachna varivestis*) larvae (mummies). Upon receipt in the laboratory, the parasitized MBB larvae were maintained in an incubator at the temperature 25±5°C, the relative humidity 70±5% and the photoperiod 14 light: 10 dark for adult emergence. The newly emerged adults were supplied with 30% honey/water (v/v) solution and acclimated for one day prior to inclusion in feeding test.

Assay: Wasp adults of one-day old were exposed to mCry51Aa2 protein (3.1 mg TIC834_16-1/mL) incorporated into 30% honey/water (v/v) solution at concentration of 200µg/mL and 400µg/mL, respectively, for a period of 20 days. Potassium arsenate (KH_2AsO_4) at a concentration of 200 µg/mL diet was used as the positive control substance to demonstrate the effectiveness of the dietary exposure system to detect toxic effects. Additionally, a water control (deionized water) and a buffer control (buffer solution) were also included in this study. All controls consisted of three replicates and mCry51Aa2 treatments included 2 replicates. Each replicate included at least 22 individual adults housed in a flask (162 cm^2 cell culture flask with vented cap, Costar® 3151, Corning Inc., Corning, NY) and provided with appropriate treatment diet in two screened feeding dishes. All test adults were hatched from the same batch on the same day and impartially included in each replicate. All test wasps were maintained in the same incubator for adult emergence. All wasps in all treatments were observed for mortality every two days at the time of diet replacement. Dead wasps, if any, were removed from the test arenas and recorded.

Statistics: A statistical analysis was not required for interpretation of the survival endpoint since results were similar for mCry51Aa2 protein treatments and the buffer control.

Results: All wasps (100%) survived in the water control and 97.2% wasps survived in buffer control, indicating no background effect on survival of test adults. Similarly, 100% and 94.2% test wasps survived in the mCry51Aa2 protein treatment at 200 µg/mL and 400 µg/mL, respectively. The survival in both mCry51Aa2 treatments was similar to or greater than the buffer control and the untreated control. In contrast, in the positive control treatment of 200 µg KH_2AsO_4/mL diet, mortality was observed at Day 4 and reached 100% by Day 12 confirming the effectiveness of the test system to detect toxic effects. These results indicate no adverse effect of 400 µg mCry51Aa2 protein/mL diet on the survival of wasp adults after 20 days of continuous dietary exposure.

H.10. Beneficial Arthropods – Order Collembola

H.10.1. Collembola, *Folsomia candida* (Family: Isotomidae)

Experimental Design: Juvenile Collembola were exposed to mCry51Aa2 protein, buffer control, untreated control, or toxic reference treatment via a diet medium. A starvation control was also included. The endpoints of the assay were an assessment of any mortality amongst the insects originally introduced into the arenas, and an assessment of their reproductive success at 28 days. The test method was adapted from OECD Guideline 232 (OECD, 2009).

Insects: The test organism (*F. candida*) was obtained from an in-house culture maintained at the contract research organization, Mambo-tox Ltd (Southampton, UK) since 2005. For the test, 9- to 10-day-old springtails were used.

Assay: A assay was carried out in which a dilution of mCry51Aa2 protein (10.5 mg TIC834_16-1/mL) was incorporated into an inactivated-yeast diet medium, for feeding to confined populations of springtails. The mCry51Aa2 protein, already held in a carbonate buffer solution, was further diluted in purified water for mixing with the yeast at a rate equivalent to 400 µg protein/g diet medium. This single application rate of the test substance was compared to an assay control (untreated diet of inactivated-yeast and purified water), a buffer control (inactivated yeast mixed with the 25 mM carbonate buffer), a blank control (no food provided), and a toxic reference diet (inactivated-yeast treated with the insect growth regulator, teflubenzuron). The various diet media were prepared and treated prior to test initiation, and then they were divided into small aliquots prior to being stored in a freezer.

The test arenas consisted of lidded glass jars, lined at the base with a solid layer of a plaster-of-Paris and charcoal substrate. Groups of 10 juvenile springtails (9-10 days old) were placed into each replicate jar (n = 4 per treatment) and a freshly-defrosted aliquant of the appropriate diet was provided. Every 2-3 days, the springtails were provided with additional treated diet, *ad libitum*, for the remainder of the experiment. At 28 days, the numbers of the original population of springtails still surviving in each test arena and the numbers of their offspring were recorded for each replicate arena.

Statistics: A statistical analysis was not required for interpretation of the survival and mean number of progeny endpoints since results were similar for mCry51Aa2 protein treatments and the buffer control. The numbers of surviving adults were used to calculate the percentage mortality of the *F. candida* originally introduced in each treatment.

Results: At 28 days, the percentage mortality for adult Collembola with the buffer and untreated assay control diets were both 0%, compared with 3% in the 400 µg/g mCry51Aa2 diet treatment. With only a 3% difference between the treatment and buffer control the result for survival in the mCry51Aa2 treatment did not require statistical analysis for interpretation of a lack of treatment effect. The blank (starvation) control resulted in 0% mortality; however, the Collembola were very small when compared to the buffer and assay control diet treatments. In the toxic reference diet treatment, 83%

mortality was recorded compared with 0% for the assay and buffer control, confirming the effectiveness of the test system to detect toxic effects.

The mean number of progeny produced per replicate (with SD) with the untreated assay control diet, the buffer control diet, and the mCry51Aa2 protein diet treatment was 170 (4.3), 161 (4.6), and 159 (13.2) progeny, respectively. With less than a 3% difference, the result for the mCry51Aa2 diet was so similar to the buffer control diet that a statistical analysis was not required for an interpretation of the results. In the blank (starvation) control, a mean of 2 progeny were produced per replicate. Also, it was noted that the original springtails added to the blank control arenas at the start of the test remained small compared to the untreated control treatment. In the toxic-reference diet treatment, the mean number of progeny produced per replicate was 4, a value so apparently different from the assay control (170) and buffer control (161) that a statistical analysis was not required to confirm that the reproductive endpoint in this assay was effective for detecting toxic effects.

These results indicate that 400 µg mCry51Aa2 protein/g inactive-yeast diet had no significant effects on the survival or reproductive capacity of the springtails. The blank (starvation) control did have a significant effect on reproduction, demonstrating that the yeast diet was necessary for the *F. candida* to grow and reproduce under these test conditions. The toxic reference diet had a significant effect on both springtail survival and reproduction, confirming that the test system was capable of detecting toxic effects through dietary exposure.

H.11. Beneficial Arthropods – Order Haplotaxida

H.11.1. Earthworm, *Eisenia andrei* (Family: Lumbricidae)

Experimental Design: For the study, a dilution of mCry51Aa2 protein was incorporated into a standard artificial soil medium, to which adult earthworms were then exposed for 14 days. Mortality was assessed over a 14-day testing period and the change in fresh weight of the worms was assessed for survivors at 14 days after treatment (DAT). The test design and methods were based on OECD Guideline 207 (OECD, 1984).

Insects: The test organism (*Eisenia andrei*) was obtained from an in-house culture maintained at the contract research organization, Mambo-tox Ltd (Southampton, UK). The worms selected for the assay had individual wet weights of 300-600 mg and a visible clitellum.

Assay: A assay was carried out in which a dilution of mCry51Aa2 protein (10.5 mg TIC834_16-1/mL) was incorporated into a standard artificial soil medium and earthworms were then exposed to the soil for 14 days. The mCry51Aa2 protein, already held in a carbonate buffer solution, was further diluted in purified water for incorporation into a soil medium at a rate equivalent to 400 µg protein/g soil dry weight. This single application rate of the test substance was compared to a buffer-treated control and a water-treated control. All of these treatments were incorporated into an artificial soil substrate containing 10% w/w peat, the moisture content of which was brought to 50% of its pre-determined maximum water-holding capacity by the addition of the individual

treatments. The treated soil was placed onto 1-L-capacity lidded jars (n = 4 per treatment). Ten adult *E. andrei* (approx. 5 months old, with a fresh weight of 300-600 mg and with a visible clitellum) were then introduced into each jar. No food was provided during the duration of the test. Mortality was assessed over a 14-day testing period and the change in fresh weight of the worms was assessed for survivors at 14 DAT.

Statistics: A statistical analysis was not required for interpretation of the survival results since 100% of the worms survived in mCry51Aa2 protein treatment.

Results: At 14 days, no worms were found to have died in either of the controls or in the test-item treatment indicating equivalent survival in control and mCry51Aa2 treatments.

At 7 and 14 DAT, all of the worms in all of the treatments appeared healthy and active.

These results indicate no adverse effect of 400 µg mCry51Aa2 protein/g soil dry weight on earthworm survival after 14 days of continuous exposure.

References for Appendix H

Bachman, P. M., Ahmad, A., Ahrens, J. E., Akbar, W., Baum, J., Brown, S., Clark, T. L., Fridley, J. M., Gowda, A., Greenplate, J. T., Jensen, P. D., Mueller, G. M., Odegaard, M. L., Tan, J., Uffman, J. P., and Levine, S.L. 2017. Characterization of the Activity Spectrum of MON 88702 and the Plant-Incorporated Protectant Cry51Aa2.834_16. PloS ONE 12(1): 12(1): e0169409. doi:10.1371/journal.pone.0169409

OECD. 1984. Page 66 in Earthworm, acute toxicity tests. Organisation for Economic Co-Operation and Development, Paris, France.

OECD. 2009. OECD guidelines for testing chemicals: Collembolan reproduction test in soil. 232. Organisation for Economic Co-operation and Development, Rome, Italy.

SAS. 2012. Software Release 9.4 (TS1M1). Copyright 2002-2012 by SAS Institute, Inc., Cary, North Carolina.

Tan, J., M.S. Paradise, S.L. Levine, P.M. Bachman, J.P. Uffman, C. Jiang and D.B. Carson. 2011. Development and survival of *Orius insidiosus* (Say) nymphs on encapsulated bee pollen-based diet in a Tier-I toxicity assay. Environmental Entomology 40:1613-1621.

Winston, M.L. 1987. Form and function: Honey bee anatomy. Pages 13-45 in The Biology of the Honey Bee. Harvard University Press, Cambridge, Massachusetts.

Appendix I. Materials and Methods for the Tiered Assessment of mCry51Aa2 on Non-Target Organisms

This appendix provides a summary of the studies conducted to assess the potential effects of mCry51Aa2 protein on non-target organisms. All the diet feeding studies were conducted using *in vitro*-produced mCry51Aa2 protein which was shown to be functionally equivalent to mCry51Aa2 protein produced *in planta*. With the exception of the earthworm and Collembola studies, all the diet feeding studies utilizing the *in vitro*-produced test substance included a diet analysis using a sensitive insect (Western tarnished plant bug, *Lygus hesperus*) to confirm that the mCry51Aa2 protein was biologically active and had the expected level of biological activity in diet. Additionally, where appropriate based upon the diet matrix, the homogeneity of the test material and stability over the period of storage was also confirmed. A dose confirmation was not appropriate for the earthworm or Collembola studies due to the degradation of mCry51Aa2 protein in the soil matrix (discussed in Section V.B.5).

I.1. Tier 1 Testing

I.1.1. Evaluation of Potential Effects of mCry51Aa2 Protein to the Earthworm *Eisenia andrei* (Oligochaeta: Lumbricidae).

The purpose of this study was to evaluate the potential effects of dietary and contact exposure of mCry51Aa2 protein on adults of the earthworm *E. andrei* over a 14 day exposure period. For this study, a dilution of mCry51Aa2 protein (test) was prepared in a buffer solution (10 mM sodium carbonate/bicarbonate, pH 10.25) and incorporated into a standard artificial soil medium following the standard methodology described in OECD guideline 207 (OECD, 1984). Four treatment were supplied to *E. andrei* for dietary exposure, including the test treatment at 2500 and 500 µg mCry51Aa2 protein/g soil dry weight, an assay (purified water) control, and a buffer control diet containing the buffer solution at the same level as the test treatment at 2500 µg mCry51Aa2 protein/g soil dry weight. Treatments were mixed into an artificial soil in replicated 1 L glass jars (test arenas/chambers). At initiation of the test, ten adult *E. andrei* were placed on the soil surface of each jar. Each treatment was replicated four times for a total of 40 earthworms exposed per treatment. All jars were maintained in an incubator at 19.6-20.3ºC with a continuous lighting of 590-780 lux. The survival, biomass and behavior of the worms was assessed at 7 and 14 days after commencement of the assay. In lieu of a positive control, the sensitivity of the *E. andrei* worm culture used for this study was previously confirmed in a separate testing at the Test Facility, in accordance with the guideline, and conducted within 12 months from the start of the study in-life phase. After 14 days, no effect on survival (100% survival in all treatments), was observed with *E. andrei* treated with mCry51Aa2 protein at a concentration equivalent to 2500 and 500 µg mCry51Aa2 protein/g soil dry weight. Additionally, the biomass of worms exposed to mCry51Aa2 protein (20.8 ± 4.0% change) was significantly increased compared to the buffer control (-4.8 ± 2.4% change) (α=0.05) and there were no adverse observed effects on worm behavior. Based on the results of assessments for mortality, behavior and change in biomass, the NOEC for mCry51Aa2 protein was concluded to be ≥ 2500 µg mCry51Aa2 protein/g soil dry weight.

I.1.2. Evaluation of Potential Dietary Effects of mCry51Aa2 Protein to the Springtail *Folsomia candida* (Collembola, Isotomidae).

The purpose of this study was to evaluate the potential effect of chronic dietary exposure to *in vitro* transcribed mCry51Aa2 protein on the survival and reproduction of the springtail, *F. candida* over a 28-day exposure period. The mCry51Aa2 protein was presented in an inactivated-yeast diet at concentrations nominally equivalent to 2500 and 500 µg mCry51Aa2 protein/g diet and fed to populations of springtails confined in test arenas. The test method was adapted from OECD Guideline 232 (OECD 2009). The test diet was compared to an assay (purified water) control diet, a buffer control diet containing the buffer solution at the same level as the mCry51Aa2 protein treatment at 2500 µg/mL and a positive control diet containing insect growth regulator, diflubenzuron at 0.1 mg a.i./g diet. The test arenas consisted of lidded glass jars, lined at the base with a solid layer of a plaster-of-Paris and charcoal substrate. Groups of 10 juvenile springtails (11 days old) were placed into each replicate jar (n = 4 per treatment) and the appropriate diet was provided for the springtails *ad libitum* throughout the in-life phase of the experiment. Prior to test initiation, the treated diets were prepared aliquoted and then stored in a freezer. Freshly-defrosted aliquots of the diet were provided every 2-3 days. The ambient conditions recorded during the dietary exposures were 18.7-20.4ºC with a 12 h photoperiod of 590-780 lux. At 28 days, the numbers of the original population of *F. candida* still surviving in each test arena and the numbers of their offspring were recorded for each replicate arena. The mortality of *F. candida* fed the assay control was 8.0%, indicating a negligible background effect and meeting the assay acceptance criteria of having less than 20% mortality. On the assay control diet, the mean number of 239 progeny were produced per replicate and these results demonstrate that consumption of the yeast diet was necessary for the springtails to grow and reproduce under the test conditions. In contrast, *F. candida* fed the positive control diet showed 60% mortality (57% corrected mortality) and a mean number of 2 progeny produced per replicate, indicating that the dietary exposure was effective in detecting toxic effects of test substance incorporated into diet. The mortality of *F. candida* fed the mCry51Aa2 treatment diet at 2500 and 500 µg/g was 8.0% and 5.0% respectively, which were not significantly different (α=0.05) from the 8.0% mortality in buffer control, confirming that both test diet treatments had no adverse effect on *F. candida*. Additionally, there was no significant difference (α=0.05) in mean number of progeny produced per replicate with 230 in buffer control diet, compared with 252 and 246 in mCry51Aa2 treatment diets at 2500 and 500 µg/g, respectively. Based on the results of assessments for mortality and reproduction, the NOEC for mCry51Aa2 protein was concluded to be $\geq$ 2500 µg protein/g diet.

I.1.3. Evaluation of the Potential Dietary Effects of mCry51Aa2 Protein on Honeybee Larvae (*Apis mellifera* L.)

The purpose of this study was to evaluate the potential dietary effects of mCry51Aa2 protein (test) on the survival and development of honey bee, *A. mellifera* larvae. Larvae of *A. mellifera,* 2 days old, were exposed to mCry51Aa2 protein at a single dose administered to the brood cell. A single dose 500 µg/mL solution, in a 10 µl aliquot of 30% (w/v) sucrose/purified water, was added to each larval cell at test initiation for a

total mass of 5.6 µg mCry51Aa2/cell[5]. In addition, an assay control treated with 10 µL of 30% (w/v) sucrose/purified water was included as well as a positive control (2000 µg potassium arsenate/mL) in a 30% (w/v) sucrose/purified water solution to confirm that the test honey bee larvae were feeding. For each treatment, there were four replicate frames with a target number of 20 larvae assessed per frame. Treatments were administered to the cells and frames were returned to the hives for incubation. Treated brood cells were mapped in each treated frame and identified on acetate overlay maps to indicate the study, hive, replicate, and treatment group numbers. Post-capping and prior to emergence, all treated frames were removed from the hives, placed into screened hive boxes in a growth chamber and maintained under a 0L: 24D photoperiod at approximately 23.3 to 24.4ºC and 56.0 to 59.0% relative humidity (RH). The endpoints measured were survival at two different life stages: dosing to cell-capping (larval stage) and cell-capping to test termination (pupal stage). There was 100% survival in both the mCry51Aa2 protein and buffer control treatments. Additionally, emergence was initiated in the test and control on the same day for the mCry51Aa2 protein treatment and the assay control (day 15), approximately 50% emergence occurred on the same day (day 16) for the mCry51Aa2 treatment and the assay control and 100% emergence was achieved on the same day (day 17) for the mCry51Aa2 protein treatment and the assay control. Behavioral observations at emergence indicated no adverse behavior or morphological effects. Survival for the positive control treatment was 0%, confirming the validity of the test system. Based on no differences in survival and development between the test and control treatments, the NOEC of the mCry51Aa2 protein for honeybee larvae was ≥5.6 µg /larvae.

I.1.4. Evaluation of Potential for Chronic Dietary Effects of mCry51Aa2 Protein on Honey Bee Adults (*Apis mellifera* L.).

The purpose of this study was to evaluate the potential dietary effects of mCry51Aa2 protein (test) on the survival of honey bee, *A. mellifera* adults in 14-day continuous feeding study. Honey bee adults, ≤ 2 days old, were exposed to three treatments that included: 500 µg/g mCry51Aa2 protein in a 50% (w/v) sucrose/purified water solution, an assay control with 50% (w/v) sucrose/purified water solution and a positive control (20 µg potassium arsenate/mL) in a 50% (w/v) sucrose/purified water solution to confirm that the test bees are feeding. For each of the three treatments there were four cages (replicates) containing a target number 20 adult bees per replicate. Each cage was provided with approximately 10 ml of appropriate treatment diet solution in two vials inserted on the top of each cage, and bees were allowed to feed *ad libitum*. The vials were replaced every two days with fresh treatment diets over the study duration of 10 days. Prior to test initiation, adult bees were starved for a maximum of two hours. Test bees for each treatment group were observed daily for mortality, abnormal behavior, and appearance. During the test period, adult bees were maintained under a 0L: 24D

[5] A single dose of 10 µl of 500 µg/g solution was added to each larval cell for a total mass of 5.6 µg mCry51Aa2/cell. The concentration of 500 µg/g mCry51Aa2 protein in the diet solution is calculated based on the density of the 30% sucrose/water (w/v) solution of 1.127 g/mL.

photoperiod, with the exception of the time during which mortality and behavior assessments were conducted. Environmental conditions were maintained at approximately 23.2 to 30.4ºC and 46.3 to 60.6% relative humidity (RH). The positive control produced 100% mortality, confirming the validity of the test system. There were no significant differences (α=0.05) in mean survival between the mCry51Aa2 protein (88.75%) and the assay control (96.25%) treatments after 10 days of continuous dietary exposure. The positive control had 100% mortality and was significantly different (α=0.05) from the assay control confirming the validity of the exposure system. The validity criteria of ≤ 20% mortality in the assay control and > 50% mortality in the positive control were met for this study. No abnormal behavior was observed in either the mCry51Aa2 protein treatment or the assay control treatment. The NOEC of the mCry51Aa2 protein for adult honeybees was ≥500 µg/g.

I.1.5. Evaluation of Potential Dietary Effects of mCry51Aa2 Protein on the Lady Beetle, *Coccinella septempunctata* (DeGeer) (Coleoptera, Coccinellidae).

The purpose of this study was to evaluate potential dietary effects of mCry51Aa2 protein on the survival, development and growth of the lady beetle, *C. septempunctata*. Five treatment diets were supplied to *C. septempunctata,* including the mCry51Aa2 protein (test) treatment diets at 2500 and 500 µg/g diet, an assay (purified water) control diet, a buffer control diet containing the buffer solution at the same level as the mCry51Aa2 protein treatment at 2500 µg/g diet, and a positive control diet containing teflubenzuron SC at 0.01 mg a.i/g diet. Each treatment was replicated three times and with a target of 30 insects per diet treatment. Larvae were exposed for a maximum of 15 days. The larvae of *C. septempunctata* originating from the same egg batch were used for each replicate. All diet treatments were maintained in an incubator with a temperature of 24.4-25.9° C, a relative humidity (RH) of 65-81% and a 16L: 8D photoperiod (Lighting of 2400-4400 lux). The endpoints measured were pre-imaginal mortality, and development time (days) to adult emergence, and adult biomass. The pre-imaginal mortality of *C. septempunctata* fed the assay control was 13.3%, indicating a negligible background effect and meeting the assay acceptance criteria of having less than 20% mortality. The mortality in the assay control was not significantly different (α=0.05) from the 23.3% mortality in buffer control, demonstrating there was no buffer effect on *C. septempunctata*. In contrast, the positive control group had 100% mortality and none of the insects in the positive control group developed to the pupa stage, confirming the validity of the test system. The pre-imaginal mortality of *C. septempunctata* fed the mCry51Aa2 treatment diet at 2500 and 500 µg/g was 20.0% and 10.0% respectively, and was not significantly different (α=0.05) compared to buffer control diet, confirming that both test diet treatments had no adverse effect on *C. septempunctata*. Additionally, there was no significant difference (α=0.05) for development time to adult emergence between the larvae fed the test treatments, assay control, or buffer control diet. For the test, assay control, and buffer control diet treatment groups the development time to adult emergence was 13.8 to 14.3 days. There was no significant difference (α=0.05) with adult biomass between the test and buffer control diets. Because of significant differences in body mass between males and females in *C. septempunctata* populations, separate analyses were performed by gender. There was no significant difference (α=0.05) between the biomass of females averaging 22.3 mg, 20.9 mg, and 20.3 mg and males averaging 21.0 mg, 21.9 mg, and 19.4 mg for the test

treatments at 2500 μg/g diet, 500 μg/ g diet, and the buffer control diet respectively. Based on the results of assessments for pre-imaginal mortality, development time to adult emergence, and adult biomass, the NOEC of the mCry51Aa2 protein for *C. septempunctata* was concluded to be ≥2500 μg/g of diet.

I.1.6. Evaluation of Potential Dietary Effects of mCry51Aa2 Protein on the Rove beetle, *Aleochara bilineata* (Coleoptera: Staphylinidae).

The purpose of this study was to evaluate potential dietary toxicity of the mCry51Aa2 protein against adults of the rove beetle, *A. bilineata*. The mCry51Aa2 protein (test) was presented in a pre-cooked and homogenized meat-based (beef) diet at concentrations nominally equivalent to 2500 and 500 μg mCry51Aa2 protein/g diet and fed to populations of adult *A. bilineata* confined in test arenas. The test diet was compared to an assay (purified water) control diet, a buffer control diet containing the buffer solution at the same level as the mCry51Aa2 protein treatment at 2500 μg/g, and a positive control diet containing dimethoate 40EC at 5 μL product/g diet. Prior to test initiation, the treated diets were prepared aliquoted and then stored in a freezer. Freshly-defrosted aliquots of the respective diet were provided on daily basis. The test arenas consisted of polystyrene boxes lined at the base with a layer of damp clean quartz sand and secured with lids that had holes covered with fine mesh netting to allow air circulation. Each treatment was replicated four times and each replicate was initiated with a target of 20 insects per diet treatment. Adult *A. bilineata* were exposed for a maximum of 21 days. All diet treatments were maintained in an incubator with a temperature of 20.0-20.7° C, a relative humidity (RH) of 65-71% and a 16L: 8D photoperiod (Lighting of 800-900 lux). The adults were observed at Day 7, 14, and 21 to record any dead adults which were removed from the test arenas at the time of observation. At Day 21, the mortality of adult *A. bilineata* fed the assay control was 7.5%, indicating a negligible background effect and meeting the assay acceptance criteria of having less than 20% mortality. In contrast, adult *A. bilineata* fed the positive control diet showed 100% mortality, indicating that the dietary exposure was effective in detecting toxic effects of test substance incorporated into diet. The mortality of adult *A. bilineata* fed the mCry51Aa2 treatment diet at 2500 and 500 μg/g was 6.3% and 7.5% respectively, which were not significantly different (α=0.05) from the 10.0% mortality in buffer control, confirming that both treatments had no adverse effect on adult *A. bilineata*. Based on the results of assessments for adult mortality, the NOEC of the mCry51Aa2 protein for *A. bilineata* was concluded to be 2500 μg/g of diet.

I.1.7. Evaluation of Potential Dietary Effects of mCry51Aa2 Protein on the Lacewing, *Chrysoperla carnea* (Stephens) *(Neuroptera, Chrysopidae)*.

The purpose of this study was to evaluate potential dietary effects of mCry51Aa2 protein on the survival of adults of the lacewing, *C. carnea*. The mCry51Aa2 protein (test) was incorporated in an artificial diet medium at concentrations nominally equivalent to 2500 and 500 μg mCry51Aa2 protein/g diet and fed to populations of adult *C. carnea* confined in test arenas. The test diet was compared to an assay (purified water) control diet, a buffer control diet containing the buffer solution at the same level as the mCry51Aa2 protein treatment at 2500 μg/g, and a positive control diet containing dimethoate 40EC at 0.1 μL product/g diet. Prior to test initiation, the treated diets were prepared, aliquoted,

and then stored in a freezer. Freshly-defrosted aliquots of the respective diet were provided three times a week. The test arenas consisted of polystyrene boxes with close-fitting lids and a sheet of fibrous tissue under the lid of each box to serve as an oviposition site. The dishes of the treated diet, and dishes of honey-water and water-only, were placed on the floor of each box. Each treatment was replicated twice, and each replicate was initiated with a target of 20 insects per diet treatment. Adult *C. carnea* were exposed for a maximum of 14 days. All diet treatments were maintained in an incubator with a temperature of 24.7-25.9° C, a relative humidity (RH) of 65-80% and a 16L: 8D photoperiod (Lighting of 3400-4200 lux). The adults were observed every 1-3 days to record any dead adults which were removed from the test arenas at the time of observation. After 14 days, the mortality of adult *C. carnea* fed the assay control was 18.4%, indicating a negligible background effect and meeting the assay acceptance criteria of having less than 20% mortality. In contrast, adult *C. carnea* fed the positive control diet showed 100% mortality, indicating that the dietary exposure was effective in detecting toxic effects of test substance incorporated into diet. The mortality of adult *C. carnea* fed the mCry51Aa2 treatment diet at 2500 and 500 µg/g was 7.5% and 20% respectively, which were not significantly different (α=0.05) from the 20.0% mortality in buffer control, confirming that both treatments had no adverse effect on adult *C. carnea*. Based on the results of assessments for adult mortality, the NOEC of the mCry51Aa2 protein for *C. carnea* was concluded to be $\geq$ 2500 µg/g of diet.

I.1.8. Evaluation of Potential Dietary Effects of mCry51Aa2 Protein on the Insidious Flower Bug, *Orius insidiosus* (Say) (Heteroptera: Anthocoridae).

Orius insidiosus Assay

The purpose of this study was to evaluate potential dietary effects of mCry51Aa2 protein on the survival and development of nymph *O. insidiosus* over 10 days of continuous exposure following the methodology described in Tan et al., (2011). Eight treatment diets were supplied to nymph *O. insidiosus* for dietary exposure, including the mCry51Aa2 protein (test) treatment diets at 13, 32, 80, 200, and 500 µg/g diet, an assay (purified water) control diet, a buffer control diet containing the buffer solution at the same level as the mCry51Aa2 protein treatment at 500 µg/g diet, and a positive control diet containing potassium arsenate at 100 µg/g diet. All dietary exposures were initiated with five-day old nymphs and each treatment consisted of a total of 30 nymphs. All test nymphs were individually housed in test arenas and supplied with appropriate treatment diet in two domes. The dome diets were replaced every two days. All test nymphs were allowed to feed *ad libitum* on the treated diet for 10 days. All diet treatments were maintained in an incubator with a temperature of 25 ± 5° C, a RH of 70 ± 10 % and a 16L: 8D photoperiod. The test nymphs were observed every day to record mortality and development. All test nymphs survived and developed to adults in both assay control and buffer control treatments. The survival of nymph *O. insidiosus* fed the assay control was 100%, indicating no background effect and met the assay acceptance criteria of having less than 20% mortality. In contrast, nymph *O. insidiosus* fed the positive control diet showed 0% survival by day 10 with 3.33% nymphs developing to adults, indicating that the dietary exposure was effective in detecting adverse effects of test substance incorporated into diet. The survival in the mCry51Aa2 protein treatments decreased with

the increase of mCry51Aa2 concentration in the diet, indicating that the effect of mCry51Aa2 protein on the survival of nymph *O. insidiosus* was concentration dependent. The survival in mCry51Aa2 protein treatments was 93.3, 80.0, 73.3, 60.0, and 53.3% for the concentrations at 13, 32, 80, 200, and 500 µg/g diet, respectively. There was no significant difference (α=0.05) in survival between mCry51Aa2 protein at a concentration of 13 µg/g diet and buffer control treatments, whereas there was a significant effect on five-day old nymph survival in all other mCry51Aa2 diet treatments compared to the buffer control. The LC_{50} value for five-day old nymphs was estimated to be >500 µg/g diet since the survival was 53.33% at the highest concentration of 500 µg/g diet. However, the development of surviving *O. insidiosus* nymphs was not affected by ingestion of mCry51Aa2 protein, as no significant differences (α=0.05) were detected among all mCry51Aa2 diet treatments and the buffer control for the mean development time to adult stage. Compared to buffer control, the mCry51Aa2 treatment at the concentration of 13 µg/g diet showed no significant differences (α=0.05) in the number of survival of the *O. insidiosus* nymphs, surviving nymphs that developed to adult stage (100% development in both treatments). Additionally, the development time for nymphs to adults were not significantly different (α=0.05) between the buffer control (9.83 days) and the 13 µg/g mCry51Aa2 treatment (9.97 days). In conclusion, the results of this study demonstrate that continuous dietary exposure to mCry51Aa2 protein for 10 days at concentrations >13 µg/g diet affected the survival, but not the development time of five-day old *O. insidiosus* nymphs, in a dose-dependent manner. However, the exposure mCry51Aa2 protein at 13 µg/g diet did not show significant effects on the survival of the nymph *O. insidiosus*, surviving nymphs that developed to adult stage, and development time for surviving nymphs to develop into adults. Therefore, the NOEC of mCry51Aa2 protein for five-day old *O. insidiosus* nymphs, is 13 µg/g diet. Additionally, the LC_{50} value of the mCry51Aa2 protein for five-day old *O. insidiosus* nymphs was estimated to be >500 µg/g diet.

Diet analysis for Confirmation of Dose, Homogeneity and Stability of the Test Substance

A diet analysis was conducted to confirm the mCry51Aa2 protein was contained in the treatment diet at the nominal concentration of 500 and 13 µg/g diet, was homogenous in the treatment diet, and was stable in the treatment diet under the conditions of storage and under the *in situ* conditions for two days in the incubator. An ELISA analysis was performed in accordance with a study specific work procedure (SSWP-1).

