S-1058 ANNUAL REPORT FOR 2014 AND PLANS FOR 2015

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ACCOMPLISHMENTS

Objective 1. Characterize and evaluate the effect of established natural enemies.

Arkansas efforts under this objective previously documented that natural enemies already established in the agroecosystems systems have provided inadequate control of fire ant, Japanese beetles and of spotted knapweed. These findings provided the impetus to consider addition of new, approved biological control agents to these systems or to research methods to enhance the efficacy of the agents already established.

Objective 2. Exploration, importation, and assessment of natural enemies for invasive pests.

Research under this objective remains focused on the newly released and/or established biological control agents for red imported fire ant and spotted knapweed. The outcome of a lab study with the fire ant biological control agents *Pseudacteon curvatus* and *P. obtusus* suggest the two species should coexist. These findings were consistent with field studies in Florida and Texas. Release sites for *Larinus minutus* indicate significant spread of the species from all release locations, with recoveries several miles from the closest release sites. The biological control agent is now broadly established across much of northwestern Arkansas. Despite prior year recoveries of the knapweed root weevil (*Cyphocleonus achates*), it was not recovered in 2014. Previously published reports that these even though weevils were well-established in an area, in some years the weevils are scarce and not commonly collected. Therefore, surveys will be intensified during 2015.

Objective 3. <u>Implementation, evaluation, and enhancement (e.g., conservation) of biological</u> <u>control</u>.

Research under the third objective has largely involved work with the fire ant and its natural enemies (decapitating phorid flies in the genus Pseudacteon) and efforts in organic strawberry production systems in high tunnels. A field study comparing *Pseudacteon* sampling methods in associated with fire ants was completed in 2014. Results indicated that sticky traps (both the Puckett and fly ribbon/sandwich trap) were effective in capturing multiple Pseudacteon species when baited with either live or dead ants. Traps baited with fire ant attractant bait to attract and capture P. curvatus and P. tricuspis were ineffective. These findings will allow more cost-effective sampling in the future to document the establishment and potential impact of the released agents. Monitoring for insects and mites on strawberries produced in high tunnels continues, following conventional (Fayetteville) and organic (Clarksville) practices. In the organic high tunnel, defoliation by cutworms was stopped after an application of Bt (Thuricide) on 6 January. Starting 14 January in Clarksville, biweekly sprays of neem (Natural Guard) followed by a JMS Stylet Oil spray on 15 April suppressed an outbreak of aphids in the organic strawberries that were causing reduced fruit size and quality (honeydew induced sooty mold and cast skins on berries and lower leaves). In cooperation with Eric Riddick (USDA-ARS Stoneville, MS) we released lady beetle (Coleomegilla maculata) eggs and larvae on 27 March and larvae and adults on 17 April to Chandler and San Andreas strawberry cultivars. By 11, 16 April and 2 May, honeydew and mold dropped to undetectable levels and there was a sustained drop in the numbers of aphids per leaflet of Chandler strawberry plants and adjacent plants of Festival, San Andreas and Camarosa.

UTILITY OF FINDINGS:

The foci of the regional research conducted in Arkansas during this reporting year involved two invasive pestiferous species, the fire ant and spotted knapweed and the evaluation of methods to assess movement of natural enemies in natural and managed ecosystems. Redistribution of effective biological control agents from other areas of the US into Arkansas remains the goal of this project. One of the knapweed agents (*Larinus minutus*) is well-established and reducing pest populations as they have done in other states. We have yet to document establishment of the other knapweed agent (*Cyphocleonus achates*), although it appears both introduced fire ant parasitoids are established. These redistributions are conducted under approved state and federal permits, and according to well-established guidelines.

The results of these research efforts were disseminated a broad array of outlets. The scientific community was informed of these findings through the numerous previously-reported publications appearing in refereed journals and through delivery of study details at scientific meetings through presentations delivered by principle investigators and graduate students. Producers (particularly in the fruit industry) were notified of project

results via grower-organized production meetings as well as those organized through the Cooperative Extension service. Results were also provided to affected private landowners through personal visitation by project personnel on numerous occasions.

WORK PLANNED FOR NEXT YEAR (2012):

Annual, comprehensive evaluations of knapweed performance continue at the long-term, static study sites to provide data on the impact of the newly introduced natural enemies. Additional surveys will also be conducted to evaluate the potential establishment of the knapweed root weevil, *Cyphocleonus achates*. If funding becomes available we may continue and expand remote sensing efforts for spotted knapweed. Studies are also being initiated to evaluate the impact of traditional (Highway Department) right-of-way weed management practices on the biology, spread and efficacy of established biological control agents.

PUBLICATIONS (2014)

- Pallipparambil, G. R., R. J. Sayler, J. P. Shapiro, J. M. G. Thomas, T. J. Kring and F. L. Goggin. 2014. *Mi-1.2*, an R gene for aphid resistance in tomato, has direct negative effects on a zoophytophagous biocontrol agent, *Orius insidiosus*. J. of Exp. Botany. doi:10.1093/jxb/eru361
- Minteer*, C. R., T. J. Kring, Y. J. Shen and R. N. Wiedenmann. 2014. Release and monitoring of *Larinus minutus* (Coleoptera: Curculionidae), a biological control agent of spotted knapweed in Arkansas. Florida Entomologist. 97(2): 662-667.

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ACCOMPLISHMENTS

OBJECTIVE 1. Characterize and evaluate the effect of established natural enemies

<u>Air potato</u>: In collaboration with the Florida Department of Agriculture and USDA/ARS, approximately 340,000 adults of the air potato leaf beetle *Lilioceris cheni* (Coleoptera: Chrysomedidae) have been released in Florida since 2012. Preliminary results of an overwintering study suggest that a biotype of the beetle from Nepal may be better adapted to winter conditions in northern Florida.

OBJECTIVE 2. Exploration, importation, and assessment of natural enemies for invasive pests

<u>Cogongrass</u>: Travelled to South Africa from 27 February to 8 March to conduct field surveys for *Acrapex* sp., a native stemborer of cogongrass. On 2 March, surveyed roadsides in Pilanesberg National Park. Unfortunately, did not find cogongrass populations at this particular location.

In collaboration with scientists at Bogor Agricultural University, West Java, Indonesia, several surveys were conducted to identify and locate areas where the gall midge *Orseolia javanica* (Diptera: Cecidomyiidae) was discovered attacking cogongrass. The survey areas included estates with annual plantations, crops areas (paddy, corn, and soybean), open areas, etc. in West Java, especially in the Districts of Bogor and Cianjur. Once cogongrass locations were identified with the symptoms of the gall midge, the coordinates were georeferenced using a GPS unit. Galled plants were then transported to the laboratory for rearing of adult midges or parasitoids. The collected insects were identified using available identification keys, and newly emerged *O. javanica* adults were used to establish a laboratory colony. Rearing of *O. javanica* currently is being conducted in laboratory or glasshouse at Bogor University, West Java, Indonesia.

Foreign exploration for natural enemies of cogongrass also was conducted in Uganda and Japan during this reporting period. In both countries, putatively host specific lepidopteran stemborers

were found; *Acrapex syscia* (Noctuidae) in Uganda and *Acrapex azumai* in Japan. Live individuals of both species were hand-carried to UF/IFAS Biological Control Research & Containment Laboratory (BCRCL), Ft. Pierce, FL, but colonies were not successfully established.

<u>Brazilian peppertree</u>: Stem thrips *Pseudophilothrips ichini* (Thysanoptera: Phlaeothripidae). Host range testing was completed in collaboration with USDA/ARS and a petition for field release was submitted for TAG review in August 2014.

Stem boring weevil *Apocnemidophorus pipitzi* (Coleoptera: Curculionidae). Continued maintaining a colony of *A. pipitzi* collected in Paraguay in 2006 using established procedures. In total, 1,169 new adults were produced in the F17 generation. The F18 generation started emerging in April 2014 and continued until January 2015. To date, 1,247 new adults were produced in the F18 generation.

An experiment was initiated in February 2014 to determine the number of progeny a single mated *A. pipitzi* female could produce during her lifetime. The mean number of adults produced per female was 23.8 ± 22 .

A study was initiated on 16 July 2014 to determine the impact of the weevil A. pipitzi on Brazilian peppertree growth. In total, 9 large potted plants were selected for the experiment. Average plant height was 153.81 cm \pm 9.98 cm, basal diameter was 5.67 cm \pm 1.04 cm, average number of leaves was 358.22 ± 99.62 and average number of stems was 3.66 ± 1.93 for each plant. Three plants were selected randomly to measure the dry weight biomass for calculating a growth index; the mean dry weight of leaves was 56.73 g, stems were 300.7 g and roots were 262 g. The plants were kept in cages (24" length \times 24" width \times 72" height) with 1 plant per cage and were maintained in a rearing room with a high intensity GroLight® located in the UF/IFAS Entomology Department Containment Laboratory (EDCL), Gainesville, FL. Each treatment cage (3 replications) received 5 male and 5 female newly emerged A. *pipitzi* adults and three cages were designated as controls. Abscised leaflets from each cage were collected once per month; leaflets with or without weevil feeding damage were counted. The average number of abscised leaflets from the control cages was 334.3 ± 296 and 672.3 ± 525 from the weevil cages. Of the total abscised leaflets in the weevil cages, 195.6 ± 116.3 or 29% exhibited weevil feeding damage. The experiment is still ongoing to monitor emergence of new adults and to see if the developing larvae are impacting stem development.

