HATCH PROJECT
Alabama Agricultural Experiment Station
Auburn University

TITLE OF PROPOSED PROJECT: Development and Implementation of Alternative Pest Management Strategies for Emerging Crop Pests in Alabama

PRINCIPAL INVESTIGATOR:

Department:

NON TECHNICAL SUMMARY

This project focuses on the management of key emerging pests of crops in Alabama, specifically pests of fruit and vegetable crops and soybean. Fruit and vegetable crops constitute an important group of horticultural crops in the U.S. with an annual market value of approximately $23 billion. Soybean is also an important crop in the U.S with an annual market value of about $42 billion. Several arthropod pests attack these crops in Alabama with the potential to cause significant economic losses to producers. The goal of this project is to develop and implement ecologically based and cost-effective integrated pest management (IPM) practices for major and emerging pests of peaches, cucurbits, crucifers and soybean. Specific objectives are to: 1) Evaluate and implement IPM tactics for key insect pests of peaches with special focus on plum curculio, plant bugs and scale insects, 2) Develop management strategies for key insect pests of cucurbits with special focus on squash bug, 3) Develop and implement organically-acceptable IPM program for key pests of crucifer crops with special focus on yellowmargined leaf beetle, *Microtheca ochroloma*, and 4) Develop alternative strategies for managing kudzu bug, *Megacopta cribraria* in soybeans. This project addresses the goals of the USDA-NIFA and the National IPM Roadmap, and will identify low-input IPM tactics that will reduce pesticide use in crop production, reduce human health risks, and minimize adverse environmental effects of use of toxic conventional insecticides.

LITERATURE REVIEW

Fruit and vegetable crops constitute an important group of horticultural crops in the United States (US) with an annual market value of approximately $23 billion (USDA, NASS, 2002). These crops are also included in the Specialty Crop Competitiveness Act of 2004 signed by President Bush on December 21, 2004. In Alabama, commercial fruit and vegetable production has a combined annual value of over $61 million (USDA, NASS, 2002). According to the 2002 USDA-NASS census publication, Alabama ranked 9th and 11th among the 50 states in the production of vegetables and fruits, respectively. The top fruit and vegetable crops in the state include peaches, pecans, tomatoes, Satsuma citrus, cucurbits, crucifers, blueberries, and strawberries.

In Alabama, as in most other parts of the US, fruit and vegetable crops are attacked by numerous species of insect and mite pests. Intense pressure from pests can severely reduce yield and profitability. Fruits and vegetables are high value crops which must meet high quality production standards, including being free from insect damage. Traditionally, conventional growers have achieved pest control in their farms through applications of broadspectrum pesticides. In many cases, repeated use of pesticides has resulted in development of pest resistance, as well as concerns over food safety, human health, and the environment. The passage of the Food Quality Protection Act in 1996 (FQPA, 1996), which has resulted in cancellations or restrictions (by the US Environmental Protection Agency) of many major pesticides, has had a negative impact on fruit production and pest management. The removal or restriction of key conventional insecticides, which historically have been used by specialty crop growers to control their major pests, has created a dire need for alternative management tactics for the major pests. In addition, removal of key conventional insecticides has also created serious concerns regarding emergence of new pests and increasing status of some minor pests in specialty crop production. These issues heighten the need to develop effective and ecologically based integrated pest management strategies (IPM) for pests of specialty crops.

The goal of this Hatch project is to develop and implement alternative pest management strategies for emerging crop pests in Alabama. This Hatch project will specifically focus on fruit (peaches), vegetable (cucurbits and crucifers), and field crops (soybeans). These crops have been selected based on various reasons including local needs and opportunities, and economic importance in Alabama. The project addresses the goals of USDA-NIFA-AFRI and is also relevant to the goals of the National IPM Roadmap by improving cost benefit analyses of adopting IPM practices in crop production. Furthermore, the research will identify low-input IPM tactics that will reduce pesticide use in fruit production, reduce human health risks, and minimize adverse environmental effects of use of toxic conventional insecticides. Thus, the project is potentially fundable by several USDA grant programs (e.g., AFRI, Specialty Crops Grants, Organic Research & Extension Initiative), and similar funding agencies such as Southern Region IPM and SARE (Sustainable Agriculture Research & Education).

Peaches

Peach production is a major industry in Alabama and many other southeastern states: over 40,000 acres of fresh and processed peaches worth $65 million are produced annually in Alabama, Georgia, and South Carolina (USDA, NASS, 2002). In Alabama alone, approximately 22 million pounds of peaches were produced in 2001 with a market value of about $10 million (Alabama Agricultural Statistics Service, 2005). The plum curculio (*Conotrachelus nenuphar*) (Coleoptera: Curculionidae) is the most serious economic pest of peaches in Alabama and other parts of the Deep South. Being a high value crop, there is zero tolerance for insect damage to peaches. As a result peach growers typically maintain a prophylactic spray schedule to produce unblemished fruit for the fresh market. For instance, Alabama peach growers utilize a calendar spray program involving applications of 5-16 insecticide sprays per season to manage plum curculio and other pests. This extensive use of insecticides has resulted in many drawbacks including pest resistance and environmental pollution. Furthermore, some of the conventional insecticides have been banned or restricted (FQPA, 1996), resulting in a dire need for alternative pest management tactics.