Confirmation of the Dose

An ELISA analysis was conducted with treatment diet at the lowest and highest concentrations used in the diet feeding assay with *O. insidiosus* (i.e. 13 and 500 µg/g, respectively). A recovery control was included within the analysis, that consisted of buffer control samples with a spiked concentration of mCry51Aa2 protein at 13 or 500 µg/g. The results of the ELISA analysis demonstrated that the mean measured concentration for the 13 µg/g diet sample was 13 µg mCry51Aa2 protein/g diet with a 95% confidence interval (CI) of 9-17 µg/g, representing a 100% of the nominal concentration. This is comparable to the recovery control which had a recovery of 12.2 µg/g with a 95% CI of 10.3-14.1 µg/g. The mean measured concentration for the 500 µg/g diet samples including first, middle, and last plate was 396 µg mCry51Aa2 protein/g

diet with a 95% CI of 338-455 µg/g, representing a 79% of the nominal concentration. This is comparable to the recovery control showing a recovery of 361 µg mCry51Aa2 protein/g diet with a 95% CI of 334-388 µg/g. The results indicated that the mCry51Aa2 protein was presented in the treatment diets at the nominal concentration of 13 and 500 µg/g diet, respectively.

Homogeneity Analysis

Homogeneity was evaluated by use of a large batch of diet formulated targeting an mCry51Aa2 protein concentration of 500 µg/g, and sampling the top, middle and bottom of the diet batch. Repeat samples of these three diet portions were analyzed by ELISA. The mean measured concentration and the associated 95% CI for the top, middle, and bottom portions of diet sample at 500 µg/g diet was 350 (241-458), 450 (292-607), and 390 (233-547) µg mCry51Aa2 protein/g diet, respectively. The overlapping 95% CIs confirmed that the test substance, mCry51Aa2 protein was homogeneously mixed in the treatment diet.

Stability Analysis

The mean measured concentration and the associated CI was 450 (292-607) µg mCry51Aa2 protein/g diet for the samples stored at -20 °C from the diet collection to the end of feeding (ST1), was 504 (122-887) µg mCry51Aa2 protein/g diet for the samples which were maintained at feeding test conditions for 2 days (ST2), and was 485 (161-809) µg mCry51Aa2 protein/g diet for the samples stored at –80 °C (ST3). The overlapped 95% CIs confirmed that the mCry51Aa2 protein in the treatment diet was stable under the conditions of storage and *in situ* in incubation.

I.1.9. Evaluation of Potential Dietary Effects of mCry51Aa2 Protein on the Parasitic Wasp, *Pediobius foveolatus* Crawford (Hymenoptera: Eulophidae)

The purpose of this study was to evaluate potential dietary effects of the mCry51Aa2 protein on the survival of adult parasitic wasp, *Pediobius foveolatus* Crawford over 20 days of continuous exposure. Five treatment diets were supplied to adult *P. foveolatus* for dietary exposure, including the mCry51Aa2 protein treatment diets at 500 and 2500 µg/mL diet, an assay control diet, a buffer control diet containing the buffer solution at the same level as the mCry51Aa2 protein treatment at 2500 µg/mL diet, and a positive control diet containing 200 µg potassium arsenate/mL diet. The base diet was a 40% honey/water solution. Exposure of adult *P. foveolatus* to the five treatment diets was replicated four times with 15 adult wasps per replicate for a total of 60 wasps per treatment. All dietary exposures were initiated with newly emerged adults after approximately 24 hours of acclimation. The adult wasps in each replicate were housed together in a single arena and allowed to feed *ad libitum* on the treated diet for 20 days. All diet treatments were maintained in an incubator at a target temperature of 25±5°C, a target RH of 70±10% and a photoperiod of 14 L: 10 D. Mortality was observed every two days at diet replacement and the dead wasps, if any, were removed from the test arenas at the time of observation. The survival of adult *P. foveolatus* fed the assay control was 96.67%, indicating a negligible background effect and meeting the assay acceptance criteria of having less than 20% mortality. In contrast, the adult *P. foveolatus* fed the positive control treatment diet showed 0% survival at Day 12, indicating that the test

system was effective in detecting toxic effects through the dietary exposure. The survival of adult *P. foveolatus* fed both the mCry51Aa2 treatment diet at 500 and 2500 µg/mL for 20 days was 98.33%, which was the same as the buffer control, confirming that both treatments had no adverse effect on *P. foveolatus* adult survival. Based on the results of assessments for adult survival, the NOEC of the mCry51Aa2 protein for the parasitic wasp, *P. foveolatus*, was ≥ 2500 µg/mL diet.

I.1.10. Evaluation of Potential Dietary Effects of mCry51Aa2 Protein the Big-eyed Bug, *Geocoris punctipes* (Hemiptera: Geocoridae), the Western Damsel Bug, *Nabis alternatus* (Hemiptera: Nabidae), and the Leafhopper Assassin Bug, *Zelus renardii* (Hemiptera: Reduviidae)

The purpose of this study was to evaluate the potential dietary effects of the mCry51Aa2 protein on the survival and development of one-day old nymphs of the big-eyed bug, *Geocoris punctipes* (Hemiptera: Geocoridae), the Western damsel bug, *Nabis alternatus* (Hemiptera: Nabidae), and the leafhopper assassin bug, *Zelus renardii* (Hemiptera: Reduviidae) following the methodology described in Tan et al., (2011). Five treatment diets were supplied to nymphs for dietary exposure, including the mCry51Aa2 protein (test) treatment diets at 400 and 4000 µg mCry51Aa2 protein/g diet, an untreated assay (purified water) control diet, a buffer control diet containing the buffer solution at the same level as the mCry51Aa2 protein treatment at 4000 µg/g diet, and a positive control diet containing potassium arsenate at 100 µg/g diet. In the case of *G. punctipes* and *N. alternatus*, different treatments used nymphs that hatched on different days because an insufficient number of nymphs could be obtained for use in all treatments simultaneously. Each treatment targeted a total of 30 to 40 individually housed nymphs. All test nymphs were supplied with appropriate treatment diet in two feeding domes and allowed to feed *ad libitum* on the treatment diet until all surviving nymphs developed to adulthood. The dome diets were replaced every two days. All treatments were maintained in an incubator with a temperature of 25±5°C, a relative humidity (RH) of 50±10% and a photoperiod of 14 h Light: 10 h Dark. The test nymphs were observed every day to record survival and development stage. The newly-emerged adults were weighted to determine adult body mass.

I.1.10.1. *Geocoris punctipes*

Two sets of dietary feeding tests were conducted with *G. punctipes*, including the untreated control (water control), positive control, buffer control, and mCry51Aa2 protein treatment at 4000 µg/g diet in Set 1. The results of the direct feeding assay are provided in Table I-1. All one-day old nymphs in the positive control treatment died after 11 days of continual exposure to the diet, confirming that the feeding exposure system is highly effective in detecting the toxic activity of test substance incorporated in the treatment diets against *G. punctipes* nymphs. The survival of *G. punctipes* nymphs fed the untreated control (water control) was 97.3%, indicating no background effect. Compared with untreated control (water control) treatment, the buffer control treatment showed 89.66% survival, indicating no buffer effect on nymph survival. The survival in the mCry51Aa2 protein treatments was 96.55% at 4000 µg/g diet and was not significantly different from the buffer control treatments (α=0.05). All surviving nymphs

completed the development into adulthood and the body mass of newly-emerged adults was not significantly different from those in the buffer control. The development time to adults was significantly prolonged, 25.31 days for buffer control versus 28.71 days for 4000 µg mCry51Aa2 protein/g diet treatment. When the nymphs were exposed to mCry51Aa2 protein at a concentration of 400 µg mCry51Aa2 protein/g diet in Set 2 where an additional buffer control was included for comparison, the results showed the same effects as the concentration of 4000 µg mCry51Aa2 protein/g diet, indicating no impact on nymph survival and body mass of newly-emerged adults, but significantly longer development time to adult by 1.20 days (Table I-1).

I.1.10.2. *Nabis alternatus*

The dietary feeding tests in Set 1 included the untreated control (water control), positive control, buffer control, and mCry51Aa2 protein treatment at 4000 µg mCry51Aa2 protein/g diet. The results of the direct feeding assay are provided in Table I-2. All one-day old nymphs in the positive control treatment died after 9 days of continual exposure to the diet, confirming that the feeding exposure system is highly effective in detecting the toxic activity of test substance incorporated in the treatment diets against *N. alternatus* nymphs. All nymphs survived and developed into adults from untreated control (water control), buffer control, and mCry51Aa2 protein treatment at 4000 µg mCry51Aa2 protein/g diet, indicating both buffer control substance and mCry51Aa2 protein had no impact on nymph survival and potential to develop to adults. The mCry51Aa2 treatment at 4000 µg mCry51Aa2 protein/g diet significantly delayed the nymph development time to adults (α=0.05), 22.06 days for the mCry51Aa2 treatment compared to 16.84 days for buffer control. The body mass of newly-emerged adults was also significantly reduced. When the nymphs were exposed to mCry51Aa2 protein at a concentration of 400 µg mCry51Aa2 protein/g diet in Set 2 where an additional buffer control was included for comparison, no impact was observed on the measurements including nymph survival and body mass of newly-emerged adults, but significantly longer development time to adult by 1.13 days (Table I-2).

I.1.10.3. *Zelus renardii*

All five treatments including untreated control (water control), buffer control, mCry51Aa2 protein treatment at 4000 and 400 µg mCry51Aa2 protein/g diet, and a positive control) were tested in one set. The results of the direct feeding assay are provided in Table I-3. All one-day old *Z. renardii* nymphs died after 3 days of continual exposure to the positive control treatment diet, indicating the feeding exposure system is highly effective in detecting the toxic activity of test substance incorporated in the treatment diets by *Z. renardii* nymphs. The survival of *Z. renardii* nymphs fed the buffer and untreated control (water control) treatments was 72.4 % and 79.3 %, respectively. The survival in the mCry51Aa2 protein treatments at 4000 and 400 µg mCry51Aa2 protein/g diet was 60% and 65.52%, respectively, none of which was significantly different from the buffer control treatment (α=0.05). All surviving nymphs developed into adults. Both the mCry51Aa2 treatments at 4000 and 400 µg mCry51Aa2 protein/g diet significantly delayed the nymph development time to adults and reduced the body mass of newly-emerged adults when compared to the buffer control treatment (Table I-3).

Table I-1. *Geocoris punctipes* Nymph Survival, Development Time to Adulthood, and Body Mass of Newly-emerged Adults

Treatment		Number of Nymphs tested	Nymph Survival %	P-value	Time of Nymph Development to Adults (Mean±SE) (d)	P-value	Body Mass of Newly-emerged Adults (Mean±SE) (mg)	P-value
Set 1	Positive control	32	0					
	Untreated control (Water control)	37	97.30	0.312*	25.92±0.32	0.198*	4.04±0.14	0.776*
	Buffer control	29	89.66	0.612	25.31±0.34	<0.001	3.99±0.11	0.254
	mCry51Aa2 at 4000 µg/g diet	29	96.55		28.71±0.45		3.78±0.15	
Set 2	Buffer control	39	100	1.000	29.13±0.17	<0.001	4.32±0.14	0.781
	mCry51Aa2 at 400 µg/g diet	40	97.50		30.33±0.30		4.26±0.14	

Note *Untreated control (water control) was compared with buffer control in Set 1.

Table I-2. *Nabis alternatus* Nymph Survival, Development Time to Adulthood, and Body Mass of Newly-emerged Adults

Treatment		Number of Nymphs Tested	Nymph Survival %	P-value	Time of Nymph Development to Adults (Mean±SE) (d)	P-value	Body Mass of Newly-emerged Adults (Mean±SE) (mg)	P-value
Set 1	Positive control	39	0					
	Water control	37	100	1.000*	16.70±0.27	0.699*	5.63±0.16	0.230*
	Buffer control	37	100	1.000	16.84±0.21	<0.001	6.03±0.28	<0.001
	mCry51Aa2 at 4000 µg/g diet	32	100		22.06±0.71		4.39±0.17	
Set 2	Buffer control	40	90.00	0.359	20.28±0.34	0.042	6.24±0.29	0.773
	mCry51Aa2 at 400 µg/g diet	38	97.37		21.41±0.42		6.35±0.26	

Note *Untreated control (water control) was compared with buffer control in Set 1.

Table I-3. *Zelus renardii* Nymph Survival, Development Time to Adulthood, and Biomass of Newly-emerged Adults

Treatment		Number of Nymphs Tested	Nymph Survival %	P-value	Time of Nymph Development to Adults (Mean±SE) (d)	P-value	Body Mass of Newly-emerged Adults (Mean±SE) (mg)	P-value
Set 1	Positive control	30	0					
	Buffer control	29	72.41		46.71±1.40		20.48±0.82	
	Untreated control (Water control)	29	79.31	0.760	43.17±0.93	0.042	20.62±0.97	0.913
	mCry51Aa2 at 4000 µg/g diet	30	60.00	0.412	62.06±1.95	<0.001	16.48±0.68	<0.001
	mCry51Aa2 at 400 µg/g diet	29	65.52	0.777	59.42±2.23	<0.001	17.63±0.73	0.013

I.1.11. Evaluation of Acute Toxicity of mCry51Aa2 Protein on the Northern Bobwhite Quail, *Collinus virginianus* (Galliformes: Odontophoridae)

The purpose of this study was to evaluate the acute toxicity of mCry51Aa2 protein administered orally to the northern bobwhite quail, *C. virginianus*. Thirty-eight weeks old birds were provided a single dose using capsules at a rate of 2500 mg of lyophilized mCry51Aa2 protein (test substance) per kilogram of body weight (mg a.i./kg) and observed for 14 days for potential adverse effects on survival and body weight. Thirty birds (10 per treatment group) were randomly divided into three groups orally administered one of three treatment group: a test dose substance (TDS), a control dose substance (lyophilized bovine serum albumin, CDS) and a negative control (empty capsules). Each dosage group was assigned two pens and birds were housed by sex (five males and five females). Birds were acclimated to the facility and cage for 12 weeks prior to study initiation. Each bird was individually weighed and dosed on the basis of milligrams of test substance or control substance per kilogram of body weight. All dosage group were maintained with a temperature of 21.9-23.3°C, a relative humidity (RH) of 42-80% and a 8L: 16D photoperiod (Lighting of approximately 183 lux). The endpoints measured were mortality, sign of toxicity, abnormal behavior, and body weight. There were no biologically relevant differences for any of the endpoints (mortality, signs of toxicity, and abnormal behavior) to bobwhite quail provided with mCry51Aa2 protein from MON 88702 as compared to those in the control groups. When compared to the negative control group and the CDS group, there were no apparent treatment-related differences in mean body weight, mean body weight changes, or feed consumption for the males or females in the TDS group. The mean body weight of the males in the TDS group was statistically higher (α=0.05) than the males in the CDS group on Day 1 and Day 7. This difference was not considered to be related to treatment as it occurred prior to dosing (Day 1) and the weight difference was maintained during the study. The acute oral LD50 value for *C. virginianus* exposed to mCry51Aa2 protein as a single oral dose was determined to be greater than 2500 mg a.i./kg, the highest dosage level tested. Based on the results of assessments for adult survival, the NOEC of the mCry51Aa2 protein for the northern bobwhite quail, *C. virginianus*, was ≥2500mg/kg body weight.

I.1.12. Evaluation of Acute Oral Gavage Toxicity of mCry51Aa2 Protein in Mice, *Mus musculus* (Rodentia: Muridae)

The purpose of this study was to evaluate the acute toxicity of mCry51Aa2 protein when given orally by gavage twice in 1 day to CD-1 mice, *M. musculus* and subsequently observed for 14 days. Male and female mice, 10 mice/sex/group were administered orally by gavage (twice in 1 day) one of three treatments: VDS (vehicle dosing solution) for Vehicle, CDS (control dosing solution) for Bovine Serum Albumin, and TDS (test dosing solution) for mCry51Aa2 protein. The animals were housed individually throughout the study in polycarbonate cages containing appropriate bedding equipped with an automatic watering valve. All dosage groups were maintained with a temperature of 69°F to 70°F (~21°C), a relative humidity (RH) of 48-52% % and a 12L: 12D photoperiod. Ten or greater air changes per hour with 100% fresh air (no air recirculation) were maintained in the animal rooms. The animals were observed for general health/mortality and

moribundity twice daily, once in the morning and afternoon, throughout the study. The following parameters and end points were evaluated in this study: clinical signs, body weights, body weight changes, food consumption, and gross necropsy findings. No mortality occurred during the study. No test substance-related clinical findings were observed. There were no mCry51Aa2-related differences in body weight, body weight gain, food consumption, or gross necropsy findings. In conclusion, there were no test substance-related effects of mCry51Aa2 when administered by oral gavage at a dose of 5000 mg/kg body weight to male and female mice. Therefore, under the conditions of this study the no observed adverse effect level (NOAEL) for mCry51Aa2 protein was 5000 mg/kg body weight.

I.2. Tier 2 Testing

I.2.1. Evaluation of the Potential Prey-mediated Effects of mCry51Aa2 Protein on Five-day Old Nymphs of the Insidious Flower Bug, *Orius insidiosus* (Say) (Heteroptera: Anthocoridae) in a Tri-trophic Feeding Test with Fall Armyworm

The purpose of this study was to evaluate the potential prey-mediated effects of mCry51Aa2 protein on the survival and development of nymphs of the insidious flower bug, *O. insidiosus* following 10-days of feeding on prey, fall armyworm (FAW), *Spodoptera frugiperda* larvae raised on an mCry51Aa2 protein treatment diet. Five-day old *O. insidiosus* nymphs were individually kept in test arenas and supplied with FAW larvae raised on three treatment diets for three days. The treatment diets included the mCry51Aa2 protein treatment diet at 2500 µg/g diet, an assay control diet, and a buffer control diet containing buffer solution at the same level as the mCry51Aa2 protein treatment. Newly-hatched FAW larvae (≤ 30 hrs old) were fed one of the three treatments diets ad libitum. After 3-day feeding, the FAW larvae were collected and placed on dry ice and frozen. Four to five thawed FAW larvae were fed to five-day old *O. insidiosus* nymphs daily over a period of 10 days. Each treatment was initiated with 40 individually housed nymphs maintained in an incubator at a target temperature of 25±5°C, a target relative humidity (RH) of 70±10% and a photoperiod of 16 L:8 D. Test nymphs were observed each day during the 10-day period to record survival and the development stage.

ELISA analysis was performed on the mCry51Aa2 treatment diet samples and FAW larvae raised on the mCry51Aa2 treatment diet. The results of the ELISA analysis indicated that the mean levels of mCry51Aa2 protein to be 1999 µg/mL in the treatment diet and 6.47 µg/g in the FAW larvae. These results confirm that a very small amount of the mCry51Aa2 protein was transferred or retained during diet ingestion by FAW larvae.

Survival of the *O. insidiosus* fed FAW larvae raised on assay control and buffer control treatment diets was 87.5% and 90.0%, respectively, meeting the assay acceptance criteria of having less than 20% mortality. The survival of the *O. insidiosus* fed FAW larvae raised on mCry51Aa2 treatment diet was 82.5% which was not significantly different (α=0.05) from the survival of buffer control. Compared to the buffer control, the mCry51Aa2 treatment showed no significant difference (α=0.05) in the number of surviving nymphs that developed to adult. Additionally, the development time for nymphs to adults was not determined to be statistically different (α=0.05) between the buffer control and the mCry51Aa2 treatment.

In conclusion, these results provide evidence that *O. insidiosus* is expected to have limited exposure to the mCry51Aa2 protein through the consumption of FAW prey, due to very limited trophic transfer and accumulation of mCry51Aa2 from MON 88702 plant tissue to herbivore prey. Therefore, due to *O. insidiosus* feeding ecology, this species is not expected to encounter levels of the mCry51Aa2 protein in the field that are sufficient to result in an adverse effect on *O. insidiosus* survival or development.

I.2.2. Evaluation of the Potential Prey-mediated Effects of mCry51Aa2 Protein on One-day Old Nymphs of the Minute Pirate Bug, *Orius majusculus* (Heteroptera: Anthocoridae) in a Tri-trophic Feeding Test with Spider Mites

The information below was provided by Michael Meissle, Principle Investigator at Agroscope, Switzerland, as part of an on-going collaboration with Monsanto. The information describes the materials, methods and results of tri-trophic feeding experiments with one-day old nymphs of *O. majusculus* and spider mites, which will be part of a future publication from Agroscope.

Methods

Cultivation of cotton

Genetically modified cotton MON 88702 and the non-transgenic near isoline DP393 were planted in 1.3 L pots and incubated in a climate chamber at 25°C, 70% relative humidity, and a 16:8 light to dark cycle.

Spider mite culture

Tetranychus urticae spider mites were provided by Syngenta Crop Protection Münchwilen AG (Stein, Switzerland). Colonies were established on *Bt* and non-*Bt* cotton plants. Spider mite colonies remained on the respective cotton type for the whole experimental period without mixing among plant types. The glasshouse cabins were temperature-regulated to approximately 25°C and supplied with additional light (16h per day).

Predatory bug culture

O. majusculus was purchased from Andermatt Biocontrol (Grossdietwil, Switzerland) and cultured at Agroscope since October 2016. The culture was maintained in the glasshouse at approximately 22ºC and additional light to ensure 16 h of light around the year.

Spider mite assay 1: Development and fecundity on Bt and non-Bt cotton

This experiment was conducted to investigate if life cycle parameters of spider mites on *Bt* cotton leaf disks differ from those on non-*Bt* cotton, with a focus on development and reproduction.

Leaf disks (2.5 cm diameter) were cut from the youngest fully developed leaves of 5-6 week-old plants (typically 6 fully developed leaves). Each leaf disk was cut from a different plant (20 *Bt* and 20 non-*Bt* plants) and placed upside down in a transparent

plastic dish (5 cm diameter, 1 cm high) covered with a ventilated lid. The plastic dishes were lined with wet cotton cosmetic pads that provided moisture to the leaf and prevented spider mites from leaving the leaf disk (method adapted from Li and Romeis 2010 and Shu et al. 2018). One female spider mite from the culture on *Bt* or non-*Bt* plants was placed on each leaf disk of the respective plant type. On the next day, females were removed and all eggs except one were destroyed (day 0). Egg hatching, survival, larval/nymphal stage, and the gender of adults was recorded daily. Spider mites were transferred to new dishes as soon as larvae hatched. Leaf disks were changed every 3-4 days. The experiment ended for males after becoming adult. Newly emerged females were paired with one male either from the experiment or from the respective culture for 2 days. Eggs were counted daily and either removed or destroyed. During the oviposition phase, eggs on leaf disks were not destroyed when the disk was changed. The old disks with eggs were incubated to determine egg fertility. After 5 days, the number of unhatched eggs was counted. The experiment ended when the last female died. The whole experiment was conducted in triplicate.

The following parameters were analyzed statistically: egg hatching rate (1st generation), days from egg laying to hatching, juvenile survival from egg hatching to adult emergence, days from egg hatching to adult emergence, gender of adult, days from female emergence to death (female longevity), number of eggs laid from female emergence to death (total fecundity), number of eggs laid per day (total number of recorded eggs divided by female longevity), and egg hatching rate (2nd generation). To assess the egg hatching rate of the 2nd generation (fertility), the proportion of unhatched eggs compared to the total number of incubated eggs was calculated for each female.

Spider mite assay 2: Development and adult measures on Bt and non-Bt cotton

A second series of assays with spider mites was conducted with the following aims: 1) the first assay showed rather high juvenile mortality (Table I-4). In this second assay, juvenile development was thus once more investigated with improved methodology to avoid handling of juveniles. 2) The experiment ended once nymphs became adults. This allowed to record additional measures of adults, such as weight and size. 3) The spider mite cultures remained on the same type of plants (*Bt* and non-*Bt*) for more than one year. In case the spider mites were sensitive to the *Bt* protein, the quality of the colony might have either degraded or the colony might have adapted to the *Bt* protein by evolution. Therefore, we tested if the origin of the mothers of the juveniles used in the assay (*Bt* or non-*Bt* culture) influenced their performance on *Bt* or non-*Bt* cotton.

Leaf disks of 6-8 weeks old plants were cut from 25 *Bt* and 25 non-*Bt* plants and placed in dishes as described previously. One female spider mite from the culture on *Bt* or non-*Bt* plants was placed on each leaf disk of the respective plant type. On the next day, females were removed (day 0). After 3 days, 2 eggs per leaf disk were cut out using fine scissors. One egg, together with the little leaf piece it was laid on, was transferred to a fresh *Bt* leaf disk and the other egg to a non-*Bt* disk. This resulted in 25 replications of each combination of plant type mother origin and plant type food (100 eggs altogether). Subsequently, egg hatching, survival, and development was recorded until adults emerged. Adults were sexed, frozen and later measured (body length and width). The weight of individual females was recorded. The weight of individual males was too low

to be measured individually with reasonable precision. Therefore, groups of 3-5 males from the same treatment were pooled and weighed as a group. For statistical analysis, the calculated average weight per male was used. This experiment was also replicated three times.

Predator assay 1: Survival on Bt and non-Bt cotton

We first established that *O. majusculus* can develop on spider mites as an exclusive food source. A preliminary assay revealed that neonates consumed in average 6 spider mites per day, increasing to more than 50 for 5th instars. All stages of spider mites (including eggs) are consumed by the predator.

Aim of the first predator assay was to test if neonates differ in their development to adulthood when exclusively fed with spider mites from *Bt* cotton or non-*Bt* cotton. In each dish, either a *Bt* or non-*Bt* cotton leaf disk (diameter 38 mm) was placed. One *O. majusculus* neonate (<24 h after hatching) was introduced to each leaf disk. Spider mites from *Bt* or non-*Bt* cotton were provided to the respective leaf disks. To ensure *ad libitum* feeding, the amount of spider mites was increased during the experiment from approximately 10 for neonates to at least 60 for 5th instars. Leaf disks, dishes and cotton pads were changed every 3-4 days to ensure continuously high exposure of the spider mites to the *Bt* protein.

Survival and developmental stage of *O. majusculus* were recorded daily until the nymphs became adults or died. Moltings were determined by the presence of exuvia. The experiment ended with adult emergence. The experiment was conducted twice with 10 replicates and another two times with 20 replicates per plant treatment (60 replicates per treatment altogether).

Predator a*ssay 2: Fecundity on Bt and non-Bt cotton*

To compare fecundity of *O. majusculus* on *Bt* vs. non-*Bt* cotton, a second assay was conducted. Because of the high mortality in the previous predator assay, *O. majusculus* neonates were raised on *Ephestia* eggs. After 5 days, each nymph was weighed and placed either on a *Bt* or a non-*Bt* cotton leaf disk as described for the previous predator assay. Spider mites of the respective cotton plant type were provided *ad libitum* and the leaf disks were changed every 3-4 days throughout the experiment. One female and one male were placed together on a larger leaf disk (diameter 54 mm) in a ventilated plastic dish (diameter 75 mm diameter, height 23mm) lined with a moist cotton pad. Males were removed once the first eggs were laid. If no eggs were laid after 4 days, the male was replaced. During the oviposition period, leaf disks were replaced every 2 days and the old disks were monitored for offspring. Numbers of fresh eggs and hatched nymphs were recorded daily until all females died. The experiment was repeated with at least 35 individuals.

Determination of mCry51Aa2 protein in leaves, spider mites, and predatory bugs

During each experimental repetition of both spider mite assays, samples of leaves (0.5 cm diameter disks) were taken directly from the plants and frozen for the determination of mCry51Aa2-protein concentrations. In addition, in the second and third repetition of the

first spider mite assay, leaf disks were incubated under the conditions of the experiment and samples were taken 4 days later. In the second spider mite assay (all repetitions), additional samples were collected after 7 days of incubation. Those matured leaf samples in comparison with the fresh leaf samples allow an estimation of the stability of the mCry51Aa2 protein during the time period between leaf disk changes.

At the end of each predator assay 1 (repetition 3 and 4) and 2 (both repetitions), leaf and spider mite samples were collected from the spider mite culture. At each time period, samples were collected from 5 groups of *Bt* cotton plants. To get an idea of the vertical distribution of the mCry51Aa2 protein, samples were taken from the lower third, the middle third and the upper third of the plants. Spider mite samples consisted of ca. 5mg, leaf samples of ca. 10 mg. Sampled plants in the spider mite culture were in the flowering or boll forming stage, but not necessarily of uniform age across the sampling periods.

To estimate the mCry51Aa2 protein concentrations in the predator, neonates of *O. majusculus* were reared on *Ephestia* eggs for 5 days. Subsequently, spider mites from the *Bt* culture were provided *ad libitum*. When the nymphs reached the 5^{th} instar (after approximately 4 days), they were fed one more day with spider mites and then 5 samples consisting of 6 nymphs each were collected for ELISA. This procedure was repeated 3 times.

We also sampled leaves of non-*Bt* plants throughout the year (5 samples) and spider mites from the non-*Bt* culture (9 samples). mCry51Aa2 protein concentrations in all non-*Bt* leaf samples were below the limit of detection (LOD) (<0.0015µg/g FW). Concentrations in spider mite samples were below the LOD when the *Bt* and non-*Bt* cultures were in separate glasshouse cabins (<0.0011µg/g FW). When both cultures were in one large glasshouse cabin, mCry51Aa2 protein was detected in spider mites from the non-*Bt* culture, but concentrations were below 1% of those from the *Bt* culture.

All samples for ELISA were weighed (fresh weight) and stored at -80 ºC. For the extraction of mCry51Aa2 protein, 1× tris-borate buffer with 0.05% Tween20 was added at a ratio of at least 500:1 buffer (µL) to sample fresh weight (mg). After adding one 3 mm tungsten carbide ball, the tissues were macerated in a Qiagen Tissue Lyser II (Qiagen, Hombrechtikon, Switzerland) fitted with 24-tube adapters for microreaction tubes (Qiagen) at 30 Hz for 2 min. Macerated samples were centrifuged at 13000 × g for 5 min and the supernatant was used either undiluted (*O. majusculus* samples and all non-*Bt* samples or diluted with 1×PBST + 0.5% BSA depending on the expected concentration in the samples (transgenic cotton leaves 500-1000×, spider mites 200×). Cry51Aa2 protein concentrations were subsequently measured with a sandwich enzyme linked immunosorbent assay (ELISA) method transferred from Monsanto, St. Louis, MO. The LOD was calculated based on 3× SD of the OD values of the blanks of each plate. At least 5 blanks were loaded on each plate.

Data analysis

Data from the spider mite and predator performance assays were analyzed with linear models (LM), generalized linear models (GLM), or mixed effects models (LMER or

GLMER) using R statistical software (R version 3.5.1, The R Foundation for Statistical Computing, Vienna, Austria). For all categorical factors, contrasts were set to orthogonal. Time data were analyzed by GLM or GLMER with Poisson distribution from the lme4 package and binomial data (sex ratio, juvenile survival, egg hatching of the 1st generation) were analyzed by GLM or GMLER with binomial distribution (logit-link function). Weight, length, width, and number of eggs were analyzed by LM or LMER. Egg hatching of the 2nd generation was analyzed by LM after arcsin-square root transformation. Effects of factors and interactions were determined from an ANOVA table with Type III sum of squares (car package).

For spider mite assay 1, applied models were full factorial for the fixed factors "Plant type food" and "Experimental repetition". Spider mite assay 2 used full factorial mixed effect models with the fixed factors "Plant type food", "Experimental repetition", and "Plant type mother origin", and the random effect "Spider mite identity". Exceptions were juvenile survival and egg hatching rate, where experimental repetition had to be modelled as a random factor. Male weight (pooled 3-5 individuals) was analyzed by LMER with fixed factors "Plant type mother origin" and "Plant type food" and random factor "Experimental repetition".

In the first predator assay, overall survival was analyzed by GLM with fixed factors "Plant type food" and "Experimental repetition". More detailed survival analysis was conducted with tools from the "Survival" package. A survival object was created (Surv-function), Kaplan-Meier estimates for *Bt* and non-*Bt* cotton were calculated (survfit), and a log-rank test was applied to test for differences between both food plant types. A survival plot was created with "ggsurvplot" from the "survminer" package. The duration of individual developmental stages were analyzed with GLMER (random factor "experimental repetition").

In the second predator assay, GLM and LM models were fitted with "Plant type food" and "Experimental repetition" as fixed factors.

Power analyses were performed to determine the detectable differences (percentage difference of detectable treatment means relative to control means) based on the means and SDs of the non-*Bt* treatment, a power of 80% and an α level of 0.05 (package pwr) (details see Shu et al. 2018).