At the request of TAG, additional no-choice host range tests were initiated during this reporting period to determine the weevil's "fundamental host range". Because of space limitations, the experiment was divided into 2 phases. Phase 1 included the following test plants: *Pistacia vera* var Kerman, *Schinus molle*, *Metopium toxiferum*, *Rhus copallium*, *R. glabra*, *R. yyphina*, *R. aromatica*, *P. chinensis*, *Toxicodendron vernex* and *Schinus terebinthifolia* (control). Each container received a stem of each test plant (~2cm diameter x 10 cm length), one newly emerged female weevil and 2 males. The weevils were fed with fresh leaves from the same plant from which the stem was obtained. The test was initiated in May 2014 with 5 replications. To date, adult weevils emerged from *R. aromatica* (5.6 ± 5.2), *M. toxiferum* (2.4 ± 2.5), *P. vera* var Kerman (0.4 ± 0.4), *S. molle* (2.6 ± 2.2), *R. copallium* (0.2 ± 0.2), *P. chinensis* (10.2 ± 7.9) and

Brazilian peppertree, *S. terebenthifolia* (10 ± 1.8) . Phase 2 was initiated in September 2014 with 10 additional plant species: *P. vera* var Kerman UCB1 (Pistacia rootstock), *Malosma laurina*, *S. polygamus*, *Comocladia dodonaea*, *T. pubescens*, *P. texana*, *R. integrifolia*, *T. radicans*, and *S. terebinthifolia* (control), also with 5 replications. Phase 2 is still in progress.

The native *R. aromatica* is one of the species from the Phase 1 tests in which *A. piptzi* laid eggs and was able to complete its life cycle. *Rhus aromatica* only occurs in the western Panhandle of Florida- Escambia and Jackson counties. Because North Florida often is subjected to freezing temperatures during winter months, a temperature tolerance experiment was conducted to see if *A. piptzi* would survive the freezing temperatures. The weather data from two weather stations that are in close proximity to these counties were obtained the FAWN website. The longest continuous freezing period for 2013-2014 (temperature ≤ 0 °C) was 53 hours in late January 2014. Newly emerged adult weevils were exposed to five Brazilian peppertree stem (~3cm diam.) in colony cages for about 2 months. Control logs were kept in the cages and the treatment logs were placed in Ziploc freezer bags, placed in a freezer for 53 hours, removed and then returned to the cages. There were 3 replications and each cage is being monitored for adult emergence. To date, 3.0 ± 2.6 adults have emerged in the control cages whereas 0 adults have emerged from the weevil-exposed stems subjected to freezing temperatures for 53 hours.

Leaflet galling psyllids, *Calophya* spp. A petition for release of the leaflet galling psyllid *Calophya latiforceps* (Hemiptera: Calophyidae) was submitted to UF/IFAS for internal review in June, but has not yet been approved for submission to TAG. Since September 2013, up to 9 generations of three leaflet galling psyllids *Calophya* spp. from Brazil were successfully reared at the BCRCL, Ft. Pierce, FL. The life cycle from egg to adult is completed in ~ 40 days.

<u>Bromeliad Weevils:</u> Monitoring of release sites for the parasitoid *Lixadmontia franki* (Diptera: Tachinidae) during this reporting period has provided no evidence of the parasitic fly's establishment.

<u>Cycad Aulacaspis Scale</u>: Eggs of the lady beetle *Phaenochilus kashaya* suffered 100% mortality when exposed to 0°C for more than 1 day. Larvae, pupae, and adults cannot tolerate 0°C for more than a few days and cannot tolerate subfreezing temperatures. Predation by *P. kashaya* adults on green lacewing eggs was very high in both no-choice and choice tests, but the green lacewing is not a predator of the cycad aulacaspis scale. A proposal for field release of *P. kashaya* was resubmitted to APHIS; a decision on the petition is pending.

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

<u>Hydrilla</u>: Laboratory host range testing of the stem miner *Cricotopus lebetis* (Diptera: Chironomidae) showed this insect is not a hydrilla specialist. Other preferred hosts included the native Canadian waterweed (*Elodea canadensis*), the exotic Brazilian elodea (*Egeria densa*) and the native southern naiad (*Najas guadalupensis*). This finding suggests that *C. lebetis* may have developed a "new association" with dioecious hydrilla after this plant became the dominant submersed weed or perhaps was introduced with monoecious hydrilla, another preferred host of the insect. To determine the field host specificity or ecological host range of *C. lebetis*, a survey was conducted from February to December 2014 at Lake Rowell, Bradford Co. FL. Samples of hydrilla and three other dominant aquatic plant species (coontail, *Ceratophyllum demersum*

water pennywort, *Hydrocotyle ranunculoides*, and water primrose, *Ludwigia* sp. were collected and returned to a IFAS/UF greenhouse in Gainesville for further processing. Of the four dominant aquatic plant species sampled, *C. lebetis* was recovered only from hydrilla and coontail during February and March.

The results of a temperature-dependent development study showed that the optimal temperatures for larval development were between 20 and 30 °C. These data were used to construct a map of the potential number of generations (~ 7-10) per year of *C. lebetis* in Florida. In conjunction with historical weather data, the distribution of *C. lebetis* in the United States was predicted. A distribution maps also was predicted using an ecological niche modeling approach by characterizing the climate at locations where *C. lebetis* is known to occur and then finding other locations with similar climate. The predicted distributions based on the two modeling approaches were not statistically different and suggested that much of the southeastern United States was climatically suitable for establishment of *C. lebetis. (Note: established of the insect was previously confirmed in Louisiana).*

The impact of egg refrigeration on larval hatch and subsequent development was examined because eggs often are refrigerated to delay development for experiments or for transportation to field release sites. Egg masses that were stored in a refrigerator at 5 °C for 1, 2, 4, 7, 14, and 21 days were tested for larval hatch rate, development to pupation and adult eclosion. Hatch rate and adult eclosion decreased significantly after 7 and 2 days at 5 °C, respectively. Pupal mortality increased significantly after 2 days. A manuscript of the findings was prepared and submitted for publication in the peer-reviewed Journal of Aquatic Plant Management.

In 2013, laboratory experiments were conducted to test a reduced-risk approach for hydrilla control by integrating selective herbivory by the insect *C. lebetis* with the fungal pathogen *Mycoleptodiscus terrestris* or the ALS inhibiting herbicide imazamox. A manuscript was prepared / submitted to the peer-reviewed Journal of Aquatic Plant Management during this reporting period.

<u>Whiteflies and Aphids</u>: Both groups are important insect pests in vegetable crops. To mitigate the use of chemical insecticides, "push-pull "strategies can be used as components of sustainable or cultural pest management. We conducted field studies using mustard plants (var. giant red mustard and caliente) as companion crops intercropped at different ratios with the target collard crops. Additionally, laboratory olfactometer tests measured the effects of plant volatiles from three varieties of mustard as whitefly repellents. We found that the giant red mustard, nemat and caliente mustard plants are promising repellents against silverleaf whiteflies, *Bemisia* spp. (Hemiptera: Aleyrodidae) thereby comprising a potential "push" component. In another field study, buckwheat was used as an attractive plant for beneficial natural enemies when intercropped with collards. Preliminary analysis combined with a separate study on sweet alyssum intercropped with lettuce revealed the most common predatory hoverflies to be *Eupeodes americanus* and *Allograpta obliqua*. "Push-pull" strategies can be complemented with natural enemy refuges as cultural management techniques in farmscaping towards sustainable management of whiteflies and aphids.

Asian Citrus Psyllid: Research was conducted to improve mass rearing and quality control of

Tamarixia radiata (Waterston), a eulophid parasitoid, for use in managing Asian citrus psyllid, *Diaphorina citri* Kuwayama (Homoptera: Psyllidae) (ACP), in citrus groves. Improvements in the quality of host plants and host insects will increase efficiency and productivity of mass rearing facilities, and yield high quality *T. radiata* wasps. *Tamarixia radiata* rearing was improved by narrowing ACP oviposition time to obtain greater proportions of desirable early 5th instar hosts. Insectary ACP can be selected for larger nymph size for the mass production of *T. radiata*. This results in larger female wasps with higher fecundity and survivorship. Differences in sex ratios produced by the different mass rearing facilities in California and Florida (85% females in CA, 75% females in FL) show that substantial gains can be made in the mass rearing. It is assumed that the quality of a female wasp is determined by her size; this was quantified in the laboratory.

Bromeliad Weevils: Development of the Mexican bromeliad weevil (MBW), Metamasius callizona, occurred at 18-30°C. At 15°C; there was no survival beyond second instar. At 35°C, no eggs hatched. The numbers of eggs collected at 22-33°C were statistically similar at 0.3-0.5 eggs per day per female. At 15 and 18°C, MBW oviposited infrequently; at 35°C, it oviposited infrequently and died prematurely. In a cold tolerance study, half of the test cohort adults held at 0°C for 1 day survived, 20% survived 2 days at 0°C, and no adults survived 4 days at 0°C. Of the test cohort larvae, 60% held at 0°C for 1 day survived, 20% survived 2 days at 0°C, and no larvae survived 4 days at 0°C. Leaves from four host plants were compared for MBW oviposition rate, development time from egg to pupa, and adult size. The oviposition rate in pineapple leaves was greater than all other host types. The oviposition rate in Central American Tillandsia utriculata was similar to that in Florida T. utriculata and Tillandsia fasciculata, but the oviposition rate was greater in Florida T. *utriculata* than in T. *fasciculata*. None of the larvae that were fed leaves from Central American T. utriculata or T. fasciculata developed beyond third instar. Larvae developed almost twice as fast on pineapple leaves than on leaves from Florida T. *utriculata*; development times were similar from pupation to adult emergence. Adult MBW reared on pineapple leaves were significantly larger than those raised on Florida T. utriculata leaves. These studies allow us to understand the demographics of MBW in Florida and hypothesize why it is a pest in Florida but not in Central America.

<u>Yellowmargined Leaf Beetle</u>: A laboratory study evaluated the effect of four concentrations (0.1, 0.5, 1.0, and 2.0 g per 100 ml of distilled water) of the entomopathogenic fungus *Isaria fumosorosea* on adult yellowmargined leaf beetle (YMLB), *Microtheca ochroloma*, mortality and leaf consumption by the beetle. There were no significant differences in adult mortality among the treatments. However, damage on plants sprayed with 0.5 and 1.0 g per 100 ml of distilled water was significantly less than that on the control plants. Control plants had on average 3.7% more damage than the treated plants.