Despite its economic importance, relatively little research has been conducted on the ecology and behavior of plum curculio. Plum curculio pressure can be intense, but existing control measures are quite variable from region to region. Since 2005, the PI has been working on the ecology and management of this pest in Alabama peaches. These studies have quantified the seasonal phenology of the pest in Alabama and identified potential pest management tactics. In one study, we evaluated the effectiveness of two widely used trap types (pyramid versus Circle traps) and commercially available synthetic lures for monitoring the pest in two peach orchards in Alabama during 2008 and 2009. The results showed that pyramid traps baited with a mixed component lure consisting of benzaldehyde (fruit volatile) and grandisoic acid (aggregation pheromone of plum curculio) was the best available monitoring system for the pest (Akotsen-Mensah et al., 2010). Another study showed that three properly timed sprays of insecticides applied during the peaks of the phenology of plum curculio could provide effective control of the pest similar to the control provided by the conventional calendar-based insecticide application approach in which 6-12 sprays are applied per season (Akotsen-Mensah et al., 2011a). In yet another study, we used trap capture data of plum curculio in unbaited pyramid traps recorded annually from 2000 to 2009 in an unmanaged peach orchard in central Alabama to determine its seasonal phenology and develop predictive degree-day models for the pest. The results showed that the biofix (date at which degree-day accumulation begins) and lower temperature threshold for the best-fit model were 1 January and 10°C, respectively. The models predicted the first and peak trap captures of plum curculio to occur at mean cumulative degree-days of 105.8 ± 10 and 225.5 ± 16, respectively (Akotsen-Mensah et al., 2011b). After more than a decade-long research effort, we have realized that it is practically impossible to obtain one hundred percent pest control to meet the market standard of zero tolerance for insect damage to peaches. Therefore, in this Hatch project we will develop outside the box concepts using cutting edge post-harvest technology that would enable growers to meet consumer expectation for blemish-free peaches. We will also focus on the ecology and management of stink bugs (Pentatomidae) and leaffooted bugs (Coreidae), which are emerging pests of fruit crops in the southern US. These sucking bugs have now become important pests of orchards partly because of changes in pesticide use. We will also focus on the San Jose scales, *Quadraspidiotus perniciosus* (Comstock), which is an emerging pest of fruit crops, in the southern US. Several factors may have contributed to recent outbreaks of San Jose scales in the region including advances in IPM programs; especially programs that utilized fewer insecticide sprays, failure to apply dormant oils, and a string of warm falls and mild winters in recent years. We will compare effectiveness of different application of various reduced risk insecticides (before blooming and summer) against San Jose scales in peaches.

Cucurbits

Cucurbit crops, in particular squash, cucumbers, and pumpkins, are high value crops in Alabama. Curcubit production has increased significantly in recent years to meet the growing demand by consumers. The squash bug, *Anasa tristis* (De Geer), has consistently been identified by growers as the most destructive insect pest of cucurbits in the region. Both adults and nymphs (immatures) feed on leaves, fruits, and vines by sucking the sap and causing severe yield losses. The squash bug overwinters as adult in the soil or ground litter. When temperatures reach above 70° F, overwintered adults emerge, immediately seek out cucurbit host plant on which they feed, mate, and deposit egg masses on the underside of leaves. Feeding on leaves first appears as yellow spots which later turn black. Feeding on fruits can cause scars, sunken spots, and early rot render the fruit unmarketable. The feeding damage also cause fruit rot during storage. Under severe infestation, the entire plant may wilt above the point of feeding, and die in a condition known as "Anasa wilt". More importantly, squash bugs are vectors of a newly recognized disease of cucurbit crops - cucurbit yellow vine disease (CYVD) which is caused by phloem colonizing bacterium, *Serratia marcescens* Bizio. Melons, watermelon, and pumpkins are susceptible to this disease and results in complete crop failure. Adult squash bugs harbor the bacterium while going into diapause and can infect new plants the following spring.
Cucurbit growers in the region are heavily dependent on broad-spectrum synthetic insecticides to control squash bugs but are still very difficult to manage as they are prone to develop resistance to insecticides and adults have unusually hardy exoskeleton. In addition, broad-spectrum insecticides that are effective against squash bugs are now being regulated, in part because such insecticides kill not only the target pest, but also many other non-target insects, including pollinators and biocontrol agents. As cucurbits rely entirely on pollinators for fruit set, the negative influence on pollinator's population would drastically impact cucurbit crop yield.

