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Mission Statement

Southern Insect Management Research Unit
USDA-ARS, Mid South Area
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The mission of the Southern Insect Management Research Unit (SIMRU) is to generate new knowledge of arthropod pest biology, ecology and management and integrate this knowledge into contemporary farming systems that will promote economical and environmentally stable pest management practices for the southern U.S.

The vision of SIMRU is to be a recognized center of innovation for negating agricultural pest problem through deployed scientific knowledge of pest biology, ecology and management options.

Disclaimer and Purpose of Report

This report summarizes progress made on research objectives for 2013 and plans for research activities in 2014.

Many of the results are preliminary and others are being released through established channels. Therefore, this report is not intended for publication and should not be referred to in literature citations.

Intent of this report is to give the reader an overview of the Southern Insect Management Research Unit (SIMRU) activities. The activities (progress and plans) address the research unit mission. Formal annual reports of research progress as submitted to the CRIS system are included in the summary.
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Overall Summary and Perspective of 2013 SIMRU Activities

As with the Agency and the Mid South Area, 2013 was a year of transition and change for SIMRU. A number of significant changes in personnel occurred during the year including Ryan Jackson’s departure to work for Syngenta and Gordon Snodgrass’ announcement of retirement effective in January 2014. Nathan Little joined SIMRU as a CAT 2 scientist, and Katherine Parys was hired as a CAT 1 scientist working on insect ecology. Yu Cheng Zhu, Sandy West and the entire Zhu Laboratory were detailed to work on honey bee toxicology under the supervision of John Adamczyk at the USDA ARS Southern Horticulture Research Unit in Poplaville, Mississippi. Calvin Pierce was hired as a Biological Science Research Technician in O.P. Perera’s Laboratory, and Jasmine Warren was hired on a term appointment with Clint Allen. Donny Adams and Larry Adams provided important leadership for field research programs in 2013. This was extremely important given the departure of Cat 1 scientists.

A new research farm was leased from Prewitt Farms south of Leland, and field work began in 2013. Owen Houston, Phil Powell and the entire group of SIMRU field research worked cooperatively to establish this new research location. A SIMRU “Pigweed Party” was held in June to weed plots, and almost all SIMRU employees actively participated or supported the effort. This volunteer effort and cooperation was used as an example of SIMRU team work in the FY2014 ARMPS presentation.

Sixteen new student interns were hired in 2013, and poster presentations of summer activities for 27 students was organized in August. A number of SIMRU scientists served as Acting RL of the unit during 2013 when Randy Luttrell was on detail to National Programs and later in year when he was assigned as Acting RL of BCPRU. Interviews for the vice-Blanco position were held in late December. Dr. Nathan Little and Dr. Miriam Hay-Roe were candidates for the position.

Visitors to SIMRU during the year include Ryan Kurtz of Cotton Incorporated, Mike Brewer of Texas A&M University, Craig Wilson with the USDA Future Scientist Program, Doug Street with Monsanto, and Jeanette Martinez, Kristine Edwards and Mike Caprio from Mississippi State University. The unit continued active collaborations with Jeff Gore, Don Cook and Fred Musser from Mississippi State University, and with Tahir Rashid from Alcorn State University.

Cathy Warren, Sakinah Parker, Yolanda Harvey, Pria Chatakondi, and Julian Henry continued their studies for college degrees, and several SIMRU employees supported out-reach activities in the community. Maribel Portilla, Henry Winters and Essanya Winder provided insects for the USDA Future Scientist Program throughout the year. Tabatha Nelson, Chad Roberts and Essanya Winder participated in the Mississippi State University Insect Rearing Shortcourse.

Overall, SIMRU remains a cohesive research unit with significant collaboration and an understanding of the importance and value of the unit’s mission. Given the personnel changes and the transitions with the Agency, the internal cooperation and focus on the unique mission and vision of the unit is increasingly important. During 2014, SIMRU expects to add additional scientific expertise, and the unit expects to relocate to renovated laboratory and office space in April 2014.
Research Project:  *Insecticide Resistance Management and New Control Strategies for Pests of Corn, Cotton, Sorghum, Soybean, and Sweet Potato*

**Project Scientists:** Randall Luttrell (Lead Scientist), Clint Allen, Katherine Parys, Maribel Portilla, OP Perera, Gordon Snodgrass, Yu Cheng Zhu  
**Project Number:** 6402-22000-063-00D  
**Start Date:** Sep 01, 2010  
**End Date:** Aug 31, 2015  
**Project Type:** Appropriated

**Objectives:** The long-term objective of this project is to develop an improved understanding of how the changing cropping landscape impacts insecticide resistance development and management of various insect pest species in order to increase profitability and sustainability of mid-South row crops. Objective 1: Improve tarnished plant bug control and insecticide resistance management by gaining new information on the pest’s ecology and biology using multi-disciplinary approaches, e.g. molecular genetic tools, stable carbon isotope analysis, gene expression and proteomics, and insecticide resistance assays coupled with field sampling. Objective 2: Determine the effect of bollworm ecology (corn earworm) on resistance to pyrethroid insecticides by developing and utilizing genetic markers linked to resistance traits, stable carbon isotope analysis, gossypol detection in adult insects, and insecticide resistance monitoring. Objective 3: Develop pest control strategies for the U.S. Mid-South’s Early Soybean Production System by determining accurate treatment thresholds, understanding the impact of changing cropping systems on farm-scale pest ecology, and developing effective insecticide resistance management practices for the stink bug complex, three-cornered alfalfa hopper, bean leaf beetle and soybean looper. Objective 4: Improve low input systems of pest control for sweet potato by evaluating the efficacy and proper use of newly registered insecticides to enhance their integration with crop rotation and other low cost control strategies.

**Approach:** We plan to improve tarnished plant bug control and insecticide resistance management by gaining new information on the pest’s ecology and biology using multi-disciplinary approaches. Analytical techniques, such as stable carbon isotope analysis, will be used to determine the influence of C4 host plants, such as field corn or pigweed, on populations of tarnished plant bug adults infesting cotton fields. This information will identify sources of tarnished plant bugs that may lead to alternative control measures prior to infestations into cotton fields. Tarnished plant bug populations will be monitored for resistance to various classes of insecticides commonly used by mid-South producers. This will provide real-time information to decision makers that will allow them to adjust their control recommendations based on the type of resistance that is found in their area of the mid-South. Detoxification enzyme activity surveys will be conducted in an effort to correlate and quantify insecticide resistance levels in field populations of the tarnished plant bug. Molecular genetics techniques will be conducted on tarnished plant bug populations that could lead to assays to evaluate the extent of field resistance in tarnished plant bug populations and provide input for insect management decisions. We also plan to determine the effect of bollworm ecology (corn earworm) on resistance to pyrethroid insecticides. Analytical techniques, such as stable carbon isotope analysis and a gossypol
detection technique, will be used to determine the impact of bollworm larval plant host on pyrethroid resistance levels measured in adults collected from pheromone traps. Molecular genetics tools will be used to identify candidate genes and biological pathways associated with insecticide resistance in bollworm populations. Successful identification of loci associated with insecticide resistance and the development of genetic markers for those will provide a method to obtain quantitative estimates of field evolved resistance by estimating the allele frequencies via population studies. We will also develop pest control strategies for the U.S. Mid-South’s Early Soybean Production System by determining accurate treatment thresholds and developing effective insecticide resistance management practices for the stink bug complex and bollworm. Field studies will be conducted to evaluate treatment thresholds for stink bugs and bollworms in early season soybeans. Stink bug populations will be monitored for potential resistance to various classes of insecticides, and this effort will provide real-time information to decision makers regarding the proper use of insecticides for control of these pests. We also plan to improve low input systems of pest control for sweet potato by evaluating the efficacy and proper use of newly registered insecticides to enhance their integration with crop rotation and other low cost control strategies. Field and laboratory studies will be conducted to determine the impact of crop rotation on populations of insect pests of sweet potatoes, as well as information of insecticide efficacy and proper application techniques.