An open-field study evaluated guidelines for releasing nymphs of the spined soldier bug (SSB), *Podisus maculiventris*, on organically grown bok choy. In three trials on two organic farms during two years, three predator release rates were tested: 0, 3, and 6 nymphs per plant at the first sign of pest adults. The number of YMLB larvae and adults per plant in plots receiving SSB was significantly less than control plots in some weeks. Releases of SSB lowered plant damage by the pest and plants with SSB bugs suffered less proportional leaf area loss compared to control plants. Timely releases of first- and second-instar SSB are capable of reducing larval populations

of YMLB. This control tactic will result in less leaf damage and better commodity product for organic growers. The predators should be purchased at the first sign of adult YMLB and released within five days of receipt.

<u>Ambrosia Beetles</u>: Adult female redbay ambrosia beetles (RAB), *Xyleborus glabratus* were exposed separately to two strains of *I. fumosorosea*, one strain of *Metarhizium brunneum*, and one strain of *Beauveria bassiana*. Contact with any of the entomopathogenic fungi resulted in death of all RAB females within a few days. *Beauveria bassiana* and *M. brunneum* killed RAB females faster. Female RAB dipped in *B. bassiana* and *M. brunneum* had the highest number of viable spores attached to their bodies. No significant differences were observed in the mortality of beetles that were dipped in fungal spore solutions and then offered avocado logs as substrate, compared to beetles that were allowed to walk on logs previously treated separately with the different fungi. This is the first study to demonstrate that entomopathogenic fungi are potential biological control agents against adult RAB.

Two sets of bioassays were conducted to determine the time required for each of three commercial formulations of *B. bassiana*, *I. fumosorosea*, and *M. brunneum* to cause mortality of the ambrosia beetles *Xylosandrus crassiusculus* and *Xyleborus volvulus*. Spore densities of the treatments were all 2,400,000 spores per ml. Contact with all of the entomopathogenic fungi resulted in death of all females. Mean survival ranged from 5.5 and 5.8 days (*B. bassiana*) to 17.1 and 7.8 days (*I. fumosorosea*) for *X. crassiusculus* and *X. volvulus*, respectively. *Beauveria bassiana* killed the beetle females faster, followed by *M. brunneum*, and then *I. fumosorosea*. *Xylosandrus crassiusculus* females dipped in *B. bassiana* had the highest number of viable spores attached to their bodies, with no differences between *M. brunneum* and *I. fumosorosea*. *Xyleborus volvulus* females dipped *in I. fumosorosea* had significantly more viable spores attached to their bodies, followed by *M. brunneum* and then *B. bassiana*. This is the first study to demonstrate that entomopathogenic fungi are potential biological control agents of *X. crassiusculus* and *X. volvulus*.

<u>Sri Lanka weevil</u>: Cold tolerance studies on adults of the Sri Lanka weevil, Myllocerus undecimpustulatus undatus, and analysis of historical collection data were conducted to determine its potential distribution within Florida and spread to comparable climate zones in the United States. An experiment was designed that more closely resembles actual cold events in Florida and other potential distribution areas. Adult weevils collected in August were evaluated with three different treatments: a control maintained at 20°C; a sustained cold exposure (SEC), weevils were acclimated to -5°C, where they were exposed for 10 continual hours, then returned to 20°C; multiple cold exposure (MEC) weevils were immediately reduced from 20°C to -5°C, held for 2 hours, then returned to 20°C for 22 hours, and this was repeated 4 times. Mortality showed little variation between the control and multiple exposures II through V, whereas sustained exposure resulted in higher mortality. Leaf area consumption varied little between the control and multiple exposures II and III, IV and V are similar, whereas weevils under sustained exposure consumed less. This sustained exposure may have other negative impacts on fecundity and longevity.

<u>Mosquitoes</u>: Research was conducted on the potential for the essential amino acid methionine as a biorational mosquito larvicide. Assays were conducted with three pestiferous mosquito

species of three genera: *Culex tarsalis*, *Anopheles quadrimaculatus* and *Aedes albopictus*. Non-target organisms were tested including the honey bee (*Apis mellifera*), a fish (fatheaded minnow, *Pimephales promelas*), and the water flea (*Daphnia magna*).

UTILITY OF FINDINGS

<u>Hydrilla</u>: (a) Because of its broad host range, the use of the midge *C. lebetis* as an augmentative biological control agent of hydrilla will be restricted to Florida and Louisiana where populations of the insect already are established. (b) Cold storage of eggs destined for field release should be avoided. If eggs do require storage, then the number released should be increased to compensate for cold-induced mortality. (c) Incorporating proven biological controls like the fungal pathogen Mt and the hydrilla stem miner *C. lebetis* into an integrated weed management strategy could reduce overreliance onherbicides and provide a more sustainable solution to the hydrilla problem.

<u>Cogongrass:</u> Identification of natural enemies is the first step towards classical biological control of cogongrass. The discovery of potentially useful insect natural enemies in West Java, Uganda and Japan provides a foundation for future efforts to examine the biologies of selected species. For instance, once a vigorous colony of the gall forming midge *O. javanica* is established in West Java, the biology and behavior of the insect will be investigated, and shipments of galled plant material will be sent to the BCRCL, Ft. Pierce, FL, to obtain insects for host range tests.

<u>Brazilian peppertree</u>: Significant progress has been made towards the release of two biological control agents in Florida; the completion of a field release petition for the thrips *Pseudophilothrips ichini*, and near completion of a release petition for the psyllid *Calophya latiforceps*. Results of the host range tests and impact study will determine if another petition requesting field release of the stem boring weevil *A. pipitzi* is warranted. Colonies of psyllids are being maintained for species description (*Calophya* sp.), host range and temperature tolerance studies (*C. terebinthifolii, Calophya* sp.) and/or anticipation of field release (*C. latiforceps*).

<u>Air potato</u>: Studies on the overwintering biology of two biotypes of *Lilioceris cheni* may prove useful in developing release strategies at different latitudes in Florida.

<u>Whiteflies and aphids</u>: These research areas relate to the use of "push-pull" strategies to control whiteflies and other insect pests in vegetables in an integrated pest management program.

<u>Asian Citrus Psyllid:</u> Optimize mass rearing to produce the greatest number of high quality of females for the lowest possible cost of production.

<u>Bromeliad Weevils</u>: Establishment of the Honduran parasitic fly *L. franki* will save populations of native Florida bromeliad species which are currently threatened and endangered due to the presence of the Mexican bromeliad weevil in the state. Information from biological studies of the weevil will help us better understand the ecology of the pest, as well as improve our method for mass-rearing *L. franki*. The studies on development and host-plant interactions of the weevil allow us to understand the demographics of MBW in Florida and hypothesize why it is a pest in Florida but not in Central America.

<u>Cycad Aulacaspis Scale</u>: Introduction and establishment of ladybeetle *P.kashaya* will potentially control populations of the cycad aulacaspis scale on ornamental cycads in Florida and native cycads in Guam.

<u>Yellowmargined Leaf Beetle</u>: Development of augmentation biological control technology with field releases of insectary-reared predatory stinkbugs and green lacewings will provide organic growers a needed management tactic for controlling the leaf beetle on crucifer crops. Initial on-farm experiments with preliminary results from data analysis indicate that augmentative releases of the predatory spined soldier bug can have a significant effect in the control of yellowmargined leaf beetle populations in organically grown crucifers.

<u>Ambrosia Beetles</u>: The research on entomopathogenic fungal strains will determine those that are infective against the red bay ambrosia beetle under field conditions, cause epizootics, and reduce brood production in infested natural areas and commercial avocado groves. Biological studies will provide greater insight into biological control agent/pest interactions.

<u>Mosquitoes</u>: The results demonstrated that methionine was an effective larvicide for all three species and was particularly toxic to *Anopheles quadrimaculatus*. Therefore, methionine has potential for use as a tool for mosquito IPM. As an essential amino acid, methionine is natural and likely to be non-toxic to vertebrates. Non-target assays confirmed that methionine had little to no effect on honey bees, which is critically important for the use of a potential pesticide in the natural environment.

WORK PLANNED FOR NEXT YEAR (2015)

<u>Hydrilla</u>: (a) Continue surveys at other lakes (e.g., Lake Istokpoga, Highlands Co., FL) for additional field hosts of *C. lebetis*. (b) Conduct a mesocosm study in collaboration with Aquatic Vegetation Control, Martin Co., FL, in which harvesting of topped-out hydrilla will be combined with follow-up herbivory by the naturalized meristem mining midge *C. lebetis*. (Note: this project was planned for 2014 but had to be postponed because the midge colony crashed). (c) Investigate competitive interactions between hydrilla and the native eelgrass, *Vallisneria americana*, as influenced by *C. lebetis* herbivory. This mescosm study will determine if midge feeding activity can reduce the competitiveness of hydrilla in favor of the native eelgrass. (d) In a greenhouse study, the maximum water depth (down to 270 cm) that larvae of the midge *C. lebetis* can migrate in the water column to locate hydrilla will be determined using extruded acrylic tubes filled with well water and hydrilla. As part of a new federal grant, a field research and demonstration project will be initiated in collaboration with LAKEWATCH and the USDA to expand on our laboratory research. This mesocosm study will assess the efficacy of combining *C. lebetis* with the fungus Mt and the herbicide imazamox.

<u>Cogongrass</u>: Continue overseas exploration for natural enemies of cogongrass in Africa, Indonesia and Japan.

<u>Brazilian peppertree</u>: Submit petitions for the release of the psyllid *C. latiforceps* and perhaps the weevil *A. pipitzi* to the Techical Advisory Group of USDA/APHIS.

<u>Air potato</u>: Continue to mass rear and release the leaf beetle *Lilioceris cheni* for biological control of air potato.

<u>Whiteflies and aphids</u>: Evaluate the use of annual and perennial plants as natural enemy refuges to enhance the effectiveness of biological control agents in suppressing populations of invasive whiteflies and other insect pests. Test the effect of botanical products on generalist predators and lepidopteran insect pests. Intra-guild predation of the latter also will be studied.

