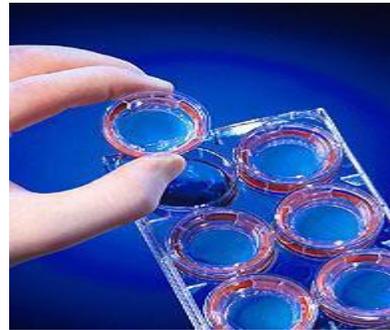


**United States Department of Agriculture  
Biotechnology Risk Assessment Grants Program  
Annual Project Director's Meeting**



USDA APHIS-BRS  
Riverdale, Maryland  
June 4, 2015



United States Department of Agriculture  
National Institute of Food and Agriculture



United States  
Department of  
Agriculture

National  
Institute of Food  
and Agriculture

# **USDA Biotechnology Risk Assessment Grants Program Annual Project Director's Meeting**

Welcome to the Annual Project Director's (PD) Meeting for the USDA Biotechnology Risk Assessment Grants (BRAG) Program. This year's meeting includes awardees of proposals submitted in fiscal years 2011, 2012, 2013, and 2014.

Authority for the BRAG program is contained in section 1668 of the Food, Agriculture, Conservation, and Trade Act of 1990 (i.e., 1990 Farm Bill) and amended in section 7210 of the Farm Security and Rural Investment Act of 2002 (i.e., 2002 Farm Bill). In the Food, Conservation, and Energy Act of 2008 (i.e., 2008 Farm Bill), the authority was not repealed, so the BRAG program continued its role in supporting risk assessment research related to biotechnology. In accordance with the legislative authority in the 2002 Farm Bill, the BRAG program supports research designed to identify and develop appropriate management practices to minimize physical and biological risks associated with genetically engineered (GE) animals, plants, and microorganisms. The USDA's National Institute of Food and Agriculture (NIFA) and Agricultural Research Service (ARS) jointly administer the BRAG program.

The main purpose of the BRAG program is to support the generation of new information that will assist Federal regulatory agencies in making science-based decisions about the effects of introducing into the environment GE organisms, including plants, microorganisms (including fungi, bacteria, and viruses), arthropods, fish, birds, mammals and other animals excluding humans. Investigations of effects on both managed and natural environments are relevant. The BRAG program accomplishes its purpose by providing Federal regulatory agencies with scientific information relevant to regulatory issues.

The overall goal of the PD Meetings is to improve post-award management of competitive grants administered by NIFA and ARS. It is the intent that these meetings will enhance communication and interaction between USDA Program Staff and BRAG awardees. In turn, this will assist Program Staff in identifying success stories resulting from USDA-

sponsored research in the BRAG program and facilitate the reporting of important impacts resulting from the most successful research through communications with Congress, the Secretary and Undersecretary of Agriculture, USDA administrators, federal regulators, the scientific community, commodity groups and other stakeholders, and the general public. It is critical to identify and highlight these impacts in order to maintain funding in USDA's biotechnology risk assessment program areas, as well as to continue the recent trend of increased Congressional budget appropriations to USDA competitive grant programs that have occurred since 2008. Conducting annual meetings for awardees is just one of several approaches being implemented by NIFA to improve post-award management.

A second purpose of this meeting is to foster communication among awardees in this program and federal regulators, such as USDA Animal and Plant Health Inspection Service, U.S. Environmental Protection Agency, and the U.S. Food and Drug Administration, which have scientific interests in risk assessment research. It is anticipated that the sharing of information and the ensuing dialogue that will occur in this informal setting will allow all awardees to benefit from the experiences of their colleagues and yield greater opportunity for successful completion of their BRAG awards. In addition, it is expected that improved communication among BRAG awardees will result in better sharing of limited resources and the development of new fruitful collaborations.

We look forward to a highly successful and productive meeting, and we eagerly anticipate continued progress on your BRAG awards.

Respectfully,

Shing F. Kwok, National Program Leader  
202-401-6060  
[skwok@nifa.usda.gov](mailto:skwok@nifa.usda.gov)

Lakshmi Matukumalli, National Program Leader  
202-401-1766  
[lmatumalli@nifa.usda.gov](mailto:lmatumalli@nifa.usda.gov)

Jack Okamuro, National Program Leader  
301-504-5912  
[jack.okamuro@ars.usda.gov](mailto:jack.okamuro@ars.usda.gov)

Desirée Abrams, Program Specialist  
202-401-5046

## TABLE OF CONTENTS

Description	Pages
<b>SCHEDULE</b> .....	<b>5</b>
<b>POSTER LIST</b> .....	<b>6</b>
<b>PROJECTS AWARDED BY YEAR</b> .....	<b>8</b>
<b>PROJECT REEPORTS (ordered by year and PD last name)</b> .....	<b>12</b>
Fostering Coexistence: Industry-Driven Field and Landscape on Pollen-Mediated Gene Flow in Genetically Engineered Alfalfa (Greene) .....	12
Antibody-Based Paratransgenics for Pierce’s Disease: Advanced Methods for Transmission Blocking and Environmental Monitoring (Durvasula) .....	16
TALEN-Mediated Chromosome Targeting for Monosexing and Genetics Containment in Livestock (Fahrenkrug) .....	19
Risk from Field-Evolved Resistance to Bt Corn by Western Corn Rootworm (Gassmann) .....	21
Genomic Approaches for Bt Resistance Risk Assessment and Improvement of Regulatory Triggers (Gould) .....	26
An Adaptive Framework for Non-Target Risk Assessment of RNAi Based, Insect Resistant GM Crops (Lundgren) .....	30
Transmission Genetics of Sorghum to Johnsongrass Gene Transfer (Paterson) .....	35
Gene Flow Networks and Potential Invasiveness of Perennial Biofuel Grasses (Miscanthus) (Snow) .....	38
Molecular Genetic Basis of Insect Resistance to Bt-Crops (Wang) .....	43
Linking Pollinator Behavior to Gene Flow to Reduce Gene Flow Risk over the Landscape (Brunet) .....	47
Assessing the Impact of Gene Replacement and Genetic Modification in a Crop Species at the Whole Genome Level (Douches) .....	50
Monitoring the Dispersal of Genetically Engineered Organisms and their Byproducts Using Light Transmission Spectroscopy (Egan).....	53

Silencing of Naturally Occurring Genes Controlling Seed Dormancy to Reduce Fitness of Transgene-Contaminated Weedy Rice (Gu) .....	56
Assessing the Risk of Transgene Escape via Pollen Flow in Carrot (Mandel).....	59
Switchgrass Bioconfinement: Delayed Flowering, Selective Male and Seed-Sterility, and Conditional Total Bioconfinement (Stewart) .....	65
Genome-Wide Assessment of Off-Target Effect and Removal of Transgenes Associated with TALEN-based Gene Editing in Plant (Yang).....	68
Resistance Risk Assessment for Seed Mixture Refuges with Pyramided Bt Corn (Carriere) .....	71
Targeted Gene Knockout of Reproductive Genes of Catfish with Hormone Therapy to Restore Fertility (Dunham) .....	74
Extended Pest Migration in Bt Versus Non-Transgenic Crops: Impacts on Risk Assessment and Bt Resistance Dissemination (Jurat-Fuentes). 76	
Risk Assessment for Plant Incorporated Insecticidal Products on Non Target Aquatic Invertebrates (Lamp) .....	80
Impact of Transgenic Bt Crops on <i>Helicoverpa Zea</i> Ecology and Subsequent Resistance Risk (Reisig) .....	84
Assessing Phenotypic Variations in Soybean Seed Protein and Oil Traits Using GFP as a Reporter in Both Mutagenesis and Transgenomic Approach (Schmidt).....	87
Efficacy and Ecological Impacts of Transgenic Containment Technologies in Poplar (Strauss) .....	91
<b>QUESTIONS FOR DISCUSSION.....</b>	<b>95</b>
<b>APPENDIX .....</b>	<b>96</b>

**USDA**  
**Biotechnology Risk Assessment Grants (BRAG) Program**  
**Project Director's Meeting**

**June 4, 2015**

USDA-APHIS-BRS Headquarters  
Oklahoma Memorial Conference Center  
4700 River Road  
Riverdale, MD 20737

- |                         |   |
|-------------------------|---|
| <b>8:30 - 9:00 AM</b>   | <b>Arrival and Poster Setup</b>   |
| <b>9:00 - 9:15 AM</b>   | Welcome<br>Shing Kwok – USDA-NIFA   |
| <b>9:15 - 9:35 AM</b>   | Research Priorities for Plant Pest Risk Assessment<br>John Turner and Sally McCammon– USDA-APHIS-BRS  |
| <b>9:35 - 9:55 AM</b>   | EPA Overview of Biotechnology Products and Research Needs<br>John Kough – EPA   |
| <b>9:55 - 10:15 AM</b>  | Environmental Assessment of GE Animals at FDA<br>Evgenij Evdokimov – FDA  |
| <b>10:15 - 10:30 AM</b> | <b>Break</b>  |
| <b>10:30 -10:50 AM</b>  | Fostering Coexistence: Industry-driven Field and Landscape Research on Pollen-mediated Gene Flow in Genetically Engineered Alfalfa<br>Stephanie Greene – USDA-ARS, Washington |
| <b>10:50 - 11:10 AM</b> | An Adaptive Framework for Non-Target Risk Assessment of RNAI-Based, Insect Resistant GM Crops<br>Jonathan Lundgren – USDA-ARS, South Dakota                                   |
| <b>11:10 - 11:30 AM</b> | TALEN-mediated Chromosome Targeting for Monosexing and Genetic Containment in Livestock –<br>Tad Sonstagar - Recombinetics Inc.   |
| <b>11:30 - 1:00 PM</b>  | <b>Lunch - On Your Own</b>  |
| <b>1:00 - 1:20 PM</b>   | Molecular Genetic Basis of Insect Resistance to Bt-Crops<br>Ping Wang – Cornell University  |

- 1:20 - 1:40 PM** Assessing the Impact of Gene Replacement and Genetic Modification Methods in a Crop Species at the Whole Genome Level  
David Douches – Michigan State University
- 1:40 - 2:00 PM** Genome-wide Assessment of Off-Target Effect and Removal of Transgenes Associated with TALEN-based Gene Editing in Plant  
Bing Yang – Iowa State University
- 2:00 - 3:00 PM** Discussion
- 3:00 - 3:15 PM** **Break**
- 3:15 - 5:00 PM** Poster Session

## 2015 BRAG PD Meeting Poster List

#	Name	Institution	Presentation Title
1	Johanne Brunet	USDA-ARS, WI	Linking Pollinator Behavior to Gene Flow to Reduce Gene Flow Risk over the Landscape
2	Brad Coates	USDA-ARS, IA	Risks from Field-Evolved Resistance to Bt Corn by Western Corn Rootworm
3	Rex Dunham	Auburn University	Targeted Gene Knockout of Reproductive Genes of Catfish with Hormone Therapy to Restore Fertility
4	Ravi Durvasula	New Mexico VA Healthcare System	Antibody-based Paratransgenics for Pierce's Disease: Advanced Methods for Transmission Blocking and Environmental Monitoring
5	Scott Egan	Rice University	Monitoring The Dispersal Of Genetically Engineered Organisms and Their Byproducts Using Light Transmission Spectroscopy
6	Fred Gould	North Carolina State University	Genomic Approaches for Bt Resistance Risk Assessment and Improvement of Regulatory Triggers
7	Xing-You Gu	South Dakota State University	Silencing of Naturally Occurring Genes Controlling Seed Dormancy to Reduce Fitness of Transgene-Contaminated Weedy Rice
8	Juan Jurat-Fuentes	University of Tennessee, Knoxville	Extended Pest Migration in Bt Versus Non-Transgenic Crops: Impacts on Risk Assessment and Bt Resistance Dissemination
9	William Lamp	University of Maryland, College Park	Risk Assessment for Plant Incorporated Insecticidal Products on Non-Target Aquatic Invertebrates
10	Jennifer Mandel	University of Memphis	Assessing the Risk of Transgene escape via Pollen Flow in Carrot
11	Andrew Paterson	University of Georgia, Athens	Transmission Genetics of Sorghum to Johnsongrass Gene Transfer
12	Dominic Reisig	North Carolina State University	Impact of Transgenic Bt Crops on Helicoverpa Zea Ecology and Subsequent Resistance Risk
13	Monica Schmidt	University of Arizona	Assessing Phenotypic Variations in Soybean Seed Protein and Oil Traits Using GFP as a Reporter in Both Mutagenesis and Transgenomic Approaches
14	Allison Snow	Ohio State University	Gene Flow Networks and Potential Invasiveness of Perennial Biofuel Grasses (Miscanthus)
15	Neal Stewart	University of Tennessee, Knoxville	Switchgrass Bioconfinement: Delayed Flowering, Selective Male- And Seed-Sterility, And Conditional Total Bioconfinement
16	Steven Strauss	Oregon State University	Efficacy and Ecological Impacts of Transgenic Containment Technologies in Poplar
17	Bruce Tabashnik	University of Arizona	Resistance Risk Assessment for Seed Mixture Refuges with Pyramided Bt Corn

## Awarded Projects by Year

### 2011

Project Director Name	Award Number	Proposal Title
Stephanie Greene	2011-33522-30733	Fostering Coexistence: Industry-Driven Field and Landscape Research on Pollen-Mediated Gene Flow in Genetically Engineered Alfalfa

### 2012

Project Director Name	Award Number	Proposal Title
Ravi Durvasula	2012-33522-19935	Antibody-based Paratransgenics for Pierce`s Disease: Advanced Methods for Transmission Blocking and Environmental Monitoring
Scott Fahrenkrug	2012-33522-19766	TALEN-Mediated Chromosome Targeting for Monosexing and Genetic Containment in Livestock
Aaron Gassmann	2012-33522-19766	Risks from Field-Evolved Resistance to Bt Corn by Western Corn Rootworm
Fred Gould	2012-33522-19793	Genomic Approaches for Bt Resistance Risk Assessment and Improvement of Regulatory Triggers
Jonathan Lundgren	2012-33522-19728	An Adaptive Framework for Non-Target Risk Assessment of RNAI-Based, Insect Resistant GM Crops
Andrew Paterson	2012-33522-19790	Transmission Genetics of Sorghum to Johnsongrass Gene Transfer
Allison Snow	2012-33522-19961	Gene Flow Networks and Potential Invasiveness of Perennial Biofuel Grasses (Miscanthus)
Ping Wang	2012-33522-19791	Molecular Genetic Basis of Insect Resistance to Bt-Crops

**2013**

<b>Project Director Name</b>	<b>Award Number</b>	<b>Proposal Title</b>
Johanne Brunet	2013-33522-20999	Linking Pollinator Behavior to Gene Flow to Reduce Gene Flow Risk over the Landscape
David Douches	2013-33522-21090	Assessing the Impact of Gene Replacement and Genetic Modification Methods in a Crop Species at the Whole Genome Level
Scott Egan	2013-33522-21007	Monitoring the Dispersal of Genetically Engineered Organisms and Their Byproducts Using Light Transmission Spectroscopy
Xingyou Gu	2013-33522-21097	Silencing of Naturally Occurring Genes Controlling Seed Dormancy to Reduce Fitness of Transgene contaminated Weedy Rice
Jennifer Mandel	2014-33522-21826	Assessing the Risk of Transgene Escape via Pollen Flow in Carrot
Neal Stewart	2013-33522-20997	Bioconfinement: Delayed Flowering, Selective Male and Seed-Sterility, And Conditional Total Bioconfinement
Bing Yang	2013-33522-21091	Genome-Wide Assessment of Off-Target Effect and Removal of Transgenes Associated With TALEN-based Gene Editing in Plant

**2014**

<b>Project Director Name</b>	<b>Award Number</b>	<b>Proposal Title</b>
Yves Carriere	2014-33522-22214	Resistance Risk Assessment for Seed Mixture Refuges with Pyramided Bt Corn
Rex Dunham	2014-33522-22263	Targeted Gene Knockout of Reproductive Genes of Catfish with Hormone Therapy to Restore Fertility
Juan Luis Jurat-Fuentes	2014-33522-22215	Extended Pest Migration in Bt versus Non-Transgenic Crops: Impacts on Risk Assessment and Bt Resistance Dissemination
William Lamp	2014-33522-22220	Risk Assessment for Plant Incorporated Insecticidal Products on Non Target Aquatic Invertebrates
Dominic Reisig	2014-33522-22265	Impact of Transgenic Bt Crops on Helicoverpa Zea Ecology and Subsequent Resistance Risk
Monica Schmidt	2014-33522-22531	Assessing Phenotypic Variations in Soybean Seed Protein and Oil Traits Using GFP as a Reporter in Both Mutagenesis and Transgenomic Approach
Steven Strauss	2014-33522-22216	Efficacy and Ecological Impacts of Transgenic Containment Technologies in Poplar

Please note: The listing of projects in the table above are all active projects (without a no-cost extension) in the BRAG program. For a full list of projects (research and conference) funded by the BRAG program, please go to this link:

<http://cris.nifa.usda.gov/cgi-bin/starfinder/0?path=fastlink1.txt&id=anon&pass=&search=GC=HX&format=WEBTITLESG>.

<b>Title:</b>	<b>Fostering coexistence: industry-driven field and landscape research on pollen-mediated gene flow in genetically engineered alfalfa</b>		
<b>Sponsoring Agency</b>	NIFA	<b>Project Status</b>	CHANGED
<b>Funding Source</b>	Non Formula	<b>Reporting Frequency</b>	Annual
<b>Accession No.</b>	226415	<b>Grants.gov No.</b>	GRANT10813008
<b>Project No.</b>	WNW-2011-02242	<b>Proposal No.</b>	2011-02242
<b>Project Start Date</b>	09/01/2011	<b>Project End Date</b>	08/31/2016
<b>Reporting Period Start Date</b>	09/01/2013	<b>Reporting Period End Date</b>	08/31/2014
<b>Submitted By</b>	Stephanie Greene	<b>Date Submitted to NIFA</b>	09/02/2014

**Program Code:** HX

**Program Name:** Biotechnology Risk Assessment

**Project Director**

Stephanie Greene

509.786.9265

stephanie.greene@ars.usda.gov

**Recipient Organization**

AGRICULTURAL RESEARCH SERVICE

800 BUCHANAN ST, RM 2020

Berkeley, CA 947101105

DUNS No. 136650657

**Performing Department**

Agricultural Research Service

**Co-Project Directors**

Boydston, Rick

Walsh, Doug

Martin, Ruth

**Departments**

Irrigated Ag. Res. & Ext. Center

Agricultural Research Center

Agricultural Research Service

**Non-Technical Summary**

On January 27, 2011, USDA-APHIS announced the complete deregulation of glyphosate-resistant alfalfa, colloquially known as Roundup-Ready alfalfa (RRA). Grower demand for RRA seed surged immediately, and acreage of RRA hay and seed is predicted to increase rapidly. Following a previous period of RRA deregulation (2005-2007), transgenes have been detected in conventional alfalfa, suggesting that current practices are not sufficiently protective to mitigate gene flow from RRA to conventional alfalfa and alfalfa seed. Certain key markets for U.S.-produced alfalfa hay and seed, including many export markets and the organic market, have little to no tolerance for the presence of transgenes. This project seeks to broaden our understanding of pollen flow biology and how it might influence the movement of the RRA transgene into conventional fields. Through nine specific research objectives, we intend to assess the role of feral alfalfa in transgene transmission; the impact of pollinators on pollen-mitigated gene flow; and the flow of transgenes from genetically engineered RRA hay fields to conventional alfalfa seed production fields in different environments. Our trans-disciplinary (genetics, molecular biology, entomology, weed science), multi-state (Washington, Oregon, California, Idaho), multi-institutional (USDA-ARS, Washington and Oregon; Washington State University) team brings a comprehensive skill set to the task of formulating science-based strategies for co-existence of GE alfalfa, conventional alfalfa, and other crops. Data we will gather in pursuit of these nine objectives should provide substantial information to help ensure hay and seed production for both GE sensitive and non-sensitive markets can continue to prosper in the United States.

**Accomplishments**

**Major goals of the project**

With the deregulation of Roundup-Ready alfalfa (RRA) in February 2011, there is an urgent need to complete and implement coexistence strategies to protect the export seed market and other alfalfa markets that are sensitive to the adventitious presence (AP) of transgenic traits. Extended conversations with alfalfa producers and breeding companies have led to the following objectives of our project: (1) to examine how leaf cutter and alkali bees transmit RRA across commercial seed fields and how that will impact proposed harvest strategies that separate seed for non AP- and AP- sensitive markets and to examine the persistence of RRA pollen in honeybee hives; (2) to characterize fitness parameters such as seed production, seed dormancy and viability, longevity in the seed bank, seedling establishment and plant persistence, in feral and feral-RRA hybrid alfalfa to determine how important and to what extent control strategies are required; (3) to track RRA transgene flow from RRA hay and seed production fields planted during the previous deregulation (seed fields were removed in 2007) into

feral alfalfa to understand the role feral alfalfa plays as a transgene reservoir and vector for long distance transgene dispersal; (4) to study the transmission of the RR transgene from RRA hay fields to conventional seed fields to refine isolation distances by taking into account landscape variables. Our objectives support the following goals: (1) understand the role feral alfalfa plays in transmitting the RRA transgene in important seed and hay areas in the states of Washington, Idaho and California; (2) test the relative efficacy of current stewardship practices in limiting the movement of the CP4 EPSPS transgene into the environment; (3) develop a greater understanding of the role pollinators play in transgene flow. Anticipated outputs include conducting and analyzing experiments and surveys that will allow us to confirm, refine and build on current management recommendations for isolating hay and seed fields, controlling feral alfalfa and managing pollinators to support GE-sensitive and non-sensitive alfalfa production in the United States

### What was accomplished under these goals?

In 2014 we accomplished the following activities in support of our project objectives:

Objective 1- Developed methods to isolate DNA from bees, alfalfa seeds and pollen provision balls for qPCR testing to look at various pollinators and their role in RR gene flow. Identified positive pollen from bee studies. We have determined using RR pollen as a biomarker that alkali bees are flying at least 2.3 miles while foraging during late bloom. We have documented increased incidence of RR seed associated with leaf cutting bee domiciles in conventional alfalfa seed field grown in close proximity to RR seed fields. We have completed a comprehensive survey of the "other" types of potential pollinating bees within and in close proximity to alfalfa seed fields in Washington State. We have completed the field phase of our honeybee objective. The lab phase is underway.

Objective 2- Implemented two field trials to evaluate fitness (seed longevity and ability to establish) of RR-alfalfa and conventional alfalfa. Alfalfa was seeded in small plots Nov. 2013 and seed packets buried. Plots were monitored for alfalfa emergence monthly throughout the year in both trials. We conducted the third year of a field trial evaluating the effects of auxin inhibitor herbicides on alfalfa seed development and viability. Seed was collected two weeks following herbicide treatments to conduct germination and emergence tests.

Objective 3- To support feral plant occurrence modeling, we obtained elevation, slope, and aspect data from the USGS National Elevation Dataset (NED) at a 30 x 30 m spatial resolution. Precipitation, maximum, and minimum temperature was obtained from PRISM. We obtained the latitude and longitudes of historic seed fields from Forage Genetics Inc. In June Monsanto provided data on the proximity of historic hay fields. We have concluded a preliminary analysis but have found that the data provided by Monsanto has limited value. We are currently negotiating with Monsanto to provide data that will allow us to implement an appropriate analysis, while still protecting the confidentiality of their hay growers.

Objective 4) We developed a seedling assay to quantify the occurrence of transgenic seedlings in the sink seedlots collected in 2013. We have assayed seedlots from key fields collected in Walla Walla and conducted a preliminary analysis. We are continuing to conduct assays, and we just concluded a second year of data collection from Walla Walla.

### What opportunities for training and professional development has the project provided?

Graduate students Natalie Boyle, Amber Vinchesi, and Amelia Jordan have all attended regional or national meetings of the Entomological Society of America. Post doctoral research associate, Sandya Kesoju attended and presented at the North American Alfalfa Improvement Conference, Lethbridge, CA July 2014.

### How have the results been disseminated to communities of interest?

Newsletters, field days, written reports to alfalfa seed organizations and presentations at industry and scientific meetings.

### What do you plan to do during the next reporting period to accomplish the goals?

We will be completing and writing a paper on our feral alfalfa survey work. We will complete our seedling assays on the field gene flow studies and begin to analyze the data. We will complete the final year of RR-alfalfa fitness testing at two sites, including burial of seed packets and recording alfalfa emergence. Both seed longevity and ability of plants to establish and persist in irrigated and non-irrigated sites will be determined. Longevity of honey bee pollen will be determined.

### Participants

#### Actual FTE's for this Reporting Period

Role	Non-Students or faculty	Students with Staffing Roles			Computed Total by Role
		Undergraduate	Graduate	Post-Doctorate	
Scientist	0	0	1.2	1	2.2
Professional	0.1	0	0	0	0.1
Technical	5.6	0.5	0	0	6.1

**Actual FTE's for this Reporting Period**

Role	Non-Students or faculty	Students with Staffing Roles			Computed Total by Role
		Undergraduate	Graduate	Post-Doctorate	
Administrative	0.1	0	0	0	0.1
Other	0	0	0	0	0
Computed Total	5.8	0.5	1.2	1	8.5

**Student Count by Classification of Instructional Programs (CIP) Code**

{NO DATA ENTERED}

**Target Audience**

Target audience reached during this reporting period included alfalfa hay and seed growers, alfalfa seed companies, and scientists focused on alfalfa seed and hay production and bee pollination

**Products**

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Published	2014	NO

**Citation**

Vinchesi, A.C. and D.B. Walsh. Quadrat Method for Assessing the Population Abundance of a Commercially Managed Native Soil-nesting Bee, *Nomia melanderi* Hymenoptera: Halictidae) in Proximity to Alfalfa Seed Production in the Western USA. *J. Econ. Entomology* 107:1695-1699.

Type	Status	Year Published	NIFA Support Acknowledged
Other	Published	2013	NO

**Citation**

Vinchesi, A. & D. Walsh. 2013. Leafcutter Bee Flight Monitoring. Western Alfalfa Seed Growers Newsletter.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2014	YES

**Citation**

Boyle, N. & D. Walsh. Estimating the foraging range of the alfalfa leafcutting bee (*Megachile rotundata*) using transgenic pollen as a marker. Pacific Branch of the Entomological Society, Tucson, AZ.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2014	YES

**Citation**

Jordan, A., R. Zack, & D. Walsh. 2014. Ufo's in alfalfa (*Medicago sativa*): Unveiling the pollinators. Pacific Branch of the Entomological Society, Tucson, AZ.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2013	YES

**Citation**

Boyle, N., & D. Walsh. 2013. Evaluating gene flow facilitated by the alfalfa leafcutting bee, *Megachile rotundata*, in alfalfa seed production. Entomological Society of America. Austin, TX.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2013	YES

**Citation**

Vinchesi, A. & D. Walsh. 2013. Measuring the foraging distance of the alkali bee, *Nomia melanderi*, using transgenic pollen as a marker. Entomological Society of America. Austin, TX.

Type	Status	Year Published	NIFA Support Acknowledged
Theses/Dissertations	Published	2014	NO

**Citation**

Jordan, A. 2014. A QUALITATIVE SURVEY OF NATIVE BEES IN AND NEAR ALFALFA (MEDICAGO SATIVA) SEED FIELDS IN WASHINGTON STATE. Washington State University

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2014	YES

**Citation**

Kesoju K, Greene S, Martin R, Boydston R 2014. Modeling feral alfalfa (*Medicago sativa* subsp. *sativa* L.) occurrence using topographical and environmental variables. North American Alfalfa Improvement Conference, Lethbridge, Canada, July 9, 10, 2014.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2014	YES

**Citation**

Greene S, Kesoju S, Martin R, Evans M, Boydston R, Walsh D. 2014. Ensuring coexistence of GE and non-GE alfalfa: status of current research efforts. North American Alfalfa Improvement Conference, Lethbridge, Canada, July 9, 10, 2014.

**Other Products****Product Type**

Data and Research Material

**Description**

1. Western Alfalfa Winter Seed Conference, Las Vegas, NV Jan. 25, 2014 – presented results of auxin inhibitor herbicide trials and their effects on developing alfalfa seed germination and emergence.
2. Washington Alfalfa Seed Commission Growers Meeting, Pasco, WA Feb. 20, 2014 –Presented results of auxin inhibitor herbicide trials and their effects on developing alfalfa seed germination and emergence.
3. Alfalfa Seed Grower Field Days, Touchet & Warden WA, June 2014- presented update on pollinator and gene flow studies

**Changes/Problems**

{Nothing to report}

<b>Title:</b>	<b>Antibody-based Paratransgenics for Pierce's Disease: Advanced Methods for Transmission Blocking and Environmental Monitoring</b>		
<b>Sponsoring Agency</b>	NIFA	<b>Project Status</b>	ACTIVE
<b>Funding Source</b>	Non Formula	<b>Reporting Frequency</b>	Annual
<b>Accession No.</b>	230151	<b>Grants.gov No.</b>	
<b>Project No.</b>	NMW-2012-01635	<b>Proposal No.</b>	2012-01635
<b>Project Start Date</b>	09/01/2012	<b>Project End Date</b>	08/31/2016
<b>Reporting Period Start Date</b>	09/01/2013	<b>Reporting Period End Date</b>	08/31/2014
<b>Submitted By</b>	Ravi Durvasula	<b>Date Submitted to NIFA</b>	11/08/2014

**Program Code:** HX

**Program Name:** Biotechnology Risk Assessment

**Project Director**

Ravi Durvasula  
505-265-1711  
ravi.durvasula@va.gov

**Recipient Organization**

BIOMEDICAL RESEARCH INSTITUTE OF NEW  
1501 SAN PEDRO DR SE # 14  
Albuquerque, NM 871085153  
DUNS No. 807430764

**Performing Department**

Medicine Services

**Co-Project Directors**

{NO DATA ENTERED}

**Departments**

{NO DATA ENTERED}

**Non-Technical Summary**

Paratransgenic strategies are under development for control of Pierce's disease transmission by sharpshooter vectors. Critical elements of the approach have been refined. *Pantoea agglomerans*, a symbiotic bacterium of *H. vitripennis* that maintains physical proximity to the causative agent *Xylella fastidiosa*, has been characterized and genetically transformed. Putative antibodies have been designed to disrupt *Xylella* transmission. Field dispersal approaches for genetically modified *Pantoea* are being modeled. The paratransgenic platform might have far-reaching applications in control of agricultural vector-borne diseases and similar approaches are under development for control of whitefly, thrip and locust-borne diseases. Field application of these technologies- still a future prospect- mandates rigorous risk assessment and mitigation strategies. The team of Durvasula, Kang and Miller has been pioneering paratransgenic approaches for over a decade. In this proposal, we introduce a dual risk mitigation strategy involving (1) an entirely novel molecule, the REDantibody, as a tool for both transmission blockade AND environmental monitoring of GMO spread and (2) a novel bioencapsulation approach with tunable nano-materials for containment of GMO release into the environment. The unique properties of this antibody- stability, embedded fluorescence, visual colorimetric detection and adaptability to a variety of pathogen repertoires- will be coupled with advanced microencapsulation techniques for bacterial containment and transgene stability to create a singular technology that could propel field application of paratransgenic control for Pierce's disease and, perhaps, other devastating vector-borne diseases of agriculture, worldwide.

