**S-1073 ANNUAL REPORT FOR 2018 AND PLANS FOR 2019**

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## ACCOMPLISHMENTS

1. To discover, assess, and release new biological control agents

   Since 2010, Drs. Don Steinkraus, Bryan Petty and Donn Johnson (University of Arkansas) have collaborated with Michigan State University (Dr. David Smitley and Erica Hotchkiss) and APHIS (Dr. Phillip Lewis) on a Farm Bill project titled: Facilitating Spread of the Microsporidian Pathogen, *Ovavesicula popilliae*, for Long-Term Suppression of Japanese Beetle (JB). In Michigan for several years from 2010 on, adult JBs were trapped at golf courses in July. At least 50% of the 100 JBs dissected from each location were infected by *O. popilliae*. Over 1,000 dead infected beetles were sent to each cooperator in 17 states for introducing the pathogen to their location. Protocol was followed to meet the APHIS permit requirements. In Arkansas, *O. popilliae* infected adult JB were released in soil under turf at four sites (2 in Fayetteville, 1 in Rogers, and 1 in Westfork). Annually in July at each site (including 2019), we collected at least 100 live adult JBs in a screen capture arena attached under a dual-lure (JB sex pheromone + floral lure) baited yellow funnel JB trap. One trap was placed in each release site and at a non-release site on the University of Arkansas Agricultural Research & Extension Center-Fayetteville, AR. Each sample of 100 JB was frozen for 48 hrs in vials of saline, labeled with site name and GPS coordinates, and shipped overnight to Michigan State University. In Arkansas, the percentage infections of adult JBs by *O. popilliae* was only 1.4% in Fayetteville in 2017 compared to 1% in Fayetteville and 3.2% in Rogers in 2018. The 2019 samples will be dissected this fall to record the percentage of adult JB infected with *O. popilliae* per Arkansas site.

   With the cooperation of APHIS field staff and MSU, introduce *O. popilliae* to eight major cargo-plane airports in the Eastern and Midwestern United States where Japanese beetle has been a problem, and to two golf courses in Colorado.

***Progress through December 2018:*** In 2017 USDA APHIS inspectors and University entomologists were contacted from 10 different states to collaborate on this project. At each site beetles were collected and shipped to Michigan State University according to the protocol in the APHIS permit granted in spring of 2017. Dissection of these beetles and pathogen analysis began in August 2017 and was completed by April of 2018 (Table 1). Results from the pathogen analysis indicate a high level of infection in Michigan at sites where *O. popilliae* was introduced 15 years earlier. All of the Midwest and Eastern USA locations, with the exception of Indianapolis, tested positive for *O. popilliae*, with levels of infection varying from 1 to 50%. We only received 20 beetles from Indianapolis, so when we receive more beetles in 2018 we may find it there, also. At 11 of 19 sites located in states west of the Mississippi River, we did not detect *O. popilliae* (Table 1). Two of the eight sites west of the Mississippi River where we found the pathogen were at locations in Colorado where it had been introduced two years earlier. At the remaining six locations where *O. popilliae* was found in western states, less than 5% of the beetles were infected. One of those locations was near the Portland International Airport where it has recently been determined that all of the beetles trapped there are introductions from the same summer. This strongly supports our approach of making sure *O. popilliae* is established at major airports in the eastern United States, so that some infected beetles will go everywhere that Japanese beetle is accidentally transported.

In 2018 APHIS inspectors and university researchers collected Japanese beetles in June, July or August and sent us at least 100 adult beetles from 27 different locations in 12 states. Most of the collection sites were located near key cargo airports which are indicated by green print in Table 1. About 50% of some 4,000 beetles have been dissected for pathogen diagnosis, and we expect to complete all dissections by the end of April, 2019.

Japanese beetles were collected in Michigan at two sites where *O. popilliae* is epizootic for shipping to 17 locations in 8 states. An average of 3,000 beetles were sent to each location in August. Fifty beetles from each of the two sources of infected beetles were dissected and found to be 35% and 65% infected with *O. popilliae.*

Dan Hulbert, a post-doctorate research scientist working on this project has made excellent progress in developing a qPCR assay for detecting the pathogen in homogenized beetles using Sybr Green chemistry. He designed primers for use in the assay and cloned the resulting fragment into a plasmid, so it could be sequenced and verification of the PCR target (16S-like gene of *O. popillae*) could be made. The plasmids with the fragment insert were also used in absolute standard curve experiments to determine cycle thresholds for a linear dynamic range of target DNA concentrations. He also tested the assay with about 100 beetles that were first dissected for visual diagnosis before he ran the qPCR. The qPCR results agree well with the visual diagnoses.

2. To characterize and evaluate the impact of native and introduced biocontrol agents-
   a. Determine the success of the introduction of *O. popilliae* in 2016 at two golf course sites in Colorado. Note: this objective is now expanded to five golf courses where the pathogen is being introduced plus five control sites. ***Progress in 2018:*** Traps were set, and beetles collected at three golf courses in Colorado. At two of these golf courses, *O. popilliae* was introduced as dead infected beetles in 2016. The third golf course is a control site where *O. popilliae* was not introduced. Whitney Cranshaw at Colorado State

University set traps, collected beetles and froze them in July of 2017. The dead beetles were shipped to Michigan State University where they were dissected for pathogen analysis. *O. popilliae* was found in 2 of 81 beetles collected from Flat Iron Golf Course, and in 5 of 83 collected from Pueblo Golf Course. No infected beetles were found at the Pueblo Zoo site. Therefore, *O. popilliae* was found at two locations were dead infected were introduced in 2016, and not at the third location where it had not been introduced.

In 2018 seven new golf course sites were added, for a total of five introduction sites and five control sites. Infected beetles from Michigan were sent in July to Colorado for the introduction sites. Dr. Cranshaw collected beetles from all 10 locations and sent them to Michigan for pathogen analysis.

b. Quantify the impact of *O. popilliae* infection on the growth and development of Japanese beetle larvae, and on their successful pupation and emergence as adults. ***Progress in 2018: Methods.*** Japanese beetle grubs were collected in October 2017 from two locations: Eastern Hills golf course where *O. popilliae* is well established and the Hancock Center at MSU where *O. popilliae* was not found. A golf course cup-cutter was used to pull 15 cm-deep turf and soil cores which were inserted into a snug-fitting plastic sleeve pot with drainage holes in the bottom. The sleeve-pot with the soil and turf core was then placed back in the same hole it was taken from. Because Japanese beetle grubs stay below the soil surface the sleeve-pots keep the grubs contained in the turf and soil core. Three grubs were added to each sleeve-pot, for a total of 72 grubs per treatment, and 288 grubs in the experiment. The four treatments of this experiment are (1) grubs collected from the Hancock Center that were put in sleeve pots at the Hancock Center, (2) grubs from the Hancock Center put in sleeve pots at Eastern Hills, (3) grubs from Eastern Hills put into sleeve-pots at the Hancock Center, and (4) grubs from Eastern Hills put into sleeve-pots at Eastern Hills (Table 2). ***Results and Discussion.*** All of the infected Japanese grubs collected at Eastern Hills and placed into sleeve-pots at the Hancock Center died in the time period from October 2017 to May 2018. The results were similar for grubs collected at Eastern Hills and placed into sleeve-pots at Eastern Hills (an estimated 34% infected in October and only 3.6% of the same grubs infected in May). Overall, survival of healthy grubs at a location without *O. popilliae* was 78% compared with a survival rate of 37.6% for grubs at a location where *O. popilliae* is active (Table 2). By comparing the survival of grubs collected from locations with and without *O. popilliae* we can conclude that most of the mortality observed from October to May was caused by the pathogen, either directly or by weakening grubs enough to make them susceptible to secondary infections. Also, *O. popilliae* infections that were initiated earlier in the season (Aug. to September), so that they had progressed to a level that was detectable when grubs were dissected in October, caused greater mortality (95 – 100%) from October to May than infections that were initiated after the middle of October. This can be determined by comparing the infection rate and survival of grubs moved in October from a site without *O. popilliae* to a site where the pathogen was active (28.3% infection, Treatment 2) with the survival of grubs collected at a site without *O. popilliae* and placed back into the same site (0% infection, Treatment 1). Although 28.3% of the grubs in Treatment 2 became infected the survival rate of those grubs from October to May was 68.0%, only slightly less than the survival rate of healthy grubs (78.0%). Another useful outcome of this experiment is that we were able to infect healthy grubs by putting them into soil from a site where *O. popilliae* is active (Eastern Hills).

3. To develop integrated pest management programs that have a biological control component

4. To develop augmentation and conservation biological control tactics, especially to improve the quality of agricultural habitats for pollinators

## UTILITY OF FINDINGS

In the future, there may need to be additional releases of *O. popilliae* or another natural enemy to increase the rate of natural control of JB in Arkansas and adjacent, JB-infested states. Since the first detection of JB in Ft. Smith, Lowell and Fayetteville, AR in 1997, JB has spread concentrically outward. By 2019, JB is reported in damaging numbers in the northern quarter of Arkansas, throughout Missouri, in the eastern and central portions of Kansas and northeast Oklahoma.