A major goal of this Hatch project is to develop ecologically sound alternative management tactics against squash bugs in Alabama. Specifically, we will evaluate various host plants of squash bugs with the aim of identifying most attractive plant species which can be used as perimeter trap crop to manage early migrating, overwintering population of squash bugs. The management tactics developed in this project will be applicable to both conventional and organic cucurbit production systems.

Crucifers

Crucifer crops are important vegetable crops in Alabama. For instance, annual production of cabbage and collards in Alabama is estimated at 3,500 acres, with a value of about $3 million (Williams and Dangler, 1992). Organic production of crucifer vegetable crops is also on the rise in Alabama. However, pest management is a major factor limiting the expansion of the organic vegetables in the state. Cruciferous crops are attacked by a wide variety of insect pests in Alabama including diamondback moth (*Plutella xylostella*), cabbage looper (*Trichoplusia ni*), imported cabbageworm (*Pieris rapae*), armyworms (*Spodoptera* spp.), cutworms (*Agrotis* spp.), and flea beetles (*Phyllotreta* spp.). However, the yellowmargined leaf beetle (*Microtheca ochroloma*) (Coleoptera: Chrysomelidae), is arguably the most devastating insect pest of organic crucifers in the state and other parts of the southern region. Organic vegetable growers in the state have repeatedly ranked this insect as the most damaging and difficult to control. The PI has been collaborating with vegetable growers to develop organic research projects that address their critical research needs. Growers were surveyed about their greatest obstacles to organic production, and pest and disease management were listed most often as issues of greatest concern. Through grants from Southern Region IPM Center and USDA-NIFA, the PI's lab has established research projects focusing on the development of organically-acceptable IPM tactics against organic crucifer vegetable pests in the state. These studies have identified and evaluated several potential IPM tactics in crucifer crops (Balusu and Fadamiro, 2011a, b, 2013). In one study, we evaluated effectiveness of turnip as trap crop for managing yellowmargined leaf beetle in organic crucifer production. The results showed that perimeter trap cropping with turnip attracted beetles away from the cabbage cash crop, and also resulted in significantly lower feeding damage on the cabbage crop compared to control plots without turnip trap crop (Balusu et al., 2015). Another study showed that volatile organic compounds released by turnips serve as attractants for the beetle. Synthetic blend of crucifer specific compounds from turnip was shown to be highly attractive to adult beetles in the field test.

This Hatch project will build upon the success of previous research work. Specifically, we will conduct on-farm integration of effective tactics identified in previous research to develop and implement cost-effective and environmentally sound alternative strategies for managing pests of organic crucifer crops in Alabama.

Soybeans

Soybeans rank second, after corn, among the most planted field crops in the U.S with an estimated annual market value of about $41.8 billion (USDA-NASS, 2013). Nationally, Alabama ranks 12th in soybean production with an annual value of over $1 billion (USDA- NASS, 2010). The soybean acreage in Alabama has expanded rapidly from 1.5 million acres in 2005 to 4.5 million acres in 2009 (Alabama Agricultural Statistics, 2011). Pest management strategies have been developed for many soybean insect pests such as caterpillars and stink bugs. However, the invasive kudzu bug has recently emerged as the top yield-limiting pest of soybean in the southeastern U.S.

Native to Asia, the kudzu bug, *Megacopta cribraria* (F.) (Heteroptera: Plataspidae), was first detected in the U.S. in Georgia in 2009. Its distribution since then has been rapidly expanding across the Southeast, and is currently established in 14 states including South

Carolina, Alabama, North Carolina, Florida, Tennessee, Mississippi, Virginia, Kentucky, Maryland, and Delaware. Both nymphs and adults aggregate in large numbers, and feed on tender stems or leaves resulting in significant yield loss. In addition to the severe crop loss caused to soybean, *M. cribraria* infestation also impacts international trade and commerce. For instance, the Honduran government has banned all agricultural imports from Alabama, Georgia, and South Carolina due to detection of dead *M. cribraria* adults in a container shipment. As the threat posed by this invasive insect species is rapidly increasing, no effective control strategies other than chemical insecticides are currently available to help soybean farmers manage this exotic pest in the region. These toxic chemical insecticides are harmful to beneficial insects that may help mitigate kudzu bug and other soybean pests. Moreover, no local natural enemies are available to keep this bug under control, and the effectiveness of the newly discovered parasitoid wasps (*Strongygaster triangulifera* and *Paratelenomous saccharalis*) remains unknown. As a consequence, farmers have continued to rely heavily on insecticide sprays to control kudzu bug. This increased use of chemical pesticides can result in the development of pest resistance.