**Accomplishments**

**Major goals of the project**

The overall aim of this four-year collaborative project involving University of New Mexico, Queen Mary College University of London in the UK and University of California at Riverside is to develop an advanced paratransgenic approach with recombinant antibodies directed at the transmission of *Xylella fastidiosa*, the causative agent of Pierce's disease, by the arthropod vector, *Homalodisca vitripennis*. This proposal will build upon ongoing paratransgenic studies of *H. vitripennis* (USDA/CSREES BRAG 2004-39454-15205, USDA/APHIS 08-8500-0510-GR and USDA/BRAG 2010-33120-21852) with particular focus on (1) development of recombinant antibodies that disrupt transmission of *Xylella* (2) engineering fluorescent antibodies that permit environmental monitoring of genetically modified symbiotic bacteria of *H. vitripennis* and (3) incorporation of microencapsulation technology to minimize unwanted environmental release of genetically transformed bacteria. An Environmental Monitoring System (EMS) for engineered organisms via embedded fluorescent tags will be developed as a platform technology that can be used in a variety of transgenic and paratransgenic systems for control of vector-borne agricultural diseases. This risk mitigation strategy will derive from the recently patented affinity fluorescent protein REDantibody described by two of the investigators on this proposal (Durvasula and Kang). The coupling of the

Environmental Monitoring System (EMS) with second-generation bioencapsulation techniques (derived from USDA/BRAG 2010-33120-21852) will result in the most advanced paratransgenic approach to date. This dual risk mitigation strategy, aimed at reducing environmental spread of transgenic organisms while providing robust tools to track their release and spread, is designed for field applications of paratransgenic control. Indeed, the Pierce's Disease application will be the prototype for this method of pathogen control. However, several other paratransgenic strategies aimed at agricultural and human diseases, under development in our laboratories, will be propelled toward field use as a result of this platform. Specific Aim 1: To express a recombinant REDantibody that recognizes key surface-exposed epitopes of *Xylella fastidiosa*, causative agent of Pierce's Disease, via *Pantoea agglomerans* E325, a symbiotic bacterium of the Glassy Winged Sharpshooter (GWSS), *Homalodisca vitripennis* Specific Aim 2a: To demonstrate, in closed-cage settings, the uptake of *P. agglomerans* transformed with REDantibody by *H. vitripennis* and colonization of the anterior cibarium of the arthropod Specific Aim 2b: To demonstrate, in closed-cage settings, the efficacy of selected REDantibodies in blocking transmission of *X. fastidiosa* by *H. vitripennis* Specific Aim 3: To develop a dual risk mitigation strategy that employs (1) fluorescent and colorimetric properties of REDantibodies within an Environmental Monitoring System (EMS) that tracks genetically modified bacteria in the rhizosphere and (2) second generation bioencapsulation technology using gated nano-materials to prevent escape of engineered bacteria into the environment

#### What was accomplished under these goals?

Five mice were commercially inoculated with heat inactivated *X. fastidiosa*. Spleens from these animals were harvested when an appropriate titer was achieved. Total RNA was extracted from one of the harvested spleens. Using PCR methodologies, we subsequently generated a library of single chain antibody (scFv) sequences with a T7 promoter and Kozak sequence on the 5' end and a Streptavidin tag on the 3' end from the extracted RNA. In vitro transcription and translation was performed with these PCR products. As each of the PCR products lack a stop codon, the translated antibodies are linked to the mRNA. These complexes were mixed individually with the 12 pre-selected surface-exposed antigen targets of *X. fastidiosa*. The Sterptavidin tag was utilized to pull down the antibody-antigen mRNA complexes. These experiments yielded a number of putative scFv sequences. These second round of enrichment is currently underway to further limit the size of the library.

#### What opportunities for training and professional development has the project provided?

{Nothing to report}

#### How have the results been disseminated to communities of interest?

The approach and preliminary results were presented as a poster at the annual USDA Biotechnology Risk Assessment Grants (BRAG) Program Project Director's Meeting, Riverdale, MD on June 5, 2014.

#### What do you plan to do during the next reporting period to accomplish the goals?

During the next period, we will:

1. Confirm the binding specificity of the scFv's against targeted surface-exposed epitopes of *X. fastidiosa*
2. Insert monomeric red fluorescent protein between the heavy and light chains of scFv's that bind with high specificity to *X. fastidiosa* to generate inherently fluorescent molecules, or REDantibodies, that would permit environmental monitoring of *P. agglomerans*, the symbiont of *H. vitripennis*
3. Verify that the *P. agglomerans* expressed REDantibodies can further disrupt transmission of *X. fastidiosa*

#### Participants

##### Actual FTE's for this Reporting Period

Role	Non-Students or faculty	Students with Staffing Roles			Computed Total by Role
		Undergraduate	Graduate	Post-Doctorate	
Scientist	2	0	0	2	4
Professional	0	0	0	0	0
Technical	0	0	0	0	0
Administrative	0	0	0	0	0
Other	0	0	0	0	0
Computed Total	2	0	0	2	4

#### Student Count by Classification of Instructional Programs (CIP) Code

Undergraduate	Graduate	Post-Doctorate	CIP Code
		2	26.02 Biochemistry, Biophysics and Molecular Biology.

**Target Audience**

1. Biotechnologists with a focus on environmental applications
2. Entomologist with interest in control of vector-borne disease

**Products**

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Other	2014	YES

**Citation**

Arora A, Forshaw A, Pesko K, Quintero-Hernandez V, Hurwitz i, Kang A, Miller T, and Durvasula R. Antibody-based Paratransgenics for Pierce's Disease: Advanced Methods for Transmission Blocking and Environmental Monitoring. Poster presentation at USDA Biotechnology Risk Assessment Grants (BRAG) Program Project Director's Meeting, Riverdale, MD 20737. June 5, 2014.

**Other Products**

{Nothing to report}

**Changes/Problems**

{Nothing to report}

<b>Title:</b>	<b>TALEN-mediated chromosome targeting for monosexing and genetic containment in livestock</b>		
<b>Sponsoring Agency</b>	NIFA	<b>Project Status</b>	ACTIVE
<b>Funding Source</b>	Non Formula	<b>Reporting Frequency</b>	Annual
<b>Accession No.</b>	229821	<b>Grants.gov No.</b>	
<b>Project No.</b>	MINW-2012-01628	<b>Proposal No.</b>	2012-01628
<b>Project Start Date</b>	08/01/2012	<b>Project End Date</b>	07/31/2015
<b>Reporting Period Start Date</b>	08/01/2013	<b>Reporting Period End Date</b>	07/31/2014
<b>Submitted By</b>	Scott Fahrenkrug	<b>Date Submitted to NIFA</b>	07/24/2014

**Program Code:** HX

**Program Name:** Biotechnology Risk Assessment

**Project Director**

Scott Fahrenkrug

612-727-2000

scott@recombinetics.com

**Recipient Organization**

RECOMBINETICS, INC.

1246 UNIVERSITY AVE W STE 301

SAINT PAUL, MN 55104

DUNS No. 829874523

**Performing Department**

{NO DATA ENTERED}

**Co-Project Directors**

Carlson, Daniel

Hackett, Perry

**Departments**

{NO DATA ENTERED}

**Non-Technical Summary**

Genetic engineering could provide for dramatic improvements in the sustainability of agricultural animal production. The use of genetically engineered animal products would be facilitated by methods that 1) reduce the risk of GE animals to the environment; 2) reduce the potential for transfer of transgenes beyond breeding stock; and 3) demonstrate that GE animals will not have an effect on wild species. We hypothesize that editing of the pig genome can be used to develop lines of animals that either produce only females, or lines of pigs that fail to undergo sexual maturation unless managed in a breeding facility with pre-established protocols for puberty-induction. Monosexing and infertility can be used to effectively control the dispersion of genetics from engineered animals. Such control will facilitate the introduction of engineered animals into the U.S. Biomedical and Food Agriculture marketplace.

**Accomplishments**

**Major goals of the project**

Genetic engineering can be used to develop lines of pigs that either produce only females, or that are incapable of undergoing sexual maturation without intervention. We will use TAL-effector nucleases (TALENs) to direct either single-basepair changes, or to direct integration of expression cassettes to specific genetic loci in pig genome. In our first aim we will assess two loci on the swine Y-chromosome for their target-ability and amenability to express transgenes intended to disrupt male sperm function. Boars containing this modification should only be capable of producing daughters. For aim two we will implement a method for TALEN-mediated, reversible sterilization based on targeted inactivation of a gene required for maturation. Pigs treated in this way are predicted to remain pre-pubertal and infertile unless treated with a compound to induce sexual maturation. The consequence will be that the animals will only be able to be propagated in a breeding facility with pre-established protocols for puberty induction.

**What was accomplished under these goals?**

**Y-chromosome targeting.** Gene targeting of the Y chromosome was accomplished in the last reporting period using a variety of homology repair templates and strategies. These strategies all depended on either a selectable marker, or visual marker transgene to identify correctly targeted colonies. During the 2014 period, we were able to successfully target the Y chromosome without the aid of selectable or visual markers. This is critical for applications where tissue specific transgenes are desired without a having a ubiquitously express marker transgene in close proximity. Using this approach, we introduced EGFP into 3' of the SRY locus under the control of the late spermatogenesis promoter, SP10. The cells were used for cloning in early July 2014, and parturition is expected in early November.

**Reversible sterilization.** The KissR homozygous knockout males were born in March, 2014. A total of 18 live piglets were produced from 3 embryo transfers, and all piglets are of good health. Now nearly 4 months of age, there is strong evidence of the expected phenotype of hypogonadotropic hypogonadism. Testicles are extremely small, nearly impalpable.

**What opportunities for training and professional development has the project provided?**

This project has required extensive research into the biology of puberty and treatment regimens for hypogonadotropic hypogonadism. It has also encouraged partnership with medical doctors for advice on treatments that are typically only used for humans.

**How have the results been disseminated to communities of interest?**

The results will be published when complete, otherwise shared with members of the community that can provide input on study methods.

**What do you plan to do during the next reporting period to accomplish the goals?**

**Y-chromosome targeting.** The Y-targeted boar clones will be 7-8 months of age by July, 2015. By that time it is possible to have early semen collections for EGFP analysis. In addition, hemi-castration may be conducted on select individuals for IHC detection of EGFP in the appropriately staged spermatids.

**Reversible sterilization.** Coincident with the expected timeline of puberty, a portion of the pigs will receive gonadotropin treatments to measure influence on GnRH response, serum testosterone, testicle size and spermatogenesis.

**Participants**

**Actual FTE's for this Reporting Period**

Role	Non-Students or faculty	Students with Staffing Roles			Computed Total by Role
		Undergraduate	Graduate	Post-Doctorate	
Scientist	0.5	0	0	0	0.5
Professional	0	0	0	0	0
Technical	0	0	0	0	0
Administrative	0	0	0	0	0
Other	0	0	0	0	0
Computed Total	0.5	0	0	0	0.5

**Student Count by Classification of Instructional Programs (CIP) Code**

{NO DATA ENTERED}

**Target Audience**

Livestock genetics companies that need to protect their genetics from environmental escape. This could include pigs, cattle, goats, sheep and aquaculture. In addition, the KissR pigs may be of interest for pre-clinical testing of novel treatments for hypogonadotropic hypogonadism.

**Products**

{Nothing to report}

**Other Products**

{Nothing to report}

**Changes/Problems**

{Nothing to report}

<b>Title:</b>	<b>Risks from Field-Evolved Resistance to Bt Corn by Western Corn Rootworm</b>		
<b>Sponsoring Agency</b>	NIFA	<b>Project Status</b>	ACTIVE
<b>Funding Source</b>	Non Formula	<b>Reporting Frequency</b>	Annual
<b>Accession No.</b>	230280	<b>Grants.gov No.</b>	GRANT11452114
<b>Project No.</b>	IOW05330	<b>Proposal No.</b>	2012-01630
<b>Project Start Date</b>	09/01/2012	<b>Project End Date</b>	08/31/2015
<b>Reporting Period Start Date</b>	09/01/2013	<b>Reporting Period End Date</b>	08/31/2014
<b>Submitted By</b>	Cathy Good	<b>Date Submitted to NIFA</b>	11/24/2014

**Program Code:** HX

**Program Name:** Biotechnology Risk Assessment

**Project Director**

Aaron Gassmann  
515-451-0568  
aaronjg@iastate.edu

**Recipient Organization**

IOWA STATE UNIVERSITY OF SCIENCE AND  
1350 BEARDSHEAR HALL  
Ames, IA 500112025  
DUNS No. 005309844

**Performing Department**

{NO DATA ENTERED}

**Co-Project Directors**

Coates, Brad

**Departments**

{NO DATA ENTERED}

**Non-Technical Summary**

Transgenic corn producing insecticidal toxins from the bacterium *Bacillus thuringiensis* (Bt) has been adopted rapidly by farmers, providing control of key insect pests and reducing use of conventional insecticides. The development of Bt resistance by pests is the single greatest threat to this technology. A key pest controlled by Bt corn is the western corn rootworm *Diabrotica virgifera virgifera*. Beginning in 2009, field populations of western corn rootworm were identified in Iowa with resistance to corn that produces Bt toxin Cry3Bb1. Because resistance may spread and develop independently in other regions of the United States, these first cases of resistance present a risk Bt corn containing Cry3Bb1 and to other types of Bt corn, if resistance to Bt toxin Cry3Bb1 also confers resistance to other commercialized Bt toxins. Additionally, these first cases of resistance highlight the potential vulnerability of other types of Bt corn targeting western corn rootworm. Currently, there is uncertainty about how to best measure resistance and the rate at which resistance will spread. A multidisciplinary research team has been assembled to address these risks and uncertainties. We are proposing research that is directly relevant to the biotechnology risk assessment grant's stated purpose of generating new information to assist federal regulatory agencies in making science-based decisions. We will determine the best method for measuring resistance and will test for cross resistance to other Bt toxins. The potential for resistance to spread and for the independent evolution of resistance will be evaluated in part by measuring whether or not, in the absence of Bt corn, Bt-resistant insects are at a disadvantage relative to susceptible insects (i.e. whether or not fitness costs of resistance are present). We also will measure the inheritance of resistance to understand how effective refuges may be at delaying resistance. We will apply multiple genomic tools to develop molecular markers and to identify genes associated with resistance, which will enable monitoring and detection of resistance before it reaches levels that threaten Bt corn in additional fields.

**Accomplishments**

**Major goals of the project**

A multidisciplinary team has been assembled that will study Bt resistance from the level of the gene to the population, and in doing so, will assess the risks that field-evolved resistance to Cry3Bb1 corn presents to single-trait and pyramided-trait technologies. These goals will be accomplished by completing the following objectives. 1) Measure resistance and cross resistance in the laboratory and in the field; 1a) Test which of several laboratory methods best characterizes resistance in the field; 2b) Measure resistance and cross resistance in fields with a history of cultivation of Bt corn and documented injury to Bt corn by western corn rootworm; 2) Measure inheritance of resistance and fitness costs of resistance for strains of Cry3Bb1-resistant western corn rootworm collected from the field; 3) Conduct quantitative trait locus analysis based on single nucleotide polymorphisms to identify candidate genes and markers associated with Bt resistance in western corn rootworm; 4) Analyze the midgut transcriptome for western corn rootworm strains with field-evolved Bt resistance 4a) test for the

mechanisms of resistance; 4b) develop molecular markers Outputs from this research will include field experiments, laboratory experiments, and molecular analysis. Through objective 1, we will conduct experiments in fields with Bt-resistant western corn rootworm to test the level of resistance and cross resistance, which will directly assess the risk to other single traits and pyramided traits. Furthermore, by testing several laboratory and greenhouse bioassays we will provide critical information on how to best characterize resistance. In objective 2, we will conduct laboratory experiments with field-collected, Cry3Bb1-resistant strains to test the inheritance of resistance and to measure fitness costs of resistance, both of which will quantify risks associated with the persistence of resistance in the field, and the rate at which resistance may spread or evolve independently in other populations. Molecular markers developed through objectives 3 and 4 will enable resistance to be monitored in the field before it reaches levels that cause field failures. Elucidating the molecular basis of resistance also will provide a better understanding of potential cross resistance and provide fundamental knowledge that may be applied to combat resistant populations with new technologies.

### **What was accomplished under these goals?**

Genetic engineering of crop plants to produce insecticidal toxins derived from the bacterium *Bacillus thuringiensis* (Bt), is a recently developed and highly efficacious technology. In addition to providing a simple solution for managing populations of harmful insect pests, Bt crops often lead to reductions in the use of broader spectrum conventional insecticides, which in turn reduces the environmental footprint of agriculture. The most significant threat to the success of Bt crops is the evolution of Bt resistance by pest insects. Western corn rootworm is the most serious pest of corn in the Midwest and is currently managed by planting of Bt corn. Recent cases of Bt resistance by western corn rootworm raise concerns about the long-term viability of this technology. Research conducted under this grant over the past year has been focused on understanding the extent of Bt resistance among field populations of western corn rootworm, the likelihood that Bt resistance will arise in additional populations and persist once present, and the molecular basis of this resistance. Knowledge generated from this grant will aid in preserving the effectiveness of Bt corn for management of western corn rootworm, and more broadly, will enable Bt crops to be applied in a more sustainable manner for management of other insect pests.

Bioassays were conducted on over a dozen populations of western corn rootworm sampled from Iowa and Nebraska. Data from these bioassays found resistance to two of the four currently commercialized Bt toxins (mCry3A and Cry3Bb1). Data on these cases of resistance and the occurrence of cross-resistance will enable farms and technology providers to adjust their management practices to mitigate the effects of resistance on crop yields.

Additional experiments were conducted to compare bioassay approaches for measuring resistance. While work is still ongoing in this effort, the goal is to provide industry, regulators and other scientists with comparative data on bioassay approaches for measuring Bt resistance in western corn rootworm.

Data on fitness costs of resistance and inheritance of resistance among field populations were collected. These data will aid in predicting the rate at which resistance will develop across the landscape and the extent to which resistance will persist within populations once established. Two complementary approaches are underway to understand the molecular mechanisms of resistance. Data from these experiments will enable more efficient monitoring of resistance based on molecular markers, and may provide new approaches to manage Bt-resistant pests by targeting those genes responsible for resistance. One approach we are taking to cast light on the molecular mechanism of resistance is the identification of quantitative trait loci associated with resistance. To this end, initial crosses between male and female western corn rootworm were conducted to establish families with segregating traits for resistance to Cry3Bb1. Resulting F1 progeny were mated en masse and subsequent F2 feed either non-Bt corn or Cry3Bb1 corn, where survivors of the latter group were assigned a resistant phenotype. Subsequent extraction of genomic DNA and sequencing will enable marker identification. A second approach was to study genes specific to the insect midgut, which is the site of activity for Bt toxins. High throughput sequencing of mRNA ("RNA-Seq") data have been comprehensively analyzed to identify differentially-expressed genes between Cry3Bb1-resistant and Cry3Bb1-susceptible western corn rootworm larvae after 4 or 8 hours of feeding on Cry3Bb1 corn or non-Bt corn. Very few genes were differentially expressed after 4 hours of feeding but more were differentially expressed after 8 hours. Genes encoding a cadherin and an aminopeptidase N were expressed at a higher level in the resistant population, whereas genes encoding several metalloprotease enzymes and papilin protease inhibitors were expressed at a lower level in the resistant population. Several genes encoding a variety of ATP binding cassette (ABC) transporters were differentially expressed: one was expressed at a higher level in the resistant population and nine were expressed at a lower level in the resistant population.

### **What opportunities for training and professional development has the project provided?**

Two Ph.D. students and one M.S. student have received training as part of this grant. One Ph.D. student was given hands-on training in the analysis of high-throughput sequencing data, and a second Ph.D. student conducted bioassays to measure patterns of resistance and cross-resistance among field populations. An M.S. student conducted research on the fitness costs and inheritance of resistance. These graduate students presented their results at scientific conferences including the National Meeting of the Entomological Society of America, and have contributed to the writing of peer-reviewed publications from this research.

**How have the results been disseminated to communities of interest?**

Data from this research contributed to ten presentations at scientific conferences, including the national meeting of the Entomological Society of America and the European Congress of Entomology. In Iowa, results contributed to outreach presents at 1) Iowa State University's Integrated Crops Management Conference, 2) Iowa Soybean Association's On-Farm Network Conference, and 3) Ohio Agribusiness Association Industry Conference. Data were shared with various industry stakeholders. Slides based on this research were made available to Dr. Erin Hodgson, Iowa State University Extension Entomologist, and to Iowa State University field agronomists for use in their meetings with clientele. In Nebraska, results were presented to the Independent Crop Consultant Association and at meetings with farmers.

**What do you plan to do during the next reporting period to accomplish the goals?**

Research will continue under all objectives. For objective one, bioassay will be conducted to assess resistance of field populations to Bt corn. For objective two, experiments will be run to measure fitness costs and inheritance of resistance in diapausing and non-diapausing strains. Inbred lines developed during year two will be used in QTL mapping of resistance traits as described under objective three. For objective four, temporal expression profiles of differentially expressed genes will be investigated by quantitative PCR. The RNA-Seq data set will be analyzed to identify sequence polymorphisms associated with Bt resistance.

**Participants****Actual FTE's for this Reporting Period**

Role	Non-Students or faculty	Students with Staffing Roles			Computed Total by Role
		Undergraduate	Graduate	Post-Doctorate	
Scientist	0.1	0	0	0	0.1
Professional	0.3	0	1.3	0	1.6
Technical	0	0.5	0	0	0.5
Administrative	0	0	0	0	0
Other	0	0	0	0	0
Computed Total	0.4	0.5	1.3	0	2.2

**Student Count by Classification of Instructional Programs (CIP) Code**

Undergraduate	Graduate	Post-Doctorate	CIP Code
3	3		01.00 Agriculture, General.

**Target Audience**

The target audience included agricultural companies, biotechnology companies, extension specialists, farmers, members of the scientific community, regional agronomists, regulators, and the general public.

**Products**

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2014	YES

**Citation**

Rault, L., Miller, N., Wang, H., Gassmann, A. and Siegfried, B. 2014. Expression profile by next-generation sequencing of western corn rootworm (*Diabrotica virgifera virgifera*) neonates exposed to Bt toxin Cry3Bb1. International Working Group on Ostrinia and other Maize Pests. Chicago. Illinois (poster).

Type	Status	Year Published	NIFA Support Acknowledged
Other	Published	2014	YES

**Citation**

Meinke, L., D. Wangila, R. Wright, T. Hunt, and G. Kruger. 2014. UNL documents shift in corn susceptibility to rootworms in Nebraska. CropWatch Newsletter. Univ. Nebraska-Lincoln Extension. 10 April 2014.

Type	Status	Year Published	NIFA Support Acknowledged
Other	Published	2014	YES

**Citation**

Meinke, L., D. Wangila, R. Wright, T. Hunt, and G. Kruger. 2014. Corn rootworm management update. pp. 30-34 in: 2014 Proceed. Crop Protection Clinics. Univ. Nebraska-Lincoln Extension.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2014	YES

**Citation**

Meinke, L.J., B.D. Siegfried, and D.S. Wangila. Adaptation by *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) to management practices in Nebraska: Historical and current perspectives. Presented as part of symposium "Western corn rootworm management in Europe and the United States: recent developments and challenges". European Congress of Entomology. York, UK. 7 August 2014.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2014	YES

**Citation**

Wangila, D.S. and L.J. Meinke. Field-evolved resistance to Bt toxin Cry3Bb1 and mCry3A in Nebraska western corn rootworm populations. North Central Branch- Entomological Society of America annual meeting. Des Moines, IA. 10 March 2014.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2014	YES

**Citation**

Meinke, L.J. Rootworm Bt trait/insecticide combinations: Potential impact on western corn rootworm biology and management. Presented as part of symposium "Advances and challenges in western corn rootworm management". North Central Branch- Entomological Society of America annual meeting. Des Moines, IA. 12 March 2014.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2014	YES

**Citation**

Wangila, D.S. and L.J. Meinke. Susceptibility of western corn rootworm (*Diabrotica virgifera virgifera*, LeConte, Coleoptera: Chrysomelidae) populations collected in Nebraska to Bt corn events. Poster presentation: International working group on *Ostrinia* and other maize pests, Chicago, Ill. 14-17 April 2014.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2014	YES

**Citation**

Meinke, L.J., Hunt, T., Wright, R., Kruger, G., Wangila, D., Miwa, K. Rootworm-Bt trait/insecticide combinations: Potential impact on western corn rootworm larval control and adult production in trait failure vs. non-failure history fields. Presented as part of the symposium "Corn rootworm management: current status, challenges & novel strategies". Entomological Society of America national meeting, Austin, TX. 10 November 2013.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2014	YES

**Citation**

Wangila, D.S. and L.J. Meinke. Susceptibility of western corn rootworm (*Diabrotica virgifera virgifera*, LeConte, Coleoptera: Chrysomelidae) populations collected in Nebraska to Bt corn events. Poster presentation: Entomological Society of America national meeting, Austin, TX. 11 November 2013.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2014	YES

**Citation**

Gassmann, A.J., Clifton, E.H., Dunbar, M.W., Hoffmann, A.M., Ingber, D.A., Jakka, S., Petzold-Maxwell, J.L., Rudeen, M., and Shrestha, R.B. 2014. Bt resistance in western corn rootworm: A model for understanding pest management with Bt crops that are not high dose. International Working Group on Ostrinia and other Maize Pests. Chicago. Illinois.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2014	YES

**Citation**

Gassmann, A.J., Petzold-Maxwell, J.L., Clifton, E.H., Dunbar, M.W., Hoffmann, A.M. and Ingber, D.A. 2013. Resistance of western corn rootworm to Bt corn: data from the laboratory and field. Entomological Society of America. Austin, Texas, USA.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2014	YES

**Citation**

Gassmann, A.J., Clifton, E.H., Dunbar, M.W., Hoffmann, A.M., Ingber, D.A., Jakka, S., Petzold-Maxwell, J.L., Rudeen, M., and Shrestha, R.B. 2014. Bt resistance in western corn rootworm: a model for understanding pest management with Bt crops that are not high dose. North Central Branch Meeting, Entomological Society of America. Des Moines, Iowa, USA.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2014	YES

**Citation**

Gassmann, A.J. 2014. Bt resistance and western corn rootworm: challenges and considerations for managing a pest with less than a high-dose Bt crop. European Congress of Entomology. York, England.

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Published	2014	YES

**Citation**

Gassmann, A.J., Petzold-Maxwell, J.L., Clifton, E.H., Dunbar, M.W., Hoffmann, A.M., Ingber, D.A. and Keweshan, R.S. 2014. Field-evolved resistance by western corn rootworm to multiple *Bacillus thuringiensis* toxins in transgenic maize. Proceedings of the National Academy of Sciences USA 111:5141-5146.

**Other Products**

**Product Type**

Audio or Video

**Description**

Gassmann, A.J. 2014. Resistance evolution and IRM for rootworm. Plant Management Network.

**Changes/Problems**

{Nothing to report}

<b>Title:</b>	<b>Genomic Approaches for Bt Resistance Risk Assessment and Improvement of Regulatory Triggers</b>		
<b>Sponsoring Agency</b>	NIFA	<b>Project Status</b>	ACTIVE
<b>Funding Source</b>	Non Formula	<b>Reporting Frequency</b>	Annual
<b>Accession No.</b>	229537	<b>Grants.gov No.</b>	GRANT11046442
<b>Project No.</b>	NC09243	<b>Proposal No.</b>	2012-01637
<b>Project Start Date</b>	09/01/2012	<b>Project End Date</b>	08/31/2015
<b>Reporting Period Start Date</b>	09/01/2013	<b>Reporting Period End Date</b>	08/31/2014
<b>Submitted By</b>	Dawn Piercy	<b>Date Submitted to NIFA</b>	09/03/2014

**Program Code:** HX

**Program Name:** Biotechnology Risk Assessment

**Project Director**

F Gould  
919-515-1647  
fred\_gould@ncsu.edu

**Recipient Organization**

NORTH CAROLINA STATE UNIVERSITY  
2701 SULLIVAN DR STE 240 CAMPUS BX 7514  
Raleigh, NC 276957003  
DUNS No. 042092122

**Performing Department**

Entomology

**Co-Project Directors**

{NO DATA ENTERED}

**Departments**

{NO DATA ENTERED}

**Non-Technical Summary**

The US government views insecticidal properties of the bacterium, *Bacillus thuringiensis* (Bt), as a "public good" and has taken actions to ensure that toxin genes from this organism that are moved into transgenic crops are used in a manner that decreases the risk of pests evolving resistance and eroding this public good. Organic farmers have used this special insecticide for over 50 years. The US-EPA and USDA concluded that monitoring for resistance could improve resistance management practices and decrease resistance risk. However, current monitoring methods are inadequate. We will use new genomic tools in concert with field data to better assess both the current extent of Bt resistance in *Heliothis virescens* and *Helicoverpa zea* moths and the rate at which resistance is increasing, if at all. This will be accomplished by developing tools that detect changes in the frequencies of alleles of candidate Bt resistance genes and also detect changes in genetic sequences that confer Bt resistance but are not in genomic regions associated with currently identified Bt resistance candidate genes. We have annually archived thousands of samples of *H. virescens* and *H. zea* from 1993 until 2011, and will use these valuable samples to predict future changes in resistance from past patterns of change and current planting patterns of Bt cultivars. While the proposed research focuses on Bt resistance, the tools developed could also be used to improve monitoring of resistance to future insecticidal crop traits as well as for monitoring weed resistance relevant to transgenic, herbicide tolerant crops.

**Accomplishments**

**Major goals of the project**

We will use genomic tools in concert with field data to better assess both the current extent of Bt resistance and the rate at which resistance is increasing, if at all. This will be accomplished by developing genomic tools that detect changes in the frequencies of alleles of candidate Bt resistance genes. Furthermore, the genomic techniques developed will be able to detect changes in genetic sequences that confer Bt resistance but are not in genomic regions associated with currently identified Bt resistance candidate genes. This work will be possible because our lab has been archiving samples of *H. virescens* and *H. zea* from Mississippi and Louisiana from 1993 to the present. Other areas have been sampled, but not as regularly. Our lab-strains of *H. virescens* with known genes for resistance to Bt toxins will serve as positive controls for the robustness of the genomic techniques. Specific Goals: 1) Address the untested hypotheses that; a) field populations of *Helicoverpa zea* are accumulating Bt resistance genes more rapidly than populations of *Heliothis virescens*, b) the rate at which Bt resistance is evolving in *H. zea* has decreased since the introduction of dual toxin cultivars, c) in both *H. virescens* and *H. zea* alleles for resistance to pyrethroids have decreased in frequency. 2) Assess the risk of future emergence of economically important Bt resistance in field populations based on time series analysis of allele frequencies from archived material and the newly implemented resistance management requirements for multi-toxin Bt cultivars. Supporting Objectives: 1) Determine if there have been changes in the frequency of alleles of any candidate genes for Bt or pyrethroid resistance in *H. zea* and *H.*

virescens between 1993 and 2012. 2) Determine if there are genomic signatures of response to Bt selection in regions of the genome that are not associated with Bt resistance candidate genes. 3) If genomic changes are found in *H. zea* based on objectives 1 and/or 2, collect surviving larvae from Bt and non-Bt corn to determine if survivors from Bt corn show enrichment in specific alleles. 4) Use all data from objectives 1, 2, and 3 to predict future levels of resistance to Bt cultivars.