## WORK PLANNED FOR NEXT YEAR (2019)

None in Arkansas by Donn Johnson. He retires after 31 December 2019.

## PUBLICATIONS (2018)

None

## Presentations (2019):

Austin Goldsmith. Isolation of native strains of *Metarhizium anisopliae* and their efficacy for the control of *Ambylomma americanum in lab bioassays.* Entrance research seminar on 24 June 2019.

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## ACCOMPLISHMENTS

Objective 1. To discover, assess, and release new biological control agents.

*Brazilian peppertree*. Over the past 20 years, several insect herbivores of Brazilian peppertree (*Schinus terebinthfolia* Raddi, Anacardiaceae) have been investigated as potential biological control agents. Two natural enemies were recommended for field release in 2016: the psyllid *Calophya latiforceps* Burckhardt (Hemiptera: Calophyidae) and a thrips *Pseudophilothrips ichini* Hood (Thysanoptera: Phlaeothripidae). Release permits are expected in 2019.

Hypersensitive response by Brazilian peppertree to feeding by *Calophya* psyllids. Plants can exhibit different mechanisms to defend themselves against herbivores. Several species rely on physical structures such as thorns and hairs. When these structures are not present, plants like Brazilian peppertree often produce secondary chemical defenses for protection. When the immatures of *Calophya* spp. begin to feed on Brazilian peppertree, the plant is stimulated to produce salicylic acid, which causes the formation of necrotic tissue around the point of feeding, and results in the premature death of the insects. The goal of this experiment is to gain a better understanding of the physiological processes involved in of the production of chemical defenses in Brazilian peppertree, the time frame when chemical defenses are inititated, and if there is a systemic response in the whole plant or if it is only a localized effect. During the Summer and Fall semesters of 2018, all the leaf tissue needed to study the systemic response was collected and analysis of chemicals produced by Brazilian peppertree in response to psyllid herbivory was analyzed in Gainesville with GC-MS (Gas Chromatography, Mass Spectrometry) at the USDA ARS CMAVE laboratory. Results are pending.

Ovipositional preference and development of *Calophya latiforceps* on male and female Brazilian peppertree plants. Attempts at colonizing *Calophya* spp. in a laboratory environment have been challenging. One possibility is that female plants of the dioecious Brazilian peppertree may be better defended chemically from attack by the psyllid than their male counterparts. Male-biased herbivory was recently documented for the stem boring weevil *Apocnemidophorus*

*pipitzi* (Faust), another Brazilian peppertree natural enemy (see Cuda et al. 2018). This study aims to determine the extent to which dioecy affects psyllid survival. The objectives of this project are: (i) investigate preference of *C. latiforceps* to oviposit on male or female plants from five different geographic areas in Florida, and (ii) monitor development/survival to adulthood on male and female plants. In progress.

Host specificity of *Calophya terebinthifolii* Burckhardt & Basset and *Calophya lutea* Burckhardt (Hemiptera: Calophyidae). Host range testing for candidate biological control agents for Brazilian peppertree (*Schinus terebinthifolia*), *Calophya lutea* and *C. terebinthifolii*, has been completed. Both insect species will only complete development on Brazilian peppertree.

Cold hardiness of *Calophya latiforceps* and *Calophya terebinthifolii* adults on Brazilian peppertree. During studies of potential biological control agents, cold hardiness experiments are necessary because winter temperatures are a major source of insect mortality and could limit the use of agents from tropical regions. Two leaf gall-inducing insects, *C. latiforceps* and *C. terebinthifolii* that were discovered in different climatic regions in Brazil are being investigated for release in Florida. The objective of this study was to determine the influence of cold temperatures on adult survival. The results showed that *C. terebinthifolii* had a higher tolerance to all temperatures tested. However, based on climate history of Florida and the current distribution of Brazilian peppertree, both *C. latiforceps* and *C. terebinthifolii* could establish in the areas where BP is present.

Mass rearing Brazilian peppertree weevil *Apocnemidophorus pipitzi*. Continued maintaining a colony of the stem-boring weevil *A. pipitzi* in the Entomology Department Containment Laboratory (EDCL). During this reporting period, a total of 2,014 adults were produced (1,015 males, 1,017 females). The sex ratio of 1.00 indicates normal colony reproduction.

*Cogongrass*. Cogongrass (*Imperata cylindrica* (L.) P. Beauv.; Poaceae) is a federal listed noxious weed that occurs in Florida and other southeastern states. This invasive grass infests cattle pastures, golf courses, lawns, and thrives in poor soil conditions such as ditch banks, roadside and railroad rights-of-way as well as reclaimed phosphate-mining areas. Biological control using natural enemies from the native range of cogongrass has received little attention and no biological control agents have been introduced anywhere in the world. The Indonesian gall midge *Orseolia javanica* Kieffer and van Leeuwen-Reijinvaan (Diptera: Cecidomyiidae) is a potential biological control agent of cogongrass. However, it is not known whether *O. javanica* will develop and reproduce on the FL peninsula or Gulf coast clones of cogongrass. The goal of this preliminary study in Indonesia is to determine the extent to which the gall midge *O. javanica* is pre-adapted to Florida cogongrass by comparing its performance on Florida plants with the West Java cogongrass, its natural host. In progress.

*Earleaf acacia*. Field surveys have been conducted in Northern Australia to find potential biological control agents for the invasive tree *Acacia auriculiformis* A.Cunn. ex Benth., Fabaceae. Several insect species have been selected as promising candidates for biological control of *A. auriculiformis*: *Trichilogaster* sp. nov. (Hymenoptera: Pteromalidae), *Melanterius* sp. (Coleoptera: Curculionidae), and *Calomela intemerata* (Coleoptera: Chrysomelidae). Each of these species is considered promising due to high densities in the field and success of their

congeners in plant biological control programs.

Objective 2. To characterize and evaluate the impact of native and introduced agents.

*Brazilian peppertree*. Evaluation of the impact of local insect herbivores on the growth and reproduction of Brazilian peppertree at two sites in Florida were conducted. There were no statistical differences in plant height, basal diameter, number of branches, or number of fruits between insecticide treated and control plants in either of the two study sites over the 4 years of the study. This study serves as justification for the need for classical biological control in this system.

*Invasive mole crickets*. Invasive mole crickets are damaging pests of turfgrass and forage crops. With funding from USDA-NIFA ($90K), my lab partnered with Florida cattlemen and IFAS research stations to study the effects of an introduced parasitic nematode, *Steinernema scapterisci*, on two invasive mole cricket species over time. We found the parasite has established and dispersed throughout north-central Florida, and that mole crickets remain abundant and appear to have become up to 10% larger. We speculate that mole cricket-parasite interactions have selected for larger, more fit mole crickets, which has implications for future management and further dispersal into the U.S. This work has resulted in a peer-reviewed research publication currently in review with *Biological Invasions.*

Objective 3. To develop integrated pest management programs that have a biological control component.

*Hydrilla*. The invasive aquatic weed hydrilla [*Hydrilla verticillata* (L. f.) Royle] causes serious environmental and economic impacts. When left unmanaged, this aquatic weed creates damaging infestations that choke out native plants, clog flood control structures, and impede navigation and recreation. In Florida, millions of dollars are spent annually to control large infestations of hydrilla. However, during the past 15 years, hydrilla developed resistance to fluridone and endothall, two of the most commonly used herbicides approved for aquatic use. Since 2010, we have been testing a novel IPM approach for hydrilla control. Our IPM system integrates selective insect herbivory by the hydrilla tip miner *Cricotopus lebetis* Sublette (Diptera: Chironomidae) with a disease-causing fungal pathogen [*Mycoleptodiscus terrestris* Gerd.) Ostaz.] (Mt), and low concentrations of the herbicide imazamox, an acetolactate synthase (ALS) inhibitor recently registered for aquatic use. Field testing was performed in limnocorrals (1 m diam. x 1 m depth) installed in three ponds at the UF/IFAS Center for Aquatic and Invasive Plants.
Although results varied seasonally, the tip miner *C. lebetis* and fungus Mt together or in combination with the herbicide imazamox significantly reduced hydrilla biomass compared to the untreated controls. Midge specific tip damage was evident in all treatments inoculated with the midge, which confirmed the insect was compatible with the fungus Mt and herbicide. A significant reduction in turions also was observed in all treatment combinations during the fall season. Overall, these findings indicate that a combination of different biological and chemical tactics can be used to effectively manage hydrilla.