**Summary:** For tarnished plant bug (TPB), contribution of pigweed and corn to adult populations within cotton fields can now be estimated using stable isotope analyses. Initial estimates indicate that ~80-90% of TPB adults collected from cotton fields in the Mississippi Delta during late June and early July completed nymphal development on a C4 host, primarily field corn. This may provide an opportunity to manage TPB populations prior to movement into cotton. Insecticide resistance monitoring in TPB populations indicated that control issues with acephate and pyrethroids will continue. TPB populations continue to be susceptible to thiamethoxam, imidacloprid, and novaluron. MS producers and consultants utilize this information when selecting insecticides for TPB control. TPB transcriptome sequence reads and their assemblies were made available to the public by depositing in the National Center for Biotechnology Information database. More than 200 xenobiotic processing gene transcripts were identified, and sequences were deposited in the database. Over 20,000 polymorphic genetic markers in expressed gene transcripts were also identified. Analysis of 6,688 genes of TPB using microarray revealed 6 esterase, 3 P450, and one glutathione S-transferase (GST) gene(s) that were significantly up-regulated. Economic and ecological impacts of wide-scale adoption of Bt cotton and insecticide use on grower profits and perceived risks of bollworm (BW) damage are being measured. These impacts have potential influence on BW resistance evolution to insecticides and Bt toxins. Pyrethroid susceptibility estimates in BW across the cotton belt did not vary from those generated during the previous three years; however, pyrethroid susceptibility estimates over the last 4 years have generally decreased compared to estimates generated during the late 1990’s. No patterns of pyrethroid or Bt susceptibility were identified in BW collected from different cropping landscapes. BW genomic sequences (n=392) containing xenobiotic processing genes and insecticide targets were identified by sequencing a BCA library. Over 23,000 microsatellite markers were identified from the genome of the BW. Sequencing and assembly of the BW transcriptome were completed, and microarrays were developed for gene expression analysis. In soybeans, stink bug species were surveyed across the MS Delta to document temporal and spatial dynamics of stink bug populations. Late-instar stink bug nymphs
(green, southern green, and redbanded) were approximately twice as tolerant as adults when tested against organophosphorus or pyrethroid insecticides. Early stage nymphs should be targeted for insecticidal control. Tracking wireworm populations in sweetpotato and corn is providing new information on the impact of these soil-inhabiting pests on sweetpotato damage and crop rotation influences. Preplant applications of chlothianidin, chlorpyrifos, and imidaclorpid reduced sugarcane beetle damage to sweetpotato in a large field cage study. Chlorpyrifos applications had no effect on reniform nematode populations in a study with insecticides applied alone and in combination with potassium N-methyldithiocarbamate.
Research Project: *Control of Tarnished Plant Bugs by Biocontrol and Other Methods*

**Project Scientists:** Vacant (Lead Scientist), Randall Luttrell, Maribel Portilla  
**Project Number:** 6402-22000-064-00D  
**Project Type:** Appropriated  
**Start Date:** Jan 03, 2011  
**End Date:** Jan 02, 2015

**Objectives:** Determine the effect of temperature and reproductive state on susceptibility of tarnished plant bugs to *Beauveria (B.) bassiana* (ARSEF 8889). Determine the effect of exposure to insect growth regulators (IGRs) and *B. Bassiana* (ARSEF 8889) on immature tarnished plant bug survival. Determine the effect of host plant and application timing (season) on susceptibility of tarnished plant bugs treated with ARSEF 8889 and IGRs (in situ).

**Approach:** The effect of temperature and reproductive state on the susceptibility of tarnished plant bugs to *Beauveria (B.) bassiana* (ARSEF 8889) will be determined in replicated laboratory tests. The two reproductive states tested will be normal reproductive adults and nymphs and diapausing adults and nymphs that produce diapausing adults. Temperatures tested will range from 10°C to 30°C. Insect growth regulators (IGRs) will be tested with nymphs in replicated laboratory tests to determine which IGRs are effective and the rate at which to use them. The most effective IGR(s) will be tested in laboratory tests in combination with ARSEF 8889 to determine the most effective combination treatment. Results from the laboratory tests will be tested in the field in replicated tests in cotton (for in-season plant bug control) and in the fall and winter on wild host plants (for control of the diapausing overwintering generation). The effect of IGRs and ARSEF 8889 treatment on beneficial arthropod populations will be evaluated in the field tests and with additional laboratory tests.

**Summary:** The effectiveness of ARSEF 8889 (NI8) against reproductive and diapausing tarnished plant bug (TPB) was evaluated in several laboratory tests at temperatures from 10° to 30°C. The fungus was found to be equally effective against reproductive and diapausing TPB with higher infection rates at the warmer temperatures. A replicated small plot test in cotton was conducted during July 2010. Treatments tested were NI8, NI8 + novaluron, novaluron, and an untreated check. Results were inconclusive because of adult movement between the plots. A large replicated test in cotton is currently being conducted in which plot size is one acre. This should allow us to have a valid evaluation of the results. A preliminary test was conducted in October and November 2010 in which NI8 was used alone and in combination with novaluron to kill overwintering TPB adults and nymphs on pigweed and tall goldenrod. Beneficial arthropods found on the wild hosts were also identified and their populations estimated before and after treatment. The novaluron and novaluron plus NI8 treatments were very effective in reducing numbers of nymphs on both wild hosts with infection rates as high as 41.5%. Infection rates among adults with NI8 were as high as 77.7%. This test will be conducted in October 2011 using replicated plots. The most abundant beneficial arthropods on both wild hosts were spiders in the families Thomisidae (crab spiders) and Salticidae (jumping spiders). Crab spiders made up 48.5% of the total number of beneficial arthropods while jumping spiders were 22.2%. Crab spiders and jumping spiders were evaluated in laboratory tests for susceptibility to NI8 infection. Crab spiders were mostly immune while a low percentage of jumping spiders were infected. Green lacewing, ladybird beetle and big-eyed adults were also tested in the laboratory for susceptibility to NI8. Infection rates were low for these predators.
ARSEF 8889 was evaluated for tarnished plant bug (TPB) control in cotton in 2011. The test was conducted under extreme conditions in that the temperature exceeded 90°F on every day of the test and the TPB population in the cotton was well established and above the treatment threshold in every plot when the test began. None of the treatments controlled the TPB population including a standard treatment with dicrotophos or acephate (tests with a glass-vial bioassay showed that the population was highly resistant to pyrethroid and organophosphate insecticides). The test will be repeated in 2012 and will be changed so that the first treatment application is made earlier when below threshold numbers are present. A second application will be made one week after the first application. Deposition of spores on different locations in the plants will also be determined and used to evaluate spray coverage and residual effectiveness for TPB control. A field test using ARSEF 8889 to control TPB in corn when it tassels and in soybeans during bloom will be conducted. Field tests in the fall to evaluate the use of ARSEF 8889 and novaluron for control of overwintering TPB on wild hosts will again be conducted. In a cooperative effort between SIMRU and the Boyce Thompson Institute, the genome of Beauveria bassiana has been sequenced and will be published this year.
Research Project: Effect of Resistance on Insect Pest Management in Transgenic Cotton

Project Scientists: OP Perera (Lead Scientist), Kerry Clint Allen, Nathan Little, Randall Luttrell
Project Number: 6402-22000-065-00D
Project Type: Appropriated
Start Date: April 26, 2011
End Date: March 31, 2016

Objectives: Determine the impact of a changing cropping landscape on host plant ecology and insect resistance management practices for bollworm using analytical techniques. Determine gene flow and migration patterns by analyzing tobacco budworm and bollworm populations in temporal and spatial scales using genetic and/or empirical/mathematical approaches. Identify possible mechanisms of resistance to Bt toxins by profiling gene expression patterns and develop a marker based genetic linkage map.

Approach: More than 95% of the second generation bollworm within each growing season utilizes field corn as a host. Impact of corn plants expressing multiple Bt toxins on the bollworm populations will be studied by comparing historical pheromone trap data with current and future population estimates influenced by increased acreages of Bt corn expressing multiple Bt toxins. Stable carbon isotope analysis will be used to identify bollworms using corn as a host plant. Influence of local cropping landscape on bollworm populations will be studied using sentential plots of conventional and Bt corn and cotton and early maturing soybeans. Large field cages will be used to evaluate the impact of pyramided-gene Bt corn hybrid/refuge system on resistance management strategies. Expressed genes of tobacco budworm and bollworm will be identified by transcriptome sequencing, and genetic markers developed from polymorphic nucleotide regions will be used in ecological genetic studies of tobacco budworm and bollworm populations. Gene expression profiles will be used to identify biological processes involved in physiological response to ingestion of Bt toxins. Markers developed for candidate loci associated with resistance to Bt toxins will be used to estimate allele frequencies in natural populations. Genetic loci under selection will be identified using statistical methods. A genetic linkage map of the bollworm developed using polymorphic markers will be used to study inheritance of loci of interest to Bt resistance.

Summary: This is the first report for the project 6402-22000-065-00D that started in April 2011. Field and laboratory studies have been initiated, and insect samples are being collected and held for future analyses. Twelve-month milestones will be complete upon the end of the cropping season.