### What was accomplished under these goals?

In March of 2014 we completed RAD library preparation and sequencing of susceptible and Bt resistant laboratory-reared *Heliothis virescens* (n = 45 per population). Marker generation for these colony strains, where genes under selection are known, serves as an important demonstration of the ability to use this technique for detecting signatures of directional selection. In total, we developed over 2000 polymorphic markers that could be used to examine population-level divergence due to selection. A greater percentage of these markers have low nucleotide diversity in our resistant strain relative to that of our susceptible strain (Figure 1), which is indicative of either directional selection or genetic drift. We are continuing to examine these markers for other indicators of directional selection. Our methods, and preliminary results have been presented in a number of venues, including the National Entomological Society meeting in 2013, and NCSU for the Post-doctoral Association and the Program in Genetics. We expect to submit this work for publication by November of 2014. To determine where these markers are found positionally in the genome, we used RAD-seq to develop markers for a mapping family of 97 progeny. We selected 659 high quality molecular markers (bp = 350), and used them to develop a dense *H. virescens* genetic map (total length = 1919.5 cM; Figure 2) containing 33 linkage groups. Markers from our colony strains were aligned to this genetic map in a downstream analysis. We have examined nucleotide diversity estimates along 3 chromosomes: two contain genes known to contribute to resistance, and one is a control, where selection should be identical between strains (add LG figures here?). One challenge that we've faced is that the ability to detect a signature of selection is influenced by the distance between markers. Despite the density of our map, there are an average of 560 KB between our markers, based on the estimated *H. virescens* genome size of 375 MB. To overcome these challenges, we are sequencing the genome of *H. virescens*, which will allow us to improve the density of markers in our genomic scan for selection. We have Illumina data in hand, and will begin the process of genome assembly in September 2014. Following the methodology of the *Heliconius* genome project, we plan to use our linkage map as a scaffold for our *H. virescens* genome assembly. More recently, we constructed and sequenced 3 additional RAD-seq libraries of field-collected *H. virescens*. These libraries were prepared using individuals from two different field sites in the years 1997, 2007 and 2012 (representing pre- and post-deployment of Bt crops). Sequence data from these libraries will also be aligned to our draft genome sequence prior to analysis. We are currently preparing RAD-seq libraries for *Helicoverpa zea*, and plan to use these data for an interspecific comparison with *H. virescens*. We aim to have these data in hand by December of 2014, and submit this work for publication in spring of 2015.

### Figure Captions

Figure 1: Distribution of nucleotide diversity estimates for marker sequences from susceptible (above) and Bt resistant (below) *Heliothis virescens* populations.

Figure 2: RAD-seq linkage map for *Heliothis virescens*

### What opportunities for training and professional development has the project provided?

The postdoc on this project, Megan Fritz, has learned a huge amount about quantitative genetics and bioinformatics. She has done a great job of helping other students, staff, and faculty to understand more about RADtag analysis by developing a journal club on the subject.

She has also trained two additional undergraduates in the lab on molecular techniques in 2013-2014.

This past summer Megan has also trained a local middle school teacher.

### How have the results been disseminated to communities of interest?

Talk at ESA meeting in Winter 2013.

Information transferred to middle school teacher is now on u-tube and will be used by other teachers.

### What do you plan to do during the next reporting period to accomplish the goals?

We are in the midst of writing one paper on the results with lab populations and with development of the linkage map. We are moving on to analysis of wild populations.

### Participants

**Actual FTE's for this Reporting Period**

Role	Non-Students or faculty	Students with Staffing Roles			Computed Total by Role
		Undergraduate	Graduate	Post-Doctorate	
Scientist	0.1	0	0	1	1.1
Professional	0	0	0	0	0
Technical	0.5	0	0	0	0.5
Administrative	0	0	0	0	0
Other	0	0	0	0	0
Computed Total	0.6	0	0	1	1.6

**Student Count by Classification of Instructional Programs (CIP) Code**

{NO DATA ENTERED}

**Target Audience**

We are still in the midst of our data collection and analysis. We have presented one talk about the work at the Entomological Society of America meeting in 2013.

We gave an oral presentation at the BRAG meeting in spring 2014.

We have also helped with training other NCSU staff on the use of RADtag next generation sequencing and analysis

**Products**

{Nothing to report}

**Other Products**

{Nothing to report}

**Changes/Problems**

In going through our oldest field samples, we found that in some cases the DNA was too degraded for RADtag analysis. We will first analyze samples from 2001 through 2014 with RADtags and then use PCR to pull out alleles of interest in the older samples.

<b>Title:</b>	<b>AN ADAPTIVE FRAMEWORK FOR NON-TARGET RISK ASSESSMENT OF RNAI-BASED, INSECT RESISTANT GM CROPS</b>		
<b>Sponsoring Agency</b>	NIFA	<b>Project Status</b>	ACTIVE
<b>Funding Source</b>	Non Formula	<b>Reporting Frequency</b>	Annual
<b>Accession No.</b>	229526	<b>Grants.gov No.</b>	GRANT11050008
<b>Project No.</b>	SDW-2012-01639	<b>Proposal No.</b>	2012-01639
<b>Project Start Date</b>	09/01/2012	<b>Project End Date</b>	08/31/2015
<b>Reporting Period Start Date</b>	09/01/2013	<b>Reporting Period End Date</b>	08/31/2014
<b>Submitted By</b>	Jonathan Lundgren	<b>Date Submitted to NIFA</b>	12/02/2014

**Program Code:** HX

**Program Name:** Biotechnology Risk Assessment

**Project Director**

Jonathan Lundgren

605-693-5211

jonathan.lundgren@ars.usda.gov

**Recipient Organization**

AGRICULTURAL RESEARCH SERVICE

2150 CENTRE AVE BLDG D

Fort Collins, CO 805268119

DUNS No. 837350560

**Performing Department**

NCARL

**Co-Project Directors**

{NO DATA ENTERED}

**Departments**

{NO DATA ENTERED}

**Non-Technical Summary**

Unique aspects of RNAi-based insect resistant crops challenge the current approach to risk assessment of genetically modified (GM) crops to non-target organisms. One primary concern is that the stability of double-stranded and small interference RNA (dsRNA and siRNA; the basis of RNAi) within the environment is unclear and it is unknown what components of food webs within agroecosystems are directly exposed to the dsRNA. This is particularly important because the small nucleotide sequences targeted by specific siRNAs can be expressed by many organisms, thus dramatically increasing the number of potential targets of the insecticide relative to Bt-based GM crops and pesticides. In short, current knowledge gaps prevent predicting which species are actually at risk of toxicity. We will pair novel genetic methods for examining food webs and genome sequencing with traditional approaches used to establish exposure pathways to develop an exposure-based framework for assessing which species are at risk of ingesting insecticidal RNAi, especially as produced by GM crops. The overall goal of this proposal is to determine the likelihood of exposure to and toxicity of interference RNA to a corn-based arthropod food web. Specifically, this research will establish which species are at risk through consuming dsRNA containing corn tissue under field conditions, and whether dsRNAs are transferred to higher trophic levels via consuming herbivorous prey. The research will establish crucial infrastructure that can be used to establish risk of both existing RNAi-based GM crops and pesticides as well as future constructs. Specific Objectives: 1) Use PCR-based gut content analysis to establish trophic linkages to corn within an arthropod community, 2) Establish whether dsRNA passes to higher trophic levels (predators and parasitoids) via consuming herbivores, and 3) Sequence the genomes of key taxa from corn to determine whether sequence homologies exist that place these organisms at risk from crop-produced dsRNA. Diets of a corn arthropod community will be analyzed by searching in their stomachs for corn DNA. From this, a food web will be created that can help predict which species are exposed to GM corn plants and RNAi. Next, in the laboratory we will feed RNAi to plant-feeding insects (mites, caterpillars, and aphids), and determine whether the RNA persists in the herbivores and can thereby affect predators and parasitoids. Based on these datasets, we will select five species that are highly exposed to RNAi-expressing corn plants, and sequence their entire genomes. From this, we can predict whether these species are at risk of non-target effects of new RNAi molecules. This project will produce the necessary infrastructure to evaluate the exposure and potential toxicity of future RNAi-based GM crops and pesticides to a suite of ecologically relevant non-target species in a format that is adaptive and transparent to the public.

**Accomplishments**

**Major goals of the project**

The overall goal of this proposal is to determine the likelihood of exposure to and toxicity of interference RNA to a corn-based arthropod food web. Specifically, this research will establish which species are at risk through consuming dsRNA containing corn tissue under field conditions, and whether dsRNAs are transferred to higher trophic levels via consuming herbivorous

prey. The research will establish crucial infrastructure that can be used to establish risk of both existing RNAi-based GM crops as well as future constructs. Specific Objectives 1. Use PCR-based gut content analysis to establish trophic linkages to corn within an arthropod community. 2. Establish whether dsRNA passes to higher trophic levels (predators and parasitoids) via consuming herbivorous prey. 3. Sequence the genomes of key taxa from corn to determine whether sequence homologies exist that place these organisms at risk from crop-produced dsRNA.

### What was accomplished under these goals?

This project has developed a generalizable protocol for evaluating the risks of novel insecticidal agents in agroecosystems. The protocol seeks to use faunistic collections within the target crop system to assess the likelihood of exposure to the insecticidal agent, which can then be used to guide efforts at understanding what potential hazards the insecticidal agent poses for non-target organisms. This project focused on a new generation of RNAi-based transgenic insecticidal agents that have not yet received extensive attention from independent risk-assessment agencies.

During the growing season of 2013, cornfields were visited on 10 separate farms in eastern South Dakota. One cornfield was sampled at each farm on two separate visits (late June and late July/early August). All arthropods were collected via whole-plant counts, 50 cm x 50 cm quadrats on the soil surface, and 10-cm-deep soil cores placed in Berlese funnels. From these collections, a community database is being established, which will allow a detailed evaluation of community and food web interactions within corn ecosystems in this region of the state.

A total of 5245 specimens (representing 322 morphotaxa) was collected in 2013. Of these, 767 specimens (representing 39 morphotaxa) were analyzed via qPCR analysis to determine whether corn DNA could be found in their guts, signifying a trophic linkage to corn and demonstrating the potential for exposure to any plant-incorporated insecticidal agents that might be used in a cornfield. Of 767 specimens tested, 38 specimens (representing 10 species) tested positive for corn DNA. Notably, the highest rate of positive results came from adult green lacewings (12/34, 35%, of specimens tested), a common natural enemy of aphids and other small pests. Adult western corn rootworms also tested positive at high rates (9/35, 26%, of specimens tested), as did another natural enemy, the minute pirate bug, Orius (5/54, 9%, of specimens tested).

Field collections were repeated in 2014, this time with 8 farms (many returning from the previous year). A total of 6044 specimens has been collected, representing at least 161 morphotaxa (although many more morphotaxa are suspected and await sorting).

Additionally, experiments to evaluate potential tritrophic routes of exposure have been initiated, using *Rhopalosiphum padi* aphids as the prey for *Coleomegilla maculata* lady beetles. Aphids have been fed western corn rootworm dsRNA in a 20% sucrose/water solution, and the presence of putative siRNAs that result from Dicer cleavage of the target dsRNA has been evaluated using a short RNA analysis system called Mir-X. This analysis is ongoing. Preparations and preliminary trials have also begun for experiments to feed dsRNA-fed aphids to lady beetles, and to feed dsRNA-fed cutworm larvae to carabid beetles.

#### Part I: Survey of insects in corn ecosystems

2013:

- 10 farms surveyed (6 organic, 3 untreated/refuge, 1 conventional GMO)
- 5245 specimens collected
- 322 morphospecies identified (average richness: 15.2 species/site/date)
- 767 specimens (39 species) screened for corn DNA
- 38 specimens (10 species) tested positive for corn DNA
- 12/34 (35%) *Chrysoperla* adults tested positive
- 5/54 (9%) *Orius* adults tested positive
- Only 2/107 (<2%) *Rhopalosiphum* aphids tested positive
- 0/83 thrips tested positive

2014:

- 8 farms surveyed twice (all organic)
- 6044 specimens collected

### What opportunities for training and professional development has the project provided?

{Nothing to report}

### How have the results been disseminated to communities of interest?

See the presentations that listed below in this report. We are actively disseminating the results of this work to stakeholders and end-users.

Mogren, C., M. M. Bredeson, K. T. Nemecek, **J. G. Lundgren**. Using diversity to decrease the risks of plant-incorporated pesticides to pollinators. 12<sup>th</sup> International Symposium for Plant-Pollinator Relationships, Ghent, Belgium.

**Lundgren, J. G.** 2014. Balancing insecticide risks and forage production on farms to promote honey bees in South Dakota. SD Beekeepers Annual Meeting, Aberdeen, SD. (50 attendees)

Mogren, C. and **J. G. Lundgren**. 2014. Evaluating the risks posed by RNAi crops to pollinators in an agricultural landscape. IWGO Meeting, Chicago, IL

**Lundgren, J. G.** 2014. Conservation of beneficial species in corn fields and rethinking insecticide use in modern cropping systems. Ohio Conservation Tillage Association annual meeting, Ada, OH. (800 attendees)

**Lundgren, J. G.** 2014. Rethinking insecticide use in modern cropping systems. National No-tillage Conference, Springfield, IL.

Bredeson, M. **J. G. Lundgren.** 2014. Corn insect communities of eastern South Dakota. IWGO meeting, Chicago, IL.

Welch, K. D., **J. G. Lundgren.** 2014. Assessing risks to non-target arthropods via molecular analysis of trophic webs. Entomological Society of America annual meeting, Portland, OR.

#### What do you plan to do during the next reporting period to accomplish the goals?

The laboratory assays are underway to determine the toxicity of pesticidal RNAs to non-target organisms through prey. We are working on coordinating with the i5K initiative to sequence ecologically relevant non-target species for future RNAi assessments.

#### Participants

##### Actual FTE's for this Reporting Period

Role	Non-Students or faculty	Students with Staffing Roles			Computed Total by Role
		Undergraduate	Graduate	Post-Doctorate	
Scientist	0	0	0	1	1
Professional	0	0	0	0	0
Technical	0	1	1	0	2
Administrative	0	0	0	0	0
Other	0	0	0	0	0
Computed Total	0	1	1	1	3

#### Student Count by Classification of Instructional Programs (CIP) Code

Undergraduate	Graduate	Post-Doctorate	CIP Code
1	1	1	01.03 Agricultural Production Operations.

#### Target Audience

We have communicated our research project to fellow scientists, regulators (EPA, EFSA, USDA-APHIS), farmers, bee keepers, and the general public.

To reach the audiences, we delivered science-based presentations at professional and trade meetings, and provided specific presentations to the EPA and EFSA at their request.

#### Products

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Other	2014	YES

#### Citation

Mogren, C., M. M. Bredeson, K. T. Nemecek, J. G. Lundgren. Using diversity to decrease the risks of plant-incorporated pesticides to pollinators. 12th International Symposium for Plant-Pollinator Relationships, Ghent, Belgium.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Other	2014	YES

#### Citation

Lundgren, J. G. 2014. Science on the efficacy of neonicotinoid seed treatments and their effects on non-target organisms. U.S. Environmental Protection Agency Office of Pesticide Programs, Washington, DC.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Other	2014	YES

**Citation**

Lundgren, J. G. 2014. Balancing insecticide risks and forage production on farms to promote honey bees in South Dakota. SD Beekeepers Annual Meeting, Aberdeen, SD. (50 attendees)

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Other	2014	YES

**Citation**

Mogren, C. and J. G. Lundgren. 2014. Evaluating the risks posed by RNAi crops to pollinators in an agricultural landscape. IWGO Meeting, Chicago, IL

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Other	2014	YES

**Citation**

Lundgren, J. G. 2014. Conservation of beneficial species in corn fields and rethinking insecticide use in modern cropping systems. Ohio Conservation Tillage Association annual meeting, Ada, OH. (800 attendees)

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Other	2014	YES

**Citation**

Lundgren, J. G. 2014. Rethinking insecticide use in modern cropping systems. National No-tillage Conference, Springfield, IL. (1000 attendees)

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Other	2014	YES

**Citation**

Bredeson, M. J. G. Lundgren. 2014. Corn insect communities of eastern South Dakota. IWGO meeting, Chicago, IL.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Other	2014	YES

**Citation**

Welch, K. D., J. G. Lundgren. 2014. Assessing risks to non-target arthropods via molecular analysis of trophic webs. Entomological Society of America annual meeting, Portland, OR.

**Other Products****Product Type**

Audio or Video

**Description**

An animated video targeting non-scientists on what RNAi is, and what pesticidal RNAi risks might be characterized as.

Funds from the USDA-NIFA grant were not used to produce this video, but our expertise was consulted by the film maker.

<http://vimeo.com/97012992>

**Changes/Problems**

The laboratory studies have been delayed because we included a second field season for the food web analysis. The reason for the second season was because the first 10 farms varied substantially in the communities that were collected, and we wanted to get a stronger sample from the anthesis-phase of the growing season. Although this slowed progress on the laboratory study, we still anticipate completing the project goals in a reasonable time frame.

<b>Title:</b>	<b>Transmission genetics of sorghum to Johnsongrass gene transfer</b>		
<b>Sponsoring Agency</b>	NIFA	<b>Project Status</b>	ACTIVE
<b>Funding Source</b>	Non Formula	<b>Reporting Frequency</b>	Annual
<b>Accession No.</b>	229653	<b>Grants.gov No.</b>	GRANT11040909
<b>Project No.</b>	GEO-2012-01658	<b>Proposal No.</b>	2012-01658
<b>Project Start Date</b>	09/01/2012	<b>Project End Date</b>	08/31/2015
<b>Reporting Period Start Date</b>	09/01/2013	<b>Reporting Period End Date</b>	08/31/2014
<b>Submitted By</b>	La Kesha Clark	<b>Date Submitted to NIFA</b>	09/03/2014

**Program Code:** HX

**Program Name:** Biotechnology Risk Assessment

**Project Director**

Andrew Paterson  
706-583-0162  
paterson@uga.edu

**Recipient Organization**

UNIVERSITY OF GEORGIA RESEARCH  
200 D.W. BROOKS DRIVE  
Athens, GA 306025016  
DUNS No. 004315578

**Performing Department**

Plant Genome Mapping Laboratory

**Co-Project Directors**

Cox, Thomas

**Departments**

{NO DATA ENTERED}

**Non-Technical Summary**

Building on prior BRAG-supported results and engaging new resources, integrative genetic, phenotypic, and ecological/evolutionary studies are proposed to provide baseline information about: the expected fate and stability (persistence) of sorghum (*S. bicolor*) transgenes that escape into Johnsongrass (*S. halepense*); the efficacy of genetic techniques to restrict gene transfer such as the targeting of transgenes to specific genomic islands of differentiation that are recalcitrant to interspecific gene flow; and the efficacy of mitigation measures to limit the spread of introgressed transgenes such as linkage to alleles that reduce fitness in the wild. The primary focus of this proposal is BRAG program area 3, Gene Transfer to Domesticated and Wild Relatives, while also addressing elements of program area 1, Management Practices to Minimize Environmental Risk.

**Accomplishments**

**Major goals of the project**

Our goal is to reveal the genome-wide transmission genetics of gene transfer between sorghum and Johnsongrass (*S. halepense*), one of the worlds most noxious weeds and a paradigm for the potential dangers of crop-weed introgression. Since *S. bicolor* ( $2n=2x=20$ ) and *S. halepense* ( $2n=4x=40$ ) differ in ploidy, gene transfer between these species utilizes unreduced gametes formed by sorghum (reviewed in Warwick and Black, 1983; Tang and Liang, 1988). Using the fully-sequenced reference genotype, BTx623, we have produced *S. bicolor* x *S. halepense* tetraploid F1 hybrids and their F2-selfed progeny that closely mimic the early-generation progeny from natural crosses between these species that would lead to transgene escape. Objectives including genetic mapping will clarify the transmission genetics of each region of the genome in this population; QTL mapping will clarify the relationship of specific chromosomal regions to traits that are important to the fitness of *S. halepense* in the wild; and targeted resequencing will provide complementary fine-scale evidence toward precise delineation of loci or small regions responsible for genomic incompatibilities or QTL effects. The overall outcome of these integrative genetic, phenotypic, and ecological/evolutionary studies will be to provide objective and comprehensive baseline information about the expected fate and stability (persistence) of sorghum transgenes that escape into Johnsongrass; evaluation of the efficacy of genetic techniques to restrict gene transfer such as the targeting of transgenes to specific genomic islands of differentiation that are recalcitrant to interspecific gene flow; and mitigation measures to limit the spread of introgressed transgenes such as linkage to domestication genes or other genes that reduce fitness in the wild (Gressel and Al-Ahmad, 2006).

**What was accomplished under these goals?**

Genetic mapping of the *S. bicolor* x *S. halepense* tetraploid F2 population is in progress, with a framework of about 100 SSRs mapped. Additional reduced-representation sequencing (for genotyping-by-sequencing) is being analyzed.

Extensive phenotyping was performed both in Georgia and in Kansas on F3 progeny rows from the F2 plants, including the spectrum of morphological and physiological traits proposed as well as regrowth after overwintering. Substantial differences between the locations, particularly regarding winter survival (in 2013 >90% in GA, about 24% in KS; in 2014 ~30% in GA, very low in KS) are expected to be valuable for genetic analysis. In the Georgia location, we also added a measurement of biomass produced from regrowth after overwintering based on a harvest on 31 July 2013 (approximately the midpoint of the growing season) a second measurement at the end of the growing season, to provide a further assessment of perenniality (the ability to sustain similar levels of biomass over multiple harvests may be a valuable complement to 'regrowth' measurements that primarily assessed winter survival). These additional measures are not meaningful in 2014 due to low winter survival.

#### What opportunities for training and professional development has the project provided?

Several postdoctoral scientists (particularly Uzay Sezen, Changsoo Kim, and Tae-ho Lee) and graduate students (particularly Hui Guo and Wenqian Kong) funded by a variety of sources including this award have been engaged in genetic mapping, phenotyping, and data analysis. Many technicians and part-time student workers in the PIs' lab also assisted with phenotyping.

#### How have the results been disseminated to communities of interest?

Presentations at BMG, PAG and BRAG meetings (see description of the target audience(s) reached).

#### What do you plan to do during the next reporting period to accomplish the goals?

We consider that we are on track, indeed a little ahead of schedule, based on the proposed timeline. Notification of a probable award was early enough that we were able to plant experimental populations in time for the 2012 growing season, which permitted us to gain some time relative to the proposed timetable. With most of the proposed phenotypic data now in hand (earlier than anticipated), our focus during the next funding period is to complete genetic mapping and associated data analysis.

#### Participants

##### Actual FTE's for this Reporting Period

Role	Non-Students or faculty	Students with Staffing Roles			Computed Total by Role
		Undergraduate	Graduate	Post-Doctorate	
Scientist	0.1	0	0	0	0.1
Professional	0	0	0	2	2
Technical	0	0	0	0	0
Administrative	0.1	0	0	0	0.1
Other	0	2	0	0	2
Computed Total	0.2	2	0	2	4.2

#### Student Count by Classification of Instructional Programs (CIP) Code

{NO DATA ENTERED}

#### Target Audience

The PI presented results at a 'convening' organized by the Bill and Melinda Gates Foundation in December of 2013, one workshop talk and one poster were presented at PAG in January of 2014, and one poster was presented at the BRAG PI meeting in June of 2014, in each case addressing other scientists, program personnel, and decision-makers.

**Products**

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Submitted	2014	YES

**Citation**

U. Uzay Sezen, Jacob N. Barney, Dan Z. Atwater, Gary A. Pederson, Jeffrey F. Pederson, J. Mike Chandler, T. Stan Cox, Sheila Cox, Peter Dotray, David Kopec, Steven E. Smith, Jill Schroeder, Cheryl Fiore, Steven D. Wright, John A. Roncoroni, Yuannian Jiao, Wenqian Kong, Valorie Goff, Susan Auckland, Lisa Rainville, Gary J. Pierce, Cornelia Lemke, Rosana Compton, Christine Phillips, Alexandra Kerr, Matt Mettler, Andrew H. Paterson 201# US naturalization of a post-Columbian invasive, Sorghum halepense. Submitted.

**Other Products****Product Type**

Other

**Description**

A workshop talk was given at PAG in January of 2014: Progress in Breeding for Perenniality in Sorghum.

**Product Type**

Other

**Description**

A poster was given at PAG in January of 2014: Population Genetic Analysis of Naturalized US Populations of a Post-Columbian Invasive, Sorghum halepense

**Product Type**

Other

**Description**

A poster was given at the BRAG PI meeting in June of 2014.

**Changes/Problems**

Not applicable

<b>Title:</b>	<b>Gene flow networks and potential invasiveness of perennial biofuel grasses (<i>Miscanthus</i>)</b>		
<b>Sponsoring Agency</b>	NIFA	<b>Project Status</b>	ACTIVE
<b>Funding Source</b>	Non Formula	<b>Reporting Frequency</b>	Annual
<b>Accession No.</b>	229850	<b>Grants.gov No.</b>	GRANT11047486
<b>Project No.</b>	OHOW-2012-01659	<b>Proposal No.</b>	2012-01659
<b>Project Start Date</b>	08/01/2012	<b>Project End Date</b>	07/31/2015
<b>Reporting Period Start Date</b>	08/01/2013	<b>Reporting Period End Date</b>	07/31/2014
<b>Submitted By</b>	Allison Snow	<b>Date Submitted to NIFA</b>	11/04/2014

**Program Code:** HX

**Program Name:** Biotechnology Risk Assessment

#### **Project Director**

Allison Snow  
614-292-3445  
snow.1@osu.edu

#### **Recipient Organization**

OHIO STATE UNIVERSITY, THE  
1960 KENNY RD  
Columbus, OH 432101016  
DUNS No. 832127323

#### **Performing Department**

Evol Ecology and Org Biology

#### **Co-Project Directors**

Miriti, Maria

#### **Departments**

Dept of Evol Ecology & Org Bio

#### **Non-Technical Summary**

With the planned introduction of transgenic perennial grasses such as *Miscanthus* as biofuel cultivars, data on the extent and consequences of gene flow via pollen and seeds are needed for environmental risk assessment. Because these crops are relatively new, and because experimental transgenes must be confined during early field trials, it is essential to understand the baseline ecology and genetics of the cultivars and their weedy relatives. In addition, more information is needed to assess the long-term consequences of releasing novel, transgenic cultivars. *Miscanthus* species, which are leading candidates for bioenergy production, are non-native cultivars that are widely grown as ornamentals and have naturalized to become invasive in some areas of the USA. Surprisingly little is known about the population ecology of naturalized populations or their ability to hybridize with bioenergy and ornamental cultivars. The future trajectory of how quickly these taxa will spread is not known, and the current distribution and abundance of feral populations may be in a state of flux and could be affected by the large-scale cultivation of bioenergy cultivars. Ecological information about the potential for *Miscanthus* to become weedy when planted for bioenergy is urgently needed by USDA's Biotechnology Regulatory Services. Industry-sponsored field trials with transgenic *Miscanthus* are ongoing and deregulation could be proposed in the next few years. Seeded, nontransgenic cultivars also could be released soon. Non-sterile *Miscanthus* cultivars with improved agronomic traits are being developed, including transgenic lines field-tested in 2011. Biofuel cultivars may become naturalized and could hybridize with feral and ornamental *M. sinensis* and *M. sacchariflorus*. However, little is known about the ecology and distribution of free-living populations of *Miscanthus*, which we refer to as "feral" or "naturalized" interchangeably. We plan to address the following information gaps regarding feral populations by using field surveys, common garden experiments, and mathematical models that integrate key life-cycle data for these plants, with parallel approaches in Ohio and Iowa: 1) Gene flow characterization, including population genetic structure, ability to hybridize, pollen-limited seed production, and pollen dispersal distances, and 2) Fitness comparisons among feral, cultivar, and hybrid biotypes, including studies of seed germination, seed dormancy, and seed longevity; ability to start new populations in seed addition experiments; and clonal growth and competitive ability. **OUTCOMES** - Our findings will be useful for establishing isolation distances for field trials, managing volunteer plants from field trials, and evaluating larger-scale ecological consequences, if any, of gene flow from biofuel crops to feral populations.

#### **Accomplishments**

##### **Major goals of the project**

*Miscanthus* offers many advantages as a biofuel crop, but quantitative studies are needed to examine gene flow and the potential for new cultivars to become invasive, including the potential for transgenic traits to exacerbate weed problems if naturalized plants become too abundant. Industry-sponsored field trials with transgenic *Miscanthus* are ongoing and deregulation could be proposed in the next few years. Seeded, nontransgenic cultivars also could be released soon. Non-

sterile *Miscanthus* cultivars with improved agronomic traits are being developed, including transgenic lines field-tested in 2011. Biofuel cultivars may become naturalized and could hybridize with feral and ornamental *M. sinensis* and *M. sacchariflorus*. In the short term, research is needed to provide regulatory agencies with findings that are relevant to the design of small- and medium-scale field trials prior to deregulation. This includes data on sexually compatible, naturalized populations, pollen and seed dispersal, and fitness characteristics of feral and hybrid progeny. The proposed research will help fill this gap by examining gene flow, population dynamics, and the relative competitive ability of *Miscanthus* cultivars, naturalized biotypes, and their hybrids in a variety of locations and environmental conditions. Fitness traits will be measured in both cropping and non-cropping (marginal lands) ecosystems. EXPECTED OUTPUTS: Our findings will be useful for establishing isolation distances for field trials, managing volunteers from field trials, and evaluating larger-scale ecological consequences, if any, of gene flow from biofuel crops to feral populations. Information and expertise needed by APHIS-BRS for regulatory oversight of the safe development of transgenic perennial grasses for biofuel production. Specifically, we plan to disseminate our results via scientific presentations, peer-reviewed publications, and information for farmers and natural research managers.

#### **What was accomplished under these goals?**

During the past year, we learned a great deal about the potential for gene flow among *Miscanthus* species, in terms of shared ploidy levels, overlapping flowering times, and the ability of cultivars to establish feral populations. This research involved two major field experiments, one with seed addition treatments and the other with a common garden approach and competition treatments. Results from these experiments will be obtained in the fall of 2014, after two growing seasons. So far, our results suggest that feral *M. sacchariflorus* is restricted to northern regions of the USA, while feral *M. sinensis* occurs mainly in southern regions, but appears to be spreading northward. We also continued studies of the genetic diversity and spread of *M. sacchariflorus* and *M. sinensis* using molecular markers. Due to limited funds and personnel, we did not attempt to study the distance of pollen movement from source populations, per se. However, we were able to show that isolated plants and small populations of *M. sinensis* appear to be pollen limited. We have begun working on publications based on all of these results.

#### **What opportunities for training and professional development has the project provided?**

The project provided training and professional development for two postdoctoral researchers, 3 graduate students, and 4 undergraduates, one of whom completed a senior honors thesis.

#### **How have the results been disseminated to communities of interest?**

##### **Meetings/presentations:**

Ibrahim T.A., M.N. Miriti, A.A. Snow, E.A. Heaton, D.J. Palik, C. Bonin, E. Mutegi, and H. Chang. Relative competitive ability of feral and cultivated biotypes of *Miscanthus* spp.: implications for new biofuel cultivars. Botanical Society of America Annual Meeting, Boise, Idaho, July 28, 2014.

Bonin, C., E. Heaton, M. Miriti, and A. Snow. 2014. Gene flow networks and potential invasiveness of perennial biofuel grasses (*Miscanthus*). Biotechnology Risk Assessment Grant Annual Project Director's Meeting. Riverdale, MD.

Bonin, C.L. and E.A. Heaton. 2014. *Miscanthus sacchariflorus* - biofuel parent or new weed? 74th Midwest Fish and Wildlife Conference. Kansas City, MO.

Bonin, C., E. Heaton, and J. Barb. 2013. *Miscanthus sacchariflorus* - biofuel parent or new weed? ASA-CSSA-SSSA International Annual Meeting. Tampa, FL.

Heaton E.A., Schulte L.A, Brandes E., Muth D., Snow A., Miriti M., Bonin C. & Milster F. (2014) *Miscanthus* and switchgrass cropping systems - ecophysiology to landscape. American Society of Plant Biology 4<sup>th</sup> Pan-American Congress on Plants and Bioenergy, Guelph, Canada.

Bonin C., VanLoocke A., Mitchell R. & Heaton E.A. (2014) A coupled field and modeling approach for quantifying the environmental impacts of genetic improvements in switchgrass. Agro-IBIS Workshop, Ames, IA.

Heaton E.A., Schulte L.A, Brandes E., Darr M., Hu G., Wang L. & Milster F. (2014) Putting the Pieces Together. Mosaic Seminar Series, University of Minnesota, St. Paul, MN.

Heaton E.A. (2014) Biomass for energy? Pros and cons in the big picture. Graduate Program in Sustainable Agriculture Colloquium, Iowa State University, Ames, IA.