*Sweet potato whitefly*. The sweetpotato whitefly is a serious pest of vegetable crops. A cultural

method using repellent plants or products may control whiteflies. We studied the behavior of the whitefly using an odor detecting equipment, a video recorder and a behavior analysis software. The whiteflies were exposed to different colors, plant oils and entire plants. We found measurable movement of whitefly adults towards cucumber plants and the colors yellow and green. However, when mustard odors were added, whiteflies were no longer attracted, and at times were repelled. Results showed highest total distances traversed and rate of insect movement in hot pepper wax, mustard oil and the cucumber controls. Time allocation analysis showed attraction towards cucumber and hot pepper wax, as well as aversion to garlic oil and mustard oil. These studies suggest mustard, mustard oil and garlic oil for use as repellents in a "push" component of a "push-pull" management strategy to control insect pests.

*Golf course insect pests*. Urbanization is commonly associated with habitat loss and declining beneficial insects. With more golf courses than any U.S. state, Florida is well-positioned to strategically utilize them as ecologically functional green spaces. I have partnered with industry stakeholders to develop methods for increasing pollinating and predatory insects, translating to a 50% increase in biological control of golf course insect pests and reducing maintenance inputs.

Objective 4. To develop augmentation and conservation biological control tactics, especially to improve the quality of agricultural habitats for pollinators.

*Alligatorweed*. In June 2018, the Land Manager for Jekyll Island State Park located near Brunswick, GA, was interested in obtaining some alligator weed flea beetles as an alternative to herbicide control of alligator weed. The flea beetle was introduced into Florida in 1964 for biological control of the invasive alligator weed that is native to South America. Within a few years, alligator weed ceased to be a major problem at most sites in Florida where the beetle was released. However, the beetle often will not establish at northern sites that are occasionally subjected to freezing temperatures. The solution to the problem involved collecting the beetle where it is established in Florida and making augmentative releases at Jekyll Island. On the morning of 9 July 2018, ~ 110 beetles were collected from one of the ponds located at the UF/IFAS Center for Aquatic and Invasive Plants. The beetles were placed in a cooler and transported to Jekyll Island where they were released the same day. By October, the alligator weed exhibited significant feeding damage by the beetle, and several adults were observed on the plants. If the winter is not too cold in southeast Georgia, there is good chance the beetle will establish permanently at the site because of its proximity to the temperature-moderating effects of the Atlantic Ocean.

*Trichogramma wasps*. Wasps in the genus *Trichogramma***,** because of their ability to control a wide range of Lepidoptera, are probably the most widely produced and released beneficial insect. The parasitoids are most commonly produced on eggs of the Mediterranean flour moth, *Ephestia kuehniella*. Efficient rearing of *Trichogramma* spp. depends on the quality of host eggs that can affect the acceptance of eggs for parasitism by female wasps, growth and development of the parasitoids, and the quality and sex ratio of the parasitoid progeny. It was determined that *E. kuehniella* produced significantly larger eggs when the larvae were fed a nutritionally enhanced diet versus adequate or minimal diets, and *T. brassicae* oviposited more eggs on the larger host eggs. Emergence of wasps was equivalent from *E. kuehniella* eggs regardless of size; however, more female wasps were produced from the larger eggs. Additionally, wasps from larger eggs

were more capable of flying but the tests must be repeated.

*Golf course insect pests*. See Objective 3.

*Predatory mites*. The objectives of the experiment were to evaluate the different release methods of predatory mites, *Amblyseius* swirskii (Athias-Henriot) (Acari: Phytoseiidae) and the effects of squash (*Cucurbita pepo* L.) cultivars on *A. swirskii* and whitefly (*Bemisia tabaci* Middle East Asia Minor 1) densities in an organic field. The experiment was conducted in Citra, Florida (University of Florida-Plant Science Research and Education Unit). Seeds from three different cultivars-Zephyr, Eight ball, and Sunburst were planted on October 3rd, 2018. Following four release methods were used for *A. swirskii*- i) Approximately 1ml of barn containing 40 *A. swirskii* was sprinkle on top of the squash leaves, ii) Approximately 1ml of barn containing 40 *A. swirskii* was placed on base of the stem, iii) Sachets (Swirskii Ulti-Mite, Koppert Biological System) containing 250 *A. swirskii* were placed on alternative plant, and iv) Control (without *A. swirskii*). *A. swirskii* was released only one time on 3rd week after planting. There was a total of 12 treatment combinations with 4 release methods and 3 cultivars, which were replicated 4 times in split plot in Randomized Complete Block Design. Weekly in-situ observations were made in the field for adult whiteflies, starting from 4th week of planting for consecutive five weeks. Overall, a higher number of whiteflies was found on Zephyr and Sunburst than Eight ball. Also, leaf sampling was conducted every week after 4th until 10th weeks. Sprinkle on leaves release method showed the higher number of *A. swirskii* on leaves than by sachets and stem methods. Control had the lowest number of *A. swirskii.* A higher number of eggs and nymphs of whiteflies was found on Zephyr and Sunburst than on Eight ball. A higher number of plants showing virus symptoms and silverleaf disorder symptoms also was found on Zephyr followed by Sunburst and then Eight ball. Zephyr also had the highest yield than Sunburst and Eight ball.

The objective of this experiment was to compare preventative, whole plot, and spot treatment releases of *Neoseiulus californicus* (McGregor) predatory mites for management of the twospotted spider mite (TSSM), *Tetranychus urticae* Koch. The experiment was conducted at the Citra Plant Science Research and Education Unit near Citra Florida in the variety 'Festival'. There were 5 replicates of 4 treatments. Weekly leaf samples were collected from 14 Nov 2017 until 12 March 2018. *Neoseiulus californicus* mites were released at the preventative rate of 25 per $m^2$ into the preventative treatment plots on 16 Nov 2017. *Neoseiulus californicus* mites also were released into the whole plot and spot treatment plots on 11 Jan 2018 at the rate of 1 predatory mite per 10 TSSM. In the whole plot treatment, predatory mites were released over the entirety of each plot. In the spot treatment, predatory mites were released onto 5 groups of eight plants in each plot. The 4th treatment was an untreated control. In the preventative treatment plots, TSSM numbers never rose above an average of 2 mites per leaf or 3 eggs per leaf. This was the only treatment with significantly fewer TSSM mites and eggs compared with the control. There were numerically fewer TSSM and TSSM eggs in the whole plot and spot treatments compared with the control and these two treatments performed similarly. There were no differences in yield among treatments. In conclusion, preventative releases of *N. californicus* appear to be the better method to reduce TSSM in strawberries.

## UTILITY OF FINDINGS

*Brazilian Peppertree*. Brazilian peppertree is one of the worst upland invasive weed species in Florida. The lack of control by insects and other arthropods present in the invaded range of Brazilian peppertree justify the need for classical biological control. Biological control agents for Brazilian peppertree are awaiting release permits or are currently being developed.
The release of biocontrol agents (the psyllid *C. latiforceps* and the thrips *P. ichini*) will reduce herbicide use and contribute to the sustainable management of this highly invasive weed in Florida and other states were Brazilian peppertree is invasive.

*Earleaf acacia*. Earleaf acacia continues to spread in peninsular Florida. Continued native range exploration for new potential agents will continue and host-range testing for agents currently in colony will be conducted.

*Cogongrass*. Control of cogongrass relies primarily on mowing and herbicide applications. Biological control has received little attention and no biological control agents have been introduced anywhere in the world. The exploration for natural enemies of cogongrass over the past several years has identified several natural enemies that are putative specialists (e.g., the gall midge *O. javanica* and noctuid stemborers of the genus *Acrapex).*

*Hydrilla*. The Florida Fish and Wildlife Conservation Commission recently issued a moratorium on aquatic weed control in Florida waterbodies until further notice due to the negative public perception of herbicides such as glyphosate. Effective management of hydrilla could be implemented in Florida by integrating the fungus *M. terrestris* (Mt) and/or the herbicide imazamox with the stem mining midge *C. lebetis*. However, our research has shown that (a) post hatch midge survival will influence rearing and release protocols; neonates cannot be held past eclosion; (b) midges will persist in the field after a sufficient number of augmentative releases, and (c) because larvae and pupae of the hydrilla midge *C. lebetis* are vulnerable to fish predation, release numbers should compensate for this effect.

*Trichogramma wasps*. This study determined that the most expensive *E. kuehniella* larval diet result in the production of eggs that are the most acceptable to *Trichogramma brassicae* (Hymenoptera: Trichogrammatidae) for parasitism and produce high quality progeny. Therefore, the enhanced diet is the most cost-effective.

*Sweet potato whitefly* These research areas will complement cultural techniques such as use of "push-pull" strategies to control insect pests in vegetables in an integrated pest management program.