Results

Spider mite assay 1: Development and fecundity on Bt cotton

Table I-4. Life Cycle Parameters of Spider Mites (*Tetranychus urticae*) Raised on Leaf Disks of Bt (mCry51Aa2) and Near-isogenic Non-*Bt* cotton Plants

	Bt	Non-*Bt*	Statistic *Bt*/non-*Bt*	% det. diff.
Egg hatching 1st gen. (%)	98.3 (60)	100 (60)	GLM, Chi^2<0.001, p=1.0	-
Egg hatching time (days)	3.9 ± 0.05 (59)	3.9 ± 0.05 (60)	GLM, Chi^2=0.007, p=0.9	5.3
Juvenile survival (%)	66 (59)	79 (58)	GLM, Chi^2=2.74, p=0.1	20.2
Juvenile dev. time (days)	5.1 ± 0.11 (39)	5.2 ± 0.13 (46)	GLM, Chi^2=0.04, p=0.8	10.3
Gender (% females)	67 (39)	52 (46)	GLM, Chi^2=2.13, p=0.1	27.6
Female longevity (days)	9.7 ± 0.65 (23)	8.9 ± 0.65 (24)	GLM, Chi^2=0.42, p=0.5	29.7
Total fecundity (# eggs)	53.8 ± 5.98 (24)	48.3 ± 6.15 (24)	LM, $F_{1,42}$=0.15, p=0.7	51.6
Daily fecundity (# eggs)	6.3 ± 0.38 (24)	5.9 ± 0.41 (24)	LM, $F_{1,42}$=0.37, p=0.5	28.4
Egg hatching 2nd gen. (%)	98.7 ± 0.49 (23)	96.4 ± 1.34 (23)	LM[$], $F_{1,40}$=0.65, p=0.4	-

[$] statistics performed with arcsin(sqrt) transformed data

Values are presented as means ± SE (N). Egg hatching time, juvenile development time and female longevity were analyzed with generalized linear models (GLM) assuming Poisson distribution, juvenile survival and gender with GLM assuming binomial distribution (logit link function), and fecundity with linear models (LM). The models were full factorial with plant type (Bt/non-Bt) and experimental repetition (run 1-3) as categorical factors. Repetition as well as interactions were not significant for any analysis (p>0.05). Detectable differences are based control data.

Spider mite assay 2: Development and adult measures on Bt cotton

Table I-5. Life Cycle Parameters of Spider Mites (*Tetranychus urticae*) Raised on Leaf Disks of Bt (mCry51Aa2) and Near-isogenic Non-*Bt* Cotton Plants

	Bt	Non-*Bt*	Statistic *Bt*/non-*Bt*	Sig. factors[$]	% det. diff.
Egg hatching 1st gen. (%)[#]	98.5 (134)	96.3 (134)	GLMER, Chi^2=0.005, p=0.9		6.3
Egg hatching time (days)	4.1 ± 0.02 (132)	4.1 ± 0.02 (129)	GLMER, Chi^2=0.001, p=1.0		2.4
Juvenile survival (%)[#]	91.7 (132)	92.3 (129)	GLMER, Chi^2=0.71, p=0.4		9.1
Juvenile dev. time (days)	5.0 ± 0.06 (121)	5.0 ± 0.07 (119)	GLMER, Chi^2=0.01, p=0.9	R	5.5
Gender (% females)	48.8 (121)	57.1 (119)	GLMER, Chi^2=2.28, p=0.1		17.6
Male length (µm)	292.1 ± 1.95 (61)	294.6 ± 1.70 (49)	LMER, Chi^2=0.012, p=0.9	R	2.3
Female length (µm)	405.8 ± 5.01 (54)	403.8 ± 4.51 (66)	LMER, Chi^2=0.68, p=0.4	M×P, M×P×R	4.5
Male width (µm)	166.0 ± 1.20 (61)	168.3 ± 1.23 (49)	LMER, Chi^2=1.41, p=0.2	M, M×P	2.9
Female width (µm)	249.0 ± 1.62 (54)	249.9 ± 1.66 (66)	LMER, Chi^2=0.51, p=0.5		2.7
Male weight(µg)*	3.6 ± 0.18 (17)	3.8 ± 0.12 (13)	LMER, Chi^2=1.19, p=0.3		12.8
Female weight (µg)	12.5 ± 0.43 (54)	13.0 ± 0.44 (65)	LMER, Chi^2=1.15, p=0.3		13.4

* calculated from groups of pooled males (3-5 individuals per replicate). Fixed factors: plant type mother origin, plant type food; random factor: experimental repetition

[#] experimental repetition as random factor in the model

[$] R: Experimental repetition, M: Plant type mother origin, P: Plant type food

Values are presented as means ± SE (N). Egg hatching time and juvenile development time were analyzed with generalized linear mixed effects models (GLMER) assuming Poisson distribution, egg hatching rate, juvenile survival and gender with GLMER assuming binomial distribution (logit link function), and length, width, and weight data with linear mixed effects models (LMER). Unless otherwise stated, the models were full factorial with plant type food (*Bt*/non-*Bt*), experimental repetition (run 1-3), and plant type mother origin as categorical factors and mother ID as random factor. Statistic of the comparison plant type food are presented in detail, other significant fixed factors and interactions are listed. Detectable differences are based control data.

Predator assay 1: Survival on Bt and non-Bt cotton

The overall survival of *O. majusculus* was reduced when fed prey from *Bt* compared to prey from non-*Bt* cotton (Chi^2 = 59.6, p<0.0001). No effect of the 4 experimental repetitions was observed and there was no interaction between plant type and experimental repetition.

More detailed survival analysis confirmed the lower survival on *Bt* cotton (Log-rank test, Chi^2 = 44, p < 0.0001, Figure 1A).

In the non-*Bt* treatment, nymphs became adults between day 10 and 13. In the *Bt* treatment, only 6 nymphs molted into adults (days 13-16). Because of the high mortality in the *Bt* treatment, the developmental time of each nymphal stage was analyzed separately. Development time in the *Bt* treatment was generally longer in all nymphal stages. This difference was confirmed statistically for instar 1 and 3 (Table I-6).

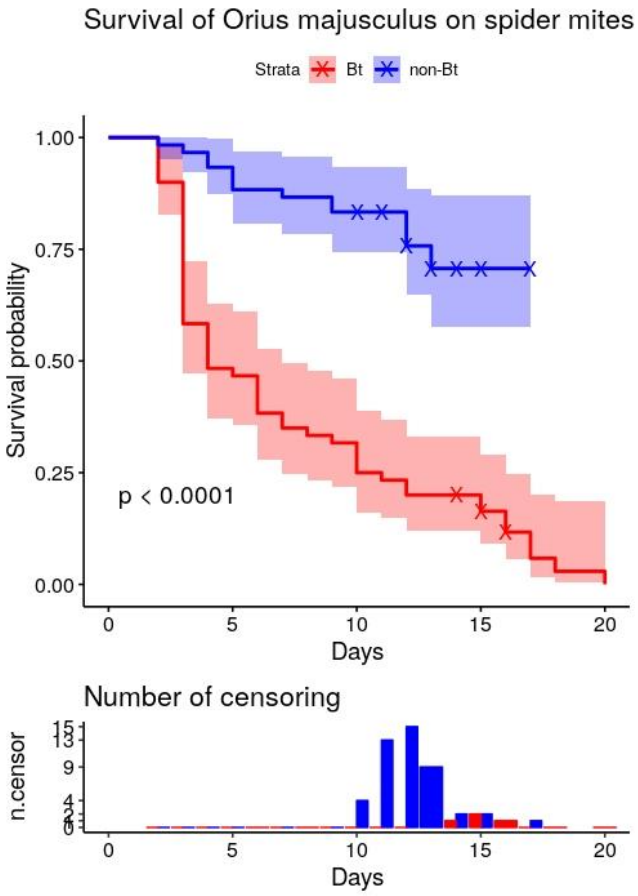


Figure I-1. Survival of *O. majusculus* in MON 88702 and DP393 Spider Mite/Leaf Treatments

Top panel: Survival of *Orius majusculus* when fed exclusively spider mites from *Bt* (red) or non-*Bt* cotton (blue). Kaplan-Meier survival estimates. Bottom panel: Number of nymphs becoming adults (censoring = becoming adults) on each day of the experiment. Data are pooled from 4 experimental repetitions.

Table I-6. *Orius majusculus* Fed Exclusively with Spider Mites from *Bt* or Non-*Bt* Cotton

Instar	*Bt*	Non-*Bt*	Statistic
1	3.4 ± 0.23 (28)	2.6 ± 0.07 (58)	Chi^2=4.6, p=0.03
2	2.4 ± 0.15 (17)	1.8 ± 0.07 (52)	Chi^2=2.1, p=0.15
3	2.9 ± 0.31 (16)	1.8 ± 0.10 (52)	Chi^2=7.2, p=0.01
4	2.8 ± 0.26 (11)	2.2 ± 0.07 (50)	Chi^2=1.4, p=0.24
5	4.5 ± 0.22 (6)	3.7 ± 0.09 (46)	Chi^2=0.9, p=0.34

Duration of each nymphal instar [mean days ± SE (N)]. Statistical comparisons were conducted with generalized linear models with Poisson distribution.

Predator assay 2: Fecundity

Table I-7. *Orius majusculus* Fecundity Assay in Which Nymphs and Adults were Fed Spider Mites Exclusively from Day 5 Onwards. Values are Means ± SE (N)

	Bt	Non-*Bt*	Statistic *Bt*/non-*Bt*	Sig. factors	% det. diff.
Start weight 5d (µg)	191.4 ± 3.99 (42)	187.5 ± 3.45 (31)	LM, $F_{1,69}$=0.1, p=0.8	R	7.4
Start weight fem. 5d (µg)	189.8 ± 6.54 (16)	186.4 ± 4.48 (20)	LM, $F_{1,32}$=0.03, p=0.9	R	9.8
Juvenile survival (%)*	95.2 (42)	100 (31)	GLM, Chi^2=2.3, p=0.1		-
Juvenile dev. time (days)	6.2 ± 0.14 (39)	6.1 ± 0.17 (31)	GLM, Chi^2=0.2, p=0.6	R	11.1
Gender (% females)	41.0 (39)	64.5 (31)	GLM, Chi^2=4.5, p=0.03	R	31.2
Female weight (µg)	461.6 ± 11.49 (16)	537.3 ± 14.50 (20)	LM, F1,32=15.3, p=0.0004		11.0
Female longevity (days)	15.5 ± 2.02 (16)	24.6 ± 2.14 (19)	GLM, Chi^2=24.5, p<0.0001	R, R×P	35.3
Fecund females (%)	84.6 (13)	77.8 (18)	GLM, Chi^2=1.1, p=0.3		33.4
Preoviposition time (days)	5.8 ± 0.70 (11)	5.8 ± 0.97 (14)	GLM, Chi^2=0.08, p=0.8	R×P	68.8
Total fecundity (# eggs)	14.5 ± 4.47 (11)	67.6 ± 8.36 (14)	LM, $F_{1,21}$=46.0, p<0.0001	R, R×P	50.9
Daily fecundity (# eggs)	0.9 ± 0.25 (11)	2.9 ± 0.28 (14)	LM, $F_{1,21}$=36.8, p<0.0001	R	40.0
Egg hatching (%)	85.1 ± 3.63 (9)	92.4 ± 0.84 (14)	LM$^{\$}$, $F_{1,19}$=2.1, p=0.2		-

* juveniles that died 1 or 2 days after the start of the experiment were excluded from analysis and assumed to have died because of handling.

$ statistics performed with arcsin(sqrt) transformed data

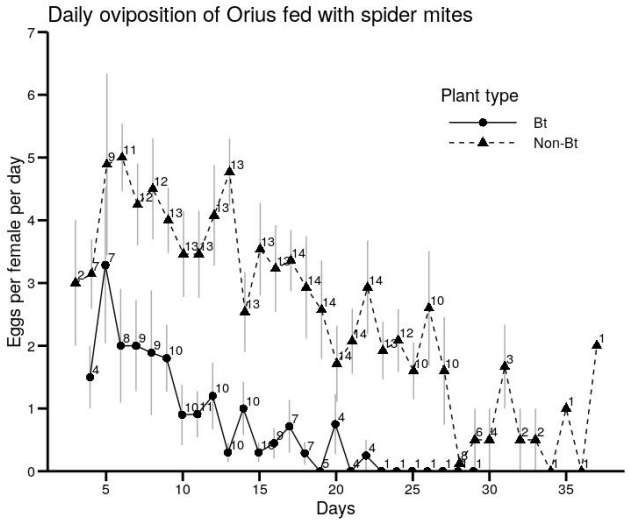


Figure I-2. Mean daily oviposition per female of Orius majusculus when fed exclusively spider mites from 5d old nymphs (reared on *Bt* or non-*Bt* cotton) Numbers in the plot represent N of each data point. Grey vertical lines represent SE.

mCry51Aa2 protein measurements

Table I-8 shows the mCry51Aa2 protein concentrations of the leaf samples that were collected during the spider mite assays. Matured leaves contained the mCry51Aa2 protein in the same order of magnitude as fresh leaves. Some measurements showed a decrease (up to 54%), others even an increase (12%) in concentration.

Table I-8. mCry51Aa2 Protein Concentrations of Fresh and Matured Leaf Disks Collected During the Spider Mite Assays

	Collection date	Condition	N	Median [min – max]	Change
Assay 1, rep. 1	23.10.2017	Fresh	11	111 [72 – 141]	
Assay 1, rep. 2	21.11.2017	Fresh	20	151 [91 – 357]	
	30.11.2017	Fresh	21	190 [119 – 383]	
	4.12.2017	Matured (4d)	18	166 [120 – 270]	-13%
Assay 1, rep. 3	11.1.2018	Fresh	19	241 [171 – 311]	
	25.1.2018	Fresh	14	290 [206 – 480]	
	29.1.2018	Matured (4d)	14	134 [113 – 228]	-54%
Assay 2, rep. 1	9.4.2018	Fresh	5	108 [84 – 155]	
	16.4.2018	Matured (7d)	5	121 [100 – 135]	+12%
Assay 2, rep. 2	7.5.2018	Fresh	5	185 [143 – 238]	
	14.5.2018	Matured (7d)	5	143 [107 – 196]	-22%
Assay 2, rep. 3	4.6.2018	Fresh	5	163 [143 – 217]	
	11.6.2018	Matured (7d)	5	169 [95 – 191]	+4%

The mCry51Aa2 concentrations are given as median in µg/g fresh weight with minimum and maximum values. Number of samples given (N), each sample analyzed once on ELISA plate.

Table I-9. mCry51Aa2 Protein Concentrations of Leaves and Spider Mites Collected During the Assays with *O. majusculus*

	Collection date	Leaves bottom	middle	top	Spider mites bottom	middle	top
Assay 1, rep. 3	11.12.2017	126 [108 – 157]	169 [140 – 205]	262 [228 – 570]	39 [34 – 46]	53 [48 – 55]	60 [49 – 87]
Assay 1, rep. 4	26.1.2018	125 [110 – 175]	167 [154 – 186]	180 [167 – 220]	20 [15 – 25]	23 [17 – 31]	27 [18 – 31]
Assay 2, rep. 1	16.4.2018	446 [407 – 600]	399 [280 – 911]	440 [348 – 476]	79 [67 – 90]*	55 [38 – 65]*	85 [49 – 128]
Assay 2, rep. 2	5.6.2018	162 [93 – 168]	253 [179 – 338]	226 [212 – 283]	11 [10 – 16]	16 [12 – 25]	25 [15 – 27]

* bottom: only 2 samples, middle: 4 samples available

The mCry51Aa2 protein concentrations are given as median in µg/g fresh weight with minimum and maximum values. N = 5 samples for all values unless stated otherwise, each sample analyzed in duplicate on the ELISA plate.

Table I-10. mCry51Aa2 Protein Concentrations Measured in *Orius majusculus* 5th Instars that were Fed With Spider Mites from the *Bt* Cotton Culture for Approximately Five Days

Collection date	Median [min – max]
17.1.2017	0.13 [0.01 – 0.42]
10.2.2018	0.23 [0.06 – 0.39]
16.4.2018	0.30 [0.14 – 1.90]

The mCry51Aa2 concentrations are given as median in µg/g fresh weight with minimum and maximum values. N = 5 samples, each consisting of 6 specimens. Each sample was analyzed in duplicate on the ELISA plate.

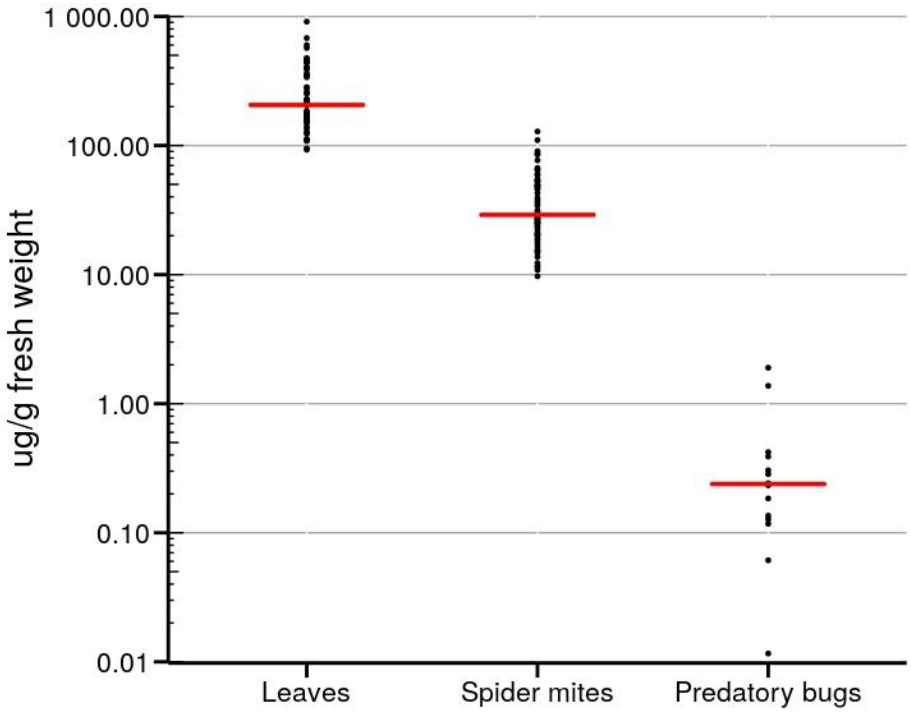


Figure I-3. mCry51Aa2 Concentrations in Leaves, Spider Mites and Predatory Bugs

For spider mites and bugs, all available values were used. For leaves, all values of samples taken together with the spider mite samples were used. More details of the individual samples see Tables I-9 and I-10.

I.2.3. Evaluation of the Potential Prey-mediated Effects of mCry51Aa2 Protein on One-day Old Nymphs of the Insidious Flower Bug, Orius insidiosus (Say) (Heteroptera: Anthocoridae) in a Tri-trophic Feeding Test with Spider Mites

The purpose of this study was to evaluate the potential effects of the mCry51Aa2 protein on the survival and development of one-day old nymphs of the insidious flower bug, *Orius insidiosus* (Say) (Heteroptera: Anthocoridae) through tri-trophic feeding interactions with MON 88702-fed two spotted spider mites, *Tetranychus urticae* (Acari: Tetranychidae). Approximately one-day-old *O. insidiosus* nymphs were individually placed into test arenas assigned to one of two dietary treatments. Test arenas consisted of 6 cm-diameter Petri dishes with either MON 88702 or conventional control DP393 cotton leaves pressed over three moistened filter papers. The two treatments consisted of MON 88702- or DP393-fed *T. urticae*, in addition to MON 88702 or DP393 cotton leaf tissue in the test arena. Twenty to fifty *T. urticae* were considered optimal to ensure feeding was *ad libitum* over this period, but avoid buildup of silk which could entrap the *O. insidiosus* nymphs. Test arenas were housed in an incubator set to a temperature of 25± 5 °C, 70± 10% RH, and a photoperiod of 16L: 8D. Survival and development time of *O. insidiosus* nymphs was evaluated daily for both treatments. Throughout the duration of the assay, leaf tissue and *T. urticae* were replaced every three days to ensure *ad libitum* availability of prey items (*T. urticae*) and leaf tissue until all *O. insidiosus* individuals had completed development.

The expression levels of the mCry51Aa2 protein in MON 88702 cotton leaf tissue and the stability of the protein under study conditions were determined by a validated immunoassay following the same process as described in Appendix C.6.2. The results demonstrated the mCry51Aa2 protein level in leaf tissue ranged from 234 – 270 mCry51Aa2 protein/g fwt. This demonstrates the protein was stable in MON 88702 leaf tissue during three days of incubation under study conditions. Protein levels in *T. urticae* reared on MON 88702 and DP393 cotton were quantified using an immunoassay and following the methods as described in Appendix C.6.1. The average level of mCry51Aa2 protein in MON 88702-reared *T. urticae* was 25 µg/g fwt.

O. insidiosus survival was significantly reduced in MON 88702 spider mite/leaf treatments compared to DP393 spider mite/leaf treatments (22.2% vs 88.9%, respectively; $\alpha = 0.05$). Development of *O. insidiosus* was significantly prolonged in MON 88702 treatments compared to DP393 when *T. urticae* were provided as prey (13.5 vs 12.0 days, respectively; $\alpha = 0.05$). Therefore, the results of this study demonstrate that the survival and development of the *O. insidiosus* nymphs was negatively affected in a tri-trophic scenario where *O. insidiosus* feeding was limited to MON 88702 leaf tissue and *T. urticae* prey.

I.3. Tier 3 Testing: Evaluation of the Potential Prey-mediated Effects of mCry51Aa2 Protein on One-day Old Nymphs of the Insidious Flower Bug, *Orius insidiosus* (Say) (Heteroptera: Anthocoridae) in a Tri-trophic Feeding Test with Spider Mites and Mediterranean Flour Moth Eggs

The purpose of this study was to evaluate the potential effects of the mCry51Aa2 protein on the survival and development of one-day old nymphs of the insidious flower bug, *Orius insidiosus* (Say) (Heteroptera: Anthocoridae) through tri-trophic feeding interactions with MON 88702-fed two spotted spider mites, *Tetranychus urticae* (Acari: Tetranychidae), and Mediterranean flour moth eggs, *Ephestia kuehniella* (Lepidoptera: Pyralidae). This was defined as a tier 3 study since it approximated more realistic, choice feeding scenarios for the predator *O. insidiosus*. Approximately one-day-old *O. insidiosus* nymphs were individually placed into test arenas assigned to one of several different exposure treatments described below. Test arenas consisted of 6 cm-diameter Petri dishes with either MON 88702 or conventional control DP393 cotton leaf tissue pressed over three moistened filter papers. Test arenas were housed in an incubator set to a temperature of 25± 5 °C, 70± 10% RH, and a photoperiod of 16L: 8D. The expression levels of the mCry51Aa2 protein in MON 88702 cotton leaf tissue and the stability of the protein under study conditions were determined by a validated immunoassay for which the results are described in Appendix I.2.3, and demonstrate that the protein was stable in MON 88702 leaf tissue during incubation under study conditions.

<u>*O. insidiosus* + leaf tissue</u>

Within this first experimental set up, baseline survival of *O. insidiosus* nymphs was assessed in the following treatments: MON 88702 cotton leaf tissue, conventional control DP393 cotton leaf tissue, *E. kuehniella* egg and green bean, dry filter paper and wet filter paper. *O. insidiosus* survival and development were assessed daily for ten days. The *E. kuehniella* eggs and green beans were replaced, as needed, over the ten-day assay period. This allowed the assessment of survivability of *O. insidiosus* under various conditions to define its ability to survive on green tissue alone.

Following assay initiation, all *O. insidiosus* nymphs in the wet and dry filter paper treatments died within one day, indicating that simply supplying hydration was not sufficient for survival. The following experiment determined that all insects in both the MON 88702 and DP393 leaf tissue only treatments died within two days, demonstrating that the addition of green tissue does not increase the potential for survival of *O. insidiosus* in and of itself. All *O. insidiosus* nymphs in the *E. kuehniella* eggs and green bean treatment survived and developed into adults by Day 10, indicating that the feeding behaviors of *O. insidiosus* requires prey items beyond leaf or other alternatives or hydration to survive.

<u>*O. insidiosus* + leaf tissue + *E. kuehniella* eggs</u>

Within this experimental set up, approximately one-day-old *O. insidiosus* nymphs were individually placed into test arenas containing either MON 88702 or DP393 leaf tissue. One scoop of *E. kuehniella* eggs using an 8mm-diameter spoon was added at initial set up of the arenas. All leaf tissue and *E. kuehniella* eggs were replaced every three days to

ensure *O. insidiosus* fed *ad libitum* throughout the duration of the assay. *O. insidiosus* survival and development were assessed daily until all *O. insidiosus* individuals had completed development.

There was no significant difference in survival (82.4% vs 87.5%, respectively; $\alpha = 0.05$) or development (10.1 days for both treatments) of *O. insidiosus* fed *E. kuehniella* eggs placed on MON 88702 or DP393 leaf tissue, respectively.

O. insidiosus + leaf tissue + *E. kuehniella* eggs + *T. urticae*

Within this experimental set up, approximately one-day-old *O. insidiosus* nymphs were individually placed into test arenas containing either MON 88702 or DP393 leaf tissue. MON 88702- or DP393-fed *T. urticae* were added to the respective test arenas. Twenty to fifty *T. urticae* and approximately One scoop of *E. kuehniella* eggs using an 8mm-diameter spoon was added at initial set up of arenas, and all leaf tissue, *T. urticae* and *E. kuehniella* eggs were replaced every three days to ensure *O. insidiosus* was fed *ad libitum* throughout the duration of the assay. The expression levels of the mCry51Aa2 protein in MON 88702-fed cotton *T. urticae* was determined by an immunoassay (Appendix I.2.3). *O. insidiosus* survival and development were assessed daily until all *O. insidiosus* individuals had completed development.

There was no difference in *O. insidiosus* survival or development when provided a combination of *E. kuehniella* eggs and MON 88702- or DP393- fed *T. urticae* between MON 88702 and DP393 test arenas, respectively (85.7% and 10.4 days).

I.4. Tier 4 Testing

The main purpose of this field study was to assess the relative impact of MON 88702 on abundance of predatory Hemiptera, compared to a conventional control. Additional observations on pest abundance were also made.

I.4.1. Study Site Descriptions and Experimental Design

Field trials were established in 2018 at six sites in the U.S. that provided a range of environmental and agronomic conditions representative of U.S. cotton growing regions (Table I-11). The field cooperator at each field site was familiar with cotton growth and production. The study was established at each site in a randomized complete block design with three replications. Plot and row dimensions are described in Table I-12.

General trial maintenance, such as fertilizer, irrigation, and other management practices were applied as necessary throughout the season. All maintenance operations were performed uniformly across all plots.

I.4.2. Materials and Treatments

At each site MON 88702 and its conventional control (DP393) were planted. DP393 has a genetic background similar to the test material with the exception of the biotechnology-derived trait. The identities of MON 88702 and conventional control DP393 seed were verified by event-specific polymerase chain reaction analyses.

A total of five treatments was established at each site (Table I-13). Treatment 1 consisted of DP393 plots treated with the broad spectrum insecticide, acephate, which is used to control plant bugs and concurrently reduce natural enemy densities (Asiimwe et al., 2014). Two sites (AZMA and NCRC) used acephate in combination with pyrethroids as treatment 1 based on local recommended guidelines for Lygus management (Table I-14). Separate treatments were established where a selective insecticide regime was applied to both MON 88702 (treatment 3) and the conventional control DP393 (treatment 2). The selective insecticides used were flonicamid, imidacloprid and sulfoxaflor, which are known to provide effective control against plant bugs but have minimal to no effect on beneficial arthropods (Asiimwe et al., 2014; Catchot, 2019) (Table I-14). All broad spectrum and selective insecticide applications were made at or near the local established thresholds and local recommended rates were used whenever the applications were made. Untreated MON 88702 (treatment 5) and the conventional control DP393 (treatment 4) were also established, in which no insecticides were applied across the entire growing season.

I.4.3. Arthropod Collections

Weekly samples of beneficial arthropods as well as pest species were collected from all the plots over the course of the growing season, initiated at early squaring through cut-out for a total of 10 sampling times over the course of the season. All collections were conducted prior to high-noon and by the same field personnel at each site. Sampling was conducted using different collection methods.

Vertical Beat Sheet Collections

A vertical beat sheet method was used to determine nymph and adult abundance of the beneficial Hemiptera (*Orius* spp., *Geocoris* spp., *Nabis* spp., *Zelus* spp., predatory stink bug) across all sampling times and sites (Drees and Rice, 1985). The abundance of aphids, whiteflies, Lygus, cotton fleahopper and aphids was also conducted using the vertical beat sheet method. All taxa were identified to at least genus level and separate counts of nymphs and adults were monitored. For Lygus and cotton fleahoppers, nymph counts were made on 3rd-5th instars (large nymphs) due to ease of identification of these stages between these two closely related taxa. A general category of mirid pest nymphs (1st and 2nd instar Lygus and cotton fleahoppers) were also evaluated. At each arthropod collection, a total of six arthropod sub-samples, one from each row, was collected in the center of each plot (rows 8-13).

Sweep Net Collections

Lygus, cotton fleahopper and stink bug densities were also determined using a 38 cm diameter sweep net. Fifty sweeps were made in each plot, 25 on each row, to determine triggers for insecticide applications and to monitor overall treatment effects on their densities. Total counts (combining all life stages) of Lygus, cotton fleahopper and stink bugs were monitored in the sweep nets.

Visual Collections

Spider mite and thrips densities were monitored by a visual inspection of the 4th mainstem node leaf from 10 randomly selected plants per plot, and using a 10X hand lens, counting the number of spider mites and thrips on the underside of the leaf, differentiating eggs from the other developmental stages. Bollworm densities were also assessed by examining the terminals of 10 randomly selected terminals per plant for number of live larvae.

Table I-11. Field Site Locations and Site Codes

Site Code	County/Parish, State
AZMA	Pinal, AZ
AZYU	Yuma, AZ
LACH	Rapides, LA
MSGV	Washington, MS
NCRC	Edgecombe, NC
TXUV	Uvalde, TX

Table I-12. Field Information

Site Code[1]	Planting Date[2]	Planting Rate (seeds/m^2)	Rows / Plot	Inter-row Distance (m)	Row Length (m)	Plot Width (m)
AZMA	05/22/2018	11.0	18	1.02	17.4	18.3
AZYU	05/30/2018	12.3	20	1.07	18.3	21.3
LACH	06/07/2018	12.9	20	1.02	18.3	20.3
MSGV	05/29/2018	13.7	20	0.97	18.3	19.2
NCRC	05/28/2018	14.3	20	0.91	18.3	18.3
TXUV	05/30/2018	12.8	20	1.02	18.3	20.42

[1] Site code: AZMA = Pinal county, AZ; AZYU = Yuma county, AZ; LACH = Rapides parish, LA; MSGV = Washington County, MS; NCRC = Edgecombe County, NC; TXUV = Uvalde County, TX.

Table I-13. Treatment Descriptions

Treatment Code	**Treatment**
1	DP393 conventional insecticide regime
2	DP393 minimal insecticide regime, selective insecticides
3	MON 88702 minimal insecticide regime, selective insecticides
4	DP393 untreated
5	MON 88702 untreated

Table I-14. Insecticide Applications for Lygus and Cotton Fleahopper Control in a 2018 U.S. Tier 4 Field Study

Site Code[1]	Application date	Insecticide Applied	Application rate	Material Name (Treatment Code)
AZMA	7/27/2018	Acephate	1156 g a.i/ha	DP393 (1)
		Flonicamid	133 g a.i/ha	DP393 (2), MON 88702 (3)
	8/17/2018	Acephate	1156 g a.i/ha	DP393 (1)
		Sulfoxaflor	106 g a.i/ha	DP393 (2), MON 88702 (3)
	9/5/2018	Acephate+Fenpropathrin	1156 g a.i/ha + 750 g a.i/ha	DP393 (1)
		Flonicamid	196 g a.i/ha	DP393 (2), MON 88702 (3)
AZYU	8/18/2018	Acephate	1120 g a.i/ha	DP393 (1)
	8/26/2018	Flonicamid	196 g a.i/ha	DP393 (2), MON 88702 (3)
	9/11/2018	Acephate	1120 g a.i/ha	DP393 (1)
		Sulfoxaflor	106 g a.i/ha	DP393 (2), MON 88702 (3)
LACH	8/22/2018	Acephate	1120 g a.i/ha	DP393 (1)

Table I-14. Insecticide Applications for Lygus and Cotton Fleahopper Control in a 2018 U.S. Tier 4 Field Study (continued)

Site Code[1]	Application date	Insecticide Applied	Application rate	Material Name (Treatment Code`)
MSGV	7/24/2018	Acephate	911 g a.i/ha	DP393 (1)
		Sulfoxaflor	106 g a.i/ha	DP393 (2)
	8/2/2018	Sulfoxaflor	106 g a.i/ha	MON 88702 (3)
	8/14/2018	Acephate	911 g a.i/ha	DP393 (1)
		Sulfoxaflor	106 g a.i/ha	DP393 (2), MON 88702 (3)
NCRC	7/16/2018	Acephate	1121 g a.i/ha	DP393 (1)
	8/9/2018	Acephate+Dicrotophos	1121 g a.i/ha+560 g a.i/ha	DP393 (1)
		Sulfoxaflor	158 g a.i/ha	DP393 (2)
	8/25/2018	Sulfoxaflor	158 g a.i/ha	MON 88702 (3)
TXUV	7/11/2018	Acephate	560 g a.i/ha	DP393 (1)
		Imidacloprid	140 g a.i/ha	DP393 (2), MON 88702 (3)
	8/22/2018	Flonicamid	175 g a.i/ha	MON 88702 (3)

[1] Site code: AZMA = Pinal county, AZ; AZYU = Yuma county, AZ; LACH = Rapides parish, LA; MSGV = Washington County, MS; NCRC = Edgecombe County, NC; TXUV = Uvalde County, TX.