Transcriptome of tobacco budworm was assembled using over 20 million nucleotide sequence reads. All nucleotide reads and assemblies were submitted to public databases. Glass slide microarrays containing 44,000 features were developed using curated transcriptome sequences. BW transcriptome sequencing using Roche 454 and Illumina platforms was completed. Assembly and bioinformatic analyses were performed to identify expressed genes in the transcriptome assembly. Nucleotide sequence reads were re-assembled (overlaid) using curated reference gene sequences to identify single nucleotide polymorphisms. Nucleotide reads and assemblies of tobacco budworm and bollworm were submitted to public databases.
Primary cell cultures generated from susceptible YDK strain and resistant KCB and CXC strains of tobacco budworm were treated with Cry1Ac and Cry2Ab toxins. Treated and control cells were harvested and total RNA and proteins were extracted. RNA extractions are being used in RNA-Seq based gene expression profiling experiments. Protein fractions were subjected to two-dimensional differential gel electrophoresis to identify differentially expressed proteins. The protein spots identified were excised from the genes and were submitted to a core facility for mass spectrometry based determination of amino acid sequences. Laboratory screening of bollworm larvae collected from transgenic corn plants was initiated to select a bollworm line resistant to Cry1Ac. Bollworm larvae collected from various field sites were used to establish laboratory strains to obtain genetic material for mapping studies.

Results from the initial year of this project indicated that field corn was still the primary host of H. zea during the growing season even though greater than 50% of the corn planted in the southern U.S. produces one or multiple Bt proteins. Bt corn hybrids that produce multiple Bt proteins suppress H. zea larval numbers and kernel damage, but yields do not necessarily reflect this. Laboratory colonies of H. zea were established from light trap samples taken in concentrated areas of corn, cotton and soybean production in the Mississippi Delta and assayed for susceptibility to Cry1Ac. Variable responses were obtained but no clear association with the different crop areas was evident.

Corn fields were surveyed after harvest to assess the density and maturity of volunteer corn in the fall as well as the abundance of corn earworm in non-Bt corn at various growth stages. Greenhouse and field trials examining the efficacy of various crosses of Bt and non-Bt corn varieties on bollworm were conducted during last year and are being repeated during 2012.

Helicoverpa zea colonies with increased tolerance to Cry2Ab and Cry1Ac were established from insects collected from field locations in the Mississippi Delta. Back-cross genetic mapping populations were developed by mating these colonies with insects from a susceptible laboratory colony maintained at SIMRU and DNA extractions were completed from 96 insects for use in a marker based mapping study.

Nucleotide sequences from 14 DNA pools containing 36,500 clones of a bacterial artificial chromosome (BAC) library were obtained using high-throughput sequencing technology. The sequence reads were assembled to identify candidate Bt resistance genes and genetic markers associated with them. Nucleotide sequences from bollworm BAC clones and transcriptome are also being used to complete assembly and annotation of the bollworm genome by CSIRO, Australia.

Laboratory colonies of Bt resistant and susceptible Old World bollworm, Helicoverpa armigera, exposed to Cry1Ac were used to study the time course of midgut gene expression. RNA extracted from midguts of treated insects was used to obtain short nucleotide reads for RNA-Seq profiling. Validation of differential expression patterns observed in RNA-Seq experiments is in progress.

Computer model simulations examining the risk of corn earworm resistance development on corn expressing two Bt genes versus those expressing a single gene were carried out. At least
350 replicate simulations with randomly drawn parameters were completed for each of four risk assessments. When dual-gene Bt-cotton, planted with a natural refuge and single-gene corn planted with a 50% refuge was simulated, resistance to both toxins simultaneously never occurred within 30 years, but in 38.5% of simulations, resistance evolved to toxin present in single-gene Bt-corn (Cry1A). When both corn and cotton were simulated as dual-gene products, cotton with a natural refuge and corn with a 20% refuge, 3% of simulations evolved resistance to both toxins simultaneously within 30 years, while 10.4% of simulations evolved resistance to the Cry1A toxin.
2013 Research Program Accomplishments

Adams Research Program

USDA-ARS, Southern Insect Management Research Unit (SIMRU) and LSU AgCenter collaborated for the second year to evaluate six insecticide regimes for efficacy against sugarcane beetles in sweetpotato. These studies were conducted in large cages at the SIMRU location in Stoneville, MS. Beauregard sweetpotatoes were transplanted to four row plots in the 1/8 acre field cages (14 plots per cage). Preplant and layby treatments were applied with an ATV two row boom sprayer calibrated to deliver 12 GPA. Weekly treatments were applied with a backpack two row boom sprayer calibrated to deliver 2.5 gallons to 1/8 acre. Treatments were arranged in a RCB design and replicated four times. Sweetpotatoes were harvested from the two center rows of each plot. Yield, quality and insect damage were recorded and analyzed. (Larry Adams, Randall Luttrell and Tara Smith)

During 2013 SIMRU submitted yield and quality results from four check lines and four numbered research varieties, in cooperation with researchers from Louisiana State University, North Carolina State University and Mississippi State University, to the National Sweetpotato Collaborators Group Variety Trials for the NSCG Annual Report. (L. C. Adams, R. G. Luttrell and Chris Johnson)

In 2013 USDA-ARS, Southern Insect Management Research Unit completed a second year of an on-farm sweetpotato demonstration study to illustrate the value in taking soil samples for nematode populations and applying a nematicide and a preplant incorporated insecticide to control soil insects attacking the developing sweetpotato roots. Two Mississippi Delta locations, Sanders Farm, Mound Bayou, MS and Alcorn State University Research Farm, Mound Bayou, MS, were transplanted with Beauregard 14 slips in mid May. Treatments were applied approximately two weeks prior to transplanting and included Lorsban, K-Pam, Lorsban and K-Pam, a grower managed field and an untreated control. Soil samples were taken preplant to determine nematode populations. All locations were harvested at ~135 days. Yield, quality and insect damage were recorded and analyzed. (L. C. Adams, C. Johnson)

Trap, chart, summarize and report results of insect pheromone trapping of the H. zea (21st year) and H. virescens (22nd year). We continue to work with SIMRU scientist in the Bacillus thuringiensis resistance monitoring program. The results of the long term trapping of these pests illustrate the decline of population dynamics since the introduction of transgenic crops in the Mississippi Delta. (L. C. Adams, C. Johnson)

Allen Research Program

A survey of looper and stink bug species inhabiting soybeans in Mississippi was continued during the 2013 growing season. Soybean fields were sampled in Bolivar, Coahoma, Holmes, Leflore, Sharkey, Sunflower, Washington, and Warren Counties in MS. Fields were sampled with a sweep net and looper larvae obtained from sampling were placed on artificial diet and
reared to adult for species identification. Fairly high densities of soybean looper, *Chrysodeixis includens*, and gold looper, *Argyrogramma verruca*, larvae were collected in a soybean field in Stone County on June 15th, but the first soybean looper larva was not collected in the Delta until July 15th in Holmes County, MS. The only other looper species collected in the Delta after July 15th was a single gold looper larva collected from Warren Co. on August 12. The majority of the stink bug species observed this year were green, *Chinavia hilaris* and brown, *Euschistus servus*. The southern green stink bug, *Nezara viridula*, was collected in later planted soybean during August and September in some locations. The redbanded stink bug, *Piezodorus guildinii* was collected in low numbers, especially in Warren County during June and populations increased in this county into September. Kudzu bugs were collected in Warren County in low numbers in June and reached over one per sweep in later planted beans in this area. (C. Allen, L. Andrews)

An evaluation of sprayed and unsprayed plots for lepidopteran pests in early and late planted soybean and the subsequent impact on yield within production fields was examined. Studies were conducted in Bolivar, Coahoma, Holmes, Leflore, Sharkey, Sunflower, Warren and Washington Counties. At most locations, an early and late planting date of soybean was used for the study. At the R2-R3 growth stages, an automatic insecticide application of either a Karate (lambda-cyhalothrin) or Prevathon (rynaxypyr) insecticide was made on plots at all locations. Subsequent applications on threshold levels of lepidopteran insects were planned, but low populations at most locations prohibited additional applications. Overall, low numbers of insects were observed in sprayed and unsprayed plots and little differences in yields were observed. This work will be continued to examine the economic benefits of insecticide applications at varying densities of larvae (bollworms or loopers) (C. Allen, L. Andrews, D. Adams).

Both corn earworm, *Helicoverpa zea*, and tobacco budworm, *Heliothis virescens*, (heliothines) can inhabit soybean. Because the immature stages of these insects are difficult to distinguish in the field, many times insecticides are applied without knowing which species or composition of species is present in a given field. During 2012, soybean fields were sampled throughout the MS Delta and heliothine larvae were collected and placed on artificial diet. Larvae were allowed to develop into pupae or adults for identification by species. The largest collections were made in Coahoma and Leflore Counties during the first two weeks of August, and populations were relatively low during other periods of the growing season. Overall, 221 larvae were collected and 144 of these were identified to species. Only 11% (16 larvae) of the heliothine larvae identified were tobacco budworm and the remainder were corn earworm. (C. Allen, L. Andrews, D. Adams).