*Golf course insect pests*. Our work with invasive mole crickets and their introduced parasites has advanced our understanding of classical biological control and generated insights into host-parasite interactions over extended time and space. Our results have generated data for future grant proposals and research projects. Our work creating golf course conservation habitats has generated a significant amount of interest among the Florida golf industry, but also generated guidelines that should improve golf course IPM programs throughout Florida and the Southeast. This work has also generated data to fuel future grant proposals and publications.
*Predatory mites*. New projects.

## WORK PLANNED FOR NEXT YEAR (2019)

*Brazilian Peppertree*. Upon receipt of release permits for the thrips *P. ichini* and the psyllid *C. latiforceps,* field release, establishment, and impact studies are planned. Integration of biological control with chemical control studies are also planned. Submission of TAG petitions for the remaining *Calophya* species will be written and submitted. Colonies of the thrips *P. ichini*, the weevil *A. pipitzi,* and the *Calophya* psyllids are being maintained for field release, temperature tolerance, and competition studies (*C. terebinthifolii*, *Calophya lutea*). Brazliain peppertree dioecy experiments with *Calophya* spp. will be completed by the next reporting period.

*Cogongrass*. If funding is available, efforts will be directed at developing effective rearing methods for the Indonesian gall midge *O. javanica* so that laboratory-based host range testing can be initiated. Pursue additional funding for classical biological control of cogongrass

*Earleaf acacia*. Continued native range exploration for new potential agents will continue and host-range testing for agents currently in colony will be conducted.

*Trichogramma wasps*. The research has been concluded and a publication is being written for the Journal of Insect Science.

*Sweet potato whitefly*. We will evaluate the effects of attractive refuge flowering plants in attracting beneficial insects to control insect pests in vegetables in high tunnel systems.

*Golf course insect pests*. Continue research and extension projects associated with creating conservation habitats on golf courses for IPM, conservation biological control, and pollinator conservation. Publish two peer-reviewed research publications about creating conservation habitats on golf courses. Publish two peer-reviewed publications about classical biological control of invasive mole crickets. Pursue additional funding for conservation biological control and classical biological control of invasive mole crickets.

*Predatory mites*. Continue field evaluations of both mite species.

## PUBLICATIONS (2018)

*Refereed*

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**S-1073 ANNUAL REPORT FOR 2018 AND PLANS FOR 2019**

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## ACCOMPLISHMENTS

1. To discover, assess, and release new biological control agents

2. To characterize and evaluate the impact of native and introduced biocontrol agents

*a. Parasitoids and predators of peanut pests in agricultural landscapes*- Current control methods for the peanut burrower bug, *Pangaeus bilineatus* (Say), primarily based on insecticides have been unsuccessful. In addition, the over use of insecticides in attempts to control this pest can cause serious problems with other pests. This has been observed in peanut systems in GA, and is a concern for growers because this can lead to heavy reliance on insecticides or calendar sprays, when things go wrong. This indicates that biological control is very important for peanut systems and is operating well most of the time, but secondary pests do become a problem, which indicates that some of the biocontrol agents are being removed in certain situations.

In the first year of this study, the Schmidt lab in collaboration with Mark Abney collected pests, predators and parasitoids from commercial peanut fields in 2019. We designed a spatially balanced design of sites to understand the effects of the amount of peanut production in the landscape to other land cover areas in relation to populations of natural enemies and biocontrol services provided. We sampled over 40 fields in one week to provide an initial view of these communities (i.e. within field spatial 3 locations, two transects, 40 fields for a total of 240 arthropod community samples). Currently we are processing samples and will repeat in 2020. Predators collected were preserved at -20°C in the freezers in the Schmidt lab. These will be screened for the presence of pests using DNA technology used in the Schmidt lab. These data will then be related to farming practices, and land-use/ landcover data, which will allow us to predict when biocontrol is working the best. We will note: irrigation, covercrop use, and any pest management tools used – insecticides/herbicides, and basics on soil types for fields sampled.

b. *Characterize and evaluate the use of cover crops for promoting biological control of early and late season pests in cotton*-This is a project with seed funding from the GA Cotton Commission and Cotton Inc. The overall goals were to assess the biocontrol services and production value of crops grown under different cover cropping systems. We expanded on this project to look at how arthropod communities are being shaped by cover cropping, and have presented multiple papers with one manuscript in review and one in preparation.

3. To develop integrated pest management programs that have a biological control component

4. To develop augmentation and conservation biological control tactics, especially to improve the quality of agricultural habitats for pollinators
    a. *Agriculture and solar synergies*-Working with the InSpire funded DOE project, we are setting up a set of experimental plots to assess the synergies of solar and habitat provisioning (i.e. wildflower habitats) for promoting biological control communities, and pollinators in agricultural landscapes. This is a multi-year, multistate project. And, GA is one node forming in this network. We are currently managing the site using cover crops or tillage or a combination, and no insect samples have occurred in this year.
    b. *Attracting pollinators and natural pest managers: screening plants for ease of use and maintenance*-In collaboration with Bodie Pennisi and Joshua Dawson, we have established 3 sites containing replicated mono-culture native wildflower plots. We have over 30 species represented at each location with upwards of 8 replicates for each plant type. In 2017-2019, we have taken samples over the season of pollinators and biocontrol species attracted to the wildflowers. We have held workshops to help educate the urban landscape clientele on choosing plants and also for pest management. We have presented data on attraction at multiple conferences, and are now in the process of preparing publications from these first two years of data collection. For the Schmidt lab aspect, we are very interested in the biocontrol potential of integrating the right species into vegetable and cotton producing landscapes (compatibility), and ease of management to boost biocontrol services.

**UTILITY OF FINDINGS**

Although there are many studies of the effects of landscape composition on pests and natural enemies, very few are conducted in subtropical southeast US systems. Our goal is to understand the compositional changes of communities and link these to area wide management strategies. We must first understand the shape of arthropod communities.

Cover cropping provides many benefits to agroecology systems (i.e. erosion prevention, soil fertility and structure, and weed control). Our work is helping to advance the effects on insect pest management and biodiversity related ecosystem services. In the first years of our study in cotton cover cropping systems we have overall positive effects of cover crops on natural enemy communities, and possible applications of cover crops to improve biological control as part of integrated pest management program. There is seasonality to the effects of cover crops on biological control communities, and in particular strong early season effects on predator communities correspond with reductions of pest pressure into the late season.

**WORK PLANNED FOR NEXT YEAR (2019-2020)**

2. To characterize and evaluate the impact of native and introduced biocontrol agents
    a. *Parasitoids and predators of peanut burrower bug and caterpillar pests in agricultural landscapes*-We will repeat the same procedures in 2019 for 2020. We will select sites in peanut that are very close to the locations for 2019. Peanut growers in GA usually rotate their crops, and we anticipate being able to old our spatial design pretty close with site near the 2019. All other procedures will follow 2019.

3. To develop integrated pest management programs that have a biological control component
    a. *Evaluating synergies of plant traits with biological control*-
    This is a SSARE funded project that came to life based on synergies in the S1073 team. This brings together three states and three institutions across the S1073 network. The project just began September 2019, and will continue for the next three years. The overall goals are to understand the interactions between plant traits, biodiversity provisioning and pest management. The three institutions involved are Clemson University (JC Chong), Cornell University (Martha Mutschler), and University of Georgia (Jason Schmidt). In next year, we will repeat trials for fall with 3 acyl sugar lines and current industry standards. We have partnered with other UGA researchers and extension specialists to build a larger component that originally proposed. We will test traits in key seasonal periods, the fall, and the spring. Each presents unique pest challenges and also associated possibilities for natural pest management through biological control.
    b. *Evaluating current pest management in organic onions on biocontrol communities and how best to manage for promoting biocontrol services in organic onion*-This will be a new project. The overall project is funded by USDA NIFA ORG program. The Schmidt lab will add additional data collection to understand predator-prey interactions in the onion systems of GA under this project to boost the overall outputs proposed in the NIFA grant for this project. We will collect predators and assess services provided on pests using molecular gut content analysis.
    *c. Characterize and evaluate the use of cover crops for promoting biological control of early and late season pests in cotton*- We will seek regional and federal funding for this work. We have reached a point in our studies where we are ready to expand this project and incorporate a larger scale to both the research and extension components that will lead to further integration into production tool kits for sustainable crop production.
4. To develop augmentation and conservation biological control tactics, especially to improve the quality of agricultural habitats for pollinators
    a. *Agriculture and solar synergies*-Working with the InSpire funded DOE project, we are setting up a set of experimental plots to assess the synergies of solar and habitat provisioning (i.e. wildflower habitats) for promoting biological control communities, and pollinators in agricultural landscapes. This is a multi-year, multistate project. And, GA is one node forming in this network. Wildflower areas will be planted and we will sample for ability of wildflowers to boost biodiversity in these systems. Also assess costs of production of biodiversity and diversity related services in relation to habitat management in solar systems.

b. *Attracting pollinators and natural pest managers: screening plants for ease of use and maintenance* – We will publish the work from the last 2 years this year, and into the current design of plants we will add new species for testing of attractiveness. Our discussion and design will be moving towards creating our own mixes for different situations (i.e. urban landscapes meadows, backyard garden, turf and agricultural settings).