The goal of this Hatch project is to protect the soybean industry from this rapidly invading pest by developing environmentally sustainable, alternative management tactics. Specifically, in this Hatch project I propose to develop and evaluate trap cropping tactics with highly attractive host plant identified in our preliminary studies, and develop pheromone-based lures to detect/monitor and manage kudzu bug in the Southern U.S. It is expected that this project will ultimately ensure continued expansion and profitability of the soybean industry in the South by developing environmentally sustainable tactics for managing kudzu bugs.

GOALS AND OBJECTIVES

In Alabama, as in most other parts of the US, fruit and vegetable crops are attacked by numerous species of insect and mite pests. Intense pressure from pests can severely reduce yield and profitability. Fruits and vegetables are high value crops which must meet high quality production standards, including being free from insect damage. Traditionally, conventional growers have achieved pest control in their farms through applications of broadspectrum pesticides. In many cases, repeated use of pesticides has resulted in development of pest resistance, as well as concerns over food safety, human health, and the environment. The passage of the Food Quality Protection Act in 1996 (FQPA, 1996), which has resulted in cancellations or restrictions (by the US Environmental Protection Agency) of many major pesticides, has had a negative impact on fruit production and pest management. The removal or restriction of key conventional insecticides, which historically have been used by specialty crop growers to control their major pests, has created a dire need for alternative management tactics for the major pests. In addition, removal of key conventional insecticides has also created serious concerns regarding emergence of new pests and increasing status of some minor pests in specialty crop production. These issues heighten the need to develop effective and ecologically based integrated pest management strategies (IPM) for crop pests. The goal of this Hatch project is to develop and implement alternative pest management strategies for emerging crop pests in Alabama. This Hatch project will specifically focus on fruit (peaches), vegetable (cucurbits and crucifers), and field crops (soybeans). These crops have been selected based on various reasons including local needs and opportunities, and economic importance in Alabama. The project addresses the goals of USDA-NIFA-AFRI and is also relevant to the goals of the National IPM Roadmap by improving cost benefit analyses of adopting IPM practices in crop production. Furthermore, the research will identify low-input IPM tactics that will reduce pesticide use in crop production, reduce human health risks, and minimize adverse environmental effects of use of toxic conventional insecticides. The specific objectives of this Hatch project are to: 1) Evaluate and implement IPM tactics for key insect pests of peaches with special focus on plum curculio, plant bugs and scale insects, 2) Develop management strategies for key insect pests of cucurbits with special focus on squash bug, 3) Develop and implement organically-acceptable IPM program for key pests of crucifer crops with special focus on yellowmargined leaf beetle, *Microtheca ochroloma*, and 4) Develop alternative strategies for managing kudzu bug, *Megacopta cribraria* in soybeans.

PROCEDURES AND METHODS

The proposed project will involve laboratory, greenhouse and field studies. Laboratory experiments will be conducted at the Department of Entomology & Plant Pathology, Auburn University (AU). Greenhouse studies will be conducted at the AU Plant Science greenhouse, while field experiments will primarily be conducted at AAES research stations and select commercial farms in Alabama. The PI's lab is equipped with the necessary facility for this research including analytical tools such as gas chromatography (GC), gas chromatography coupled electroantennogram detection (GC-EAD), and gas chromatography-coupled mass spectroscopy (GC-MS), behavioral and electroantennogram (EAG) systems.

Objective 1. Develop, evaluate and implement IPM tactics for managing key insect pests of peaches with special focus on plum curculio, plant bugs and scale insects.

Several studies will be conducted under this objective including:
1) Evaluation of targeted insecticide spray program for San Jose scales in peaches
2) Develop electronic sensor technology which is compatible with grading/packing lines to detect internal fruit damage caused by plum curculio in real time.

1) Evaluation of targeted insecticide spray program for San Jose scale in peaches.

Experiments will be conducted at the Chilton County Research & Extension Center (CREC) and select commercial peach orchards in Alabama to evaluate the utility of targeted insecticide spray programs for managing San Jose scale. A wide range of products ranging from less expensive dormant oil to highly expensive advanced chemistry insecticides are commercially available for managing San Jose scale in peaches. However, little is known about the efficacy and cost-effectiveness of these products. In this study, we will compare the effectiveness of before-bloom and summer schedules of various insecticides against San Jose scale. The following insecticides presently labeled for peaches will be evaluated in the spray program: i) Spirotetramat (Movento®) – belongs to the tetramic acid chemical class and has unique mode of action classified as a Lipid Biosynthesis Inhibitor (LBI), ii) Pyriproxyfen (Esteem® 35 WP) – a insect growth regulator, iii) Buprofezin (Centaur®)- a insect growth regulator, and iv) Dormant oil. The insecticides will be tested at recommended field rates singly or in combinations at different numbers of applications per season (spray programs). Each test plot will consist of at least 100 trees. Six inner trees within will be tagged for subsequent data collection. Plots will be separated by a minimum buffer 10 m.