Heaton E.A. (2013) Energy Crops in Iowa. Graduate Program in Sustainable Agriculture Colloquium, Iowa State University, Ames, IA.

Milster F. & Heaton E.A. (2013) Introduction to dedicated energy crops with a focus on Miscanthus. Grower Interest Meeting, University of Iowa, Iowa City, IA.

Heaton E.A., Schulte L.A. & Milster F. (2013) Integrating food and fuel production in the Corn Belt. Kohn Lecture Series, University of Iowa, Iowa City, IA.

Heaton E.A., Schulte L.A. & Wilson D.M. (2013) Integrating food and fuel: how to manage a 2G crop portfolio. BioFuelNet Canada Annual Meeting, Montreal, CA.

#### Extension and Outreach Presentations:

Heaton E.A., Schulte-Moore L.A., Brandes E., Muth D., Bonner I., Cafferty K. & Milster F. (July 24, 2014) Is it over after stover? Dedicated energy crops in Iowa. Union of Concerned Scientists and Great Plains Institute Joint Summit on Cellulosic Biofuels, Ames, IA, 120 participants.

Heaton E.A. & Milster F. (January 29, 2014) The biomass power partnership: replacing coal with dedicated energy crops at the University of Iowa. 2014 Iowa State University Crop Advantage Series Workshops, Iowa City, IA, 17 participants.

Heaton E.A. & Milster F. (January 23, 2014) The biomass power partnership: replacing coal with dedicated energy crops at the University of Iowa. 2014 Iowa State University Crop Advantage Series Workshops, Waterloo, IA, 26 participants.

Heaton E.A. & Milster F. (January 10, 2014) The biomass power partnership: replacing coal with dedicated energy crops at the University of Iowa. 2014 Iowa State University Crop Advantage Series Workshops, Burlington, IA, 12 participants.

Heaton E.A., Schulte-Moore L.A., Helmers M., Liebman M. & Milster F. (December 6, 2013) Producing food, feed and energy: How can agriculture do it all? 25<sup>th</sup> Annual Integrated Crop Management Conference. Iowa State University, Ames, IA; 182 participants.

Heaton E.A. (August 14, 2013) Bioenergy crop research. Congressional Aide field tour. Boone, IA, 53 participants.

Heaton E.A. (August 8, 2013) Bioeconomy media tour. Boone, IA, 18 participants.

#### Popular Press Coverage (E. A. Heaton):

1. Government officials take biofuels tour (2014) Sara Parks. Iowa EPSCoR press release. <http://iowaepscor.org/news/2014/govtour>
2. Study shows better yield potential for Miscanthus in Iowa (2014) Meghan Sapp. Biofuels Digest. <http://www.biofuelsdigest.com/bdigest/2014/07/28/study-shows-better-yield-potential-for-miscanthus-in-iowa/>
3. Miscanthus to play a major role in Iowa agriculture (2014) Staff. The Daily Fusion. <http://dailyfusion.net/2014/07/miscanthus-iowa-agriculture-30804/>
4. Miscanthus to play a major role in Iowa ag (2014) Staff. Morning Ag Clips. [https://www.morningagclips.com/miscanthus-to-play-a-major-role-in-iowa-ag/?utm\\_content=articles&utm\\_campaign=NLCampaign&utm\\_source=Newsletter&utm\\_term=newsletteredition&utm\\_medium=email](https://www.morningagclips.com/miscanthus-to-play-a-major-role-in-iowa-ag/?utm_content=articles&utm_campaign=NLCampaign&utm_source=Newsletter&utm_term=newsletteredition&utm_medium=email)
5. Miscanthus would yield more biomass than originally thought (2014) Staff. Iowa Farmer Today, August 2, 2014.
6. Iowa State University agronomist says Miscanthus would yield more biomass than originally thought in Iowa soil (2014) Fred Love. Iowa State University press release. <http://www.news.iastate.edu/news/2014/07/23/miscanthus2014>
7. Cropping biofuels: Iowa State research looks at growing bioenergy crops (2014) Jerry Perkins. Biofuels Journal. <http://edition.pagesuite-professional.co.uk/launch.aspx?pbid=4d63415d-a95c-43f0-937d-3d7fe7e4fc52>
8. University of Iowa is planting Giant Miscanthus (2014) Staff. Wallace's Farmer. <http://farmprogress.com/story-university-iowa-planting-giant-miscanthus-9-112044>
9. Miscanthus an option for bioenergy (2014) Matt Kelley. Radiolowa. <http://www.radioiowa.com/2014/06/02/miscanthus-an-option-for-bioenergy/>
10. Miscanthus: crop of the future? (2014) Rick Fredericksen. Iowa Public Radio. <http://iowapublicradio.org/post/miscanthus-crop-future>
11. University of Iowa grows Miscanthus grass for renewable energy goals (2014) Sara Agnew. Iowa City Press Citizen. <http://www.press-citizen.com/story/news/education/university-of-iowa/2014/05/07/ui-grows-miscanthus-grass-renewable-energy-goals/8831579/>
12. Biomass upstarts (2014) Becky Mills. My Farm Life. <http://www.myfarmlife.com/crops/biomass-upstarts/3/>
13. Project seeks crop systems to profit farmers, clean air (2014) Staff. Iowa Farmer Today. [http://www.iowafarmertoday.com/news/crop/project-seeks-crop-systems-to-profit-farmers-clean-air/article\\_a2536fb6-9a37-](http://www.iowafarmertoday.com/news/crop/project-seeks-crop-systems-to-profit-farmers-clean-air/article_a2536fb6-9a37-)

14. Project seeks cropping systems that profit farmers, provide food and fuel and scrub carbon out of the air (2014) Lynn Laws & Ed Adcock. Iowa State University press release. <http://www.ag.iastate.edu/news/>.
15. A garden of marvels (2014) Ruth Kassinger. 416 pp. William Morrow, publisher. <http://www.amazon.com/Garden-Marvels-Discovered-Flowers-Secrets/dp/0062048996>  
- Heaton advised the author on ecophysiological limits to plant productivity.
16. Masterminding Miscanthus (2014) Anna Simet. Biomass Magazine. <http://www.biomassmagazine.com/articles/9937/masterminding-miscanthus>.
17. From recipe to scientific formula (2014) Chris Hanson. Biomass Magazine. <http://www.biomassmagazine.com/articles/9933/from-recipe-to-scientific-formula>.
18. Biomass appeal – SWCC students gain advantage by studying energy crops (2013) Stephanie Finley. Southwest Iowa AgMag, Shaw Media. Fall 2013, pp 42-46.
19. Biofuels on the verge (2013) Herman Trabish. Greentech Media.
20. Alternative crops for alternative fuels (2013) Becky Mills. BALE magazine.
21. Miscanthus (2013) Richard Banks. Farm Life, a Massey Ferguson publication. Includes 20-minute video interview at <http://www.myfarmlife.com/asides/growing-miscanthus/>.

### What do you plan to do during the next reporting period to accomplish the goals?

During the next and final reporting period, we plan to complete all of the field and laboratory work, analyze the data, present the results at professional meetings, and prepare manuscripts for publication.

### Participants

#### Actual FTE's for this Reporting Period

Role	Non-Students or faculty	Students with Staffing Roles			Computed Total by Role
		Undergraduate	Graduate	Post-Doctorate	
Scientist	0	0	1	0.8	1.8
Professional	0	0	0	0	0
Technical	0	0.6	0	0	0.6
Administrative	0	0	0	0	0
Other	0	0	0	0	0
Computed Total	0	0.6	1	0.8	2.4

### Student Count by Classification of Instructional Programs (CIP) Code

Undergraduate	Graduate	Post-Doctorate	CIP Code
		2	01.11 Plant Sciences.
	3		01.11 Plant Sciences.
4			01.11 Plant Sciences.

### Target Audience

Other researchers and regulatory agencies - reached with publications and presentations at meeting.  
Growers and extension agents in Iowa - reached with presentations and publicity by E. A. Heaton

### Products

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Published	2014	YES

### Citation

Bonin, C.L., E.A. Heaton, and J. Barb. 2014. Miscanthus sacchariflorus – biofuel parent or new weed? Global Change Biology Bioenergy. DOI: 10.1111/gcbb.12098.

Type	Status	Year Published	NIFA Support Acknowledged
Theses/Dissertations	Published	2014	YES

**Citation**

Verhoff, S. 2014. Pollen Limits Seed Set in Small Populations of Feral *Miscanthus sinensis*, an Ornamental Grass with Invasive Potential." Senior Honors Thesis, Ohio State University Knowledge Bank, <http://hdl.handle.net/1811/59923>

**Other Products**

{Nothing to report}

**Changes/Problems**

We were not able to set up experiments for studying the distance over which pollen is carried by wind from source populations to wild plants at varying distances from the source. This portion of the project was deleted due to limited funds and personnel, logistical challenges, and the long period of time needed for completion. However, we were able to study pollen limitation of seed set using extant populations that vary in size and density.

<b>Title:</b>	<b>Molecular Genetic Basis of Insect Resistance to Bt-Crops</b>		
<b>Sponsoring Agency</b>	NIFA	<b>Project Status</b>	ACTIVE
<b>Funding Source</b>	Non Formula	<b>Reporting Frequency</b>	Annual
<b>Accession No.</b>	229615	<b>Grants.gov No.</b>	GRANT11049879
<b>Project No.</b>	NYG-621575	<b>Proposal No.</b>	2012-01645
<b>Project Start Date</b>	09/01/2012	<b>Project End Date</b>	08/31/2015
<b>Reporting Period Start Date</b>	09/01/2013	<b>Reporting Period End Date</b>	08/31/2014
<b>Submitted By</b>	Donna Loeb	<b>Date Submitted to NIFA</b>	10/24/2014

**Program Code:** HX

**Program Name:** Biotechnology Risk Assessment

**Project Director**

Ping Wang

315-787-2348

pw15@cornell.edu

**Recipient Organization**

CORNELL UNIVERSITY, INC

373 PINE TREE RD

Ithaca, NY 148502820

DUNS No. 872612445

**Performing Department**

Entomology

**Co-Project Directors**

{NO DATA ENTERED}

**Departments**

{NO DATA ENTERED}

**Non-Technical Summary**

Since 1996, genetically engineered crops (GE-crops) with insecticidal genes from the soil bacterium *Bacillus thuringiensis* (Bt) (Bt-crops) have been rapidly adopted in the US and the acreage of Bt-crops worldwide has reached near 60 million hectares with proven economic and environmental benefits. However, the widespread adoption of Bt-crops greatly increases the selection pressure for development of insect resistance to Bt toxins, which is the primary risk to the long-term future of Bt-based biotechnology and environmentally sustainable pest management programs. To date, cases of insect resistance to Bt toxins in the field or greenhouses have been reported in six Lepidoptera and one Coleoptera species, and increasing frequencies of Bt-resistant individuals in populations of four insect pests have been observed in regions where Bt-cotton has been planted. Therefore, there is an urgent need to assess and manage the risk of development of Bt-resistance in insects, which critically relies on understanding the genetic basis of Bt resistance evolved in insects in agricultural systems. Identification of the genes and genetic mechanisms conferring insect resistance to Bt-crops is particularly important for effective and efficient monitoring of Bt resistance in insect populations and for development of regulatory framework for insect-resistant GE-crops. However, molecular genetic basis of Bt-resistance has not been identified in any insects that have developed resistance in an agricultural setting, conferring resistance to Bt-crops. *T. ni* is one of the seven insect species that has developed resistance to Bt in agricultural systems upon selection pressure with Bt products. It is a significant pest of agriculture with an exceptionally broad and diverse range of host plants, including at least 160 plants in 36 families. The Bt-resistance mechanism selected in *T. ni* confers resistance not only to Bt toxins on artificial diet but also to Bt-broccoli and commercial Bt-cotton varieties. Therefore, the Bt-resistant strains of *T. ni* provide us with a unique and timely opportunity to identify the molecular genetic basis of insect resistance to Bt-crops. This project is designed to understand the molecular genetic basis of resistance to Bt toxin Cry1Ac in *T. ni* in order to generate new information and provide tools important for assessment and management of the risk of development of insect resistance to Bt-crops in agriculture. Knowledge obtained from this project will assist federal regulatory agencies in making science-based decisions about the effects of introducing into the environment genetically engineered organisms.

**Accomplishments**

**Major goals of the project**

Development of Bt-resistance in insect populations associated with the planting of Bt-crops has been reported in five insect pests since the introduction of Bt-crops in agriculture. Moreover, the frequencies of individuals resistant to Bt toxins in populations of four insect pests have been observed to be drastically increased in areas where Bt-cotton has been extensively planted. Evidently, the risk of resistance development in insect populations in response to the widespread adoption of Bt-crops requires urgent attention, and assessment of resistance development is a very important component of the regulatory framework for insect-resistant GE-crops. We have used the Bt-resistant cabbage looper, *Trichoplusia ni*, populations evolved

in commercial greenhouses to establish a unique biological system to study the mechanism of Bt-resistance which is selected in agriculture and confers high-level of resistance to commercial Bt-crops. We have recently identified that the biochemical basis for Cry1Ac resistance in *T. ni* is alteration of APN (aminopeptidase N) expression by a trans-regulatory yet to be known mechanism, and confirmed that the genetic basis for the resistance is different from those currently known in some laboratory-selected insects. With the unique *T. ni* strains established and the research foundation built, we have a unique opportunity to study the molecular genetic basis of Bt resistance evolved in an agricultural system and to identify molecular markers for assessment and management of the risk of insect resistance to Bt-crops, which will contribute important information for regulators to make science-based decisions on GE-crops in agriculture. In this project, we will focus on understanding the molecular genetic basis of resistance in *T. ni* to Bt toxin Cry1Ac, which is the primary insecticidal toxin in current commercial Bt-crops to target Lepidoptera pests, by 1) cloning the ABC transporter gene ABCC2 and determining the association of ABCC2 with Cry1Ac-resistance, 2) identifying genes and molecular markers associated with Cry1Ac-resistance, and 3) identifying mutations and altered expression of midgut genes associated with Cry1Ac-resistance. Understanding on the molecular genetic basis of Cry1Ac resistance in *T. ni* acquired from this project will contribute knowledge towards assessment and management of the risk of Bt-resistance in agriculture.

#### What was accomplished under these goals?

At the end of this second year of the project, all the aims that are proposed to be accomplish in Year 1 and Year 2 were reached. To summarize, 1) both the cDNA and genomic DNA of the ABC transporter gene ABCC2 in *T. ni* have been fully sequenced and the Cry1Ac resistance gene has been further confirmed to be in the same genetic linkage group as the ABCC2 gene. In addition to SNP identification in the ABCC2 gene between susceptible and Cry1Ac resistant *T. ni* populations, expression of the ABCC2 gene was also quantitatively examined to compare the expression of ABCC2 gene in the resistant and susceptible populations. 2) *T. ni* genome sequencing was initiated at the start of this project. The *T. ni* genome was sequenced and assembled. Genes in the genomic region where Cry1Ac resistance is mapped to were identified. 3) *T. ni* midgut transcriptome was sequenced in depth and assembled, and the *T. ni* midgut transcriptome assembly was further improved by combining RNA-seq data from *T. ni* larvae and cells. Various *T. ni* midgut RNA-seq libraries were made and sequenced for analysis of midgut expressed genes and their potential association with Cry1Ac resistance in *T. ni*.

#### What opportunities for training and professional development has the project provided?

This project provided opportunities for training to 1 postdoctoral associate, 4 graduate students. The project is also an excellent opportunity for professional development for the PI and a research support specialist on the research team.

#### How have the results been disseminated to communities of interest?

The results from our research were disseminated to the communities of interest by our presentations in international and national academic conferences and symposia, as well as outreach events to reach the general public.

#### What do you plan to do during the next reporting period to accomplish the goals?

The project has progressed as proposed in this reporting period and the proposed aims for the first 2 years of this project are reached and new observations have been made. We will continue this project as planned in the proposal, and develop research hypotheses with our new observations made in this project to fully reach the goals of this project.

#### Participants

##### Actual FTE's for this Reporting Period

Role	Non-Students or faculty	Students with Staffing Roles			Computed Total by Role
		Undergraduate	Graduate	Post-Doctorate	
Scientist	0.3	0	1.2	1	2.5
Professional	0.3	0	0	0	0.3
Technical	0	0	0	0	0
Administrative	0	0	0	0	0
Other	0	0	0	0	0
Computed Total	0.6	0	1.2	1	2.8

#### Student Count by Classification of Instructional Programs (CIP) Code

Undergraduate	Graduate	Post-Doctorate	CIP Code
---------------	----------	----------------	----------

Undergraduate	Graduate	Post-Doctorate	CIP Code
0	3	1	01.11 Plant Sciences.

### Target Audience

The target audience of this project includes researchers and technologists in academia and industry in the areas of insect pathology, agricultural biotechnology and Bt-resistance management. Audience of this project also includes insect pest management professionals and growers, students and postdoctoral trainees in relevant areas, relevant government agencies, and the general public.

In this reporting period, target audience was reached through presentations of our research project and results from the project in academic meetings, including 5 presentations in international and national academic conferences and invited seminars, 1 presentation in this USDA funding agency workshop. General audience was also reached by outreach activities in local community and schools.

This project also provided training opportunities to 1 postdoctoral researcher and 3 students.

### Products

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Published	2014	YES

### Citation

Tian, J.C., Long, L.P., Wang, X.P., Naranjo, S.E., Romeis, J., Hellmich, R.L., Wang, P., Shelton, A.M., 2014. Using resistant prey demonstrates that Bt plants producing Cry1Ac, Cry2Ab, and Cry1F have no negative effects on *Geocoris punctipes* and *Orius insidiosus*. *Environ. Entomol.* 43, 242-251.

Type	Status	Year Published	NIFA Support Acknowledged
Book Chapters	Awaiting Publication	2015	YES

### Citation

Wang, P. (in press). Mechanism of Cry1Ac resistance in cabbage looper - A resistance mechanism selected in insect populations in an agricultural environment. In: M. Soberon, Y. Gao and A. Bravo (eds.) *Bt Resistance: Molecular Characterization and Strategies for Preserving Effectiveness*. CAB International. (in press)

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Other	2013	NO

### Citation

Tetreau, G. 2013. How environmentally-safe are bioinsecticides? The case study of the mosquitocidal Bti. Invited Presentation, Department of Entomology, Cornell University, Ithaca, NY. Nov 25, 2013.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Other	2013	YES

### Citation

Wang, P. 2013. Variation of the midgut cadherin in the cabbage looper, *Trichoplusia ni*. Annual Meeting of the Entomological Society of America, Austin, TX. Nov. 10-13, 2013.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Other	2013	YES

### Citation

Wang, P. 2013. ABC transporter-associated resistance to *Bacillus thuringiensis* toxins in insects. Annual Meeting of the Entomological Society of America, Austin, TX. Nov. 10-13, 2013.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Other	2013	YES

**Citation**

Tetreau, G., Song, X., Chen, Y-R., Gao, S., Fei, Z., Blissard, G. and Wang, P. 2013. Midgut transcriptome of the cabbage looper, *Trichoplusia ni*. Annual Meeting of the Entomological Society of America, Austin, TX. Nov. 10-13, 2013.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Other	2014	YES

**Citation**

Wang, P. 2014. Molecular genetic basis of insect resistance to Bt-crops. USDA NIFA Biotechnology Risk Assessment Grants Program Annual Project Director's Meeting, Riverdale, MD. Jun 5, 2014.

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Other	2014	YES

**Citation**

Wang, P. 2014. Understanding insecticidal Bt toxins: mode of action and mechanisms of resistance. Invited special seminar series, National Key Laboratory for Biological Control, Zhongshan University, Guangzhou, China. Jan 11, 2014.

**Other Products**

{Nothing to report}

**Changes/Problems**

{Nothing to report}

<b>Title:</b>	<b>Linking pollinator behavior to gene flow to reduce gene flow risk over the landscape</b>		
<b>Sponsoring Agency</b>	NIFA	<b>Project Status</b>	ACTIVE
<b>Funding Source</b>	Non Formula	<b>Reporting Frequency</b>	Annual
<b>Accession No.</b>	1000602	<b>Grants.gov No.</b>	
<b>Project No.</b>		<b>Proposal No.</b>	2013-03551
<b>Project Start Date</b>	09/01/2013	<b>Project End Date</b>	08/31/2017
<b>Reporting Period Start Date</b>	09/01/2013	<b>Reporting Period End Date</b>	08/31/2014
<b>Submitted By</b>	Johanne Brunet	<b>Date Submitted to NIFA</b>	08/28/2014

**Program Code:** HX

**Program Name:** Biotechnology Risk Assessment

**Project Director**

Johanne Brunet  
608-265-3587  
johanne.brunet@ars.usda.gov

**Recipient Organization**

AGRICULTURAL RESEARCH SERVICE  
1815 N UNIVERSITY ST  
Peoria, IL 616043902  
DUNS No. 136635104

**Performing Department**

Agriculture

**Co-Project Directors**

Clayton, Murray

**Departments**

Department of Plant Pathology

**Non-Technical Summary**

The types and acreages planted to genetically-engineered (GE) crops keep increasing. Despite the large number of insect-pollinated crops and the fact that GE varieties are available or being developed for many of these crops, the understanding of how insect pollinators affect the movement of genes via pollen (gene flow) is lagging in comparison to how wind pollination influences gene movement. A better understanding of the factors that affect gene flow in insect-pollinated systems would improve our predictions of gene flow risk and promote the development of management strategies to reduce gene flow. This would increase the potential for coexistence of agricultural crops and reduce the risk of increased invasiveness and decreased genetic diversity of wild populations. A main goal of this research is to link pollinator behavior to gene flow in order to better predict gene flow in insect-pollinated crops. Because pollinator type and landscape attributes have been shown to influence pollinator movements and gene flow, we examine the impact of these factors on the movement of three pollinator types in alfalfa patches of two different sizes or patches planted at different distances from one another. Analyses of these data will highlight the factors that affect pollinator movements. This information will be used to simulate pollinator movements and combined with pollen deposition curves or seed curves, will help better predict pollen movement or gene flow, respectively. The model will be tested using gene flow data obtained experimentally for distinct pollinators in specific landscape settings. A better understanding of the factors that affect gene flow in insect-pollinated systems will not only help us better predict the risk of gene flow, but will also help identify the critical factors mediating gene flow and thus serve as a foundation for improving seed purity Best Management Practices (BMPs). Our approach will help facilitate coexistence among different insect-pollinated production systems in the US.

**Accomplishments**

**Major goals of the project**

The overall goal of this research project is to develop and validate a model of gene flow by insect pollinators at the landscape level. The model links pollinator foraging behavior to gene flow. The specific objectives include (1) to determine the features of the landscape that affect pollinator movements; (2) to determine whether differences exist among pollinators in their movements between flowers while foraging; (3) to examine how pollinator type affect pollen deposition and seed curves; (4) to develop a simulation-based model of pollinator movements for distinct pollinators in different landscapes using the empirical data collected in (1-2) ; (5) to combine data on pollen deposition and seed curves to the pollinator movement model to capture pollen and gene flow; and (6) to validate the model using empirically collected gene flow data in distinct landscapes.

**What was accomplished under these goals?**

Field data on the impact of isolation distances on pollinator movements for three types of pollinators, bumble bees, honey bees and leaf cutting bees were gathered over the summer months (for objectives 1 and 2).  
 Data on residence or the number of flowers visited during a foraging bout in small and in large patches and in patches at different isolation distances were collected over the summer months (for objectives 1 and 2).  
 First-phase of development of the model of pollinator movements within patches of alfalfa was completed (for objective 4).  
 Crosses to produce alfalfa plants with 3 copies of the GUS gene for the experiments on pollen deposition and seed curves were performed (for objective 3).

**What opportunities for training and professional development has the project provided?**

The project has provided training and mentoring for one graduate student involved full time on this project and has contributed to the mentoring of 2 other graduate students who helped with summer data collection.  
 The project has provided training and mentoring for one undergraduate student who worked over the academic year and during the summer months on this project.

**How have the results been disseminated to communities of interest?**

Phone conference with Wisconsin county agents on pollinators and genetically modified crops.  
 Participated (booth on pollination) at two large events to introduce the general public to pollinators and their impact in agriculture. One event reached over 1,000 people (Science Expeditions) and the other over 200 people (Urban Horticulture day at West Madison Agricultural Station).  
 Poster presentation at the NIFA PI meeting

**What do you plan to do during the next reporting period to accomplish the goals?**

Continue development of the pollinator movement models.  
 Gather field data on pollinator movements in alfalfa patches of different sizes and at different isolation distances.  
 Statistical analyses of the field data collected this year.  
 Continue crosses to obtain plants with 3 GUS alleles for the pollen deposition experiments.  
 Use the results of the field data on pollinator movements in patches of sizes and isolation distances to guide the design of the gene flow experiments.

**Participants****Actual FTE's for this Reporting Period**

Role	Non-Students or faculty	Students with Staffing Roles			Computed Total by Role
		Undergraduate	Graduate	Post-Doctorate	
Scientist	0.1	0.7	1	0	1.8
Professional	0	0	0	0	0
Technical	0.3	0	0	0	0.3
Administrative	0	0	0	0	0
Other	0	0	0	0	0
Computed Total	0.4	0.7	1	0	2.1

**Student Count by Classification of Instructional Programs (CIP) Code**

{NO DATA ENTERED}

**Target Audience**

Wisconsin County Agents: Phone Conference on pollinators and genetically modified crops.  
 General public: Booth on pollinator identification, methods of preservation, ecosystem services including roles in agriculture for Science Expeditions at UW-Madison (Audience: 1,000 visitors) and at West Madison Agricultural Station Horticulture field

day (200 visitors).

**Products**

{Nothing to report}

**Other Products****Product Type**

Data and Research Material

**Description**

First year of data collection on pollinator movements in alfalfa patches planted at different distances. These data will be used in the development of the pollinator movement model to help predict gene flow. This model will benefit regulatory agencies, farmers and people and agencies interested in the flow of transgenes between crop fields and in the coexistence of the biotechnology and the conventional, export and organic markets.

**Product Type**

Models

**Description**

First phase of development of the simulation model to describe the movement of pollinators within alfalfa fields. This model will benefit regulatory agencies, farmers and people and agencies interested in the flow of transgenes between crop fields and in the coexistence of the biotechnology and the conventional, export and organic markets.

**Product Type**

Educational Aids or Curricula

**Description**

Guest lectures on pollination biology. To increase awareness and knowledge about pollinators and their role in agriculture, developed and presented lectures on pollination biology for graduate and undergraduate students at the University of Wisconsin in Madison.

**Product Type**

Other

**Description**

Mentored 1 graduate and 1 undergraduate students working on this project during the year and 2 more graduate students over the summer months.

**Changes/Problems**

{Nothing to report}

<b>Title:</b>	<b>Assessing The Impact Of Gene Replacement And Genetic Modification Methods In A Crop Species At The Whole Genome Level</b>		
<b>Sponsoring Agency</b>	NIFA	<b>Project Status</b>	ACTIVE
<b>Funding Source</b>	Non Formula	<b>Reporting Frequency</b>	Annual
<b>Accession No.</b>	1000441	<b>Grants.gov No.</b>	GRANT11359482
<b>Project No.</b>	MICL05078	<b>Proposal No.</b>	2013-03570
<b>Project Start Date</b>	09/01/2013	<b>Project End Date</b>	08/31/2017
<b>Reporting Period Start Date</b>	09/01/2013	<b>Reporting Period End Date</b>	08/31/2014
<b>Submitted By</b>	Linda Haubert	<b>Date Submitted to NIFA</b>	08/25/2014

**Program Code:** HX

**Program Name:** Biotechnology Risk Assessment

**Project Director**

David Douches  
517-884-6946  
douchesd@msu.edu

**Recipient Organization**

MICHIGAN STATE UNIVERSITY  
426 AUDITORIUM RD RM 2  
East Lansing, MI 488242601  
DUNS No. 193247145

**Performing Department**

Plant Soil & Microbial Science

**Co-Project Directors**

Voytas, Daniel  
Buell, Carol

**Departments**

Department of Genetics, Cell Biology and  
Development  
Plant Biology

**Non-Technical Summary**

New methods to engineer crop plants are emerging that have the potential to provide unparalleled specificity in genome modification. However, quantitative data in crop species of potential off-target effects in the genome following targeted genome modification are essential for informed risk assessments of engineered plants. This research is focused on collecting quantitative, whole genome sequence and expression data as well as replicated field trial phenotypic data on a panel of 64 potato lines engineered using three distinct genome modification methods - Agrobacterium transformation and two targeted gene editing methods (CRISPR/Cas and TALENs). For comparison, data will also be gathered from potato lines mutagenized with a conventional chemical mutagen. Importantly, the three genome modification strategies and conventional mutagenesis will create plants with the same phenotype, namely herbicide resistance due to mutations introduced into the acetolactate synthase gene. This proposal addresses the BRAG priority area "Comparison between Transformation-associated Genomic Variation and Genomic Variation Introduced by Non-genetic Engineering Approaches in Plants". This study is relevant to environmental risk assessment and the federal regulatory process.

**Accomplishments**

**Major goals of the project**

This proposal addresses the BRAG priority area "Comparison between transformation-associated genomic variation and genomic variation introduced by non-genetic engineering approaches in plants" as well as "Comparison of the types and frequencies of nucleic acid changes introduced into plant genomes via genetic insertion techniques versus other plant breeding techniques". This proposed study is relevant to environmental risk assessment and the federal regulatory process and should provide data that will quantify the potential of off-target effects that may occur from newer genetic engineering technologies in comparison to current methods for induced mutation.

Our specific objectives are to:

**Objective 1:** Generate independent herbicide resistant lines using *Agrobacterium*-mediated transformation, CRISPR/Cas and TALEN-mediated gene modification for a total of 48 independent lines. In parallel, we will generate 16 independent herbicide resistant lines using ethyl methanesulfonate (EMS).

**Objective 2:** Perform whole genome sequencing in the 48 genetically modified lines and 16 EMS lines to assess off-target effects on the DNA sequence.

**Objective 3:** Perform whole transcriptome gene profiling in the set of 64 lines to assess the impact of genetic modification or induced variation on expression patterns at the whole transcriptome level.

**Objective 4:** Assess agronomic phenotypes of the 48 genetically modified and 16 EMS lines in the field to determine phenotypic effects relevant to agriculture.

#### What was accomplished under these goals?

Viable protoplasts from two diploid lines of potato MSX914-10 and chc523-3, are being routinely isolated and transformed with control constructs ( $n \geq 10$ ; 35S::eGFP) resulting in transformation rates averaging  $>50\%$ . MSX914-10 protoplasts have been transformed with six different site-specific nucleases targeting the acetolactate synthase (*ALS*) genes in *S. tuberosum*. These nucleases consist of four separate TALE-Nucleases, and two CRISPR/Cas9 nucleases. Assessment of the efficacy of the nucleases via amplification of the *ALS* locus followed by high-throughput sequencing is ongoing. An additional six site-specific nucleases (SSNs: two TALEN and four CRISPR/Cas9 reagents) have been developed and will be transformed into the diploid lines protoplasts to assess efficacy as described above. The most effective reagent from each class of SSN will be used to create the herbicide-resistant plant lines. Six plasmid constructs that encode SSNs and donor molecules that will convert the *ALS* locus into the chlorosulfuron- and imazamox- resistant version have been developed. The additional six SSN/donor molecule combination plasmids are in progress.

Recently, a new protocol has been developed for regeneration of calli from protoplasts. MSX914-10 and chc523-3 show signs of callus growth under the newly developed protocol. This protocol is still undergoing optimization.