Little Research Program

Transgenic cottons have never produced a high dose of toxin from the bacterium *Bacillus thurengensis* (Bt) for control of bollworms. Even second-generation Bt cottons that produce multiple Bt proteins often sustain economic damage from this pest. Therefore, supplemental insecticide applications for bollworm control are commonly made in Bt cotton. Field studies were conducted in Stoneville, MS to evaluate supplemental bollworm control in two types of cotton: conventional and Bollgard II. Each cultivar was scouted independently, and insecticide applications of the nuclear polyhedrosis virus (NPV) of *Helicoverpa zea* (Gemstar®), lambda-
cyhalothrin (Karate Z®), or chlorantraniliprole (Prevathon®) were made when a larval threshold of 4 larvae per 100 plants was met. Data analyses are being conducted to determine whether non-Bt cottons can be grown that are economically competitive with Bt cottons relative to bollworm control. (R. Luttrell, N. Little)

Black light traps were used to collect bollworms and tobacco budworms for laboratory studies on the susceptibility of these insects to Bt toxins. Twelve light traps were run twice a week for a period of eight weeks starting in July. A total of 319 live bollworms and 2 live tobacco budworms were collected from light traps. These insects were brought to the laboratory, mated, allowed to lay eggs, and the F2 generation of neonate larvae were utilized in multiple bioassays to determine the extent of susceptibility to various Bt toxins and conventional insecticides. (N. Little, R. Luttrell, K. Parys, C. Roberts, K. Dixon, M. Mullen)

Luttrell Research Program

Transgenic cottons have never produced a high dose of toxin from the bacterium Bacillus thuringiensis (Bt) for control of bollworms. Even second-generation Bt cottons that produce multiple Bt proteins often sustain economic damage from this pest. Therefore, supplemental insecticide applications for bollworm control are commonly made in Bt cotton. Field studies were conducted in Stoneville, MS to evaluate supplemental bollworm control in two types of cotton: conventional and Bollgard II. Each cultivar was scouted independently, and insecticide applications of the nuclear polyhedrosis virus (NPV) of Helicoverpa zea (Gemstar®), lambda-cyhalothrin (Karate Z®), or chlorantraniliprole (Prevathon®) were made when a larval threshold of 4 larvae per 100 plants was met. Bt cotton plots required fewer insecticide applications than conventional cotton plots. Yields did not significantly differ among treatments, although there was a numerical trend for higher yields in Bt cotton plots. Additional economic study of net cost and benefits is planned, and additional replication of the study is needed to address variable results. (R. Luttrell, N. Little)

Monitoring of field populations of bollworm and tobacco budworm for resistance to Bt toxins has traditionally relied on laboratory bioassays with Bt toxins provided by industry cooperators. Results have been variable with limited association of bioassay results to actual survival of insects on toxin producing plants. Additional research is needed with paired laboratory and field experiments. This requires a source of toxin for assays and current industry sources are limited. Studies were conducted in 2013 to confirm baseline susceptibilities of bollworm, tobacco budworm and fall armyworm to two commercial formulations of Bacillus thuringiensis, Dipel and Javelin, that include a mixture of different Bt toxins. Previous published work indicated correlations of activity of Dipel, Javelin, Cry1Ac and Cry1Ab across different field colonies of bollworm and tobacco budworm. The baseline studies conducted in 2013 will be used as reference points to further investigate these relationships and determine if Dipel or Javelin can be used as a tool to measure susceptibility to Cry1Ac expressed in most Bt crops. (R. Luttrell, N. Little, K. Dixon, M. Mullen).

A small-plot field study was conducted in 2013 to measure the activity of different Beauveria bassiana treatments on control of tarnished plant bug. A report by M. Portilla et al. describes the study of infection rates and impact of the entomopathogenic fungus on tarnished plant bugs. Additional observations were made on plant damage and insect densities in the cotton plots
surrounded by mustard and corn barriers. The mustard was used as a trap crop to build populations of tarnished plant bugs. When the cotton reached pinhead square stage, the mustard was mowed releasing high densities of the bugs. The movement of the bugs to the cotton plots and the surrounding corn was monitored, and subsequent impacts on bug densities and damage to cotton were determined by monitoring fruit retention. These data are being compared to a 2012 study to further quantify the movement of bugs and refine potential use of the technique in field research. (R. Luttrell, N. Little, O. Houston, M. Portilla, L. Adams, C. Johnson).

Parys Research Program

Novaluron is a relatively new insecticide used to control insects in cotton fields across the Mississippi Delta. Populations of adult tarnished plant bugs, *Lygus lineolaris* (Palisot de Beauvois), were collected from 20 field locations brought to the lab to found colonies. Each of the individual colonies was reared on meridic diet, and resulting eggs were removed. The F1 generation was reared on broccoli before being transferred onto meridic diet. During the 3rd instar, each population was evaluated for response to novaluron through both a diet incorporated assay and contact assay. (K. Parys, R. Jackson, C. Allen, C. Roberts)

The introduction of transgenic crops containing Bt proteins has changed the quantity and type of pesticides applied in agricultural fields each year. Several new plant technologies are expected to be released into the market in the next few years, including the possibilities of Bt soybeans and cotton plants expressing a gene for the control of *Lygus* spp. To monitor the potential impact of these new varieties on non-target organisms, we collected baseline data on arthropod community structure. Pitfall and flight intercept traps were used in both cotton and soybean fields to collect both ground and flying insects. (K. Parys, N. Little, C. Roberts)

The last host plant list available for *L. lineolaris* was published in 1986, over 25 years ago. We used online resources to compile an updated list of host plant records in peer-reviewed literature. (K. Parys, G. Snodgrass)

Perera Research Program

A handful of genes associated with mechanisms conferring of tolerance to *Bacillus thuringiensis* (Bt) toxins in corn earworm and tobacco budworm have been identified. These genes include aminopeptidases, membrane-bound alkaline phosphatase, cadherins, and ABC transporters. Although the contribution of individual genes have been characterized to some extent, Bt toxin tolerance in these insects seems to involve interactions between multiple genetic loci. In order to perform genetic association studies on Bt tolerance, high-throughput genetic maker capture system was developed for corn earworm using genome sequences obtained from bacterial artificial chromosomes. Capture oligonucleotides were developed for approximately 3,900 markers and successfully used to identify markers from a test genome. (O. Perera)

Old-world cotton bollworm (*Helicoverpa armigera*) has invaded Brazil and a few neighboring countries in South America. Due to climatic conditions optimal for development of this invasive pest in North America, it is an imminent threat to US agriculture. A PCR based assay capable of separating cotton bollworm, corn earworm, and tobacco budworm was developed and used to screen genomic DNA extracted from field collected insects. The assay is sensitive enough to
identify corn earworm using a single partially developed egg. Over 1,000 insects collected from various locations in 2002, 2005, and 2011 have been screened. Insects collected in 2013 are currently being processed. (O. Perera, C. Allen, C. Pierce, P. Chatakindi).

The perception and discrimination of odorants in insects require odorant-binding proteins (OBPs). OBPs bind odorant molecules and facilitate triggering of receptor combinations specific to the odorant class. To gain a better molecular understanding of olfaction in the agronomic pest *Lygus lineolaris*, the tarnished plant bug, OBP repertoire was identified using a transcriptomics-based. In total, 33 putative transcripts, including the previously reported *Lygus* antennal protein, were identified based on the characteristic and/or sequence similarity with annotated orthologous sequences from other species. [O. Perera, G. Snodgrass, C. Pierce, J. Hull (USDA-ARS, Maricopa, AZ)].