Applications will be timed to coincide with either male flight (monitored with pheromone traps) or crawler activity. The relative abundance of crawlers in various treatments will be measured electric black sticky-tape trap. Two stick-tape traps will be placed on branches 5 cm diameter that have scale infestation. The tape traps will check at weekly throughout the season.

Fruit damage assessment will be done monthly in each plot by randomly collecting 50 fruits (25 each from the outer and inner canopy) from each of the 6 designated middle trees for a total of 300 fruits per plot. Percent fruit damage will be calculated using a simple ratio of number of fruits showing damage to the total number of fruit evaluated. Data will be analyzed using (ANOVA) followed by Tukey-Kramer HSD ($P < 0.05$).

2) Develop electronic sensor that would be compatible with grading/packing lines to detect internal fruit defects in real time.

Volatile organic compounds from infested fruit may serve as indicators of internal fruit defects nondestructively. First the volatile signal and other related attributes that act as indicators for internal fruit defects will be characterized. Second, the sensors to detect and measure signature volatile cues or volatile classes will be developed. The volatile profiles of peach fruit infested by plum curculio will be compared with undamaged fruit to identify internal fruit defect indicators.

Objective 2. Develop management strategies for key insect pests of cucurbits with special focus on squash bug.

The long term goal of this project is to enhance economic viability of cucurbit vegetable production in Alabama and the south by developing effective pest management tools to the growers. Specifically, this proposal will develop effective low-input pest management tactics such as trap crops for managing squash bug in cucurbit production.

1) Evaluate host plant preference and identify attractive trap crops for squash bug

This study aimed at assessing the attractiveness of different cucurbit varieties to squash bug with an aim to identify effective trap crop. We will evaluate eight types of winter quash from three species of cucurbits in comparison to butternut squash. The most attractive host plants identified in this study will be further evaluated as perimeter trap crop in commercial field trials. The squash bug number, per cent crop damage; in a perimeter trap crop treatment will be compared with a conventional treatment with no border. In general, the methodology is similar (with minor modifications) to those described in Objective 2 including study of host plant preference in greenhouse cage experiments, laboratory bioassays using four-choice olfactometer, and field evaluation of the most attractive host plants as trap crops.

3) Develop and implement organically-acceptable IPM program for key pests of crucifer crops with special focus on yellowmargined leaf beetle, *M. ochroloma.*

The aim of this objective is to integrate some of the effective tactics identified in our previous research to develop organic IPM programs for crucifer crops. The following potential IPM programs will be tested involving different combinations of the best treatment for each tactic as determined in our previous studies.

1) *Integration of trap crop and biopesticides*. In this program, transplants will be established as main crop in test plots (at least 100 x 30 ft). The turnip crop treatment will be established along the borders of the plot 2 weeks before planting the main crop. The trap crop will be treated with a biopesticide (e.g., Entrust®) weekly or as necessary. The main crop will be evaluated weekly for *M. ochroloma* and other insects and crop damage. If sampling indicates significant insect pressure (e.g., 1 per plant), the main crop will be treated with Entrust®.Additional insecticide applications will be made based on insect population density as determined by insect sampling.

If additional sprays are necessary, other effective insecticides such as PyGanic will be alternated with Entrust®. At harvest, 40 plants will be randomly selected from the main crop and evaluated for crop damage.

2) *Integration of attractant and biopesticides*. The main aim of this program is to determine if the attractants identified in our previous research could be effectively used to optimize insecticide application against *M. ochroloma*. One of the main problems limiting the efficacy of insecticides against *M. ochroloma* is the fact that the beetles typically migrate *en masse* from summer aestivation sites in the fall and overwintering sites in the spring into crop fields where they aggregate and cause serious damage within a short period. This sudden "mass attack" usually catches farmers by surprise giving them little opportunity to act in a timely fashion. By the time the first insecticide spray is made the beetle density is already too high and significant crop damage may have already been inflicted. The idea is to use attractant-baited monitoring traps to determine the onset of activity and seasonal population dynamics of the pest and then use this information to better time insecticide sprays. Our hypothesis is that *M. ochroloma* attractant can be effectively used to determine onset of beetle activity and optimize application of insecticides such as Entrust® to provide a more effective control of the beetle. Test plots for this program will be established and evaluated as in Program 1 above. The only difference is that the main crop will not be bordered by a trap crop. Instead, monitoring traps (yellow sticky or bucket style traps, based on the results of Obj. 1A) baited with the attractant (single compound or mixture) will be deployed at the beginning of the planting season (spring and fall) prior to field activity of *M. ochroloma*. Traps will be deployed near the four borders of the main crop, in surrounding / adjacent volunteer plants/fields (which serve as overwintering/summer hosts for *M. ochroloma*), and in the center of the main crop. Traps will be checked at weekly intervals at the beginning of each season to determine the onset of *M. ochroloma* activity. Once the first beetle has been captured in traps located within the main crop or on the border, the first insecticide treatment (Entrust®) will be applied as described above. Additional treatments and evaluation of plants for pest density and crop damage before and during will follow the same procedures described above for Program 1.