<u>Asian Citrus Psyllid:</u> Host plant quality will be improved by selecting plant species and cultivars and improving growing conditions (media selection, irrigation and fertigation scheduling, pest and disease protection). ACP quality will be improved by determining the best arrangement of these plants (size, arrangement, age, density). An effort will be made to characterize *T. radiata* strains genetically and by means of life history traits to determine if certain strains are better adapted and more capable of suppressing ACP in Florida.

<u>Bromeliad Weevils</u>: Conduct field surveys for detecting possible establishment of *Lixadmontia franki* in natural areas where *L. franki* flies have been released. Quantify effects of temperature on the development, survival, and oviposition rate, as well as hot and cold tolerance, of a population of the Mexican bromeliad weevil native to Belize. Measure the fertility of the Mexican bromeliad weevil. Quantify host-plant effects on the oviposition rate, survivorship, and developmental time of the Mexican bromeliad weevil. Collect tissue samples from Florida and Central American forms of *Tillandsia utriculata* for DNA sampling and comparative analysis.

<u>Yellowmargined Leaf Beetle</u>: Evaluate non-crucifer extracts to repel or mask host plant odors to reduce damage caused by the beetle, and the effect of those extracts on the occurrence of predators in the field. Evaluate two cultural methods, the use of plastic mulch and the removal of dried leaves at the base of the plant, on the populations of the beetle and its predators.

<u>Ambrosia Beetles</u>: Continue bioassays to assess the infectivity and efficacy for controlling adults of the beetles *X. crassiusculus* and *X. volvulus* with three entomopathogenic fungi, *I. fumosorosea*, *B. bassiana*, and *M. brunneum*.

<u>Mosquitoes</u>: Continue experiments with the non-target organisms *Daphnia magna* and *Pimephales promelas* to clarify preliminary results.

<u>Training</u>: Disseminate information on invasive plants and their biological control to a range of audiences in Florida and elsewhere. Mentor graduate and undergraduate students on biological control of invasive plants.

PUBLICATIONS (2014)

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Ali, A. D., Harlow, J. L., Avery, P. B., and V. Kumar. Investigating the role of entomopathogens in whitefly IPM programs. Journal of Entomological Science (submitted Dec 2014).

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Diaz, R., V. Manrique, J. E. Munyaneza, V. G. Sengoda, S. Adkins, K. Hendricks, P. D. Roberts, and W. A. Overholt. 2014. Host specificity testing and examination for plant diseases reveal that the gall-forming psyllid, *Calophya latiforceps* (Hemiptera: Calophyidae), is safe to release for biological control of *Schinus terebinthifolia* (Sapindales: Anacardiaceae). Entomologia Experimentalis et Applicata 154: 1-14.

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Moorthi, P. V., Balasubramanian, C., Avery, P. B., Kubendran, T., T. Rathinakumar, T. and A. N. Banu. 2014. Pathogenicity and proteome production of *Isaria fumosorosea* (=*Paecilomyces fumosoroseus*) Wize isolates against lemon butterfly, *Papilio demoleus* (Papilionidae: Lepidoptera). African Journal of Biotechnology 13: 4176-4182.

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Patt, J. M., Chow, A., Meikle, W. G., Garcia, C., Jackson, M. A., Flores, D., Sétamou, M., Dunlap, C. A., Avery, P. B., Hunter, W. B., Mafra-Neto, A. and J. J. Adamczyk. Development of an autodisseminator for an entomopathogenic fungus, *Isaria fumosorosea*, to suppress Asian citrus psyllid, *Diaphorina citri*, in non-commercial and organic citrus. Biological Control (resubmitted July 2014).

Rogers, M., Avery, P., Ownley, B. and A. Wszelaki. Efficacy of novel biopesticides for management of cucumber beetles (Coleoptera: Chrysomelidae) on Galia muskmelons. Journal of Applied Entomology (submitted Nov 2014).

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Stratman, K. N., W. A. Overholt, J. P. Cuda, A. Mukherjee, R. Diaz, M. D. Netherland, P. C. Wilson. 2014. Temperature-dependent development, cold tolerance, and potential distribution of *Cricotopus lebetis*, a tip miner of *Hydrilla verticillata*. Journal of Insect Science 14. DOI: 10.1093/jisesa/ieu015DOI: <u>http://dx.doi.org/10.1093/jisesa/ieu015</u>.

Takasu, K., Y. Yoshiyasu, A. M. Burrell, P.E. Klein, A. Racelis, J. A. Goosby and W. A. Overholt. 2014. *Acrapex azumai* Sugi (Lepidoptera: Noctuidae) as a possible biological control agent of the invasive weed *Imperata cylindrica* (L.) Beauv. (Poaceae) in the United States. Lepidoptera Science 65: 30-35.

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Medal, J., Gandolfo, D., Gaskalla, R., Overholt, W., Diaz, R., Charudattan, R., Bustamante, N., 77 Davis, B.J., Hibbard, K., Fox, A., Díaz, J., Roda, A., Amalin, D., Hight, S., Stansly, P., Gioeli, K., Osborne, L., Seller, B., McKay, F., Usnick, S., Sudbrink, D., Cuda, J., Pitelli, R., Santana, A., Vitorino, M., Beal, L., Buss, A., Pedrosa, J.H., Bredow, E., Ohashi, D., Wikler, C. and Gravena, R. 2014. Biological control of *Solanum viarum* in Florida, USA: a successful project, p. 77-81. Proceedings of the XIV International Symposium on Biological Control of Weeds, F.A.C. Impson, C.A. Kleinjan and J.H. Hoffmann (eds), 2-7 March 2014, Kruger National Park, South Africa.

Book Chapters:

Baniszewski, J., B.R. Coon, J.P. Cuda, N.E. Harms, M.J. Grodowitz, D.H. Habeck, J.E. Hill, J. Russell, E.N.I. Weeks, 2014. Insects and fish associated with hydrilla, pp. 77-124. In J.L. Gillett-Kaufman, V. Ulrike-Lietze, E.N.I Weeks, Hydrilla Integrated Management. UF|FAS. Gainesville, FL.

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Coon B.R., Harns N.E., Grodowitz, M.J., Weeks, E.N.I., Cuda J.P. 2014. Hydrellia fly parasitic wasp, Trichopria columbiana. UF/IFAS Entomology and Nematology Featured Creatures. Available at https://edis.ifas.ufl.edu/in1040.

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and Invasive Plants. University of Florida. <u>http://plants.ifas.ufl.edu/node/133</u>. Weeks E.N.I. 2014. Hydrilla tuber weevil, *Bagous affinis*. UF/IFAS Entomology and Nematology Featured Creatures. Available at <u>https://edis.ifas.ufl.edu/in1039</u>.

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S-1058 ANNUAL REPORT FOR 2013 AND PLANS FOR 2014

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ACCOMPLISHMENTS

OBJECTIVE 1: Characterize and evaluate the effect of established natural enemies

Sustainable agriculture must provide for growing human demands for crops while minimizing impacts on ecosystems. This is a daunting challenge as agroecosystems have trended towards monocultures with intensive synthetic inputs. Moreover, agricultural landscapes often lack natural habitats that are necessary to support biodiversity. Furthermore, problems associated with agricultural intensification and land-use change may be exacerbated by climate change, which increases the frequency of disturbances, modifies the suitability of habitats, and changes the way species interact. To meet this challenge, farmers must increasingly rely on integrated pest management strategies, including biological control. Biological control of arthropods, weeds, and diseases can promote the stability and diversity of agricultural communities and aid in reducing synthetic inputs. Promoting biological control may thus help farming systems adapt to a rapidly changing world. A special issue was published in Biological Control to consider how multiple global change drivers such as agricultural intensification, land-use change, and climate change affect biological control. The paper discusses these papers and highlight concepts that remain relatively unexplored in the context of global change and biological control. Future research addressing these issues will promote biological control and enhance agricultural sustainability in a rapidly changing world.

Agroecosystems contain complex networks of interacting organisms and these interaction webs are structured by the relative timing of key biological and ecological events. Recent intensification of land management and global changes in climate threaten to desynchronize the temporal structure of interaction webs and disrupt the provisioning of ecosystem services, such as biological control by natural enemies. It is therefore critical to recognize the central role of temporal dynamics in driving predator–prey interactions in agroecosystems. Specifically, ecological dynamics in crop fields routinely behave as periodic oscillations, or cycles. Familiar examples include phenological cycles, diel activity rhythms, and crop-management cycles. The relative timing and the degree of overlap among ecological cycles determine the nature and

magnitude of the ecological interactions among organisms, and ultimately determine whether ecosystem services, such as biological control, can be provided. Additionally, the ecological dynamics in many cropping systems are characterized by a pattern of frequent disturbances due to management actions such as harvest, sowing and pesticide applications. These disturbance cycles cause agroecosystems to be dominated by dispersal and repopulation dynamics. However, they also serve as selective filters that regulate which animals can persist in agroecosystems over larger temporal scales. In a review paper published in Biological Control, key concepts and examples from the literature on temporal dynamics in ecological systems were given, and a framework to guide biological control strategies for sustainable pest management in a changing world was provided.

A major goal of gut-content analysis is to quantify predation rates by predators in the field, which could provide insights into the mechanisms behind ecosystem structure and function, as well as quantification of ecosystem services provided. However, percentage-positive results from molecular assays are strongly influenced by factors other than predation rate, and thus can only be reliably used to quantify predation rates under very restrictive conditions. We developed two statistical approaches, one using a parametric bootstrap and the other in terms of Bayesian inference, to build upon previous techniques that use DNA decay rates to rank predators by their rate of prey consumption, by allowing a statistical assessment of confidence in the inferred ranking. To demonstrate the utility of this technique in evaluating ecological data, data was tested on web-building spiders for predation on a primary prey item, springtails. Using these approaches we found that an orb-weaving spider consumes springtail prey at a higher rate than a syntopic sheet-weaving spider, despite occupying microhabitats where springtails are less frequently encountered. It is suggested that spider-web architecture (orb web vs. sheet web) is a primary determinant of prey-consumption rates within this assemblage of predators, which demonstrates the potential influence of predator foraging behavior on trophic web structure. Such modeling techniques can greatly advance the field of molecular gut-content analysis.