I.4.4. Statistical Analysis

The relative abundance of each monitored arthropod was evaluated across sites and collections using a linear mixed model. For taxa collected using the vertical beat sheet method and visual counts, abundance was evaluated separately for individual life stages (eggs, nymphs and adults, where present), and total counts (combining all life stages) of each species. For those collected using sweep nets, total abundance (i.e. combining all life stages) was evaluated. Following Ahmad et al. (2016), an inclusion criteria was established where a mean count across collection times per plot of ≥ 1 was required for each site to be included in the analysis. Secondly, an average of at least one capture per replicate was required for each collection time. Sites and/or collections with counts below the criteria were excluded from the analyses. All other data, including zero counts, were included in the analyses. In the case of certain monitored arthropods, the application of these inclusion criteria therefore enabled the analysis of their total counts but not of separate life stages.

The model, described below, reflects the design structure of the experiment, accounting for variation in counts between sites, replicates, collections, and the interaction of site and treatment. Each of these factors is modeled as a random effect, creating a broad inference space that allows results of the pairwise comparisons between treatments to be generalized to the entire growing region and season (Stroup, 2013). Key model assumptions were satisfied by assuming heterogenous variance between sites and using a square-root variance stabilizing transformation to account for the count nature of the data. Arithmetic means (untransformed) are presented below, however all pairwise comparisons between treatments were made using Least Squares (LS) means at the $\alpha = 0.05$ level of significance. LS means are, in effect, treatment means appropriately adjusted for the other effects in the model. The LS means are also presented in Tables I-28 through I-31. More precisely, they are the best, unbiased estimates of marginal means for a balanced population (Milliken & Johnson 2009). Given the complexity of the experimental design, including a degree of imbalance in the dataset, as well as the square root transformation and heterogeneous variance used in the analysis, differences between the LS means and arithmetic means calculated on the raw (untransformed) counts are expected.

It should be noted that the use of generalized linear mixed models (GLMMs) for the analysis of count data was extensively explored, making different assumptions about the distribution of the response variable (e.g., Poisson, Negative Binomial, Generalized Poisson). Furthermore, the use of zero-inflated models was investigated for addressing the overdispersion (i.e., too many zeros) that is so common with insect counts in the field. GLMMs extend linear model theory to accommodate non-normal data and random model effects. Despite a strong theoretical foundation (Breslow & Clayton 1993; Wolfinger & O'Connell 1993), GLMMs are computationally demanding when dealing with complex design and/or treatment structures, which limits their use to simple applications (Bolker et al. 2009; Stroup 2013; Ives 2015; Bates et al. 2019). This remains true despite ongoing advancements in the methods (e.g., Laplace, Quadrature) used to approximate the likelihood to estimate parameters in the GLMM. Given the complex design structure of the tier 4 field study, these challenges were expected and indeed encountered when applying GLMMs and zero-inflated models to the counts collected in

the field. The approach summarized here – a linear mixed model with transformation – is a tractable alternative (Bolker et al. 2009; Stroup 2013) that (1) accounts for the count nature of the data through the variance-stabilizing transformation; and (2) allows to consistently and accurately reflect the design and treatment structures of the field trial for all taxa (totaling 142 models when considering combined- and individual-site analyses); this second point is the most critical aspect in terms of statistical inference and making rigorous, unbiased comparisons between treatments (Stroup 2013).

Statistical power of the arthropod abundance analyses was estimated assuming a 50% difference (i.e. effect size) in the abundance of each taxonomic group. Four factors affect these power calculations, including the effect size (set at 50% of control), sample size, significance level (set at $\alpha = 0.05$), and amount of variability in the arthropod counts. Two of these factors, namely the sample size and amount of variability, differed among the arthropods that were sampled, resulting in differences in the computed level of power. Statistical power increases with sample size when the effect size, significance level, and amount of variability are held constant. On the other hand, variability inversely affects statistical power when these factors are controlled in an experiment. Thus, statistical power is low when an arthropod occurs infrequently (small sample size) and the variation of the mean difference between MON 88702 and the conventional control DP393 is large. Based on previous experience (first described in Duan et al. 2006), taxa with extremely low abundance generally have higher amounts of variability relative to the mean (e.g., CV > 100%); for those taxa, a larger sample size is needed to achieve a higher level of statistical power.

The primary utility of power analysis is for study design planning (Stroup, 2013). In this case the prospective power analyses were used to design the tier 4 field study in terms of approximate number of sites, replicates, and collections over the season to ensure sufficient power for key taxa being assessed in the case of MON 88702 cotton; *Orius* spp. and *Geocoris* spp. Statistical power in field studies is a function of several factors, including insect abundance; in general, smaller effect sizes can be observed in more abundant taxa. These more abundant taxa will also tend to play more important roles than less abundant taxa within the same guild. The 50% effect size assumed in the power analysis is an established metric for regulatory testing of field studies with non-target arthropods as documented extensively in the published literature (e.g. Blumel et al. 2000; Candolfi et al. 2000; Perry et al. 2003; Duan et al., 2006) and regulatory guidance (e.g. de Jong et al., 2010). This effect size is both ecologically-relevant, since between-season recovery is usually not impeded at this effect level (Candolfi et al. 2000), and practical for detection in the field; for many taxa, assuming a smaller effect size would place unrealistic demands on the study design (de Jong et al. 2010). Further, Naranjo (2005a; b) evaluated the relationship between abundance and biological control in the cotton agro-ecosystem and provided an estimation of effect size with biological relevancy specific to this type of study assessing predator abundance in cotton. They determined that a 50% effect size was appropriate based on previous studies comparing predator abundance in *Bt* and non-*Bt* cotton plots, that indicated this percent reduction in abundance of key predators in the cotton agro-ecosystem would result in a biologically meaningful reduction in the biological control function (Naranjo 2005a; b). Percent reductions in predator abundance below this threshold, e.g. 20%, did not result in reduced predator function, justifying the 50% threshold for these studies.

The retrospective power analysis provided for the tier 4 study conducted for the assessment of MON 88702 (Tables I-20, I-21, I-26 and I-27) confirmed that the experimental design of the study was robust. The actual data, collected across the U.S. cotton growing region, provide direct, empirical insight into whether there was a treatment effect or not. Independent of the effect size assumed in the power analysis, the mean values for the combined-site unsprayed conventional control and unsprayed MON 88702 treatments were not statistically significantly different, indicating that MON 88702 does not pose a risk to predatory Hemiptera present in the cotton agro-ecosystem.

Details of the linear mixed model analysis, associated power calculations, and additional individual-site evaluations are provided below.

Summary statistics

Arthropod taxa were classified in two categories: category I (abundance separating different arthropod life stages) and category II (total abundance).

PROC MEANS in SAS was used to calculate the overall abundance of each taxa by treatment and site over collection times and replicates. The results are provided in Table I-15.

In order to make a valid analysis of the treatment effect on each arthropod category, at first, a site inclusion criterion was applied separately for each category I and II: mean count per plot ≥ 1 (i.e. the mean in Table I-15 ≥1). Secondly, a minimum collection inclusion criterion was applied for each collection time after the site inclusion: each replicate had at least one capture on average. Data combinations with counts below the criterion were excluded from the following summary, the significance testing, and power analysis.

After applying the above criteria, PROC MEANS was used to calculate the by-replicate mean over collection times at each site, and then to calculate the mean and standard error for each treatment. Results of mean and standard error were then averaged over sites and displayed in Table I-16 for category II and Table I-22 for category I. Note that, standard errors in Table I-16 and Table I-22 were further divided by the square-root of the number of sites exceeding the above criteria. Results of mean and standard error for each site are displayed in Table I-18 for category II and Table I-24 for category I.

Combined-site Analysis

The following linear mixed model was used to conduct a combined-site analysis for each species:

$$y_{ijkl} = \mu + s_i + r(s)_{j(i)} + t_k + d_{l(i)} + (ts)_{ik} + e_{ijkl} \quad (1)$$

where :

y_{ijkl} = square-root of the observed count;

μ = overall mean;

s_i = random site effect;

$r(s)_{j(i)}$ = random within-site replicate effect;
t_k = fixed material effect;
$d_{l(i)}$ = random within-site collection effect;
$(ts)_{ik}$ = random interaction effect of site and material;
e_{ijkl} = residual.

PROC MIXED was used to fit model (1) to the data. A square-root transformation was used to account for the nature of the count data. Variation heterogeneity assumption was further improved by assuming heterogeneous residual variation among sites. Residual plots were visually inspected, and assumptions of normality and variance homogeneity among treatments were found to be satisfied reasonably well. Pairwise comparisons among treatments were defined within the procedure and tested using t-tests. The results of the comparisons between treatments are displayed in Table I-17 for category II, and Table I-23 for category I. Least Square means and standard errors are listed in Table I-28 for category II and Table I-30 for category I.

Individual-site Analysis

The following linear mixed model was used to conduct an individual-site analysis for each species:

$$y_{jkl} = \mu + r_j + t_k + d_l + e_{jkl} \quad (2)$$

where :

y_{jkl} = square-root of the observed count;

μ = overall mean;

r_j = random replicate effect;

t_k = fixed material effect;

d_l = random collection effect;

e_{jkl} = residual.

PROC MIXED was used to fit model (2) to the data at each site. Again, a square-root transformation was used to account for the nature of the count data. Heterogeneity assumption was further enhanced by assuming heterogeneous residual variation among collections. Residual plots were visually inspected, and assumptions of normality and variance homogeneity among materials were found to be satisfied reasonably well. Pairwise comparisons among treatments were defined within the procedure and tested using t-tests. The significance of the comparisons at the 0.05 significance level are displayed along with simple mean differences in counts in Table I-19 for category II and Table I-25 for category I. Least Square means and standard errors are listed in Table I-29 for category II and Table I-31 for category I.

Power Analysis

Statistical power was estimated assuming a 50% difference (i.e., effect size) in the abundance of each taxonomic group (Blumel et al. 2000; Perry et al. 2003), following the method introduced by Duan et al. (2006).

A customized SAS algorithm executing the following steps was used to calculate statistical power for detecting a 50% difference in the test treatment mean count relative to the control (Treatment = 04) at the $\alpha = 0.05$ level of significance.

- Estimate least square mean of the control (Treatment = 04) in the square-root scale and relevant random variation of the control mean from the LSMEANS and COVPARMS statements after fitting the mixed model (1) for the across-site analysis and model (2) for the by-site analysis;
- Applying the estimated control mean and variation to estimate the difference in a square-root scale corresponding to 50% of the control mean in count;
- Estimate standard error and the degrees of freedom of the treatment difference in a square-root scale using the LSMEANS statement;
- Estimate power using the above estimates in a non-central t-distribution.

Results of the combined-site power analyses are listed in Table I-20 for category II and Table I-26 for category I. Results of the individual-site power analysis are listed in Table I-21 for category II and Table I-27 for category I.

Table I-15. Overall Abundance By Site and Treatment for All Arthropod Taxa Collected Using Three Different Collection Methods: Total Counts over Collection Times and Replicates, and Overall Arithmetic Mean over Treatments, Collections, Replicates, and Treatments

Method	Taxa	Site	01	02	03	04	05	Overall Mean
BeatSheet	Aphids	AZMA	7	4	3	9	3	0.2
BeatSheet	Aphids	AZYU	1	1	0	1	3	0.0
BeatSheet	Aphids	LACH	6230	6253	5672	6500	5925	203.9
BeatSheet	Aphids	MSGV	4696	1725	2410	1762	2265	85.7
BeatSheet	Aphids	NCRC	1980	783	742	854	734	34.0
BeatSheet	Aphids	TXUV	88	58	27	28	27	1.5
BeatSheet	Cotton Fleahopper Adults	AZMA	20	25	26	63	44	1.2
BeatSheet	Cotton Fleahopper Adults	AZYU	37	70	54	117	58	2.2
BeatSheet	Cotton Fleahopper Adults	LACH	1	0	0	0	0	0.0
BeatSheet	Cotton Fleahopper Adults	MSGV	1	0	1	0	0	0.0
BeatSheet	Cotton Fleahopper Adults	NCRC	2	4	2	6	4	0.1
BeatSheet	Cotton Fleahopper Adults	TXUV	62	118	55	177	121	3.6
BeatSheet	Cotton Fleahopper Large Nymphs	AZMA	21	57	47	189	170	3.2
BeatSheet	Cotton Fleahopper Large Nymphs	AZYU	30	49	61	106	65	2.1
BeatSheet	Cotton Fleahopper Large Nymphs	LACH	0	0	0	0	0	0.0
BeatSheet	Cotton Fleahopper Large Nymphs	MSGV	1	1	0	6	1	0.1
BeatSheet	Cotton Fleahopper Large Nymphs	NCRC	0	3	1	7	5	0.1
BeatSheet	Cotton Fleahopper Large Nymphs	TXUV	257	287	107	471	431	10.4
BeatSheet	Euschistus servus (adults)	AZMA	3	8	4	14	13	0.3
BeatSheet	Euschistus servus (adults)	AZYU	5	0	13	3	7	0.2
BeatSheet	Euschistus servus (adults)	LACH	2	2	0	1	1	0.0

Table I-15. Overall Abundance By Site and Treatment for All Arthropod Taxa Collected Using Three Different Collection Methods: Total Counts over Collection Times and Replicates, and Overall Arithmetic Mean over Treatments, Collections, Replicates, and Treatments (continued)

Method	Taxa	Site	01	02	03	04	05	Overall Mean
BeatSheet	Euschistus servus (adults)	MSGV	2	5	10	7	5	0.2
BeatSheet	Euschistus servus (adults)	NCRC	3	2	3	2	2	0.1
BeatSheet	Euschistus servus (adults)	TXUV	0	0	0	1	0	0.0
BeatSheet	Geocoris (nymphs)	AZMA	40	96	69	193	169	3.8
BeatSheet	Geocoris (nymphs)	AZYU	54	84	191	246	190	5.1
BeatSheet	Geocoris (nymphs)	LACH	20	22	30	19	32	0.8
BeatSheet	Geocoris (nymphs)	MSGV	112	159	127	277	216	5.9
BeatSheet	Geocoris (nymphs)	NCRC	23	56	39	68	72	1.7
BeatSheet	Geocoris (nymphs)	TXUV	23	27	37	62	34	1.2
BeatSheet	Geocoris pallens (adults)	AZMA	8	23	14	17	20	0.5
BeatSheet	Geocoris pallens (adults)	AZYU	38	87	127	145	132	3.5
BeatSheet	Geocoris pallens (adults)	LACH	5	6	16	8	6	0.3
BeatSheet	Geocoris pallens (adults)	MSGV	89	129	122	172	153	4.5
BeatSheet	Geocoris pallens (adults)	NCRC	6	10	19	23	27	0.6
BeatSheet	Geocoris pallens (adults)	TXUV	0	0	0	0	0	0.0
BeatSheet	Geocoris punctipes (adults)	AZMA	11	42	63	85	51	1.7
BeatSheet	Geocoris punctipes (adults)	AZYU	0	0	0	4	0	0.0
BeatSheet	Geocoris punctipes (adults)	LACH	0	0	0	0	0	0.0
BeatSheet	Geocoris punctipes (adults)	MSGV	1	4	0	4	2	0.1
BeatSheet	Geocoris punctipes (adults)	NCRC	0	0	1	0	0	0.0
BeatSheet	Geocoris punctipes (adults)	TXUV	57	48	59	86	81	2.2

Table I-15. Overall Abundance By Site and Treatment for All Arthropod Taxa Collected Using Three Different Collection Methods: Total Counts over Collection Times and Replicates, and Overall Arithmetic Mean over Treatments, Collections, Replicates, and Treatments (continued)

Method	Taxa	Site	01	02	03	04	05	Overall Mean
BeatSheet	Lygus Adults	AZMA	53	46	25	44	23	1.3
BeatSheet	Lygus Adults	AZYU	32	42	46	64	24	1.4
BeatSheet	Lygus Adults	LACH	5	19	21	15	11	0.5
BeatSheet	Lygus Adults	MSGV	51	32	39	72	42	1.6
BeatSheet	Lygus Adults	NCRC	21	23	25	36	19	0.8
BeatSheet	Lygus Adults	TXUV	0	0	0	0	0	0.0
BeatSheet	Lygus Large Nymphs	AZMA	99	58	12	108	42	2.1
BeatSheet	Lygus Large Nymphs	AZYU	36	10	15	29	8	0.7
BeatSheet	Lygus Large Nymphs	LACH	44	87	35	69	50	1.9
BeatSheet	Lygus Large Nymphs	MSGV	128	106	58	194	89	3.8
BeatSheet	Lygus Large Nymphs	NCRC	99	110	47	139	63	3.1
BeatSheet	Lygus Large Nymphs	TXUV	0	0	1	1	1	0.0
BeatSheet	Nabis (nymphs)	AZMA	0	1	1	5	0	0.0
BeatSheet	Nabis (nymphs)	AZYU	0	7	23	23	9	0.4
BeatSheet	Nabis (nymphs)	LACH	1	0	1	0	0	0.0
BeatSheet	Nabis (nymphs)	MSGV	9	13	13	15	13	0.8
BeatSheet	Nabis (nymphs)	NCRC	5	11	3	14	13	0.3
BeatSheet	Nabis (nymphs)	TXUV	0	0	0	0	0	0.0
BeatSheet	Nabis alternatus (adults)	AZMA	0	0	0	0	0	0.0
BeatSheet	Nabis alternatus (adults)	AZYU	16	19	22	27	23	0.7
BeatSheet	Nabis alternatus (adults)	LACH	1	0	0	1	0	0.0
BeatSheet	Nabis alternatus (adults)	MSGV	2	7	2	2	5	0.2

Table I-15. Overall Abundance By Site and Treatment for All Arthropod Taxa Collected Using Three Different Collection Methods: Total Counts over Collection Times and Replicates, and Overall Arithmetic Mean over Treatments, Collections, Replicates, and Treatments (continued)

Method	Taxa	Site	01	02	03	04	05	Overall Mean
BeatSheet	Nabis alternatus (adults)	NCRC	2	6	4	9	4	0.2
BeatSheet	Nabis alternatus (adults)	TXUV	0	1	0	0	1	0.0
BeatSheet	Nezara viridula (Adults)	AZMA	0	0	0	1	0	0.0
BeatSheet	Nezara viridula (Adults)	AZYU	1	3	4	0	1	0.1
BeatSheet	Nezara viridula (Adults)	LACH	1	2	3	0	2	0.1
BeatSheet	Nezara viridula (Adults)	MSGV	16	16	25	20	20	0.6
BeatSheet	Nezara viridula (Adults)	NCRC	5	3	3	9	3	0.2
BeatSheet	Nezara viridula (Adults)	TXUV	0	0	0	1	0	0.0
BeatSheet	Orius (nymphs)	AZMA	28	61	50	71	84	2.0
BeatSheet	Orius (nymphs)	AZYU	49	64	86	91	47	2.2
BeatSheet	Orius (nymphs)	LACH	41	51	53	54	59	1.7
BeatSheet	Orius (nymphs)	MSGV	65	70	58	89	59	2.3
BeatSheet	Orius (nymphs)	NCRC	86	116	100	126	139	3.8
BeatSheet	Orius (nymphs)	TXUV	1271	1144	1104	1180	1360	40.4
BeatSheet	Orius insidiosus	AZMA	0	0	0	0	0	0.0
BeatSheet	Orius insidiosus	AZYU	0	0	0	1	0	0.0
BeatSheet	Orius insidiosus	LACH	0	0	0	0	0	0.0
BeatSheet	Orius insidiosus	MSGV	0	0	0	0	0	0.0
BeatSheet	Orius insidiosus	NCRC	0	0	0	0	0	0.0
BeatSheet	Orius insidiosus	TXUV	99	120	135	173	157	4.6
BeatSheet	Orius tristicolor	AZMA	87	103	125	105	90	3.4
BeatSheet	Orius tristicolor	AZYU	95	131	107	102	80	3.4

Table I-15. Overall Abundance By Site and Treatment for All Arthropod Taxa Collected Using Three Different Collection Methods: Total Counts over Collection Times and Replicates, and Overall Arithmetic Mean over Treatments, Collections, Replicates, and Treatments (continued)

Method	Taxa	Site	01	02	03	04	05	Overall Mean
BeatSheet	Orius tristicolor	LACH	30	17	29	27	15	0.8
BeatSheet	Orius tristicolor	MSGV	51	61	47	78	54	2.0
BeatSheet	Orius tristicolor	NCRC	82	124	86	88	120	3.3
BeatSheet	Orius tristicolor	TXUV	1126	1403	1287	1464	1669	46.3
BeatSheet	Other Nabis (Adults)	AZMA	4	6	5	12	4	0.2
BeatSheet	Other Nabis (Adults)	AZYU	0	.	.	.	.	0.0
BeatSheet	Other Nabis (Adults)	MSGV	0	0	0	0	0	0.0
BeatSheet	Other Nabis (Adults)	NCRC	0	0	0	0	0	0.0
BeatSheet	Other Nabis (Adults)	TXUV	0	0	0	0	0	0.0
BeatSheet	Other Zelus (Adults)	AZMA	0	0	0	0	0	0.0
BeatSheet	Other Zelus (Adults)	MSGV	0	0	0	0	0	0.0
BeatSheet	Other Zelus (Adults)	NCRC	0	0	0	0	0	0.0
BeatSheet	Podisus (adults)	AZMA	0	0	0	0	0	0.0
BeatSheet	Podisus (adults)	AZYU	0	0	0	0	0	0.0
BeatSheet	Podisus (adults)	LACH	1	1	1	0	0	0.0
BeatSheet	Podisus (adults)	MSGV	11	7	14	12	13	0.4
BeatSheet	Podisus (adults)	NCRC	1	1	0	0	0	0.0
BeatSheet	Podisus (adults)	TXUV	0	0	0	0	0	0.0
BeatSheet	Podisus (nymphs)	AZMA	0	0	0	0	0	0.0
BeatSheet	Podisus (nymphs)	AZYU	0	5	1	0	6	0.1
BeatSheet	Podisus (nymphs)	LACH	6	9	3	7	2	0.2
BeatSheet	Podisus (nymphs)	MSGV	14	21	23	29	29	0.8

Table I-15. Overall Abundance By Site and Treatment for All Arthropod Taxa Collected Using Three Different Collection Methods: Total Counts over Collection Times and Replicates, and Overall Arithmetic Mean over Treatments, Collections, Replicates, and Treatments (continued)

Method	Taxa	Site	01	02	03	04	05	Overall Mean
BeatSheet	Podisus (nymphs)	NCRC	6	9	6	1	16	0.3
BeatSheet	Podisus (nymphs)	TXUV	0	0	0	0	0	0.0
BeatSheet	Small Mirid Nymphs	AZMA	45	66	88	72	76	2.3
BeatSheet	Small Mirid Nymphs	AZYU	80	74	95	130	81	3.1
BeatSheet	Small Mirid Nymphs	LACH	53	85	67	70	86	2.4
BeatSheet	Small Mirid Nymphs	MSGV	89	76	64	65	62	2.4
BeatSheet	Small Mirid Nymphs	NCRC	34	40	37	53	36	1.3
BeatSheet	Small Mirid Nymphs	TXUV	127	144	59	167	170	4.4
BeatSheet	Stink bug (nymphs)	AZMA	5	23	16	27	15	0.6
BeatSheet	Stink bug (nymphs)	AZYU	1	1	3	2	3	0.1
BeatSheet	Stink bug (nymphs)	LACH	3	3	5	0	2	0.1
BeatSheet	Stink bug (nymphs)	MSGV	3	10	30	9	15	0.4
BeatSheet	Stink bug (nymphs)	NCRC	8	27	27	29	21	0.7
BeatSheet	Stink bug (nymphs)	TXUV	1	0	3	7	33	0.3
BeatSheet	Whiteflies	AZMA	3105	2573	1721	2008	1686	74.0
BeatSheet	Whiteflies	AZYU	2647	1855	1274	1763	1030	57.1
BeatSheet	Whiteflies	LACH	4	12	5	11	5	0.2
BeatSheet	Whiteflies	MSGV	1381	1116	1094	873	771	34.9
BeatSheet	Whiteflies	NCRC	13	25	17	14	24	0.6
BeatSheet	Whiteflies	TXUV	2810	3251	4829	6150	4386	158.7
BeatSheet	Zelus (nymphs)	AZMA	25	76	51	128	92	2.5
BeatSheet	Zelus (nymphs)	AZYU	5	9	19	17	13	0.4

Table I-15. Overall Abundance By Site and Treatment for All Arthropod Taxa Collected Using Three Different Collection Methods: Total Counts over Collection Times and Replicates, and Overall Arithmetic Mean over Treatments, Collections, Replicates, and Treatments (continued)

Method	Taxa	Site	01	02	03	04	05	Overall Mean
BeatSheet	Zelus (nymphs)	LACH	9	0	14	2	11	0.2
BeatSheet	Zelus (nymphs)	MSGV	0	1	0	0	0	0.0
BeatSheet	Zelus (nymphs)	NCRC	0	0	0	0	0	0.0
BeatSheet	Zelus (nymphs)	TXUV	49	61	34	52	30	1.5
BeatSheet	Zelus renardii (adult)	AZMA	6	6	6	12	8	0.3
BeatSheet	Zelus renardii (adult)	AZYU	4	7	12	5	7	0.2
BeatSheet	Zelus renardii (adult)	LACH	0	0	0	0	0	0.0
BeatSheet	Zelus renardii (adult)	MSGV	0	0	0	0	0	0.0
BeatSheet	Zelus renardii (adult)	NCRC	0	0	0	0	0	0.0
BeatSheet	Zelus renardii (adult)	TXUV	6	6	5	10	5	0.2
SweepNet	Fleahopper	AZMA	108	132	134	231	224	5.5
SweepNet	Fleahopper	AZYU	36	81	83	128	84	2.7
SweepNet	Fleahopper	LACH	10	4	10	7	0	0.2
SweepNet	Fleahopper	MSGV	0	0	0	0	0	0.0
SweepNet	Fleahopper	NCRC	0	0	0	0	0	0.0
SweepNet	Fleahopper	TXUV	84	94	88	147	128	3.6
SweepNet	Lygus	AZMA	110	99	74	130	94	3.4
SweepNet	Lygus	AZYU	142	97	131	174	84	4.2
SweepNet	Lygus	LACH	85	94	91	81	70	2.8
SweepNet	Lygus	MSGV	236	158	156	282	177	6.7
SweepNet	Lygus	NCRC	92	81	92	112	97	3.2
SweepNet	Lygus	TXUV	1	1	1	0	1	0.0

Table I-15. Overall Abundance By Site and Treatment for All Arthropod Taxa Collected Using Three Different Collection Methods: Total Counts over Collection Times and Replicates, and Overall Arithmetic Mean over Treatments, Collections, Replicates, and Treatments (continued)

Method	Taxa	Site	01	02	03	04	05	Overall Mean
SweepNet	Stink bugs	AZMA	1	2	5	3	2	0.1
SweepNet	Stink bugs	AZYU	5	6	11	7	6	0.2
SweepNet	Stink bugs	LACH	17	17	8	4	9	0.4
SweepNet	Stink bugs	MSGV	13	18	20	15	13	0.5
SweepNet	Stink bugs	NCRC	15	19	9	23	25	0.6
SweepNet	Stink bugs	TXUV	3	0	0	0	1	0.0
Visual	Bollworm (larvae)	AZMA	0	0	0	0	0	0.0
Visual	Bollworm (larvae)	AZYU	0	0	0	0	0	0.0
Visual	Bollworm (larvae)	LACH	13	12	17	24	13	0.1
Visual	Bollworm (larvae)	MSGV	4	6	8	5	6	0.0
Visual	Bollworm (larvae)	NCRC	0	2	3	4	2	0.0
Visual	Bollworm (larvae)	TXUV	1	2	2	1	1	0.0
Visual	Spider mites (eggs)	AZMA	0	1	0	0	0	0.0
Visual	Spider mites (eggs)	AZYU	0	0	0	0	0	0.0
Visual	Spider mites (eggs)	LACH	0	0	0	0	0	0.0
Visual	Spider mites (eggs)	MSGV	0	0	0	0	0	0.0
Visual	Spider mites (eggs)	NCRC	28	34	12	40	28	0.1
Visual	Spider mites (eggs)	TXUV	366	658	696	300	584	1.7
Visual	Spider mites (nymphs and adults)	AZMA	0	0	0	0	0	0.0
Visual	Spider mites (nymphs and adults)	AZYU	2	0	0	0	0	0.0
Visual	Spider mites (nymphs and adults)	LACH	12	15	13	16	12	0.0
Visual	Spider mites (nymphs and adults)	MSGV	0	5	1	17	32	0.0

Table I-15. Overall Abundance By Site and Treatment for All Arthropod Taxa Collected Using Three Different Collection Methods: Total Counts over Collection Times and Replicates, and Overall Arithmetic Mean over Treatments, Collections, Replicates, and Treatments (continued)

Method	Taxa	Site	01	02	03	04	05	Overall Mean
Visual	Spider mites (nymphs and adults)	NCRC	45	71	38	32	75	0.2
Visual	Spider mites (nymphs and adults)	TXUV	365	559	638	330	499	1.6
Visual	Thrips (eggs)	AZMA	0	0	0	0	0	0.0
Visual	Thrips (eggs)	AZYU	0	0	0	1	0	0.0
Visual	Thrips (eggs)	LACH	0	0	0	0	0	0.0
Visual	Thrips (eggs)	MSGV	0	0	0	0	0	0.0
Visual	Thrips (eggs)	NCRC	1	0	52	3	2	0.0
Visual	Thrips (eggs)	TXUV	19	34	24	13	26	0.1
Visual	Thrips (nymphs and adults)	AZMA	49	42	25	51	27	0.2
Visual	Thrips (nymphs and adults)	AZYU	16	38	19	29	10	0.1
Visual	Thrips (nymphs and adults)	LACH	0	0	1	0	1	0.0
Visual	Thrips (nymphs and adults)	MSGV	16	18	13	15	6	0.0
Visual	Thrips (nymphs and adults)	NCRC	12	19	15	47	16	0.1
Visual	Thrips (nymphs and adults)	TXUV	66	56	22	39	31	0.1

Table I-16. Combined-site Treatment Arithmetic Mean and Standard Error for Category II From Three Collection Methods Meeting the Inclusion Criterion