Control lines with an herbicide resistant *ALS* transgene and herbicide resistant EMS (ethyl methanesulfonate)-induced mutations are currently being generated. The herbicide resistant *ALS* transgene consists of a modified coding sequence and native 2.5 kb upstream promoter region of one of the two *ALS* genes in *S. tuberosum* (referred to as *ALS1*). Modifications of the coding sequence include mutations previously shown to confer resistance to *ALS*-inhibiting herbicides such as chlorosulfuron and Imazamox. To date, 54 *ALS* transgene control lines in the MSX914-10 background have been generated and evaluated using a rooting assay in which high and low concentrations of Imazamox are tested. Of the 54 lines, 21 demonstrated resistance to low concentrations of Imazamox and 14 demonstrated resistance to high concentrations of Imazamox. In addition to *ALS* transgene control lines, experiments are being conducted to optimize EMS treatment and herbicide selection using selfed seed from chc523-3.

**Genome sequence and assembly of targeted diploid potato genomes.** Two diploid potato genotypes are currently being assessed for genome editing, chc523-3 and MSX914-10. To date, we have generated multiple Illumina paired end sequencing libraries for each of these two genotypes. All libraries have been sequenced, the reads have been cleaned, and an initial assembly constructed for the two genotypes. We are currently exploring additional genome assembly software and altered assembly parameters to improve the quality of the assemblies with respect to coverage and length of the scaffolds. In addition, we are in the process of generating mate pair libraries for each of these genotypes to further increase the N50 scaffold sizes.

#### What opportunities for training and professional development has the project provided?

Two graduate students are running the lab experiments. An undergraduate Honors student is assisting the graduate student at MSU. A post doc at MSU has been obtaining exposure to field plot experimentation. The MSU graduate student traveled to the University of Minnesota for additional training in genome editing techniques and procedures. He also attended the Plant Genome Stability and Change conference Asilomar Conference Center, Pacific Grove, CA.

#### How have the results been disseminated to communities of interest?

A poster on the research project was presented at the project directors meeting in Beltsville, MD June 2014. In December 2013 the genome editing research was reported at the North Central Regional Potato Breeding and Genetics meeting in Chicago, IL. An abstract was submitted to the 2014 Potato Association of America meeting in Spokane, WA. A poster was presented at the Plant Genome Stability and Change conference, Asilomar Conference Center, Pacific Grove, CA.

#### What do you plan to do during the next reporting period to accomplish the goals?

Our goal in year one is to optimize the protocols and then generate and characterize the germplasm that is edited and modified by the TALENs, CRISPRs/Cas9, EMS and Agrobacterium experiments.

#### Participants

##### Actual FTE's for this Reporting Period

Role	Non-Students or faculty	Students with Staffing Roles			Computed Total by Role
		Undergraduate	Graduate	Post-Doctorate	
Scientist	0.1	0.2	1	0	1.3

**Actual FTE's for this Reporting Period**

Role	Non-Students or faculty	Students with Staffing Roles			Computed Total by Role
		Undergraduate	Graduate	Post-Doctorate	
Professional	0	0	0	0	0
Technical	0.2	0	0	0	0.2
Administrative	0	0	0	0	0
Other	0	0	0	0	0
Computed Total	0.3	0.2	1	0	1.5

**Student Count by Classification of Instructional Programs (CIP) Code**

{NO DATA ENTERED}

**Target Audience**

Plant Biotechnologists and regulators of GM plants.

**Products**

{Nothing to report}

**Other Products****Product Type**

New Germplasm

**Description**

We have created some potato lines that have the ALS gene modified by TALENs and CRISPRs to confer herbicide resistance.

**Changes/Problems**

As an alternative to protoplast transformation, a geminivirus transformation system has been developed in *S. tuberosum* using the MSX914-10 genotype. This geminivirus virus system has been used successfully in tobacco by the Voytas lab for introducing modifications via homologous recombination (HR) and could facilitate generation of TALEN and CRISPR-modified lines. The geminivirus system is an Agrobacterium-based two component system relying on the independent action of a replicase (REP) viral enzyme to release a geminivirus replicon (GVR) containing the SSN and donor molecule. Once released, the GVR is replicated to a high copy number in the plant cell via host factors and can become the substrate for HR. Initial experiments using this system demonstrated the ability to replicate the GVR in potato leaf explants by co-transforming a REP-containing construct (35S::REP) and a GVR containing a GUS control marker (LSL-GUS). Upon demonstration of GVR replication, SSNs targeting ALS and the ALS donor molecule including a neomycin phosphotransferase II (NptII) marker fusion for kanamycin resistance were cloned into the geminivirus construct (LSL-GT) and used for transformation experiments. Kanamycin-resistant events were regenerated and screened for herbicide modifications in ALS. At least one line using CRISPR/Cas and TALENs have been verified using Sanger sequencing and are being used in crosses with chc523-3 to determine if the modifications are heritable. Additional events are currently being generated using different modes of selection (ex: Imaxamox) and will be evaluated in a similar manner.

<b>Title:</b>	<b>Monitoring The Dispersal Of Genetically Engineered Organisms And Their Byproducts Using Light Transmission Spectroscopy</b>		
<b>Sponsoring Agency</b>	NIFA	<b>Project Status</b>	ACTIVE
<b>Funding Source</b>	Non Formula	<b>Reporting Frequency</b>	Annual
<b>Accession No.</b>	1000425	<b>Grants.gov No.</b>	
<b>Project No.</b>		<b>Proposal No.</b>	2013-03556
<b>Project Start Date</b>	09/01/2013	<b>Project End Date</b>	08/31/2015
<b>Reporting Period Start Date</b>	09/01/2013	<b>Reporting Period End Date</b>	08/31/2014
<b>Submitted By</b>	Scott Egan	<b>Date Submitted to NIFA</b>	09/02/2014

**Program Code:** HX

**Program Name:** Biotechnology Risk Assessment

**Project Director**

Scott Egan

615-618-6601

egan.28@nd.edu

**Recipient Organization**

UNIVERSITY OF NOTRE DAME DU LAC

940 GRACE HALL

Notre Dame, IN 465560000

DUNS No. 824910376

**Performing Department**

Biological Sciences

**Co-Project Directors**

Feder, Jeffrey

Howard, Scott

Ruggiero, Steven

Tanner, Carol

Tank, Jennifer

Lodge, David

**Departments**

Biological Sciences

Electrical Engineering

Physics

**Non-Technical Summary**

Understanding and monitoring the distribution and dispersal of genetically engineered organisms and their byproducts is a critical component of the safe and responsible use of transgenic technology. However, we currently lack the ability to rapidly and adequately track the movement of genetically engineered organisms and their byproducts in the environment, even though research has demonstrated that they can escape their intended ranges. Our multidisciplinary team, which includes evolutionary biologists, ecologists, physicists, and biogeochemists, will address this critical challenge by working to increase our ability to detect genetically engineered organisms and their byproducts in the environment. We will adapt a novel technology called Light Transmission Spectroscopy, which has the ability to identify and accurately measure in real-time the size, shape, and number of small particles suspended in fluid at the nanometer scale (1 nanometer equals 1 billionth of a meter). Light Transmission Spectroscopy has demonstrated high sensitivity and greater size resolution than competing technologies, which we will use to address two specific and pressing needs in genetically engineered detection: the potential dispersal of genetically engineered fish and the byproducts of genetically engineered maize.

To detect genetically engineered fish dispersal, we will combine Light Transmission Spectroscopy with another developing technology, environmental DNA (eDNA). eDNA is a species surveillance tool that recognizes a unique advantage of aquatic environmental sampling: Water often contains microscopic bits of tissue in suspension, including the scales of fish, the exoskeletons of insects and the sloughed cells and tissues of aquatic species. These tissue fragments can be filtered from water samples, and then a standard DNA extraction is performed on the filtered matter. Then, we will develop the Light Transmission Spectroscopy device to detect the DNA of a specific species. To detect the byproducts of genetically engineered maize, we will take a similar approach. Here, the byproduct is the Bt toxin produced by genetically engineered maize. The Bt toxin is intended to kill insect pests feeding on the maize, but can leach into nearby streams. The Bt toxin can also be sampled from aquatic environments by filtering. Then, we will develop the Light Transmission Spectroscopy device to detect the Bt toxin. Overall, Light Transmission Spectroscopy exhibits the potential to be a field ready device that can generate rapid and highly accurate detection results, even when a target is at low densities.

## Accomplishments

### Major goals of the project

Understanding and monitoring the dispersal of genetically engineered (GE) organisms and their byproducts is a critical component of the safe and responsible use of transgenic technology in the environment. However, we currently lack the ability to rapidly and adequately track the movement of GE organisms and their byproducts in the environment, even though previous work has demonstrated that GE organisms can escape their intended range and their byproducts can disperse off of agricultural fields and throughout river networks. Our multidisciplinary project and team will address this critical challenge described in program area (2) ?Methods to Monitor Dispersal of GE Organisms? within the Biotechnology Risk Assessment Research Grants (BRAG) Program to increase our fundamental ability to detect GE organisms and GE byproducts in the environment. Our approach combines advances in environmental, biological, and nanoscale technologies, to address this challenge through the development of environmental sampling strategies and rapid, field-based detection devices that can identify GE organisms and their byproducts using target-specific genetic variation and target-specific protein binding properties. We will adapt Light Transmission Spectroscopy (LTS) technologies, which have been applied to DNA based detection of aquatic invasive species, to the question of GE monitoring and dispersal in aquatic environments. LTS has the ability to identify and accurately measure in real-time the size, shape, and number of nanoparticles suspended in fluid at 106 times the sensitivity and 5 times the size resolution of competing technologies. LTS is applicable for species detection by measuring nanoparticles that specifically bind to target species DNA and therefore grow in size. We propose to address two pressing needs in detection of GE organisms and their byproducts using LTS field and agricultural settings to safeguard natural resources and biodiversity. Specifically, we will address two critical questions in the proposed research: (1.) Can eDNA combined with LTS be applied to GE organism detection in aquatic environments in the same way it has pioneered the detection of aquatic invasive species? (2.) Can we detect GE specific proteins, such as Cry1Ab from GE maize, using LTS and protein-specific antibodies? The LTS technology exhibits the potential to be a field ready device that can generate rapid and highly accurate detection results, even when a target is at low densities. Moreover, this work will have far reaching applications beyond the specific GE organisms tested here, as these studies can work as examples for detection of any organism or byproduct given straightforward modifications of the approaches proposed here. We will conduct a sequential and complementary set of experiments that apply the LTS technology to an ongoing agricultural and societal challenge, the dispersal of GE organisms and GE byproducts beyond intended boundaries. We plan on addressing two dimensions of detection in aquatic environments, target proteins and DNA specific variation, using two different GE organisms as examples of how the approach can be employed for any GE organisms or byproducts. First, we will consider the GE fish, the AquaAdvantage salmon (AquaBounty Technologies), which is currently under review from the FDA. This is an Atlantic salmon (*Salmo salar*) that has been modified by the addition of a growth hormone regulating gene from a Pacific Chinook salmon (*Oncorhynchus tshawytscha*) and a promoter from an ocean pout (*Zoarces americanus*). These transferred genes enable the AquaAdvantage salmon to grow year round instead of only during spring and summer and increases the speed at which the fish grows, without affecting its ultimate size or other qualities. However, concerns have been raised about the possible escape of the GM salmon and how that will affect native stocks, with studies demonstrating the ability of the GM salmon to outcompete wild fish. Thus, there is a critical need for an accurate, rapid, and field ready detection platform for the detection of this GM fish prior to its introduction and commercial use. The second challenge we will address with LTS is the detection of Cry1Ab protein produced by Bt maize. Insect-resistant transgenic maize is now planted throughout North American and globally, with nearly 23 million hectares in the US. One of the more common insect-resistant GE maize varieties expresses the insecticidal Cry1Ab protein from *Bacillus thuringiensis* (hereafter Bt maize) to resist crop damage by the European corn borer (*Ostrinia nubilalis*). Cry1Ab protein is expressed throughout the tissues of Bt maize, and after maize is harvested, the protein remains detectable in terrestrial detritus for up to seven months. The potential effects of Bt maize detritus on non-target terrestrial organisms and ecosystems have been studied. Recent work has found that maize detritus enters, is processed, and can be transported within streams flowing adjacent to crop fields and laboratory trials suggested that consumption of Bt maize detritus may affect stream dwelling invertebrates, but may be dependent on duration of leaching of submerged detritus. Thus the potential effects of Bt maize on aquatic ecosystems have attracted a great deal of attention recently, despite only a handful of studies exploring this topic. To test the LTS approach on GE byproducts, using Bt maize, we will use an antibody marker specific to the Bt maize Cry1Ab protein to monitor its dispersal through aquatic environments. Our overall goal is to develop field-based technologies using application of cutting edge LTS techniques to improve GE organism detection in aquatic environments, giving stakeholders the tools they need to monitor the distribution and dispersal, even at very low densities and concentrations.

### What was accomplished under these goals?

In the first year of this grant, which we are reportin on here, we have made great intital progress. This progress involved partially funding two new PhD graduate students (one in Biology and one in Physics/Electrical Engineering). With the hiring of these two graduate students, we could begin experiments, including eDNA experiments with salmonids and carp, as well as eProtein experiments with Cry1Ab and GM maize. Our intiiial experiments in the lab and using our controlled stream facility at the Cary Institute have gone well. We are in the process of analyzing data from these experiments now, while also planning our next set of experiments. Our LTS device seems to be working well detecting eDNA and eProtein signals, with the latter

being a very new result for the field of protein detection.

### What opportunities for training and professional development has the project provided?

Our project is providing training and professional development for two graduate students, as they are actively participating in experimental design and the execution of experiments, data analysis, protein and genetic work, as well as other molecular biology techniques. This project is naturally interdisciplinary, and the students participating in this work are receiving a broad training in integrative and interdisciplinary work.

### How have the results been disseminated to communities of interest?

We presented an initial poster at the NIFA-USDA-BRAG annual PI meeting, which included some initial results of the eDNA and eProtein results.

### What do you plan to do during the next reporting period to accomplish the goals?

We plan on continuing the experiments described in our initial proposal, including additional lab and field experiments on eDNA and eProtein detection using Light Transmission Spectroscopy. These experiments will include the detection and sensitivity of eDNA to detect salmonid fish, similar to GM salmon, and eProtein detection for the Cry1Ab protein produced by GM maize. These experiments will include lab based experiments in aquaria, an experimental stream system, and field experiments in areas known to harbor target organisms.

### Participants

#### Actual FTE's for this Reporting Period

Role	Non-Students or faculty	Students with Staffing Roles			Computed Total by Role
		Undergraduate	Graduate	Post-Doctorate	
Scientist	0.8	0	0.5	0	1.3
Professional	0	0	0	0	0
Technical	0	0	0	0	0
Administrative	0	0	0	0	0
Other	0	0	0	0	0
Computed Total	0.8	0	0.5	0	1.3

### Student Count by Classification of Instructional Programs (CIP) Code

{NO DATA ENTERED}

### Target Audience

Our research effort extends to many different audiences, including farmers, natural resource managers, persons interested in biotechnology, and NIFA employees, as well as other members of the federal government involved with GMOs.

### Products

{Nothing to report}

### Other Products

{Nothing to report}

### Changes/Problems

Natural to studies that press the limits of detection, we have run into challenges. One initial problem in protein detection in the lab, which we have consulted with local biochemist at the University of Notre Dame, was that the protein and the antibody were clumping in our solutions as we were trying to experiment with detection and dilutions series to test for sensitivity. We have adopted the suggestions of our colleagues, including adding an additional chemical to help avoid clumping, as well as mechanical solutions using sonication to break up clumped products. We are now moving forward after solving this initial problem.

<b>Title:</b>	<b>Silencing of naturally occurring genes controlling seed dormancy to reduce fitness of transgene-contaminated weedy rice</b>		
<b>Sponsoring Agency</b>	NIFA	<b>Project Status</b>	ACTIVE
<b>Funding Source</b>	Non Formula	<b>Reporting Frequency</b>	Annual
<b>Accession No.</b>	1000577	<b>Grants.gov No.</b>	GRANT11359941
<b>Project No.</b>	SD00G466-13	<b>Proposal No.</b>	2013-03572
<b>Project Start Date</b>	09/01/2013	<b>Project End Date</b>	08/31/2017
<b>Reporting Period Start Date</b>	09/01/2013	<b>Reporting Period End Date</b>	08/31/2014
<b>Submitted By</b>	Cy Wang	<b>Date Submitted to NIFA</b>	07/16/2014

**Program Code:** HX

**Program Name:** Biotechnology Risk Assessment

**Project Director**

Xingyou Gu  
605-688-6908  
xingyou.gu@sdstate.edu

**Recipient Organization**

SOUTH DAKOTA STATE UNIVERSITY  
2201 ADMINISTRATION LANE  
Brookings, SD 570070001  
DUNS No. 929929743

**Performing Department**

Plant Science

**Co-Project Directors**

{NO DATA ENTERED}

**Departments**

{NO DATA ENTERED}

**Non-Technical Summary**

Cross-pollination between genetically modified crops and local wild relatives, or gene flow, may create fitness-enhanced weeds that combine the wild with transgenic characteristics to exacerbate weed problems in agriculture. Seed dormancy, a key adaptive trait controlled by genes, disseminates germination across seasons, making weeds persist in agro-ecosystems. This project aims to develop a transgenic mitigating (TM) strategy to reduce the risk of gene flow by linking to a primary transgene with RNA interference (RNAi) structures to silence seed dormancy genes prevailing in weeds. Because of the linkage with reduced dormancy or increased germination uniformity, transgene-containing weeds would be less persistent in the soil seed bank and also relatively easy to eliminate by agronomic practices. The seed dormancy genes SD7-1, SD7-2 and SD12 cloned from weedy "red" rice will be used to design the silencing structures, which will be ligated with the herbicide resistance gene Bar on the same vectors to transform a rice cultivar. Transgenic lines will be selected to cross with isogenic lines for the dormancy genes to determine the silencing effects under controlled conditions. Selected transgenic lines will be also crossed with weedy rice to evaluate fitness of the transgene-containing weed genotypes under field conditions across generations. Expected outcomes include new knowledge about the efficacy of the TM approach in the rice crop system, techniques that could be extended to or modified for the other crop systems to prevent the rising of fitness-enhanced weeds, and purified transgenic materials that will be disseminated for in-depth analysis of gene flow.

**Accomplishments**

**Major goals of the project**

The goal of this project is to develop a transgenic mitigation (TM) strategy by silencing naturally occurring seed dormancy genes prevailing in wild/weed populations to lower the risk of "geneflow" from genetically modified (GM) crops in agro-ecosystems. The objectives of this project are:

- 1) To develop transgenic lines of rice using a built-in construct that contain an herbicide-resistant gene and RNA interference (RNAi) structure(s) silencing one to three seed dormancy genes cloned from weedy red rice;
- 2) To determine effects of the built-in constructs on germination uniformity, herbicide resistance, and other adaptive traits in a nearly isogenic background under controlled conditions; and,
- 3) To evaluate fitness of the transgene-containing weed genotypes under field conditions across generations.

**What was accomplished under these goals?**

1. Developed three types of tandem constructs to completely link the herbicide resistance transgenic gene Bar with one, two, and three RNAi structures, respectively. These RNAi structures are inverted repeat sequences (IRS) cloned from the seed dormancy genes SD7-1, SD7-2 and SD12.

2. Completed transformation for the three types of tandem constructs using the japonica type of cultivated rice Nipponbare as the recipient and obtained transgenic T0 plants.
3. Analyzed T0 plants for copy numbers of the transgenic structures by Southern Blot and for resistance to the commercial herbicide glyphosate. Transgenic lines with the IRS for SD7-1 (Bar::IRS\_SD7-1) or the IRSs for both SD7-1 and SD12 (Bar::IRSs\_SD7-1+SD12) were advanced to the T2 generation.
4. Developed hybrids between the selected IRS\_SD7-1 transgenic T0 plants and the isogenic line for the SD7-1 functional allele (ILSD7-1) and obtained hybrid (Bar::IRS\_SD7-1/ILSD7-1) F1 plants and their F2 segregating populations.
5. Conducted genetic analysis for one F2 population from the Bar::IRS\_SD7-1/ILSD7-1 cross to identify linkage effects of the BAR::IRS\_SD7-1 tandem construct on seed dormancy, red pericarp color (SD7-1's pleiotropic effect) and herbicide resistance.
6. Developed hybrids between the selected IRSs\_SD7-1+SD12 transgenic T0 plants and two accessions of weedy rice and obtained hybrid (Bar::IRSs\_SD7-1+SD12/weedy rice) F1 plants.

#### What opportunities for training and professional development has the project provided?

One postdoctoral research fellow worked for 60% of his time on the project to receive training for plant genetics and molecular biology and for advising graduate students in PD's lab.

One graduate student worked for about 30% of her time on the project to receive training for plant genetics and molecular biology.

#### How have the results been disseminated to communities of interest?

Part of the results from the research in the first year was released to the U.S. Rice Community by an oral presentation in The 35<sup>th</sup> Rice Technical Working Group Meeting held in New Orleans, LA, in February 2014. The abstract for the presentation was published in the conference symposium.

A poster developed based on the latest results of the project was present in the USDA-NIFA BRAG Program Directors' Meeting.

#### What do you plan to do during the next reporting period to accomplish the goals?

1. Continue to advance the transgenic plants and the transgene/weedy rice hybrids developed in the first year to the higher generations for the following experiments.
2. Select transgenic lines to map the insertion loci on the rice genome by TAIL-PCR and linkage mapping to track the transgene mitigating effects across generations.
3. Compare differences of the Bar::IRSs\_SD7-1+SD12 construct for effects on seed dormancy between tropic and temperate ecotypes of weedy rice in F2 populations.
4. Evaluate effects of the Bar::IRSs\_SD7-1+SD12 construct on seed germinability in soil under controlled conditions.
5. Develop hybrids between selected Bar::IRSs\_SD7-1+SD7-2+SD12 transgenic plants and two accessions of weedy rice to evaluate effects of silencing the three seed dormancy genes on the fitness of transgene-contaminated weedy rice.

#### Participants

##### Actual FTE's for this Reporting Period

Role	Non-Students or faculty	Students with Staffing Roles			Computed Total by Role
		Undergraduate	Graduate	Post-Doctorate	
Scientist	0.3	0	0.3	0.6	1.2
Professional	0	0	0	0	0
Technical	0	0	0	0	0
Administrative	0	0	0	0	0
Other	0	0	0	0	0
Computed Total	0.3	0	0.3	0.6	1.2

#### Student Count by Classification of Instructional Programs (CIP) Code

{NO DATA ENTERED}

#### Target Audience

{Nothing to report}

### Products

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Published	2014	YES

### Citation

Gu, X.-Y., H. Ye, J. Feng. 2014. Silencing naturally occurring genes controlling seed dormancy to reduce fitness of transgene-contaminated weedy rice. USDA-NIFA Project Directors' Meeting for Biotechnology Risk Assessment Grants Program, USDA-APHIS, Oklahoma Memorial Conference Center, MD, June 5, 2014.

Ye, H., J. Feng, X.-Y. Gu. 2014. "Mitigating risk of gene-flow from transgenic cultivars to weedy rice by silencing seed dormancy genes", The 35th Rice Technical Working Group Meeting, New Orleans, LA., Feb. 18-21, 2014

Type	Status	Year Published	NIFA Support Acknowledged
Other	Published	2014	YES

### Citation

Gu Xing-You, "Genetics of seed dormancy from natural variation to genes in rice" Invited lecture by Department of Plant Pathology & Crop Physiology, Louisiana State University, Feb. 19, 2014.

### Other Products

{Nothing to report}

### Changes/Problems

{Nothing to report}

<b>Title:</b>	<b>Assessing the risk of transgene escape via pollen flow in carrot</b>		
<b>Sponsoring Agency</b>	NIFA	<b>Project Status</b>	COMPLETE
<b>Funding Source</b>	Non Formula	<b>Reporting Frequency</b>	Annual
<b>Accession No.</b>	1000458	<b>Grants.gov No.</b>	GRANT11354085
<b>Project No.</b>		<b>Proposal No.</b>	2013-03560
<b>Project Start Date</b>	09/01/2013	<b>Project End Date</b>	08/31/2016
<b>Reporting Period Start Date</b>	09/01/2013	<b>Reporting Period End Date</b>	08/31/2016
<b>Submitted By</b>	Jennifer Mandel	<b>Date Submitted to NIFA</b>	02/07/2014

**Program Code:** HX

**Program Name:** Biotechnology Risk Assessment

**Project Director**

Jennifer Mandel

706-583-5510

jmandel@plantbio.uga.edu

**Recipient Organization**

UNIVERSITY OF GEORGIA RESEARCH

200 D.W. BROOKS DR

Athens, GA 306025016

DUNS No. 004315578

**Performing Department**

Plant Biology

**Co-Project Directors**

McCauley, David

McCauley, David

**Departments**

Department of Biological Sciences

Biological Sciences

**Non-Technical Summary**

Gene flow between genetically modified (GM) crops and their wild relatives has the potential to enhance weediness and/or invasiveness of wild species. In the United States, invasive species have major negative impacts on natural ecosystems, leading to billions of dollars per year in economic and environmental damages, and invasive species are often responsible for the displacement and/or extirpation of native species. Given that crop plants and their sexually-compatible wild relatives often overlap in terms of geographic proximity and flowering time, the likelihood of gene escape can be quite high. The placement of a transgene into the organellar (chloroplast or mitochondrial) genome of a plant has been suggested as a means of reducing the risk of pollen-mediated transgene escape from GM crops to wild relatives because these genomes are usually transmitted solely through seed and not pollen (that is, the chloroplast and mitochondria are maternally transmitted). Carrot lines carrying transgenes conferring a variety of beneficial traits are being developed and organellar placement of such transgenes has been proposed to reduce the risk of transgene escape via pollen to wild and weedy carrot. Maternal transmission of organellar genomes is, however, far from universal, and mathematical models have suggested that even low levels of transmission in pollen may be sufficient for the establishment and spread of transgenes in the wild. In carrot, we have found preliminary evidence that the chloroplast and/or mitochondrial genomes may sometimes be paternally transmitted thus allowing a transgene to potentially escape via pollen flow to its highly inter-fertile weedy relative, wild carrot (Queen Anne's Lace). We plan to use multiple approaches to assess the likelihood and degree of paternal transmission of organellar genomes in carrot. The proposed research will greatly improve our understanding of the potential for the paternal transmission of organellar genomes to allow transgene escape via crop-wild hybridization. Beyond providing insight into specific risks associated with crop-wild gene flow in carrot, this project will also inform risk assessment efforts in other crop systems.

**Accomplishments**

**Major goals of the project**

The specific goals of this project are to:

- (1) characterize the level of heteroplasmy in natural populations of wild carrot and evaluate the potential for paternal leakage and cytoplasmic gene flow;
- (2) directly measure the frequency of paternal leakage and heteroplasmy in controlled crosses between cultivated and wild carrot;
- (3) investigate historical levels of crop-wild gene flow across the range of carrot cultivation, thereby providing baseline estimates of gene flow for use in mathematical models aimed at assessing the risk of transgene escape;
- (4) implement and expand upon predictive models of the spread of cytoplasmic transgenes in wild populations.

**What was accomplished under these goals?**

A series of plant collections from wild populations of *Daucus carota* (Queen Anne's Lace) have been made along the eastern coast of the United States. When possible, both leaves and seeds were collected to facilitate the goals of Objectives 1 and 2 of this grant: characterize the level of heteroplasmy in natural populations of wild carrot and use indirect methods to evaluate the potential for paternal leakage and cytoplasmic gene flow, and characterize the level of heteroplasmy in natural populations of wild carrot and use indirect methods to evaluate the potential for paternal leakage and cytoplasmic gene flow. DNA extraction of these wild individuals is underway. In addition, the assays for quantifying heteroplasmy in *D. carota* have been designed, ordered, obtained, and calibration experiments of the assays have begun. With respect to Objectives 3 and 4, no progress has been made to date. Work on these objectives will continue at the PD's new institution.

**What opportunities for training and professional development has the project provided?**

Since this project had just begun in September 2013, and the project is being transferred to the University of Memphis where the PD is now an Assistant Professor, no training and professional developments have occurred at this time. These opportunities will occur as the project continues in the PD's new institution.

**How have the results been disseminated to communities of interest?**

Since this project had just begun in September, and the project is being transferred to the University of Memphis where the PD is now an Assistant Professor, no results have been disseminated at this time. Results will be disseminated to communities of interest as the project continues in the PD's new institution.

**What do you plan to do during the next reporting period to accomplish the goals?**

{Nothing to report}

**Participants****Actual FTE's for this Reporting Period**

Role	Non-Students or faculty	Students with Staffing Roles			Computed Total by Role
		Undergraduate	Graduate	Post-Doctorate	
Scientist	1.2	0	0	0	1.2
Professional	0	0	0	0	0
Technical	0	0	0	0	0
Administrative	0	0	0	0	0
Other	0	0	0	0	0
Computed Total	1.2	0	0	0	1.2

**Student Count by Classification of Instructional Programs (CIP) Code**

{NO DATA ENTERED}

**Target Audience**

The target audiences for this research were ecological geneticists and biotechnology regulators. This research was also relevant for seed producers who wish to minimize crop-to-crop transgene dispersal.

**Products**

{Nothing to report}

**Other Products**

{Nothing to report}

**Changes/Problems**

The PD has moved to the University of Memphis (Assistant Professor of Biological Sciences). There has been no modification of the scientific work or plan for this grant. The grant is being transferred to the University of Memphis.

<b>Title: Assessing the Risk of Transgene escape via pollen flow in carrot</b>			
<b>Accession No.</b>	1002531	<b>Sponsoring Institution</b>	National Institute of Food and Agriculture
<b>Project No.</b>		<b>Project Status</b>	ACTIVE
<b>Funding Source</b>	Non Formula	<b>Proposal No.</b>	2014-01446
<b>Grants.gov No.</b>	GRANT11692035	<b>DUNS Number</b>	055688857
<b>Start Date</b>	12/15/2013	<b>End Date</b>	12/14/2016
<b>Award Number</b>	2014-33522-21826	<b>Award Amount</b>	\$ 494,740
<b>Award Date</b>	09/05/2014	<b>Award Fiscal Year</b>	2014
<b>Submitted By</b>	Jennifer Mandel	<b>Date Submitted to NIFA</b>	01/30/2014

**Program Code** HX

**Program Name**

Biotechnology Risk Assessment

**Project Director**

Jennifer Mandel

901-678-5130

jmandel@memphis.edu

**Performing Organization/Institution**

UNIVERSITY OF MEMPHIS, THE

315 ADMINISTRATION BLDG

Memphis, TN 381523302

**Performing Department**

Biological Sciences

**Co-Project Directors**

McCauley, David

**Departments**

Biological Sciences

**Collaborating/Partnering States**

{NO DATA ENTERED}

**Collaborating/Partnering Countries**

{NO DATA ENTERED}

**Collaborating/Partnering Organizations**

University of Memphis

**Non-Technical Summary**

Gene flow between genetically modified (GM) crops and their wild relatives has the potential to enhance weediness and/or invasiveness of wild species. In the United States, invasive species have major negative impacts on natural ecosystems, leading to billions of dollars per year in economic and environmental damages, and invasive species are often responsible for the displacement and/or extirpation of native species. Given that crop plants and their sexually-compatible wild relatives often overlap in terms of geographic proximity and flowering time, the likelihood of gene escape can be quite high. The placement of a transgene into the organellar (chloroplast or mitochondrial) genome of a plant has been suggested as a means of reducing the risk of pollen-mediated transgene escape from GM crops to wild relatives because these genomes are usually transmitted solely through seed and not pollen (that is, the chloroplast and mitochondria are maternally transmitted). Carrot lines carrying transgenes conferring a variety of beneficial traits are being developed and organellar placement of such transgenes has been proposed to reduce the risk of transgene escape via pollen to wild and weedy carrot. Maternal transmission of organellar genomes is, however, far from universal, and mathematical models have suggested that even low levels of transmission in pollen may be sufficient for the establishment and spread of transgenes in the wild. In carrot, we have found preliminary evidence that the chloroplast and/or mitochondrial genomes may sometimes be paternally transmitted thus allowing a transgene to potentially escape via pollen flow to its highly inter-fertile weedy relative, wild carrot (Queen Anne's Lace). We plan to use multiple approaches to assess the likelihood and degree of paternal transmission of organellar genomes in carrot. The proposed research will greatly improve our understanding of the potential for the paternal transmission of organellar genomes to allow transgene escape via crop-wild hybridization. Beyond providing insight into specific risks associated with crop-wild gene flow in carrot, this project will also inform risk assessment efforts in other crop systems.