OBJECTIVE 2: Exploration, importation, and assessment of natural enemies for invasive pests

N/A

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

A broad range of environmental conditions likely regulate predator–prey population dynamics and impact the structure of these communities. Central to understanding the interplay between predator and prey populations and their importance is characterizing the corresponding trophic interactions. The structure of the community of natural enemies preying upon the squash bug, *Anasa tristis*, a herbivorous cucurbit pest that severely hinders organic squash and pumpkin production in the United States, was examined using molecular gut analysis. Primer pairs were designed to examine the effects of organic management practices on the strength of these trophic connections and link this metric to measures of the arthropod predator complex density and diversity within an experimental open-field context. Replicated plots of butternut squash were randomly assigned to three treatments and were sampled throughout a growing season. Rowcover treatments had significant negative effects on squash bug and predator communities. In total, 11% of predators were found to have preyed on squash bugs, but predation varied over the season between predator groups (coccinellids, geocorids, nabids, web-building spiders and hunting spiders). Through the linking of molecular gut-content analysis to changes in diversity and abundance, these data delineate the complexity of interaction pathways on a pest that limits the profitability of organic squash production.

UTILITY OF FINDINGS

Research utilizing molecular tools to study foraging behavior of generalist predators has enhanced our understanding of the mechanisms of foraging and the role of alternative prey biodiversity in biological control. Ultimately our ability to discern the strength of all trophic linkages in agricultural food webs, particularly those with alternative prey and intraguild predators that potentially disrupt levels of biological control, will contribute to identifying the role of indigenous natural enemies in management of pest species. New research will examine how field borders influence biological control in cucurbit and soybean crops and further refine the use of mathematical approaches with molecular gut analysis for food web research.

WORK PLANNED FOR NEXT YEAR (2015)

Research protocols utilizing molecular techniques to study food web structure will continue to be developed to identify the structure of predator-prey food webs in agroecosystems and examine the effects of alternative prey and intraguild interactions in biological control. Research will continue to examine the site-specific hunting strategies of predators in soybeans, wheat, alfalfa and corn.

PUBLICATIONS (2014)

Journal Articles

- Whitney, T.D., Philip, B.J., Harwood, J.D. (2014). Tradeoff in two winter-active wolf spiders: increased mortality for increased growth. *Entomologia Experimentalis et Applicata*, 153, 191-198.
- Welch, K.D., Schofield, M.R., Harwood, J.D. (2014). Comparing rates of springtail predation by web-building spiders using Bayesian inference. *Molecular Ecology*, 23, 3814-3825.
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- Symondson, W.O.C., Harwood, J.D. (2014). Special issue on molecular detection of trophic interactions: Unpicking the tangled bank. *Molecular Ecology*, 23, 3601-3604.
- Welch, K.D., Harwood, J.D. (2014). Temporal dynamics of natural enemy-pest interactions in a changing environment. *Biological Control*, 75, 18-27.
- Crowder, D.W., Harwood, J.D. (2014). Promoting biological control in a rapidly changing world. *Biological Control*, 75, 1-7.

Book Chapters

Addendum: Theses, Articles in Press, Abstracts, Unpublished Reports, etc.:

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S-1058 ANNUAL REPORT FOR 2013 AND PLANS FOR 2014

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ACCOMPLISHMENTS

Objective 1. Characterize and evaluate the effect of established introduced natural enemies.

The musk thistle head weevil, *Rhinocyllus conicus* Froelich, was introduced into Northwest Louisiana in 1975 for control of musk thistle, *Carduus nutans* L. Initially released near Hanna, LA (Red River Parish) only one subsequent survey was conducted in 1976 by the researchers who made that initial release. Beginning in 1994, periodic surveys (2001, 2004, 2008, and 2010) of musk thistle in Northwest Louisiana were conducted to determine the range expansion of the musk thistle weevil. Beginning in 2004 the surveys were expanded into southwestern Arkansas. Since our initial survey in 1994, *R. conicus* has continued to expand its range north and weevil numbers continue to increase in areas of expansion.

Although another survey with emphasis on expansion into southwestern Arkansas was planned for 2014, other priorities prevented time for the survey.

Objective 3. Implementation, evaluation, and enhancement of biological control.

The salvinia weevil, *Cyrtobagous salviniae* Calder and Sands, was imported into the US for control of the invasive aquatic fern, giant salvinia (*Salvinia molesta* Mitchell). This weevil was released into Cross Lake in March of 2013 to investigate its potential as a biocontrol agent in Northwest Louisiana. The winter of 2013/2014 was unusually cold for Northwest Louisiana. During one 3-day period from 5 Jan to 8 Jan 2014, 40 continuous hours of below freezing temperatures were recorded in the Shreveport/Bossier City area. Because of the low temperatures experienced during the winter, no weevils were recovered from any of the 2013 release sites during the spring of 2014. Additionally, the giant salvinia was severely impacted by the cold temperatures and surviving salvinia could only be found in the primary stage of growth and only sparsely around cypress trees and within protected areas of alligatorweed and other aquatic plants.

A project was initiated in Sept 2014 to determine if adding mulch on top of weevil infested giant salvinia would improve the temperature profile at the water surface and thus improve winter survival of the salvinia weevil.

Cross Lake. On 16 Sept 2014, 6 floating enclosures made from PVC pipe were established on the west end of Cross Lake in Shreveport, LA. The outer portions of the enclosures were made of 4-inch diameter PVC and were 4' X 4' square. Enclosures were anchored to the bottom with rope and cider blocks to prevent movement. The inner cage was made of 1-inch diameter PVC covered with plastic-coated wire mesh. Two Hobo data loggers (Onset Computer Corporation, Bourne, MA 02532) were placed in each enclosure to monitor temperature, one approximately an inch above the water surface and one an inch below the surface.

On 24 Sept 2014, approximately 288 salvinia weevils were released into each of the 6 enclosures. Weevils and salvinia were from the LSU AgCenter rearing facility in Houma, LA. On 15 Oct 2014, 4 of the enclosures were sampled to determine the weevil population and adult weevil numbers averaged 12.8 weevils / kg of giant salvinia. On 12 Nov 2014, just prior to the first winter freeze, two of the enclosures were mulched with approximately 6 inches of pine straw and the inner cages on two were covered with plastic.

Enclosures were sampled for weevils again on 12 Nov, 16 Dec 2014, and 20 Jan 2015. Temperature data from all Hobo data loggers were also downloaded on 12 Nov 2014 and 20 Jan 2015.

In the non-mulched enclosures, salvinia weevil numbers declined after 15 Oct and remained low through 20 Jan 2015. During this period, the lowest air temperature recorded was 19° F on 8 Jan and a total of 169 hours of below freezing temperatures were recorded.

Low temperatures in the pine straw mulched enclosures were similar to the un-mulched enclosures although only 153 hours of below freezing temperatures were recorded. By the first sample date after mulching (16 Dec 2014), most of the pine straw and some of the giant salvinia mat had sunk. This exposed the Hobos to ambient air temperature and resulted in air temperatures in the mulched and non-mulched enclosures being very similar. Although the enclosures were re-mulched on 22 Dec, that mulch and even more salvinia had sunk by the 20 Jan 2015 sample date. In these enclosures weevil numbers peaked at 15.2 weevils / kg salvinia on 16 Dec before declining to zero on 20 Jan.

Weevils in the plastic covered enclosures also peaked on 16 Dec at 28.0 weevils /kg salvinia. Temperatures in these enclosures generally remained above 40° F. Temperature extremes (highs and lows) were greatly moderated in the plastic covered enclosures from 12 Nov until 21 Dec. On the 20 Jan sample date, it was found that both plastic covered enclosures had suffered considerable damage to the plastic from animal activity.

Lake Bistineau. On Lake Bistineau in Northwest Louisiana, 4 floating enclosures were established in a giant salvinia infested area on 3 Nov 2014. These enclosures were similar to those used on Cross Lake but with only the outer floating 4-inch PVC pipe. Weevils and salvinia came from the Red River Research Station's greenhouse weevil rearing facility and weevil numbers were low and the salvinia was not as well developed

as that obtained from Houma, LA. Two of the enclosures were mulched with pine straw on 11 Nov and two were left un-mulched. No plastic covered enclosures were used on Lake Bistineau. Enclosures were sampled for weevils on 15 Dec 2014 and 20 Jan 2015.

No weevils were recovered on any of the sample dates from Lake Bistineau. This is likely the result of low weevil numbers at infestation, poor condition of the salvinia, and the late infestation date (3 Nov). Because of this, the salvinia mat within the enclosures remained intact during the sampling period as did the pine straw mulch. Figure 1 shows the moderating effect of the pine straw mulch on air temperatures within the mulch compared with the non-mulched enclosures.

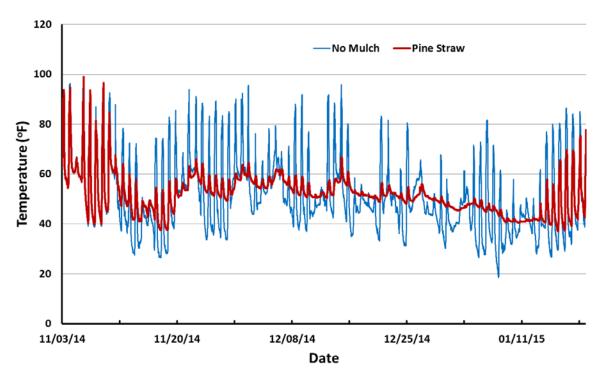


Figure 1. Air temperature in mulched and non-mulched enclosures on Lake Bistineau. Pine straw mulch applied to two enclosure on 11 Nov 2014.

UTILITY OF FINDINGS

The musk thistle head weevil continues to survive and spread across northwest Louisiana and into southwestern Arkansas since its release in 1975. Monitoring biological control agents after their initial release is important to our understanding of these agents and their ability to establish and spread within their targets host range.

The salvinia weevil has been a successful biological control for giant salvinia worldwide. In northern Louisiana, efforts to establish the weevil have been less successful because of winter temperatures that can kill off the weevils but not the giant salvinia. It is hoped that current studies might yield promising techniques that might improve the salvinia weevil's survival during winters in north Louisiana.