Method	Category II	N (Rep)	01	02	03	04	05
BeatSheet	Total Aphids	12	131.4(9.17)	90.8(3.96)	89.7(8.71)	93.6(5.48)	90.9(4.45)
BeatSheet	Total Cotton Fleahoppers	9	5.7(0.36)	8.1(1.07)	4.6(0.35)	15.1(0.71)	12.0(0.91)
BeatSheet	Total Geocoris	18	2.8(0.19)	4.5(0.25)	5.3(0.32)	8.0(0.43)	6.8(0.35)
BeatSheet	Total Lygus	15	4.4(0.28)	4.4(0.19)	2.6(0.18)	6.1(0.37)	2.9(0.16)
BeatSheet	Total Nabis	6	0.8(0.22)	1.6(0.27)	1.8(0.27)	2.1(0.42)	1.5(0.38)
BeatSheet	Total Orius	18	19.3(1.70)	21.4(1.18)	20.1(0.41)	22.7(1.11)	24.3(0.73)
BeatSheet	Total Predatory Stink bugs	3	1.3(0.37)	1.7(0.59)	2.3(0.75)	2.4(0.20)	2.2(0.72)
BeatSheet	Total Stink bugs	3	1.5(0.38)	2.0(0.66)	4.4(0.96)	2.4(0.36)	3.1(0.94)
BeatSheet	Total Whiteflies	12	105.5(5.01)	93.5(9.54)	96.2(7.02)	113.5(6.36)	83.7(7.46)
BeatSheet	Total Zelus	6	2.9(0.60)	4.4(0.26)	2.6(0.18)	6.0(0.44)	3.8(0.51)
SweepNet	Total Cotton Fleahoppers	9	2.9(0.21)	4.0(0.25)	4.0(0.21)	6.5(0.73)	5.6(0.49)
SweepNet	Total Lygus	15	5.0(0.26)	3.9(0.20)	4.0(0.17)	5.8(0.30)	3.8(0.27)
Visual	Total Spider mites	6	16.0(4.08)	26.0(5.40)	26.3(1.20)	13.9(2.70)	23.3(5.89)
Visual	Total Thrips	8	2.3(0.16)	2.3(0.17)	3.1(1.14)	2.9(0.24)	1.9(0.29)

Table I-17. Results of Combined-site Significance Testing for Category II From Three Collection Methods Meeting the Inclusion Criterion: Estimated Difference and Standard Error in Square-root Scale, t value, p value, and Significance (Sig at 0.05, (denoted by "*"))

Method	Comparison	Category II	Estimate	Standard Error	DF	t Value	p Value	Sig at 5%
BeatSheet	01 vs 02	Total Aphids	1.8	0.56	9.21	3.16	0.011	*
BeatSheet	01 vs 02	Total Cotton Fleahoppers	-0.6	0.29	7.86	-2.07	0.073	
BeatSheet	01 vs 02	Total Geocoris	-0.5	0.17	19.1	-3.11	0.006	*
BeatSheet	01 vs 02	Total Lygus	-0.1	0.16	14.9	-0.35	0.731	
BeatSheet	01 vs 02	Total Nabis	-0.5	0.19	3.97	-2.43	0.072	
BeatSheet	01 vs 02	Total Orius	-0.4	0.12	16.6	-3.78	0.002	*
BeatSheet	01 vs 02	Total Predatory Stink bugs	-0.1	0.26	64	-0.57	0.567	
BeatSheet	01 vs 02	Total Stink bugs	-0.2	0.28	52	-0.75	0.455	
BeatSheet	01 vs 02	Total Whiteflies	0.9	0.87	8.91	1.03	0.330	
BeatSheet	01 vs 02	Total Zelus	-0.5	0.35	4.24	-1.42	0.224	
BeatSheet	01 vs 04	Total Aphids	1.6	0.56	9.21	2.82	0.020	*
BeatSheet	01 vs 04	Total Cotton Fleahoppers	-1.9	0.29	7.86	-6.34	<.001	*
BeatSheet	01 vs 04	Total Geocoris	-1.2	0.17	19.1	-7.18	<.001	*
BeatSheet	01 vs 04	Total Lygus	-0.4	0.16	14.9	-2.44	0.028	*
BeatSheet	01 vs 04	Total Nabis	-0.6	0.19	3.97	-2.89	0.045	*
BeatSheet	01 vs 04	Total Orius	-0.5	0.12	16.6	-4.44	<.001	*
BeatSheet	01 vs 04	Total Predatory Stink bugs	-0.5	0.26	64	-2.01	0.049	*
BeatSheet	01 vs 04	Total Stink bugs	-0.4	0.28	52	-1.42	0.160	
BeatSheet	01 vs 04	Total Whiteflies	0.7	0.87	8.91	0.80	0.445	
BeatSheet	01 vs 04	Total Zelus	-1.1	0.35	4.24	-3.19	0.031	*
BeatSheet	01 vs 05	Total Aphids	1.7	0.56	9.21	3.05	0.013	*
BeatSheet	01 vs 05	Total Cotton Fleahoppers	-1.3	0.29	7.86	-4.30	0.003	*
BeatSheet	01 vs 05	Total Geocoris	-1.0	0.17	19.1	-5.97	<.001	*
BeatSheet	01 vs 05	Total Lygus	0.4	0.16	14.9	2.42	0.029	*
BeatSheet	01 vs 05	Total Nabis	-0.4	0.19	3.97	-2.06	0.110	

Table I-17. Results of Combined-site Significance Testing for Category II From Three Collection Methods Meeting the Inclusion Criterion: Estimated Difference and Standard Error in Square-root Scale, t value, p value, and Significance (Sig at 0.05) (continued)

Method	Comparison	Category II	Estimate	Standard Error	DF	t Value	p Value	Sig at 5%
BeatSheet	01 vs 05	Total Orius	-0.4	0.12	16.6	-3.53	0.003	*
BeatSheet	01 vs 05	Total Predatory Stink bugs	-0.3	0.26	64	-1.07	0.290	
BeatSheet	01 vs 05	Total Stink bugs	-0.7	0.28	52	-2.41	0.019	*
BeatSheet	01 vs 05	Total Whiteflies	1.8	0.87	8.91	2.03	0.073	
BeatSheet	01 vs 05	Total Zelus	-0.5	0.35	4.24	-1.44	0.220	
BeatSheet	02 vs 03	Total Aphids	0.2	0.56	9.21	0.31	0.764	
BeatSheet	02 vs 03	Total Cotton Fleahoppers	0.4	0.29	7.86	1.22	0.258	
BeatSheet	02 vs 03	Total Geocoris	-0.2	0.17	19.1	-1.18	0.252	
BeatSheet	02 vs 03	Total Lygus	0.5	0.16	14.9	3.30	0.005	*
BeatSheet	02 vs 03	Total Nabis	-0.1	0.19	3.97	-0.30	0.780	
BeatSheet	02 vs 03	Total Orius	0.1	0.12	16.6	0.87	0.398	
BeatSheet	02 vs 03	Total Predatory Stink bugs	-0.3	0.26	64	-1.18	0.242	
BeatSheet	02 vs 03	Total Stink bugs	-0.7	0.28	52	-2.67	0.010	*
BeatSheet	02 vs 03	Total Whiteflies	0.3	0.87	8.91	0.30	0.773	
BeatSheet	02 vs 03	Total Zelus	0.3	0.35	4.24	0.91	0.412	
BeatSheet	02 vs 04	Total Aphids	-0.2	0.56	9.21	-0.34	0.743	
BeatSheet	02 vs 04	Total Cotton Fleahoppers	-1.2	0.29	7.86	-4.27	0.003	*
BeatSheet	02 vs 04	Total Geocoris	-0.7	0.17	19.1	-4.06	<.001	*
BeatSheet	02 vs 04	Total Lygus	-0.3	0.16	14.9	-2.09	0.054	
BeatSheet	02 vs 04	Total Nabis	-0.1	0.19	3.97	-0.46	0.669	
BeatSheet	02 vs 04	Total Orius	-0.1	0.12	16.6	-0.66	0.517	
BeatSheet	02 vs 04	Total Predatory Stink bugs	-0.4	0.26	64	-1.43	0.157	
BeatSheet	02 vs 04	Total Stink bugs	-0.2	0.28	52	-0.67	0.505	
BeatSheet	02 vs 04	Total Whiteflies	-0.2	0.87	8.91	-0.23	0.824	
BeatSheet	02 vs 04	Total Zelus	-0.6	0.35	4.24	-1.77	0.148	
BeatSheet	02 vs 05	Total Aphids	-0.1	0.56	9.21	-0.11	0.917	

Table I-17. Results of Combined-site Significance Testing for Category II From Three Collection Methods Meeting the Inclusion Criterion: Estimated Difference and Standard Error in Square-root Scale, t value, p value, and Significance (Sig at 0.05) (continued)

Method	Comparison	Category II	Estimate	Standard Error	DF	t Value	p Value	Sig at 5%
BeatSheet	02 vs 05	Total Cotton Fleahoppers	-0.7	0.29	7.86	-2.23	0.057	
BeatSheet	02 vs 05	Total Geocoris	-0.5	0.17	19.1	-2.85	0.010	*
BeatSheet	02 vs 05	Total Lygus	0.4	0.16	14.9	2.77	0.014	*
BeatSheet	02 vs 05	Total Nabis	0.1	0.19	3.97	0.38	0.724	
BeatSheet	02 vs 05	Total Orius	0.0	0.12	16.6	0.25	0.809	
BeatSheet	02 vs 05	Total Predatory Stink bugs	-0.1	0.26	64	-0.49	0.625	
BeatSheet	02 vs 05	Total Stink bugs	-0.5	0.28	52	-1.66	0.103	
BeatSheet	02 vs 05	Total Whiteflies	0.9	0.87	8.91	1.00	0.343	
BeatSheet	02 vs 05	Total Zelus	-0.0	0.35	4.24	-0.01	0.989	
BeatSheet	03 vs 04	Total Aphids	-0.4	0.56	9.21	-0.65	0.533	
BeatSheet	03 vs 04	Total Cotton Fleahoppers	-1.6	0.29	7.86	-5.49	<.001	*
BeatSheet	03 vs 04	Total Geocoris	-0.5	0.17	19.1	-2.88	0.010	*
BeatSheet	03 vs 04	Total Lygus	-0.9	0.16	14.9	-5.38	<.001	*
BeatSheet	03 vs 04	Total Nabis	-0.0	0.19	3.97	-0.16	0.880	
BeatSheet	03 vs 04	Total Orius	-0.2	0.12	16.6	-1.53	0.145	
BeatSheet	03 vs 04	Total Predatory Stink bugs	-0.1	0.26	64	-0.25	0.802	
BeatSheet	03 vs 04	Total Stink bugs	0.6	0.28	52	1.99	0.052	
BeatSheet	03 vs 04	Total Whiteflies	-0.5	0.87	8.91	-0.53	0.611	
BeatSheet	03 vs 04	Total Zelus	-0.9	0.35	4.24	-2.68	0.052	
BeatSheet	03 vs 05	Total Aphids	-0.2	0.56	9.21	-0.42	0.686	
BeatSheet	03 vs 05	Total Cotton Fleahoppers	-1.0	0.29	7.86	-3.45	0.009	*
BeatSheet	03 vs 05	Total Geocoris	-0.3	0.17	19.1	-1.67	0.111	
BeatSheet	03 vs 05	Total Lygus	-0.1	0.16	14.9	-0.53	0.606	
BeatSheet	03 vs 05	Total Nabis	0.1	0.19	3.97	0.68	0.535	
BeatSheet	03 vs 05	Total Orius	-0.1	0.12	16.6	-0.62	0.542	
BeatSheet	03 vs 05	Total Predatory Stink bugs	0.2	0.26	64	0.69	0.493	

Table I-17. Results of Combined-site Significance Testing for Category II From Three Collection Methods Meeting the Inclusion Criterion: Estimated Difference and Standard Error in Square-root Scale, t value, p value, and Significance (Sig at 0.05) (continued)

Method	Comparison	Category II	Estimate	Standard Error	DF	t Value	p Value	Sig at 5%
BeatSheet	03 vs 05	Total Stink bugs	0.3	0.28	52	1.00	0.320	
BeatSheet	03 vs 05	Total Whiteflies	0.6	0.87	8.91	0.70	0.500	
BeatSheet	03 vs 05	Total Zelus	-0.3	0.35	4.24	-0.92	0.406	
BeatSheet	04 vs 05	Total Aphids	0.1	0.56	9.21	0.23	0.822	
BeatSheet	04 vs 05	Total Cotton Fleahoppers	0.6	0.29	7.86	2.04	0.076	
BeatSheet	04 vs 05	Total Geocoris	0.2	0.17	19.1	1.21	0.241	
BeatSheet	04 vs 05	Total Lygus	0.8	0.16	14.9	4.86	<.001	*
BeatSheet	04 vs 05	Total Nabis	0.2	0.19	3.97	0.84	0.449	
BeatSheet	04 vs 05	Total Orius	0.1	0.12	16.6	0.91	0.377	
BeatSheet	04 vs 05	Total Predatory Stink bugs	0.2	0.26	64	0.94	0.350	
BeatSheet	04 vs 05	Total Stink bugs	-0.3	0.28	52	-0.99	0.328	
BeatSheet	04 vs 05	Total Whiteflies	1.1	0.87	8.91	1.23	0.250	
BeatSheet	04 vs 05	Total Zelus	0.6	0.35	4.24	1.76	0.150	
SweepNet	01 vs 02	Total Cotton Fleahoppers	-0.2	0.15	6.47	-1.40	0.208	
SweepNet	01 vs 02	Total Lygus	0.2	0.11	13.1	2.23	0.043	*
SweepNet	01 vs 04	Total Cotton Fleahoppers	-0.8	0.15	6.47	-5.38	0.001	*
SweepNet	01 vs 04	Total Lygus	-0.2	0.11	13.1	-1.55	0.144	
SweepNet	01 vs 05	Total Cotton Fleahoppers	-0.5	0.15	6.47	-3.32	0.014	*
SweepNet	01 vs 05	Total Lygus	0.2	0.11	13.1	2.11	0.054	
SweepNet	02 vs 03	Total Cotton Fleahoppers	0.0	0.15	6.47	0.08	0.941	
SweepNet	02 vs 03	Total Lygus	-0.0	0.11	13.1	-0.22	0.831	
SweepNet	02 vs 04	Total Cotton Fleahoppers	-0.6	0.15	6.47	-3.99	0.006	*
SweepNet	02 vs 04	Total Lygus	-0.4	0.11	13.1	-3.79	0.002	*
SweepNet	02 vs 05	Total Cotton Fleahoppers	-0.3	0.15	6.47	-1.92	0.099	
SweepNet	02 vs 05	Total Lygus	-0.0	0.11	13.1	-0.12	0.907	
SweepNet	03 vs 04	Total Cotton Fleahoppers	-0.6	0.15	6.47	-4.06	0.006	*

Table I-17. Results of Combined-site Significance Testing for Category II From Three Collection Methods Meeting the Inclusion Criterion: Estimated Difference and Standard Error in Square-root Scale, t value, p value, and Significance (Sig at 0.05) (continued)

Method	Comparison	Category II	Estimate	Standard Error	DF	t Value	p Value	Sig at 5%
SweepNet	03 vs 04	Total Lygus	-0.4	0.11	13.1	-3.57	0.003	*
SweepNet	03 vs 05	Total Cotton Fleahoppers	-0.3	0.15	6.47	-2.00	0.089	
SweepNet	03 vs 05	Total Lygus	0.0	0.11	13.1	0.10	0.924	
SweepNet	04 vs 05	Total Cotton Fleahoppers	0.3	0.15	6.47	2.06	0.081	
SweepNet	04 vs 05	Total Lygus	0.4	0.11	13.1	3.67	0.003	*
Visual	01 vs 02	Total Spider mites	-0.7	0.69	3.93	-0.95	0.399	
Visual	01 vs 02	Total Thrips	0.1	0.38	7.96	0.18	0.862	
Visual	01 vs 04	Total Spider mites	0.1	0.69	3.93	0.12	0.910	
Visual	01 vs 04	Total Thrips	-0.1	0.38	7.96	-0.39	0.710	
Visual	01 vs 05	Total Spider mites	-0.7	0.69	3.93	-1.05	0.355	
Visual	01 vs 05	Total Thrips	0.2	0.38	7.96	0.44	0.669	
Visual	02 vs 03	Total Spider mites	-0.3	0.69	3.93	-0.47	0.662	
Visual	02 vs 03	Total Thrips	0.1	0.38	7.96	0.37	0.720	
Visual	02 vs 04	Total Spider mites	0.7	0.69	3.93	1.07	0.348	
Visual	02 vs 04	Total Thrips	-0.2	0.38	7.96	-0.56	0.588	
Visual	02 vs 05	Total Spider mites	-0.1	0.69	3.93	-0.10	0.924	
Visual	02 vs 05	Total Thrips	0.1	0.38	7.96	0.26	0.798	
Visual	03 vs 04	Total Spider mites	1.1	0.69	3.93	1.54	0.200	
Visual	03 vs 04	Total Thrips	-0.4	0.38	7.96	-0.94	0.377	
Visual	03 vs 05	Total Spider mites	0.3	0.69	3.93	0.37	0.731	
Visual	03 vs 05	Total Thrips	-0.0	0.38	7.96	-0.11	0.918	
Visual	04 vs 05	Total Spider mites	-0.8	0.69	3.93	-1.17	0.309	
Visual	04 vs 05	Total Thrips	0.3	0.38	7.96	0.83	0.431	

Table I-18. Individual-site Treatment Arithmetic Mean and Standard Error for Category II From Three Collection Methods Meeting the Inclusion Criterion

Method	Category II	Site	01	02	03	04	05
BeatSheet	Total Aphids	LACH	259.5(25.50)	260.5(18.91)	236.3(41.23)	270.7(22.65)	246.8(9.45)
BeatSheet	Total Aphids	MSGV	173.8(27.93)	63.7(3.15)	89.2(22.32)	65.1(8.04)	83.8(19.37)
BeatSheet	Total Aphids	NCRC	82.5(13.51)	32.6(6.19)	30.9(5.88)	35.5(12.63)	30.5(6.22)
BeatSheet	Total Aphids	TXUV	9.8(6.45)	6.4(3.44)	2.2(0.22)	2.9(0.56)	2.7(0.58)
BeatSheet	Total Cotton Fleahoppers	AZMA	1.5(0.07)	3.4(0.52)	2.9(0.42)	10.3(0.71)	8.7(0.55)
BeatSheet	Total Cotton Fleahoppers	AZYU	2.4(0.37)	4.3(1.05)	4.3(0.45)	8.1(1.11)	4.5(1.40)
BeatSheet	Total Cotton Fleahoppers	TXUV	13.1(1.40)	16.7(3.97)	6.5(0.94)	26.9(1.84)	22.8(2.80)
BeatSheet	Total Geocoris	AZMA	2.0(0.26)	5.4(0.72)	4.9(1.00)	9.8(0.94)	8.0(0.26)
BeatSheet	Total Geocoris	AZYU	3.1(0.80)	5.7(1.00)	10.6(1.87)	13.2(1.79)	10.7(3.27)
BeatSheet	Total Geocoris	LACH	1.1(0.24)	1.1(0.13)	2.3(0.59)	1.6(0.64)	2.3(0.35)
BeatSheet	Total Geocoris	MSGV	6.7(0.54)	9.7(0.90)	8.3(1.02)	15.1(1.29)	12.4(0.83)
BeatSheet	Total Geocoris	NCRC	1.0(0.19)	2.2(0.15)	2.0(0.09)	3.0(0.50)	3.3(0.38)
BeatSheet	Total Geocoris	TXUV	2.8(0.79)	2.8(0.80)	3.5(0.13)	5.4(1.20)	4.2(0.06)
BeatSheet	Total Lygus	AZMA	6.0(0.94)	4.3(0.40)	1.5(0.29)	6.3(0.44)	2.6(0.31)
BeatSheet	Total Lygus	AZYU	3.1(1.09)	2.4(0.08)	2.9(0.74)	4.2(1.04)	1.2(0.55)
BeatSheet	Total Lygus	LACH	2.7(0.25)	5.7(0.51)	3.0(0.17)	4.5(0.59)	3.1(0.24)
BeatSheet	Total Lygus	MSGV	6.0(0.43)	4.6(0.25)	3.2(0.32)	8.9(1.14)	4.4(0.38)
BeatSheet	Total Lygus	NCRC	4.3(0.44)	4.9(0.90)	2.7(0.50)	6.5(0.89)	3.0(0.24)
BeatSheet	Total Nabis	AZYU	0.9(0.37)	1.6(0.40)	2.6(0.61)	2.9(0.74)	1.8(0.64)
BeatSheet	Total Nabis	MSGV	0.8(0.25)	1.6(0.36)	1.1(0.17)	1.3(0.43)	1.3(0.43)
BeatSheet	Total Orius	AZMA	3.8(0.41)	5.5(0.61)	5.8(0.09)	5.9(0.30)	5.8(0.92)
BeatSheet	Total Orius	AZYU	4.8(1.19)	6.5(0.49)	6.4(0.68)	6.5(0.20)	4.2(0.72)
BeatSheet	Total Orius	LACH	2.8(0.52)	2.8(0.34)	3.2(0.08)	3.3(0.44)	3.1(0.18)
BeatSheet	Total Orius	MSGV	6.3(0.92)	7.1(0.34)	5.8(0.56)	9.1(0.83)	6.2(0.29)
BeatSheet	Total Orius	NCRC	5.6(0.25)	8.0(0.30)	6.2(0.15)	7.1(0.90)	8.6(0.77)
BeatSheet	Total Orius	TXUV	92.4(21.66)	98.6(15.20)	93.4(4.50)	104.2(13.67)	118.0(7.81)
BeatSheet	Total Predatory Stink bugs	MSGV	1.3(0.37)	1.7(0.59)	2.3(0.75)	2.4(0.20)	2.2(0.72)

Table I-18. Individual-site Treatment Arithmetic Mean and Standard Error for Category II From Three Collection Methods Meeting the Inclusion Criterion (continued)

Method	Category II	Site	01	02	03	04	05
BeatSheet	Total Stink bugs	MSGV	1.5(0.38)	2.0(0.66)	4.4(0.96)	2.4(0.36)	3.1(0.94)
BeatSheet	Total Whiteflies	AZMA	115.0(11.68)	95.3(2.11)	63.7(11.22)	74.4(7.45)	62.4(2.71)
BeatSheet	Total Whiteflies	AZYU	98.0(10.83)	68.7(24.61)	47.1(11.83)	65.2(16.91)	38.1(19.74)
BeatSheet	Total Whiteflies	MSGV	91.9(6.00)	74.4(7.60)	72.8(9.10)	58.1(4.12)	51.4(11.87)
BeatSheet	Total Whiteflies	TXUV	117.1(11.55)	135.5(42.04)	201.2(23.99)	256.3(22.40)	182.8(25.34)
BeatSheet	Total Zelus	AZMA	1.6(0.49)	4.1(0.36)	2.8(0.36)	7.4(0.91)	5.0(0.63)
BeatSheet	Total Zelus	TXUV	4.3(1.20)	4.7(0.36)	2.5(0.14)	4.6(0.33)	2.7(0.82)
SweepNet	Total Cotton Fleahoppers	AZMA	4.0(0.13)	4.9(0.65)	5.0(0.64)	8.4(0.78)	8.2(0.91)
SweepNet	Total Cotton Fleahoppers	AZYU	1.6(0.22)	3.6(0.21)	3.8(0.33)	5.8(1.90)	4.0(0.64)
SweepNet	Total Cotton Fleahoppers	TXUV	3.1(0.72)	3.5(0.46)	3.3(0.13)	5.4(1.13)	4.6(0.98)
SweepNet	Total Lygus	AZMA	4.0(0.28)	3.6(0.52)	2.7(0.41)	4.8(0.60)	3.4(0.30)
SweepNet	Total Lygus	AZYU	6.6(0.72)	4.3(0.62)	5.7(0.47)	8.0(1.53)	3.9(1.37)
SweepNet	Total Lygus	LACH	3.4(1.08)	3.8(0.15)	3.5(0.26)	3.2(0.44)	2.8(0.58)
SweepNet	Total Lygus	MSGV	7.9(0.52)	5.3(0.46)	5.2(0.55)	9.4(0.62)	5.9(0.64)
SweepNet	Total Lygus	NCRC	3.1(0.35)	2.7(0.44)	3.1(0.23)	3.7(0.22)	3.2(0.18)
Visual	Total Spider mites	NCRC	4.9(1.16)	6.9(2.54)	3.1(0.57)	4.5(1.67)	6.4(2.19)
Visual	Total Spider mites	TXUV	27.1(10.38)	45.1(12.73)	49.4(2.83)	23.3(5.96)	40.1(14.45)
Visual	Total Thrips	AZMA	3.1(0.29)	2.6(0.36)	1.7(0.29)	3.4(0.21)	1.8(0.64)
Visual	Total Thrips	NCRC	0.3(0.22)	0.2(0.08)	5.4(5.17)	3.3(0.14)	1.3(0.51)
Visual	Total Thrips	TXUV	3.6(0.33)	4.2(0.45)	2.1(0.45)	2.2(0.91)	2.5(0.33)

Table I-19. Results of Individual-site Significance Testing For Category II From Three Collection Methods Meeting the Inclusion Criterion: Simple Mean Difference in Count and Statistical Significance at the 0.05 Significance Level (denoted by "*"). A Missing Value Implies Abundance at the Site Below the Inclusion Criterion

Method	Comparison	Category II	AZMA	AZYU	LACH	MSGV	NCRC	TXUV
BeatSheet	01 vs 02	Total Aphids			-1.0	110.1	49.8*	3.3
BeatSheet	01 vs 02	Total Cotton Fleahoppers	-1.9*	-1.9*				-3.6*
BeatSheet	01 vs 02	Total Geocoris	-3.4*	-2.6*	-0.1	-3.0*	-1.2*	-0.0
BeatSheet	01 vs 02	Total Lygus	1.8	0.7	-3.0*	1.4	-0.5	
BeatSheet	01 vs 02	Total Nabis		-0.7		-0.8		
BeatSheet	01 vs 02	Total Orius	-1.6*	-1.7*	-0.1	-0.8	-2.4*	-6.2
BeatSheet	01 vs 02	Total Predatory Stink bugs				-0.4		
BeatSheet	01 vs 02	Total Stink bugs				-0.5		
BeatSheet	01 vs 02	Total Whiteflies	19.7	29.3*		17.5		-18.4
BeatSheet	01 vs 02	Total Zelus	-2.6*					-0.3
BeatSheet	01 vs 04	Total Aphids			-11.2	108.7	47.0*	6.9
BeatSheet	01 vs 04	Total Cotton Fleahoppers	-8.8*	-5.7*				-13.8*
BeatSheet	01 vs 04	Total Geocoris	-7.9*	-10.1*	-0.5	-8.4*	-2.1*	-2.6*
BeatSheet	01 vs 04	Total Lygus	-0.2	-1.0	-1.8*	-2.9*	-2.1	
BeatSheet	01 vs 04	Total Nabis		-2.0*		-0.5		
BeatSheet	01 vs 04	Total Orius	-2.0*	-1.7*	-0.5	-2.8	-1.5*	-11.8*
BeatSheet	01 vs 04	Total Predatory Stink bugs				-1.1*		
BeatSheet	01 vs 04	Total Stink bugs				-0.9		
BeatSheet	01 vs 04	Total Whiteflies	40.6	32.8*		33.8*		-139.2*
BeatSheet	01 vs 04	Total Zelus	-5.9*					-0.3
BeatSheet	01 vs 05	Total Aphids			12.7	90.0	52.0*	7.1
BeatSheet	01 vs 05	Total Cotton Fleahoppers	-7.2*	-2.1*				-9.7*
BeatSheet	01 vs 05	Total Geocoris	-6.0*	-7.7*	-1.3*	-5.6*	-2.3*	-1.4
BeatSheet	01 vs 05	Total Lygus	3.4	1.9*	-0.4	1.6	1.3	
BeatSheet	01 vs 05	Total Nabis		-0.9		-0.5		
BeatSheet	01 vs 05	Total Orius	-2.0*	0.6	-0.3	0.2	-3.0*	-25.6*

Table I-19. Results of Individual-site Significance Testing For Category II From Three Collection Methods Meeting the Inclusion Criterion: Simple Mean Difference in Count and Statistical Significance at the 0.05 Significance Level (denoted by "*"). A Missing Value Implies Abundance at the Site Below the Inclusion Criterion (continued)

Method	Comparison	Category II	AZMA	AZYU	LACH	MSGV	NCRC	TXUV
BeatSheet	01 vs 05	Total Predatory Stink bugs				-0.9		
BeatSheet	01 vs 05	Total Stink bugs				-1.6		
BeatSheet	01 vs 05	Total Whiteflies	52.6	59.9*		40.5*		-65.7
BeatSheet	01 vs 05	Total Zelus	-3.4*					1.7
BeatSheet	02 vs 03	Total Aphids			24.2	-25.4	1.7	4.2
BeatSheet	02 vs 03	Total Cotton Fleahoppers	0.5	0.1				10.2
BeatSheet	02 vs 03	Total Geocoris	0.5	-4.9*	-1.2*	1.4	0.2	-0.7
BeatSheet	02 vs 03	Total Lygus	2.8*	-0.4	2.7*	1.4*	2.2*	
BeatSheet	02 vs 03	Total Nabis		-1.0		0.5		
BeatSheet	02 vs 03	Total Orius	-0.4	0.1	-0.4	1.3	1.8	5.2
BeatSheet	02 vs 03	Total Predatory Stink bugs				-0.6		
BeatSheet	02 vs 03	Total Stink bugs				-2.4*		
BeatSheet	02 vs 03	Total Whiteflies	31.6	21.6		1.6		-65.8
BeatSheet	02 vs 03	Total Zelus	1.3					2.2
BeatSheet	02 vs 04	Total Aphids			-10.2	-1.4*	-2.9	3.6
BeatSheet	02 vs 04	Total Cotton Fleahoppers	-6.9*	-3.8*				-10.2*
BeatSheet	02 vs 04	Total Geocoris	-4.5*	-7.5*	-0.5	-5.4*	-0.8	-2.6*
BeatSheet	02 vs 04	Total Lygus	-2.0*	-1.8	1.2	-4.3*	-1.6	
BeatSheet	02 vs 04	Total Nabis		-1.3		0.3		
BeatSheet	02 vs 04	Total Orius	-0.4	0.0	-0.5	-2.0	0.9	-5.6
BeatSheet	02 vs 04	Total Predatory Stink bugs				-0.7		
BeatSheet	02 vs 04	Total Stink bugs				-0.4		
BeatSheet	02 vs 04	Total Whiteflies	20.9	3.5		16.3		-120.8*
BeatSheet	02 vs 04	Total Zelus	-3.3*					0.1
BeatSheet	02 vs 05	Total Aphids			13.7	-20.1	2.2	3.8
BeatSheet	02 vs 05	Total Cotton Fleahoppers	-5.3*	-0.2				-6.1*

Table I-19. Results of Individual-site Significance Testing For Category II From Three Collection Methods Meeting the Inclusion Criterion: Simple Mean Difference in Count and Statistical Significance at the 0.05 Significance Level (denoted by "*"). A Missing Value Implies Abundance at the Site Below the Inclusion Criterion (continued)

Method	Comparison	Category II	AZMA	AZYU	LACH	MSGV	NCRC	TXUV
BeatSheet	02 vs 05	Total Geocoris	-2.6	-5.0*	-1.2	-2.6	-1.1	-1.4*
BeatSheet	02 vs 05	Total Lygus	1.6	1.2*	2.6*	0.2	1.8	
BeatSheet	02 vs 05	Total Nabis		-0.2		0.3		
BeatSheet	02 vs 05	Total Orius	-0.3	2.3*	-0.3	0.9	-0.6	-19.4
BeatSheet	02 vs 05	Total Predatory Stink bugs				-0.5		
BeatSheet	02 vs 05	Total Stink bugs				-1.1		
BeatSheet	02 vs 05	Total Whiteflies	32.9	30.6*		23.0		-47.3
BeatSheet	02 vs 05	Total Zelus	-0.9					2.0
BeatSheet	03 vs 04	Total Aphids			-34.4	24.0*	-4.6	-0.7
BeatSheet	03 vs 04	Total Cotton Fleahoppers	-7.3*	-3.9*				-20.3*
BeatSheet	03 vs 04	Total Geocoris	-5.0*	-2.6	0.7	-6.8*	-1.1	-1.9*
BeatSheet	03 vs 04	Total Lygus	-4.8*	-1.3	-1.5	-5.6*	-3.8*	
BeatSheet	03 vs 04	Total Nabis		-0.3		-0.2		
BeatSheet	03 vs 04	Total Orius	-0.0	-0.0	-0.1	-3.3*	-0.9	-10.8
BeatSheet	03 vs 04	Total Predatory Stink bugs				-0.1		
BeatSheet	03 vs 04	Total Stink bugs				2.0		
BeatSheet	03 vs 04	Total Whiteflies	-10.6	-18.1		14.7		-55.0
BeatSheet	03 vs 04	Total Zelus	-4.7*					-2.1*
BeatSheet	03 vs 05	Total Aphids			-10.5	5.4*	0.5	-0.4
BeatSheet	03 vs 05	Total Cotton Fleahoppers	-5.8*	-0.3				-16.3*
BeatSheet	03 vs 05	Total Geocoris	-3.1*	-0.1	0.0	-4.1*	-1.3	-0.7
BeatSheet	03 vs 05	Total Lygus	-1.2*	1.6	-0.1	-1.1	-0.4	
BeatSheet	03 vs 05	Total Nabis		0.8		-0.2		
BeatSheet	03 vs 05	Total Orius	0.0	2.2*	0.1	-0.4	-2.4*	-24.6
BeatSheet	03 vs 05	Total Predatory Stink bugs				0.1		
BeatSheet	03 vs 05	Total Stink bugs				1.3		