**Goals / Objectives**

Gene flow between genetically modified (GM) crops and their wild relatives has the potential to enhance weediness and/or

invasiveness of wild species. In angiosperms, cytoplasmic transgene placement has been proposed to reduce the risk of transgene escape due to the maternal transmission of organellar genomes; however, this maternal inheritance is far from universal. We propose to investigate the potential for the escape of cytoplasmic transgenes from cultivated into wild carrot, where we have already found indirect evidence of paternal leakage in the form of organellar heteroplasmy. Our specific aims are to:

- (1) characterize the level of heteroplasmy in natural populations of wild carrot and evaluate the potential for paternal leakage and cytoplasmic gene flow;
- (2) directly measure the frequency of paternal leakage and heteroplasmy in controlled crosses between cultivated and wild carrot;
- (3) investigate historical levels of crop-wild gene flow across the range of carrot cultivation, thereby providing baseline estimates of gene flow for use in mathematical models aimed at assessing the risk of transgene escape;
- (4) implement and expand upon predictive models of the spread of cytoplasmic transgenes in wild populations.

Beyond providing insight into specific risks associated with crop-wild gene flow in carrot, where numerous GM lines have been developed and organellar gene containment has been proposed as a means for reducing the risk of gene escape, this project will also inform risk assessment efforts in other systems. This work is thus highly relevant to the stated goals of the Program, especially with regard to Program Area 3, "Gene Transfer to Domesticated and Wild Relatives."

### Methods

Field, greenhouse, and laboratory methods will be employed. We will also use quantitative PCR, statistical software, and mathematical modeling.

### Target Audience

The target audiences for this research are ecological geneticists and biotechnology regulators. This research is also relevant for seed producers who wish to minimize crop-to-crop transgene dispersal.

### Products

Several peer-reviewed publications will result from the funded research. At least one graduate student, one research technician, and at least 2 undergraduates will be trained.

### Expected Outcomes

The placement of a transgene into the organellar (chloroplast or mitochondrial) genome of a plant has been suggested as a means of reducing the risk of pollen-mediated transgene escape from GM crops to wild relatives because these genomes are usually transmitted solely through seed and not pollen (that is, the chloroplast and mitochondria are maternally transmitted). In carrot, we have found preliminary evidence that the chloroplast and/or mitochondrial genomes may sometimes be paternally transmitted thus allowing a transgene to potentially escape via pollen flow to its highly inter-fertile weedy relative, wild carrot (Queen Anne's Lace). The work we have begun will improve our understanding of the potential for the paternal transmission of organellar genomes to allow transgene escape via crop-wild hybridization.

### Keywords

Daucus ~carrot ~cpDNA ~crop ~gene flow ~heteroplasmy ~mtDNA ~paternal leakage ~wild

### Estimated Project FTEs For The Project Duration

Role	Non-Students or Faculty	Students with Staffing Roles			Computed Total by Role
		Undergraduate	Graduate	Post-Doctorate	
Scientist	2.9	0.5	2.0	0.0	5.4
Professional	0.0	0.0	0.0	0.0	0.0
Technical	0.0	0.0	0.0	0.0	0.0
Administrative	0.0	0.0	0.0	0.0	0.0
Other	0.0	0.0	0.0	0.0	0.0
Computed Total	2.9	0.5	2.0	0.0	5.4

Animal Health Component 0 %

### Activities

### Research Effort Categories

Accession No. 1002531

Project No.

Research 100 %  
Extension 0 %  
Education 0 %

Basic 100 %  
Applied 0 %  
Developmental 0 %

**Classification**

Knowledge Area (KA)	Subject of Investigation (SOI)	Field of Science (FOS)	Percent
201	1452	1060	100

**Knowledge Area**

201 - Plant Genome, Genetics, and Genetic Mechanisms

**Subject Of Investigation**

1452 - Carrot

**Field Of Science**

1060 - Biology (whole systems)

<b>Title:</b>	<b>Switchgrass Bioconfinement: Delayed Flowering, Selective Male- And Seed-Sterility, And Conditional Total Bioconfinement</b>		
<b>Sponsoring Agency</b>	NIFA	<b>Project Status</b>	ACTIVE
<b>Funding Source</b>	Non Formula	<b>Reporting Frequency</b>	Annual
<b>Accession No.</b>	1000875	<b>Grants.gov No.</b>	GRANT11358669
<b>Project No.</b>	TEN2013-03566	<b>Proposal No.</b>	2013-03566
<b>Project Start Date</b>	09/01/2013	<b>Project End Date</b>	08/31/2016
<b>Reporting Period Start Date</b>	09/01/2013	<b>Reporting Period End Date</b>	08/31/2014
<b>Submitted By</b>	Carrera Romanini	<b>Date Submitted to NIFA</b>	11/26/2014

**Program Code:** HX

**Program Name:** Biotechnology Risk Assessment

**Project Director**

Charles Stewart

865-974-6487

nealstewart@utk.edu

**Recipient Organization**

UNIVERSITY OF TENNESSEE

2621 MORGAN CIR 103M

Knoxville, TN 379964540

DUNS No. 133891015

**Performing Department**

Plant Sciences

**Co-Project Directors**

{NO DATA ENTERED}

**Departments**

{NO DATA ENTERED}

**Non-Technical Summary**

Genes that are engineered into crops typically serve the purpose of adding value to the crop or product. There remains a concern among regulators of biotechnology, some scientists and citizens, about gene flow from biotech crops into other non-engineered plants, including free-living wild plants and weeds. In a crop such as switchgrass, where wild switchgrass exists in the areas of cultivation, the gene flow concern can be addressed by engineering plants so that they do not flower, or that pollen, seed, or both are non-viable. We will test three different systems to achieve these endpoints. The first uses a gene that delays flowering 'microRNA 156,' that also increases biomass production. The second system utilizes an enzyme to degrade DNA that will be targeted to be produced specifically in pollen or seed. This DNA chopper--the restriction enzyme 'EcoRI'--has proven to render male sterility in a test plant system and thus, it will be applied to switchgrass. The third system to limit gene flow is to produce a switchable system--a 'synthetic circuit' in engineered plants. This circuit will allow plants to flower and set seed when someone wants them to reproduce, say, when breeding to produce seed to sell to farmers, but be activated to limit gene flow in production fields. The systems could be combined to increase effectiveness of gene flow limitation. Taken together these systems will advance our technological toolkit and knowledge to limit gene flow in engineered crops.

**Accomplishments**

**Major goals of the project**

Goals / Objectives

The overall goal of the project is to develop systems to bioconfine transgenes into the intended host of switchgrass. Specific objectives of the project include the following:

1. Delayed or no flowering. Switchgrass plants engineered for moderate overexpression of microRNA156 (miR156), which renders delayed or no flowering, will be field-tested.
2. Male- and seed-sterility. A restriction endonuclease, EcoRI, will be specifically expressed in pollen or seed tissues and the efficacy for ablating target cells will be assessed, first in rice as a grass model and then translated to switchgrass.
3. Conditional total transgene bioconfinement. A novel system in which EcoRI-based seed sterility is repressible by chemical treatment for breeding (in trans) will be developed and assessed in rice and then translated to switchgrass.

**What was accomplished under these goals?**

1. Delayed or no flowering. Switchgrass plants engineered for moderate overexpression of microRNA156 (miR156), which renders delayed or no flowering, will be field-tested.

We have established one field experiment in a regulated site and have obtained a USDA APHIS BRS release-into-the-environment permit to establish a second site. The first site requires that panicles be removed as a bioconfinement mechanism. However, one transgenic event did not flower at all and another produced very few panicles. Based on early data, both of these transgenic events appeared to produce biomass comparable to the nontransgenic control line.

2. Male- and seed-sterility. A restriction endonuclease, EcoRI, will be specifically expressed in pollen or seed tissues and the efficacy for ablating target cells will be assessed, first in rice as a grass model and then translated to switchgrass.

Greenhouse and field experiments confirm that EcoRI is able to completely ablate transgenic tobacco in pollen cells when under the control of the tomato LAT52 promoter. To translate this result to rice and switchgrass, a total of 7 pollen-specific and 6 embryo-specific promoters have been identified and 5 of these, controlling the expression of the EcoRI gene, have been transformed into rice. We found that the remainder of these were impossible to maintain in various strains of *Agrobacterium tumefaciens* strains. We hypothesize that 'leaky' expression is allowing EcoRI to be expressed and killing *Agrobacterium*. Therefore, we constructed the EcoRI gene with an intron and have 9 new constructs that are stable in *Agrobacterium*.

3. Conditional total transgene bioconfinement. A novel system in which EcoRI-based seed sterility is repressible by chemical treatment for breeding (in <sup>trans</sup>) will be developed and assessed in rice and then translated to switchgrass.

We have identified three inducible promoters and are characterizing these to couple with the best cassetted above to make a syntheticcircuit-based GURT system. We have 5 glyphosate-inducible promoters under testing.

#### What opportunities for training and professional development has the project provided?

Postdoc Yanhui Peng has received training and he also helping to mentor and undergraduate researcher and a new graduate student who is supported from other sources.

#### How have the results been disseminated to communities of interest?

{Nothing to report}

#### What do you plan to do during the next reporting period to accomplish the goals?

We will establish new plants in the field for the miR156 study and analyze the gene expression and flowering phenotypes.

For Objectives 2 and 3, we will continue to build constructs and test them in rice, with the best ones going into switchgrass in year 2 of the grant.

#### Participants

##### Actual FTE's for this Reporting Period

Role	Non-Students or faculty	Students with Staffing Roles			Computed Total by Role
		Undergraduate	Graduate	Post-Doctorate	
Scientist	0	0	0	1	1
Professional	0	0	0	0	0
Technical	0	0	0	0	0
Administrative	0	0	0	0	0
Other	0	0	0	0	0
Computed Total	0	0	0	1	1

##### Student Count by Classification of Instructional Programs (CIP) Code

Undergraduate	Graduate	Post-Doctorate	CIP Code
		1	01.11 Plant Sciences.

#### Target Audience

The primary audience is government regulators and other scientists; ultimately the audience will consist of the concerned populace. The research will primarily initially be disseminated mainly through professional presentations and internationally recognized peer-reviewed journals, such as Plant Biotechnology Journal, BMC Biotechnology and Plant Cell Reports. The readers of these journals are scientists and regulators. The research will also be incorporated in various courses and outreach activities. Interviews will be granted with journalists that also serve as a conduit to disseminate research findings.

### Products

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Published	2013	YES

### Citation

Sang, Y., R.J. Millwood, C.N. Stewart Jr. 2013 Gene use restriction technologies for transgenic plant bioconfinement. Plant Biotechnology Journal 11:649-658.

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Published	2013	YES

### Citation

Ellstrand, N.C., P. Meirmans, J. Rong, D. Bartsch, A. Ghosh, T.J. de Jong, P. Haccou, B.-R. Lu, A.A. Snow, C.N. Stewart, Jr., J.L. Strasburg, P.H. van Tienderen, K. Vrieling, D. Hooftman. 2013. Introgression of crop alleles into wild or weedy populations. Annual Review of Ecology, Evolution, and Systematics 44: 325-345.

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Published	2014	NO

### Citation

Gressel, J., C.N Stewart, Jr., L.V. Giddings, A.J. Fischer, J.C. Streibig, N.R. Burgos, A. Trewavas, A. Merotto, Jr., C.J. Leaver, K. Ammann, V. Moses, A. Lawton-Rauh. 2014. Overexpression of epsps transgene in weedy rice: insufficient evidence to support multiple conclusions about biosafety. New Phytologist 202:360-362.

### Other Products

{Nothing to report}

### Changes/Problems

{Nothing to report}

<b>Title:</b>	<b>Genome-wide assessment of off-target effect and removal of transgenes associated with TALEN-based gene editing in plant</b>		
<b>Sponsoring Agency</b>	NIFA	<b>Project Status</b>	ACTIVE
<b>Funding Source</b>	Non Formula	<b>Reporting Frequency</b>	Annual
<b>Accession No.</b>	1000366	<b>Grants.gov No.</b>	GRANT11360081
<b>Project No.</b>		<b>Proposal No.</b>	2013-03588
<b>Project Start Date</b>	09/01/2013	<b>Project End Date</b>	08/31/2016
<b>Reporting Period Start Date</b>	09/01/2013	<b>Reporting Period End Date</b>	08/31/2014
<b>Submitted By</b>	Bing Yang	<b>Date Submitted to NIFA</b>	12/03/2014

**Program Code:** HX

**Program Name:** Biotechnology Risk Assessment

**Project Director**

Bing Yang

Associate Professor

515-294-2968

byang@iastate.edu

**Recipient Organization**

IOWA STATE UNIVERSITY OF SCIENCE AND

1350 BEARDSHEAR HALL

Ames, IA 500112025

DUNS No. 005309844

**Performing Department**

Genetics, Dev, & Cell Biology

**Co-Project Directors**

Spalding, Martin

Fei, Shuizhang

**Departments**

Genetics, Development and Cell Biology

{NO DATA ENTERED}

**Non-Technical Summary**

This proposal will address the risks associated with TALEN-based genetic engineering in crop plants. The hypotheses underlying this work are that TALENs, fusion proteins of TAL effectors and the DNA cleavage domain of endonuclease FokI, are capable of introducing precise, targeted genomic modifications in crops and that the TALEN constructs can be completely removed through genetic segregation. Our published and preliminary data demonstrate, as a proof-of-concept, that TALENs can create site-specific gene changes and intended phenotypes in rice and that the transgenes are undetectable by PCR in the modified genomes after genetic segregation. Thus, our experiments are designed to assess the broad applicability of TALEN technology to polyploid switchgrass and to use the rice model to thoroughly assess potential unintended genotypic and phenotypic effects of TALENs, as well as the unequivocal removal of TALEN transgenes from the modified genomes. We will use whole-genome sequencing to compare the genome of wild-type rice with those of rice mutants that have undergone two rounds of TALEN-mediated gene editing, to identify and quantify potential risks associated with any promiscuous activity of TALENs and residues of TALEN transgenes in the modified rice. Finally, developing a better assessment of the TALEN-associated risks will provide a broad foundation for future crop engineering efforts directed at genomic modification in rice, provide comprehensive insight into the applicability of TALEN technology to other crop and bioenergy plants, and assist federal regulatory agencies in determining whether TALEN technology merits either an exemption from regulatory oversight or a less rigorous regulatory process.

**Accomplishments**

**Major goals of the project**

1. Establish a pipeline of TALEN-based gene editing and assess its robustness and general applicability in crop plants by focusing on rice and switchgrass.
2. Assess potential off-target mutations caused by TALENs in modified rice genomes by comparing seven modified genomes against the parental reference genome.
3. Assess the removal of the TALEN transgenes from the modified rice genome through genetic segregation at the genomic level.
4. Analyze potential phenotypic variations in rice plants associated with TALEN-based gene editing by investigating a number of morphological traits (plant height, tiller number, dry biomass, etc.).

**What was accomplished under these goals?**

We have made progress in the following areas of the Major goals:

**1. Establish a pipeline of TALEN-based gene editing and assess its robustness and general applicability in crop plants by focusing on rice and switchgrass.**

1-1. We have established a pipeline of TALEN-based gene editing in rice. The pipeline includes (1) design and engineer any novel TALEN genes using a "Gold gate" modular assembly method with a TAL repeat library we have developed, (2) introduce TALEN constructs into rice embryogenic callus cells and generate transgenic rice lines through tissue culture and transformation, (3) identify TALEN-mediated gene editing in primary transgenic plants and their progeny, and (4) characterize the TALEN-mediated mutant plants molecularly and physiologically.

To assess its robustness of this pipeline for TALEN-mediated gene editing, we chose 15 rice SWEET (sugar transporter) genes for TALEN-mediated gene editing. We have made 15 TALEN constructs each targeting one SWEET gene, and have obtained edited plants for 7 SWEET genes, but have not obtained edited plants from 8 TALEN constructs. For those constructs that generated gene editing, the frequency ranged from about 15% to 70%.

1-2. We have also established a workable protocol for switchgrass tissue culture and Agrobacterium-mediated transformation. Construction of TALEN plasmids targeting three switchgrass genes is in progress.

1-3. We have expanded our effort to successfully establish a CRISPR/Cas9 system for gene editing in rice (Zhou et al. *Nucleic Acids Research* 42:10903-10914). This endeavour will allow us to compare the two most advanced gene editing or mutagenesis technologies in rice and probably in switchgrass.

**2. Assess potential off-target mutations caused by TALENs in modified rice genomes by comparing seven modified genomes against the parental reference genome.**

We have whole genome sequenced 8 rice lines including one parental line Kitaake and seven lines that contained the TALEN-mediated edit in the promoters of SWEET11 and SWEET14. The sequencing work with Illumina and PacBio technologies has been done and data analysis for off-target mutations caused by TALENs is in progress.

**3. Assess the removal of the TALEN transgenes from the modified rice genome through genetic segregation at the genomic level.**

The assessment of the removal of TALEN-transgenes from the above mentioned 7 lines will be completed with analysis of whole genome sequencing data in Objective 2.

**4. Analyze potential phenotypic variations in rice plants associated with TALEN-based gene editing by investigating a number of morphological traits (plant height, tiller number, dry biomass, etc.).**

The propagation of seed from those rice mutant lines for phenotypic variation analysis is in progress.

**What opportunities for training and professional development has the project provided?**

This grant has provide training opportunities for two graduate students and one undergraduate student as well as one scientist to work on the most advance biotechnologies that provides promise in basic and applied science in agriculture.

**How have the results been disseminated to communities of interest?**

We have published one article and submitted another article for publication related to this project. The publication is one of the most efficient ways to disseminate our results among the scientific community. The PIs have also presented our effort and results in several scientific conferences and university lectures.

**What do you plan to do during the next reporting period to accomplish the goals?**

We will continue to work on the objectives we proposed based on the proposed timetable.

**Participants****Actual FTE's for this Reporting Period**

Role	Non-Students or faculty	Students with Staffing Roles			Computed Total by Role
		Undergraduate	Graduate	Post-Doctorate	
Scientist	1	1	2	0	4
Professional	0	0	0	0	0
Technical	0	0	0	0	0
Administrative	0	0	0	0	0
Other	0	0	0	0	0
Computed Total	1	1	2	0	4

**Student Count by Classification of Instructional Programs (CIP) Code**

Undergraduate	Graduate	Post-Doctorate	CIP Code
1	2		01.11 Plant Sciences.

**Target Audience**

The target audience includes the scientific community as our work has been presented as the invited talks in several scientific meetings and also includes graduate/undergraduate students as our efforts reached classrooms in the forms of guest lectures and seminars in and out of ISU.

**Products**

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Under Review	2014	YES

**Citation**

Si Nian Char, Erica Unger-Wallace, Bronwyn Frame, Sarah A. Briggs, Marcy Main, Martin H. Spalding, Erik Vollbrecht, Kan Wang, Bing Yang. Heritable site-specific gene mutagenesis using TALENs in maize. Plant Biotechnology Journal

Type	Status	Year Published	NIFA Support Acknowledged
Journal Articles	Published	2014	NO

**Citation**

Zhou H, Liu B, Weeks DP, Spalding MH, Yang B (2014) Large chromosomal deletions and heritable small genetic changes induced by CRISPR/Cas9 in rice. Nucleic Acids Research 42(17):10903-10914.

**Other Products**

{Nothing to report}

**Changes/Problems**

{Nothing to report}

<b>Title: Resistance Risk Assessment for Seed Mixture Refuges with Pyramided Bt Corn</b>			
<b>Accession No.</b>	1003673	<b>Sponsoring Institution</b>	National Institute of Food and Agriculture
<b>Project No.</b>		<b>Project Status</b>	ACTIVE
<b>Funding Source</b>	Non Formula	<b>Proposal No.</b>	2014-02978
<b>Grants.gov No.</b>	GRANT11608612	<b>DUNS Number</b>	806345617
<b>Start Date</b>	09/01/2014	<b>End Date</b>	08/31/2017
<b>Award Number</b>	2014-33522-22214	<b>Award Amount</b>	\$ 497,520
<b>Award Date</b>	08/07/2014	<b>Award Fiscal Year</b>	2014
<b>Submitted By</b>		<b>Date Submitted to NIFA</b>	07/15/2014

**Program Code** HX

**Program Name**

Biotechnology Risk Assessment

**Project Director**

Yves Carriere

520-626-8329

ycarrier@ag.arizona.edu

**Performing Organization/Institution**

{NO DATA ENTERED}

null, null null

**Performing Department**

Entomology

**Co-Project Directors**

Tabashnik, Bruce

**Departments**

Entomology

**Collaborating/Partnering States**

AZ

**Collaborating/Partnering Countries**

{NO DATA ENTERED}

**Collaborating/Partnering Organizations**

ABOR University of Arizona

**Non-Technical Summary**

Evolution of pest resistance is the most serious risk threatening the continued efficacy of transgenic pyramided Bt crops that produce two or more toxins. Empirical data are urgently needed to assess the relative risk of resistance associated with two currently used types of refuge: seed mixtures and "structured" refuges. Structured refuges are blocks of non-Bt plants grown near Bt plants. Seed mixtures yield a random array of Bt plants and non-Bt plants side-by-side within fields. To allow regulators to better assess the risks associated with seed mixtures versus structured refuges of pyramided Bt corn, we will test *H. zea* larvae of three different genotypes (resistant, susceptible and heterozygous) in laboratory and field experiments on commercially available pyramided Bt corn producing Cry1A.105 and Cry2Ab. Generated data will allow regulators to evaluate risks associated with current resistance management mandates and enhance the regulatory framework for mitigating the risk of pest resistance to pyramided Bt crops.

**Goals / Objectives**

This project will provide key data that regulators need for science-based assessment of the risk of pest resistance to pyramided Bt corn with two different types of refuges: seed mixtures and block refuges ("structured refuges"). To achieve this goal, we will conduct laboratory and field experiments with *H. zea* larvae of three different genotypes (Bt-resistant, Bt-susceptible and heterozygous) and widely-used pyramided Bt corn producing Cry1A.105 and Cry2Ab targeting lepidopteran pests to accomplish the following objectives:

- 1) Determine genotype-specific larval survival and dominance of resistance with seed mixtures for pyramided Bt corn;
- 2) Determine genotype-specific larval survival and dominance of resistance with structured refuges for pyramided Bt corn;
- 3) Determine effects of genotype-specific larval movement in the field between Bt and non-Bt corn plants in seed mixtures with pyramided Bt corn;
- 4) Determine the presence of Cry1A.105 and Cry2Ab in kernels from corn grown in the field in seed mixtures and structured

refuges;

5) Develop and apply resistance risk assessment models using results from Objectives 1-4.

### Methods

There are currently no published empirical data evaluating how seed mixtures of non-Bt and Bt corn pyramids affect survival of target pests with different resistance genotypes. To obtain the data regulators need to improve risk assessment for seed mixtures of pyramided Bt corn, we will test *H. zea* larvae of three different resistance genotypes on field-grown ears of non-Bt and pyramided Bt corn from seed mixtures. There are currently no published empirical data evaluating how pests with different resistance genotypes perform on non-Bt corn and pyramided Bt corn grown in blocks. To provide a rigorous comparison between seed mixtures and structured refuges, we will simultaneously test *H. zea* larvae of three different resistance genotypes on field-grown ears of non-Bt corn and pyramided Bt corn from seed mixtures (Objective 1) and from pure blocks (Objective 2).

One of the concerns about seed mixtures is that larval movement between Bt plants and nearby non-Bt plants will accelerate evolution of resistance. Although modeling results show that resistance evolves faster if larval movement increases the fitness of individuals with resistance alleles relative to susceptible individuals, experimental evaluation of this concept has been limited. Therefore, we will conduct a field experiment to examine genotypic-specific movement of *H. zea* larvae between corn plants in seed mixtures, which will provide critical data for assessing the risk of resistance to pyramided Bt corn (Objective 3).

We expect extensive pollen-mediated gene flow between Bt and non-Bt corn in seed mixtures and little or no such gene flow between blocks of Bt and non-Bt corn separated by >300 m. In particular, movement of pollen from Bt corn to nearby non-Bt corn can produce a mosaic of Bt and non-Bt kernels in ears of non-Bt corn plants. To enable interpretation of observed patterns of larval performance (Objectives 1-3), we will determine the percentage of kernels producing Cry1A.105 and Cry2Ab in each of the four types of ears examined in Objectives 1-3 (Bt block, non-Bt block, Bt RIB, and non-Bt RIB).

Computer simulations have been used to compare durability of seed mixtures of pyramided and non-Bt crops to durability of separate blocks of these crops. However, the critical data are not currently available for comparing the risk of resistance between seed mixtures and structured refuges for key target pests. Specifically, effects of gene flow and inter-plant movement on relative fitness of genotypes in seed mixtures have not been investigated. For the first time, we will develop models based on experimental data for a key pest of corn tested in seed mixtures vs. structured refuges of a commercially grown Bt crop pyramid to examine the risk of resistance in seed mixtures and blocks of Bt and non-Bt corn.

### Target Audience

Target audiences include EPA regulators, farmers that plant seed mixtures of Bt corn, members of the biotech industry, and scientists working in the field of resistance management for Bt crops and insecticides.

### Products

**The overall rationale and significance of the research proposed here is that it will evaluate, for the first time, key parameters required to compare the risk of resistance to Bt corn with refuges in either seed mixtures or blocks.**

### Expected Outcomes

This project will generate knowledge of resistance to improve assessment and management of the risk of resistance to pyramided Bt crops. This project will be the first to provide critical data for improved risk assessment for seed mixtures of pyramided Bt corn. In particular, we will provide the first data for any target pest on genotype-specific larval survival and dominance of resistance to Bt crop pyramids in seed mixtures vs. block refuges. This project will also provide the first assessment of *H. zea* larval movement between Bt and non-Bt corn plants in seed mixtures in the field.

### Keywords

Bt corn ~corn earworm ~gene flow ~larval movement ~resistance ~seed mixtures ~transgenic crop ~risk assessment ~biotechnology

**Estimated Project FTEs For The Project Duration**

Role	Non-Students or Faculty	Students with Staffing Roles			Computed Total by Role
		Undergraduate	Graduate	Post-Doctorate	
Scientist	0.0	0.0	0.0	0.0	0.0
Professional	0.0	0.0	0.0	0.0	0.0
Technical	0.0	0.0	0.0	0.0	0.0
Administrative	0.0	0.0	0.0	0.0	0.0
Other	0.0	0.0	0.0	0.0	0.0
Computed Total	0.0	0.0	0.0	0.0	0.0

**Animal Health Component** 0 %

**Activities**

<b>Research</b>	100 %
<b>Extension</b>	0 %
<b>Education</b>	0 %

**Research Effort Categories**

<b>Basic</b>	0 %
<b>Applied</b>	100 %
<b>Developmental</b>	0 %

**Classification**

Knowledge Area (KA)	Subject of Investigation (SOI)	Field of Science (FOS)	Percent
211	3110	1070	100

**Knowledge Area**

211 - Insects, Mites, and Other Arthropods Affecting Plants

**Subject Of Investigation**

3110 - Insects

**Field Of Science**

1070 - Ecology

<b>Title: Targeted Gene Knockout of Reproductive Genes of Catfish with Hormone Therapy to Restore Fertility</b>			
<b>Accession No.</b>	1003876	<b>Sponsoring Institution</b>	National Institute of Food and Agriculture
<b>Project No.</b>	ALA016-4-14020	<b>Project Status</b>	ACTIVE
<b>Funding Source</b>	Non Formula	<b>Proposal No.</b>	2014-02966
<b>Grants.gov No.</b>	GRANT11610455	<b>DUNS Number</b>	066470972
<b>Start Date</b>	09/01/2014	<b>End Date</b>	08/31/2018
<b>Award Number</b>	2014-33522-22263	<b>Award Amount</b>	\$ 498,923
<b>Award Date</b>	08/25/2014	<b>Award Fiscal Year</b>	2014
<b>Submitted By</b>	Art Appel	<b>Date Submitted to NIFA</b>	07/30/2014

**Program Code** HX

**Program Name**

Biotechnology Risk Assessment

**Project Director**

Rex Dunham  
334-844-9121  
dunhara@auburn.edu

**Performing Organization/Institution**

AUBURN UNIVERSITY  
107 SAMFORD HALL  
Auburn University, AL 368490001

**Performing Department**

School of Fisheries

**Co-Project Directors**

Peatman, Eric

**Departments**

Fisheries and Allied Aquacultu

**Collaborating/Partnering States**

AL

**Collaborating/Partnering Countries**

{NO DATA ENTERED}

**Collaborating/Partnering Organizations**

Auburn University

**Non-Technical Summary**

Methodology is needed to have absolute confinement of genetically engineered fish (GMOs). One of the most promising technologies to accomplish this is to gene edit key hormones needed for reproduction, which sterilizes the fish. Once these fish are produced they can only breed if the aquaculturist introduces the reproductive hormones to the fish artificially and this only temporarily restores fertility. Thus, any genetically engineered fish that are gene edited for these key reproductive genes cannot breed if they were to escape, eliminating the possibility of any permanent damage or genetic change to fish populations in the natural environment. This will allow safe application of genetically engineered fish for the benefit of society without the possibility of environmental damage.

**Goals / Objectives**

Our long-term goal is to accomplish reversible transgenic sterility in fish, specifically catfish, to reproductively confine and prevent the establishment of transgenic or domestic genotypes in the natural environment. The eventual application is the stacking of technologies, transgenic fish possessing the transgene of interest as well as the sterilization construct or a sterilizing mutation, to accomplish reproductive confinement.-

Specific objectives- 1) accomplish knockout of gonadotropin- releasing hormone (GnRH), luteinizing hormone (LH) or follicle stimulating hormone (FSH) by targeted deletion using transcription activator-like (TAL) effectors combined with the nuclease domain of FokI restriction enzyme, TAL effector nucleases (TALENs), with zinc finger nucleases (ZFNs) or with clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 system in channel catfish, 2) predict successful sterility by measuring steroid sex hormones, 3) confirm the sterility of GnRH, LH and FSH knockout channel catfish, 4) compare effectiveness of restoring fertility to GnRH, LH and FSH knockout channel catfish with synthetic GnRH (luteinizing hormone releasing hormone analogue, LHRHa), LH and FSH, respectively, and 5) evaluate potential pleiotropic effects of knockout of GnRH, LH and FSH on the growth, survival, disease resistance, seinability and carcass traits of channel catfish.

**Methods**

GnRH, LH and FSH genes will be gene edited in catfish by using ZFN, TALEN and CRISPR technologies. Recombinant or synthetic forms of these hormones will be used to restore fertility and spawn these fish.

**Target Audience**

Scientists, regulatory agencies and the aquaculture industry

**Products**

Products include a technology to have absolute reproductive confinement of fish, scientific papers and catfish that can only breed when hormone therapy is applied by the aquaculturist.

**Expected Outcomes**

Gene editing will produce catfish that cannot produce key reproductive hormones which will genetically sterilize these fish. Hormone therapy will be applied and fertility temporarily restored upon command. This technology can then be applied for absolute biological confinement of genetically engineered fish and other genotypes of fish when desirable.