WORK PLANNED FOR NEXT YEAR (2015)

A musk thistle head weevil survey will be conducted in 2015 with emphasis on southwestern Arkansas and southeastern Oklahoma. Surveying southeastern Oklahoma had been planned for 2013, but thunder storms and tornadic weather in Oklahoma at the time of our survey prevented work there.

In 2015, new salvinia weevil releases will be made on Cross Lake and monitored through the year. Additional mulching experiments will be planned and started in the early fall of 2015. This may include examining other materials for mulching as well as varying the depth of pine straw mulch applied to the giant salvinia.

PUBLICATIONS (2014)

None.

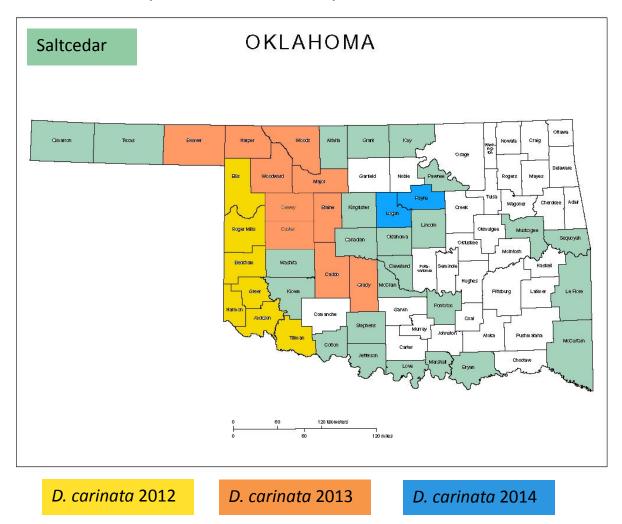
S-1058 ANNUAL REPORT FOR 2014 AND PLANS FOR 2015

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ACCOMPLISHMENTS

Objective 1. Characterize and evaluate the effect of established natural enemies.

Diorhabda species have continued spreading throughout Oklahoma, and were documented in Payne and Logan counties in 2014 (See Figure below). Our primary objective is to describe the impact of native predators on *Diorhabda carinulata* populations as they move into Oklahoma. Preliminary preference and suitability studies indicate that common Coccinellidae and *Zelus tetracanthus* will readily feed on *Diorhabda* but only *Zelus* will survive.



Objective 2. Exploration, characterization and assessment of natural enemies for invasive species.

NA

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

Completed 4th year of studies evaluating natural enemy abundance and movement within winter canola landscapes. Studies are designed to evaluate the impact of this expanding biofuel crop on insect predators, and pollinators. Newly summarized data indicate that canola and winter wheat are significant sources of natural enemies including competing aphid parasitoids. In winter canola, results from an exclusion experiment revealed that predators and parasitoids are capable of preventing cabbage aphids from reaching economic injury levels.

Field studies documented that *Diaeretiella rapae* (M'intosh) (Hymentoptera: Braconidae) is the primary aphid parasitoid in winter canola fields in Oklahoma. Parasitism rates have increased in recent years and this may be attributed to an overall increase in the abundance of this species.

Laboratory studies were completed investigating host-plant-aphid responses of *Diaeretiella rapae*. Results indicated that chemical cues from mummies are critical for emerging adults and their ability to find aphids on host plants. Additionally, parasitism results indicate that *D. rapae* are likely adapted to utilizing aphids on *Brassica* plants.

Laboratory studies were completed investigating the effects of insecticide treated green peach aphids on the survival and development of Coccinellidae. We also investigated competitive interactions among Coccinellidae on insecticide treated canola plants. Flonicamid and Sulfoxaflor were the narrow spectrum insecticides used in this study. Results indicated that Flonicamid treated aphids had no effect on survival or development of Coccinellidae. Feeding on Sulfoxaflor treated aphids, however, 1) increased development times of *Coccinella septempunctata* and only males survived feeding assays, and 2) decreased size of male *Hippodamia convergens. Coccinella septempunctata* were more susceptible to intraguild predation when aphid resources were suppressed by either insecticide, but the effect was most pronounced in microcosms treated with Sulfoxaflor.

UTILITY OF FINDINGS:

Diorhabda carinulata populations are moving into Oklahoma at a rapid pace. We will continue to evaluate whether native natural enemies pose a significant threat to *D. carinulata* and if suppression of *Tamarix* in Oklahoma is being disrupted. Natural enemies in intensively managed winter canola are contributing towards aphid suppression, but frequent insecticide usein canola and competition among natural enemies in wheat/canola landscapes may be reducing their impact in canola.

WORK PLANNED FOR NEXT YEAR (2015)

Continue characterization of generalist predator assemblages associated with *D. carinulata* throughout western Oklahoma. Studies on the suitability of *D. carinulata* as a prey source for generalist predators will be completed. Continue studies describing intraguild dynamics among insect predators and parasitoids in winter wheat and canola systems in the Southern Plains.

PUBLICATIONS (2014)

Elliott, N. C., G. F. Backoulou, K. L. Giles and T. A. Royer. 2014. Aphids and parasitoids in wheat and nearby canola fields in central Oklahoma. Southwestern Entomol. 39: 23-28.

Robideau, X. M. 2014. Effects of insecticide treated green peach aphids (*M. Persicae*) on the survival and development of Coccinellidae. M.S. Thesis, Oklahoma State University.

Ferguson, M. E. 2014. The effect of chemical cues on host finding and host acceptance behavior of Diaeretiella rapae (M'intosh) (Hymentoptera: Braconidae). M.S. Thesis, Oklahoma State University.

S-1058 ANNUAL REPORT FOR 2014 AND PLANS FOR 2015

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ACCOMPLISHMENTS

Objective 1. Characterize and evaluate the effect of established introduced natural enemies.

The natural enemy complex of the lecanium scales, *Parthenolecanium corni* and *Parthenolecanium quercifex*, in the urban landscape of GA (Kris Braman, UGA), SC (JH Chong, Clemson), NC (Steve Frank, NCSU) and VA (Peter Schultz, VT) was characterized from 2009-2014. A total of 21 parasitoid species and 12 coleopteran and neuropteran predators were documented, of which *Coccophagus lycimnia* (Aphelinidae), *Blastothrix* spp. (Encyrtidae), *Encyrtus* spp. (Encyrtidae), *Metaphycus* spp. (Encyrtidae), *Eunotus* sp. (Pteromalidae), *Pachyneuron* sp. (Pteromalidae), *Chrysoperla rufilabris* (Chrysopidae), *Hyperaspis signata* sp. group (Coccinellidae) and *Anthribus nebulosus* (Anthribidae) were found to be the most numerous. Species diversities of the parasitoids and predators were largely similar across the four states. Among the major parasitoids, *C. lycimnia* was the only species emerged from scale insect nymphs, whereas other species emerged from adults. The natural enemies were active from early March to September. Parasitism rate ranged from 27 to 92%, whereas predation rate was difficult to assess. All parasitoid species that attacked adults were able to reduce the fecundity of the scale insects.

Objective 2. Exploration, importation, and assessment of natural enemies for invasive pests.

No specific project was conducted in 2013.

Objective 3. Implementation, evaluation, and enhancement of biological control.

Compatibility of a novel miticide, cyflumetofen (Sultan), with the predatory mites *Amblyseius swirskii* and *Phytoseiuslus persimilis* was evaluated in greenhouses in CA (Jim Bethke, UC Extension), FL (Lance Osborne, UF) and SC (J.-H. Chong, Clemson). The study found that cyflumetofen did not cause elevated acute and residual mortality in both predatory mite species. The new miticide also did not cause a reduction in the reproduction of *A. swirskii*. The project demonstrated that cyflumetofen can be used with biological control in greenhouse ornamental plant production system.

UTILITY OF FINDINGS

The results of this project were disseminated mainly through extension workshops and presentations, as well as extension articles in trade journals and newsletters. This project showed that the peak activity periods of the parasitoids and predators of *Parthenolecanium* spp. coincide with the timing of scale insect crawler emergence. Current recommendation for the management of *Parthenolecanium* spp. includes application of contact insecticides against the crawlers in late May to mid-June. Considering the potentially detrimental impact of foliar applications of contact insecticides on the natural enemies, we suggest that the current recommendation to be modified to reduce the non-target impact of applications. Growers and landscape care professionals targeting lecanium scales should not apply contact insecticides. Instead, an indirect application (i.e. soil drench, soil injection, trunk spray and granule) of systemic neonicotinoids (acetamiprid, clothianidin, dinotefuran, imidacloprid and thiamethoxam) should be applied in May to achieve reduction of the population of nymphs and to avoid direct impact on the natural enemies. This recommendation is applicable to all states studied (GA, NC, SC and VA) because the natural enemy assemblage and activity periods are similar or closely aligned.

WORK PLANNED FOR NEXT YEAR (2015)

Study on the compatibility of cyflumetofen with *A. swirskii* and *P. persimilis* will be repeated in the spring of 2015.

PUBLICATIONS (2015)

Peer-reviewed articles:

Chong, J.-H. and E. R. Camacho. 2014. Distribution, host plants and life history of *Melanaspis deklei* (Hemiptera: Coccoidea: Diaspididae) in South Carolina, U.S.A. Acta Zoologica Bulgarica, Supplement 6: 13-19.

Rameshkumar, A., J. S. Noyes, J. Poorani, and J.-H. Chong. 2013. Description of a new species of *Anagyrus* Howard (Hymenoptera: Chalcidoidea: Encyrtidae), a promising biological control agent of the invasive Madeira mealybug, *Phenacoccus madeirensis* Green (Hemiptera: Sternorrhyncha: Pseudococcidae). ZooTaxa 3717: 76-84.

Trade journal articles:

Chong, J.-H. 2015. Wax myrtle scale, *Melanaspis deklei*. The South Carolina Nurseryman. January/February 2015, pp. 32-33.