The control program will be a similar plot managed using standard organic pest management practices typically used by the cooperating grower (e.g.,no usetrap crops, attractants or biocontrol).

Objective 2. Develop alternative strategies for managing kudzu bug (*Megacopta cribraria*) in soybeans.

This objective will focus on the chemical ecology and development of IPM tactics for kudzu bug. We propose to evaluate host preference of kudzu bug and identify chemical basis for the observed preference. Specific components of this objective include:

1) Evaluate host plant preference and identify attractive trap crops for M. cribraria*.*

The goal of this study is to develop of an efficient trap cropping system for managing *M. cribraria* in soybean/legume crop production. The study will involve laboratory, greenhouse and field studies.

<u>*Greenhouse cage experiments to study host plant preference:*</u> Multiple-choice experiments will be conducted in the greenhouse (Auburn University Plant Sciences greenhouse facility) to further evaluate host preference of *M. cribraria* by comparing host plant attractancy. Using the protocols successfully utilized in our preliminary tests, several different legume varieties and cultivars, including fordhook limabean, speck bean, and Jackson butterbean will be

tested against soybean in a large cage (2 × 2 × 4 ft). Four of the selected host plant species (treatments) will be placed in each corner of the cage (i.e. four host plants will be simultaneously tested in each cage). The position of each treatment in the cage will be determined randomly and rotated during each replication. A group of 25 pairs of field collected adults starved for 24 hours will be released at the center of the cage by opening petri dish lid. The experiment will be replicated four times. Each cage will be examined daily for a period of two weeks to evaluate host preference by recording number of adult bugs on each host plant. Data will be analyzed using repeated measures multivariate analysis of variance (MANOVA).

<u>*Behavioral bioassay (four-choice olfactometer):*</u> The response of both sexes of adult *M. cribraria* to headspace volatiles of the different plant species will be tested in a four-choice olfactometer (Analytical Research Systems, Inc., Gainesville, FL = ARS) using protocols developed in our preliminary experiments. The set-up and protocols developed in the PI's laboratory have been previously described in detail (Balusu and Fadamiro, 2011). Briefly, the four-choice olfactometer consists of a square-shaped stage (2 x 12 x 12") on four 6" legs. The orifices of the olfactometer are connected through Teflon-glass tubes connectors to four pumps on an air delivery system equipped with a vacuum pump (ARS). Purified and humidified air is drawn at a constant rate of 100 mL/min through the extending orifices (arms) on the four sides of the stage and removed by suction via the vacuum pump through the central orifice of the olfactometer at the rate of 500 mL/min. The olfactometer stage will be housed within a paper box (~30 x 30 x 40"), the inside of which will be lined with white paper for uniform light diffusion. The box will be positioned under two fluorescent lights (~200 lux) for uniform lighting. The olfactometer will be carefully rinsed with hexane after each test. Each test will be replicated six times. Female and male bugs starved for 24 hours will be tested in all behavioral bioassays. Bioassays will be conducted at ~ 25 ± 1° C and 40-60% relative humidity. Data obtained will be analyzed using ANOVA followed by Tukey

<u>*Evaluation of trap crops for managing M. cribraria:*</u>

Our interest in the potential use of trap crop for managing *M. cribraria* is further spurred by our preliminary findings which demonstrated strong preference of *M. cribraria* for speck bean over soybean. The idea is to use speck bean or another highly attractive plant to deter migrating kudzu bugs or lure them away from the main (soybean) crop. Trap crop (speck bean) will be planted along the perimeters (borders) of the test plot. We hypothesized that early perimeter plantings of speck bean will serve to trap and arrest first generation of *M. cribraria* adults that migrate from kudzu plant and prevent them from moving into the main crop. Trap crop test plots will be established on research stations and two soybean grower's farms in early May before the migration of first generation *M. cribraria*. Each test plot will be ~ 100 ft long x 30 ft wide (15 rows, ~100 plants per row). Two treatments will be evaluated at each location: i) perimeter planting of speck bean along the four borders of the plot two weeks before planting main crop, and ii) main crop without perimeter planting of trap crop (control). Kudzu bugs will be controlled in the trap crop by using pyrethroid insecticide spray. Plots will be evaluated weekly by sampling 10 randomly selected plants within the main crop for *M. cribraria* densities. The average number of nymphs and adults of *M. cribraria* per plant will be compared using ANOVA followed by Tukey-Kramer HSD test ($P < 0.05$).

2) Identify plant-based semiochemical attractants for M. cribraria.