**Keywords**

CRISPR ~Catfish ~Gene Knockout ~Reproductive Genes ~TALEN ~ZFN ~risk assessment ~biotechnology

**Estimated Project FTEs For The Project Duration**

Role	Non-Students or Faculty	Students with Staffing Roles			Computed Total by Role
		Undergraduate	Graduate	Post-Doctorate	
Scientist	2.0	0.0	3.0	1.0	6.0
Professional	1.0	0.0	0.0	0.0	1.0
Technical	0.0	0.0	0.0	0.0	0.0
Administrative	0.0	0.0	0.0	0.0	0.0
Other	0.0	0.0	0.0	0.0	0.0
Computed Total	3.0	0.0	3.0	1.0	7.0

**Animal Health Component** 20 %

**Activities**

<b>Research</b>	100 %
<b>Extension</b>	0 %
<b>Education</b>	0 %

**Research Effort Categories**

<b>Basic</b>	65 %
<b>Applied</b>	35 %
<b>Developmental</b>	0 %

**Classification**

Knowledge Area (KA)	Subject of Investigation (SOI)	Field of Science (FOS)	Percent
301	320	1080	70
303	320	1080	30

**Knowledge Area**

301 - Reproductive Performance of Animals; 303 - Genetic Improvement of Animals

**Subject Of Investigation**

0320 - Watersheds

**Field Of Science**

1080 - Genetics

<b>Title: Extended pest migration in Bt versus non-transgenic crops: impacts on risk assessment and Bt resistance dissemination</b>			
<b>Accession No.</b>	1003821	<b>Sponsoring Institution</b>	National Institute of Food and Agriculture
<b>Project No.</b>	TEN2014-02995	<b>Project Status</b>	ACTIVE
<b>Funding Source</b>	Non Formula	<b>Proposal No.</b>	2014-02955
<b>Grants.gov No.</b>	GRANT11610723	<b>DUNS Number</b>	133891015
<b>Start Date</b>	09/01/2014	<b>End Date</b>	08/31/2017
<b>Award Number</b>	2014-33522-22215	<b>Award Amount</b>	\$ 492,396
<b>Award Date</b>	08/15/2014	<b>Award Fiscal Year</b>	2014
<b>Submitted By</b>	Carrera Romanini	<b>Date Submitted to NIFA</b>	07/28/2014

**Program Code** HX

**Program Name**

Biotechnology Risk Assessment

**Project Director**

Juan Luis Jurat Fuentes

865-974-5931

jurat@tennessee.edu

**Performing Organization/Institution**

{NO DATA ENTERED}

null, null null

**Performing Department**

Entomology and Plant Pathology

**Co-Project Directors**

Nagoshi, Rodney

Huang, Fangneng

Meagher, Robert

**Departments**

Behavior and Biocontrol

Entomology

{NO DATA ENTERED}

**Collaborating/Partnering States**

FL

LA

**Collaborating/Partnering Countries**

{NO DATA ENTERED}

**Collaborating/Partnering Organizations**

Louisiana State University Agricultural Center

USDA-ARS

USDA-ARS, CMAVE

**Non-Technical Summary**

Transgenic crops producing insecticidal proteins from the bacterium *Bacillus thuringiensis* (Bt crops) represent currently more than 70% of corn and cotton acreage in the U.S., yet this high adoption rates question their sustainability and ecological safety. Recent evidence supports that insects that are able to feed on Bt crops and survive display increased migratory tendencies, suggesting important unintended effects by Bt crops compared to non-transgenic plants on the abundance and dispersal of insect pests. This temporal and geographical abundance of insect pests determines the severity of economic losses due to insect feeding in agricultural systems, yet these hazards have been previously overlooked in Bt crop risk assessments. This project addresses this critical knowledge gap by determining the effects on migratory behavior induced by feeding on Bt compared to non-transgenic crops. The project uses the fall armyworm (FAW) as insect pest model due to its economic importance, its well-characterized migratory behavior, and recent reports of field FAW resistance to Bt corn. Our first objective will compare migratory behavior (both speed and distance) in FAW moths that were fed as larvae on Bt corn/cotton or non-transgenic plants. This comparison will determine if feeding on Bt crops contributes to increased migration of insect pests. In Objective 2 we will develop a novel assay to discriminate between FAW that are susceptible or resistant to Bt corn. This method will be used in Objective 3 to determine if resistant FAW display enhanced migratory behavior and if FAW resistance to Bt corn has disseminated from Puerto Rico into southeastern U.S. to threaten corn production. The project is

expected to assess potential unintended risks of Bt crops related to insect pest abundance and movement, which will allow for the design of practices to minimize these risks and maintain the societal, health and environmental benefits of Bt crops.

### Goals / Objectives

The main goal of this project is to evaluate effects of Bt crops as suboptimal hosts on dispersal behavior of *Spodoptera frugiperda* (fall armyworm, FAW) as a previously ignored hazard of Bt versus non-transgenic crops. The rationale behind the choice of FAW as model is based on it being an economically important insect pest with well-characterized migratory behavior and documented to evolve field resistance to Bt corn. Our first objective will compare dispersal propensity in flight mills of FAW moths, including FAW resistant to Bt corn, that were reared as larvae on Bt versus non-Bt cotton or corn plants. In our second objective we plan to develop a diagnostic assay to detect the presence of the allele responsible for resistance to transgenic Bt corn expressing the Cry1Fa toxin in FAW. Upon validation of the method with known resistant strains, we will apply it to FAW populations recently reported as resistant from corn-growing regions in the Southeastern U.S.A. In our third objective we plan to evaluate risks implied by increased FAW dispersal on the potential spread of field-evolved resistance to Bt corn to migratory destinations in the U.S. by capitalizing on new and decade-long FAW genetic material collections.

### Methods

The project will test the effect on the dispersal of fall armyworm (FAW) in larvae feeding on transgenic Bt crop events as suboptimal hosts compared to non-transgenic isolines, and its impact on the spread of field-evolved FAW resistance to Bt corn to the southeastern U.S. In Objective 1 will determine the effect of suboptimal feeding on long-flight propensity in susceptible and Bt-resistant strains of FAW. As suboptimal hosts we plan to use Bt corn events producing the Cry1Ab toxin displaying low activity against FAW, or the Cry1Fa toxin which is active against FAW but non-toxic to field-evolved resistant FAW. Similarly, we will use Bt cotton events producing Cry1A.105 and Cry2Ab2 or Cry1Ac and Cry1Fa toxins as predicted to have low activity against resistant FAW larvae. Effects of suboptimal feeding on migration will be tested using 12-hr flight mill tests of tethered moths from larvae fed the diverse transgenic hosts compared to control isolines. Flight parameters (duration, distance, velocity) of newly emerged unfed adults will be measured to determine effects of suboptimal feeding on flight propensity and capacity. This Objective will include efforts in training a graduate student in the agricultural sciences, one of the outcomes of this project, to perform and analyze flight mill studies as part of his/her Thesis project. Progress in this Objective will be evaluated through the analysis of resulting data.

Objective 2 will focus on development of a novel DNA-based assay to detect the allele involved in resistance to transgenic Bt corn in field-resistant FAW from Puerto Rico. The work will capitalize on preliminary data identifying the gene linked to FAW field-evolved resistance against Bt corn to develop a sensitive assay discriminating homozygous from heterozygous genotypes for resistance. This assay, one of the outcomes of the project, will then be used to determine if recent reports of field FAW resistance to Bt corn in southeastern U.S. represent migrants from resistant populations in Puerto Rico or distinct genetic events. Milestones in the evaluation of progress in this Objective include the development of the DNA-based assay and the use of this method to test FAW collected in southeastern U.S. for resistance. Field FAW populations to be used in this Objective have already been acquired and are available for testing.

Objective 3 in this project is focused on identifying migratory patterns of FAW using previously-developed haplotype markers and Bt-resistance markers develop in Objective 2. While FAW is only able to overwinter in the southern U.S., its infestation range extends to Canada, representing a classic model with annual long-distance migration to northern destinations involving successive and progressively expanding broods. Haplotype markers have been developed to differentiate the two main overwintering populations (Florida and Texas) in the U.S. These markers will be used in conjunction with the DNA-based assay from Objective 2 in discriminating field-collected FAW from locations in the Caribbean and southeastern U.S. according to overwintering population and resistance genotype. This Objective will also capitalize on more than a decade of archived genetic FAW collections, allowing for a historical description of resistance dissemination from Puerto Rico into mainland U.S.A. Bioassays will also be used to detect resistance in case diverse resistance mechanisms exist among field populations from diverse geographies. Because each annual FAW migration from overwintering locations can be considered an independent event, model predictions can be easily tested in subsequent years. Evaluation of progress in this Objective will include classification of all collected FAW samples according to overwintering population, resistance to Bt corn, and allele involved in resistance. The first milestone will be testing of archived genetic FAW material, followed by testing of recent collections. The data from completion of this Objective will allow for future development of predictive models to estimate risks of resistance dissemination to diverse geographical regions, which will in turn impact choices of transgenic crops to use in those regions.

### Target Audience

**Target audiences** reached during the completion of the project include graduate students in the agricultural sciences, research staff and laboratory directors from national and international research groups in academia and government, as well as researchers from biopesticide and biotechnology companies. Information from our project is relevant to conventional and organic farmers, as much as dissemination of resistance to Bt crops would potentially affect the effective use of Bt pesticides. Data from completion of our project also targets regulators at government agencies involved in risk assessment and design of practices and mandates to minimize unintended effects of transgenic crops in the agricultural environment.

The project will also include efforts to deliver laboratory instruction to graduate students and postdoc involved in the completion of the project through short visits to laboratories in the network of collaborative groups responsible for the project.

### Products

Activities in the project will include conducting and analyzing experiments, training and mentoring a graduate student (PhD) assigned to the project, and assessments of the existence of fall armyworm (FAW) resistant to transgenic Bt corn in mainland USA and of the impact of suboptimal feeding on transgenic Bt corn in migratory behavior of insect pests.

Expected products include data on migration of FAW resistant to transgenic Bt corn, methods to detect the allele involved in resistance to transgenic Bt corn and determine its prevalence in field populations, patent applications protecting this method to discriminate FAW according to the resistance genotype, extended collections of genetic FAW material from field populations, and at least four publications presenting data from the project in international peer-reviewed journals. The project will also foster a new collaborative network of scientists working in FAW migratory behavior and resistance to transgenic Bt corn in the Southeastern USA. An additional product will be the graduation of a graduate student assigned to the project with a PhD degree in agricultural science (Plants, Soils and Insects).

### Expected Outcomes

Data from completion of this project are expected to lead a Change in Knowledge as they will dramatically increase our understanding of the impact of transgenic insecticidal crops as suboptimal hosts on the migratory behavior of insect pests. This information will assist regulatory agencies in the US and abroad involved in risk assessment to lead a Change in Action by guiding the assessment and development of practices that would minimize unintended effects of transgenic crops on insect pest migration. Moreover, data from our project will lead a Change in Knowledge to develop sensitive, economical and high throughput resistance monitoring methods that will be used in determining if insects resistant to transgenic crops display altered migratory behavior that may have resulted in increased dissemination of resistance.

### Keywords

Bt corn ~Spodoptera frugiperda ~insect dispersal ~migration ~resistance ~risk assessment ~biotechnology ~migration

### Estimated Project FTEs For The Project Duration

Role	Non-Students or Faculty	Students with Staffing Roles			Computed Total by Role
		Undergraduate	Graduate	Post-Doctorate	
Scientist	0.3	0.8	3.0	0.0	4.1
Professional	0.0	0.0	0.0	0.0	0.0
Technical	0.0	0.0	0.0	0.0	0.0
Administrative	0.0	0.0	0.0	0.0	0.0
Other	0.0	0.0	0.0	0.0	0.0
Computed Total	0.3	0.8	3.0	0.0	4.1

Animal Health Component 0 %

### Activities

Research 90 %  
 Extension 0 %  
 Education 10 %

### Research Effort Categories

Basic 40 %  
 Applied 60 %  
 Developmental 0 %

### Classification

Knowledge Area (KA)	Subject of Investigation (SOI)	Field of Science (FOS)	Percent
211	3110	1130	100

### Knowledge Area

211 - Insects, Mites, and Other Arthropods Affecting Plants

**Subject Of Investigation**

3110 - Insects

**Field Of Science**

1130 - Entomology and acarology

<b>Title: Risk Assessment for Plant Incorporated Insecticidal Products on Non Target Aquatic Invertebrates</b>			
<b>Accession No.</b>	1003785	<b>Sponsoring Institution</b>	National Institute of Food and Agriculture
<b>Project No.</b>	2014-02983	<b>Project Status</b>	ACTIVE
<b>Funding Source</b>	Non Formula		
<b>Grants.gov No.</b>	GRANT11608129	<b>Proposal No.</b>	2014-02983
		<b>DUNS Number</b>	790934285
<b>Start Date</b>	09/01/2014	<b>End Date</b>	08/31/2017
<b>Award Number</b>	2014-33522-22220	<b>Award Amount</b>	\$ 499,996
<b>Award Date</b>	08/07/2014	<b>Award Fiscal Year</b>	2014
<b>Submitted By</b>	Theresa Simmons	<b>Date Submitted to NIFA</b>	07/24/2014

**Program Code** HX

**Program Name**

Biotechnology Risk Assessment

**Project Director**

William Lamp  
301-405-3959  
lamp@umd.edu

**Performing Organization/Institution**

{NO DATA ENTERED}  
null, null null

**Performing Department**

Entomology

**Co-Project Directors**

Dively, Galen  
Wang, Qin  
Hooks, Cerruti

**Departments**

Entomology  
Nutrition and Food Science

**Collaborating/Partnering States**

MD

**Collaborating/Partnering Countries**

{NO DATA ENTERED}

**Collaborating/Partnering Organizations**

University of Maryland

**Non-Technical Summary**

Plant-incorporated protectants (PIPs) have been found in streams draining agricultural land. Transgenic plant debris is the major source of these PIPs, and thus their entry into waterways may adversely affect the ecosystem services provided by flowing waters. Invertebrates are common in these aquatic systems, contribute to the decomposition of plant debris, and may be exposed to PIPs in the crop debris because of PIP persistence after crop senescence. We intend to focus on identifying and reducing risks of genetically-modified (GM) crops to aquatic systems by the development of (1) a Tier 1 approach for testing hazard effects of PIPs on individual invertebrate species, (2) a landscape approach for measuring effects on aquatic communities and ecosystem processes, and (3) an exposure approach for reducing PIP persistence in the environment. Specifically, we intend to 1) refine protocols and test PIPs using artificial food suitable for aquatic invertebrates that are adapted for shredding plant debris, 2) perform a landscape-level assessment of GM versus non-GM crops on the running water ecosystems draining cropland, and 3) measure degradation rates of PIPs across genes, varieties, and post-harvest crop management. Transgenic corn will serve as our model. We will assess the direct impact of PIPs fed to non-target aquatic organisms. In addition, we will provide a rigorous, landscape-level test of non-target effects of PIPs on ecosystem services in running waters. Finally, we will determine if PIP persistence can be reduced by post-harvest crop management practices.

**Goals / Objectives**

Plant-incorporated protectants (PIPs) have been found in streams draining agricultural land. Transgenic plant debris is the major source of these PIPs, and thus their entry into waterways may adversely affect the ecosystem services provided by flowing waters. Invertebrates are common in these aquatic systems, contribute to the decomposition of plant debris, and may

be exposed to PIPs in the crop debris because of PIP persistence after crop senescence. We intend to focus on identifying and reducing risks of genetically-modified (GM) crops to aquatic systems by the development of (1) a Tier 1 approach for testing hazard effects of PIPs on individual invertebrate species, (2) a landscape approach for measuring effects on aquatic communities and ecosystem processes, and (3) an exposure approach for reducing PIP persistence in the environment. Specifically, our objectives are to:

- 1) refine protocols and test PIPs using artificial food suitable for aquatic invertebrates that are adapted for shredding plant debris,
- 2) perform a landscape-level assessment of GM versus non-GM crops on the running water ecosystems draining cropland, and
- 3) measure degradation rates of PIPs across genes, varieties, and post-harvest crop management.

## Methods

**Objective 1.** Refine protocols and test PIPs using artificial food suitable for aquatic invertebrates that are adapted for shredding plant debris. Our first step is to develop cultures, at least from egg or early larval instar, for a range of aquatic invertebrates, common in agricultural streams and ditches. All species are shredders, capable of consuming corn leaf material in streams and ditches. We will use species that are widespread, and likely to be exposed to corn leaf material in the Midwest. Because their life cycle is timed with the input of leaves into streams in the fall, our tests will occur between fall and spring on early instars of immatures. We will need to adjust the protocol for the size, environmental conditions (e.g. temperature), growth rate, and feeding behavior of specific species.

Experiments will be run for each species to ensure consumption of target chemicals adhered to the aquatic food. Known toxins, e.g., insecticides, will be used in dose response experiments as a test of the ability to expose aquatic insects through oral feeding. Insecticide levels in the water will be assessed through the course of the experiment. Although both zein and CS nanoparticles work well to encapsulate rhodamine and subsequently coat the artificial food, it is possible to further optimize the nanoparticle formulations to achieve the better encapsulation and coating on feed. Based on our previous research, we can further combine these two coatings together into the nanoparticle formulation.

Our goal of a test of purified Bt protein will include positive (known toxin) and negative (no added chemical) treatments. Specific experimental conditions and replication size may depend on individual species. Dose response levels will be used appropriate to up to 20x field exposure. Measurements will include lethal and sublethal endpoints, such as growth rates and behavior. Levels of target protein will be checked using quantitative ELISA and bioassay approaches.

**Objective 2.** Perform a landscape-level assessment of GM versus non-GM crops on the running water ecosystems draining cropland. In the fall, 2014, we will locate, discuss, and evaluate potential farms as sites for the study. Primary ditches, with no tributaries, and with similar histories (clean-out period, mowing, other management) will be selected. We will select two primary ditches (i.e., no tributaries) with similar histories (clean-out period, mowing, other management) from each of the five farms. Sampling will commence in November (before the addition of our corn debris) to establish "before" conditions of the ditches. Corn representing the SmartStax variety (known to demonstrate Bt persistence) and its near non-Bt isolate, will be grown in a randomized plot design during 2015 and 2016. Bags of dried crop debris, labelled by variety and plot replicate, will be stored in a barn until placement in the ditches. The input of Bt corn debris versus the input of the non-Bt corn debris will be randomly applied to the paired ditches. The timing and amount of debris input will be based on published reports; we will make additions to the ditches based on weight of corn debris per stream length, and on a frequency of once every 3-4 weeks from post-harvest to the end of March. Debris input will be done in the 2015-16 as well as the 2016-17 post-harvest crop seasons. Data will be collected in the BACI design (before-after-control-impact). Data after the 2014 harvest will be used as "before" data, while data from 2015 and 2016 will be used as "after" data. Data from the non-Bt ditch will serve as the "control", while data from the Bt corn supplemented ditch will serve as the "impact". Each ditch will be divided into three sections along its length, and monthly samples described below will be randomly located within each section as three subsamples. Data to be collected include: 1) Invertebrate taxonomic composition and density, 2) Leafpack colonization and decomposition, and 3) Geomorphic and biogeochemical conditions.

Analysis of variance will be used to test for significant differences between the community structure metrics for the leaf treatments across the paired ditches within the BACI design. We are particularly interested to determine if the after-impact Bt treatment differs from the before-impact Bt treatment and the before/after-control non-Bt treatments. Additional multivariate analyses will be run as appropriate to determine environmental patterns at the subsample level in response to the treatments.

**Objective 3.** Measure degradation rates of PIPs across genes, varieties, and post-harvest crop management. To better understand the factors affecting the post-harvest fate of Bt proteins, we will determine the bioactivity of proteins expressed in crop residue of a SmartStax hybrid under different post-harvest conditions. The experiment will be set up in 2014 using a split-plot design with SmartStax hybrid and its non-expressing, near-isoline as a main plot factor and residue management as a subplot factor. Four blocks of a no-till field will be planted with subplots measuring 12 rows by 600 ft with a 30 ft border area between each plot. Each strip will be divided into four subplots, each 150 ft long, to accommodate four post-harvest conditions: 1) heavy disking to incorporate crop residue; 2) rotary mowing to reduce surface residue; 3) planting a rye winter cover crop; and 4) leaving crop residue standing (undisturbed). To facilitate sampling of degrading tissue from the heavy disked subplots, litter bags containing plant tissue collected at harvest will be buried in the soil and removed at each sampling

time. Samples will be stored until used for bioassay and compared with ELISA. After the 2014 experiment, we will evaluate our results and design additional experiments for 2015 and 2016 with new varieties, persistent Cry genes, and cropping practices. We will use a bioassay approach as well as quantitative ELISA to examine the persistence of Bt bioactivity in post-harvest crop debris. The presence of the Cry1Ab, Cry1F, and Cry2Ab2 proteins will be detected individually by a specific ELISA test kit, whereas a feeding bioassay will measure the growth inhibition of the pyramided proteins in corn leaves on ECB. This target pest has been used as a sensitive indicator of the toxin since Bt corn was first developed. Details of the bioassay are previously published. Although experiments will vary, direct comparisons of both types of leaf tissue from corn plots will serve as positive and negative controls to substantiate bioactivity and also quantify the level of sublethal responses after environmental exposure. Each of the objectives address one of the potential links that determine risk of non-target effects of crop PIPs in aquatic systems: (1) response of the non-target organisms to the PIP (Tier 1 tests), (2) ecosystem-level functional effects of PIPs, and (3) post-harvest practices to enhance degradation of biological activity of PIPs as a means to mitigate exposure. Each year, we will assess the status of our experiments and modify methods as needed including sample size based on statistical power, study site locations, target organisms, and duration of sampling/experiments.

### Target Audience

Our audience includes:

1. Companies that are developing and producing transgenic crops with plant-incorporated protectants.
2. Regulators who are concerned about non-target effects of genetically modified crops on aquatic ecosystems.
3. Ecotoxicologists and other scientists who measure the exposure and effects of human-produced chemicals in the environment.

### Products

For our first objective, one of the key conclusions of our research to date is the need for a Tier 1 type laboratory test for determining non-target effects of PIPs on aquatic invertebrates. In addition to Cry proteins, new PIPs are in development that will require Tier 1 testing. The use of corn leaves as a food source is not sufficient to demonstrate the effect of a PIP because of tissue-mediated changes in GMO plants. Thus, we propose developing an artificial diet for aquatic detritivores that can produce measurable differences in feeding when plant secondary compounds are included. Tests are underway to develop a Tier 1 test using zein and CS to encapsulate flaked food. Our goal is to develop a protocol for an aquatic, oral toxicity test for appropriate shredder invertebrates (e.g., selected species of caddisflies, Insecta: Trichoptera) by end of the project.

We will also perform a landscape-level assessment of GM versus non-GM crops on the running water ecosystems draining cropland. The standard protocol for comparing decomposition and colonization of PIP versus non-PIP crop debris is to place the two types of debris in separate bags and place them in the same stream. Experimentally, these two bag types are not independent and thus this type of experiment is not a strictly valid approach to measure ecosystem-level processes in flowing water. Instead, we propose to use whole streams (or ditches) as our experimental units for comparing Bt versus non-Bt corn debris input. In addition, we will focus on agricultural ditches on the Eastern Shore of Maryland, as these types of habitats are most likely to receive a large influx of crop debris. In the study, we will compare the artificial input of corn crop debris from a Bt field and its near non-Bt iseline into separate, paired agricultural ditches on replicated organic farms. Five farms will be selected for this study and will serve as blocks in the design. The ditches on organic farms will have not yet been exposed to transgenic crop debris, so we can execute a before-after-control-impact (BACI) design. Key measurements of ecosystem structure and function will be measured, including invertebrate community composition and change, organic decomposition and deposition, and changes in nutrient levels.

The exposure of Bt proteins in crop residue left on corn fields after harvest will depend on cultivation practices, different residue management practices, and whether winter cover crops are used after harvest. Our goal is to develop and educate growers on techniques to decrease the persistence of the Cry proteins, with an emphasis on cover cropping techniques. In areas of the corn belt with heavy clay soils, fall plowing or heavy disking is a common practice after harvest and this incorporates crop residue directly into the soil, thus increasing chances of microbial degradation. In contrast, crop residue remains on the surface in no-till cultivation systems, either left undisturbed or managed by using rotary or flail mowers to shorten tall stubble. The latter practice increases the amounts of fine residue material and places crop residue in closer contact with the soil surface. In production areas where growers use winter cover crops for conservation purposes, seeding occurs shortly after harvest using no-till drill planters that incorporate a small percentage of the crop residue into the soil. The cover crop itself may influence the degradation rate of the remaining proteins.

We will report our findings and extend results at various regional and national professional meetings. We will also prepare publications aimed at both the scientist and non-scientist audiences. We will employ and train undergraduate and graduate students as part of the project.

### Expected Outcomes

1. Develop a standardized protocol for assessing the effect of plant-incorporated protectants on leaf-feeding aquatic invertebrates.
2. Test the potential of a nontarget effect of Bt corn on stream ecosystem function using a rigorous landscape-level approach.

3. Determine the persistence of Bt bioactivity in senesced corn and develop agricultural practices to reduce its persistence.

### Keywords

Bt corn ~GMO ~aquatic insects ~non-target effects ~oral toxicity ~risk assessment ~stream ecology ~biotechnology

### Estimated Project FTEs For The Project Duration

Role	Non-Students or Faculty	Students with Staffing Roles			Computed Total by Role
		Undergraduate	Graduate	Post-Doctorate	
Scientist	0.8	0.0	2.3	0.0	3.1
Professional	0.0	0.0	0.0	0.0	0.0
Technical	2.5	1.8	0.0	0.0	4.3
Administrative	0.0	0.0	0.0	0.0	0.0
Other	0.0	0.0	0.0	0.0	0.0
Computed Total	3.3	1.8	2.3	0.0	7.4

Animal Health Component 0 %

### Activities

Research 100 %  
 Extension 0 %  
 Education 0 %

### Research Effort Categories

Basic 100 %  
 Applied 0 %  
 Developmental 0 %

### Classification

Knowledge Area (KA)	Subject of Investigation (SOI)	Field of Science (FOS)	Percent
112	210	1150	10
112	1510	1150	10
112	3110	1070	10
112	3110	1150	10
133	210	1150	10
133	1510	1150	10
133	3110	1070	10
133	3110	1150	10
205	1510	1150	10
205	3110	1150	10

### Knowledge Area

112 - Watershed Protection and Management; 133 - Pollution Prevention and Mitigation; 205 - Plant Management Systems

### Subject Of Investigation

0210 - Water resources; 1510 - Corn (for sweetcorn use 1480); 3110 - Insects

### Field Of Science

1070 - Ecology; 1150 - Toxicology

<b>Title: Impact of transgenic Bt crops on Helicoverpa zea ecology and subsequent resistance risk</b>			
<b>Accession No.</b>	1003925	<b>Sponsoring Institution</b>	National Institute of Food and Agriculture
<b>Project No.</b>	NC09262	<b>Project Status</b>	ACTIVE
<b>Funding Source</b>	Non Formula	<b>Proposal No.</b>	2014-02969
<b>Grants.gov No.</b>		<b>DUNS Number</b>	042092122
<b>Start Date</b>	09/01/2014	<b>End Date</b>	08/31/2018
<b>Award Number</b>	2014-33522-22265	<b>Award Amount</b>	\$ 500,000
<b>Award Date</b>	09/22/2014	<b>Award Fiscal Year</b>	2014
<b>Submitted By</b>	Dawn Piercy	<b>Date Submitted to NIFA</b>	08/05/2014

**Program Code** HX

**Program Name**

Biotechnology Risk Assessment

**Project Director**

Dominic Reisig  
252-793-4428  
dominic\_reisig@ncsu.edu

**Performing Organization/Institution**

NORTH CAROLINA STATE UNIVERSITY  
2701 SULLIVAN DR STE 240 CAMPUS BX 7514  
Raleigh, NC 276950000

**Performing Department**

Entomology

**Co-Project Directors**

Greene, Jeremy  
Reay-Jones, Francis  
Gore, Jeffrey  
Musser, Fred  
Cook, Donald  
Caprio, Michael

**Departments**

{NO DATA ENTERED}  
DREC  
BCH-EPP  
Entomology

**Collaborating/Partnering States**

MS  
SC

**Collaborating/Partnering Countries**

{NO DATA ENTERED}

**Collaborating/Partnering Organizations**

Clemson University  
Mississippi State University

**Non-Technical Summary**

This grant seeks to strategically target the gaps in knowledge related to Integrated Resistance Management. This has direct implications on policy makers who will decide the suitability of current and future Bt crops in the system. We propose an investigation for Helicoverpa zea, a primary and major pest of many US field crops, including corn, cotton and soybean. This pest is impacted in different ways by Bt corn and cotton. If Bt soybeans are registered and planted in the US, this could radically change the system. Our proposal endeavors to target major gaps in the knowledge of the biology of H. zea in the system (corn, cotton and soybean). A specific concern is the potential for this pest to develop resistance to Bt with or without Bt soybeans.

**Goals / Objectives**

Our goals and objectives are to: 1) Investigate the development of H. zea based on fecundity of moths emerging from corn producing single and pyramided Bt toxins for use in Integrated Resistance Management (IRM) modeling; 2) Characterize the development and survival of H. zea and injury to different cotton tissue types and different Bt protein combinations for use in IRM modeling; 3) Identify feeding preference and performance of H. zea in situ on soybean (currently a potential key refuge crop

and likely Bt crop in the future) for different stages of plant development and larval instars; and 4) Create a refined model of the evolution of resistance for *H. zea* in complex landscapes.

## Methods

**Obj. 1)** Small plot experiments will be established using genetically-related Bt and non-Bt corn hybrids in NC, SC, and MS. The purpose of testing the Bt hybrids is to have information from representative single and pyramided-Bt proteins that are, not only targeted for lepidopteran pests, but that are currently planted in the southern U.S. *Helicoverpa zea* pupae will be collected from each hybrid and reared to produce moths. These moths will be mated and fecundity will be measured by counting viable eggs laid from females. This will serve as a proxy to measure fecundity (the ability of *H. zea* to reproduce when raised from various Bt corn hybrids).

**Obj. 2)** Cotton representing all the commercially-available Bt varieties, as well as a non-Bt variety, will be planted in small plots during May at NC, SC and MS. Tissue injury will be quantified by methodically measuring injury on different reproductive tissue types (squares, flowers, and bolls) once *H. zea* is present in the plots.

At each sampling period, if present, leaf tissue, squares, flowers, and bolls will be randomly collected from five plants in each plot. Fresh tissue will be immediately transferred to coolers on ice and shipped to Plymouth, NC. Bt expression will be measured using standard commercially available sandwich enzyme linked immunosorbance assay (ELISA) kits. Once 60% of bolls have opened, 20 injured bolls from each plot will be assessed for end-of-season injury.

To identify the groups of variables most important to measure injury, statistical analyses will be performed to create models for how *H. zea* injures cotton reproductive tissue. The result will be at least three models—one for squares, one for flowers, and one for bolls— that incorporate the most important measurements for predicting larval development. Separate models will be created for each tissue injury type, if data are available, to see if bollworms preferentially feed, or are eliminated from, feeding on various types of tissue among varieties. Furthermore, these will be compared with the Bt tissue expression data gathered from the ELISA assays.

**Obj. 3)** Small plot replicated experiments in soybeans will be established in NC, SC and MS. Soybeans will be planted at one-month intervals from the beginning through the end of the planting window. Plots will be created to target an overlap of soybeans attractive to *H. zea* and major egg-laying events. Sampling will begin during the initial flowering stages and when major *H. zea* flights are observed in the Carolinas. In the Midsouth, generations are less distinct. Sampling will begin at flowering in MS and when *H. zea* is present based on the experience of the PIs.

Larvae will be recorded based on plant position and tissue type on which they are found. Flowers, pods with undeveloped seed (if available) and pods with fully developed seed, will be collected from fifty plants during each sampling period. Plant parts from each tissue type and node will be quantified, carefully cut from the plant, and placed in 70% ethanol. The number of nodes will vary by location, planting date, and time that *H. zea* infests the plots. Performance of *H. zea* on different tissue types will be analyzed using statistical analyses, with stadium and tissue types as fixed effects and location as random effect.