Chong, J.-H. 2014. Scale insects: The banes of ornamentals. The South Carolina Nurseryman. September/October 2014, pp. 26-28.

Robayo Camacho, E. and J.-H. Chong. 2014. Camellia scale, *Lepidosaphes camelliae*. The South Carolina Nurseryman May/June 2014, pp. 18-19.

Chong, J.-H. and E. Robayo Camacho. 2014. Oak lecanium scale, *Parthenolecanium quercifex*. The South Carolina Nurseryman January/February 2014, pp. 18-19.

S-1058 ANNUAL REPORT FOR 2014 AND PLANS FOR 2015

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ACCOMPLISHMENTS

The following information details the major activities completed and specific objectives met during 2014. Efforts primarily focused on biological control activities directed against two invasive pests - hemlock woolly adelgid, *Adelges tsugae*, and emerald ash borer, *Agrilus planipennis*. Research also was conducted on walnut twig beetle, *Pityophthorus juglandis* (vector of *Geosmithia morbida*, which causes thousand cankers disease on black walnut), as well as life history studies on a new parasitoid species, *Theocolax n.* sp., of this beetle. Biological control activities also were initiated against kudzu bug, *Megacopta cribraria*. Significant results, including major findings, developments, or conclusions, and key outcomes are provided.

Objective 1. Characterize and evaluate the effect of established introduced natural enemies. A Biological Control Demonstration Site has been established near Cosby, TN, in the Great Smoky Mountains National Park (GRSM). Multiple species of natural enemies of hemlock woolly adelgid were released into four large, tree cages (one species/cage). The four predator species were: Laricobius nigrinus (already established at the site), L. osakensis – northern Japan strain, L. osakensis - southern Japan strain (both strains of L. osakensis were released as larvae), Sasajiscymnus tsugae, and Scymnus coniferarum (both S. tsugae and S. coniferarum were released as adults). Trees within these cages and within this area were sampled every week (beginning late September) for individuals of each species. In beat-sheet sampling conducted in Fall 2013 and Spring 2014, L. nigrinus and the native species L. rubidus were collected regularly. No L. osakensis or S. coniferarum were collected, and only one S. tsugae was collected in beat-sheet sampling in Fall 2013. Emergence traps were placed under six trees, and L. nigrinus and L. rubidus were collected from several traps. Two L. osakensis were collected from traps under one of the release trees. Recovery from emergence traps indicates that this introduced species was able to feed as larvae, undergo larval development on the plant, drop and pupate successfully in the soil, and emerge as adults. This Site is intended to serve as a resource and educational tool for other land managers interested in incorporating biological controls into their management programs directed against hemlock woolly adelgid.

To reduce the spread and effect of this invasive pest and preserve hemlock in its native range, releases of the introduced predators *S. tsugae* and *L. nigrinus* have been conducted throughout the GRSM. These two predators have been recovered from several areas of release. Several significant findings have resulted from research on these adelgid predators in the GRSM. In

areas where *S. tsugae* has been recovered, regression analysis indicated that recovery of *S. tsugae* was associated with elevation of the release sites and average maximum temperature seven days following release, and normalized difference vegetation index values were higher in *S. tsugae* recovery sites than non-recovery sites which indicates that hemlocks are healthier in *S. tsugae* recovery sites than non-recovery sites. Additionally, relatively higher hemlock mortality was observed in non-recovery sites than recovery sites, and percent crown transparency, percent live crown, and percent branch dieback were also significant with presence of *S. tsugae*. Populations of *L. nigrinus* at a release site that was monitored weekly for three years have been steadily increasing. Also, a method for identifying numbers of *L. nigrinus* per tree based on emergence was identified and showed strong population densities over time. Finally, these two predatory species have been observed to coexist in some field release sites, and this coexistence may enhance their impact on adelgid populations by providing prolonged feeding. These findings, in conjunction with persistence of hemlocks in several areas of GRSM, indicate management efforts incorporating introduced biological control agents against *A. tsugae* can protect eastern hemlock.

A cooperative project was developed to implement a multi-state, regional approach to assess the establishment and impact of *L. nigrinus* in areas where it has been released and recovered. Colleagues on this assessment include Joe Elkington (Massachusetts), Scott Salom (Virginia), Bud Mayfield and Richard McDonald (North Carolina), and Jerome Grant and Greg Wiggins (Tennessee). Three sites (Blackberry Farm, Elkmont Campground, and the Biological Control Demonstration Site) were selected and established in Tennessee in Fall 2014. At each site five study trees were selected and tree health characteristics (canopy density, canopy transparency, percent canopy dieback, and live crown ratio) were assessed for each tree. On each tree, selected branchlets were caged and excluded from predators, and other branchlets remained uncaged and exposed to predation. One additional branchlet was selected and caged but the cage was left open as a means of exposing adelgids to predation while accounting for cage effects. For each branchlet, the new growth for all twigs emanating from the stem was measured and the adelgids on the new growth were counted and recorded. Data will be collected in 2015 to quantify predation of *L. nigrinus* in a field setting and enhance understanding of the impact of this predator on hemlock woolly adelgid across a wide geographical area.

Objective 2. Exploration, importation and assessment of natural enemies for invasive pests. No international activities were conducted during this reporting period; a trip was planned to collect additional predatory beetles of hemlock woolly adelgid from hemlock in Japan. However, this trip was cancelled to enable efforts to focus on additional rearing and evaluation of previously collected organisms. Preparations are underway to rear and assess an exotic parasitoid, *Spathius galinae*, of emerald ash borer in our Beneficial Insects Containment Laboratory at the University of Tennessee. This Asian parasitoid has gone through the evaluation process at Quarantine Labs in the northeastern U.S. and is seeking approval for release. We will be conducting geographical synchrony and compatibility studies in the southeastern U.S.

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

As part of efforts to implement biological control against invasive species, the Lindsay Young Beneficial Insects Laboratory at the University of Tennessee continued to rear and release natural enemies of the hemlock woolly adelgid. Several challenges in rearing predatory beetles during 2014 resulted from reduced food quantity and quality of their prey (hemlock woolly adelgid), which was attributed to the 'Polar Vortex.' The following information details specific rearing and release activities during 2014:

Sasajiscymnus tsugae

Production: Fewer *S. tsugae* were reared in 2014, as more effort (and prey) went to producing other beetles; 114,718 eggs were harvested, producing 60,549 adults. **Releases:** 11,766 released in GRSM, 11,191 in South Cherokee National Forest, 8,030 in Catoosa Wildlife Management Area, and 7,399 on the future Cumberland Trail. **Notes:** About 16,000 beetles were held in storage to be used in 2015; about 2,000 beetles will be sent to Young-Harris College to augment rearing colonies.

Laricobius nigrinus

Production: Due to lack of adelgid quality in 2014 and the need to rear as many *L. osakensis* as possible, fewer *L. nigrinus* were reared. 13,822 larvae were placed in soil aestivation containers; more than 4,000 adults emerged (33% survival). **Releases:** 595 released in GRSM, 825 in South Cherokee National Forest, 755 in Catoosa Wildlife Management Area, 387 in Piney Falls State Natural Area, 171 on the future Cumberland Trail, and 655 at Cumberland Gap National Historic Park. **Notes:** 112 beetles were provided to U.S. F.S. for research; ca. 100 remain in lab for rearing.

Laricobius osakensis (Kansai)

Production: 13,385 mature larvae were harvested and placed in soil aestivation containers; ca. 3,000 adults emerged (ca. 28% survival, which was similar to 2013). **Releases:** Almost all releases were made at two sites: GRSM (921) and South Cherokee Nat'l. Forest (781); 271 were released at Laurel-Snow State Natural Area. **Notes:** 222 were shipped to Univ. GA for colony establishment; 28 were shipped to VA Tech for research; ca. 700 remain in lab for rearing.

Scymnus coniferarum

Production: By Jan. 2014, this colony had less than 40 beetles, which were shipped to Virginia Tech for research. **Releases:** None. **Notes:** Trees at 2013 release site in Catoosa Wildlife Management Area were sampled in June 2014; no *S. coniferarum* recovered.

As part of continued efforts to implement biological control, research was expanded to improve application, assessment, and implementation of biological control tools against emerald ash borer. Specific objectives are to: 1) assess new technologies to establish and evaluate incidence and seasonal emergence of three introduced parasitoids (*Oobius agrili* – egg parasitoid; and *Spathius agrili* and *Tetrastichus planipennisi* – larval parasitoids), 2) evaluate alternate rearing strategies to enhance their production, rearing, and/or establishment, 3) assess incidence of native parasitoids, such as *Atanycolus hicoriae*, and 4) assess impact of parasitoid releases via new technologies on tree health and survival.

Parasitoid release sites and sites where field cage studies could be implemented were identified. Three parasitoid species continued to be released at new locations, as well as re-released at several previous release sites. Tree health characteristics (dbh, canopy density and transparency, percent live crown, crown position, and crown class) were assessed; trees were felled/monitored for parasitoids; pan traps continue to be deployed to assess parasitoid establishment.

We continued to assess the potential to rear and/or establish parasitoids in field cages to enhance release and establishment, as well as assess overwintering of borer parasitoids. *S. agrili* adults began emerging from barrels on 4 Aug. and ended on 19 Aug.; sex ratio was 1:1.5 (M:F). No *T. planipennisi* or *O. agrili* were observed in barrels. In a study using naturally-grown ash saplings in cages as a field insectary, no parasitoids emerged. Additionally, no parasitoid larvae of any species were observed. For larvae recovered, development was slowed, and no larvae developed past second instar. It is unclear why parasitism was not observed. Borer emergence/cage averaged four adults/cage, which is low. Perhaps uncharacteristically cold temperatures during Jan. and Feb. 2014 ('Polar Vortex') caused mortality of many borers and parasitoids.