A microsatellite markers were used in a population genetic study of the tarnished plant bug (TPB) by analyzing over 1,000 insects collected from five locations near Stoneville, MS at three time points in the 2006 growing season. Statistical genetic analyses identified two genetic clusters in the study locations. The proportions of the insect categorized in each genetic cluster changed over time. Several factors, including genetic drift and migration may be responsible for the changes in the genetic makeup of the TPB populations. [O. Perera, J. Gore (MS State), G. Snodgrass]

**Portilla Research Program**

Two isolates of *B. bassiana* including the commercial strain GHA and the Mississippi Delta native NI8 strain were evaluated in the field for pathogenicity and infectivity against TPB. Effects of application times and solar radiation on mortality and sporulation were evaluated. In order to evaluate pathogenicity by direct spray, two-d old TPB adults from a laboratory colony were placed in cages located on the top part of cotton plants in the field prior to spraying *B. bassiana* strains with a multi-sprayer tractor calibrated to deliver 6.5 x 10^{12} spores / acre. Detailed observations were made on the effect of solar radiation by releasing 2-d old TPB adults in cages with sprayed branches of cotton plants cut 0, 1, and 2 days after morning and night applications. Differences on mortality and sporulation on TPB exposed to sprayed cotton branched for 24 hours were significant among treatments. Mortality and sporulation drastically decreased from 1.7-fold by the next day to 5.6-fold by the second day after *B. bassiana* NI8 night application and a reduction of 1.5-fold by the next day to 8.2-fold by the second day in morning application. Less than 10% sporulation was found two days after *B. bassiana* application for both strains. Overall, these results indicated that *B. bassiana* application resulted in decreased survival of TPB regardless of the isolates by direct spray or by contact. However, the superior performance of the Delta native strain NI8 was observed in all treatments applications and times of evaluation. An important obstacle to the efficacy of each isolates is their inability to survive exposure to solar radiation, which affects the use of this entomopathogenic fungus for the control of the TPB. However, the >50% mortality of TPB adults obtained by direct spray or by contact should make this fungi an attractive alternative for TPB control. (M. Portilla, G. Snodgrass, R Luttrell and T. Ramsey)
The spores of two strains (NI8 and GHA) entomopathogenic fungi, *B. bassiana* was evaluated for their compatibility with two surfactants; Bio-plastic (Based-formulation) and the commercial available Tween-80 (polyoxyethylene sorbitan monoleate). Under laboratory conditions mortality and infection of TPB varied with surfactant concentration. The optimal surfactant concentration for Bio-plastic was 1%. Using 1% and 0.4% concentration of Bio-plastic and Tween-80, respectively; mortality and infection of TBP by direct spray and by contact of *B. bassiana* was evaluated. Fifty plots (0.068 acre each) were planted with cotton on late May 2013 and sprayed (Cone-Tip Quick TXVS12) with a multi-sprayer tractor on July 2013 with a concentration of 6.5x10^{12} spores /acre of both *B. bassiana* strains and a control. Two application morning and night were applied. Thirty 2-d old TPB adults from laboratory colony were placed in cages located on the top part of the cotton plants in the field prior to spraying with the *B. bassiana* strains. A total of 150 cages were used in the 50 plots (3 cages / plot). Adults were collected immediately after sprayed by knocking down individually into a solo cup with solid diet. Dead insects were kept in the same cup and were daily checked for sporulation. Adults TPB were held in an environmental room at 27°C, 65% RH, and 12: 12 (L: D) h photoperiod and checked daily for ten days. Differences in mortality were observed where the control showed the lowest mortality and infection. Significant differences were observed among treatments, and superior performance was observed in plots sprayed with NI8+Tween-80. (M. Portilla, R Luttrell, Cesare Accinelli, H. Abbas, G. Snodgrass and T. Ramsey)

Laboratory studies were carried out to determine the effect of different temperatures on two strains of *B. bassiana* activity (NI8-GHA) against *L. lineolaris* and the temperature requirements for conidial germination for both *B. bassiana* strains. Higher mortality and sporulation of NI8 has been obtained from 12°C to 30°C, low mortality and low sporulation were found at 10°C, and low mortality and none sporulation was obtained at 4 and 7°C for this strain. Significantly low mortality and low sporulation of GHA strain was found at all temperatures when compared with NI8 and low mortality and none sporulation were found at 10 °C and lower temperatures for GHA strain. LC_{50} and LD_{50} were estimated. These results suggested that NI8 could minimize overwinter population of Lygus. LC_{50} and LD_{50} estimated in this experiment at low temperatures will be used in weed plots during winter 2013. (M. Portilla, G. Snodgrass, R Luttrell and T. Ramsey)

Pathogenicity of *Beauveria bassiana* against Kudzu bug was carried out. New strain of *B. bassiana* was isolated from Kudzu bugs (Georgia) and was sent to ARSEF collection in Ithaca. Two preliminary bioassays were conducted using three strains of *B. bassiana* (Kudzu, NI8, and GHA strains) at four different concentrations (10^6, 10^7, 10^8, and 10^9). In each essay were used mixed-sex kudzu bugs adults that were collected in the field (South Carolina) and maintained at NBCL, Mississippi on fresh kudzu plants collected from a small kudzu patch in Stoneville MS. LC_{50} value were obtained with the commercial strain GHA. Differences in mortality and sporulation were found among kudzu bugs ages. Higher mortality and sporulation was observed in kudzu bugs treated with *B. bassiana* strain NI8. (M. Portilla, R Luttrell, W. Jones, Wayne Gardener, Nick Seiter, G. Snodgrass, and T. Ramsey).

Laboratory studies will continue to determine resistance of *L. lineolaris* to the entomopathogenic fungus *B. bassiana*. Progeny from infected survival adults have been treated for 22 generation and some resistance has been found. Small percentage of adults of generation ninth to twenty
survived the application. Differences in adult survival among generation were observed based on the LIFETEST Log-rank statistic procedure of SAS. (M. Portilla, G. Snodgrass, R Luttrell and T. Ramsey)

The second year of a large-field cages study was carried out to compare cotton under TPB high and no infestation. Cages were sprayed with *B. bassiana* (6.5x10^{12}). Results indicated that *B. bassiana* can effectively reduce >50% of adult population compared to the control. Over 30 thousand adults of laboratory TPB colony per cages (3 cages each year) were used for this experiment. (M. Portilla, G. Snodgrass, R. Luttrell, and Tabatha Nelson).

The Insect Rearing Unit was able to produce over 50 thousands eggs per day per specie of Lepidopterans (ZEA, VIR, and FAW) and the production can be increased if needed. Different stages of insect (eggs, larvae, pupae, and adults), mixed dry diet, and cupped diet were provided to several USDA scientists in order to complete their research projects. Over 10 thousands first instar larvae of ZEA inoculated individually on diet solo cups were provided in 2013 for the Future Scientist Program. Those insects were shipped for teaching purpose and distributed to thousand of school kids across the U. S. (Henry Winter, Essanya Winder, M. Portilla, R. Luttrell, and Tabatha Nelson)

Preliminary laboratory studies have been conducted for life table constructions and demographic parameters estimation for TPB using small cotton plants. High mortality of first to fourth instar has been obtained. I am looking for solutions. (M. Portilla, R. Luttrell)

Snodgrass Research Program

**Insecticide resistance to acephate (Orthene) and pyrethroids (permethrin) in tarnished plant bug populations in 19 locations in the Delta of AR, LA, and MS.** The average LC50 value for the test populations with acephate decreased from 11.77 ug/vial found in 2012 to 7.86 ug/vial in 2013. Six of the populations were highly resistance (RR50>3.0), while three populations were tolerant (RR50 2.5-3.0). In 2012, 14 of 26 locations had populations with RR50>3.0. The mean mortality obtained in testing populations for pyrethroid resistance with a discriminating-dose bioassay in 2012 was 65.1% for the 21 populations tested (<70% mortality is highly resistant). The mean mortality found for the 19 populations tested in 2013 was 53.6%. These results showed that resistance to pyrethroids (a recessive trait) can vary from location to location and year to year. Orthene resistance declined in 2013, and this was probably caused by increased use of some new insecticides.

**Changes in resistance levels to the neonicotinoid insecticide thiomethoxam (Centric) in field populations of tarnished plant bug in the Midsouth.** Using a bioassay developed by SIMRU in 2005, tarnished plant bug populations from mostly the same 20-25 locations in the Delta of AR, LA, and MS have been tested for resistance to thiamethoxam since 2007. Prior to 2012, little change in resistance was detected. In 2011, the average LC50 value for 25 locations was 1.07 ug/vial. In 2012, the average LC50 was 2.00 ug/vial. This was an almost 100% increase in the average LC50 value. However, in 2013 the average LC50 declined to 1.49 ug/vial which is very close to the LC50 value (1.50 ug/vial) for susceptible plant bugs from Crossett, AR. The highest RR50 value was only 1.45 ug/vial.
Examination gene regulation in tarnished plant bug using microarray: Tarnished plant bugs collected from cotton fields in Mississippi and Arkansas were treated with imidacloprid, dicrotophos, permethrin, thiometoxam, cyfluthrin, and oxamyl at 10-29× higher concentration and 4× higher spray volume than those used for LC50. RNA was extracted from these samples and a lab colony as a control. ds-cDNA was synthesized, and was used as probe after labeling to hybridize with tarnished plant bug cDNA gene chip containing 7446 genes. Microarray data were analyzed using ArrayStar.