**Obj. 4)** The potential development of *H. zea* will be modeled using computer simulations. In the first simulation, soybean will be compared as a natural host component when simulating grower practices from 2003 versus current grower practices. Data from objective 3 on soybean feeding preferences will be an important component in refining model parameters. In the second simulation, the risk of evolution of *H. zea* resistance using current grower practices with and without Bt-soybean will be compared.

## Target Audience

The primary audience will be the EPA and other policy makers for topics concerning plant-incorporated biotechnology. A secondary audience will be other scientists who read the peer-reviewed published information from these studies.

## Products

We propose to fill in relevant gaps in knowledge of pest biology and ecology to address Bt-plant-incorporated protectant resistance management plans. This will provide policy makers a scientific framework for decision-making relevant to Bt crops. Outputs will be scientific papers in peer-reviewed journals that address these gaps.

## Expected Outcomes

The best available information is generally centered around the original target pests of plant-incorporated Bt (i.e., *Ostrinia nubilalis*, *Heliothis virescens*, and *Diabrotica virgifera virgifera*). When Bt was initially released, *Helicoverpa zea* was originally considered a non-target pest. It is currently completely, partially, or not managed using Bt in the corn, cotton, soybean system. This has the potential to lead for Bt resistance in populations of *H. zea*.

The EPA incorporates a strategy entitled Insect Resistance Management (IRM) when making regulatory decisions. IRM for Bt crops relies on modeling, which is dependent on theory as well as empirical evidence. However, mathematical models can only be as good as the data that are used to parameterize them. Therefore, using IRM as a strategy to delay resistance is only effective if underlying model assumptions are met.

Our proposed work will provide scientific data for *H. zea* to fill gaps in the knowledge base related to resistance modeling. Furthermore, we propose to **provide two new models** that incorporate this new knowledge. One model will

evaluate soybean as a natural refuge for H. zea to delay Bt resistance comparing current grower practices in 2003 to current practices. The second model will compare the risk of the evolution of H. zea resistance to Bt using current grower practices with and without Bt-soybean.

Outcomes from the development of these models will be more policy makers that are more scientifically informed on the pros and cons of resistance management, especially pertaining to the relationship between Bt crops and the important pest H. zea. This will provide federal regulators with independent and relevant information on the impact of Bt current and possible future Bt crops in the system. We hope that this provides a more rigorous scientific framework to guide policies going forward.

**Keywords**

BRAG;Risk Management; mitigate; Bt crops; IRM; H.zea; cotton; soybean; larval instars; resistance ~biotechnology ~resistance ~larval instars ~soybean ~cotton ~Helicoverpazea ~integrated resistance management ~Bt crops ~mitigate ~risk management ~risk assessment ~Helicoverpa zea

**Estimated Project FTEs For The Project Duration**

Role	Non-Students or Faculty	Students with Staffing Roles			Computed Total by Role
		Undergraduate	Graduate	Post-Doctorate	
Scientist	6.0	0.0	3.0	0.0	9.0
Professional	0.0	0.0	0.0	0.0	0.0
Technical	0.0	3.0	0.0	0.0	3.0
Administrative	0.0	0.0	0.0	0.0	0.0
Other	0.0	0.0	0.0	0.0	0.0
Computed Total	6.0	3.0	3.0	0.0	12.0

**Animal Health Component** 0 %

**Activities**

**Research** 100 %  
**Extension** 0 %  
**Education** 0 %

**Research Effort Categories**

**Basic** 80 %  
**Applied** 20 %  
**Developmental** 0 %

**Classification**

Knowledge Area (KA)	Subject of Investigation (SOI)	Field of Science (FOS)	Percent
211	1820	1130	40
211	1510	1130	30
211	1710	1130	30

**Knowledge Area**

211 - Insects, Mites, and Other Arthropods Affecting Plants

**Subject Of Investigation**

1510 - Corn (for sweetcorn use 1480); 1710 - Upland cotton; 1820 - Soybean

**Field Of Science**

1130 - Entomology and acarology

<b>Title: Assessing Phenotypic Variations in Soybean seed protein and oil traits using GFP as a reporter in both mutagenesis and transgenomic approach</b>			
<b>Accession No.</b>	1004036	<b>Sponsoring Institution</b>	National Institute of Food and Agriculture
<b>Project No.</b>		<b>Project Status</b>	ACTIVE
<b>Funding Source</b>	Non Formula	<b>Proposal No.</b>	2014-02988
<b>Grants.gov No.</b>	GRANT11610544	<b>DUNS Number</b>	806345617
<b>Start Date</b>	09/01/2014	<b>End Date</b>	08/31/2018
<b>Award Number</b>	2014-33522-22531	<b>Award Amount</b>	\$ 447,183
<b>Award Date</b>	09/04/2014	<b>Award Fiscal Year</b>	2014
<b>Submitted By</b>		<b>Date Submitted to NIFA</b>	08/11/2014

**Program Code** HX

**Program Name**

Biotechnology Risk Assessment

**Project Director**

Monica Schmidt

520-626-1643

monicaschmidt@email.arizona.edu

**Performing Organization/Institution**

UNIVERSITY OF ARIZONA

888 N EUCLID AVE

Tucson, AZ 857190000

**Performing Department**

Plant Sciences

**Co-Project Directors**

Herman, Eliot

**Departments**

School of Plant Sciences

**Collaborating/Partnering States**

{NO DATA ENTERED}

**Collaborating/Partnering Countries**

{NO DATA ENTERED}

**Collaborating/Partnering Organizations**

Arizona Board of Regents The University of Arizona

**Non-Technical Summary**

Soybean is one of the world's most important protein and oil crops. Genetic engineering and mutagenesis are two primary means to introduce/enhance agronomic traits in crop species. Considerable variation of phenotypes is observed in both transgenically modified and mutagenized crops. We propose to investigate the effects on soybean seed composition and gene expression encountered by these two methods. Specifically, the level of expression of an inserted cassette, GFP-HDEL, will be assessed across 100 independently produced transgenic soybean lines and related to copy number, genome insertion site as well as the protein/ oil content. In parallel, a stable GFP-HDEL transgenic soybean line will be subjected to chemical mutagenesis generating 100 mutagenized lines showing altered GFP expression subsequently analyzed for protein/oil content. Overall the lines generated by either approach will be compared for GFP expression and collateral phenotypic alterations in protein/ oil seed content by NMR analysis. Lines from either treatment deemed to have the most altered protein/oil content by NMR will be further investigated by extensive proteomic analysis (2D gels) and oil compositional analysis (lipidomics profiling). Through these parallel approaches we will test the range of seed phenotypic variation that can be produced from transgenics or mutagenesis that will yield data to establish the comparative improvement potential in soybean using these approaches.

**Goals / Objectives**

Through parallel transgenomics and mutagenesis approaches we will test the range of seed phenotypic variation that can be produced from these alternative methods to introduce genetic variation into plant genomes. Data yielded from this study will be used to establish the comparative improvement potential in soybean using these approaches.

**Methods**

Transgenomics Objective: In this proposal the seed-specific ER/targeted and retained GFP expression cassette will be used to produce at least 100 independent expressing transgenic soybean lines that will provide the variations of insertion sites needed

to assess how the position effect influences the seed protein output trait. Using an already constructed and tested expression glycinin-GFP HDEL storage protein proxy cassette will eliminate the variables and time associated with de novo cloning and vector construction as well as qualifying any new reporter for use as a marker for investigating the position effect and mutation. The glycinin/GFP cassette involves the enhanced GFP open reading frame flanked by a 5' 20 bp ER targeted signal from *Arabidopsis* chitinase gene and a 3' HDEL sequence to retain the protein in the ER. The regulatory elements are from glycinin, one of the dominant storage proteins that regulates seed-specific expression. With this GFP cassette in hand already in a vector that also contains a constitutive expression hygromycin resistance cassette producing the 100 transgenic soybean lines to explore the position effect can be undertaken immediately. Somatic embryos will be induced from immature cotyledons isolated from 'jack' plants grown in the greenhouse.

Once the 100 independent lines expressing GFP are produced and growing in the greenhouse, tissue from the primary plants will be used to determine the number of copies inserted into the genome as well as chromosomal location. The exact chromosomal location of each insertion site in all the GFP lines will facilitate characterization of possible positional effects on phenotype as transformation involves random integration within the genome. PCR-based methods have been established to determine the site of gene integration and since the soybean genome is elucidated and assembled the insertion sites can readily be mapped for each line of GFP cassette placement (Rosenthal, 1992; Bryda and Bauer, 2010). Positional effects are often the cause of expression level variation between lines so collecting chromosomal location and correlating that with the level of GFP expression in lines would be informative. Inverse PCR is a common technique used to determine chromosomal site integration of a transgene and will be used to determine the site(s) within all 100 GFP expressing transgenic lines in this project.

Number of chromosomal insertions and their specific site of integration will be correlated with the level of GFP expression. To investigate alteration of seed traits as collateral unintended consequences homozygous GFP seeds will also be assessed for changes in seed composition, specifically protein and oil content. Bradford assays will be used to estimate overall seed protein content and samples will be sent out for nondestructive NMR analysis for % protein/oil/moisture. The most interesting 20 lines out of the total 100 independent events will be assessed by 2D gels of total soluble proteins extracted from the seeds and analyzed by spot volume software (ie PDQuest 2D analysis). Lines determined to have altered seed composition will simultaneously be analyzed for the complete lipid profile, initially by gas chromatography/mass spectroscopy (GC/MS) data and if those results indicate an alteration from nontransgenic soybean's oil composition of 14% saturated/ 24% oleic acid (18:1)/ 54% linoleic acid (18:2)/ 7% linoleic acid (18:3) then samples will be sent to the Kansas Lipidomics Research Center ([www.k-state.edu/lipid/lipidomics/](http://www.k-state.edu/lipid/lipidomics/)).

#### Mutagenesis Objective:

The second goal of this project will investigate the effects of mutagenesis on the production of unintended phenotypes using the transgene encoded expression of GFP as a seed storage protein proxy.

To be able to correlate the data collected from the mutagenized population to the transgenomic portion of the project we will again use the same GFP-HDEL cassette and focus on subjecting a single already characterized GFP stably transformed soybean line with EMS to generate a mutagenized population. The GFP mutagenized population will ultimately be assessed for the expression of the stable GFP transgene and alterations in seed protein and oil content and composition. A key aspect for the production of a mutagenized population is the balance of obtaining actual mutations in the genome while simultaneously maintaining viability/fertility. Typically a series of EMS concentrations are used to determine this optimum concentration. We will use the GFP-HDEL seeds of an already characterized stable transgenic soybean line as the seed stock. Batches of 1,000 fresh GFP-HDEL seeds will be treated with either 25, 37.5, 50 mM EMS for 9 hrs (McCallum et al., 2000ab; Cooper et al., 2008). plant will be allowed to reach maturity. In this manner, each M1 plant will only give rise to a single M2 line, so that each individual in the M2 population descended from a different mutagenized parental line. As the seed stock of the mutagenized population contains a seed-specific GFP expression cassette, we will visually screen seeds from M1 plants to selectively choose seeds with a visual phenotype. That is, seeds displaying an alteration in the inserted seed GFP expression will be selected to move into the M2 generation. We will continue to screen M1 lines by fluorescent stereo microscopy and visually compared to the seed stock parental line for the degree of GFP expression. If visual inspection isn't sensitive enough, we will chip a small amount of tissue off the side of the seeds opposite the embryo, extract total protein and perform GFP quantification using a fluorometer and by immunoassay (ELISA) that permits rapid screening of a large number of samples using commercially available GFP as a standard. ELISA assays are among the standard immunoassay procedures in the PIs' laboratories. Both over and under expressing lines will be selected to produce a total of 100 mutagenized M2 lines. By using a seed-specific inserted trait as a marker we will be able to select for lines that contain mutations that affect seed phenotypes. Through this approach 100 M2 lines will be enriched for seed mutations.

To assess altered phenotypes in the M2 lines we will perform an analysis used to assess the transgenomic lines previously described in goal one. The analysis of the M2 seeds will parallel the assays performed on the transgenomic seeds. GFP will be

quantitated from seed lysates by fluorometry. Total protein of the seeds will be estimated by Bradford assays and both protein and oil content determined by nondestructive NMR. As with the transgenomic lines, the top 20 lines that show an alteration in either/both protein or oil as determined by deviation from the wild-type levels will be selected for comprehensive proteomic and triglyceride analysis. The proteomic analysis will initially consist of 2D gel analysis. If any differential spots are observed then LC/MS analysis the spots of interest will be used to determine the proteins identification. If a large number of differences are observed the 2D gel analysis will be supplemented with additional MuDPIT analysis. The mass spectroscopy will yield a comprehensive catalog of proteins that comprise that line's seed proteome. Similarly mutant lines that exhibit an altered oil content by NMR assay will be further analyzed by GC/MS and lipidomic profiling. If no lines exhibit an altered NMR determined oil content then mutant lines can be screened by GC/MS analysis to determine oil composition changes. The top 20 lines shown to have either altered overall oil content or oil composition will be subjected to extensive lipidomics analysis.

### Target Audience

The results of this project have utility for major classes of stakeholder.s Understanding the potential variability of output traits is central to creating optimized output traits. Many of the most significant output traits for food and feed are seed traits and this project will analyze the potential insertion site and mutational variability. The result obtained will be of broad interest to both academic and industry researchers that use either transgenic or induced mutation approaches to improve plants. Regulating transgenic plants and traits is a global legal requirement, while mutational approaches are largely exempted from regulation. From a policy view the potential variations of output traits resulting from transgene insertion and mutation is a key consideration in the US science-based regulatory regime. The data produced by this project is fundamental to continuing to refine regulatory requirements and as a consequence the data produced from this project is of significance to the government regulators and their stakeholders in industry and public.

### Products

The deliverable of the transgenomic portion of this proposed project is 100 independent lines produced with the same seed-specific GFP cassette and for each line the level of GFP expression will be determined by fluorometry, and both the number of site integrations and chromosomal position of each insert determined in the genome by inverse PCR, and seed composition changes determined by proteomic analysis (Bradford, NMR, 2D gels and/or MuDPIT) and oil analysis (NMR, GC/MS and/or lipidomic analysis). A total analysis of combined data will advance the interrelationship of insertion event (genome) with the output trait or phenome advancing the body of information on the position effect in a key crop, soybean. The resulting data will encompass the effect of chromosomal position on transgene expression and the effect(s) of transgene expression on collateral gene expression giving rise to unintended altered phenotypes. Further the results will detail the impact of varying the seed specific expression of GFP on the two dominant seed composition traits in soybean, protein and oil.

The deliverable of the mutagenic portion of this proposed project is 100 independent mutant lines produced from the same seed-specific GFP stable transgenic soybean line. As with the transgenomic portion, each mutant line will have the level of GFP determined by fluorometry and seed composition changes determined by proteomic analysis (Bradford, NMR, 2D gels and/or MuDPIT) and oil analysis (NMR, GC/MS and/or lipidomic analysis). The compiled data will advance the interrelationship of mutagenesis with soybean seed output traits.

### Expected Outcomes

Worldwide, soybean is the most important source of edible vegetable oil and high quality protein. Approximately 25% of the world's edible oils and 75% of the world's protein meal are from soybean (Soystats, 2011). Oil and protein are then understandably the major economic products from soybean seed. Protein meal largely goes to animal feed. By 2050, estimates are 9 billion people on the planet and a need to increase animal feed almost 250%. Oil has a number of uses, cooking, cosmetics, industrial uses, and biofuels. As the source of 79% of edible oils, soybeans play a significant role in the American diet. One in every 4 deaths in the US is due to heart disease. Coronary heart disease is the most common type of heart disease and it is the leading cause of death for Americans. Coronary heart disease costs the US nearly \$109 billion/yr considering the health care costs, medications and loses of productivity. Foods manufactured with a healthier soybean oil will contribute to the reduction of the incidence of coronary heart disease in the US. Soybean improvements are sought by stakeholder industry that comprises the "Better Bean" initiative that includes conventional breeding as well transgenic and mutational approaches. As a crop that can be manipulated soybean has intrinsic characteristics of a lack of related species in its primary production sites in North and South America coupled with an enclosed self-fertilizing crop there is essentially no pollen flow even to adjacent plants. These characteristics make soybean a highly desirable target for improvement and deployment. The lack of relatives and pollen flow limit some of the common risk concerns associated with many other crops. Collateral effects and potential variation of output trait remains as a variable for both transgenic and mutational approaches of crop improvement. The research outlined in this proposal will directly evaluate these remaining variables.

All crop improvement efforts hinge on genetic variation existing with plant genomes. This genetic variation ultimately is derived from one of two sources: mutations or transgenics. This project will investigate to what extent unintended genetic variations occur during these two processes in the two most important seed traits in soybean: protein and oil. Through the results of this project data will be obtained that can be used to evaluate the variation of traits that result from transgenic and mutational approaches. This data will serve to evaluate the potential collateral effects for manipulating seed traits and soybeans and provide a direct assessment of the risk and safety of modifying soybeans.

### Keywords

Soybean ~mutagenesis ~seed composition ~transgenomics

### Estimated Project FTEs For The Project Duration

Role	Non-Students or Faculty	Students with Staffing Roles			Computed Total by Role
		Undergraduate	Graduate	Post-Doctorate	
Scientist	0.0	2.0	0.0	1.0	3.0
Professional	0.0	0.0	0.0	0.0	0.0
Technical	0.0	0.0	0.0	0.0	0.0
Administrative	0.0	0.0	0.0	0.0	0.0
Other	0.0	0.0	0.0	0.0	0.0
Computed Total	0.0	2.0	0.0	1.0	3.0

Animal Health Component 0 %

### Activities

Research	100 %
Extension	0 %
Education	0 %

### Research Effort Categories

Basic	50 %
Applied	50 %
Developmental	0 %

### Classification

Knowledge Area (KA)	Subject of Investigation (SOI)	Field of Science (FOS)	Percent
201	1820	1080	100

### Knowledge Area

201 - Plant Genome, Genetics, and Genetic Mechanisms

### Subject Of Investigation

1820 - Soybean

### Field Of Science

1080 - Genetics

<b>Title: Efficacy and ecological impacts of transgenic containment technologies in poplar</b>			
<b>Accession No.</b>	1003836	<b>Sponsoring Institution</b>	National Institute of Food and Agriculture
<b>Project No.</b>	OREZFES-869	<b>Project Status</b>	ACTIVE
<b>Funding Source</b>	Non Formula	<b>Proposal No.</b>	2014-02989
<b>Grants.gov No.</b>	GRANT11610664	<b>DUNS Number</b>	053599908
<b>Start Date</b>	09/01/2014	<b>End Date</b>	08/31/2017
<b>Award Number</b>	2014-33522-22216	<b>Award Amount</b>	\$ 499,999
<b>Award Date</b>	08/19/2014	<b>Award Fiscal Year</b>	2014
<b>Submitted By</b>	Sandra Larsen	<b>Date Submitted to NIFA</b>	07/29/2014

**Program Code** HX

**Program Name**

Biotechnology Risk Assessment

**Project Director**

Steven Strauss

541-737-6578

steve.strauss@oregonstate.edu

**Performing Organization/Institution**

{NO DATA ENTERED}

null, null null

**Performing Department**

Forest Ecosyst & Society

**Co-Project Directors**

Betts, Matthew

Klocko, Amy

**Departments**

Forest Ecosystem Science Prg

Forest Ecosystems and Society

**Collaborating/Partnering States**

OR

**Collaborating/Partnering Countries**

{NO DATA ENTERED}

**Collaborating/Partnering Organizations**

Oregon State University

**Non-Technical Summary**

The movement of genes from genetically engineered plants to wild or feral populations is a cause of ecological uncertainty and social concern. It also presents substantial challenges to biotechnology regulatory bodies, who must decide if the impacts are acceptable or not. Because forest trees are weakly domesticated, have wild relatives, and pollen or seeds can spread widely, trees are especially problematic. However, plantation trees are often vegetatively propagated, making fertile flowers unnecessary for commercial use. Thus, genes that induce complete sterility could provide strong and simple mitigation of dispersal, simplifying regulatory decisions. We propose to study the efficacy, stability, and ecological impacts of genes that control floral development as tools for mitigating or preventing transgene spread. We established a plantation of transgenic poplars that contain 19 different types of genes that modify the expression of several well known floral development genes. We will: 1) study the effect on vegetative and floral structure; 2) identify genes which lead to strong sterility or floral modification; 3) analyze the stability of gene effects over years; 4) assess stability of observed changes to vegetative and floral structure; and 5) through intensive literature review, evaluate the likely ecological impacts of floral modification on biological diversity in relation to plantation establishment effects themselves. Our results will inform a variety of developing containment technologies, including the rapidly evolving gene editing methods, and help to lead to methods for genetic containment that satisfy regulatory bodies and address the majority of public concerns.

**Goals / Objectives**

Analyze the floral phenotypes and gene expression in a population of transgenic poplar trees containing potential sterility-inducing genes. Thorough literature review consider possible ecological impacts of various sterility genes.

Specifically:

- 1) study the spectrum of vegetative, floral, and seed capsule morphology in a population of 311 insertion events in early

flowering poplar genotype 6K10;

- 2) identify constructs and/or gene targets which lead to strong sterility or floral modification;
- 3) analyze the stability of transgene expression or target gene suppression (RNAi) over years;
- 4) assess stability of observed vegetative, floral, and seed development phenotypes; and
- 5) through intensive literature review, evaluate the likely ecological impacts of floral modification on biological diversity in relation to plantation establishment effects.

## Methods

**Phenotypes.** We will continue our annual measurements of stem diameter to assess vegetative growth vigor. In addition, we will carefully survey all plants for variation in vegetative morphology; if variation is visually observed we will measure the phenotype in all trees within those constructs and a sample of non-transgenic controls. Some traits of interest that we will survey include leaf shape and chlorophyll density, year of first flowering, timing of vegetative and floral bud-burst in spring, timing of bud set, and color of fall foliage. We are particularly interested in leaf color and curling, as these traits can be indicative of plant stress. We will also monitor trees in the event of unpredictable environmental stresses, such as field flooding, hard freezes, windstorms, high temperatures, drought, or pest damage.

**Sample collection for floral screening.** We will prescreen for constructs and events of interest by collecting and flushing unopened floral buds. We will collect branches that bear floral buds just prior to bud flushing (they can be identified by the size, shape, and angle of their buds relative to the branch (Fig. 3; Stanton and Villar 1996). This approach will allow the branches to be held in a cold room until ready for analysis. We aim to complete this analysis in time to observe events of interest as they flower in the field.

**Floral phenotypes.** In our screens, we will gather general morphological data for all trees, paying particular attention to traits that are relevant to female-sterility. These include: Presence/absence of flowers, presence/absence of carpels or carpel modification, leafy/vegetative flowers, development of capsules with seed, and their dehiscence. We will photograph the catkins and capsules in the field for future trait quantification (such as catkin size and color). For constructs showing potentially sterile events, we will fix field collected catkins and mature capsules for later microscopic analysis. We will collect flowers from at least two highly modified events and a comparable number of events with normal morphology, as well as from non-transgenic controls. In addition to collections for microscopy, we will snap freeze a set of flowers for future RNA extraction and target gene quantification. A set of fixed flowers and those sampled directly after field flushing will be analyzed microscopically using the Keyence digital microscope system (VHX-1000). We will focus on traits relevant for assessing female floral-sterility including: presence/absence of ovules, presence/absence of cotton and seeds, in bisexual or male flowers, the development of anthers and pollen.

For those constructs showing potentially sterile phenotypes, we will also section a set of flowers to gain detailed information on the cellular scale. These flowers will be fixed for sectioning and analysis using a Leica DM 5000 computerized fluorescence optical microscope located in the Electron Microscopy Facility at Oregon State University.

## RNA extraction and qPCR

We will use qPCR to assay gene suppression of our selected target genes and to determine levels of transgene expression of our DNM and Ovexp genes. We use our StepOne qPCR machine (see facilities and equipment statement) to check both the efficiency and specificity (using melt-curve analysis) of all primer pairs. Because we will be comparing gene expression among genotypes within tissue types (leaves or floral), we will employ a single internal control gene, a poplar ubiquitin (UBQ, Potri.001G418500). This constitutively-expressed gene is commonly employed in qPCR as an internal control, and has been validated to be highly appropriate for this purpose in a study of several possible internal control genes in poplar (Brunner et al. 2004). We will sequence the control gene as well, to allow for the design of accurate control primers. We will extract RNA using modifications to the standard Qiagen RNeasy protocol that are routinely used in our laboratory. After removal of residual contaminating DNA using DNase enzyme, precise quantification and quality determination will be made using the nanodrop instrument at Oregon State University's Center for Genome Research and Biocomputing (CGRB) Core Lab.

**Quantitative analysis.** We will collect growth data on all 6K10 trees in the plantation and use ANOVA to assess if constructs have a differential effect on vegetative development, or differ from controls. To improve statistical precision, we first will map phenotypes in regards to tree location within the field and statistically correct growth data for major patterns of variation (e.g., by Kriging, covariance analysis based on row/column position, and/or the use of various geographically weighted regression programs such as GWR). Because we will be intensively screening trees for constructs that have modified floral morphology in at least two events, and by comparing them to at least two events without such modified morphology, we expect to be contrasting phenotypes that are in most cases qualitatively, not just quantitatively, different. Thus, only a small number of observations should be required to detect a statistical difference. After checking for normality and possibly transforming variables, we will use a linear mixed model to analyze phenotypes for all of the chosen events and constructs, and examine 95% confidence intervals for each event to ensure that differentiated events were chosen. We will also examine, for each trait, if variance among trees within row-plots are significantly different from that among row-plots using likelihood ratio tests; when row-plots are not significant, as expected for floral phenotypes (typically highly heritable), all the trees can be used as independent observations. This method is a common practice in statistical analysis (e.g., Stroup 2013). We will measure organ sizes for this

analysis (e.g., carpel length), however if organs show presence/absence phenotypes we will employ attribute tests such as Fisher's Exact test.

**Analysis of ecological impacts of containment technologies.** Our broad hypothesis is that the relative impacts of floral modification on biodiversity are modest relative to other plantation forest management techniques. We intend to address this through a review manuscript addressing three components: First, we will summarize the literature evaluating changes in species richness and abundance associated with land conversion from agricultural or native forest to plantations, which will serve as a comparator to evaluate the relative effects of floral modifications. Second, we will examine the life history characteristics of species associated with poplar and eucalypts plantations, identifying those likely to be affected by removal or alteration of floral resources, and evaluating the degree to which they appear reliant on those resources. Finally, we will identify knowledge gaps and propose priorities for future research to understand ecological impacts of transgenic trees.

We will outline future research priorities for understanding ecological effects of transgenic floral modification on biodiversity, including the identification of knowledge gaps, particularly for species of conservation concern. This is likely to include research to better understand plantation impacts, and to understand what types of genetic modifications to floral tissues--and what types of mitigations through silviculture and landscape management--are likely to be most effective in minimizing ecological impacts. The main product of this work will be a detailed literature analysis, published in a major and peer-reviewed forestry or wildlife journal, such as Forest Ecology and Management.

### Target Audience

Plant and forest geneticists, biotechnologists, and breeders.

Policy makers and regulators concerned with transgenic poplars and other trees.

Wildlife biologists and plantation managers.

### Products

Our expectation is that by screening the large number of constructs and insertion events produced, we will identify a number of constructs and events that have informative sterility phenotypes. These can be made available or copied in other genotypes and species.

Our analysis of ecological impacts will inform efforts to mitigate these impacts and obtain regulatory or market approval.

### Expected Outcomes

In addition to the construct and ecological information products, outcomes will include:

By comparing the level of phenotypic modification to gene expression or suppression, we should be able to confirm that the phenotypes observed are associated with gene expression modifications.

By studying gene expression over multiple years, we will be able to assess the stability of gene expression and suppression.

By studying vegetative and floral gene expression, we will assess the extent to which vegetative measures are predictive of floral phenotypes, which could save years in research and commercial development.

Additionally, by monitoring seed formation from selected events for multiple years, we will be able to determine the reliability of floral-sterility achieved from each construct and event.

### Keywords

Genetic Containment; Sterility; RNAi; Populus; Flowering ~risk assessment ~biotechnology

### Estimated Project FTEs For The Project Duration

Role	Non-Students or Faculty	Students with Staffing Roles			Computed Total by Role
		Undergraduate	Graduate	Post-Doctorate	
Scientist	0.0	0.0	0.3	2.4	2.7
Professional	0.3	0.0	0.0	0.0	0.3
Technical	0.0	2.2	0.0	0.0	2.2
Administrative	0.0	0.0	0.0	0.0	0.0
Other	0.0	0.0	0.0	0.0	0.0
Computed Total	0.3	2.2	0.3	2.4	5.2

**Animal Health Component 0 %**

**Activities**

Research	90 %
Extension	0 %
Education	10 %

**Research Effort Categories**

Basic	25 %
Applied	75 %
Developmental	0 %

**Classification**

Knowledge Area (KA)	Subject of Investigation (SOI)	Field of Science (FOS)	Percent
201	670	1080	100

**Knowledge Area**

201 - Plant Genome, Genetics, and Genetic Mechanisms

**Subject Of Investigation**

0670 - Short rotation woody crops, including holiday trees

**Field Of Science**

1080 - Genetics

## QUESTIONS FOR DISCUSSION

1. Absolute containment to prevent gene flow during research trials are severe barriers to field studies.
  - a. Is there, or can there be, regulatory flexibility to allow AP that might result, at least with familiar markers, gene transfer vectors, and genes (including intra/cisgenics).
  - b. How can this be moved forward formally?
2. Is EPA seeking to exempt or streamline PIPs (compared to other biopesticides) based on RNAi given their high specificity?
  - a. Or to resurrect its cisgenic/intragenic proposal?
3. Is USDA seeking to close the “loophole” for non-regulated transgenics? That is, biolistic based, non-pathogen sequences?
4. Have organisms modified by gene editing technology started to be considered in regards to regulations and risk assessment?
5. Does the USDA plan to regulate new transgenics that don't use “traditional” recombinant DNA techniques such as Agrobacterium?
6. How do regulators distinguish between results from resistance models that predict different results?
7. If Aquabounty has met all of the FDA requirements, why have their salmon not been approved for sale by FDA?
  - a. What is the delay?
  - b. How will this affect future applications?
8. Would USDA regulate a corn cultivar that had a high level of drought resistance based on engineering that did not involve any plant pest genes?
9. Will USDA regulate a new cultivar that has the same construct (that includes a plant pest gene) as an already deregulated cultivar but with a different insertion event?

## **APPENDIX: Appropriate Acknowledgment of Your NIFA Award**

The Biotechnology Risk Assessment Grant (BRAG) program plays an essential role in fulfilling the mission of the National Institute of Food and Agriculture and the Agricultural Research Service. Proper acknowledgment of your USDA BRAG funding in published manuscripts, presentations, press releases, and other communications is critical for the success of our USDA's programs. This includes proper acknowledgment of the Program and agencies, as well as that of the Department and grant number (Please note that the '####-#####-#####' below refers to your award number and not your proposal number).

We expect you to use the following language to acknowledge NIFA support, as appropriate:

'This project was supported by Biotechnology Risk Assessment Grant Program competitive grant no. ####-#####-##### from the USDA National Institute of Food and Agriculture and Agricultural Research Service.'

We also expect that you will use our agency's identifier in all of your slide and poster presentations resulting from your BRAG award. The identifier is sent to you twice annually for at least 2 years after the termination date of your grant.



**United States  
Department of  
Agriculture**

Please alert us of significant findings, publications, news releases, and other media coverage of your work. With your permission, we may highlight your project in a national impact story or news release. If your research is featured on the cover of a scientific journal, we can showcase the cover as well.

Examples of these publications can be found at:  
[www.nifa.usda.gov/newsroom/newsroom.html](http://www.nifa.usda.gov/newsroom/newsroom.html).