Major findings of biological control efforts against emerald ash borer included: 1) successful overwintering of *S. agrili* in the southern U.S. was observed in tree cage studies at two locations, 2) large tree cages yielded highly successful overwintering of *S. agrili* in all tree cages at one study site, 3) artificially infesting tree cages with additional borer adults greatly enhanced parasitism and overwintering of *S. agrili*, 4) a Protocol to enhance overwintering and establishment of *S. agrili* using large tree cages was developed, 5) a Preliminary Protocol to assess establishment of *S. agrili* at specific release sites was developed, 6) a potential rearing-enhancing procedure for larval parasitoids was identified, and 7) no *T. planipennisi* were recovered from any release area or study site.

UTILITY OF FINDINGS:

Biological control is crucial to controlling hemlock woolly adelgid and emerald ash borer as it is the only viable management tool for hemlocks and ash over vast areas, and also has the potential to permanently reduce population densities of hemlock woolly adelgid and emerald ash borer. Therefore, improving and expanding mass production of biological control agents, both predaceous beetles and parasitoids, is of utmost importance. Discovering and utilizing new release areas, especially those on the western and southern edges of expansion of both invasive forest pests in Tennessee, will enable personnel to better plan biological control releases to improve the chances of reducing populations of these invasive pests in newly-infested areas, which should ultimately reduce the damage that they cause in forest and urban settings.

Hemlock woolly adelgid and emerald ash borer have killed hundreds of thousands of trees throughout the eastern U.S., which cause tremendous economic, aesthetic, and environmental losses. For example, the loss of eastern hemlock as a 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 GRSM. 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 complex of natural enemies has been released against the hemlock woolly adelgid in the southeastern U.S. These species complement each other, as they feed on various life stages and

at varying times of the year. Two predator species are established at numerous locations in the southeastern U.S.; research at the University of Tennessee has demonstrated that both species can survive together on the same infested hemlock tree. Predator compatibility will elicit a more efficient and successful program for control of the hemlock woolly adelgid. Research on recovery and establishment of introduced predators in GRSM continues to provide valuable information about *S. tsugae* and *L. nigrinus* establishment in the Park, as well as expectations for recovery.

A new invasive insect threat (emerald ash borer) has been documented in 30 counties in Tennessee. 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 the University of Tennessee 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. The use of large tree cages has been successfully integrated to enhance establishment of at least one introduced biological control agent of emerald ash borer. Results of this research have demonstrated that these introduced natural enemies can overwinter and survive in Tennessee. The ultimate goal is to provide sufficient mortality of emerald ash borer to protect trees and minimize adverse environmental impacts.

WORK PLANNED FOR NEXT YEAR (2015):

Objective 1. Characterize and evaluate the effect of established introduced natural enemies.

1) Continue to assess coexistence of introduced predatory beetles of hemlock woolly adelgid

and assess interactions with native predatory species.

- 2) Continue to assess a Biological Control Demonstration Project to evaluate mass releases of multiple species of natural enemies of hemlock woolly adelgid.
- 3) Cooperate in a multi-state assessment of the impact of the introduced predator *L. nigrinus* following mass field releases on populations of adelgid and tree health.
- 4) Continue to investigate the use of whole-tree cages to promote and evaluate establishment, reproduction, and survival of *L. osakensis*.
- 5) Continue to assess establishment of field populations of S. tsugae and L. nigrinus.
- 6) Continue to assess incidence of established native biological control agents of emerald ash

borer and walnut twig beetle in southern climates.

- 7) Assess recovery and establishment of *S. agrili* and *T. planipennisi* in barrels containing ash wood collected from release sites, as well as dissection of felled trees.
- 8) Finalize and field test Overwintering and Establishment Protocol for emerald ash borer using tree cages.
- 9) Develop and field test Preliminary Establishment Assessment Protocol.
- 10) Continue to monitor open release sites for establishment of egg and larval parasitoids of emerald ash borer.
- 11) Continue developmental studies on the parasitoid, *Theocolax* sp., on walnut twig beetle.

Objective 2. Exploration, importation and assessment of natural enemies for invasive pests.

1) Continue to evaluate impact of multiple species complexes of natural enemies on

hemlock woolly adelgid and tree characteristics using whole-tree canopy enclosures.

- 2) Continue to assess the Japanese predator *Laricobius osakensis* against hemlock woolly adedgid in southern climates.
- 3) Evaluate, release and assess a new introduced parasitoid (*S. galinae*) of emerald ash borer in southern climates.
- 4) Investigate potential introduced species for importation and evaluations against insect pests and weeds in the new University of Tennessee Beneficial Insect Containment Laboratory.

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

- 1) Continue to improve rearing methodologies for predators (emphasizing *L. osakensis*) of hemlock woolly adelgid; rear large numbers for field release.
- 2) Continue to mass rear and release predator species against the hemlock woolly adelgid.
- 3) Locate sites to establish as a field insectary for rearing large numbers of predatory beetles.
- 4) Continue to investigate the use of whole-tree cages to promote and evaluate establishment, reproduction, and survival of *L. osakensis*.
- 5) Continue and expand releases and evaluations of parasitoids against emerald ash borer.
- 6) Assess new cage protocols for establishing and/or rearing parasitoids of emerald ash borer (continue to assess novel ways to enhance release and establishment).

PUBLICATIONS (2014)

Regional (Joint) Publications: None.

State Station or Agency Publications: None

Journal Articles:

Hooie, N., G. Wiggins, P. Lambdin, J. Grant, S. Powell, and J. Lelito. 2015. Native parasitoids and recovery of *Spathius agrili* from areas of release against emerald ash borer in eastern Tennessee, USA. Biocontrol Science and Technology 25:345-351.

Other Publications (Abstracts and Proceedings):

Grant, J., K. J. Copley, S. D. Powell, and G. J. Wiggins. 2014. Emerald ash borer activities in Tennessee: Three years after.... Proceedings of the 55th Southern Forest Insect Work Conference, New Orleans, LA, July 23-26, 2013, pp. 32-33.

Grant, J. F., S. D. Powell, G. J. Wiggins, and K. J. Copley. 2014. Biological control activities and distribution of emerald ash borer in Tennessee. Proceedings of the 24th USDA Interagency Research Forum on Invasive Species, Annapolis, MD, January 8-11, 2013, pg. 73.

Grant, J. F. and P. L. Lambdin. 2014. Invasive European plant species in the Southern Appalachians, USA: Potential targets for collaboration and cooperation in a new beneficial insects quarantine laboratory. Proc. 4th International Symposium on Weeds and Invasive Plants (Agricultural Weeds and Plant Invaders), Montpellier, France, May 18-23, 2014, pg. 143. Grant, J. F., G. J. Wiggins, and P. L. Lambdin. 2014. Biological control of introduced European musk thistle in the Southern Appalachians, USA: A 25-year assessment of benefits and risks. Proc. 4th Int'l. Symp. on Weeds and Invasive Plants, Montpellier, France, May 18-23, pg. 142.

Hakeem, A., J. Grant, G. Wiggins, P. Lambdin, R. Rhea, F. Hale, and D. Buckley. 2014. Using remote sensing with on-site assessments to determine associations between tree health, tree mortality, and predators of hemlock woolly adelgid. Proceedings of the 55th Southern Forest Insect Work Conference, New Orleans, LA, July 23-26, 2013, pp. 46-47.

Hakeem, A., J. Grant, P. Lambdin, G. Wiggins, F. Hale, D. Buckley, and R. Rhea. 2014. Environmental factors affecting establishment of *Sasajiscymnus tsugae*, an introduced predator of hemlock woolly adelgid. Proc. 24th USDA Interagency Research Forum on Invasive Species, Annapolis, MD, Jan. 8-11, 2013, pg. 74.

Hooie, N., P. Lambdin, J. Grant, G. Wiggins, S. Powell, and J. Lelito. 2014. Native parasitoids and recovery of *Spathius agrili* from areas of release against emerald ash borer in eastern Tennessee. Proc. 56th Southern Forest Insect Work Conf., Charleston, SC, July 22-25, pp. 30-31.

Nix, K. A., P. L. Lambdin, J. F. Grant, M. Windham, and P. Merten. 2014. Survey for potential natural enemies for control of thousand cankers disease in east Tennessee. Proceedings of the 55th Southern Forest Insect Work Conference, New Orleans, LA, July 23-26, 2013, pp. 45.

Wiggins, G., J. Grant, R. Rhea, A. Hakeem, P. Parkman, and P. Lambdin. 2014. Biological control of hemlock woolly adelgid in Tennessee: Twelve years later. Proceedings of the 56th Southern Forest Insect Work Conference, Charleston, SC, July 22-25, pg. 25.

Wiggins, G., J. Grant, R. Rhea, P. Parkman, J. Webster, E. Benton, and P. Lambdin. 2014. Establishment of a hemlock woolly adelgid biological control demonstration site in the Great Smoky Mountains National Park. Proceedings of the 56th Southern Forest Insect Work Conference, Charleston, SC, July 22-25, pp. 32-33.

Wiggins, G., J. Grant, P. Lambdin, N. Hooie, and J. Lelito. 2014. Parasitoids to the rescue?: Biological control efforts against emerald ash borer in Tennessee. Proceedings of the 55th Southern Forest Insect Work Conference, New Orleans, LA, July 23-26, 2013, pp. 46.

Addendum: Theses, Articles in Press, Abstracts, Unpublished Reports, etc.: Hooie, N. A. 2014. The seasonality of two parasitoids (*Spathius agrili* and *Tetrastichus planipennisi*) of the emerald ash borer, *Agrilus planipennis*, and a survey for native natural enemies of the Emerald Ash Borer in eastern Tennessee. University of Tennessee Thesis, 53 pp.

Hakeem, A., J. F. Grant, Wiggins, G. J., P. L. Lambdin, and J. R. Rhea. 2014. Contributing factors impacting establishment and recovery of *Sasajiscymnus tsugae*: Implications of predator establishment on hemlock health. USDA Interagency Research Forum on Invasive Species, Jan. 7-10, Annapolis, MD. (In Press)

Wiggins, G., J. Grant, N. Hooie, P. Lambdin, and J. Lelito. 2014. Parasitoids collected from ash species in Tennessee: Implications for biological control of emerald ash borer in the southern U.S. USDA Interagency Research Forum on Invasive Spp., Jan. 7-10, Annapolis, MD. (In Press)