Determination of Esterase and p450 Enzyme Activity for 2012 Tarnished Plant Bug Collection: Cropscape was used to select areas of interest based on the following criteria within the last five years: 1) grew only cotton in the area; 2) grew only soybean, rice or corn; 3) rotated the crops between cotton and soybean, rice or corn. Fifteen sites were selected and visited once a month between the months of May 2012 and Sept 2012. If the bug population allowed, collections were made in order to perform bioassay tests with selected insecticides (Bidrin and Centric). An appropriate number of TPB’s were also frozen at -80°C. The frozen samples are being tested for esterase and P450 activity in order to correlate bioassay results with enzyme assay results.

Molecular cloning of full cDNA sequences of cytochrome P450 from tarnished plant bug. Partial cDNA sequences were obtained from cDNA library sequencing. There sequences were assembled into at least eleven different P450s. To examine and compare any structural difference of the P450s between insecticide-resistant and -susceptible strains, reverse transcription and 5’/3’-RACE (rapid amplification of cDNA end) were applied to clone full P450 cDNA sequences. Currently, at least seven of the P450s have full coding region, and the other four may be only a little shorter.

Characterization of resistance mechanisms in a laboratory colony of TPB. Field populations of TPB were collected from Delta regions and selected a few times each year with acephate at 160 mg/L. This colony could maintain 2.7-fold resistance to acephate in laboratory. Synergist tests indicated that both esterase inhibitor TPP and oxidase inhibitor PBO could synergize toxicity of selected organophosphate, pyrethroid, and neonicotinoid insecticides. Results suggested that TPB populations originally collected from fields in Delta may developed cross or multiple resistance to different insecticides.

Conducted microarray analyses of gene expressions among a Bt-resistant, -susceptible strains of fall armyworm.

Designed and optimized dose response bioassay methods for evaluating acute and sub-lethal toxicity of commonly used pesticides to workers of honey bees. Substantial improvements have been made in bee handling and natural mortality reduction.
2014 Research Plans

Adams Research Plans

Participate in the 2014 NSCG Sweetpotato variety trials. (L. C. Adams and C. Johnson)

Monitor and report populations of *H. zea* and *H. virescens* in the Mississippi Delta through pheromone trapping. (L. C. Adams and C. Johnson)

Continue collaborative research with Alcorn State University scientists studying sweetpotato insect identification, sampling and damage in the Mississippi Delta. (L. C. Adams, R. G. Luttrell and Tahir Rashid)

Extend on-farm sweetpotato research with several small growers in the Mississippi Delta. Organize an informal spring meeting with interested growers to present recent USDA-ARS, SIMRU sweetpotato research and objectives. Identify areas that USDA-ARS, SIMRU could serve in a role to help these small farmers in their production efforts. (L. C. Adams, R. G. Luttrell and C. Johnson)

Allen Research Plans

The spatial and temporal distribution of various stink bug and lepidopteran species in the MS Delta will continue to be examined. (C. Allen, L. Andrews, N. Little)

Temporal and spatial dynamics of the tarnished plant bug in soybean across the MS Delta will be examined. (C. Allen, L. Andrews, K. Parys)

The species composition of bollworm and tobacco budworms in early and late planted soybeans in the MS Delta will be examined. (C. Allen, L. Andrews, D. Adams)

An evaluation of sprayed and unsprayed plots for lepidopteran pests in early and late planted soybean and the subsequent impact on yield within production fields will be conducted. (C. Allen, D. Adams, L. Andrews, R. Luttrell)

An examination of cold-tolerances of lepidopteran and hemipteran insect pests in the MS Delta and implications to overwintering survival will be examined. (C. Allen, L. Andrews, K. Parys)

Little Research Plans

Bt toxins are widely utilized in transgenic cotton and corn to control larval pests. Not only do these toxins cause larval mortality, but larvae surviving on these toxins exhibit delayed development. The widespread adoption of multiple Bt crops throughout the Mississippi Delta may reduce the number of generations of bollworms that feed on cotton. Therefore, pyramided-gene Bt corn will be evaluated in field cages for its ability to slow the development of bollworms. (N. Little, D. Adams)
Pyramided-gene Bt cotton varieties will be grown in sentinel plots throughout the MS Delta to evaluate their efficacy for bollworm control. Biological agents, conventional insecticides, and non-treated plots of the same variety will be used to compare economic feasibility of bollworm control. Bt varieties and treatments will also be compared to non-Bt varieties at these sites. (N. Little, D. Adams, C. Allen, and R. Luttrell)

Increased public concerns about lapses in the control of the cotton bollworm and the efficacy of transgenic cotton expressing multiple toxins from the bacterium Bt have prompted studies on resistance. The primary means of quantifying insect resistance to Bt crops, other than genetic profiling, is through the use of bioassays with meridic diet, which can be amended with multiple configurations of Bt proteins. This study will quantify crystalline Bt toxins present in diets amended with lyophilized cotton tissue and the extent of their decay over time in bioassays. (N. Little, O.P. Perera)

**Luttrell Research Plans**

In 2014, a bioassay laboratory will be established in SIMRU to routinely conduct diet incorporation, diet overlay and topic bioassays of noctuid larvae exposed to biological toxins, microbial and chemical insecticides. The purpose of the laboratory will be to provide quantitative assays for resistance monitoring, collaboration of field efficacy measurements and investigation of new biocides. (R. Luttrell, K. Dixon, M. Mullen, N. Little).

**Parys Research Plans**

Evaluate relationships between insecticide resistance and landscape using NASS’s Cropdata Layers in GIS. (K. Parys, K. Renken)

Continuing screening tarnished plant bug populations for baseline levels of resistance to novel chemistries (K. Parys, C. Allen, C. Roberts)

Continuing collecting insects using flight intercept traps and pitfall traps for baseline community data in cotton and soybean fields (K. Parys, G. Snodgrass, C. Roberts)

**Perera Research Plans**

Polymorphic DNA markers will be used to develop genetic maps of corn earworm using backcross families. Bt toxin tolerant corn earworm strains will be used to conduct marker-based association studies to identify loci associated with Bt toxin tolerance. In addition, insects collected from field locations will be analyzed to determine the species identity. (O. Perera, C. Allen, N. Little).
**Portilla Research Plans**

Laboratory studies will be continued in order to obtain life tables and growth rates estimations for TPB using small cotton plants.

Possible new strain of *Aspergillus flavus* will be isolated from *L. lineolaris* and will be bioassayed to evaluate possible pathogenetic action on TPB adults. Bioassays will be conducted to compare the virulence with four isolates of *A. flavus*. LC\(_{50}\)s and LD\(_{50}\)s will be estimated.

LC\(_{50}\) values estimated for *B. bassiana* against TPB at low and high temperatures under laboratory conditions will be used in the field. Plots planted with preferred weedy hosts of TPB will be treated during 2014.

Several field colonies of TPB form specific location (resistant to insecticides) will be established for further research.

The insect rearing unit will continue its production in order to provide material for researchers and for the Future Scientist Program.

**Zhu Research Plans**

Examination of acute toxicity, synergistic/antagonistic interactions, and sublethal impact of commonly used pesticides on honey bees using bioassay, biochemical, and molecular approaches (Zhu and collaborators, ARS Stoneville, MS)
2013 Trust Fund or Reimbursable Cooperative Agreements

**Project Title:** Screening the USDA Gemplasm Collection For Resistance To The Redbanded Stink Bug (Piezodorus Guildinii) and Its Associated Pathogenic yeast

**Agreement No.:** 58-6402-3-034R  
**ARS Investigator:** Clint Allen  
**Project Funded By:** Texas A&M Univ.  
**Project Investigator:** Bert White  
**Project State Date:** 01/01/2013  
**Project End Date:** 12/31/2013

**2013 Accomplishments:**

A rearing protocol for redbanded stink bug, *Piezodorus guildinii*, under controlled conditions was developed by the Texas A&M Commerce group and these insects were used to screen soybean seed from the USDA soybean germplasm collection. During the past two years, 2505 soybean lines were screened for resistance to redbanded stink bug adult feeding in laboratory tests, but the development of a field-tolerant genotype has fallen short of expectations and needs further study.