Table I-19. Results of Individual-site Significance Testing For Category II From Three Collection Methods Meeting the Inclusion Criterion: Simple Mean Difference in Count and Statistical Significance at the 0.05 Significance Level (denoted by "*"). A Missing Value Implies Abundance at the Site Below the Inclusion Criterion (continued)

Method	Comparison	Category II	AZMA	AZYU	LACH	MSGV	NCRC	TXUV
BeatSheet	03 vs 05	Total Whiteflies	1.3	9.0		21.4		18.5
BeatSheet	03 vs 05	Total Zelus	-2.2*					-0.2
BeatSheet	04 vs 05	Total Aphids			23.9	-18.7	5.0	0.2
BeatSheet	04 vs 05	Total Cotton Fleahoppers	1.5	3.6*				4.1*
BeatSheet	04 vs 05	Total Geocoris	1.8	2.4	-0.7	2.7	-0.3	1.2*
BeatSheet	04 vs 05	Total Lygus	3.6*	3.0*	1.4	4.5*	3.4*	
BeatSheet	04 vs 05	Total Nabis		1.1		0.0		
BeatSheet	04 vs 05	Total Orius	0.1	2.2	0.2	2.9*	-1.5	-13.8
BeatSheet	04 vs 05	Total Predatory Stink bugs				0.2		
BeatSheet	04 vs 05	Total Stink bugs				-0.7		
BeatSheet	04 vs 05	Total Whiteflies	11.9	27.1*		6.7		73.5*
BeatSheet	04 vs 05	Total Zelus	2.4					1.9*
SweepNet	01 vs 02	Total Cotton Fleahoppers	-0.9	-2.0*				-0.4
SweepNet	01 vs 02	Total Lygus	0.4	2.3	-0.4	2.6*	0.4	
SweepNet	01 vs 04	Total Cotton Fleahoppers	-4.4*	-4.2*				-2.3*
SweepNet	01 vs 04	Total Lygus	-0.8	-1.4	0.2	-1.5	-0.7	
SweepNet	01 vs 05	Total Cotton Fleahoppers	-4.2	-2.4*				-1.5
SweepNet	01 vs 05	Total Lygus	0.6	2.7*	0.6	2.0*	-0.2	
SweepNet	02 vs 03	Total Cotton Fleahoppers	-0.1	-0.1				0.2
SweepNet	02 vs 03	Total Lygus	0.9	-1.4	0.3	0.1	-0.4	
SweepNet	02 vs 04	Total Cotton Fleahoppers	-3.6*	-2.2				-1.9*
SweepNet	02 vs 04	Total Lygus	-1.2*	-3.8*	0.6	-4.1*	-1.0	
SweepNet	02 vs 05	Total Cotton Fleahoppers	-3.3	-0.3				-1.1
SweepNet	02 vs 05	Total Lygus	0.3	0.4	1.0	-0.6	-0.5	
SweepNet	03 vs 04	Total Cotton Fleahoppers	-3.5*	-2.0				-2.1*
SweepNet	03 vs 04	Total Lygus	-2.1*	-2.4	0.3	-4.2*	-0.7	

Table I-19. Results of Individual-site Significance Testing For Category II From Three Collection Methods Meeting the Inclusion Criterion: Simple Mean Difference in Count and Statistical Significance at the 0.05 Significance Level (denoted by "*"). A Missing Value Implies Abundance at the Site Below the Inclusion Criterion (continued)

Method	Comparison	Category II	AZMA	AZYU	LACH	MSGV	NCRC	TXUV
SweepNet	03 vs 05	Total Cotton Fleahoppers	-3.2*	-0.2				-1.3
SweepNet	03 vs 05	Total Lygus	-0.6	1.8	0.7	-0.7	-0.2	
SweepNet	04 vs 05	Total Cotton Fleahoppers	0.3	1.9				0.8*
SweepNet	04 vs 05	Total Lygus	1.4	4.1*	0.4	3.5*	0.5	
Visual	01 vs 02	Total Spider mites					-2.0	-18.0
Visual	01 vs 02	Total Thrips	0.5				0.2	-0.6
Visual	01 vs 04	Total Spider mites					0.3	3.7
Visual	01 vs 04	Total Thrips	-0.2				-2.9*	1.3*
Visual	01 vs 05	Total Spider mites					-1.5	-13.0
Visual	01 vs 05	Total Thrips	1.4				-1.0	1.1*
Visual	02 vs 03	Total Spider mites					3.7	-4.3*
Visual	02 vs 03	Total Thrips	0.9				-5.3	2.1*
Visual	02 vs 04	Total Spider mites					2.3	21.7
Visual	02 vs 04	Total Thrips	-0.7				-3.1*	2.0*
Visual	02 vs 05	Total Spider mites					0.5	5.0
Visual	02 vs 05	Total Thrips	0.9				-1.2*	1.7
Visual	03 vs 04	Total Spider mites					-1.4	26.1*
Visual	03 vs 04	Total Thrips	-1.6				2.2*	-0.1
Visual	03 vs 05	Total Spider mites					-3.3	9.3*
Visual	03 vs 05	Total Thrips	-0.1				4.1*	-0.4
Visual	04 vs 05	Total Spider mites					-1.9	-16.8
Visual	04 vs 05	Total Thrips	1.6				1.9*	-0.2

Table I-20. Statistical Power of the Combined-site Analysis for Arthropod Category II From Three Collection Methods with a Minimum Detectable Difference of 50% of the Control Mean (Treatment = "04")

Method	Category II	N (Rep)	Mean (Treatment=04)	Power (%)
BeatSheet	Total Aphids	12	111.79	78.9
BeatSheet	Total Cotton Fleahoppers	9	14.78	70.3
BeatSheet	Total Geocoris	18	7.76	93.3
BeatSheet	Total Lygus	15	5.99	90.1
BeatSheet	Total Nabis	6	1.93	27.9
BeatSheet	Total Orius	18	23.64	100.0
BeatSheet	Total Predatory Stink bugs	3	2.61	31.5
BeatSheet	Total Stink bugs	3	2.59	25.1
BeatSheet	Total Whiteflies	12	104.69	58.4
BeatSheet	Total Zelus	6	6.58	25.3
SweepNet	Total Cotton Fleahoppers	9	6.58	90.2
SweepNet	Total Lygus	15	5.62	99.3
Visual	Total Spider mites	6	22.08	16.9
Visual	Total Thrips	9	3.23	19.6

Table I-21. Statistical Power of the Individual-site Analysis for Arthropod Category II From Three Collection Methods with a Minimum Detectable Difference of 50% of the Control Mean (Treatment = "04")

Method	Category II	Site	N (Rep)	Mean (Treatment=04)	Power (%)
BeatSheet	Total Aphids	LACH	3	272.87	100.0
BeatSheet	Total Aphids	MSGV	3	105.31	100.0
BeatSheet	Total Aphids	NCRC	3	38.38	83.3
BeatSheet	Total Aphids	TXUV	3	5.26	15.5
BeatSheet	Total Cotton Fleahoppers	AZMA	3	10.18	78.3
BeatSheet	Total Cotton Fleahoppers	AZYU	3	8.11	70.6
BeatSheet	Total Cotton Fleahoppers	TXUV	3	25.68	98.9
BeatSheet	Total Geocoris	AZMA	3	9.21	92.2
BeatSheet	Total Geocoris	AZYU	3	11.50	90.1
BeatSheet	Total Geocoris	LACH	3	1.65	21.2
BeatSheet	Total Geocoris	MSGV	3	13.94	93.2
BeatSheet	Total Geocoris	NCRC	3	2.89	60.0
BeatSheet	Total Geocoris	TXUV	3	5.84	73.0
BeatSheet	Total Lygus	AZMA	3	6.56	67.5
BeatSheet	Total Lygus	AZYU	3	3.95	43.1
BeatSheet	Total Lygus	LACH	3	4.76	43.8
BeatSheet	Total Lygus	MSGV	3	8.03	85.6
BeatSheet	Total Lygus	NCRC	3	6.00	65.7
BeatSheet	Total Nabis	AZYU	3	2.73	32.6
BeatSheet	Total Nabis	MSGV	3	1.13	16.2
BeatSheet	Total Orius	AZMA	3	5.87	84.3
BeatSheet	Total Orius	AZYU	3	6.48	79.7
BeatSheet	Total Orius	LACH	3	3.27	48.8
BeatSheet	Total Orius	MSGV	3	9.17	74.7
BeatSheet	Total Orius	NCRC	3	8.07	89.7
BeatSheet	Total Orius	TXUV	3	110.89	100.0
BeatSheet	Total Predatory Stink bugs	MSGV	3	2.72	28.6
BeatSheet	Total Stink bugs	MSGV	3	2.76	28.7
BeatSheet	Total Whiteflies	AZMA	3	83.62	99.3
BeatSheet	Total Whiteflies	AZYU	3	63.38	77.7
BeatSheet	Total Whiteflies	MSGV	3	61.09	45.7
BeatSheet	Total Whiteflies	TXUV	3	224.01	92.0
BeatSheet	Total Zelus	AZMA	3	7.46	70.9
BeatSheet	Total Zelus	TXUV	3	5.92	36.6
SweepNet	Total Cotton Fleahoppers	AZMA	3	8.27	83.7

Table I-21. Statistical Power of the Individual-site Analysis for Arthropod Category II From Three Collection Methods with a Minimum Detectable Difference of 50% of the Control Mean (Treatment = "04") (continued)

Method	Category II	Site	N (Rep)	Mean (Treatment=04)	Power (%)
SweepNet	Total Cotton Fleahoppers	AZYU	3	4.92	40.9
SweepNet	Total Cotton Fleahoppers	TXUV	3	5.51	73.2
SweepNet	Total Lygus	AZMA	3	5.00	75.0
SweepNet	Total Lygus	AZYU	3	7.75	57.0
SweepNet	Total Lygus	LACH	3	3.15	33.5
SweepNet	Total Lygus	MSGV	3	8.88	96.9
SweepNet	Total Lygus	NCRC	3	3.45	64.2
Visual	Total Spider mites	NCRC	3	5.33	23.2
Visual	Total Spider mites	TXUV	3	34.70	58.7
Visual	Total Thrips	AZMA	3	2.51	29.4
Visual	Total Thrips	NCRC	3	4.86	43.9
Visual	Total Thrips	TXUV	3	2.35	24.6

Table I-22. Combined-site Treatment Arithmetic Mean and Standard Error for Category I From Three Collection Methods Meeting the Inclusion Criterion

Method	Category I	N (Rep)	01	02	03	04	05
BeatSheet	Aphids	12	131.4(9.17)	90.8(3.96)	89.7(8.71)	93.6(5.48)	90.9(4.45)
BeatSheet	Cotton Fleahopper Adults	9	1.7(0.19)	3.2(0.30)	2.0(0.24)	5.1(0.41)	3.3(0.46)
BeatSheet	Cotton Fleahopper Large Nymphs	9	4.7(0.40)	5.9(0.93)	3.1(0.39)	11.3(0.61)	9.9(0.78)
BeatSheet	Geocoris Adults	12	1.7(0.13)	2.8(0.28)	3.3(0.22)	4.3(0.33)	3.7(0.24)
BeatSheet	Geocoris Nymphs	15	1.9(0.23)	3.2(0.22)	3.6(0.26)	6.2(0.51)	5.0(0.37)
BeatSheet	Lygus Adults	9	2.2(0.26)	1.9(0.08)	1.8(0.33)	2.9(0.38)	1.5(0.25)
BeatSheet	Lygus Large Nymphs	12	3.7(0.22)	3.7(0.20)	1.5(0.13)	5.1(0.35)	2.4(0.14)
BeatSheet	Orius Adults	15	11.5(0.92)	14.4(0.81)	13.3(0.22)	15.0(0.68)	16.2(0.27)
BeatSheet	Orius Nymphs	18	10.8(1.19)	10.6(0.71)	10.1(0.37)	11.4(0.78)	12.4(0.73)
BeatSheet	Predatory Stink bugs	3	1.3(0.37)	1.7(0.59)	2.3(0.75)	2.4(0.20)	2.2(0.72)
BeatSheet	Small Mirid Nymphs	18	3.2(0.21)	3.8(0.34)	3.2(0.21)	4.3(0.34)	4.1(0.29)
BeatSheet	Whiteflies	12	105.5(5.01)	93.5(9.54)	96.2(7.02)	113.5(6.36)	83.7(7.46)
BeatSheet	Zelus Nymphs	6	3.1(0.62)	4.7(0.39)	2.7(0.26)	5.6(0.50)	3.9(0.51)
SweepNet	Fleahopper	9	2.9(0.21)	4.0(0.25)	4.0(0.21)	6.5(0.73)	5.6(0.49)
SweepNet	Lygus	15	5.0(0.26)	3.9(0.20)	4.0(0.17)	5.8(0.30)	3.8(0.27)
Visual	Spider mite eggs	3	15.3(6.22)	27.0(6.45)	29.0(7.06)	12.3(3.38)	24.3(8.52)
Visual	Spider mites	6	9.4(2.34)	14.4(3.24)	14.7(1.67)	8.0(1.31)	13.3(3.45)
Visual	Thrips	5	3.0(0.14)	2.7(0.29)	1.4(0.22)	2.6(0.39)	1.6(0.31)

Table I-23. Results of Combined-site Significance Testing for Category I From Three Collection Methods Meeting the Inclusion Criterion: Estimated Difference and Standard Error in Square-root Scale, t value, p value, and Significance (Sig at 0.05)

Method	Comparison	Category I	Estimate	Standard Error	DF	t Value	p Value	Sig at 5%
BeatSheet	01 vs 02	Aphids	1.8	0.56	9.21	3.16	0.011	*
BeatSheet	01 vs 02	Cotton Fleahopper Adults	-0.4	0.14	276	-3.00	0.003	*
BeatSheet	01 vs 02	Cotton Fleahopper Large Nymphs	-0.5	0.33	7.78	-1.37	0.208	
BeatSheet	01 vs 02	Geocoris Adults	-0.5	0.14	11.6	-3.82	0.003	*
BeatSheet	01 vs 02	Geocoris Nymphs	-0.6	0.18	15.6	-3.13	0.007	*
BeatSheet	01 vs 02	Lygus Adults	0.1	0.22	9.03	0.62	0.550	
BeatSheet	01 vs 02	Lygus Large Nymphs	-0.1	0.15	10.5	-0.69	0.504	
BeatSheet	01 vs 02	Orius Adults	-0.4	0.13	11.7	-3.05	0.010	*
BeatSheet	01 vs 02	Orius Nymphs	-0.4	0.13	18.6	-2.83	0.011	*
BeatSheet	01 vs 02	Predatory Stink bugs	-0.1	0.26	64	-0.57	0.567	
BeatSheet	01 vs 02	Small Mirid Nymphs	-0.2	0.15	18.3	-1.10	0.286	
BeatSheet	01 vs 02	Whiteflies	0.9	0.87	8.91	1.03	0.330	
BeatSheet	01 vs 02	Zelus Nymphs	-0.7	0.39	4.12	-1.71	0.161	
BeatSheet	01 vs 04	Aphids	1.6	0.56	9.21	2.82	0.020	*
BeatSheet	01 vs 04	Cotton Fleahopper Adults	-1.0	0.14	276	-7.05	<.001	*
BeatSheet	01 vs 04	Cotton Fleahopper Large Nymphs	-1.6	0.33	7.78	-4.75	0.002	*
BeatSheet	01 vs 04	Geocoris Adults	-1.0	0.14	11.6	-7.18	<.001	*
BeatSheet	01 vs 04	Geocoris Nymphs	-1.2	0.18	15.6	-6.59	<.001	*
BeatSheet	01 vs 04	Lygus Adults	-0.1	0.22	9.03	-0.62	0.551	
BeatSheet	01 vs 04	Lygus Large Nymphs	-0.5	0.15	10.5	-2.94	0.014	*
BeatSheet	01 vs 04	Orius Adults	-0.3	0.13	11.7	-2.64	0.022	*
BeatSheet	01 vs 04	Orius Nymphs	-0.5	0.13	18.6	-4.06	<.001	*
BeatSheet	01 vs 04	Predatory Stink bugs	-0.5	0.26	64	-2.01	0.049	*
BeatSheet	01 vs 04	Small Mirid Nymphs	-0.3	0.15	18.3	-2.00	0.061	
BeatSheet	01 vs 04	Whiteflies	0.7	0.87	8.91	0.80	0.445	

Table I-23. Results of Combined-site Significance Testing for Category I From Three Collection Methods Meeting the Inclusion Criterion: Estimated Difference and Standard Error in Square-root Scale, t value, p value, and Significance (Sig at 0.05) (continued)

Method	Comparison	Category I	Estimate	Standard Error	DF	t Value	p Value	Sig at 5%
BeatSheet	01 vs 04	Zelus Nymphs	-1.0	0.39	4.12	-2.70	0.052	
BeatSheet	01 vs 05	Aphids	1.7	0.56	9.21	3.05	0.013	*
BeatSheet	01 vs 05	Cotton Fleahopper Adults	-0.5	0.14	276	-3.12	0.002	*
BeatSheet	01 vs 05	Cotton Fleahopper Large Nymphs	-1.3	0.33	7.78	-3.78	0.006	*
BeatSheet	01 vs 05	Geocoris Adults	-0.8	0.14	11.6	-5.75	<.001	*
BeatSheet	01 vs 05	Geocoris Nymphs	-1.0	0.18	15.6	-5.37	<.001	*
BeatSheet	01 vs 05	Lygus Adults	0.4	0.22	9.03	1.70	0.123	
BeatSheet	01 vs 05	Lygus Large Nymphs	0.3	0.15	10.5	1.64	0.130	
BeatSheet	01 vs 05	Orius Adults	-0.3	0.13	11.7	-2.08	0.061	
BeatSheet	01 vs 05	Orius Nymphs	-0.5	0.13	18.6	-3.46	0.003	*
BeatSheet	01 vs 05	Predatory Stink bugs	-0.3	0.26	64	-1.07	0.290	
BeatSheet	01 vs 05	Small Mirid Nymphs	-0.2	0.15	18.3	-1.51	0.149	
BeatSheet	01 vs 05	Whiteflies	1.8	0.87	8.91	2.03	0.073	
BeatSheet	01 vs 05	Zelus Nymphs	-0.6	0.39	4.12	-1.61	0.182	
BeatSheet	02 vs 03	Aphids	0.2	0.56	9.21	0.31	0.764	
BeatSheet	02 vs 03	Cotton Fleahopper Adults	0.2	0.14	276	1.56	0.121	
BeatSheet	02 vs 03	Cotton Fleahopper Large Nymphs	0.2	0.33	7.78	0.68	0.517	
BeatSheet	02 vs 03	Geocoris Adults	-0.2	0.14	11.6	-1.12	0.284	
BeatSheet	02 vs 03	Geocoris Nymphs	-0.0	0.18	15.6	-0.07	0.946	
BeatSheet	02 vs 03	Lygus Adults	0.1	0.22	9.03	0.63	0.544	
BeatSheet	02 vs 03	Lygus Large Nymphs	0.8	0.15	10.5	5.02	<.001	*
BeatSheet	02 vs 03	Orius Adults	0.1	0.13	11.7	0.99	0.343	
BeatSheet	02 vs 03	Orius Nymphs	0.1	0.13	18.6	0.90	0.382	
BeatSheet	02 vs 03	Predatory Stink bugs	-0.3	0.26	64	-1.18	0.242	
BeatSheet	02 vs 03	Small Mirid Nymphs	0.1	0.15	18.3	0.57	0.574	

Table I-23. Results of Combined-site Significance Testing for Category I From Three Collection Methods Meeting the Inclusion Criterion: Estimated Difference and Standard Error in Square-root Scale, t value, p value, and Significance (Sig at 0.05) (continued)

Method	Comparison	Category I	Estimate	Standard Error	DF	t Value	p Value	Sig at 5%
BeatSheet	02 vs 03	Whiteflies	0.3	0.87	8.91	0.30	0.773	
BeatSheet	02 vs 03	Zelus Nymphs	0.4	0.39	4.12	1.09	0.336	
BeatSheet	02 vs 04	Aphids	-0.2	0.56	9.21	-0.34	0.743	
BeatSheet	02 vs 04	Cotton Fleahopper Adults	-0.6	0.14	276	-4.06	<.001	*
BeatSheet	02 vs 04	Cotton Fleahopper Large Nymphs	-1.1	0.33	7.78	-3.38	0.010	*
BeatSheet	02 vs 04	Geocoris Adults	-0.5	0.14	11.6	-3.35	0.006	*
BeatSheet	02 vs 04	Geocoris Nymphs	-0.6	0.18	15.6	-3.46	0.003	*
BeatSheet	02 vs 04	Lygus Adults	-0.3	0.22	9.03	-1.24	0.246	
BeatSheet	02 vs 04	Lygus Large Nymphs	-0.3	0.15	10.5	-2.24	0.047	*
BeatSheet	02 vs 04	Orius Adults	0.1	0.13	11.7	0.40	0.693	
BeatSheet	02 vs 04	Orius Nymphs	-0.2	0.13	18.6	-1.24	0.232	
BeatSheet	02 vs 04	Predatory Stink bugs	-0.4	0.26	64	-1.43	0.157	
BeatSheet	02 vs 04	Small Mirid Nymphs	-0.1	0.15	18.3	-0.90	0.382	
BeatSheet	02 vs 04	Whiteflies	-0.2	0.87	8.91	-0.23	0.824	
BeatSheet	02 vs 04	Zelus Nymphs	-0.4	0.39	4.12	-0.99	0.376	
BeatSheet	02 vs 05	Aphids	-0.1	0.56	9.21	-0.11	0.917	
BeatSheet	02 vs 05	Cotton Fleahopper Adults	-0.0	0.14	276	-0.12	0.901	
BeatSheet	02 vs 05	Cotton Fleahopper Large Nymphs	-0.8	0.33	7.78	-2.40	0.044	*
BeatSheet	02 vs 05	Geocoris Adults	-0.3	0.14	11.6	-1.92	0.080	
BeatSheet	02 vs 05	Geocoris Nymphs	-0.4	0.18	15.6	-2.24	0.040	*
BeatSheet	02 vs 05	Lygus Adults	0.2	0.22	9.03	1.08	0.307	
BeatSheet	02 vs 05	Lygus Large Nymphs	0.4	0.15	10.5	2.34	0.040	*
BeatSheet	02 vs 05	Orius Adults	0.1	0.13	11.7	0.97	0.352	
BeatSheet	02 vs 05	Orius Nymphs	-0.1	0.13	18.6	-0.63	0.534	
BeatSheet	02 vs 05	Predatory Stink bugs	-0.1	0.26	64	-0.49	0.625	

Table I-23. Results of Combined-site Significance Testing for Category I From Three Collection Methods Meeting the Inclusion Criterion: Estimated Difference and Standard Error in Square-root Scale, t value, p value, and Significance (Sig at 0.05) (continued)

Method	Comparison	Category I	Estimate	Standard Error	DF	t Value	p Value	Sig at 5%
BeatSheet	02 vs 05	Small Mirid Nymphs	-0.1	0.15	18.3	-0.41	0.689	
BeatSheet	02 vs 05	Whiteflies	0.9	0.87	8.91	1.00	0.343	
BeatSheet	02 vs 05	Zelus Nymphs	0.0	0.39	4.12	0.10	0.923	
BeatSheet	03 vs 04	Aphids	-0.4	0.56	9.21	-0.65	0.533	
BeatSheet	03 vs 04	Cotton Fleahopper Adults	-0.8	0.14	276	-5.61	<.001	*
BeatSheet	03 vs 04	Cotton Fleahopper Large Nymphs	-1.4	0.33	7.78	-4.06	0.004	*
BeatSheet	03 vs 04	Geocoris Adults	-0.3	0.14	11.6	-2.23	0.047	*
BeatSheet	03 vs 04	Geocoris Nymphs	-0.6	0.18	15.6	-3.39	0.004	*
BeatSheet	03 vs 04	Lygus Adults	-0.4	0.22	9.03	-1.87	0.094	
BeatSheet	03 vs 04	Lygus Large Nymphs	-1.1	0.15	10.5	-7.27	<.001	*
BeatSheet	03 vs 04	Orius Adults	-0.1	0.13	11.7	-0.58	0.571	
BeatSheet	03 vs 04	Orius Nymphs	-0.3	0.13	18.6	-2.13	0.047	*
BeatSheet	03 vs 04	Predatory Stink bugs	-0.1	0.26	64	-0.25	0.802	
BeatSheet	03 vs 04	Small Mirid Nymphs	-0.2	0.15	18.3	-1.47	0.159	
BeatSheet	03 vs 04	Whiteflies	-0.5	0.87	8.91	-0.53	0.611	
BeatSheet	03 vs 04	Zelus Nymphs	-0.8	0.39	4.12	-2.08	0.104	
BeatSheet	03 vs 05	Aphids	-0.2	0.56	9.21	-0.42	0.686	
BeatSheet	03 vs 05	Cotton Fleahopper Adults	-0.2	0.14	276	-1.68	0.094	
BeatSheet	03 vs 05	Cotton Fleahopper Large Nymphs	-1.0	0.33	7.78	-3.08	0.016	*
BeatSheet	03 vs 05	Geocoris Adults	-0.1	0.14	11.6	-0.80	0.441	
BeatSheet	03 vs 05	Geocoris Nymphs	-0.4	0.18	15.6	-2.17	0.046	*
BeatSheet	03 vs 05	Lygus Adults	0.1	0.22	9.03	0.45	0.662	
BeatSheet	03 vs 05	Lygus Large Nymphs	-0.4	0.15	10.5	-2.69	0.022	*
BeatSheet	03 vs 05	Orius Adults	-0.0	0.13	11.7	-0.02	0.985	
BeatSheet	03 vs 05	Orius Nymphs	-0.2	0.13	18.6	-1.53	0.143	

Table I-23. Results of Combined-site Significance Testing for Category I From Three Collection Methods Meeting the Inclusion Criterion: Estimated Difference and Standard Error in Square-root Scale, t value, p value, and Significance (Sig at 0.05) (continued)

Method	Comparison	Category I	Estimate	Standard Error	DF	t Value	p Value	Sig at 5%
BeatSheet	03 vs 05	Predatory Stink bugs	0.2	0.26	64	0.69	0.493	
BeatSheet	03 vs 05	Small Mirid Nymphs	-0.1	0.15	18.3	-0.98	0.340	
BeatSheet	03 vs 05	Whiteflies	0.6	0.87	8.91	0.70	0.500	
BeatSheet	03 vs 05	Zelus Nymphs	-0.4	0.39	4.12	-0.99	0.378	
BeatSheet	04 vs 05	Aphids	0.1	0.56	9.21	0.23	0.822	
BeatSheet	04 vs 05	Cotton Fleahopper Adults	0.6	0.14	276	3.93	<.001	*
BeatSheet	04 vs 05	Cotton Fleahopper Large Nymphs	0.3	0.33	7.78	0.97	0.359	
BeatSheet	04 vs 05	Geocoris Adults	0.2	0.14	11.6	1.43	0.179	
BeatSheet	04 vs 05	Geocoris Nymphs	0.2	0.18	15.6	1.23	0.238	
BeatSheet	04 vs 05	Lygus Adults	0.5	0.22	9.03	2.32	0.045	*
BeatSheet	04 vs 05	Lygus Large Nymphs	0.7	0.15	10.5	4.58	<.001	*
BeatSheet	04 vs 05	Orius Adults	0.1	0.13	11.7	0.56	0.583	
BeatSheet	04 vs 05	Orius Nymphs	0.1	0.13	18.6	0.60	0.555	
BeatSheet	04 vs 05	Predatory Stink bugs	0.2	0.26	64	0.94	0.350	
BeatSheet	04 vs 05	Small Mirid Nymphs	0.1	0.15	18.3	0.49	0.631	
BeatSheet	04 vs 05	Whiteflies	1.1	0.87	8.91	1.23	0.250	
BeatSheet	04 vs 05	Zelus Nymphs	0.4	0.39	4.12	1.09	0.333	
SweepNet	01 vs 02	Fleahopper	-0.2	0.15	6.47	-1.40	0.208	
SweepNet	01 vs 02	Lygus	0.2	0.11	13.1	2.23	0.043	*
SweepNet	01 vs 04	Fleahopper	-0.8	0.15	6.47	-5.38	0.001	*
SweepNet	01 vs 04	Lygus	-0.2	0.11	13.1	-1.55	0.144	
SweepNet	01 vs 05	Fleahopper	-0.5	0.15	6.47	-3.32	0.014	*
SweepNet	01 vs 05	Lygus	0.2	0.11	13.1	2.11	0.054	
SweepNet	02 vs 03	Fleahopper	0.0	0.15	6.47	0.08	0.941	
SweepNet	02 vs 03	Lygus	-0.0	0.11	13.1	-0.22	0.831	

Table I-23. Results of Combined-site Significance Testing for Category I From Three Collection Methods Meeting the Inclusion Criterion: Estimated Difference and Standard Error in Square-root Scale, t value, p value, and Significance (Sig at 0.05) (continued)

Method	Comparison	Category I	Estimate	Standard Error	DF	t Value	p Value	Sig at 5%
SweepNet	02 vs 04	Fleahopper	-0.6	0.15	6.47	-3.99	0.006	*
SweepNet	02 vs 04	Lygus	-0.4	0.11	13.1	-3.79	0.002	*
SweepNet	02 vs 05	Fleahopper	-0.3	0.15	6.47	-1.92	0.099	
SweepNet	02 vs 05	Lygus	-0.0	0.11	13.1	-0.12	0.907	
SweepNet	03 vs 04	Fleahopper	-0.6	0.15	6.47	-4.06	0.006	*
SweepNet	03 vs 04	Lygus	-0.4	0.11	13.1	-3.57	0.003	*
SweepNet	03 vs 05	Fleahopper	-0.3	0.15	6.47	-2.00	0.089	
SweepNet	03 vs 05	Lygus	0.0	0.11	13.1	0.10	0.924	
SweepNet	04 vs 05	Fleahopper	0.3	0.15	6.47	2.06	0.081	
SweepNet	04 vs 05	Lygus	0.4	0.11	13.1	3.67	0.003	*
Visual	01 vs 02	Spider mite eggs	-0.9	1.22	115	-0.78	0.437	
Visual	01 vs 02	Spider mites	-0.5	0.38	153	-1.33	0.185	
Visual	01 vs 02	Thrips	0.1	0.22	136	0.58	0.562	
Visual	01 vs 04	Spider mite eggs	0.2	1.22	115	0.14	0.887	
Visual	01 vs 04	Spider mites	0.1	0.38	153	0.31	0.755	
Visual	01 vs 04	Thrips	0.4	0.22	136	1.85	0.067	
Visual	01 vs 05	Spider mite eggs	-0.9	1.22	115	-0.76	0.446	
Visual	01 vs 05	Spider mites	-0.7	0.38	153	-1.87	0.063	
Visual	01 vs 05	Thrips	0.5	0.22	136	2.32	0.022	*
Visual	02 vs 03	Spider mite eggs	-0.8	1.22	115	-0.69	0.490	
Visual	02 vs 03	Spider mites	-0.3	0.38	153	-0.78	0.439	
Visual	02 vs 03	Thrips	0.6	0.22	136	2.62	0.010	*
Visual	02 vs 04	Spider mite eggs	1.1	1.22	115	0.92	0.358	
Visual	02 vs 04	Spider mites	0.6	0.38	153	1.64	0.102	
Visual	02 vs 04	Thrips	0.3	0.22	136	1.27	0.207	

Table I-23. Results of Combined-site Significance Testing for Category I From Three Collection Methods Meeting the Inclusion Criterion: Estimated Difference and Standard Error in Square-root Scale, t value, p value, and Significance (Sig at 0.05) (continued)