**PUBLICATIONS (2018)**

*Peer reviewed articles*

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Schmidt JM, Graham C, Shapiro-Ilan D, Sparks A, & Riley D. 2018. Virulence of entomopathogenic nematodes and fungi to cowpea curculio larvae, *Chalcodermus aeneus* (Coleoptera: Curculionidae). Journal of Entomological Science. 53(2): 152-161.

*Theses*

Xavier S. Evaluating the context dependencies of floral provisioning for beneficial arthropods.

*Presentations - Academic*

2018 Schmidt JM. Enhancing the multifunctionality of agro-forestry landscapes. Department of Entomology, Penn State University. College Station, PA.

2018 Schmidt JM, Alabady M, Wang Z, Olson D, & Coffin A. Cross-system biodiversity exchange: Implications of crop rotation for arthropod communities. ESA, ESC and ESBC Joint meeting. Vancouver, Canada, BC

2018 Schmidt JM. Diversity of biological control challenges and systems in the southeast. Southeastern Branch ESA, Orlando, Fl.

2018 Bowers C, Toews MD, Gaskin J, & Schmidt JM. Do cover crops structure natural enemy communities and trophic interactions in cotton agroecosystems?. Annual Meeting of the Entomological Society of America and Joint ESC and ESBC. Vancouver, Canada, BC.

2018 Bowers C, Gaskin J, Toews M, & Schmidt JM. Cover cropping to promote spider diversity in cotton agroecosystems. Annual Meeting of the American Arachnological Society. Ypsilanti, MI.

2018 Schmidt JM. Cotton food webs: implications of spiders as natural pest managers. In Annual Meeting of the American Arachnological Society. Ypsilanti, MI.

*Presentations-Extension*

2018 **Schmidt JM.** Cover crops and beneficial insects. Cotton GA Commission field day.

2018 **Schmidt JM.** Cover crops and beneficial insects. Cotton and peanut field day. Midville, GA

2018 **Schmidt JM.** Farming with beneficial insects. Xerces Society, Americus, GA.

2018 **Schmidt JM.** Show me the predators: unraveling the enemies of Georgia Blueberry pests. In Southeast Regional Fruit and Vegetable Conference Regional

**S-1073 ANNUAL REPORT FOR 2018 AND PLANS FOR 2019**

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## ACCOMPLISHMENTS

1. To discover, assess, and release new biological control agents

   *Giant salvinia*: Initiated a colony of a cold tolerant population of the salvinia weevil in quarantine. The goal of this project was to establish a cold tolerant population of the salvinia weevil at the LSU quarantine. A collecting trip was conducted in June 2018 to Uruguay by Rachel Watson. Eight hundred weevils were imported to LSU. During summer and fall of 2018, efforts were made to increase the population of weevils in quarantine. In October of 2018 there were F1 generation emergences which were reared through the fall, although there are not enough emergences to successfully establish a colony in maximum security.

   *Air potato*: Documented the establishment and impact of the air potato beetle in central and south Louisiana. Air potato (*Dioscorea bulbifera*) is an invasive vine from Asia. As 2018, air potato has been reported in 16 parishes in Louisiana. Since 2016, we have been releasing the biological control agent *Lilioceris cheni*, in central and south Louisiana. During 2018, we monitored the establishment rates and impact of this beetle. We found that the cold temperatures experienced in Spring 2018 delayed the emergence of overwintering adults. Due to the minimal resurgence of beetles by June 2018, we had to make more releases at four locations in Louisiana. Monitoring at the Grand Isle revealed that air potato grew rapidly in the early summer and became a major problem by the fall due in part to the minimal impact of the beetle. To let people know about the biological control program, we made several presentations and talked with park rangers, extension agents and local land managers. In cooperation with Dr. Veronica Manrique from Southern University, a PhD student was recruited to lead the research on overwintering ecology of the air potato beetle and impact of the biological control program in Louisiana.

2. To characterize and evaluate the impact of native and introduced biocontrol agents

*Conyza*: This collaborative project with the Australian government will help Louisiana to understand the ecology of this weed. Specifically, we will understand whether *Conyza* is reservoir of crop pests and plant pathogens; and determine potential biological control agents for Australia.

*Roseau cane die-offs*: Determined the population dynamics of the roseau cane scale and its parasitoids in Louisiana. During 2018, the population dynamics of the roseau cane scale was studied at four transects in the Mississippi River Delta (MRD). The number of scales per stem were counted at 36 sites at the MRD every two months. Results demonstrated that despite of the severe cold days experienced in December 2017 and January 2018, the density of scales increased in the spring and reached outbreak conditions by August 2018. Mean number of scales per meter of stem in August fluctuated between 100 and 140. Based on this outcome, we know that cold winter days or short freezing events do not affect the population growth of the scale in southern Louisiana. Crawler activity was monitored using sticky traps in three sites located in southern Louisiana. We found crawlers as early was March and rapid increase in numbers from June to August. There was a rapid decline in crawlers numbers from September to December. By the fall 2018, the scale was present in almost every stem of roseau cane at the MRD, demonstrating its adaptation for rapid colonization and population growth.

*Crapemyrtle bark scale*: Finished studies on the development time, host range and cold tolerance of the crapemyrtle bark scale. Laboratory studies revealed that 27.5 °C was the optimum temperature for egg hatching with the shortest time (10 d) and the highest hatching rate (95%). The developmental time for *A. lagerstroemiae* from nymph to prepupa and gravid female was 56 d and 68 d at 30 °C, respectively. Five plant species besides crapemyrtle (*Lagerstroemia* indica × fauriei L.) were able to support the immature development and reproduction of A. lagerstroemiae under no-choice conditions, including *Lawsonia inermis* L., *Heimia salicifolia* Link, *Punica granatum* L., *Lythrum alatum* Pursh (all Lythraceae), and *Callicarpa americana* L. (Lamiaceae). These results suggest that the crapemyrtle bark scale is an oligophagous species capable of maintaining populations in several plant species. Due to the temperate origin of CMBS, there was a need to understand its potential distribution in United States. Using laboratory experiments, we described the thermal tolerance of CMBS and constructed a map with the isothermal lines. For example, the super cooling point of nymphs collected in summer was higher than those collected in fall (−21 vs. −27°C), and the exposure time leading to Lt90 at 0°C was also different, which were 8 versus 50 h comparing nymphs collected in summer versus fall. Based on these results we predict that the CMBS is likely to be limited by cold temperatures along the 43° N latitude. The physiology of the CMBS was studied in collaboration with Dr. Roger Laine at the LSU Department of Chemistry. We characterized the biochemical changes of CMBS over the growing season. Results suggested that water content was lower in winter and early spring than in summer and early fall (40.8% vs. 63.3%). The proportions of the fatty acids in phospholipids were similar over seasons, but in TAG, shorter chain fatty acids (from C6:0 to C10:0) increased in winter as longer chain fatty acids (from C14:0 to C18:0) decreased. Results from this study provided an understanding on how CMBS overwinters.

3. To develop integrated pest management programs that have a biological control component

   *Giant salvinia*: Determined the impact of the herbicide 2,4-D on the salvinia weevil. Plant managers use different herbicides to control giant salvinia and other unwanted plants in Louisiana. However, limited information is available on the susceptibility of the salvinia weevil to direct and indirect application of the herbicide 2,4-D. During 2018, we evaluated the effect of 2,4-D and nonionic surfactant on adult salvinia weevil survivorship, and determined how different application rates of herbicide affect salvinia weevil survivorship. We found that the application of 2,4-D directly to salvinia weevil resulted in 9% mortality, whereas surfactant alone or in combination with the herbicide resulted in mean insect mortality of 21%. In an outdoors experiment, the application of 2,4-D alone and in combination with surfactant to giant salvinia infested with the salvinia weevil resulted in 9 to 23% mortality, and the application of surfactant alone or in combination with herbicide resulted in 9 to 19% indirect mortality. These results suggest that 2,4-D has limited negative impacts on the salvinia weevil. Control of giant salvinia might continue when using these two methods of control. A peer-reviewed publication of this research was published in May 2018.

4. To develop augmentation and conservation biological control tactics, especially to improve the quality of agricultural habitats for pollinators

   *Crapemyrtle bark scale*: Developed research protocols to understand the impact of entomopathogens on the crapemyrtle bark scale. The goal of this project was to evaluate biorational products for control of the crapemyrtle bark scale. In 2018, we established research plots at the Hammond Research Center and the LSU Burden Botanical Garden. Crapemyrtle trees were planted in summer 2018. Survey of scales and natural enemies were conducted in summer and fall of 2018 in north (Shreverport), Central (Baton Rouge) and south (Houma) Louisiana. The objective of these surveys were to document the densities and diversity of natural enemies in different urban environments. We found the the lady beetles *Chilocorus cacti*, *Hyperaspis bigeminata* (Coleoptera: Coccinellidae) and the minute pirate bug, *Orius insidiosus* (Hemiptera: Anthocoridae) were the most common natural enemies associated with the scale. Based on these results, the non-target impact of entomopathogens will be evaluated on these two coccinellids.