Redbanded stink bugs were collected from Mississippi and other areas of the mid-South and sent to Steve Clough at the University of Illinois and these stink bugs were examined for the presence of detrimental microbes and other pathogenic yeasts. Microbes found in the stink bugs included nine bacterial genus (possibly 15 different species) and four different fungal genus. Many of these are known to be associated with plants or insects, but none are known to be soybean pathogens.
2013 Specific Cooperative Agreement Research Accomplishments

Project Title: Low input systems of pest control for sweetpotato in the Mississippi Delta

Alcorn State University
Agreement No.: 58-6402-1-614
Project Investigator: Tahir Rashid
ARS Investigator: R. G. Luttrell
Project State Date: 8/1/2011
Project End Date: 8/31/2014

2013 Accomplishments:

Effect of Prohexadione-Ca on yield and insect damage to sweetpotatoes
The experiment was repeated during 2013 to study the effect of Pro-Ca on 4 sweetpotato cultivars, ‘Beauregard’, ‘Covington’, ‘O’Henry’, and ‘Puerto Rican’. Research plots were planted at ASU Extension/Research Demonstration Farm and Technology Transfer Center, Mound Bayou, MS. Each plot consisted of 2 rows of raised beds with 4 replications. Each row contained 10 plants with 1.5 ft between plants within the row and 3.5 ft between rows. Pro-Ca treatments were applied with a 5 gal backpack sprayer at 810 mg L⁻¹ (140 g a.i. ha⁻¹) mixed with 1 mL L⁻¹ of crop oil concentrate and 1mL L⁻¹ urea ammonium nitrate. Control plots were sprayed with water mixed with 1 mL L⁻¹ of crop oil concentrate and 1mL L⁻¹ urea ammonium nitrate. Treatments were applied twice during the season. All treatment plots were harvested. Random sweetpotato samples were evaluated for insect damage. Overall insect damage to the roots differed significantly among different sweetpotato cultivars. Individual white grub (Phyllophaga spp) damage and multiple insect-damage also significantly differed among different cultivars. ‘O’Henry’ had the least grub damage. Spotted cucumber beetle (Diabrotica undecimpunctata) damage did not differ among different varieties. Wireworm larval feeding damage was significantly higher in non-treated plots across all sweetpotato varieties. Yield differences are being evaluated. Further experiments will determine if selection of a resistant sweetpotato variety and manipulation of the vegetative growth of sweetpotato plant can reduce the insect feeding damage.

Monitoring of sweetpotato weevil in southern Mississippi
Sweetpotato weevil pheromone traps were installed near sweetpotato research plots in ASU main campus (Claiborne County, MS). Each of 4 traps was placed 50 ft apart from adjacent trap. The trap was fitted on a 4-ft cane just above the plant canopy and loaded with 120 μg of sweetpotato weevil lure. Traps were checked every 2 weeks and the pheromone was replaced every 4 weeks. No weevil was detected in the traps during the entire season until harvest.

2014 Research Plans:

Soil traps containing corn bait will be installed in commercial and research sweetpotato plots to collect wireworm larvae. Each treatment bait will be dispensed in a 10 cm diameter and 20 cm deep hole. The hole will be filled with soil and covered with plastic. The sampling experimental area will be divided into 4 blocks each with 10 rows X 10 m. Each block will have 4 sampling points, each point approximately 5 m apart. The samples will be collected weekly by digging each trap site and brought back to the laboratory for counting and identification.
Identified larvae of wireworms (*Conoderus vespertinus*) will be used in toxicity bioassays to test efficacy of different percentages of treatment concentrations of biological compounds (imported fire ant (*Solenopsis invicta*) alkaloids and extract of *Monormorium minimum*).
Project Title: Bt risk assessment for lepidopterous pests of cotton

Mississippi State University

Agreement No.: 58-6402-8-313

Project State Date: 7/1/2008

Project End Date: 7/1/2013

Project Investigator: Jeffery Gore

ARS Investigator: Randall Luttrell

2013 Accomplishments:

Bollworm larvae were collected from Non-Bt and VT3P field corn from 2011 and 2013. Pupal duration and pupal weights were determined for the parental generation. Backcrosses and reciprocal crosses were made and the offspring neonates were subjected to dose mortality bioassays on lyophilized Bollgard II cotton tissue. Male bollworm larvae collected from VT3P field corn had a longer pupal duration compared to males collected from Non-Bt field corn. Female pupal duration was not significantly different for individuals collected from non-Bt field corn and VT3P field corn. Populations collected from VT3P field corn had higher pupal weights than larvae collected from Non-Bt field corn. In 2011, progeny from females reared on VT3P field corn had a higher LC50 compared to progeny resulting from females reared on Non-Bt field corn regardless of paternal host. In 2012, progeny from all reciprocal crosses and the backcross larvae of non-Bt had similar LC50 values. The progeny from the VT3P backcross had a higher LC50 value than all other crosses. Based on these results, bollworms collected from VT3P field corn are healthier and more robust than those collected on non-Bt field corn. These results suggest that there are multiple minor genes that influence bollworm survival on Bollgard II cotton. Those genes may be associated with resistance traits, but are most likely influencing overall fitness of larvae that develop on VT3P field corn. Results of these experiments will be important for developing resistance management plans in areas where dual-Bt toxin corn hybrids and dual-Bt toxin cottons are grown in close proximity. (B. Von Kanel, J. Gore, D. Cook, R. Jackson, A. Catchot, and F. Musser: Agreement No. 58-6402-8-313).

An experiment was conducted from 2010 to 2013 to evaluate the impact of insecticide applications on dual toxin Bt cottons. Non-Bt, Widestrike, and Bollgard II cotton varieties were planted in a split-plot design with 4 replications. Each variety had a sprayed and unsprayed treatment. The sprayed plots were treated, as needed, with Prevathon, Belt, or Tracer to control lepidopteran insect pests. Overall, lepidopteran densities varied by year. Moderate populations were observed in 2010 and 2011. In contrast populations were very high in 2012 and very low in 2013. Overall, insecticide applications significantly reduced the numbers of damaged squares and bolls on non-Bt, Bollgard II and Widestripe cottons. In years where bollworm populations were moderate to high, significant yield improvement was observed from foliar applications. In contrast, no differences in yield were observed when populations were low. These results demonstrate that dual-gene Bt cottons are not immune to injury from bollworm. As a result, management of bollworms in dual-gene Bt cottons with foliar insecticides may be an important component for resistance management (J. Gore and D. Cook: Agreement No. 58-6402-8-313).

An experiment was conducted in 2012 to determine the impact of sequential low rate applications of Prevathon on bollworm injury and yield in Bollgard II cotton. The rates of Prevathon used included 1.5, 3.0, and 6.0 fl oz/A. These treatments were co-applied with a tarnished plant bug application when tarnished plant bugs reached the current economic
threshold of 3 per 5 row ft. Each rate was sprayed with either every tarnished plant bug application or every other application and compared to Prevathon and Belt sprayed at their normal use rates (27 fl oz/A for Prevathon and 3 fl oz/A for Belt). A total of six applications were made for tarnished plant bugs in this trial. Therefore, treatments 1-3 were sprayed six times and treatments 4-6 were sprayed three times. Treatments 7 and 8 were sprayed one time when bollworm densities reached threshold. All of the insecticide treatments reduced square and boll injury from bollworm in Bollgard II cotton compared to the unsprayed plots. Applications of 3.0 fl oz/A of Prevathon sprayed six times and 6.0 fl oz/A Prevathon sprayed three and six times provided similar levels of control to that observed with Prevathon sprayed at threshold with 27 fl oz/A. All insecticide treatments resulted in higher yields than untreated Bollgard II. These results suggest that low rates of Prevathon can be sprayed sequentially beginning prior to flowering and reduce the impact of bollworms in Bollgard II cotton. This approach can reduce input costs for growers and may serve as an effective resistance management tool in dual-gene cottons. Lower rates of 3-6 fl. oz/A applied throughout the season may result in a third toxin being present in dual-gene cottons throughout the season and further reduce the selection pressure on the Bt genes. These preliminary results will need to be repeated and refined before specific recommendations can be made (J. Gore, A. Adams, and D. Cook: Agreement No. 58-6402-8-313).

A colony of bollworms was collected from Bollgard II cotton and given to SIMRU for further testing (J. Gore and D. Cook: Agreement No. 58-6402-8-313).

Insecticide resistance in all insect pests of cotton threatens the economic viability of dual gene Bt cottons. Increased costs of foliar insecticides associated with resistant insects compounded with technology fees of dual-gene cottons results in a significant economic cost for growers. Surveys of cotton aphid susceptibility to neonicotinoid insecticides were conducted from 2008 to 2013. Cotton aphids were collected from commercial cotton fields across Mississippi, Louisiana, Arkansas, and Tennessee. Additional populations were tested from Texas and North Carolina. Aphids were collected from fields where control failures with foliar neonicotinoids were reported. Additional collections were made from cotton fields in these states where no foliar applications of neonicotinoid insecticides had been made. Leaf dip bioassays were used to measure their susceptibility to neonicotinoids. Cotton aphids collected from fields where control failures had occurred showed significant levels of resistance each year. Mean LC50 values averaged across all collections increased from 12.75 ppm in 2010 to 60.06 in 2011 and 27.0 in 2012. All of the populations tested in 2012 showed moderate to high levels of resistance to thiamethoxam regardless collection site (sprayed or unsprayed field) (J. Gore and D. Cook: Agreement No. 58-6402-8-313).