Method	Comparison	Category I	Estimate	Standard Error	DF	t Value	p Value	Sig at 5%
Visual	02 vs 05	Spider mite eggs	0.0	1.22	115	0.02	0.988	
Visual	02 vs 05	Spider mites	-0.2	0.38	153	-0.54	0.590	
Visual	02 vs 05	Thrips	0.4	0.22	136	1.73	0.085	
Visual	03 vs 04	Spider mite eggs	2.0	1.22	115	1.62	0.109	
Visual	03 vs 04	Spider mites	0.9	0.38	153	2.42	0.017	*
Visual	03 vs 04	Thrips	-0.3	0.22	136	-1.35	0.180	
Visual	03 vs 05	Spider mite eggs	0.9	1.22	115	0.71	0.481	
Visual	03 vs 05	Spider mites	0.1	0.38	153	0.24	0.814	
Visual	03 vs 05	Thrips	-0.2	0.22	136	-0.88	0.380	
Visual	04 vs 05	Spider mite eggs	-1.1	1.22	115	-0.91	0.366	
Visual	04 vs 05	Spider mites	-0.8	0.38	153	-2.18	0.030	*
Visual	04 vs 05	Thrips	0.1	0.22	136	0.47	0.642	

Table I-24. Individual-site Treatment Arithmetic Mean and Standard Error for Category I From Three Collection Methods Meeting the Inclusion Criterion

Method	Category I	Site	01	02	03	04	05
BeatSheet	Aphids	LACH	259.5(25.50)	260.5(18.91)	236.3(41.23)	270.7(22.65)	246.8(9.45)
BeatSheet	Aphids	MSGV	173.8(27.93)	63.7(3.15)	89.2(22.32)	65.1(8.04)	83.8(19.37)
BeatSheet	Aphids	NCRC	82.5(13.51)	32.6(6.19)	30.9(5.88)	35.5(12.63)	30.5(6.22)
BeatSheet	Aphids	TXUV	9.8(6.45)	6.4(3.44)	2.2(0.22)	2.9(0.56)	2.7(0.58)
BeatSheet	Cotton Fleahopper Adults	AZMA	0.7(0.15)	1.3(0.06)	1.3(0.15)	3.1(0.62)	2.0(0.33)
BeatSheet	Cotton Fleahopper Adults	AZYU	1.5(0.23)	2.9(0.53)	2.3(0.56)	4.3(1.31)	2.3(1.00)
BeatSheet	Cotton Fleahopper Adults	TXUV	2.8(0.62)	5.3(0.97)	2.4(0.56)	8.0(0.21)	5.5(1.08)
BeatSheet	Cotton Fleahopper Large Nymphs	AZMA	0.8(0.04)	2.3(0.46)	1.9(0.54)	7.7(1.09)	6.9(0.40)
BeatSheet	Cotton Fleahopper Large Nymphs	AZYU	1.3(0.54)	1.9(0.73)	2.5(0.31)	4.0(0.04)	2.6(0.56)
BeatSheet	Cotton Fleahopper Large Nymphs	TXUV	12.1(1.49)	13.6(3.63)	5.0(1.15)	22.1(2.04)	20.2(3.09)
BeatSheet	Geocoris Adults	AZMA	0.6(0.18)	2.2(0.27)	2.6(0.47)	3.4(0.38)	2.4(0.34)
BeatSheet	Geocoris Adults	AZYU	1.3(0.12)	2.9(1.02)	4.2(0.91)	5.0(0.43)	4.4(1.18)
BeatSheet	Geocoris Adults	MSGV	3.0(0.52)	4.4(0.48)	4.1(0.32)	5.9(1.01)	5.2(0.20)
BeatSheet	Geocoris Adults	TXUV	1.9(0.26)	1.8(0.48)	2.1(0.10)	3.1(0.84)	3.0(0.21)
BeatSheet	Geocoris Nymphs	AZMA	1.3(0.19)	3.2(0.56)	2.3(0.70)	6.4(0.94)	5.6(0.27)
BeatSheet	Geocoris Nymphs	AZYU	1.8(0.68)	2.8(0.38)	6.4(1.13)	8.2(1.86)	6.3(2.09)
BeatSheet	Geocoris Nymphs	MSGV	4.0(0.39)	5.8(0.63)	4.7(0.79)	10.2(1.28)	7.9(0.70)
BeatSheet	Geocoris Nymphs	NCRC	0.8(0.13)	2.5(0.05)	1.7(0.14)	2.5(0.67)	3.2(0.67)
BeatSheet	Geocoris Nymphs	TXUV	1.6(1.21)	1.9(0.79)	3.0(0.14)	3.7(0.96)	1.8(0.38)
BeatSheet	Lygus Adults	AZMA	2.5(0.75)	2.1(0.20)	1.3(0.43)	2.1(0.28)	1.1(0.36)
BeatSheet	Lygus Adults	AZYU	1.4(0.22)	2.3(0.11)	2.4(0.87)	3.2(1.15)	1.2(0.59)
BeatSheet	Lygus Adults	MSGV	2.6(0.39)	1.3(0.11)	1.8(0.39)	3.6(0.53)	2.2(0.36)
BeatSheet	Lygus Large Nymphs	AZMA	4.4(0.48)	2.6(0.30)	0.4(0.08)	4.9(0.63)	1.9(0.27)
BeatSheet	Lygus Large Nymphs	LACH	2.4(0.22)	4.6(0.47)	1.9(0.40)	3.7(0.31)	2.6(0.22)

Table I-24. Individual-site Treatment Arithmetic Mean and Standard Error for Category I From Three Collection Methods Meeting the Inclusion Criterion (continued)

Method	Category I	Site	01	02	03	04	05
BeatSheet	Lygus Large Nymphs	MSGV	4.3(0.48)	3.5(0.09)	1.9(0.15)	6.5(1.22)	3.0(0.27)
BeatSheet	Lygus Large Nymphs	NCRC	3.6(0.53)	4.1(0.74)	1.7(0.44)	5.1(0.66)	2.3(0.39)
BeatSheet	Orius Adults	AZMA	3.5(0.46)	4.1(0.79)	5.0(0.30)	4.0(0.41)	3.6(0.61)
BeatSheet	Orius Adults	AZYU	3.2(0.75)	4.4(0.03)	3.6(0.38)	3.4(0.38)	2.7(0.38)
BeatSheet	Orius Adults	MSGV	2.7(0.29)	3.3(0.19)	2.6(0.56)	4.3(0.55)	2.9(0.24)
BeatSheet	Orius Adults	NCRC	2.7(0.38)	4.1(0.35)	2.9(0.35)	2.9(0.43)	4.0(0.40)
BeatSheet	Orius Adults	TXUV	45.3(8.42)	56.3(7.69)	52.5(0.91)	60.5(5.80)	67.6(1.38)
BeatSheet	Orius Nymphs	AZMA	1.3(0.25)	3.3(0.51)	2.7(0.10)	3.7(0.10)	4.4(0.62)
BeatSheet	Orius Nymphs	AZYU	1.6(0.47)	2.1(0.52)	2.9(0.34)	3.0(0.19)	1.6(0.33)
BeatSheet	Orius Nymphs	LACH	2.0(0.35)	2.7(0.45)	2.6(0.15)	2.7(0.35)	3.1(0.36)
BeatSheet	Orius Nymphs	MSGV	4.3(0.77)	4.3(0.48)	3.6(0.12)	5.6(1.40)	3.8(0.61)
BeatSheet	Orius Nymphs	NCRC	2.9(0.42)	3.9(0.12)	3.3(0.20)	4.2(0.46)	4.6(0.99)
BeatSheet	Orius Nymphs	TXUV	52.9(15.20)	47.5(8.37)	45.9(4.54)	49.0(8.97)	56.7(7.80)
BeatSheet	Predatory Stink bugs	MSGV	1.3(0.37)	1.7(0.59)	2.3(0.75)	2.4(0.20)	2.2(0.72)
BeatSheet	Small Mirid Nymphs	AZMA	2.1(0.72)	3.4(0.29)	4.6(0.91)	3.6(0.48)	3.7(1.08)
BeatSheet	Small Mirid Nymphs	AZYU	2.8(0.23)	2.7(0.42)	3.5(0.97)	4.7(1.22)	2.9(0.62)
BeatSheet	Small Mirid Nymphs	LACH	2.3(0.59)	3.7(0.78)	3.0(0.16)	3.1(0.29)	3.7(0.36)
BeatSheet	Small Mirid Nymphs	MSGV	3.7(0.49)	3.1(0.51)	2.6(0.25)	2.5(0.63)	2.5(0.25)
BeatSheet	Small Mirid Nymphs	NCRC	1.4(0.44)	2.4(0.68)	2.4(0.22)	3.3(0.71)	2.2(0.55)
BeatSheet	Small Mirid Nymphs	TXUV	7.0(0.60)	7.8(2.29)	2.8(0.63)	8.8(1.70)	9.4(1.39)
BeatSheet	Whiteflies	AZMA	115.0(11.68)	95.3(2.11)	63.7(11.22)	74.4(7.45)	62.4(2.71)
BeatSheet	Whiteflies	AZYU	98.0(10.83)	68.7(24.61)	47.1(11.83)	65.2(16.91)	38.1(19.74)
BeatSheet	Whiteflies	MSGV	91.9(6.00)	74.4(7.60)	72.8(9.10)	58.1(4.12)	51.4(11.87)
BeatSheet	Whiteflies	TXUV	117.1(11.55)	135.5(42.04)	201.2(23.99)	256.3(22.40)	182.8(25.34)
BeatSheet	Zelus Nymphs	AZMA	1.3(0.43)	3.8(0.53)	2.4(0.45)	6.8(0.92)	4.6(0.34)
BeatSheet	Zelus Nymphs	TXUV	4.9(1.31)	5.7(0.58)	2.9(0.29)	4.4(0.48)	3.1(1.11)
SweepNet	Fleahopper	AZMA	4.0(0.13)	4.9(0.65)	5.0(0.64)	8.4(0.78)	8.2(0.91)

Table I-24. Individual-site Treatment Arithmetic Mean and Standard Error for Category I From Three Collection Methods Meeting the Inclusion Criterion (continued)

Method	Category I	Site	01	02	03	04	05
SweepNet	Fleahopper	AZYU	1.6(0.22)	3.6(0.21)	3.8(0.33)	5.8(1.90)	4.0(0.64)
SweepNet	Fleahopper	TXUV	3.1(0.72)	3.5(0.46)	3.3(0.13)	5.4(1.13)	4.6(0.98)
SweepNet	Lygus	AZMA	4.0(0.28)	3.6(0.52)	2.7(0.41)	4.8(0.60)	3.4(0.30)
SweepNet	Lygus	AZYU	6.6(0.72)	4.3(0.62)	5.7(0.47)	8.0(1.53)	3.9(1.37)
SweepNet	Lygus	LACH	3.4(1.08)	3.8(0.15)	3.5(0.26)	3.2(0.44)	2.8(0.58)
SweepNet	Lygus	MSGV	7.9(0.52)	5.3(0.46)	5.2(0.55)	9.4(0.62)	5.9(0.64)
SweepNet	Lygus	NCRC	3.1(0.35)	2.7(0.44)	3.1(0.23)	3.7(0.22)	3.2(0.18)
Visual	Spider mite eggs	TXUV	15.3(6.22)	27.0(6.45)	29.0(7.06)	12.3(3.38)	24.3(8.52)
Visual	Spider mites	NCRC	3.8(1.15)	5.5(1.42)	3.0(0.66)	2.3(0.25)	5.8(1.82)
Visual	Spider mites	TXUV	15.0(5.48)	23.3(7.75)	26.4(4.06)	13.7(3.46)	20.8(7.94)
Visual	Thrips	AZMA	3.1(0.29)	2.6(0.36)	1.7(0.29)	3.4(0.21)	1.8(0.64)
Visual	Thrips	TXUV	2.9(0.11)	2.8(0.45)	1.0(0.35)	1.8(0.88)	1.4(0.24)

Table I-25. Results of Individual-site Significance Testing For Category I From Three Collection Methods Meeting the Inclusion Criterion: Simple Mean Difference in Count and Statistical Significance at the 0.05 Significance Level (denoted by "*"). A Missing Value Implies Abundance at the Site Below the Inclusion Criterion

Method	Comparison	Category I	AZMA	AZYU	LACH	MSGV	NCRC	TXUV
BeatSheet	01 vs 02	Aphids			-1.0	110.1	49.8*	3.3
BeatSheet	01 vs 02	Cotton Fleahopper Adults	-0.6	-1.5				-2.5*
BeatSheet	01 vs 02	Cotton Fleahopper Large Nymphs	-1.5*	-0.6				-1.5
BeatSheet	01 vs 02	Geocoris Adults	-1.5*	-1.6*		-1.4		0.1
BeatSheet	01 vs 02	Geocoris Nymphs	-1.9*	-1.0		-1.8	-1.7*	-0.3
BeatSheet	01 vs 02	Lygus Adults	0.4	-0.8		1.3*		
BeatSheet	01 vs 02	Lygus Large Nymphs	1.8		-2.2*	0.7	-0.5	
BeatSheet	01 vs 02	Orius Adults	-0.6	-1.2*		-0.6	-1.4*	-10.9
BeatSheet	01 vs 02	Orius Nymphs	-2.0*	-0.5*	-0.7	0.1	-1.0	5.3
BeatSheet	01 vs 02	Predatory Stink bugs				-0.4		
BeatSheet	01 vs 02	Small Mirid Nymphs	-1.3	0.1	-1.4*	0.5	-1.0	-0.8
BeatSheet	01 vs 02	Whiteflies	19.7	29.3*		17.5		-18.4
BeatSheet	01 vs 02	Zelus Nymphs	-2.5*					-0.8
BeatSheet	01 vs 04	Aphids			-11.2	108.7	47.0*	6.9
BeatSheet	01 vs 04	Cotton Fleahopper Adults	-2.4*	-2.8*				-5.2*
BeatSheet	01 vs 04	Cotton Fleahopper Large Nymphs	-6.8*	-2.8*				-10.0*
BeatSheet	01 vs 04	Geocoris Adults	-2.8*	-3.7*		-2.9*		-1.2
BeatSheet	01 vs 04	Geocoris Nymphs	-5.1*	-6.4*		-6.2*	-1.7*	-2.1*
BeatSheet	01 vs 04	Lygus Adults	0.4	-1.7		-1.0		
BeatSheet	01 vs 04	Lygus Large Nymphs	-0.5*		-1.3*	-2.2	-1.6	
BeatSheet	01 vs 04	Orius Adults	-0.6	-0.3		-1.6	-0.2	-15.1*
BeatSheet	01 vs 04	Orius Nymphs	-2.3*	-1.4*	-0.7	-1.3	-1.3*	3.9
BeatSheet	01 vs 04	Predatory Stink bugs				-1.1*		
BeatSheet	01 vs 04	Small Mirid Nymphs	-1.6	-1.9	-0.9	1.2	-1.9	-1.8*
BeatSheet	01 vs 04	Whiteflies	40.6	32.8*		33.8*		-139.2*
BeatSheet	01 vs 04	Zelus Nymphs	-5.6*					0.4

Table I-25. Results of Individual-site Significance Testing For Category I From Three Collection Methods Meeting the Inclusion Criterion: Simple Mean Difference in Count and Statistical Significance at the 0.05 Significance Level (denoted by "*"). A Missing Value Implies Abundance at the Site Below the Inclusion Criterion (continued)

Method	Comparison	Category I	AZMA	AZYU	LACH	MSGV	NCRC	TXUV
BeatSheet	01 vs 05	Aphids			12.7	90.0	52.0*	7.1
BeatSheet	01 vs 05	Cotton Fleahopper Adults	-1.3*	-0.9				-2.7*
BeatSheet	01 vs 05	Cotton Fleahopper Large Nymphs	-6.0*	-1.4*				-8.1*
BeatSheet	01 vs 05	Geocoris Adults	-1.7*	-3.1*		-2.2*		-1.0
BeatSheet	01 vs 05	Geocoris Nymphs	-4.3*	-4.5*		-3.9*	-2.4*	-0.2
BeatSheet	01 vs 05	Lygus Adults	1.4*	0.3		0.4		
BeatSheet	01 vs 05	Lygus Large Nymphs	2.5		-0.2	1.3*	1.3	
BeatSheet	01 vs 05	Orius Adults	-0.1	0.5		-0.2	-1.3	-22.3*
BeatSheet	01 vs 05	Orius Nymphs	-3.1*	0.1	-1.1	0.5	-1.8*	-3.8*
BeatSheet	01 vs 05	Predatory Stink bugs				-0.9		
BeatSheet	01 vs 05	Small Mirid Nymphs	-1.6	-0.1	-1.4*	1.1	-0.8	-2.4*
BeatSheet	01 vs 05	Whiteflies	52.6	59.9*		40.5*		-65.7
BeatSheet	01 vs 05	Zelus Nymphs	-3.3*					1.8
BeatSheet	02 vs 03	Aphids			24.2	-25.4	1.7	4.2
BeatSheet	02 vs 03	Cotton Fleahopper Adults	0.0	0.7				3.0
BeatSheet	02 vs 03	Cotton Fleahopper Large Nymphs	0.4	-0.6				8.6
BeatSheet	02 vs 03	Geocoris Adults	-0.4	-1.3		0.4		-0.4
BeatSheet	02 vs 03	Geocoris Nymphs	0.9	-3.6*		1.1	0.8	-1.1
BeatSheet	02 vs 03	Lygus Adults	0.8	-0.1		-0.5		
BeatSheet	02 vs 03	Lygus Large Nymphs	2.1*		2.7*	1.6*	2.3*	
BeatSheet	02 vs 03	Orius Adults	-1.0	0.8		0.7	1.3	3.7
BeatSheet	02 vs 03	Orius Nymphs	0.7	-0.7	0.2	0.7	0.5	1.7
BeatSheet	02 vs 03	Predatory Stink bugs				-0.6		
BeatSheet	02 vs 03	Small Mirid Nymphs	-1.2	-0.8	0.7	0.5	0.0	4.9
BeatSheet	02 vs 03	Whiteflies	31.6	21.6		1.6		-65.8
BeatSheet	02 vs 03	Zelus Nymphs	1.3					2.8

Table I-25. Results of Individual-site Significance Testing For Category I From Three Collection Methods Meeting the Inclusion Criterion: Simple Mean Difference in Count and Statistical Significance at the 0.05 Significance Level (denoted by "*"). A Missing Value Implies Abundance at the Site Below the Inclusion Criterion (continued)

Method	Comparison	Category I	AZMA	AZYU	LACH	MSGV	NCRC	TXUV
BeatSheet	02 vs 04	Aphids			-10.2	-1.4*	-2.9	3.6
BeatSheet	02 vs 04	Cotton Fleahopper Adults	-1.8*	-1.3				-2.7*
BeatSheet	02 vs 04	Cotton Fleahopper Large Nymphs	-5.3*	-2.2*				-8.5*
BeatSheet	02 vs 04	Geocoris Adults	-1.2*	-2.1*		-1.4		-1.3*
BeatSheet	02 vs 04	Geocoris Nymphs	-3.2*	-5.4*		-4.4*	-0.0	-1.8
BeatSheet	02 vs 04	Lygus Adults	0.1	-0.9		-2.3*		
BeatSheet	02 vs 04	Lygus Large Nymphs	-2.3*		0.9	-2.9	-1.1	
BeatSheet	02 vs 04	Orius Adults	0.0	0.9		-0.9	1.2	-4.2*
BeatSheet	02 vs 04	Orius Nymphs	-0.3	-0.9	0.1	-1.3	-0.3	-1.4
BeatSheet	02 vs 04	Predatory Stink bugs				-0.7		
BeatSheet	02 vs 04	Small Mirid Nymphs	-0.2	-2.0	0.5	0.6	-0.9	-1.1
BeatSheet	02 vs 04	Whiteflies	20.9	3.5		16.3		-120.8*
BeatSheet	02 vs 04	Zelus Nymphs	-3.1*					1.2
BeatSheet	02 vs 05	Aphids			13.7	-20.1	2.2	3.8
BeatSheet	02 vs 05	Cotton Fleahopper Adults	-0.7	0.6				-0.1
BeatSheet	02 vs 05	Cotton Fleahopper Large Nymphs	-4.5*	-0.8				-6.6*
BeatSheet	02 vs 05	Geocoris Adults	-0.2	-1.5		-0.7		-1.2
BeatSheet	02 vs 05	Geocoris Nymphs	-2.4*	-3.5		-2.1	-0.8	0.2
BeatSheet	02 vs 05	Lygus Adults	1.0*	1.1*		-0.9		
BeatSheet	02 vs 05	Lygus Large Nymphs	0.7		2.1*	0.6	1.7	
BeatSheet	02 vs 05	Orius Adults	0.5	1.7*		0.4	0.1	-11.4
BeatSheet	02 vs 05	Orius Nymphs	-1.1	0.6	-0.4	0.5	-0.8	-9.1
BeatSheet	02 vs 05	Predatory Stink bugs				-0.5		
BeatSheet	02 vs 05	Small Mirid Nymphs	-0.3	-0.2	-0.0	0.6	0.3	-1.6
BeatSheet	02 vs 05	Whiteflies	32.9	30.6*		23.0		-47.3
BeatSheet	02 vs 05	Zelus Nymphs	-0.8					2.6

Table I-25. Results of Individual-site Significance Testing For Category I From Three Collection Methods Meeting the Inclusion Criterion: Simple Mean Difference in Count and Statistical Significance at the 0.05 Significance Level (denoted by "*"). A Missing Value Implies Abundance at the Site Below the Inclusion Criterion (continued)

Method	Comparison	Category I	AZMA	AZYU	LACH	MSGV	NCRC	TXUV
BeatSheet	03 vs 04	Aphids			-34.4	24.0*	-4.6	-0.7
BeatSheet	03 vs 04	Cotton Fleahopper Adults	-1.8*	-2.0				-5.7*
BeatSheet	03 vs 04	Cotton Fleahopper Large Nymphs	-5.8*	-1.5				-17.1*
BeatSheet	03 vs 04	Geocoris Adults	-0.8	-0.7		-1.8		-1.0
BeatSheet	03 vs 04	Geocoris Nymphs	-4.1*	-1.8		-5.5*	-0.8	-0.7
BeatSheet	03 vs 04	Lygus Adults	-0.8	-0.8		-1.8*		
BeatSheet	03 vs 04	Lygus Large Nymphs	-4.5*		-1.8*	-4.5*	-3.4*	
BeatSheet	03 vs 04	Orius Adults	1.0	0.1		-1.7*	-0.1	-8.0
BeatSheet	03 vs 04	Orius Nymphs	-1.0	-0.2	-0.1	-2.0	-0.9*	-3.1
BeatSheet	03 vs 04	Predatory Stink bugs				-0.1		
BeatSheet	03 vs 04	Small Mirid Nymphs	0.9	-1.2	-0.1	0.1	-0.9	-6.0*
BeatSheet	03 vs 04	Whiteflies	-10.6	-18.1		14.7		-55.0
BeatSheet	03 vs 04	Zelus Nymphs	-4.4*					-1.6
BeatSheet	03 vs 05	Aphids			-10.5	5.4*	0.5	-0.4
BeatSheet	03 vs 05	Cotton Fleahopper Adults	-0.7	-0.1				-3.1
BeatSheet	03 vs 05	Cotton Fleahopper Large Nymphs	-5.0*	-0.1				-15.2*
BeatSheet	03 vs 05	Geocoris Adults	0.2	-0.2		-1.1		-0.8
BeatSheet	03 vs 05	Geocoris Nymphs	-3.3*	0.0		-3.2	-1.5	1.3
BeatSheet	03 vs 05	Lygus Adults	0.2	1.2		-0.4		
BeatSheet	03 vs 05	Lygus Large Nymphs	-1.5*		-0.7	-1.0	-0.6	
BeatSheet	03 vs 05	Orius Adults	1.5	0.9		-0.3	-1.1	-15.1
BeatSheet	03 vs 05	Orius Nymphs	-1.7	1.3*	-0.6	-0.2	-1.3*	-10.8
BeatSheet	03 vs 05	Predatory Stink bugs				0.1		
BeatSheet	03 vs 05	Small Mirid Nymphs	0.9	0.6	-0.7	0.1	0.3	-6.6*
BeatSheet	03 vs 05	Whiteflies	1.3	9.0		21.4		18.5
BeatSheet	03 vs 05	Zelus Nymphs	-2.2*					-0.2

Table I-25. Results of Individual-site Significance Testing For Category I From Three Collection Methods Meeting the Inclusion Criterion: Simple Mean Difference in Count and Statistical Significance at the 0.05 Significance Level (denoted by "*"). A Missing Value Implies Abundance at the Site Below the Inclusion Criterion (continued)

Method	Comparison	Category I	AZMA	AZYU	LACH	MSGV	NCRC	TXUV
BeatSheet	04 vs 05	Aphids			23.9	-18.7	5.0	0.2
BeatSheet	04 vs 05	Cotton Fleahopper Adults	1.1	1.9*				2.6*
BeatSheet	04 vs 05	Cotton Fleahopper Large Nymphs	0.8	1.4*				1.9
BeatSheet	04 vs 05	Geocoris Adults	1.0	0.6		0.7		0.1
BeatSheet	04 vs 05	Geocoris Nymphs	0.8	1.9		2.3	-0.7	1.9
BeatSheet	04 vs 05	Lygus Adults	0.9	2.0*		1.4		
BeatSheet	04 vs 05	Lygus Large Nymphs	3.0*		1.2	3.5*	2.8*	
BeatSheet	04 vs 05	Orius Adults	0.5	0.8		1.4	-1.1	-7.1
BeatSheet	04 vs 05	Orius Nymphs	-0.7	1.5*	-0.4	1.8	-0.4	-7.7
BeatSheet	04 vs 05	Predatory Stink bugs				0.2		
BeatSheet	04 vs 05	Small Mirid Nymphs	-0.1	1.7	-0.6	-0.0	1.2	-0.6
BeatSheet	04 vs 05	Whiteflies	11.9	27.1*		6.7		73.5*
BeatSheet	04 vs 05	Zelus Nymphs	2.2*					1.3
SweepNet	01 vs 02	Fleahopper	-0.9	-2.0*				-0.4
SweepNet	01 vs 02	Lygus	0.4	2.3	-0.4	2.6*	0.4	
SweepNet	01 vs 04	Fleahopper	-4.4*	-4.2*				-2.3*
SweepNet	01 vs 04	Lygus	-0.8	-1.4	0.2	-1.5	-0.7	
SweepNet	01 vs 05	Fleahopper	-4.2	-2.4*				-1.5
SweepNet	01 vs 05	Lygus	0.6	2.7*	0.6	2.0*	-0.2	
SweepNet	02 vs 03	Fleahopper	-0.1	-0.1				0.2
SweepNet	02 vs 03	Lygus	0.9	-1.4	0.3	0.1	-0.4	
SweepNet	02 vs 04	Fleahopper	-3.6*	-2.2				-1.9*
SweepNet	02 vs 04	Lygus	-1.2*	-3.8*	0.6	-4.1*	-1.0	
SweepNet	02 vs 05	Fleahopper	-3.3	-0.3				-1.1
SweepNet	02 vs 05	Lygus	0.3	0.4	1.0	-0.6	-0.5	
SweepNet	03 vs 04	Fleahopper	-3.5*	-2.0				-2.1*

Table I-25. Results of Individual-site Significance Testing For Category I From Three Collection Methods Meeting the Inclusion Criterion: Simple Mean Difference in Count and Statistical Significance at the 0.05 Significance Level (denoted by "*"). A Missing Value Implies Abundance at the Site Below the Inclusion Criterion (continued)

Method	Comparison	Category I	AZMA	AZYU	LACH	MSGV	NCRC	TXUV
SweepNet	03 vs 04	Lygus	-2.1*	-2.4	0.3	-4.2*	-0.7	
SweepNet	03 vs 05	Fleahopper	-3.2*	-0.2				-1.3
SweepNet	03 vs 05	Lygus	-0.6	1.8	0.7	-0.7	-0.2	
SweepNet	04 vs 05	Fleahopper	0.3	1.9				0.8*
SweepNet	04 vs 05	Lygus	1.4	4.1*	0.4	3.5*	0.5	
Visual	01 vs 02	Spider mite eggs						-11.8
Visual	01 vs 02	Spider mites					-1.8	-8.2
Visual	01 vs 02	Thrips	0.5					0.1
Visual	01 vs 04	Spider mite eggs						2.9
Visual	01 vs 04	Spider mites					1.5	1.4
Visual	01 vs 04	Thrips	-0.2					1.1
Visual	01 vs 05	Spider mite eggs						-9.1
Visual	01 vs 05	Spider mites					-2.1	-5.8
Visual	01 vs 05	Thrips	1.4					1.4*
Visual	02 vs 03	Spider mite eggs						-2.0
Visual	02 vs 03	Spider mites					2.5	-3.1
Visual	02 vs 03	Thrips	0.9					1.8*
Visual	02 vs 04	Spider mite eggs						14.7
Visual	02 vs 04	Spider mites					3.3	9.6
Visual	02 vs 04	Thrips	-0.7					0.9
Visual	02 vs 05	Spider mite eggs						2.7
Visual	02 vs 05	Spider mites					-0.3	2.5
Visual	02 vs 05	Thrips	0.9					1.3
Visual	03 vs 04	Spider mite eggs						16.7
Visual	03 vs 04	Spider mites					0.8	12.7
Visual	03 vs 04	Thrips	-1.6					-0.8

Table I-25. Results of Individual-site Significance Testing For Category I From Three Collection Methods Meeting the Inclusion Criterion: Simple Mean Difference in Count and Statistical Significance at the 0.05 Significance Level (denoted by "*"). A Missing Value Implies Abundance at the Site Below the Inclusion Criterion (continued)

Method	Comparison	Category I	AZMA	AZYU	LACH	MSGV	NCRC	TXUV
Visual	03 vs 05	Spider mite eggs						4.7
Visual	03 vs 05	Spider mites					-2.8	5.6
Visual	03 vs 05	Thrips	-0.1					-0.4
Visual	04 vs 05	Spider mite eggs						-12.0
Visual	04 vs 05	Spider mites					-3.6	-7.1
Visual	04 vs 05	Thrips	1.6					0.4

Table I-26. Statistical Power of the Combined-site Analysis for Arthropod Category I From Three Collection Methods with a Minimum Detectable Difference of 50% of the Control Mean (Treatment = "04")

Method	Category I	N (Rep)	Mean (Treatment=04)	Power (%)
BeatSheet	Aphids	12	111.79	78.9
BeatSheet	Cotton Fleahopper Adults	9	4.99	93.9
BeatSheet	Cotton Fleahopper Large Nymphs	9	10.70	45.9
BeatSheet	Geocoris Adults	12	4.43	89.5
BeatSheet	Geocoris Nymphs	15	5.96	84.0
BeatSheet	Lygus Adults	9	2.78	37.1
BeatSheet	Lygus Large Nymphs	12	5.03	86.2
BeatSheet	Orius Adults	15	15.44	99.7
BeatSheet	Orius Nymphs	18	12.57	99.4
BeatSheet	Predatory Stink bugs	3	2.61	31.5
BeatSheet	Small Mirid Nymphs	18	4.16	85.5
BeatSheet	Whiteflies	12	104.69	58.4
BeatSheet	Zelus Nymphs	6	5.92	19.6
SweepNet	Fleahopper	9	6.58	90.2
SweepNet	Lygus	15	5.62	99.3
Visual	Spider mite eggs	3	16.98	12.3
Visual	Spider mites	6	11.87	44.3
Visual	Thrips	6	2.09	42.0

Table I-27. Statistical Power of the Individual-site Analysis for Arthropod Category I From Three Collection Methods with a Minimum Detectable Difference of 50% of the Control Mean (Treatment = "04")