The goal of this study is to isolate and identify the semiochemicals (kairomones) used by *M. cribraria* to locate host plants. The potential applications of this study include identification of attractants/repellents for *M. cribraria*. This objective will be accomplished by using a multidisciplinary approach involving integration of cutting-edge analytical, behavioral and electrophysiological techniques. These techniques have been successfully used by PI's group and have been described in details in a recent publication (e.g., Chen and Fadamiro, 2007).

Briefly, four legume plant varieties: fordhook limabean speck bean, Jackson butterbean, and soybean will be grown in the greenhouse under standard conditions for headspace volatile collection. Headspace volatiles will also be collected from these test host plants. Headspace volatile collection will follow standard protocols developed in the PI's laboratory. Individual plants (or fruit) will be placed in a headspace volatile collection chamber (ARS, Inc., Gainesville, FL) consisting of a 50 liter glass jar. A purified air stream of 1 liter/min will be passed through the jar for 24 hours. Headspace volatiles will be trapped using 100 mg of Tenax trap (Supelco) and eluted with 3 ml of solvent. The solution will be concentrated by evaporation to ~ 50 μL and stored in the freezer until use.

Behavioral bioassays. The response of both sexes of *M. cribraria* to headspace volatiles of different plants will be tested in a Y-tube olfactometer (nymphs and adults) and using standard protocols (Fadamiro and Baker, 1997). A four-choice olfactometer bioassay (preference test) will be used to test odor preference.

Electroantennogram (EAG) recordings. The EAG technique will be used to record the electrophysiological responses of *M. cribraria* to headspace volatiles obtained from different plants and fruits. The technique and protocols developed in Dr. Fadamiro's lab (Chen and Fadamiro, 2007) will be used to obtain EAG recordings from the antennae of both sexes of *M. cribraria*.

GC-EAD analyses. Headspace volatiles from the different legume test plants will be analyzed by gas chromatography coupled electroantennogram detection (GC-EAD). The GC-EAD simultaneously combines the separation power of the GC and the highly sensitive detection system of the insect antennae to determine what fraction(s) of an effluent elicit response by the insect antennae. The technique allows for concurrent correlation of GC peaks with EAG responses. Using standard protocols developed in the Dr. Fadamiro's laboratory, antennae of both sexes of *L. zonatus* will be used to obtain GC-EAD recordings in response to headspace volatiles from the different plants. Headspace volatile peaks will be compared among fruits and EAD active peaks (compounds) will be noted for further analysis and identification by gas chromatography-coupled mass spectroscopy (GC-MS). Future activities will include synthesis of GC-EAD active peaks (if they are not commercially available) and laboratory/field evaluations of biological activity of identified/synthetic compounds to determine if they constitute attractants for *M. cribraria*.

3) Identify an aggregation pheromone for M. cribraria.

The aim of this objective is to identify pheromones for M. cribraria, in particular the aggregation pheromone. The primary focus on the aggregation pheromone is based on the fact that this type of pheromone is common among the stink bugs (Michell and Mau, 1971; McBrien et al., 2002; and Aldrich et al., 2009), and may be more useful in developing attractant-based management tactics, such as mass trapping and attract-and-kill technologies. In general, the process is similar (with minor modifications) to those described in Objective 2 including volatile collection, bioassays, GC-EAD analyses and identification of biologically active peaks (peaks which elicited EAG and behavioral responses), confirmation of biological activity of identified compounds, and field testing.

OUTREACH PLAN

The ultimate goal of this project is to facilitate implementation of effective IPM tactics by fruit and vegetable growers in Alabama through education and training. This project will be conducted with the participation of growers and extension specialists (e.g., Dr. A. Majumdar,) and extension agents in the state. Participating growers will be trained on-farm on pest identification, scouting, and management practices. Research results will be disseminated via on-farm demonstrations, grower-to-grower training, Extension circulars, and presentations at grower conferences.

EXPECTED OUTCOMES

It is anticipated that this project will foster adoption of effective, environmentally friendly IPM practices by Alabama producers. Specifically, this project will result in identification of effective alternative pest management tactics for emerging crop pests in Alabama including attractants, reduced-risk insects, trap crops and cultural control tactics.

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ANTICIPATED IMPACT

This Hatch project will impact Alabama's economy by facilitating grower adoption of economically viable and ecologically sustainable IPM tactics concurrent with reduced adverse impacts of harmful pesticides in Alabama specialty fruit and vegetable crops. It is anticipated that the research will generate data for publication in high quality scientific journals and potentially result in patent registrations of novel insect attractants.

TARGET AUDIENCE

The target audience of this project are crop producers in Alabama and the Southern U.S., specifically producers of peaches, soybean and vegetable crops.