2014 Research Plans:

Project ended in 2013
Project Title: Bt risk assessment for Lepidopterous pests of cotton

Mississippi State University  
Agreement No.: 58-6402-3-017  
Project State Date: 3/01/2013  
Project End Date: 03/01/2014  
Project Investigator: Fred Musser  
ARS Investigator: Clint Allen

2013 Accomplishments:

Cotton has traditionally been the economic driver of agriculture in Mississippi and throughout much of the southern U.S. However, over time, the southern agricultural landscape has become more diverse, and cotton has become one crop option among many for most farmers. During the last several years corn has become a more abundant crop and cotton acreage has decreased in the southern states as a result of high commodity prices for corn. Corn and cotton share some pests, in particular Helicoverpa zea (corn earworm or bollworm), and the Bt toxins used in both crops are similar. Therefore, the development of Bt resistance is likely to occur based on overall exposure in the landscape rather than due to selection in a single crop. To address the overall risk of Bt resistance, the research in this project evaluated the impact of various components within the current southern agricultural landscape.

Bollworm Field Research.

Helicoverpa zea, bollworm, pyrethroid insecticide resistance monitoring was conducted during every year of this project. Resistance levels changed little from 2008 to 2012. Louisiana and Virginia were the states with the highest survival to 5 µg cypermethrin per vial. Survival during July tended to be greater than during other months of the year in all states. Average survival in Louisiana and Virginia during July averaged 43.1 and 34.1%, respectively, over the 5 years of this grant. All other states had less than 20% survival during July. Consistent high levels of survival in these two distant locations with consistent lower survival in between these locations suggest that the assays are measuring local selection rather than regional populations, or that H. zea do not move as much as previously estimated.

In a related study, the larval host plant type was identified for the moths monitored for pyrethroid resistance using carbon isotope analysis. During July, when survival to pyrethroids was highest, nearly all moths originated on C4 hosts, presumably mostly corn. The majority of moths originated from C4 hosts during most moths from all states, suggesting that grasses are the primary hosts for H. zea throughout the year. There were no differences in the proportion of hosts originating from C4 host for bollworms surviving versus those dying from an exposure to 5 µg cypermethrin per vial.

A graduate student (S. R. Vemula) completed his Ph.D. in 2009 evaluating the degree of long-distance movement of bollworm compared to local movement. He concluded that local movement was the dominant factor within a season, but long-distance movement was important between seasons.

Another graduate student (A. Babu) conducted several studies to examine the role of mixed plantings of Bt and non-Bt corn the risk of Bt resistance development in bollworm. He conducted a survey of corn fields after harvest to assess the density and maturity of volunteer corn in the fall as well as the abundance of corn earworm in non-Bt corn at various growth stages. He has
also conducted greenhouse and field trials examining the efficacy of various crosses of Bt and non-Bt corn varieties on bollworm under stressed and non-stressed environments. Findings suggest that corn seed blends will increase the risk of resistance development in *H. zea* due to Bt pollen contamination on refuge ears in the seed blend. Volunteer corn emerging after harvest can become infested with *H. zea*, but appears to contribute little to overwintering populations in central to northern MS because there are not enough heat units available for *H. zea* to complete larval development on volunteer plants. However, corn production south of this region has a long enough frost-free growing period that it could contribute to overwintering populations.

A third graduate student (B. Von Kanel) is evaluating the change in development on cotton of bollworms whose parents developed on Bt corn varieties compared to those whose parents developed on non-Bt corn varieties. He is also conducting bioassays of various crosses of these bollworm populations to try to determine the genetic basis of differences in performance. The two years of data collected so far differed in the impact of exposure to Bt corn on survival of *H. zea* on Bt cotton. Further research is planned on a continuation of this project.

**Bollworm Resistance Models**

In cooperation with EPA, models have been developed to address changes in Bt crops being planted, new Bt events being developed and changes in Bt-crop refuge requirements being implemented. In particular, models have evaluated the impact of reduced refuges and seed blends for multi-gene corn on the risk of various insects developing resistance to the Bt toxins. Multiple toxins are always superior to single toxins. However, the ideal size and configuration of the refuge depends on the behavior and movement of the insect as well as the toxicity of the events being modeled. For *H. zea*, which is quite mobile and for which the Bt toxins are not a high dose, the reduced refuge percentage coupled with the refuge being planted as a seed blend rather than a structured refuge reduces the benefits of the refuge. However, for other pests, the reduced refuge brings added benefits to growers without any practical change in the risk of resistance. The seed blend refuge is generally inferior to a properly implemented structured refuge, but superior to the poorly implemented refuges commonly observed.

A second focus for resistance modeling was to make the user interface more accessible so that researchers could input parameters of importance to them and evaluate the impact of these parameters on resistance development. This entailed working with a subcontractor to move the population genetics program from a Java platform to a Windows platform. The new application reduces the need for re-compiling and makes the program available to a broader PC community without the need for increased support or maintenance. The work is nearing completion and will be ready for beta testing soon. When the interface is complete, the model will be accessible to people without knowledge of computer programming and they will be able to adjust the parameters for the insect or technology of interest without compromising the integrity of the underlying program.

**Tarnished Plant Bug Research**

One of the benefits of Bt crops is reduced insecticide usage. However, the emergence of tarnished plant bug (TPB) as a major cotton pest in the mid-south has reduced this benefit in
cotton. Therefore we have initiated some work with TPB to improve our understanding of TPB ecology and physiology. We have examined the prevalence and impact of microsporidia. Further, we have compared development of TPB populations from different regions within MS on cotton and other crop hosts. We are also examining how TPB alter expression of several polygalacturonases depending on host plant, which could be very important for this highly polyphagous pest. This work will be completed under a continuation of this project.

**2014 Research Plans:**

Project ended in 2013
Project Title: Transgenic crop efficacy against target pests in agronomic crops

Mississippi State University
Project State Date: 08/01/2013
Project Investigator: Jeff Gore

Agreement No.: 58-6402-3-039
Project End Date: 08/01/2014
ARS Investigator: Randall Luttrell

Project Investigator: Randall Luttrell

2013 Accomplishments:

Project initiated too late in the year to collect any data during 2013

2014 Research Plans:

Assess the ongoing value of Bt crops in controlling important pests by estimating mortality of important pests from Bt transgenes being deployed in the major agronomic crops of Mississippi, and facilitate more extensive bioassays for population with unusual survival rates.
**Project Title:** Transgenic crop efficacy against target pests in agronomic crops

**Mississippi State University**
**Project State Date:** 08/01/2013
**Project Investigator:** Fred Musser

**Agreement No.:** 58-6402-3-038

**Project End Date:** 08/01/2014
**ARS Investigator:** Clint Allen

**Project Investigator:** Fred Musser

**Project State Date:** 08/01/2013

**Project End Date:** 08/01/2014

**ARS Investigator:** Clint Allen

**2013 Accomplishments:**

Project initiated too late in the year to collect any data during 2013

**2014 Research Plans:**

Assess the ongoing value of Bt crops in controlling important pests by estimating mortality of important pests from Bt transgenes being deployed in the major agronomic crops of Mississippi, and facilitate more extensive bioassays for population with unusual survival rates.
Project Title: Assembly and Annotation of *Lygus Lineolaris* (Tarnished Plant Bug) Genome

Cornell University
Project State Date: 6/1/2012
Project Investigator: Lukas Mueller

Agreement No.: 58-6402-2-719
Project End Date: 12/31/2014
ARS Investigator: OP Perera

2013 Accomplishments:

There was no activity during 2013. See the 2012 SIMRU Annual Report for the last accomplishment on this project.

2014 Research Plans:

This project will expire in December 2014; no research is plan for this project in 2014.
2013 Publications and Presentations

Publications


Presentations


Appendix A

SIMRU’s 2013 Pathway Interns/LA Appointments Employees

Ari Esters  Bailey Tubertini  Cavisha Roberson  Chris Morris  Danice Dishmon

Darshanisha Warren  Dquan Wilson  Jana Slay  John Coleman  Julian Beamon

Julian Henry  June Jones  Kristen Deangelo  Laura Sipes  Maria Benavides

Nakeshia Anderson  Priva Chatakondi  Russell Godbold  Severino Signa  Thomas Sherman