Method	Category I	Site	N (Rep)	Mean (Treatment=04)	Power (%)
BeatSheet	Aphids	LACH	3	272.87	100.0
BeatSheet	Aphids	MSGV	3	105.31	100.0
BeatSheet	Aphids	NCRC	3	38.38	83.3
BeatSheet	Aphids	TXUV	3	5.26	15.5
BeatSheet	Cotton Fleahopper Adults	AZMA	3	3.30	38.1
BeatSheet	Cotton Fleahopper Adults	AZYU	3	3.75	41.3
BeatSheet	Cotton Fleahopper Adults	TXUV	3	8.40	70.2
BeatSheet	Cotton Fleahopper Large Nymphs	AZMA	3	7.36	63.6
BeatSheet	Cotton Fleahopper Large Nymphs	AZYU	3	4.08	46.9
BeatSheet	Cotton Fleahopper Large Nymphs	TXUV	3	20.04	80.4
BeatSheet	Geocoris Adults	AZMA	3	3.42	64.1
BeatSheet	Geocoris Adults	AZYU	3	5.11	72.9
BeatSheet	Geocoris Adults	MSGV	3	5.72	78.3
BeatSheet	Geocoris Adults	TXUV	3	3.21	47.5
BeatSheet	Geocoris Nymphs	AZMA	3	6.66	74.1
BeatSheet	Geocoris Nymphs	AZYU	3	6.35	70.2
BeatSheet	Geocoris Nymphs	MSGV	3	8.79	85.9
BeatSheet	Geocoris Nymphs	NCRC	3	2.33	37.8
BeatSheet	Geocoris Nymphs	TXUV	3	4.00	22.7
BeatSheet	Lygus Adults	AZMA	3	2.10	27.7
BeatSheet	Lygus Adults	AZYU	3	2.90	34.0
BeatSheet	Lygus Adults	MSGV	3	3.61	41.3
BeatSheet	Lygus Large Nymphs	AZMA	3	5.69	54.8
BeatSheet	Lygus Large Nymphs	LACH	3	3.93	43.0
BeatSheet	Lygus Large Nymphs	MSGV	3	5.43	70.2
BeatSheet	Lygus Large Nymphs	NCRC	3	4.94	53.5
BeatSheet	Orius Adults	AZMA	3	4.09	72.6
BeatSheet	Orius Adults	AZYU	3	3.51	60.7
BeatSheet	Orius Adults	MSGV	3	4.11	47.0
BeatSheet	Orius Adults	NCRC	3	3.21	53.7
BeatSheet	Orius Adults	TXUV	3	63.81	99.9
BeatSheet	Orius Nymphs	AZMA	3	3.81	41.9

Table I-27. Statistical Power of the Individual-site Analysis for Arthropod Category I From Three Collection Methods with a Minimum Detectable Difference of 50% of the Control Mean (Treatment = "04") (continued)

Method	Category I	Site	N (Rep)	Mean (Treatment=04)	Power (%)
BeatSheet	Orius Nymphs	AZYU	3	2.84	54.8
BeatSheet	Orius Nymphs	LACH	3	2.62	27.5
BeatSheet	Orius Nymphs	MSGV	3	5.31	50.7
BeatSheet	Orius Nymphs	NCRC	3	4.73	67.8
BeatSheet	Orius Nymphs	TXUV	3	52.74	91.4
BeatSheet	Predatory Stink bugs	MSGV	3	2.72	28.6
BeatSheet	Small Mirid Nymphs	AZMA	3	3.59	28.4
BeatSheet	Small Mirid Nymphs	AZYU	3	4.04	47.1
BeatSheet	Small Mirid Nymphs	LACH	3	3.39	47.8
BeatSheet	Small Mirid Nymphs	MSGV	3	2.67	46.1
BeatSheet	Small Mirid Nymphs	NCRC	3	3.20	24.7
BeatSheet	Small Mirid Nymphs	TXUV	3	8.93	52.0
BeatSheet	Whiteflies	AZMA	3	83.62	99.3
BeatSheet	Whiteflies	AZYU	3	63.38	77.7
BeatSheet	Whiteflies	MSGV	3	61.09	45.7
BeatSheet	Whiteflies	TXUV	3	224.01	92.0
BeatSheet	Zelus Nymphs	AZMA	3	7.03	62.3
BeatSheet	Zelus Nymphs	TXUV	3	5.14	21.9
SweepNet	Fleahopper	AZMA	3	8.27	83.7
SweepNet	Fleahopper	AZYU	3	4.92	40.9
SweepNet	Fleahopper	TXUV	3	5.51	73.2
SweepNet	Lygus	AZMA	3	5.00	75.0
SweepNet	Lygus	AZYU	3	7.75	57.0
SweepNet	Lygus	LACH	3	3.15	33.5
SweepNet	Lygus	MSGV	3	8.88	96.9
SweepNet	Lygus	NCRC	3	3.45	64.2
Visual	Spider mite eggs	TXUV	3	20.69	42.5
Visual	Spider mites	NCRC	3	3.84	15.6
Visual	Spider mites	TXUV	3	19.55	59.0
Visual	Thrips	AZMA	3	2.51	29.4
Visual	Thrips	TXUV	3	1.65	20.9

Table I-28. Combined-site Least Square Means and Standard Errors in Square-root Scale for Category II Meeting the Inclusion Criterion

Method	Category II	01	02	03	04	05
BeatSheet	Total Aphids	8.2(2.09)	6.5(2.09)	6.3(2.09)	6.7(2.09)	6.5(2.09)
BeatSheet	Total Cotton Fleahoppers	1.5(0.50)	2.1(0.50)	1.8(0.50)	3.4(0.50)	2.8(0.50)
BeatSheet	Total Geocoris	1.3(0.32)	1.8(0.32)	2.0(0.32)	2.5(0.32)	2.3(0.32)
BeatSheet	Total Lygus	1.8(0.15)	1.9(0.15)	1.4(0.15)	2.2(0.15)	1.4(0.15)
BeatSheet	Total Nabis	0.6(0.19)	1.1(0.19)	1.2(0.19)	1.2(0.19)	1.0(0.19)
BeatSheet	Total Orius	2.8(1.05)	3.3(1.05)	3.2(1.05)	3.3(1.05)	3.2(1.05)
BeatSheet	Total Predatory Stink bugs	0.9(0.26)	1.0(0.26)	1.3(0.26)	1.4(0.26)	1.2(0.26)
BeatSheet	Total Stink bugs	0.9(0.38)	1.1(0.38)	1.9(0.38)	1.3(0.38)	1.6(0.38)
BeatSheet	Total Whiteflies	9.1(1.45)	8.2(1.45)	7.9(1.45)	8.4(1.45)	7.3(1.45)
BeatSheet	Total Zelus	1.2(0.34)	1.7(0.34)	1.4(0.34)	2.3(0.34)	1.7(0.34)
SweepNet	Total Cotton Fleahoppers	1.5(0.20)	1.7(0.20)	1.7(0.20)	2.3(0.20)	2.0(0.20)
SweepNet	Total Lygus	1.9(0.18)	1.7(0.18)	1.7(0.18)	2.1(0.18)	1.7(0.18)
Visual	Total Spider mites	2.7(1.71)	3.4(1.71)	3.7(1.71)	2.6(1.71)	3.5(1.71)
Visual	Total Thrips	1.2(0.29)	1.1(0.29)	1.0(0.29)	1.3(0.29)	1.0(0.29)

Table I-29. Individual-site Least Square Means and Standard Errors in Square-root Scale for Category II Meeting the Inclusion Criterion

Method	Category II	Site	01	02	03	04	05
BeatSheet	Total Aphids	LACH	11.7(3.82)	11.9(3.82)	11.9(3.82)	12.2(3.82)	12.0(3.82)
BeatSheet	Total Aphids	MSGV	6.9(2.14)	6.4(2.14)	5.9(2.14)	7.1(2.14)	6.8(2.14)
BeatSheet	Total Aphids	NCRC	5.8(1.44)	4.3(1.44)	4.6(1.44)	4.3(1.44)	4.6(1.44)
BeatSheet	Total Aphids	TXUV	2.2(0.50)	1.8(0.50)	1.2(0.50)	1.8(0.50)	1.4(0.50)
BeatSheet	Total Cotton Fleahoppers	AZMA	0.8(0.23)	1.6(0.23)	1.5(0.23)	3.0(0.23)	2.8(0.23)
BeatSheet	Total Cotton Fleahoppers	AZYU	1.1(0.31)	1.9(0.31)	1.7(0.31)	2.6(0.31)	1.7(0.31)
BeatSheet	Total Cotton Fleahoppers	TXUV	2.3(0.79)	3.0(0.79)	2.7(0.79)	4.4(0.79)	3.6(0.79)
BeatSheet	Total Geocoris	AZMA	1.1(0.23)	2.3(0.23)	2.0(0.23)	2.9(0.23)	2.6(0.23)
BeatSheet	Total Geocoris	AZYU	1.6(0.21)	2.4(0.21)	3.0(0.21)	3.2(0.21)	2.9(0.21)
BeatSheet	Total Geocoris	LACH	0.7(0.21)	0.8(0.21)	1.5(0.21)	1.1(0.21)	1.3(0.21)
BeatSheet	Total Geocoris	MSGV	2.3(0.35)	2.9(0.35)	2.7(0.35)	3.5(0.35)	3.3(0.35)
BeatSheet	Total Geocoris	NCRC	0.6(0.17)	1.3(0.17)	1.4(0.17)	1.5(0.17)	1.6(0.17)
BeatSheet	Total Geocoris	TXUV	1.4(0.29)	1.3(0.29)	1.6(0.29)	2.2(0.29)	1.8(0.29)
BeatSheet	Total Lygus	AZMA	2.0(0.22)	1.9(0.22)	0.9(0.22)	2.4(0.22)	1.6(0.22)
BeatSheet	Total Lygus	AZYU	1.5(0.24)	1.5(0.24)	1.3(0.24)	1.8(0.24)	0.8(0.24)
BeatSheet	Total Lygus	LACH	1.2(0.35)	2.2(0.35)	1.5(0.35)	1.9(0.35)	1.5(0.35)
BeatSheet	Total Lygus	MSGV	2.1(0.30)	2.1(0.30)	1.6(0.30)	2.6(0.30)	1.7(0.30)
BeatSheet	Total Lygus	NCRC	1.9(0.25)	2.0(0.25)	1.2(0.25)	2.2(0.25)	1.6(0.25)
BeatSheet	Total Nabis	AZYU	0.7(0.24)	1.1(0.24)	1.4(0.24)	1.5(0.24)	1.2(0.24)
BeatSheet	Total Nabis	MSGV	0.5(0.21)	1.1(0.21)	0.9(0.21)	0.8(0.21)	1.0(0.21)
BeatSheet	Total Orius	AZMA	1.5(0.26)	2.1(0.26)	2.3(0.26)	2.2(0.26)	2.2(0.26)
BeatSheet	Total Orius	AZYU	1.7(0.25)	2.4(0.25)	2.4(0.25)	2.4(0.25)	2.0(0.25)
BeatSheet	Total Orius	LACH	1.5(0.24)	1.4(0.24)	1.5(0.24)	1.6(0.24)	1.5(0.24)
BeatSheet	Total Orius	MSGV	2.3(0.41)	2.4(0.41)	2.2(0.41)	2.8(0.41)	2.2(0.41)
BeatSheet	Total Orius	NCRC	2.0(0.22)	2.7(0.22)	2.4(0.22)	2.7(0.22)	2.9(0.22)
BeatSheet	Total Orius	TXUV	7.8(1.91)	8.1(1.91)	8.2(1.91)	8.7(1.91)	8.8(1.91)
BeatSheet	Total Predatory Stink bugs	MSGV	0.9(0.28)	1.0(0.28)	1.4(0.28)	1.4(0.28)	1.0(0.28)

Table I-29. Individual-site Least Square Means and Standard Errors in Square-root Scale for Category II Meeting the Inclusion Criterion (continued)

Method	Category II	Site	01	02	03	04	05
BeatSheet	Total Stink bugs	MSGV	1.0(0.38)	1.2(0.38)	1.8(0.38)	1.4(0.38)	1.5(0.38)
BeatSheet	Total Whiteflies	AZMA	7.1(1.94)	6.9(1.94)	6.4(1.94)	6.6(1.94)	6.4(1.94)
BeatSheet	Total Whiteflies	AZYU	8.1(1.47)	6.6(1.47)	5.9(1.47)	6.5(1.47)	5.2(1.47)
BeatSheet	Total Whiteflies	MSGV	9.2(1.43)	7.8(1.43)	7.5(1.43)	6.9(1.43)	6.5(1.43)
BeatSheet	Total Whiteflies	TXUV	10.8(1.68)	11.3(1.68)	12.0(1.68)	13.8(1.68)	11.6(1.68)
BeatSheet	Total Zelus	AZMA	1.0(0.27)	1.7(0.27)	1.5(0.27)	2.6(0.27)	2.1(0.27)
BeatSheet	Total Zelus	TXUV	1.5(0.60)	1.5(0.60)	1.3(0.60)	2.1(0.60)	1.3(0.60)
SweepNet	Total Cotton Fleahoppers	AZMA	2.0(0.32)	2.0(0.32)	1.9(0.32)	2.6(0.32)	2.3(0.32)
SweepNet	Total Cotton Fleahoppers	AZYU	1.0(0.28)	1.7(0.28)	1.7(0.28)	2.0(0.28)	1.7(0.28)
SweepNet	Total Cotton Fleahoppers	TXUV	1.7(0.25)	1.5(0.25)	1.6(0.25)	2.1(0.25)	1.7(0.25)
SweepNet	Total Lygus	AZMA	1.8(0.19)	1.7(0.19)	1.5(0.19)	2.1(0.19)	1.7(0.19)
SweepNet	Total Lygus	AZYU	2.2(0.45)	1.7(0.45)	2.1(0.45)	2.4(0.45)	1.5(0.45)
SweepNet	Total Lygus	LACH	1.5(0.33)	1.6(0.33)	1.6(0.33)	1.4(0.33)	1.3(0.33)
SweepNet	Total Lygus	MSGV	2.7(0.30)	2.2(0.30)	2.0(0.30)	2.8(0.30)	2.2(0.30)
SweepNet	Total Lygus	NCRC	1.7(0.17)	1.5(0.17)	1.5(0.17)	1.7(0.17)	1.6(0.17)
Visual	Total Spider mites	NCRC	1.2(0.59)	1.6(0.59)	1.4(0.59)	1.5(0.59)	1.4(0.59)
Visual	Total Spider mites	TXUV	4.1(1.12)	4.6(1.12)	5.9(1.12)	4.4(1.12)	4.6(1.12)
Visual	Total Thrips	AZMA	1.3(0.22)	0.9(0.22)	1.2(0.22)	1.2(0.22)	1.0(0.22)
Visual	Total Thrips	NCRC	0.6(0.21)	0.1(0.21)	0.3(0.21)	1.9(0.21)	1.1(0.21)
Visual	Total Thrips	TXUV	1.7(0.34)	1.7(0.34)	0.9(0.34)	1.0(0.34)	1.1(0.34)

Table I-30. Combined-site Least Square Means and Standard Errors in Square-root Scale for Category I Meeting the Inclusion Criterion

Method	Category I	01	02	03	04	05
BeatSheet	Aphids	8.2(2.09)	6.5(2.09)	6.3(2.09)	6.7(2.09)	6.5(2.09)
BeatSheet	Cotton Fleahopper Adults	1.0(0.24)	1.4(0.24)	1.2(0.24)	2.0(0.24)	1.4(0.24)
BeatSheet	Cotton Fleahopper Large Nymphs	1.1(0.56)	1.6(0.56)	1.4(0.56)	2.7(0.56)	2.4(0.56)
BeatSheet	Geocoris Adults	0.9(0.18)	1.4(0.18)	1.6(0.18)	1.9(0.18)	1.7(0.18)
BeatSheet	Geocoris Nymphs	1.0(0.24)	1.6(0.24)	1.6(0.24)	2.2(0.24)	2.0(0.24)
BeatSheet	Lygus Adults	1.3(0.17)	1.2(0.17)	1.0(0.17)	1.5(0.17)	0.9(0.17)
BeatSheet	Lygus Large Nymphs	1.6(0.15)	1.7(0.15)	0.9(0.15)	2.0(0.15)	1.3(0.15)
BeatSheet	Orius Adults	2.3(0.93)	2.7(0.93)	2.6(0.93)	2.7(0.93)	2.6(0.93)
BeatSheet	Orius Nymphs	1.9(0.71)	2.3(0.71)	2.1(0.71)	2.4(0.71)	2.3(0.71)
BeatSheet	Predatory Stink bugs	0.9(0.26)	1.0(0.26)	1.3(0.26)	1.4(0.26)	1.2(0.26)
BeatSheet	Small Mirid Nymphs	1.4(0.15)	1.6(0.15)	1.5(0.15)	1.7(0.15)	1.6(0.15)
BeatSheet	Whiteflies	9.1(1.45)	8.2(1.45)	7.9(1.45)	8.4(1.45)	7.3(1.45)
BeatSheet	Zelus Nymphs	1.1(0.38)	1.8(0.38)	1.3(0.38)	2.2(0.38)	1.7(0.38)
SweepNet	Fleahopper	1.5(0.20)	1.7(0.20)	1.7(0.20)	2.3(0.20)	2.0(0.20)
SweepNet	Lygus	1.9(0.18)	1.7(0.18)	1.7(0.18)	2.1(0.18)	1.7(0.18)
Visual	Spider mite eggs	2.9(1.16)	3.8(1.16)	4.6(1.16)	2.7(1.16)	3.8(1.16)
Visual	Spider mites	2.1(1.11)	2.6(1.11)	2.9(1.11)	2.0(1.11)	2.9(1.11)
Visual	Thrips	1.5(0.17)	1.4(0.17)	0.8(0.17)	1.1(0.17)	1.0(0.17)

Table I-31. Individual-site Least Square Means and Standard Errors in Square-root Scale for Category I Meeting the Inclusion Criterion

Method	Category I	Site	01	02	03	04	05
BeatSheet	Aphids	LACH	11.7(3.82)	11.9(3.82)	11.9(3.82)	12.2(3.82)	12.0(3.82)
BeatSheet	Aphids	MSGV	6.9(2.14)	6.4(2.14)	5.9(2.14)	7.1(2.14)	6.8(2.14)
BeatSheet	Aphids	NCRC	5.8(1.44)	4.3(1.44)	4.6(1.44)	4.3(1.44)	4.6(1.44)
BeatSheet	Aphids	TXUV	2.2(0.50)	1.8(0.50)	1.2(0.50)	1.8(0.50)	1.4(0.50)
BeatSheet	Cotton Fleahopper Adults	AZMA	0.6(0.19)	0.9(0.19)	0.9(0.19)	1.7(0.19)	1.2(0.19)
BeatSheet	Cotton Fleahopper Adults	AZYU	1.0(0.29)	1.5(0.29)	1.2(0.29)	1.7(0.29)	1.1(0.29)
BeatSheet	Cotton Fleahopper Adults	TXUV	1.2(0.37)	1.8(0.37)	1.5(0.37)	2.6(0.37)	1.7(0.37)
BeatSheet	Cotton Fleahopper Large Nymphs	AZMA	0.4(0.23)	1.2(0.23)	1.1(0.23)	2.5(0.23)	2.4(0.23)
BeatSheet	Cotton Fleahopper Large Nymphs	AZYU	0.6(0.26)	1.1(0.26)	1.4(0.26)	1.7(0.26)	1.1(0.26)
BeatSheet	Cotton Fleahopper Large Nymphs	TXUV	2.1(0.83)	2.5(0.83)	2.3(0.83)	3.7(0.83)	3.6(0.83)
BeatSheet	Geocoris Adults	AZMA	0.3(0.18)	1.3(0.18)	1.4(0.18)	1.7(0.18)	1.4(0.18)
BeatSheet	Geocoris Adults	AZYU	0.8(0.21)	1.6(0.21)	2.0(0.21)	2.1(0.21)	2.0(0.21)
BeatSheet	Geocoris Adults	MSGV	1.5(0.25)	1.9(0.25)	1.8(0.25)	2.2(0.25)	2.0(0.25)
BeatSheet	Geocoris Adults	TXUV	1.1(0.24)	1.0(0.24)	1.3(0.24)	1.5(0.24)	1.4(0.24)
BeatSheet	Geocoris Nymphs	AZMA	0.7(0.22)	1.4(0.22)	1.2(0.22)	2.4(0.22)	2.3(0.22)
BeatSheet	Geocoris Nymphs	AZYU	1.3(0.19)	1.6(0.19)	2.2(0.19)	2.3(0.19)	1.8(0.19)
BeatSheet	Geocoris Nymphs	MSGV	1.9(0.28)	2.2(0.28)	2.2(0.28)	2.8(0.28)	2.5(0.28)
BeatSheet	Geocoris Nymphs	NCRC	0.5(0.19)	1.4(0.19)	1.3(0.19)	1.3(0.19)	1.5(0.19)
BeatSheet	Geocoris Nymphs	TXUV	0.8(0.43)	1.2(0.43)	1.1(0.43)	1.7(0.43)	1.1(0.43)
BeatSheet	Lygus Adults	AZMA	1.4(0.23)	1.3(0.23)	0.9(0.23)	1.2(0.23)	0.7(0.23)
BeatSheet	Lygus Adults	AZYU	1.0(0.22)	1.4(0.22)	1.0(0.22)	1.5(0.22)	0.7(0.22)
BeatSheet	Lygus Adults	MSGV	1.5(0.22)	0.8(0.22)	1.1(0.22)	1.7(0.22)	1.3(0.22)
BeatSheet	Lygus Large Nymphs	AZMA	1.4(0.23)	1.2(0.23)	0.2(0.23)	2.2(0.23)	1.4(0.23)
BeatSheet	Lygus Large Nymphs	LACH	1.2(0.33)	2.0(0.33)	1.1(0.33)	1.7(0.33)	1.3(0.33)
BeatSheet	Lygus Large Nymphs	MSGV	1.7(0.26)	1.7(0.26)	1.3(0.26)	2.1(0.26)	1.3(0.26)
BeatSheet	Lygus Large Nymphs	NCRC	1.6(0.28)	1.7(0.28)	0.9(0.28)	2.0(0.28)	1.2(0.28)
BeatSheet	Orius Adults	AZMA	1.6(0.23)	1.9(0.23)	2.1(0.23)	1.9(0.23)	1.8(0.23)

Table I-31. Individual-site Least Square Means and Standard Errors in Square-root Scale for Category I Meeting the Inclusion Criterion (continued)

Method	Category I	Site	01	02	03	04	05
BeatSheet	Orius Adults	AZYU	1.4(0.22)	1.9(0.22)	1.7(0.22)	1.7(0.22)	1.5(0.22)
BeatSheet	Orius Adults	MSGV	1.4(0.31)	1.6(0.31)	1.3(0.31)	1.8(0.31)	1.6(0.31)
BeatSheet	Orius Adults	NCRC	1.4(0.21)	1.9(0.21)	1.5(0.21)	1.6(0.21)	1.7(0.21)
BeatSheet	Orius Adults	TXUV	5.8(1.38)	6.1(1.38)	6.2(1.38)	6.7(1.38)	6.6(1.38)
BeatSheet	Orius Nymphs	AZMA	0.4(0.25)	1.6(0.25)	1.6(0.25)	1.7(0.25)	1.8(0.25)
BeatSheet	Orius Nymphs	AZYU	0.8(0.22)	1.2(0.22)	1.5(0.22)	1.4(0.22)	1.0(0.22)
BeatSheet	Orius Nymphs	LACH	1.1(0.27)	1.5(0.27)	1.4(0.27)	1.4(0.27)	1.6(0.27)
BeatSheet	Orius Nymphs	MSGV	1.8(0.33)	1.9(0.33)	1.8(0.33)	2.1(0.33)	1.7(0.33)
BeatSheet	Orius Nymphs	NCRC	1.4(0.20)	1.8(0.20)	1.6(0.20)	2.0(0.20)	2.0(0.20)
BeatSheet	Orius Nymphs	TXUV	5.1(1.55)	5.4(1.55)	5.9(1.55)	5.7(1.55)	6.1(1.55)
BeatSheet	Predatory Stink bugs	MSGV	0.9(0.28)	1.0(0.28)	1.4(0.28)	1.4(0.28)	1.0(0.28)
BeatSheet	Small Mirid Nymphs	AZMA	1.1(0.27)	1.5(0.27)	1.8(0.27)	1.6(0.27)	1.7(0.27)
BeatSheet	Small Mirid Nymphs	AZYU	1.4(0.30)	1.3(0.30)	1.5(0.30)	1.7(0.30)	1.2(0.30)
BeatSheet	Small Mirid Nymphs	LACH	1.2(0.28)	1.7(0.28)	1.5(0.28)	1.6(0.28)	1.7(0.28)
BeatSheet	Small Mirid Nymphs	MSGV	1.6(0.28)	1.5(0.28)	1.3(0.28)	1.3(0.28)	1.4(0.28)
BeatSheet	Small Mirid Nymphs	NCRC	1.0(0.29)	1.2(0.29)	1.5(0.29)	1.6(0.29)	1.3(0.29)
BeatSheet	Small Mirid Nymphs	TXUV	1.7(0.56)	2.0(0.56)	1.8(0.56)	2.5(0.56)	2.5(0.56)
BeatSheet	Whiteflies	AZMA	7.1(1.94)	6.9(1.94)	6.4(1.94)	6.6(1.94)	6.4(1.94)
BeatSheet	Whiteflies	AZYU	8.1(1.47)	6.6(1.47)	5.9(1.47)	6.5(1.47)	5.2(1.47)
BeatSheet	Whiteflies	MSGV	9.2(1.43)	7.8(1.43)	7.5(1.43)	6.9(1.43)	6.5(1.43)
BeatSheet	Whiteflies	TXUV	10.8(1.68)	11.3(1.68)	12.0(1.68)	13.8(1.68)	11.6(1.68)
BeatSheet	Zelus Nymphs	AZMA	0.8(0.29)	1.7(0.29)	1.3(0.29)	2.5(0.29)	2.0(0.29)
BeatSheet	Zelus Nymphs	TXUV	1.5(0.84)	1.9(0.84)	1.3(0.84)	1.7(0.84)	1.5(0.84)
SweepNet	Fleahopper	AZMA	2.0(0.32)	2.0(0.32)	1.9(0.32)	2.6(0.32)	2.3(0.32)
SweepNet	Fleahopper	AZYU	1.0(0.28)	1.7(0.28)	1.7(0.28)	2.0(0.28)	1.7(0.28)
SweepNet	Fleahopper	TXUV	1.7(0.25)	1.5(0.25)	1.6(0.25)	2.1(0.25)	1.7(0.25)
SweepNet	Lygus	AZMA	1.8(0.19)	1.7(0.19)	1.5(0.19)	2.1(0.19)	1.7(0.19)

Table I-31. Individual-site Least Square Means and Standard Errors in Square-root Scale for Category I Meeting the Inclusion Criterion (continued)

Method	Category I	Site	01	02	03	04	05
SweepNet	Lygus	AZYU	2.2(0.45)	1.7(0.45)	2.1(0.45)	2.4(0.45)	1.5(0.45)
SweepNet	Lygus	LACH	1.5(0.33)	1.6(0.33)	1.6(0.33)	1.4(0.33)	1.3(0.33)
SweepNet	Lygus	MSGV	2.7(0.30)	2.2(0.30)	2.0(0.30)	2.8(0.30)	2.2(0.30)
SweepNet	Lygus	NCRC	1.7(0.17)	1.5(0.17)	1.5(0.17)	1.7(0.17)	1.6(0.17)
Visual	Spider mite eggs	TXUV	2.8(0.93)	3.6(0.93)	4.3(0.93)	3.3(0.93)	3.6(0.93)
Visual	Spider mites	NCRC	1.0(0.56)	1.1(0.56)	1.6(0.56)	1.2(0.56)	1.6(0.56)
Visual	Spider mites	TXUV	3.1(0.87)	3.2(0.87)	4.0(0.87)	3.3(0.87)	3.5(0.87)
Visual	Thrips	AZMA	1.3(0.22)	0.9(0.22)	1.2(0.22)	1.2(0.22)	1.0(0.22)
Visual	Thrips	TXUV	1.5(0.25)	1.5(0.25)	0.6(0.25)	0.9(0.25)	0.9(0.25)

References for Appendix I

Asiimwe, P., S.E. Naranjo and P.C. Ellsworth. 2014. Effects of irrigation levels on interactions among *Lygus hesperus* (Hemiptera: Miridae), insecticides, and predators in cotton. Environmental Entomology 43:263-273.

Bates, D., M. Maechler, B. Bolker, S. Walker, R.H.B. Christensen, H. Singmann, B. Dai, F. Scheipl and G. Grothendieck. 2019. Linear mixed-effects models using 'Eigen' and S4. Version 1.1-21.

Bolker, B.M., M.E. Brooks, C.J. Clark, S.W. Geange, J.R. Poulsen, M.H. Stevens and J.S. White. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. Journal of the American Statistical Association 24:127-135.

Blumel, S., Aldershof, S., Bakker, F.M., Baier, F., Boller, E., Brown, K., Bylemans, D., Candolfi, M.P., Huber, B., Linder, C., Louis, F., Muther, J., Nienstedt, K.M., Oberwalder, C., Reber, B., Schirra, K.J., Ufer, A., & Vogt, H. (2000). Guidance document to detect side effects of plant protection products on predatory mites (Acari: Phytoseiidae) under field conditions: vineyards and orchards. Guidelines to Evaluate Side-effects of Plant Protection Products to Non-target Arthropods. In: Candolfi, M.P., Blümel, S., Forster, R., Bakker, F.M., Grimm, C., Hassan, S.A., Heimbach, U., Mead Briggs, M.A., Raber, B., Schmuck, R. & Vogt, H. (eds) Guidelines to evaluate side-effects of plant protection products to non-target arthropods IOBC, BART and EPPO Joint Initiative. IOBC/WPRS, Gent, pp. 145 – 153.

Breslow, N.E. and D.G. Clayton. 1993. Approximate interence in generalized linear mixed models. Journal of the American Statistical Association 88:9-25.

Candolfi, M.P., F. Bigler, D. Forti, R. Forster, A. Gathmann, S. Hassan, M. Mead-Briggs, M. Melandri, P. Neumann, E. Pasqualini, W. Powell, J.-N. Reboulet, P. Campbell, K. Romijn, B. Sechser, T. Thieme, A. Ufer, C. Vergnet, H. Vogt, U. Heimbach, R. Schmuck, G. Angeli, F. Bakker, K. Brown, G. Carli and A. Dinter. 2000. Principles for regulatory testing and interpretation of semi-field and field studies with non-target arthropods. Anzeiger fur Schadlingskunde 76:141-147.Catchot, A. 2019. Insect control guide for agronomic crops. Mississippi State University Extension, Mississippi State, MS.

de Jong, F.M.W., F.M. Bakker, K. Brown, C.J.T.J. Jilesen, C.J.A.M. Posthuma-Doodeman, C.E. Smit, J.J.M. van der Steen and G.M.A. van Eekelen. 2010. Guidance for summarising and evaluating field studies with non-target arthropods: A guidance document of the Dutch platform for the assessment of higher tier studies. RIVM. National Institute for Public Health and the Environment, The Netherlands.

Drees, B.M. and M.E. Rice. 1985. The vertical beat sheet: A new device for sampling soybeans insects. Journal of Economic Entomology 78:1507-1510.

Duan, J.J., Jiang, C., Head, G.P., Bhatti, M.A. et al. (2006) Statistical power analysis of a 2-year field study and design of experiments to evaluate non-target effects of genetically modified Bacillius thuringiensis corn. Ecological Entomology, 31, 521–531.

Ives, A.R. 2015. For testing the significance of regression coefficients, go ahead and log-transform count data. Methods in Ecology and Evolution 6:828-835.

Naranjo, S.E. 2005a. Long-term assessment of the effects of transgenic *Bt* cotton on the abundance of nontarget arthropod natural enemies. Environmental Entomology 34:1193-1210.

Naranjo, S.E. 2005b. Long-term assessment of the effects of transgenic *Bt* cotton on the function of the natural enemy community. Environmental Entomology 34:1211-1223.

OECD. 1984. Test No. 207: Earthworm, acute toxicity tests. Organisation for Economic Co-Operation and Development, Paris, France.

OECD. 2009. Test No. 232: Collembolan reproduction test in soil. Organisation for Economic Co-Operation and Development, Paris, France.

Perry, J.N., Rothery, P., Clark, S.J., Heard, M.S., & Hawes, C. (2003). Design, analysis and statistical power of the farm-scale evaluation of genetically modified herbicide-tolerant crops. Journal of Applied Ecology, 40, 17 – 31.SAS (2012). Software Release 9.4 (TS1M4). Cary, North Carolina, Copyright 2002-2012 by SAS Institute, Inc.

Stroup, W. 2013. Generalized linear mixed models: modern concepts, methods, and applications. Chapman & Hall/CRC.

Tan, J., M.S. Paradise, S.L. Levin, P.M. Bachman, J.P. Bachman, J.P. Uffman, C. Jiang, D.B. Carson. 2011. Development and survival of *Orius insidiosus* (Say) nymphs on encapsulated bee pollen-based diet in a Tier-I toxicity assay. Environmental Entomology 40:1613-1621.

Wolfinger, R. and M. O'connell. 1993. Generalization linear mixed models a pseudo-likelihood approach. Journal of statistical computation and simulation 48:3-4:233-243.