## UTILITY OF FINDINGS

*Giant salvinia*: Efforts in the research program led to the improvement of giant salvinia management in several regions of Louisiana. The establishment of the Argentinean population of the salvinia weevil in quarantine will facilitate the process of federal approval for field releases.

The expectation is that this population could survive winter temperature in North Louisiana, and thus increase the population growth earlier in the season. Research on the effect of fabrics or plant crowding on salvinia and the weevil suggested that these techniques can increase weevil overwintering survival in temperate regions. Our research on the interaction of the herbicide 2,4-D and weevils suggests that plant managers can use 2,4-D to manage broadleaf weeds with minimal impact to the weevils.

*Air potato*: In 2015, we made the first release of air potato beetles in Louisiana. The air potato beetle (*Lilioceris cheni*) is a chrysomelid specialist on air potato and approved by the USDA. Surveys in 2018 demonstrated that air potato beetle is established at every release site. Drastic reductions in air potato coverage were documented at several state and city parks. These results suggest that the beetles can be incorporated in the toolbox for land managers.

*Crapemyrtle bark scale*: Studies revealed that the scale has adaptations to expand its geographical range in United States. Our results suggest that nursery growers and homeowners in temperate regions of the United States should incorporate management tactics against this scale. Not only this scale is capable of surviving cold winters but also it can attack several tree species. During 2018, we developed protocols to assess the impact of entomopathogens in greenhouse and field settings. Due to the demand of homeowners for control methods, we need determine whether these products can be incorporated into an IPM program of the scale.

*Chinese tallowtree*: Chinese tallow tree is one of the most invasive trees in Southeastern United States. Field studies were conducted to understand the growth and local herbivory on C1hinese tallowtree before the release of two biological control agents. Long term monitoring sites were established in Louisiana. Field measurements suggested that the impact of a local leafminer seems minimal under field conditions and simulated herbivory had minimal impact on tallow growth under high soil fertility conditions. These results suggest that classical biological control of Chinese tallowtree should not be used alone but instead in combination with other tactics including herbicides and mechanical control.

*Roseau cane scale:* Roseau can die-offs were detected at the Mississippi River Delta in 2016. Due to spatial extent of the problem, a multidisciplinary team was assembled. Key efforts were made to raise awareness of this problem with different agencies and stakeholders in Louisiana. Field surveys demonstrated that the densities of an exotic scale decrease drastically in the winter and remained low until May. Outbreaks of the scale were documented in 2018 despite the severe freezes experienced at the Mississippi River Delta. Parasitoid wasps were one of the most important sources of mortality of the scale, and potential management tactics such as burning or insecticides should take in consideration their impact to parasitoids. There are four varieties of roseau cane in Louisiana, and our studies suggest that the 'European' variety is more resistant to the scale attack. Ongoing studies will determine whether this variety can be used in restoration of die-off sites.

## WORK PLANNED FOR NEXT YEAR (2019)

*Giant Salvinia*: We will try to establish a colony of the Argentinean population of the salvinia weevil in a quarantine greenhouse. Upon consultation with USDA-APHIS we will determine the feasibility and timeline of releasing this population in central and north Louisiana. We will continue the monitoring and impact of the salvinia weevil in coastal Louisiana. In collaboration with biologists at Cross Lake, we will be monitoring the population dynamics and impact of the salvinia weevil in North LA.

*Air potato*: In collaboration with Southern University, we will continue with releases and monitoring the impact of the air potato beetle. In addition, we will be conducting experiments related to the overwintering ecology of air potato beetle.

*Crapemyrtle bark scale*: The impact of commercial entomopathogens against the crapemyrtle bark scale will be studied under laboratory, greenhouse and field conditions.

*Roseau cane die-off*: We will coordinate several projects related to the roseau cane scale and the other stressors of the cane. We will continue with studies on the host range of the roseau cane scale, and on varietal resistance to stressors.

*Chinese tallowtree*: We will continue monitoring the impact of local herbivores on tallow in common garden plots at Southern University and LSU Burden. Field infestations in Central and South Louisiana will be monitored.

*Parrot feather*: Studies will be conducted on the biology, distribution, and impact of the weevil, *Parenthis vestitus*.

*Flax-leaf fleabane*: Studies will be conducted on the ecology and distribution of Flax-leaf fleabane and close relatives in Louisiana, Texas and Mississippi. Studies on the biology and host range of selected insect herbivores will be conducted under laboratory and field conditions.

## PUBLICATIONS (2018)

Burckhardt, D., Cuda, J. P., **Diaz, R**., Overholt, W. A., Prade, P., Queiroz, D., Vitorino, M. D. and G. S. Wheeler, G. S. 2018. Taxonomy of *Calophya* species associated with Brazilian peppertree (Hemiptera: Calophyidae). Florida Entomologist 101: 178-188.

Cozad, L. A. *, Harms, N., Russell, A. D., De Souza, M. and **R. Diaz**. 2018. Is wild taro a suitable target for classical control in the United States? Journal of Aquatic Plant Management 56: 1-12.

Knight, I. **, Wilson, B. E., Gill, M., Aviles, L., Cronin, J. T., Nyman, J. A., Schneider, S. A. and **R. Diaz**. 2018. Invasion of *Nipponaclerda biwakoensis* (Hemiptera: Aclerdidae) and *Phragmites australis* die-back in southern Louisiana, U.S.A. Biological Invasions 10: 2739-2744.

Smith. M., Overholt, W. A., Lake, E., **Diaz, R**., Manrique, M., Hight, S., Rohrig, E., Minteer, C., Wheeler, G., Rayamajhi, M., Bowers, K. and C. Kerr. 2018. Changes in latitude: overwintering survival

of two *Liliocerис cheni* (Coleoptera: Chrysomelidae) biotypes in Florida. Biocontrol Science and Technology 28: 293-306.

Wahl, C. *, Mudge, C. and **R. Diaz**. 2018. Does the aquatic herbicide 2,4-D and a non-ionic surfactant impact survival of salvinia weevil? Journal of Aquatic Plant Management 56: 113-119.

**S-1073 ANNUAL REPORT FOR 2018 AND PLANS FOR 2019**

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## ACCOMPLISHMENTS

1. To discover, assess, and release new biological control agents

2. To characterize and evaluate the impact of native and introduced biocontrol agents

3. To develop integrated pest management programs that have a biological control component

   A component of this objective was interaction with cooperators to test impact of tomato lines with acylsugar mediated insect resistance to determine impacts on targeted insect pests, and also on natural predators/parasites of the targeted pests, to generate the information needed to effectively and efficiently utilize acylsugar producing tomatoes within an integrated pest control system. Work to date has increased seed supply of the best acylsugar lines, which significantly reduced presence of western flower thrips and sweetpotato whiteflies, and significantly decreases likelihood of plant infection by the virus vectored by those pests (Tomato spotted wilt virus or tomato yellow leaf curl virus, respectively). The increased seed levels will support trials planned. We also were able to expand the network of cooperators, interested in using the acylsugar producing tomato lines in insect/virus trials. Since there have been reports that acylsugars might also impact fungal pathogens, acylsugar producing lines were also included in a 2019 field trial heavily inoculated with the *Alternaria tomataphila,* the pathogen that causes early blight. Data are still being collected, but data to date show slower disease development and reduced defoliation compared to normal tomato line controls. While control of fungal diseases was not a goal of this project, potentially achieving extra benefits beyond those intended is quite positive, and if acylsugars are the source of the reduced fungal disease, the trait would have additional benefits for a comprehensive IPM management strategy.

4. To develop augmentation and conservation biological control tactics, especially to improve the quality of agricultural habitats for pollinators

**UTILITY OF FINDINGS**. The findings to date will be used to inform plans for the coming year.

**WORK PLANNED FOR NEXT YEAR (2019)**

To interact with cooperators to perform acylsugar assays on tissue samples collected from their experiments, increase seed stocks of the newest lines in development, that have similar acylsugar levels, but improved horticultural characteristics, for potential use in future trials.