PARICIPANTS

Estimated Project FTEs for the Project Duration

Role	**Faculty and Non-students**	**Undergraduate**	**Graduate**	**Post-doctorate**
Scientist	3	5	4	1
Professional	0	0	0	0
Technical	1	0	0	0
Administrative	0	0	0	0
Others	0	0	0	0

ESTIMATED BUDGET (ANNUAL ESTIMATED COSTS OF FIVE-YEAR PROJECT)

Below are the estimated costs for the proposed five-year project. Please note that this estimate does not factor in expected annual increases in salaries and benefits.

<u>Salary & Wages</u>	
Project Leader (0.6 FTE)	$
Postdoctoral Research Associate	$
Research Technician	$
Graduate Students	$
Undergraduate Assistants	$
<u>Fringe Benefits</u>	
Project Leader	$
Postdoctoral Research Associate	$
Research Technician	$
Graduate Students	$
Undergraduate Assistants	$
<u>Materials & Supplies</u>	$
<u>Travel</u>	
Research Trips	$
Professional Meetings	$
<u>Publication Costs</u>	$
<u>Total per year</u>	$
Five year total	**$**

LITERATURE CITED

Akotsen-Mensah, C., R. Boozer, A. Appel, and H.Y. Fadamiro. 2011b. Seasonal phenology and development of a degree-day model for predicting activity of plum curculio, *Conotrachelus nenuphar* in Alabama peaches. Annals of Entomological Society of America 104: 192-201

Akotsen-Mensah, C., R. Boozer, and H.Y. Fadamiro. 2010. Field evaluation of traps and lures for monitoring plum curculio, *Conotrachelus nenuphar* (Coleoptera: Curculionidae) in Alabama peaches. Journal of Economic Entomology. 103: 744-753.In press.

Akotsen-Mensah, C., R. Boozer, and H.Y. Fadamiro. 2011a. Field evaluation of reduced insecticide spray programs for managing plum curculio, Conotrachelus nenuphar in Alabama peaches. Pest Management Science 68: 573-579.

Akotsen-Mensah, C., R. Boozer, and H.Y. Fadamiro. Field evaluation of reduced insecticide spray programs for managing plum curculio, *Conotrachelus nenuphar* (Coleoptera: Curculionidae) in Alabama peaches. Pest Management Science. In review.

Alabama Agricultural Statistics Annual Bulletin. 2005. http://www.aces.edu/dept/nass/bulletin/2004/pg04.htm

Balusu, R., and H.Y. Fadamiro. 2011. Evaluation of organically acceptable insecticides as stand-alone treatments and in rotation for managing yellowmargined leaf beetle, *Microtheca ochloroma* (Coleoptera: Chrysomelidae) in organic crucifer production. Pest Management Science 68: 573-579.

Balusu, R., and H.Y. Fadamiro. 2011. Host finding and acceptance preference of the yellowmargined leaf beetle, *Microtheca ochroloma* (Coleoptera: Chrysomelidae), on cruciferous crops. Environmental Entomology 40: 1471-1477.

Balusu, R., and H.Y. Fadamiro. 2013. Susceptibility of *Microtheca ochroloma* (Coleoptera: Chrysomelidae) to botanical and microbial insecticide formulations. Florida Entomologist 96: 914-921.

Balusu, R.R, M. R. Elena, E. L. Oscar, and H.Y. Fadamiro. 2015. Management of Yellowmargined leaf beetle *Microtheca ochroloma* (Coleoptera: Chrysomelidae) using turnip as trap crop. J. Econ. Entomol. 108: 2691-2701. 2015

Chen, L., and H.Y. Fadamiro. 2007. Behavioral and electroantennogram responses of phorid fly *Pseudacteon tricuspis* (Diptera: Phoridae) to red imported fire ant *Solenopsis invicta* odor and trail pheromone. Journal of Insect Behavior. 20: 267-287.

United States Department of Agriculture, Animal and Plant Health Inspection Service. 2010. Invasive insect (Bean Plataspid) poses risk to soybean crops and infests homes in southeastern states. Factsheet, October. 2 pp.

United States Department of Agriculture, National Agricultural Statistics Services. Quick Stats. 2013. http://quickstats.nass.usda.gov/data/printable/62671009-0742-3612-B96A-0C94B453097F

United States Department of Agriculture, National Agricultural Statistics Services. 2011. Alabama Agricultural Statistics. Bulletin 53. http://www.nass.usda.gov/Statistics_by_State/Alabama/Publications/Annual_Statistical_Bulletin/2011/2011AlabamaAgricultureStatistics.pdf

USDA-NASS. 2002. Summary by Market Value of Agricultural Products Sold: 2002. United States Department of Agriculture, National Agricultural Statistics Service.

Williams, L. and J. Dangler. 1992. 1992 Budgets for vegetable crop enterprises in Alabama. Special Extension Report, Alabama Cooperative Extension Service, AECBUD 1-4.