**PUBLICATIONS (2018)**

1. Ben-Mahmoud, S, JR Smeda, TM Chappell, C Stafford-Banks, CH Kaplinsky, T Anderson, MA. Mutschler, GG Kennedy, DE Ullman 2018 Acylsugar amount and fatty acid profile differentially suppress oviposition by Western flower thrips, Frankliniella occidentalis, on tomato and interspecific hybrid flowers. PlosOne doi.org/10.1371/journal.pone.0201583

**IMPACT STATEMENT:** https://www.enago.com/academy/writing-an-impact-statement-four-things-you-need-to-know/

## S-1073 ANNUAL REPORT FOR 2018 AND PLANS FOR 2019

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**ACCOMPLISHMENTS**

*Project Initiated 3-8-19. No report of accomplishments for 2018. Publications listed below summarize research from participation in multi-state project that ended 2017.

1. To discover, assess, and release new biological control agents. NA
2. To characterize and evaluate the impact of native and introduced biocontrol agents
3. To develop integrated pest management programs that have a biological control component
4. To develop augmentation and conservation biological control tactics, especially to improve the quality of agricultural habitats for pollinators

**UTILITY OF FINDINGS:** NA

**WORK PLANNED FOR NEXT YEAR (2019):** Related to objectives 2, 3, 4

*Efficacy studies with conventional and biorational insecticides will be conducted in multiple field crop systems to document effects on targeted pests and natural enemies.

*Studies describing spatial and temporal patterns of aphid parasitism and pollinators in agriculture landscapes of the Southern Plains will continue. A new study will be initiated to document aphid parasitism in organic wheat systems with the specific goal to include parasitism level into management programs, similar to that utilized in conventional wheat systems.

*Studies investigating the interaction between host-plant resistance in winter wheat and aphid parasitism will be initiated.

**PUBLICATIONS (2018)**

Elliott, N. C., M. J. Brewer and K. L. Giles. 2018. Landscape Context Affects Aphid Parasitism by *Lysiphlebus testaceipes* in Wheat Fields. Environ. Entomol. 47: 803-811.

Ferguson, M. E., K. L. Giles, M. Payton and T. A. Royer. 2018. Behavioral and Ovipositional Response of *Diaeretiella rapae* (Hymenoptera: Braconidae) to *Rhopalosiphum padi* and *Brevicoryne brassicae* in Winter Wheat and Winter Canola. Environ. Entomol. 47: 1517-1524.

# S-1073 ANNUAL REPORT FOR 2018

**Period the Report Covers**: 10/01/2017 - 09/30/2018

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## *Accomplishments:*

*(1) Characterize and evaluate the effect of established natural enemies.*
A Biological Control Demonstration Site established near Cosby, TN, in the Great Smoky Mountains National Park (GRSM) continued to be monitored for recovery and establishment of introduced predators of hemlock woolly adelgid. This Site serves as an educational tool for land managers interested in incorporating biological controls into their management programs directed against hemlock woolly adelgid. A cooperative multi-state, regional approach continues to assess establishment and impact of *Laricobius nigrinus* in areas where it has been released and recovered. States involved are MA, NC, TN and VA. Two sites (Elkmont Campground and the Biological Control Demonstration Site) were used in the 2018 study. Data collected in 2018 continued to quantify predation of *L. nigrinus* in a field setting across a wide geographical area. Data were shared with other cooperators, who compiled, analyzed, and summarized results and presented findings at various meetings. Research continues to assess establishment of three parasitoids of emerald ash borer. Extremely low numbers of two of these parasitoids have been recovered, suggesting that their establishment in the southern U.S. may be difficult due to asynchrony of adult parasitoids with larvae of emerald ash borer. An egg parasitoid has not been recovered. A fungal pathogen (*Beauveria bassiana*) and an egg parasitoid provide excellent management of kudzu bug [*Megacopta cribraria* (Hemiptera: Plataspidae)] in middle and eastern Tennessee. The egg parasitoid was recovered for the first time in Tennessee and Georgia in 2017 and was identified in 2018 as *Ooencyrtus nezarae* (Hymenoptera: Encyrtidae) on eggs of kudzu bug collected from kudzu.

*(2) Exploration, importation, and assessment of natural enemies for invasive pests.*
Efforts continue to assess the introduced *L. osakensis*, which was collected by Dr. Pat Parkman near Osaka, Japan, in December 2015, against hemlock woolly adelgid and to enhance existing laboratory populations for mass rearing. We also began conducting geographical synchrony and compatibility studies on an exotic parasitoid, *Spathius galinae*, of emerald ash borer. This project

is a cooperative effort with researchers in Massachusetts, New York, and Tennessee. Cooperators in other parts of the southern U.S. (such as Louisiana) are now involved in releasing and assessing these introduced parasitoids against emerald ash borer. A new project was initiated on biological control of crape myrtle bark scale, a new invasive pest found at several locations in middle and west Tennessee. A study is underway to assess presence, seasonality, and impact of native natural enemies of this new pest. Also, efforts are underway to collaborate with scientists at Nanjing Agricultural University, in Nanjing to identify potential natural enemies that could be evaluated for importation against this invasive pest species.

*(3) Implementation, evaluation, and enhancement (e.g., conservation) of biological control.*

**HEMLOCK WOOLLY ADELGID**

As part of efforts to implement biological control against invasive species, the Lindsay Young Beneficial Insects Laboratory (LYBIL) at the University of Tennessee continued to rear and release natural enemies of the hemlock woolly adelgid. LYBIL currently rears two predators of the hemlock woolly adelgid: *Laricobius nigrinus* and *Laricobius osakensis*. *S. tsugae* (a predator previously reared for many years) and *L. nigrinus* have been reared at LYBIL since 2003 and 2005, respectively. *L. osakensis* was reared at LYBIL in 2011, and has been reared from 2013 to the present. The following information details specific rearing and release activities during 2018 (releases occurred in Kentucky and Tennessee):

***Sasajiscymnus tsugae* –** None of this beetle species was reared and released in 2018. Efforts were focused on the two *Laricobius* spp. in 2018, with *L. osakensis* considered to be a better predator of hemlock woolly adelgid than *L. nigrinus* or *S. tsugae*.

***Laricobius nigrinus* –** Improvement in rearing *Laricobius nigrinus* continues from last year. As of September 2018, survival of mature larvae to adults was greater than 70% with more adults emerging every day. In 2017, the laboratory attained 83% survival. Average survival of the 3 previous years (2014-16) was 48%. Improved success was mainly attributed to lengthening exposure of aestivating adults to cool temperatures. More than 6,000 *L. nigrinus* have been produced so far this year, with almost 5,000 being released.

***Laricobius osakensis* –** The Kansai (southern Japan) laboratory colony of *Laricobius osakensis* has been expanded. The founding beetles of the colony were collected near Osaka in late 2015, and had only about 45 to initiate the laboratory colony in early 2016. Using these 45 adults, 2,061 adults were produced in 2016, 5,037 in 2017, and 5,896 as of late December 2018 with about 200 more emerging each week. Almost 4,300 of these beetles have been released so far, in Tennessee, Kentucky and North Carolina.

**Releases** – Almost 4,300 of these beetles have been released so far in 2018, in Tennessee, Kentucky and North Carolina. *Laricobius* adults were released in a new and promising location at the northwestern edge of the hemlock woolly adelgid's expansion, at Pickett State Forest in Tennessee. It is anticipated that this release site will become a field nursery for natural enemies. Collaboration with the Kentucky Division of Forestry continues, and they were provided with more than 1,600 *Laricobius* beetles in 2018 for release in the Daniel Boone National Forest. In addition, more than 1,200 *Laricobius* adults were provided to the U.S.D.A. Forest Service for release at their field nursery sites in North Carolina.

***EMERALD ASH BORER*** (EAB)

Following its discovery in Tennessee in 2010, introduced natural enemies of EAB

(*Spathius agrili*, n ≈ 24,000, and *Tetrastichus planipennisi,* n ≈ 84,000) were released in eastern Tennessee. To assess recovery, pan traps were collected biweekly during colder months (Nov. 2016 through December 2018) and weekly in warmer months (Apr. through Oct. 2017 and 2018). Both native and introduced parasitoids were collected from pan traps. *S. agrili* has demonstrated the ability to survive the winters in the south and emerge the following season, although they have been recovered in low numbers. Only 1 *T. planipennisi* was recovered at release sites in 2017; it is suspected that several were found in 2018 but identifications have not yet been confirmed. Phenology studies of EAB and three larval parasitoid species (*S. agrili*, *S. galinae*, and *T. planipennisi*) continued in 2018. Larval parasitoid phenology was assessed by conducting a growth-chamber study initiated May 2016 and continuing through early 2018. Similar results were found each year, with only *S. galinae* producing an F2 generation and no species producing an F3 generation. Monitoring of remaining bolts in growth chambers will continued through 2018 and did not produce any more adult parasitoids. The discovery of *S. agrili* in a release site is promising, as it indicates *S. agril*, an introduced species, can overwinter and reproduce in the south although its recovery is rare. The low recovery of *T. planipennisi* suggests that they may not establish as readily in the southern U.S. as in the northern U.S. A three-year study to assess phenological data was completed in 2018, and suggests that EAB exhibits an annual life cycle in the south as opposed to the 1½ year life cycle it displays in the northern US. This one-year life cycle suggests that small windows of opportunity exist for parasitoids to find a suitable host in the southern as opposed to the northern US. This difference in life cycle is the primary cause of lack of establishment of these introduced parasitoids in the southern US, where parasitoids and EAB are phenologically asynchronous. These life cycles also explain the relative success of these parasitoids in the north. Climate matching needs to be examined to search for parasitoid species better adapted for warmer climates. Researchers in Massachusetts are searching for natural enemies that may be better adapted for a summer climate.

***KUDZU BUG*** Research to address kudzu bug biology and ecology, as well as the incidence of a fungal pathogen *Beauveria bassiana*, continued in 2018. Kudzu bug densities were low in 2018, continuing the decline trends that were evident in 2016 and 2017. This decline is most directly related to high infection levels of the fungus and incidence of an egg parasitoid (*Ooencyrtus nezarae)*. Infection of both nymphs and adults by *B. bassiana* (from late August to late October) was extremely high late in 2017 and in 2018, but higher for nymphs. Average mortality of kudzu bug caused by *B. bassiana* peaked at 84% across six counties. This high mortality of kudzu bug populations late in the season will continue to reduce overwintering populations of kudzu bug. In 2017, an unidentified egg parasitoid species was recovered from eggs collected from kudzu. Parasitism rates were highest at the end of June, with an average of 51% parasitism of eggs across 6 counties. This parasitoid was later identified as *Ooencyrtus nezarae*. Near season-long biological control of kudzu bugs within kudzu patches appears possible. With the parasitoid active from at least June – mid September and the fungus active from late August – late October, numbers of kudzu bug can be suppressed throughout the season. These reductions also will be seen in soybean fields, as overwintering numbers of kudzu bug continue to decline or remain low.

***CRAPE MYRTLE BARK SCALE*** A new research project was began to assess incidence of crape myrtle bark scale on crape myrtle and to assess the natural enemies associated with this

invasive pest in Tennessee. The goal is to assess native biological control agents to identify niches that are not occupied by native natural enemies or to identify native natural enemies that can be augmented and enhanced to mitigate this invasive pest, which has been found at several locations in middle and west Tennessee. A study is underway to assess presence, seasonality, and impact of native natural enemies of this new pest. Also, efforts are underway to collaborate with scientists at Nanjing Agricultural University, in Nanjing to identify potential natural enemies that could be evaluated for importation against this invasive pest species.

## *Short-term Outcomes:*

This project has led to the development of a Biological Control Demonstration Site in the Great Smoky Mountains National Park which can be used to illustrate how to successfully release, evaluate and assess introduced biological control agents of hemlock woolly adelgid. In addition, opportunities have been provided to graduate students and scientists to attend forest-related meetings and other professional meetings to enhance their knowledge and skills in biological control. Project personnel participate in various outreach activities each year to promote biological control and discuss ongoing management programs. These activities help to develop a more informed and aware public knowledgeable on biological control and its role in management of pest species.

## *Outputs:*

Results have been disseminated through outreach activities (Ag Day and other public-oriented functions), presentations at workshops and seminars, and presentations at professional scientific meetings. In addition, results have been disseminated via scientific publications (such as journal articles, proceedings, etc.) and the internet. In 2018, 25+ presentations were given on biological control activities in Tennessee; these included four presentations on hemlock woolly adelgid, four presentations on emerald ash borer, five presentations on kudzu bug, three presentations on walnut twig beetle (vector of pathogen that causes thousand cankers disease), five on biological control efforts directed against forest pests or in agroecosystems, and several on crape myrtle bark scale. In addition, during this reporting period, we produced three M.S. theses (one focused on biological control of kudzu bug, one focused on biological control of emerald ash borer, and one focused on ecology and spread of walnut twig beetle), as well as one extension publication, five refereed journal articles, and six published abstracts/proceedings (see listing below).

**Products/Publications**

Hakeem, A., J. F. Grant, P. L. Lambdin, F. A. Hale, J. R. Rhea, G. J. Wiggins and C. Coots. 2018. Influence of imidacloprid and horticultural oil on spider abundance within eastern hemlock canopy in the Southern Appalachians. Environmental Entomology doi: 10.1093/ee/nvy065

Liang, W., L. Tran, J. Grant, M. Papes, G. Wiggins, R. Washington-Allen, and S. Stewart. 2018. Predicting the potential invasion of kudzu bug, *Megacopta cribraria* (Heteroptera: Plataspidae), in North and South America and determining its climatic preference. Biological Invasions doi: 10.1007/s10530-018-1743-y

Liang, W., M. Papes, L. Tran, J. Grant, R. Washington-Allen, S. Stewart, and G. Wiggins. 2018. The effect of pseudo-absence selection method on transferability of species distribution models in the context of non-adaptive niche shift. Ecological Modelling 388:1-9.

Wiggins, G., E. Benton, J. Grant, M. Kerr, and P. Lambdin. 2018. Short-term detection of imidacloprid in streams following applications in forest. Journal of Environmental Quality 47:571-578.

Yeary, W., A. Fulcher, H. Zhu, W. Klingeman, and J. Grant. 2018. Spray penetration and natural enemy survival in dense and sparse plant canopies treated with carbaryl: Implications for chemical and biological control. Journal of Environmental Horticulture 36:21-29.

**Other non-periodical, one-time publications:**
Philip Hensley. 2018, Incidence, Dispersal, and Risk Assessment of Walnut Twig Beetle, *Pityophthorus juglandis*, in Forests. M.S. Thesis, University of Tennessee.

Forest Palmer, M.S., 2018. Assessment of Introduced Parasitoids of Emerald Ash Borer as Part of an Integrated Pest Management Strategy. M.S. Thesis, University of Tennessee.

Amy Michael, M.S., 2018. Interactive Ecology of the Kudzu Bug in Eastern Tennessee. M.S. Thesis, University of Tennessee.

**Extension Publications**
Britt, K., C. Standish, J. Grant, and K. Vail. 2018. Managing Brown Marmorated Stink Bug in and Around Homes. Extension Publication W 779, University of Tennessee, 6 pp.

## *Activities:*
Developed and distributed educational displays to illustrate life cycle and impact of emerald ash borer on ash to emphasize the need for introduced biological control agents of this devastating exotic species. Several biological control agents are being mass produced for release in several states (Kentucky, North Carolina, South Carolina, and Tennessee) against hemlock woolly adelgid, and native and introduced biological control agents are being evaluated and assessed against emerald ash borer, kudzu bug, walnut twig borer, and brown marmorated stink bug.

## *Impacts:*
This project enhances the educational and research experiences of six graduate students (five M.S. and one Ph.D.) in the area of biological control, as well as providing information to undergraduate and graduate students in at least three courses. Published peer reviewed articles, M.S. thesis, research reports and scientific presentations have been produced from results of studies designed to 1) characterize and evaluate the effect of established natural enemies, and 2) implement, evaluate, and enhance (e.g., conservation) biological control. Hemlock woolly adelgid has killed hundreds of thousands of trees throughout the eastern U.S. The loss of this long-lived, foundation species in our forests has numerous ecological, environmental, social, and economical ramifications, as well as safety issues when dealing with so many dead trees in highly traveled and visited areas, such as the Great Smoky Mountains National Park. Thus, the management of this invasive insect species introduced from Japan is critical to forest health, aesthetics, safety, and the environmental sustainability of forest systems. We evaluated the establishment, spread and coexistence of two introduced predators of hemlock woolly adelgid,

and the factors responsible for their success. The results of our research provided new knowledge to enhance and revitalize biological control and management efforts against hemlock woolly adelgid. This new knowledge will improve establishment of released biological control agents, resulting in more successful release sites and improved tree health and survival. A new invasive insect threat (emerald ash borer) has been documented in 25+ counties in Tennessee, and when first found in Tennessee, represented the southernmost distribution of this insect pest in the U.S. This insect threatens to greatly reduce, if not cause the extinction of, populations of ash in the U.S., leading to tremendous economical and ecological losses. Entomologists at UTIA are at the forefront of research focusing on emerald ash borer in the southern U.S., with efforts directed at implementation of biological control to protect ash trees in forests, nurseries, and urban areas. Results of this research have demonstrated that these introduced natural enemies of emerald ash borer do not overwinter and survive in Tennessee; thus, releases in these southern areas should be limited to allow resources to be provided to more northern climates where these parasitoids are successful. Research demonstrates the need for climate matching of introduced parasitoids that are better adapted to southern climates. The ultimate goal is to provide sufficient mortality of emerald ash borer to protect trees and minimize adverse environmental impacts. Research on kudzu bug has led to the discovery of two natural enemies (egg parasitoid and fungal pathogen) which will reduce the impact of this pest on soybean production.